Nutrition, Fertility, and Human Reproductive Function

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Edited by
Kelton Tremellen
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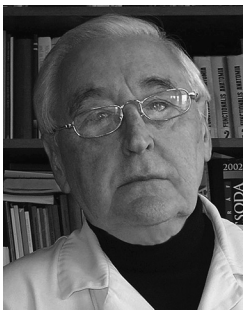
This book is dedicated to Andrew E. Czeizel, in honor of his pioneering work in the field of periconception nutritional supplements for the prevention of congenital abnormalities in children.



Professor Czeizel was born in Budapest, Hungary in 1935. Following completion of medical and scientific training (PhD), he became a clinical geneticist and headed up the Hungarian Congenital Abnormality Register, the world's first national registry for the analysis of the causes of congenital abnormalities in children. This work spurred his interest in public health initiatives that may prevent these types of abnormalities. In 1984 Professor Czeizel went on to established a preconception family planning program in Hungary that focused on getting women into optimal health both before conception and during the vital early part of pregnancy so as to optimize fetal organ development. Part of this program consisted of encouraging women to take prenatal nutritional supplements (vitamins including folate, minerals, and trace elements) in the hope that this may help prevent congenital abnormalities. It was through this service that Professor Czeizel conducted the first ever randomized controlled trial (RCT) proving that prenatal multivitamin supplements could prevent primary neural tube defects and several other major congenital abnormalities. The results of this study are discussed by Professor Czeizel in Chapter 5 of this book.

The ingredients of one of the most popular prenatal vitamin supplements (Elevit, Pronatal by Bayer) used by millions of women around the world are based on the formulation used in the pioneering Hungarian RCT. Therefore, Professor Czeizel's work has been directly instrumental in popularizing the general use of prenatal multivitamin supplements and led to the prevention of innumerable cases of congenital abnormalities around the world.

Professor Czeizel has published more than 100 original research articles and book chapters, plus served on numerous editorial boards and advisory bodies (including the WHO Centre for Control of Hereditary Diseases). Professor Czeizel is now retired from clinical practice but still maintains an active research interest in the prevention of congenital abnormalities through nutritional intervention. For this reason we dedicate this book in his honor.

Contents

Preface

Ever since Hippocrates stated "Let food be thy medicine and medicine be thy food" more than two thousand years ago, it has been apparent to all that a healthy balanced diet is essential to good health, with that benefit also extending to optimal reproductive function. This publication sets forth to address the important issue of how nutrition, both diet and nutritional supplements, can influence reproductive function in men and women, and the outcome of any resulting pregnancy.

The idea for this book was first conceived when it was realized that although there are many good books available covering the topic of diet and fertility from the perspective of the layperson, none were available for the busy clinician that covered all aspects of reproduction in a single easy to read format. Therefore we embarked on a journey to identify some of the most highly regarded researchers in the field of nutrition and reproduction, inviting them to write on the topics that they are passionate about and whose work leads the field throughout the world.

We have elected to take a "whole of life" approach to this book. From the female perspective we cover the role of nutrition from onset of menarche, to the initiation and maintenance of normal ovular function as adults, pregnancy, and then through to menopause. On the male side we cover the role of nutrition in both hormone and sperm production, but also in sexual function. Furthermore, we have included two chapters on the important role that diet and nutritional supplements play on optimizing pregnancy outcomes from the perspective of both the mother and the child. We are privileged to have Professor Andrew E. Czeizel provide us with a chapter outlining his pivotal randomized controlled trial that proved beyond doubt that prenatal multivitamin supplements can prevent serious congenital abnormalities in the child. This landmark study has advanced clinical practice, resulting in a significant drop in the number of children born with potentially lethal or life-changing congenital abnormalities. For this reason, we have dedicated this book to Professor Czeizel.

Over the course of many years of clinical practice we have noticed that patients seeking to become parents are often very interested in what they can do themselves to optimize the chances of conceiving, but also having a healthy pregnancy and child. Optimization of diet and the consumption of nutritional supplements have both become extremely popular approaches. In today's world of "Dr. Google," many patients are embarking on dietary changes that are not supported by scientific evidence, and may even be harmful, based mainly on the advice of self-proclaimed "experts" on myriad consumer websites. Therefore, the primary aim of this book is to provide the most up-to-date scientific evidence surrounding the role of nutrition in the various important reproductive processes. To facilitate easy access to this information, a Key Points Summary is included at the end of each chapter, highlighting the main "take home" messages covered in that chapter. We trust that this will make our book an easy reference point for busy physicians, naturopaths, dieticians, and nurses who are trying to advise their patients on the best nutritional path to reproductive health. It is also envisaged that this book will be a useful resource for both undergraduate and postgraduate students undertaking studies in nutrition in the context of fertility.

Some of our medical readers may be concerned by the inclusion of complementary health approaches such as Traditional Chinese Medicine (TCM) and naturopathy. However, our clinical experience suggests that a large proportion of patients consult these "alternative practitioners," often in preference to traditional medical doctors. As such, we felt it important to cover the scientific rationale behind the use of Chinese herbs and supplements, plus traditional naturopathic nutritional approaches to optimization of reproductive health. It is our belief that the inclusion of these chapters strengthens our book, both in terms of scope of interest and breadth of coverage of the topic.

A book such as this relies heavily on the goodwill and significant investment of time of all our authors. We wish to thank all of our authors for their very valued contributions. We also thank our

respective spouses, Katherine and Ian, for their support during the compilation of this book and our resulting absence from family activities. Finally, we thank our publisher, CRC Press, and more specifically Ira Wolinsky and Randy Brehm, for asking us to produce this book on a topic we both believe in so passionately.

We trust that you will enjoy reading this book and we hope that it is a useful resource for optimizing the reproductive health of your patients, and that of the next generation.

Kelton Tremellen
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Editors

Kelton Tremellen is a professor of reproductive medicine at Flinders University and a clinical director at a private reproductive medicine unit in Adelaide, South Australia. He earned his medical degree and later a PhD from the University of Adelaide before undertaking postgraduate training in obstetrics and gynecology. Dr. Tremellen is a fellow of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG), and is a board certified sub-specialist in reproductive endocrinology and infertility (CREI).

Dr. Tremellen is a member of the Fertility Society of Australia and the American Society for Reproductive Medicine. He is on the editorial board of the journal *Human Reproduction* and is an examiner for the reproductive endocrinology and infertility sub-specialty of the RANZCOG. His research interests include male infertility, recurrent miscarriage, and diminished ovarian reserve, with a key focus being on the role that nutrition plays in reproduction. Dr. Tremellen is the inventor of Menevit®, a novel fertility supplement marketed for improving sperm health in Australia and New Zealand by Bayer Consumer Care. Dr. Tremellen is also the author of numerous research articles and book chapters in the field of reproductive medicine and is a regular invited speaker at conferences on reproductive medicine.

Karma Pearce is a senior lecturer in nutrition and food sciences, University of South Australia. She has dual degrees in applied science (microbiology and applied chemistry) and adult education. Dr. Pearce gained extensive industry experience as a quality manager, food analyst, and researcher before undertaking a PhD in nutritional physiology at the University of Adelaide, where she also established an undergraduate teaching course in nutrition. Dr. Pearce accepted a tenured academic position at the University of South Australia, where she was the program director of nutrition and food sciences until 2011. In this position, she was awarded numerous prizes for outstanding contributions to teaching, including an Australian Learning and Teaching Council (ALTC) citation. She ranked in the top 1% of tertiary educators in Australia.

Dr. Pearce is a committee member of the Nutrition Society of Australia and is also a long-standing member of the Australian Institute of Food Science and Technology (AIFST), the Royal Australian Chemical Institute (RACI), and the Australian Society for Microbiology (ASM). She has an active research interest in the role of nutrition in both diabetes and reproduction. She has also researched and consulted with a number of disadvantaged minority groups in both Australia and India on the role of nutrition and health literacy in improving population health outcomes. She has authored several research papers on these subjects.

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1 Nutrition and Ovulatory Function

Jorge E. Chavarro, Audrey J. Gaskins, and Myriam C. Afeiche

CONTENTS

1.1 INTRODUCTION

Reproduction is an energetically expensive enterprise, and its costs are largely borne by females. Across species, females spend approximately 3.5 times more energy than males in gamete production [1]. Energy costs are magnified once pregnancy is achieved. The estimated energetic cost of a human pregnancy with adequate gestational weight gain is approximately 78,000 kcal [2]. From that point forward, costs associated with lactation and childrearing further tilt the energy expenditure balance toward women. An in-depth discussion of the evolution of human reproductive traits, in general, and of ovulation in particular, is outside the scope of this chapter. However, it will be useful to our discussion of the role of nutrition on ovulatory function to keep in mind that the massive energetic costs of reproduction likely exerted enormous evolutionary pressure in selecting reproductive systems capable of sensing adverse environments to prevent pregnancy in conditions of insufficient energy availability.

To address the relation between nutritional factors and ovulatory function we divide nutritional inputs into energy balance and dietary factors that might influence ovulation above and beyond their energetic contribution, and examine their relations with markers of pubertal onset, focusing on menarche and ovulatory function in adult women.

1.2 ENERGY BALANCE AND OVULATORY FUNCTION

1.2.1 Energetic Factors and Menarche

Multiple studies have documented secular trends towards earlier menarche across the world [3–10], raising speculation on the determinants of these trends, particularly because the rate of change

varies considerably between populations and appears to vary within populations according to socioeconomic conditions, or proxies thereof such as race. A long-standing hypothesis trying to explain these trends is that the overall availability of material conditions within a society strongly influences the onset of menses at a population level. Downward trends in age at menarche in Japan and Korea were reversed during World War II and the Korean War, respectively, and resumed after these conflicts ended [6,11]. Similar reversals have also been observed during World War II in Europe [12,13] and more recently during the Balkan conflict in the former Yugoslavia [14,15]. Although some of the reversal in trends toward earlier menarche could be attributed to other war-related factors such as stress, co-occurrence of trends towards earlier menarche with trends toward increasing childhood obesity and height [7,11,16], which serve as markers for energy balance during childhood, suggest that disruptions in energy availability during war could be one of the factors explaining the delay in sexual maturation associated with war.

Further support for the hypothesis that material conditions influence the timing of puberty comes from contemporary populations not exposed to war conditions. In a study among 900 girls living in southwest Ethiopia, food insecurity was strongly related to the onset of menses. Compared to girls living in food-secure households, the risk of experiencing menarche was 6% lower (95% confidence interval [CI] = –24%, 16%) for girls living in households with mild food insecurity and 50% lower (95% CI = –72%, –11%) for girls living in households with moderate to severe food insecurity [17]. Less extreme examples include well-documented correlations between markers of socioeconomic status and age at menarche. Among Colombian girls, mean age at menarche differed by approximately 1 year between extremes of a socioeconomic status variable based on parental occupation, with girls from wealthier households reaching menarche earlier than girls from more disadvantaged backgrounds [3]. In addition, girls living in larger households and smaller cities reached menarche later than girls living with fewer family members and girls living in large cities, respectively [3]. Although these relations may not be surprising in a developing country, similar relations have been observed in Europe. For example, among girls born in France between the 1920s and the 1950s, nearly identical associations of occupation-based socioeconomic status, place of residence, and family size with age at menarche are observed [18]. On the other hand, in an ethnically diverse group of girls living in New York City in the late 1990s, earlier pubertal development was related to lower maternal education, a proxy for lower socioeconomic status [19]. Although this last study is apparently at odds with the previously mentioned studies, when worldwide trends in childhood obesity (as an extreme phenotype of positive energy balance) and their relation with socioeconomic status are considered, it is clear that the apparent differences could be a function of changing patterns in the relation between childhood body weight and socioeconomic status across countries and over time. Specifically, in developing countries and in previous generations in the developed world, children from wealthier families were heavier (and more likely to be overweight) than worse-off children but this relation has recently reversed and currently in the developed world, and increasingly in the developing world, it is poverty that is related to higher body weight and obesity [20]. Therefore, the relations of socioeconomic characteristics with the timing of menarche may represent, to a large extent, the relation between prepubertal body size and sexual maturation.

The relation of childhood body size, as a marker of energy balance, and the onset of puberty has been known for a long time. By the 1960s it had been recognized that female mice from smaller litters (and hence with more food available to them compared to mice from large litters) grew faster and entered puberty earlier than female mice from large litters [21]. It was further recognized in this early work that the timing of puberty in female mice was determined by attained weight rather than chronological age [21]. Similar observations followed in humans. Most notably, work by Frisch in the 1970s documented that, although there were substantial differences in age at menarche, attained weight at menarche appeared to be invariant in multiple study populations [22–25]. Based on this work, she further hypothesized that a minimum amount of stored body fat, initially estimated as an absolute weight of 48 kg and later revised to 17% of body weight as fat [22,23,25,26], was necessary for the initiation of menses in girls. Additional work by Frisch and others in the 1970s and 1980s

documented how high levels of energy expenditure also delayed menarche. This work, focused on girls engaging in high energy expenditure activities such as professional ballet and competitive sports programs, showed drastic delays in the onset of menses associated with these activities. For example, compared to girls who did not participate in competitive sports, those who did reached menarche approximately 1 year later, and elite performers—girls who were candidates to join U.S. Olympic teams—reached menarche approximately 2 years later [27]. A similar study found that among girls who started competitive sports training (swimming and track) before menarche, each additional year of competitive training delayed the onset of menses by 5 months [28]. Likewise, ballet dancers reached menarche nearly 3 years later than control girls [29], and the prevalence of primary amenorrhea at age 18 years was 12% among girls enrolled in a professional ballet program [30].

These early findings have been extraordinarily consistent across populations and over time [31–40]. A particularly remarkable example worth highlighting is that of the correlation between prepubertal body mass index (BMI) and the incidence rate of menarche. In four separate studies the rate at which girls reached menarche was approximately 2-fold when comparing the heaviest to the leanest girls within each study [31–34]. Specifically, the hazard ratios (95% confidence intervals) for reaching menarche comparing top to bottom categories of prepubertal BMI were 2.5 (1.5, 4.3) among German girls [31], 2.2 (1.7, 2.9) among girls in California, USA [33], 2.0 (1.2, 3.4) among girls in Massachusetts, USA [32], and 2.2 (1.4, 3.6) among Canadian girls [34]. Equally consistent have been the findings on the relation between physical activity and the onset of menses, including the fact that strenuous, but not moderate, physical activity delays menarche. For example, Colombian girls who engaged in sports activities for 2 hours/day or more in the years preceding menarche reached this milestone on average 3.5 months later than girls who did not engage in sports, yet girls who engaged in sports less often did not differ from nonactive girls in the timing of menarche [3]. Similarly, Canadian girls participating in dance, ballet, gymnastics, or figure skating clubs were 50% (95% CI: –20%, –70%) less likely to have reached menarche than controls matched on date of birth [41]. This association, however, was driven by girls who were in competitive teams who were 70% (–20%, –90%) less likely to have reached menarche than all other girls in the study [41]. Likewise, among 8- to 12-year-old premenarchal girls in Germany, those in the third and fourth quartiles of time spent in sports activities at baseline were 60% (–30%, –80%) and 70% (–50%, –90%) less likely to experience menarche in the following 2 years than girls in the lowest quartile [31]. Girls in the second quartile, however, reached menarche at the same rate as the least active girls [31].

Consistency notwithstanding, this factor alone is not sufficient to conclude that the relation between adiposity and menarche is causal. In support of a causal relation, early life risk factors for childhood obesity have been related to age at menarche. Factors associated with a greater risk of childhood obesity, including pre-pregnancy overweight and obesity, excessive gestational weight gain, smoking during pregnancy, and high growth velocity during infancy [40,42–44], have been related to earlier age at menarche. In addition, changes in BMI between age 7 and 8 years were strongly related to the onset of menses among Korean girls. Specifically, girls in the highest quartile of weight gain were 6.4 (95% CI: 1.2, 34) times more likely to reach menarche before age 12 than girls in the lowest quartile of weight gain [38]. Also supportive of a causal relation were the results of a Mendelian randomization study in which a genetic risk score for high BMI, based on risk alleles identified in genome-wide association studies for BMI, was related to age at menarche. In this study, the risk of experiencing menarche before age 12 years increased by 6% (95% CI: 3%, 8%) for each additional risk allele a woman carried [45]. Lastly, the effects on menarche of a cluster randomized trial previously found to reduce childhood obesity in girls [46] also argues in favor of a causal relation. A secondary analysis of this trial limited to girls who were premenarchal at baseline found that the rate of initiation of menses was reduced by 26% (95% CI: –13%, –34%) among girls assigned to intervention schools than among those assigned to control schools [47].

The elucidation of a mechanism linking body fat stores with the hypothalamic–pituitary regulation of ovulation has further clarified the nature of the relation between adiposity and the onset of menses. Leptin, a peptide hormone produced in the adipocyte, was originally identified as a key central regulator of appetite and energy balance [48–50]. Some of the reproductive characteristics of the leptin-deficient mouse (*ob/ob*) also suggested that leptin could also be involved in the central control of hypothalamic–pituitary–gonadal (HPG) axis. Although early signs of sexual maturation take place in the female *ob/ob*, ovulation never ensues, rendering these mice sterile [51]. Morphological evaluation of the ovaries of *ob/ob* mice reveals normal numbers of primordial, primary, and secondary follicles but absence of antral follicles and corpora lutea, relative to wild-type mice [52]. Further, administration of recombinant leptin to *ob/ob* mice restores ovulation and fertility [51,52]. Recent work suggests a more specific localization of leptin's action within the hypothalamus. Targeted deletion of the leptin receptor in hypothalamic γaminobutyric acid (GABA)ergic neurons results in delayed onset of puberty and decreased fecundity but the same deletion in hypothalamic glutamatergic neurons has no impact in reproductive function [53]. In healthy children and adolescents, serum leptin concentrations are positively related to total body fat, pubertal stage [54–56], and serum concentrations of reproductive hormones [55,56], and inversely related to age at menarche [57]. Moreover, humans with genetic deficiency of leptin have a reproductive phenotype similar to that of *ob/ob* mice, and are also responsive to administration of leptin [58–61]. Interestingly, treatment with leptin does not accelerate puberty. Instead, genetically deficient humans treated with leptin undergo puberty within the expected chronological age window, suggesting that, rather than a trigger, leptin may act as a permissive factor in the progression of puberty in the presence of other leptin-independent signals [60].

Further evidence of leptin's permissive role comes from studies on the role of kisspeptin on sexual maturation and on the interactions between leptin and kisspeptin on pubertal development. As is the case with leptin, rodents and humans with loss of function mutations in the genes encoding kisspeptin (*Kiss1/KISS1*) or its receptor (*Gpr54/GPR54*) do not undergo pubertal development and have hypogonadotrophic hypogonadism [62,63]. Moreover, studies of selective deletion of leptin receptors in neurons expressing kisspeptin show that these animals have normal pubertal development, suggesting that direct leptin action on these neurons is not necessary for normal sexual maturation [64]. However, and unlike leptin, kisspeptin does not appear to be directly responsive to energy balance. For example, postnatal exposure of female rats to a high-fat diet, which results in obesity, predictably increases the frequency of irregular estrous cycles and circulating leptin levels but does not influence hypothalamic mRNA levels of *Kiss1* [65]. However, leptin does appear to influence kisspeptin to some extent, as evidenced by the relative deficiency of mRNA *Kiss1* levels in *ob/ob* mice, which is partially restored on treatment with leptin [66]. Although these findings suggest that kisspeptin may rely on leptin signaling for sensing the environment for cues on energy availability, study findings in this field are not entirely consistent. For example, recent work suggests that although leptin and kisspeptin do interact, this interaction arises only after pubertal development [67]. Although both leptin and kisspeptin are clearly important in sexual maturation, the precise nature of their interactions in the control of the onset of puberty and the maintenance of ovarian function remains an area of active investigation.

1.2.2 Energetic Factors and Ovulation in Adult Women

Most of the discussion in Section 1.2.1 on the need of a minimum amount of body fat for the initiation of menses also appears to apply to the maintenance of ovulatory cycles in sexually mature women. In fact, much of the earlier work on body weight, physical activity, and menarche also documented that leanness and strenuous exercise resulted in secondary amenorrhea and menstrual cycle irregularities [28–30]. Additional examples of how loss of adipose tissue suppresses ovulation in postmenarchal women include the well described secondary amenorrhea found in women suffering from eating disorders [68] and the seasonal patterns in fecundity present in agricultural

societies related to seasonal patterns in food availability and body weight [69]. It is currently known that the effects of undernutrition on anovulation are the result of hypothalamic dysfunction and decreased gonadotropin secretion [70], which, as discussed in Section 1.2.1, may be secondary to leptin deficiency.

Overweight and obesity, on the other end of the body weight spectrum, represent a challenge to a single mechanism governing the relation between adipose tissue stores and ovulation. If the mechanisms described for menarche and malnutrition-related secondary amenorrhea were the only ones governing how the HPG senses environmental cues related to energy stores in adipose tissue, one would expect that overweight and obese women would either have the same frequency of anovulation and infertility associated with anovulation as normal weight women or that the frequency of these conditions would be lower among women with excess body weight. This is clearly not the case. In fact, multiple studies have documented a greater frequency of anovulation and infertility among overweight and obese women than among normal weight women.

Jensen and colleagues reported that, among 10,903 Danish women who had planned their pregnancies, being overweight or obese (BMI ≥ 25 kg/m^2) was associated with an 82% (50–120%) increased risk of infertility compared to women with a BMI between 20 and 25 kg/m^2 [71]. Similarly, in a study of 7327 American women who had planned their pregnancies, being obese (BMI ≥ 30 kg/m^2) was associated with an 18% (5–28%) lower chance of pregnancy in a given cycle when compared to women with a BMI between 18.5 and 24.9 kg/m^2 [72]. Likewise, Lake and collaborators found that being obese (which was defined as BMI > 28.6 kg/m^2) at age 23 was associated with a 33% (13–48%) lower chance of conception per cycle in a group of 3327 women representative of the general UK population in comparison to lean women (BMI 18.8–23.8 kg/m^2) [73].

Other time-to-pregnancy studies have found evidence of decreased fecundity among both overweight and obese women. Hassan and colleagues found that, compared to women with a BMI between 19 and 24.9 kg/m^2, the relative risk (95% CI) of infertility for women who were either overweight or class I or class II obese was 2.2 (1.6, 3.2) and 6.9 (2.9, 16.8) for class III obese women [74]. In the largest retrospective time-to-pregnancy study to date (47,835 couples), the risk of infertility was 27% (18%, 36%) higher among overweight women, and 78% (63%, 95%) among obese women when compared to normal weight women [75]. Likewise, in the largest prospective time-to-pregnancy study conducted to date (1651 couples) the probability of becoming pregnant in any menstrual cycle decreased linearly with increasing BMI [76].

Although time-to-pregnancy studies do not provide direct evidence that problems with ovulation are the underlying cause of delayed conception, disordered ovulation has been the underlying cause of infertility most commonly associated with overweight and obesity in studies investigating specific underlying diagnoses. In a case-control study involving 308 cases of ovulatory infertility, nulligravid women who were more than 120% of their ideal body weight according to the Metropolitan Life Insurance tables had twice the risk of ovulatory infertility than nulligravid women between 85% and 120% of their ideal body weight [77]. Grodstein and collaborators obtained similar results in a case-control study of 597 nulligravid women who received a diagnosis of ovulatory infertility as their primary or secondary infertility diagnosis. In this study, women with a BMI ≥ 27 kg/m^2 had three times the risk of ovulatory infertility than women with a BMI between 20 and 24.9 kg/m^2. Furthermore, when specific pathologies were examined, the association between BMI and ovulatory infertility was strongest for women with polycystic ovary syndrome (PCOS; 6-fold greater risk) and women with hypogonadism (3.6-fold greater risk) [78].

In the Nurses' Health Study II cohort, risk of ovulatory infertility increased linearly with increasing BMI even within the range of BMI considered normal. Women with a BMI at age 18 of 22 kg/m^2 or higher were at an increased risk of ovulatory infertility compared to women with a BMI between 20 and 21.9 kg/m^2, the lowest risk BMI category. Risk increased linearly with greater BMI levels at age 18, reaching a plateau at a BMI 30 kg/m^2 or higher, which was associated with a 2.7-fold greater risk of this condition [79]. In a subsequent analysis of the cohort, there was a J-shape relation between adult BMI and risk of ovulatory infertility, similar to that described for BMI at age 18 [80,81].

However, for adult BMI the range associated with the lowest risk of infertility is slightly wider than for adolescent BMI, from 20 to 24 kg/m^2, and the risk of infertility does not appear to plateau at any BMI level [80]. In addition, overweight and obesity were not associated with infertility due to other common causes of infertility in this cohort [81] (Figure 1.1).

Obesity leads to a wide range of systemic alterations including changes in circulating levels of adipokines, reproductive hormones, and markers of endothelial dysfunction and systemic inflammation as well as metabolic disturbances in lipoprotein metabolism, glycemic control, and increased insulin resistance. This makes it difficult to distinguish obesity-induced changes that are responsible for its effects on ovulation and fertility from changes that are epiphenomena. The strongest evidence to date suggests that the effects of obesity on glycemic control and insulin sensitivity may be important factors regulating ovulation.

The strongest evidence suggesting a role of glycemic control in ovulation comes from studies of the effects of antidiabetic medications and other clinical interventions on clinical manifestations of PCOS. Oral antidiabetic medications, including thiazolidinediones [82], metformin [82], *d*-chiro-inositol [83], and acarbose [84,85] improve ovulation rates and menstrual cycle regularity among women with PCOS, clearly showing an effect of insulin resistance and glycemic control on ovulatory function. There is also some evidence that improvements in ovulatory function resulting from antidiabetic medications may translate into improved fertility in women with PCOS. In a recent randomized trial, women with PCOS planning to undergo *in vitro* fertilization (IVF) for infertility treatment were allocated to metformin (2000 mg/day) or placebo [86]. Women allocated to the metformin arm had significantly more live births (49%) than women in the placebo group (32%), but this effect was due primarily to a doubling in the spontaneous pregnancy rate before initiation of IVF in the metformin group (20% metformin vs. 10% placebo) rather than to effects of metformin on live births among the women who underwent IVF (38% metformin vs. 29% placebo) [86].

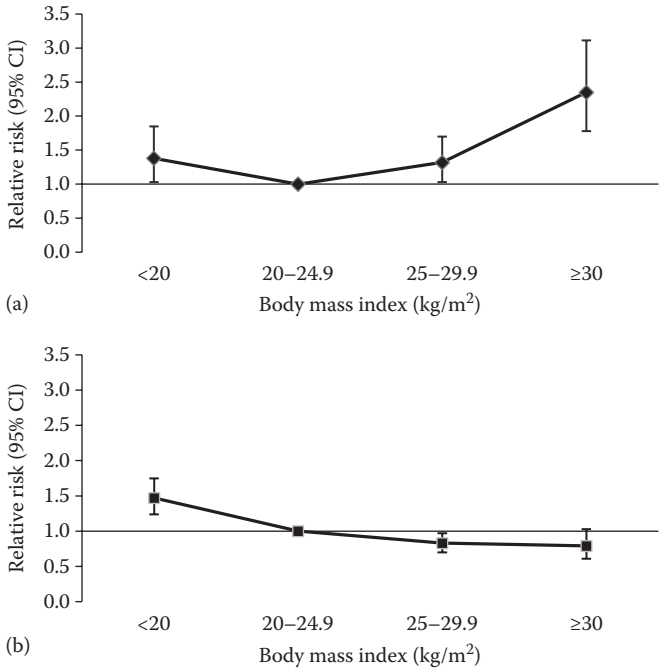

FIGURE 1.1 Body mass index in relation to (a) infertility due to ovulation disorders and (b) other causes of infertility. (Adapted from Chavarro JE et al. *Obstet Gynecol*, 110:1050–1058, 2007.)

Modest weight loss (5–10% of initial body weight) appears to have effects similar to those of antidiabetic medications among women with PCOS, as it also improves ovulatory function [87]. Among women losing weight through diet, the absolute amount of weight loss appears to be more important for improvements in reproductive function than the specific macronutrient composition of weight loss diets [88]. Similarly, among morbidly obese PCOS women undergoing bariatric surgery, the amount of weight loss appears to be related to the improvement in menstrual cyclicity [89–91].

The relations between glycemic control and insulin sensitivity and ovulatory function and fertility do not appear to be limited to women with PCOS. For example, oligomenorrheic non-PCOS infertile women are more likely to be insulin resistant and to have postprandial hyperinsulinemia than eumenorrheic infertile women [92]. Similarly, diabetic women in the general population have significantly lower fecundability than nondiabetic women [93]. Moreover, in a study of healthy pregnancy planners from the general population, HbA1c levels within the nondiabetic range were inversely related to fecundability and positively related to characteristics of PCOS (free testosterone levels and cycle irregularity) [94]. These data suggest that glycemia and insulin resistance are important determinants of ovulatory function even within nonpathologic ranges and may be the underlying physiologic mechanisms explaining the consistently observed relation between excess body weight and ovulation disorders.

1.3 DIET COMPOSITION AND OVULATORY FUNCTION

1.3.1 Diet and Menarche

Although data on the relation between specific dietary factors and the onset of menses are not as strong as the evidence showing the strong control of energy availability on ovulation, a growing literature suggests that some specific dietary factors may also influence the onset of menses. Nevertheless, the most consistent finding across studies examining the relation between diet and the onset of menses is the lack of association between dietary factors and menarche above and beyond their contribution to energy balance.

Most of the initial literature on the potential role of dietary factors on menarche focused on macronutrients (fat, protein, and carbohydrates) and most found no relation between intake of these nutrients and menarche [32,33,41,95]. For example, Koprowski and colleagues found that the hazard ratio (95% CI) of menarche comparing girls in the top and bottom quartiles of intake was 0.9 (0.7, 1.2) for total carbohydrate intake, 1.1 (0.8, 1.5) for total protein intake, and 1.0 (0.8, 1.3) for total fat intake [33]. In addition, although some studies did report associations with macronutrient intakes, associations were not consistent across studies. For example, although Merzenich and colleagues found that the risk of experiencing menarche was approximately 2-fold higher among girls in the top quartile of total fat intake [31], Koo and collaborators found exactly the opposite: the risk of menarche for girls in the top quartile of total fat intake was half of that of girls in the bottom quartile [96].

An exception worth consideration, however, has been the association between protein intake, or its primary dietary sources, with age at menarche when protein from animal and vegetable sources are considered separately. Specifically, data suggest that intake of protein from animal sources may accelerate the onset of menses whereas protein from vegetable sources may delay it [97–100]. In a study that followed 230 premenarchal girls from Southern California throughout puberty in the late 1970s and early 1980s, higher prepubertal meat intake was associated with a significantly earlier age at menarche, approximately 6 months earlier, whereas intake of nuts, beans, and legumes was associated with significantly later menarche, approximately 5 months later [97]. Similarly, animal protein intake accelerated whereas vegetable protein delayed age at menarche among girls born in the 1930s and 1940s in the Boston, USA area who were prospectively followed from birth [98]. These findings have been corroborated in recent studies. For example, in the DONALD Study

(Dortmund Nutritional and Anthropometric Longitudinally Designed Study), a prospective cohort study following German children from infancy through adulthood, girls with the highest intake of animal protein at age 5–6 years reached menarche on average 10 months earlier than girls with the lowest animal protein intake, whereas girls with the highest vegetable protein intake reached menarche on average 6 months later than girls in the lowest vegetable protein intake [99]. Similarly, in the ALSPAC Study (Avon Longitudinal Study of Parents And Children), a prospective cohort study of British children followed from pregnancy, a 1 standard deviation increase in animal protein intake at age 7 accelerated the onset of menses by 17% (95% CI: 7%, 28%); meat intake showed a similar association [100]. A possible link between protein and sexual maturation is insulin-like growth factor I (IGF-I). Intake of protein from animal sources has been longitudinally related to higher circulating levels of IGF-I later in childhood and with acceleration in somatic growth [101–104]. Whether this well-known effect of animal protein is responsible for the described relations with age at menarche remains to be determined.

Another recent intriguing finding is that of the relation between soy-based foods and soy isoflavones with age at menarche. In the ALSPAC Study, girls who were fed primarily soy-based formulas starting at or before the fourth month of life reached menarche earlier than girls raised on other infant feeding methods [105]. This association was of borderline statistical significance; the hazard ratio (95% CI) of menarche for girls with early soy exposure was 1.25 (0.92, 1.17) relative to girls who were initially fed dairy-based formulas. It should also be noted that a major problem of this study was the massive loss to follow-up, which raises concerns of selection bias. Analysis of the relation between soy and pubertal development in the DONALD Study also suggests that soy may influence the timing of puberty. Specifically, girls with the highest prepubertal (approximate age 7 years) intakes of isoflavones started breast development (attaining B2) 8 months earlier, reached peak height velocity 7 months earlier, and experienced menarche 5 months earlier than girls with the lowest intakes of isoflavones [106]. Differences in age at menarche were not statistically significant, however [106]. Although phytoestrogens are generally considered to be weak estrogens, these findings raise the possibility that the estrogenic effects of soy isoflavones may be sufficiently high to influence pubertal development. Phytoestrogens bind the estrogen receptor (ER) α with a 100–1000 times lower affinity than estradiol does [107–112]. However, phytoestrogens can bind membrane ERs with greater affinity than they bind nuclear receptors and, through the membrane receptors, induce transcription activity to the same extent that estradiol does [113]. Moreover, castrated male sheep feeding on phytoestrogen-rich pastures present development of mammary glands, lactation, and squamous metaplasia of the prostate and other accessory glands accompanied by enlargement of Cowper's gland [114]. The results from the two studies examining the relation between soy and pubertal development in humans are certainly intriguing and suggest that further examination of the relation of soy and soy-derived products with the onset of puberty is warranted.

The relation between intake of micronutrients and age at menarche has been extremely inconsistent but there are a few findings worth highlighting. In a study aimed at comprehensively assessing diet in relation to menarche, Maclure and colleagues reported a strong association between vitamin A intake and earlier menarche [32]. Specifically, the hazard ratio (95% CI) for reaching menarche comparing girls consuming more than 30,000 IU/day of vitamin A to girls consuming less than 10,000 IU/day was 5.8 (2.6, 13) [32]. Despite the surprising strength of the association (substantially stronger than the associations of BMI and height with menarche in the same study), the authors suggested that this may represent a chance finding due to multiple testing. Another intriguing finding is a recent report of an association between plasma vitamin D levels and age at menarche. In this study, 242 premenarchal girls were prospectively followed for approximately 3 years. Girls who were vitamin D deficient at baseline (25-hydroxyvitamin D [25(OH)D] < 50 nmol/L) were two times more likely to reach menarche during follow-up than girls who were vitamin D sufficient at baseline (≥75 nmol/L), even after adjustment for baseline BMI [115]. The mechanisms explaining this relation are not clear, however.

1.3.2 Diet and Ovulation in Adult Women

1.3.2.1 Macronutrients

Specific macronutrients have been proposed to affect ovulatory function through effects on insulin and glucose metabolism. As discussed in Section 1.2.2, insulin resistance, hyperinsulinemia, and hyperglycemia appear to be critical in the development of PCOS, and antidiabetic medications appear to have beneficial effects on ovulatory function and fertility in these women. These mechanisms seem to also play a role in ovulation among women with little to no clinical evidence of PCOS. Taken together these data support the notion that improvements in insulin sensitivity through diet could have similar effects.

1.3.2.1.1 Dietary Fat

Data from animal studies show that oocytes metabolize free fatty acids (FFAs) [116,117] and similar to animals, palmitic (saturated fatty acid [SFA]), stearic (SFA), and oleic (monounsaturated fatty acid [MUFA]) acids are the predominant FFAs in human ovarian follicles [118]. In animal experiments, elevated levels of follicular palmitic and stearic acids were associated with impaired oocyte maturation, suggesting that saturated FFAs could have direct adverse effects on oocytes [119]. This was confirmed recently in humans. A small study from Iran found that increased levels of saturated fatty acids and a higher ratio of SFA to polyunsaturated fatty acids (PUFAs) was negatively correlated with the number of mature oocytes [120]. Polyunsaturated fats, on the other hand, have shown beneficial effects on folliculogenesis. Studies in female cattle and other mammals have shown that diets with higher amounts of polyunsaturated fats increased the number and size of ovarian follicles, the ovulation rate, and progesterone production by the corpus luteum [121–123]. Similarly, in humans, higher intakes of PUFAs have been associated with improved metabolic and endocrine characteristics in women with PCOS [124].

In contrast to *cis* unsaturated fatty acids, consumption of *trans* fats has been associated with greater insulin resistance [125] which may adversely affect ovulatory function [126]. Results from a prospective cohort study of more than 18,000 women demonstrated that a 2% increase in energy intake from *trans* fatty acids at the expense of carbohydrates was associated with a 73% greater risk of ovulatory infertility after adjustment for potential confounders [127]. Moreover, when *trans* fats were consumed instead of monounsaturated fats the risk more than doubled.

1.3.2.1.2 Carbohydrates

Both the quality and quantity of carbohydrate in diet influence glucose metabolism, affecting insulin sensitivity in healthy individuals [128,129]. Dietary glycemic load, a summary measure of the amount and quality of carbohydrates in the diet, has been shown to be positively related to risk of ovulatory infertility [130]. Specifically, women in the highest quintile of dietary glycemic load had 92% higher risk of ovulatory infertility than women in the lowest quintile after adjustment for confounders. These results are supported by two smaller studies among women with PCOS. In the first, it was shown that higher intake of high glycemic index foods, particularly white bread and fried potatoes, was more prevalent among 30 women with PCOS than among 27 control women [131]. In a second crossover feeding trial, it was shown that when 11 women with PCOS consumed a low-carbohydrate diet (43% vs. 56% of energy) there were beneficial reductions in free testosterone levels that could subsequently enhance ovulatory function [132].

Supporting evidence for a role of carbohydrates in ovulatory function also comes from literature on vegetarian diets. Vegetarian diets have been associated with a higher incidence of menstrual irregularities and depressed levels of luteinizing hormone (LH) and estrogens [133–135]. Although it is hard to disentangle the effects of various nutrients that differ between vegetarian and nonvegetarian diets, dietary fiber has emerged as one potential explanation. Controlled trials have shown that diets high in fiber result in lower levels of estrogen throughout the menstrual cycle [136–140]. A cohort study of normally menstruating women from the United States found similar results as well

as an increased risk of incident anovulation in the women consuming >22 g/day of fiber [141]. In contrast, total fiber intake was unrelated to ovulatory infertility in a much larger cohort study [130].

1.3.2.1.3 Proteins

The amount and source of protein in the diet have been found to influence insulin sensitivity [142–145], which could subsequently influence ovulatory function. Two small randomized trials compared the effects of a hypocaloric low-protein (15% of energy) versus high-protein (30% of energy) diet on reproductive function among overweight women with PCOS [146,147]. However, in both studies, the protein content of diet had no effect on reproductive function despite improvements in menstrual regularity [146] and reductions in circulating androgens [147]. A more recent 6-month trial among PCOS women investigated the effects of a high-protein diet (>40% of energy from protein and 30% of energy from fat) versus a standard protein diet (<15% of energy from protein and 30% of energy from fat) with no calorie restriction [148]. Although replacement of carbohydrates with protein improved weight loss and glucose metabolism, there was no effect on testosterone or sex hormone binding globulin levels [148].

Data from the general population are somewhat conflicting. In a large cohort of more than 18,000 women, consumption of protein from animal sources, including chicken and red meats, was associated with an increased risk of infertility due to anovulation, whereas consuming protein from vegetable sources appeared to have the opposite effect [149]. Specifically, consuming 5% of energy as vegetable protein rather than carbohydrates was associated with a 43% lower risk of ovulatory infertility, and consuming 5% of energy as vegetable protein as opposed to animal protein was associated with a more than 50% lower risk of ovulatory infertility. Differential effects of different types of protein are consistent with the relation between protein intake and menarche discussed earlier. There are at least two not mutually exclusive mechanisms explaining differential effects of protein on ovulatory function. The presence of hormone residues in meat products, particularly in beef, is one of the purported mechanisms. Anabolic sex steroids are administered to cattle for growth promotion 60–90 days before slaughter in the United States; this practice is also approved in Canada, Australia, New Zealand, South Africa, and Japan, among other countries, but banned in the European Union [150]. The main hormones used for this purpose are estrogen, progesterone, testosterone, and three synthetic hormones (zeranol, melengestrol acetate, and trenbolone acetate). Hormone residues in edible tissues are higher in treated than in untreated animals [151,152] and there is concern that hormonal residues in edible tissues may cause adverse reproductive consequences [153]. It is also possible that the differential effect of various protein sources on insulin sensitivity could be one mechanism explaining these findings [154,155].

1.3.2.2 Micronutrients

Micronutrients have long been suspected to be key players in the process of folliculogenesis and ovulation, as evidenced from early case reports [156–164]. Many, but not all [156,157], of these reports are among women with celiac disease, a condition associated with a higher frequency of infertility and micronutrient deficiencies, most commonly of iron, folic acid, vitamin B_{12}, and vitamin D [164,165]. A growing body of literature suggests these micronutrients may be important in female ovulatory function and fertility.

1.3.2.2.1 Folate

Low folate intake has been linked to reduced cell division; disrupted methylation reactions; and increased inflammatory cytokine production, oxidative stress levels, and apoptosis, all of which could subsequently affect oocyte development [166]. Animal and human data confirm this, showing that folate is indispensable during mammalian folliculogenesis and embryogenesis. As early as the 1960s, it was shown in the immature superovulated rat that either an excess or deficiency of folates partially inhibited ovulation [167]. In rhesus monkeys, a folate-restricted diet led to irregular menstrual cycles and progressively depleted ovarian granulosa cells, which decreased preovulatory

serum estradiol and mid-luteal progesterone [168]. The authors of these articles hypothesized that folate deficiency could lead to an "ineffective" process or "abortive" attempt at cell reproduction, the functional consequences of which would be reflected in the reduced biosynthesis of the sex hormones in these germ cells.

In a prospective cohort study of U.S. reproductive-aged women, low intake of dietary synthetic folate was significantly associated with lower luteal progesterone levels and increased odds of anovulation [169]. In women attending a fertility clinic, carriers of the T allele in position 677 of the *MTHFR* gene had decreased ovarian responsiveness to follicle-stimulating hormone (FSH), fewer oocytes retrieved [170], and granulosa cells that produced less estradiol (basal and stimulated) compared to wild-type allele carriers [171]. Similar effects have been found among carriers of the *MTHFR* A1298C variant [172]. In a cohort study, women attending an IVF clinic who received a folic acid supplement had better quality oocytes and a higher degree of mature oocytes compared to women who did not receive folic acid [173]. Finally, a prospective cohort study involving more than 18,000 participants showed that women in the top two quintiles of folic acid intake (median intakes 726 mcg/day and 1138 mcg/day, respectively) were 40% less likely to develop infertility due to anovulation than women in the lowest quintile of intake (median intake 243 mcg/day) [174].

1.3.2.2.2 Vitamin D

Physiologic and experimental data in animal models strongly suggest that vitamin D plays an important role in reproduction. Expression data shows that the vitamin D receptor (VDR) is present in the ovary [175,176], the endometrium [175], and the placenta [177]. Vitamin D has also been found to stimulate the production of estradiol and progesterone in ovarian [176] and placental tissue [178], and to regulate the expression and secretion of human chorionic gonadotropin in human syncytiotrophoblasts *in vitro* [179]. Female rodents fed a vitamin D-deficient diet have reduced fertility [180,181]. Knockouts for VDR and 1α-hydroxylase, which catalyzes the hydroxylation of 25(OH)D into the biologically active $1,25(OH)_2D$, shed additional light into the role of vitamin D in reproduction. Female knockout mice have decreased fertility as a result of uterine hypoplasia, impaired follicular development, and anovulation [182–184]. Calcium supplementation partially reverses these reproductive effects in the knockout models [181,185] and in the nutritional deficiency model [181], while vitamin D supplementation reverses them in the deficiency model [180], suggesting that there may be a combination of direct effects of vitamin D deficiency and effects mediated through secondary derangements in calcium and phosphorus homeostasis.

In humans, some studies have found vitamin D levels to be inversely related to markers of hyperandrogenism among women with PCOS including total testosterone levels [186–188], free androgen index (FAI) [186,189], dehydroepiandrosterone (DHEAS) [187], and Ferriman–Gallway scores [186,188]. However, these associations are not consistent across studies [186–189], and one study found no association [190]. In addition, most of these studies failed to account for body weight, which is perhaps the strongest confounder owing to its inverse relationship to vitamin D levels in the body [191,192]. In the few studies that were able to account for body weight, the associations disappeared [189] or were of reduced magnitude [188], suggesting that the associations between vitamin D status and PCOS or its endocrine features may be partially explained by adiposity.

Further evidence for a role of vitamin D on ovulatory function can be drawn from studies on seasonal variation. Serum 25(OH)D levels are highest during the summer and autumn and lowest during winter and spring. In northern countries, where a strong seasonal contrast in luminosity exists, ovulation rates have been shown to be reduced during long dark winters [193]. Studies on vitamin D and early outcomes of IVF are conflicting, with one finding a beneficial effect of high 25(OH)D levels on pregnancy rates [194], one finding no difference [195], and one other finding a detrimental effect of high 25(OH)D levels on embryo quality and pregnancy rates [196]. Findings from the Nurses' Health Study II also do not support the beneficial effects of dietary vitamin D on ovulatory function as they found no association between vitamin D intake and risk of ovulatory infertility [197].

1.3.2.2.3 Iron

Iron status may also be important for ovulation, as highlighted by the presence of transferrin and its receptor in granulosa cells and oocytes [198–200]. It has also been reported that granulosa cells can synthesize transferrin, which may be translocated to the oocytes [200]. Although it is possible that transferrin and its receptor are redundant in the ovary or do not play an important role in local iron metabolism, it has been suggested that these proteins are essential for ovum development and are required to support the increased iron demand of the developing follicle [199]. Additional evidence for a role of iron comes from studies documenting a higher risk of infertility among women with subclinical celiac disease. Undiagnosed celiac disease is more common among women with unexplained infertility than among fertile controls [164,201]. Moreover, some of these infertile women have signs of iron deficiency including iron deficiency anemia [164] and low ferritin levels without evidence of other nutrient deficiencies [201]. Intake of nonheme iron was found to be related to lower risk of infertility due to anovulation in a large prospective cohort study [202]. Women in the highest quartile of nonheme iron intake (median intake 76 mg/day) had 40% lower risk of infertility due to anovulation than women in the lowest quintile of intake (median intake 9.7 mg/day). Heme iron intake was unrelated to fertility in this study [202].

1.3.2.3 Other Dietary Factors

No summary of the relation between diet and ovulatory function would be complete without a discussion of the two most researched nutrients—caffeine and alcohol—which have garnered attention mainly because of their proposed roles as reproductive toxicants.

1.3.2.3.1 Caffeine

A variety of pathophysiologic effects of caffeine on sex hormones and ovulatory function exist. Animal models suggest that caffeine can inhibit oocyte maturation or enhance steroid production via inhibition of phosphodiesterase [203] or may interfere with estrogen metabolism via inhibition of aromatase, the key enzyme responsible for converting androgens to estrogen [204]. Studies in women have found positive [205], inverse [206–208], and null [209] associations between caffeine intake and estradiol levels while no studies have shown effects of caffeine on ovulatory function [208,210]. Two large cohort studies also found no association between caffeine intake and risk of ovulatory infertility [211,212]. In addition, among the prospective studies on caffeine and fertility, four studies showed no relation between caffeine and fertility [213–216], one study showed slightly higher fertility among caffeine consumers [217], and two studies suggested a deleterious effect [218,219]. Taken together these results suggest that even though moderate caffeine intake may alter estrogen levels, these alterations are not within a range that affects ovulatory function or fertility.

On a related note, more studies are moving away from focusing on caffeine and instead concentrating on sugary beverages. Both human and animal studies have shown that diets high in sugars, particularly in liquid form, result in insulin resistance [220–222], which could impact ovulatory function and the pathogenesis of polycystic ovary syndrome. Two small studies found no association between soda and premenopausal reproductive hormones [205,223]; however, a larger, more recent study found that sweetened soda intake (>1 cup/day) was associated with elevated follicular free and total estradiol [224] levels, which mirrors the endocrine effects seen in animal models [225]. Despite this increase in estradiol, however, there was no impact on ovulation in this study [224]. This is in contrast to findings from the Nurses' Health Study II. In this study, women consuming two or more caffeinated soft drinks per day had a 47% greater risk of ovulatory infertility than women who consumed less than one caffeinated soft drink per week. Four additional studies have also reported significant associations between caffeinated soft drinks and decreased fecundibility [215,226–228].

1.3.2.3.2 Alcohol

In animal models, ethanol has been reported to suppress plasma estradiol, luteinizing hormone, and progesterone levels and inhibit ovulation [229–231]. Moreover, rats maintained on a 5% ethanol

liquid diet were shown to have decreased ovarian function, including cessation of estrous cycles [232]. Despite these adverse effects on ovulatory function, a separate study showed that rats that received ethanol for 16 weeks but that were not fed alcohol during their pregnancy had just as many pups per litter as controls (that were fed no alcohol) when allowed to mate [233]. Thus, although ethanol might adversely affect the menstrual cycle in animals, it does not appear to totally suppress ovarian function.

In humans, the data on alcohol and reproductive hormones give divergent results. Three separate studies by Mendelson and colleagues showed that acute alcohol intake in premenopausal women led to increased plasma estradiol levels [234–236]. Specifically, acute alcohol intake resulting in peak blood alcohol levels of 70–75 mg/dl within an hour (approximately three standard drinks) produced a 55–66% increase in plasma estradiol levels above pre-drinking levels [235]. Biologically, these results are supported. The alcohol-induced rise in estrogen levels is thought to be a consequence of the metabolic breakdown of alcohol in the liver. Enzymatic degradation of alcohol is accompanied by a change in the proportions of the two forms of the coenzyme nicotinamide-adenine dinucleotide (NAD). The accumulation of the reduced form, NADH, means that the breakdown of estradiol to estrone is less favored and so estradiol accumulates.

Whether these transient increases in estradiol translate into clinical significance is still unclear, particularly given the high intakes necessary to observe these changes. For example, although female alcoholics show a higher prevalence of menstrual cycle disturbances compared to control women, this does not translate into reduced fertility [237]. Furthermore, among the prospective studies on alcohol and fertility in the general population, three studies report decreased fertility with increasing alcohol intake [213,219,238], two studies report no association between alcohol and fertility [217,239], one study found decreased fertility with higher alcohol intake among women older than 30 years of age but the opposite association among younger women [240], and one study reported significantly decreased fertility only among slow acetylators of alcohol [216]. Thus, despite much research to date on alcohol and ovulatory function, it is not possible to draw strong conclusions and further study is clearly needed.

1.3.2.3.3 Dairy Foods

Dairy foods have been suggested as a potential reproductive toxicant through at least two mechanisms. First, lactose, the main carbohydrate in milk, is cleaved in the intestine into glucose and galactose. In animal experiments, rodents fed high amounts of galactose have decreased ovulatory rates and develop premature ovarian failure (POF) [241,242]. This observation led to the hypothesis that high intake of milk and dairy products may increase the risk of infertility due to ovulatory dysfunction in otherwise healthy women [243]. In addition, dairy foods have been suggested as an important source of environmental estrogens. Because commercial milk is a mixture of milk from cows at different stages of pregnancy [152], dairy products contain detectable amounts of estrogens and other hormones that increase during pregnancy [244,245] and account for 60–80% of intake of estrogens from foods in Western countries [246]. However, few studies have been conducted in humans [243,247,248], and their results are conflicting.

The first study to address this question compared per capita milk consumption and age-related decrease in fertility rates in 31 countries and found an inverse relation between them [243]. However, a case-control study following up on these findings [247] found that women consuming three or more glasses of milk daily had a 70% lower risk of infertility than women who did not consume milk [247]. In a subsequent prospective cohort study, no relation was found between total intake of dairy foods and risk of ovulatory infertility [248]. However, unexpected associations between intake of reduced fat dairy foods with higher risk of ovulatory infertility and between intake of full fat dairy foods with lower risk of this condition were reported [248]. Although unanticipated, the association between intake of low fat dairy and higher risk of infertility due to anovulation is consistent with the well described ability of this food to increase circulating IGF-I levels [249–251] and the potential role of the latter on PCOS [252–254]. A closer inspection of the animal models also

suggests that diets with galactose contents that are closer to relevant intakes in humans do not result in POF or other signs of ovarian damage [255]. Collectively, these data suggest that galactose is unlikely to be an ovarian toxicant at the usual intake levels of humans. Although the epidemiologic literature suggests that dairy foods may influence fertility, this literature is scarce, inconsistent, and requires further replication.

1.3.2.3.4 Soy and Phytoestrogens

There has been much interest in the potential role of soy and phytoestrogens on reproductive function given the estrogenic activity of these compounds, which has been briefly described in Section 1.3.1. However, an equally healthy dose of skepticism has ensued given that the classical estrogenic effects of phytoestrogens may be too small in comparison to endogenously produced estrogen, particularly in premenopausal women, and given the relatively low intake levels of phytoestrogens in Western populations. A meta-analysis of randomized trials evaluating the effect of soy food or purified isoflavones on reproductive hormone levels or menstrual cycle characteristics found that, among premenopausal women, soy food or isoflavone supplementation resulted in significantly lower circulating FSH and LH levels (approximately 20% lower) and significantly longer menstrual cycle length (approximately 1 day longer) [256]. It is difficult to discern the clinical significance of these findings, particularly in relation to ovulation and fertility. However a recent study suggests that other phytoestrogens may have an important impact on fecundity. In a prospective cohort study of couples trying to become pregnant, baseline levels of urinary lignans, specifically of enterodiol and enterolactone, were associated with shorter time to pregnancy; urinary isoflavone levels were not related to this outcome, however [257].

Three randomized trials of phytoestrogen supplementation among couples undergoing infertility treatment suggest that the relation between phytoestrogens and fertility may not be due to effects on ovulation. In one of these trials, 147 couples with unexplained infertility treated with clomiphene citrate (CC) for ovulation induction plus timed intercourse were randomized to receive, or not, a 120 mg/day phytoestrogen supplement between days 1 and 10 of the treatment cycle (overlapping with the CC course) [258]. Although this intervention resulted in more than doubling of the clinical pregnancy rate (13.6% vs. 36.7%), there were no apparent differences between groups ovulation parameters. There were clear differences, however, in endometrial thickness suggesting improved endometrial receptivity. Consistent with this interpretation, two trials of infertile couples undergoing intrauterine insemination (IUI) [259] or IVF [260] found that supplementation with 1500 mg/day of phytoestrogen found a dramatic effect of this intervention on higher pregnancy rates, specifically, nearly doubling pregnancy rates (16.2% vs. 30.3%) in the IVF setting [260] and close to a 5-fold increase (4.4% vs. 20%) in the IUI setting [259]. The results of the study among couples undergoing IVF is particularly telling about potential mechanisms because supplementation in this trial started after oocyte retrieval, therefore excluding any ovarian effects and pointing toward implantation instead. Although these results are certainly dramatic they warrant further evaluation. Particularly, it is important to further distinguish potential effects on ovulation versus implantation or other mechanisms and the relevance to nonsupplemented Western populations who do not have high intakes of phytoestrogens in their diets.

1.3.2.4 Overall Patterns of Diet

Although multiple dietary factors may influence ovulation, as described in Section 1.3.2.3, the combined effects of multiple dietary factors cannot always be fully characterized as the sum of their independent effects, since this usually ignores interactions between multiple foods and nutrients. Hence, investigator- or data-derived dietary patterns often provide more realistic estimates of the total impact of diet on health outcomes, including measures of ovulatory function. To date, two prospective cohort studies have examined the relation between dietary patterns and fertility. In the Nurses' Health Study II, an investigator-generated "fertility diet" score was strongly related to a lower risk of ovulatory infertility and infertility due to nonovulatory causes (Figure 1.1) [261]. The highest scores were

assigned to women with high intakes of protein from vegetable sources, full-fat dairy foods, iron, the ratio of monounsaturated to *trans* fats, and more frequent use of multivitamins; low intakes of protein from animal sources, dietary glycemic load, and low-fat dairy foods. Women with the highest 20% of all scores had 66% lower risk of ovulatory infertility and 27% lower risk of infertility due to other causes than women with the lowest 20% independently of age, parity, BMI, and other potential confounders [261]. Furthermore, the authors estimated that, assuming these relations were causal, nearly half of all infertility cases due to anovulation could be prevented by changes in diet composition alone, and two thirds of the cases could be prevented by changes, in diet composition, physical activity, and weight control [261]. The Seguimiento Universidad de Navarra (SUN) cohort, which follows university graduates in Spain, reported similar findings. Higher adherence to a "Mediterranean pattern" diet, characterized by higher intakes of vegetables, fruit, fish, poultry, low-fat dairy, and olive oil, was associated with a lower risk of seeking medical help for difficulty getting pregnant [262]. Specifically, women in the top 25% of adherence to this pattern had a 44% lower risk of difficulty getting pregnant than women in the lowest 25%. While these two studies strongly suggest that overall diet pattern have an impact on female fertility, further replication of these findings is warranted.

1.4 CONCLUSION

Reproduction requires enormous amounts of energy from females. As a result, evolutionary pressure selecting reproductive systems capable of sensing the availability of energy in the environment are to be expected. In humans, this sensing appears to take place, at least partly, by the production of leptin, an adipocyte-derive peptide hormone and its central signal modulating the activity of the gonadotropin releasing hormone pulse generator. As a result, volitional, biological, pathological, and social conditions resulting in excessive energy expenditure or large energy deficits have been consistently associated with delayed sexual maturation and the cessation of ovulation in sexually mature women. On the other hand, long-term positive energy balance resulting in overweight and obesity also influences ovulatory function through different mechanisms. Most importantly, hyperinsulinemia and insulin resistance hinder ovulation, which, in extreme cases, can be manifested as PCOS or PCOS-like phenotypes and in less extreme examples as sporadic anovulation and elevated risk of infertility due to anovulation.

The relation between dietary factors and ovulatory function beyond their contribution to energy balance has received less attention. Intake of protein from animal sources has been consistently related to earlier onset of menses. This association could reflect the well-known effect of animal protein on IGF-I. Intriguing findings relating vitamin D status and intakes of soy products and vitamin A warrant further consideration. Among adult women, factors known to influence insulin sensitivity or glycemia, primarily the macronutrient composition of diet, have been related to ovulatory function. In addition, some micronutrients including folate and other nutrients involved in the one-carbon metabolism, iron, and vitamin D have been found to influence ovulation.

We have gained substantial insights on the relation between nutritional factors and ovulatory function over the last decade, particularly about potential influences not mediated through changes in energy balance. However, the study of the role of nutritional factors on ovulatory function, and more generally on human reproduction, is still a nascent field.

1.5 KEY POINTS SUMMARY

1. Nutrition affects ovarian function through at least two mechanisms: energy balance and the influence of specific macro- and micronutrients on insulin sensitivity. Some of the associations between diet and ovarian function may involve additional, not yet fully characterized, mechanisms.
2. Across all species, females spend at least three times more energy on gamete production than males, even before considering the additional energy costs of pregnancy itself. Given

these significant energy costs associated with ovarian activity and pregnancy, it is not surprising that evolutionary pressures have produced reproductive systems that are capable of sensing and reacting to adverse environmental conditions such as inadequate or poor nutrition.

3. Multiple studies have shown that undernutrition due to food insecurity brought about by war, adverse climatic conditions, or low socioeconomic status have all have been linked with significant delays in the age of onset of menarche in girls. Furthermore, high energy expenditure associated with participation in elite sports, and an associated extremely lean body composition, has also been linked with delayed menarche. Conversely, childhood obesity has been correlated with an earlier onset of menarche. The physiology behind these observations is that once a girl reaches a critical body fat content (approximately 17% fat), the production of leptin by adipose tissue reaches a critical threshold that modulates the brain's production of kisspeptin, which in turn activates the hypothalamic–pituitary (HP) axis to start producing gonadotropins and results in sex steroid hormone production from the previously quiescent prepubertal ovary. It is these sex steroids that then produce the breast changes of puberty and onset of menstruation.
4. Extremes of energy balance, brought about by inadequate or excessive food intake or exercise, are also linked with impaired ovulation. Extreme leanness (BMI < 18) due to excessive dieting or exercise is associated with menstrual cycle irregularity, anovulation, and secondary amenorrhea. In this scenario a relative deficiency in adipose leptin production, with an associated HP axis dysfunction, is thought to be responsible. On the excess side, overweight and obesity have been conclusively linked with an increased risk of anovulatory infertility, both in polycystic ovarian syndrome (PCOS) and non-PCOS cohorts, but the correlation between BMI and anovulation is strongest for women with PCOS. Here the etiological link between adiposity and anovulation is insulin resistance, with hyperinsulinemia interfering with ovarian function. Modest weight loss (5–10% of initial weight) can normalize insulin sensitivity and result in a return to ovulation in previously anovulatory overweight women. Antidiabetic medications have shown similar effects.
5. Studies examining the effect of specific macro- and micronutrients on the onset of menarche have suggested that a high intake of animal protein is related to an earlier onset of menarche, possibly reflecting the well-known ability of animal protein to boost the body's production of the growth factor IGF-1. Interestingly, the consumption of high amounts of soy and vitamin A has also been linked with an earlier onset of menarche by some preliminary studies.
6. Macronutrient intake during adulthood has been shown to affect ovarian function. First, consumption of saturated and *trans* fats has been reported to impair oocyte maturation and quality in both animal and human studies. Second, women who consume foods with a high glycaemic index (white bread, sweets, and fried potatoes) have been shown to have an increased risk of anovulation, probably mediated by the adverse effect of insulin resistance on the ovary. Data linking protein and fiber intake with ovulatory function are not consistent, making definitive conclusions impossible.
7. Significant evidence supports a role for folate, vitamin D, and iron in ovulation. Low dietary folate intake is associated with an increased risk of anovulation and lower luteal progesterone. Conversely, boosting the body's stores of folate through supplementation has been shown to improve oocyte quality during IVF treatment production and possibly increase the chances of dizygotic (fraternal or dual ovulation) twins. Vitamin D receptors are present in the ovary and some evidence links low vitamin D action (nutritional deficiency or gene knock out models) with impaired ovulation in animals and women with PCOS. Finally, high nonheme (plant-based) iron intake has been shown to reduce the chances of anovulatory infertility.

8. Although caffeine and alcohol are commonly cited as important factors contributing to anovulation and infertility, a judicious examination of current evidence does not suggest a significant effect of either caffeine or alcohol on ovulation. High alcohol intake, however, does increase serum estrogen levels by inhibiting hepatic clearance.
9. Studies of the relation between overall dietary patterns and ovarian function are scarce but suggest that the combination of several of the dietary factors linked to improved ovulation can have major impacts on fertility, particularly when ovulation disorders are the underlying cause for infertility.

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2 The Role of Diet and Lifestyle Modification in the Treatment of Polycystic Ovary Syndrome

Rebecca L. Thomson, Helena J. Teede, Nigel K. Stepto, Lauren K. Banting, and Lisa J. Moran

CONTENTS

2.1 BACKGROUND

Polycystic ovary syndrome (PCOS) has major health implications for women across their lifespan. It is the most common female hormonal condition, affecting between 4% and 21% of women of reproductive age depending on diagnostic criteria and populations studied [1–8]. The health consequences of PCOS are significant and diverse and include reproductive dysfunction (menstrual irregularity, infertility, high androgen levels, hirsutism, and acne) [9,10], weight disorders (greater rate of weight gain and obesity) [11], metabolic disturbance (insulin resistance, abnormal lipid profiles, increased gestational and type 2 diabetes, cardiovascular disease [CVD] risk factors) [12,13], and psychological disability (depression, anxiety, quality of life, sexual dysfunction) [14–17]. The resultant health and economic burden of PCOS is significant [18,19].

2.2 DEFINITION AND DIAGNOSIS OF PCOS

The diagnostic criteria for PCOS have been controversial, and until recently, two main criteria have been applied (Figure 2.1). The 1990 National Institutes of Health (NIH) criteria included clinical

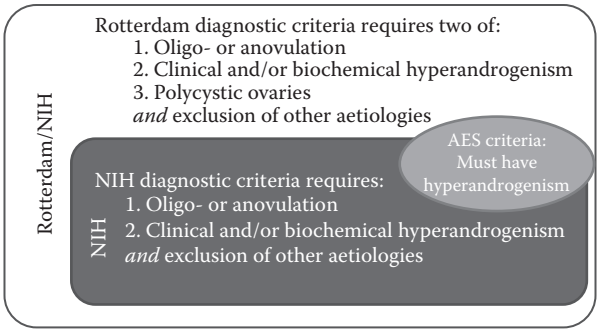

FIGURE 2.1 The Rotterdam criteria for diagnosis of polycystic ovary syndrome (PCOS). The Rotterdam criteria are inclusive of the original National Institutes of Health (NIH) criteria in that a woman diagnosed with PCOS using the NIH criteria will also meet Rotterdam criteria; however, a woman diagnosed with PCOS using Rotterdam criteria may not meet NIH criteria. (Adapted and reproduced from Teede HJ et al. *Med J Aust*., 195(6):S65–S112, 2011.)

hyperandrogenism and/or hyperandrogenemia, oligo-ovulation, and the exclusion of other causes. In 2003, the Rotterdam consensus expanded diagnostic criteria to include at least two of the following three features: clinical and/or biochemical hyperandrogenism, oligoanovulation, and polycystic ovary (PCO) morphology on ultrasound, excluding other causes [20]. Rotterdam criteria are inclusive of the original NIH criteria, but encompass milder phenotypes including those with either clinical and/or biochemical hyperandrogenism and PCO or ovulatory dysfunction with PCO. More recently, the NIH has endorsed the Rotterdam criteria [21] and there is now far greater international consensus on the diagnostic criteria. However, each of these diagnostic components needs greater definition and consistent application [22], as some studies report approximately 70% of women with PCOS remain undiagnosed [23]. Greater insights are needed into the natural history of menstrual cycles and defining when irregular cycles warrant further investigation in adolescents. PCO on ultrasound is perhaps the most controversial area in diagnosis, and the international Androgen Excess and Polycystic Ovary Society has developed new guidance on ultrasound criteria for PCOS [24]. Emerging literature suggests that serum anti-Müllerian hormone (AMH) levels may offer an alternative for assessing ovarian function in the future [25]. Finally, clinical hyperandrogenism varies across different ethnic groups, and interpretation can be hampered by treatments including laser and pharmacological therapy [10,26]. As a result of these controversies, many women remain undiagnosed, missing opportunities for prevention and early treatment, leaving women with increased risk of complications. Furthermore, health services often provide inconsistent and late-stage treatment of established disease. Action to improve diagnosis and focus on prevention including long-term assisted self-management, ongoing primary care, and consistent specialty management is now imperative.

2.3 ETIOLOGY OF PCOS

PCOS occurs in all ethnic groups, with differing prevalence depending on body weight, diet, lifestyle, and ethnic background [2,3,27]. The etiology of PCOS is yet to be fully elucidated, but there is marked inherent insulin resistance, even in lean women [28,29], which appears to be inherited. Subsequent hyperinsulinemia increases androgen production and reduces binding proteins to increase free androgen levels [30]. These hormonal abnormalities underpin the reproductive and metabolic features of PCOS [31,32] (Figure 2.2), with significant alterations in ovarian appearance and function disturbing ovulation and adversely impacting fertility.

PCOS-related insulin resistance is further exacerbated by obesity [28,33]. Obesity appears to have a bidirectional relationship in PCOS, with women predisposed to weight gain, which in turn

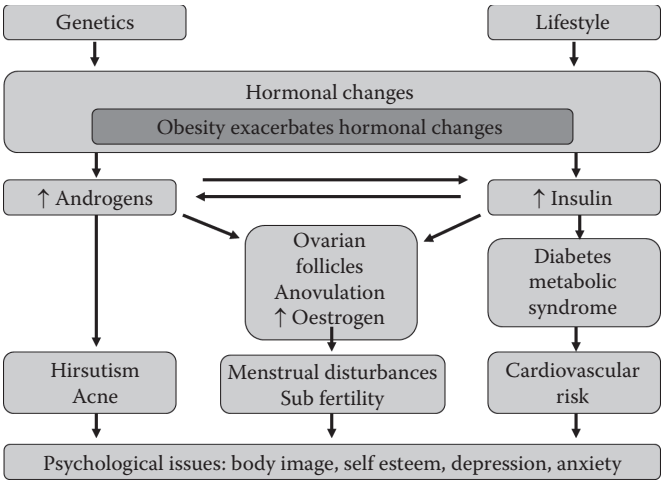

FIGURE 2.2 The etiological, hormonal and clinical features of polycystic ovary syndrome. (Adapted and reproduced from Teede HJ et al. *Med J Aust*., 195(6):S65–S112, 2011.)

exacerbates PCOS prevalence and severity [11]. Obesity is known to cause the triad of insulin resistance, low-grade chronic inflammation, and sympathetic nervous system dysfunction [34]. This triad has been noted in PCOS, independently of obesity [35], and is strongly associated with adverse metabolic outcomes, not only in PCOS, but also in obesity and diabetes. PCOS responds well to exercise [36,37] and to dietary intervention [38,39], both of which reduce insulin resistance, as further outlined in this chapter.

2.4 PROPOSED LIFESTYLE MANAGEMENT FOR PCOS

2.4.1 Definition of Lifestyle Management

Despite the recognition of the importance of overweight and obesity in worsening the presentation of PCOS, there has been a paucity of high-quality data examining the evidence for lifestyle management strategies in PCOS. Weight management can be defined as both preventing the development of overweight or obesity and treating established overweight and obesity. This can be defined more precisely as primary prevention of excess weight gain, achieving modest weight loss in those who are overweight or obese, maintaining this reduced weight loss long term, and optimizing health and reducing risk of disease whether or not weight loss is achieved [40]. The concept of lifestyle management traditionally refers to a complex multidisciplinary approach that combines dietary modification, physical activity, and behavioral interventions including attention to psychological adjustment, behavior modification, and stress management strategies [41,42]. Recent international evidence-based guidelines for the prevention of overweight and obesity in adults for the general population recommend reduced intake of energy-dense foods, fast foods, and alcoholic beverages and encouragement of increased physical activity and reduced sedentary behavior including television watching. Although weight loss targets should be individualized, a weight loss of 5–10% in individuals with a body mass index (BMI) of 25–35 kg/m^2 is recommended for reducing cardiovascular disease and metabolic risk factors, with greater weight losses (15–20%) recommended for individuals with a BMI greater than 35 kg/m^2 [40]. To achieve these goals, an individualized dietary program is recommended that includes a daily energy deficit of 600 kcal/day (2508 kJ/day) and a physical activity volume of 1800–2500 kcal/week (7560–10,500 kJ/week) or 225–300 minutes of moderate-intensity physical activity.

TABLE 2.1
Cochrane Review and Meta-Analysis Outcomes for Lifestyle Management Compared to Minimal Treatment for Anthropometric, Reproductive Nonfertility, and Metabolic Outcomes in PCOS

Outcome	Mean Difference, 95% Confidence Interval, and *P* Values	Participants, Trials
Weight	–3.47 kg, –4.94 to –2.00, $P < 0.00001$	108 participants, 2 trials
% Weight	–7.00%, –10.10 to –3.90, $P < 0.00001$	13 participants, 1 trial
Waist-to-hip ratio	–0.04, –0.07 to –0.00, $P = 0.02$	113 participants, 3 trials
Waist circumference	–1.95 cm, –3.34 to –0.57, $P = 0.006$	108 participants, 2 trials
Total testosterone	–0.27 nmol/L, –0.46 to –0.09, $P = 0.004$	144 participants, 5 trials
Ferriman–Gallwey score	–1.19, –2.35 to –0.03, $P = 0.04$	132 participants, 4 trials
Fasting insulin	–2.02 μU/mL, –3.28 to –0.77, $P = 0.002$	144 participants, 5 trials
Oral glucose tolerance test insulin	Standardized mean difference –1.32, –1.73 to –0.92, $P < 0.00001$	121 participants, 3 trials

Source: Adapted from Moran LJ et al. *Cochrane Database Syst Rev.* 2011(7);CD007506.

2.4.2 Lifestyle Management in PCOS

We recently authored a Cochrane review that examined the available randomized controlled trials assessing lifestyle interventions for weight management in PCOS [38]. Six articles were identified that examined combined lifestyle interventions [39,43,44] or structured physical activity interventions [45–47]. Considerable clinical heterogeneity was present in the studies, with sample sizes ranging from 11 to 90, durations ranging from 12 to 48 weeks, and interventions aimed at either specifically inducing or not inducing weight loss or examining lean or overweight participants. On meta-analysis, there was a greater reduction for lifestyle compared to minimal treatment for weight, % weight, waist-to-hip ratio, waist circumference, total testosterone, Ferriman–Gallwey score, fasting insulin, and oral glucose tolerance test insulin (Table 2.1). This meta-analysis highlighted the importance of lifestyle management for a range of clinical outcomes in PCOS. However, it also identified the limited and variable literature, lack of effect on outcomes such as glucose tolerance and lipid profiles, and lack of data on psychological outcomes.

In a further addition to the evidence base for lifestyle management in the treatment of PCOS, our group as part of the PCOS Australian Alliance was recently instrumental in developing the first Evidence-Based Guidelines for the Assessment and Management of PCOS [31], which were conducted in accordance with national (Australia) and international (National Institute of Clinical Excellence) criteria for best practice in clinical guideline development. The evidence-based guidelines noted that lifestyle management (single or combined approaches of diet, exercise, and/or behavioral interventions) for weight loss, prevention of weight gain, or for general health benefits should be recommended in women with PCOS (level B evidence). Specifically, this lifestyle management should target weight loss in overweight/obese women (BMI ≥25 kg/m^2) and prevention of weight gain in lean women (BMI 18.5–24.9 kg/m^2), should include both reduced dietary energy (caloric) intake and exercise, and should be first-line therapy for all women with PCOS (level C evidence).

2.4.3 Optimal Dietary Intake in PCOS

These guidelines also assessed the clinical question of the optimal diet composition of lifestyle management, which was assessed by a systematic review [48]. Six articles from five studies ($n = 137$)

were included in the review. The studies were an acute (16-day) weight maintenance intervention comparing a monounsaturated fatty acid enriched diet, a conventional healthy diet, and a low-carbohydrate diet [49]; a 12-month weight loss intervention comparing a low glycemic index (GI) or a healthy diet [50]; a 3-month weight loss and 1-month weight maintenance intervention comparing a high-protein and a standard protein diet [51,52]; a 6-month weight maintenance intervention comparing a carbohydrate-restricted and a fat-restricted diet [53]; and a 1-month weight loss intervention comparing a high- or standard protein diet [54] (Table 2.2).

There was considerable variation between the studies with regard to the characteristics of the trial, participants, or the diets studied. Some subtle differences did occur with modifying diet composition, with greater weight loss for a monounsaturated fat-enriched diet; improved menstrual regularity for a low-GI diet; increased free androgen index (FAI) for a high-carbohydrate diet; greater reductions in insulin resistance, fibrinogen, total and high-density lipoprotein cholesterol for a low-carbohydrate or low-GI diet; improved quality of life for a low-GI diet; and improved depression and self-esteem for a high-protein diet. However, for the majority of the outcomes there were largely similar decreases in weight and body composition and improvements in pregnancy rate, menstrual regularity, ovulation, hyperandrogenism, insulin resistance, lipids, and quality of life that occurred with weight loss independent of diet composition. This review concluded that weight loss should be targeted in all overweight women with PCOS through reduction of caloric intake in the setting of adequate nutritional intake and healthy food choices irrespective of diet composition. The evidence-based guidelines for the optimal diet composition of a lifestyle intervention in PCOS are based on this systematic review and report this as level C evidence.

2.4.4 Dietary Differences in Women with and without PCOS

It is also possible that women with PCOS have an elevated prevalence of overweight or obesity owing to altered energy balance such as reduced energy expenditure through physical activity or increased dietary energy intake. Increases in carbohydrate craving are reported for women with elevated androgen levels independent of PCOS status [55], indicating the potential for increased food intake in women with PCOS. In support of this, women with PCOS consumed higher amounts of high-GI foods (white bread, fried potatoes) than women without PCOS [56], and lean women with PCOS consumed less total energy than weight-matched women without PCOS, suggesting women with PCOS may need to follow more stringent dietary restrictions to maintain weight [57]. Conversely, no differences in total energy, macronutrient or micronutrient intake, or food groups were observed between lean and overweight women with or without PCOS [56–58].

In the largest study to date comparing dietary intake in women with (n = 409) and without (n = 7057) PCOS, we reported a subtly better dietary intake for women with PCOS as indicated by an elevated diet quality score, lower saturated fat and GI intake, and higher micronutrient (fiber, folate, iron, calcium, magnesium, niacin, phosphorus, potassium, sodium, vitamin E, and zinc) intake [59]. However, this improved dietary intake occurred in the context of a greater energy intake (approximately 215 kJ/day, 51 kcal/day) for the women with PCOS compared to the women without PCOS. Together these findings suggest that women with PCOS may be improving their diet quality by following healthy lifestyle recommendations from national and international guidelines and position statements [31,60] but not adequately regulating the quantity of their food intake and may benefit from further education regarding appropriate portion sizes.

2.4.5 Role of Vitamin D in PCOS Therapy

There is also increasing interest in the potential effect of specific dietary components on the presentation of PCOS. Vitamin D is a micronutrient found in only a few foods, such as fatty fish, mushrooms, egg yolks, and liver and is mostly produced endogenously when the skin is exposed to ultraviolet radiation from sunlight. Recent position statements have recommended serum

TABLE 2.2
Systematic Review of Studies Assessing Different Diet Compositions in PCOS Identified

Citation	Intervention	Comparisons	Design	Duration	Findings: Differences between Diet Groups
Douglas et al. [49]	MUFA and LC weight maintenance diet $n = 11$	Conventional healthy (ADA guidelines) weight maintenance diet $n = 11$	Crossover	16 days	LC vs. MUFA: Greater decrease in weight and insulin response to glucose. LC vs. healthy diet: Greater decrease in fasting insulin and cholesterol than standard diet. No significant differences in other outcomes.
Marsh et al. [50]	Low-GI *ad libitum* weight loss diet $n = 29$	Conventional healthy *ad libitum* weight loss diet $n = 20$	Parallel	12 months	Low-GI compared to healthy diet: Greater increases in insulin sensitivity, lower fibrinogen, increased menstrual regularity, greater improvement for emotion sub-domain of quality of life questionnaire. No significant differences in other outcomes.
Moran et al. [51]; Galletly et al. [52]	HP weight loss diet $n = 14$	SP weight loss diet $n = 14$	Parallel	4 months (3 months weight loss, 1 month weight maintenance)	SP: Decrease in HDL-C. Increase in FAI from week 12 to 16. HP: Decrease in TC:HDL-C from week 0 to 12. HP: Improvement in depression and self-esteem. No significant differences in other outcomes.
Moran et al. [53]	CC semi-*ad libitum* weight maintenance diet after weight loss $n = 14$	FC semi-*ad libitum* weight maintenance diet after weight loss $n = 9$	Parallel	8 months (2 months weight loss, 6 months weight maintenance)	No significant differences between any outcomes.
Stamets et al. [54]	HP weight loss diet $n = 13$	SP weight loss diet $n = 13$	Parallel	1 month	No significant differences between any outcomes.

Source: Adapted from Moran LJ et al. *J Acad Nutr Diet*. 2013;113(4):520–545.

Note: ADA, American Diabetes Association; CC, carbohydrate counting; FAI, free androgen index; FC, fat counting; GI, glycemic index; HDL-C, high-density lipoprotein cholesterol; HP, high protein; ISI OGTT, insulin sensitivity index following an oral glucose tolerance test; LC, low carbohydrate; MUFA, monounsaturated fatty acid; SP, standard protein; TC:HDL, total cholesterol: high density lipoprotein cholesterol.

concentrations of 25-hydroxyvitamin D (25OHD) of greater than 50 nmol/L for adults [61,62] and during pregnancy [63]. This level may need to be 10–20 nmol/L higher at the end of summer to maintain levels greater than 50 nmol/L over winter and spring. Vitamin D deficiency is common in women with PCOS, with reports of 31–85% having serum concentrations of 25OHD less than 50 nmol/L [64–71]. Vitamin D deficiency has been linked with an increased risk of obstetric complications such as preterm labor, gestational diabetes, and preeclampsia [72].

Accumulating evidence suggests a role of vitamin D in the pathophysiology of PCOS [73]. Several studies have demonstrated that vitamin D deficiency may exacerbate symptoms, including insulin resistance [64–66,74,75], infertility (ovulatory and menstrual dysfunction, impaired follicle development, and lower pregnancy success) [76–78], hirsutism and hyperandrogenism [64–67], and obesity [64–67,74]. Accumulating evidence suggests that vitamin D may be involved in the pathogenesis of PCOS through gene transcription [79] and hormonal modulation, which influences insulin metabolism [69,79] and fertility regulation [74]. A vitamin D response element exists on the human insulin receptor gene promoter, with vitamin D exposure significantly increasing the insulin receptor function [80]. Therefore there is a theoretical reason why low levels of vitamin D may be linked with PCOS through the initiation of insulin resistance. Furthermore, studies have directly correlated serum vitamin D levels with the degree of insulin resistance in women with PCOS [64–66,74]. Obesity itself has also been linked to reduced vitamin D levels, and many studies have reported inverse associations between body weight (BMI, body fat, and waist measurements) and serum 25OHD levels in women with PCOS [64–67,71,74].

There have been only a few uncontrolled and randomized controlled trials that have evaluated the effects of vitamin D supplementation in women with PCOS (Table 2.3). Two small uncontrolled studies have indicated that vitamin D therapy may have a beneficial effect on insulin resistance and insulin secretion in obese women with PCOS [68,81], and the increase in vitamin D levels was positively correlated with the improvements in insulin secretion and effectiveness [81]. Conversely, two uncontrolled pilot studies, one in relatively lean women with PCOS without severe insulin resistance [82] and one in very obese insulin-resistant women (BMI, 39 kg/m^2; fasting insulin levels, 25 μIU/ml; homeostatic model assessment of insulin resistance [HOMA-IR] scores, 6.7) with PCOS [83] found no changes in insulin resistance. A recent randomized controlled trial in vitamin D-deficient women with PCOS found that 2 months of vitamin D supplementation did not improve insulin resistance and sensitivity, assessed using fasting blood compared with placebo, despite significant increases in 25OHD levels [84]. Beta cell function assessed using HOMA increased significantly, but was not different compared with placebo [84].

Several studies have also examined the effects of vitamin D supplementation on reproductive function (Table 2.3). Two uncontrolled studies found improvements in menstrual frequency in 50% of oligo- or amenorrheic women [82] and normalized menstrual cycles in 78% of the women with menstrual dysfunction and two pregnancies [70]. Two similar randomized controlled trials investigated the effects of metformin with and without vitamin D and calcium in infertile women with PCOS and found those treated with vitamin D, calcium, and metformin had a higher number of dominant follicles [85]. They also found greater improvement in menstrual irregularities [85,86] and more pregnancies [86]; however, these were not significantly different between treatments. This suggests a potential for treatment with vitamin D and calcium to normalize cycles in women with PCOS and low levels of vitamin D.

Limited studies have looked at the effect of vitamin D supplementation on measures of hyperandrogenism (Table 2.3). Two small uncontrolled studies in women with PCOS supplemented with vitamin D combined with calcium reported clinical improvements in acne vulgaris in all three women presenting [70] and reductions in testosterone [83] but there were no changes in other clinical [70] and biochemical [83] measures of hyperandrogenism. Two other uncontrolled studies observed no changes in testosterone, sex hormone-binding globulin (SHBG), and FAI [68,82].

There is limited evidence that vitamin D supplementation may have a beneficial effect on insulin resistance and reproductive function. Most of these were small uncontrolled studies, so the data can be considered only preliminary in nature. In some studies, although vitamin D levels were

TABLE 2.3
Studies Investigating the Effect of Vitamin D Supplementation in Women with PCOS

Study	Participants	Treatment (Δ25OHD Levels)	Duration	Insulin Resistance Outcomes	Reproductive Outcomes	Hyperandrogenism Outcomes
			Uncontrolled Studies			
Kotsa et al. [81]	15 obese, insulin-resistant women with PCOS	1 mcg/day vit D 37.9–71.4 nmol/L	3 months	↑ First-phase insulin secretion, insulin effectiveness ↓ First-phase glucose AUC – Glucose, insulin, integrated AUC for insulin, insulin sensitivity		
Selimoglu et al. [68]	11 obese, insulin-resistant women with PCOS	Single dose of 300,000 IU vit D_3 42.2–92.6 nmol/L	3 weeks	↓ HOMA-IR – Fasting glucose and insulin levels		– DHEAS, total and free testosterone, androstenedione
Pal et al. [83]	12 overweight, vitamin D-deficient women with PCOS	2000 IU/day vit D_3 and 50,000 IU/week or month vit D_2 43.9–71.4 nmol/L	3 months	– Fasting insulin and glucose, HOMA-IR, QUICKI, AUC glucose and insulin		↓ Total testosterone – FAI, SHBG, androstenedione
Wehr et al. [82]	46 women with PCOS	20,000 IU/week vit D_3 69.9–130.8 nmol/L	24 weeks	↓ Fasting and stimulated glucose – Fasting and stimulated insulin, HOMA-IR	23/46 of oligo- or amenorrheic women improved menstrual frequency 4/16 women seeking pregnancy conceived	– Testosterone, free testosterone, SHBG, FAI
Thys-Jacobs et al. [70]	13 vitamin D-deficient women with PCOS	50,000 IU/week or biweekly vit D_2 + 1500 mg/day Ca 28.0 to 75–100 nmol/L	6 months		7/9 women normalized menstrual cycles 4 maintained normal menstrual cycles 2 pregnancies	Improved acne vulgaris in 3/3 women – Hirsutism, acanthosis nigricans, alopecia

Randomized Controlled Studies					
Ardabili et al. [84]	50 vitamin D-deficient women with PCOS	3 oral doses of 50,000 IU vitamin D_3 or placebo 17.2–58.4 nmol/L	2 months	– Fasting insulin and glucose, insulin sensitivity (HOMA-S, QUICKI), insulin resistance (HOMA-IR) ↓ HOMA-β in Vit D group (*ns* from placebo)	
Rashidi et al. [85]	60 infertile women with PCOS	400 IU vit D + 1000 mg Ca/day; 400 IU vit D + 1000 mg Ca + 1500 mg/day metformin; vs. 1500 mg/day metformin 25OHD not measured	3 months; 3-month follow-up		↑ Number of dominant follicles greater in Vit D + Ca + Metformin group ↓ Menstrual irregularities greater in Vit D + Ca + Metformin (*ns*) – Rates of pregnancy and menstrual regularity
Firouzabadi et al. [86]	100 infertile women with PCOS	1500 mg/day metformin with or without 100,000 IU/month vit D + 1000 mg/day Ca 32.9–61.9 nmol/L	6 months; 6-month follow-up		↑ Regular menstrual cycles (33/48 Vit D + Ca + metformin, 25/46 in metformin) 9/15 pregnancies in Vit D + Ca group

Source: Adapted from Thomson R et al. *Clin Endocrinol*. 2012;77(3):343–350.

Note: Δ25OHD, change in 25-hydroxyvitamin D levels in women supplemented with vitamin D; AUC, area under the curve; Ca, calcium; FAI, free androgen index; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of beta-cell function; HOMA-S, homeostatic model assessment of insulin sensitivity; *ns*, not significant; QUICKI, quantitative insulin sensitivity check index; Vit D, vitamin D; –, unchanged; ↓, decrease; ↑, increase.

increased, women still had suboptimal vitamin D status. There is a need for larger randomized controlled trials with higher doses to investigate further the role for vitamin D supplementation in PCOS therapy to confirm and understand better the potential benefits of vitamin D supplementation in this population. Although beneficial effects of vitamin D are largely achieved by supplementation and sunlight exposure rather than dietary intake, this highlights the potential importance of micronutrient sufficiency in the management of PCOS. Vitamin D deficiency has also been linked with adverse obstetric complications, and increasing 25OHD levels through supplementation in a general obstetric population has been shown to reduce those adverse outcomes [87]. Therefore, even if the ability of vitamin D supplements to normalize ovulation in PCOS women is under debate, adequate replacement of vitamin D in women with serum levels below 50 nmol/L makes good obstetric preventative medicine sense, especially if they are contemplating pregnancy.

2.4.6 Interaction of Diet and Exercise in PCOS Therapy

Exercise is an important contribution to lifestyle intervention in both the general population and in women with PCOS. Adolescents with PCOS reported lower duration and intensity of physical activity [88] and women with PCOS had reduced cardiopulmonary capacity as a marker of fitness and potential physical activity tolerance [89] compared to weight- or BMI-matched controls. In contrast to these findings, no differences in physical activity, aerobic exercise capacity, or muscle strength were observed between women with and without PCOS [57,58,90,91]. In our community cohort study in women with (n = 409) and without (n = 7057) PCOS, we saw no differences in total physical activity but a higher level of sedentary time for women with PCOS (approximately 0.5 hour/day) [59]. This highlights reduced sitting time as an additional therapeutic target in women with PCOS and is of clinical relevance given that each 2-hour/day increment in television watching is associated with a 23% increase in obesity and a 14% increase in type 2 diabetes risk [92].

The combination of exercise and dieting substantially increases weight loss compared with dieting or exercise alone [93,94]. Furthermore, moderate- to high-intensity aerobic exercise improves an array of health-related outcomes including body composition and reproductive (ovulation and fertility), metabolic (insulin resistance, dyslipidemia, and the development of cardiovascular disease and type 2 diabetes), and psychological (mood and well-being) outcomes [95–100]. These improvements can occur independent of weight loss [95]. Participation in regular exercise or combining exercise and dieting also provides better long-term weight maintenance [101,102]. These health benefits are reflected in a number of physical activity guidelines and position statements, which recommend 30 minutes of moderate to vigorous physical activity on most days of the week [103–105]. Resistance or weight training is also effective for improving insulin resistance and body composition and can preserve lean tissue during energy-restricted weight loss [106–108]. Furthermore, combining aerobic and resistance exercise has been reported to be more efficacious for improving insulin sensitivity and glycemic control and reducing abdominal fat in various obese groups compared with either form of exercise alone [109–112].

Despite well-established benefits of exercise training and its recommendation as a cornerstone of PCOS management [31,38,60,113], as illustrated in the conceptual model (Figure 2.3), there is limited well-controlled research evaluating the effects of exercise training and specific exercise regimens in women with PCOS. Recent reviews [114,115] and the evidence-based guidelines [31] identified 14 studies on the effects of exercise on clinical outcomes in PCOS. Only 8 were randomized control trials, mostly of low quality, with high risk of bias due to study design issues and low participant numbers. Studies were heterogeneous, including variable duration, inclusion/exclusion criteria, extent of caloric restriction, and type/intensity of exercise ranging from unsupervised walking to structured/supervised exercise programs.

Six exercise-only cohort studies have shown improvements in fitness [36,37,116,117], body composition (waist-to-hip ratio [116], total body and abdominal fat mass, and visceral fat [36]), body

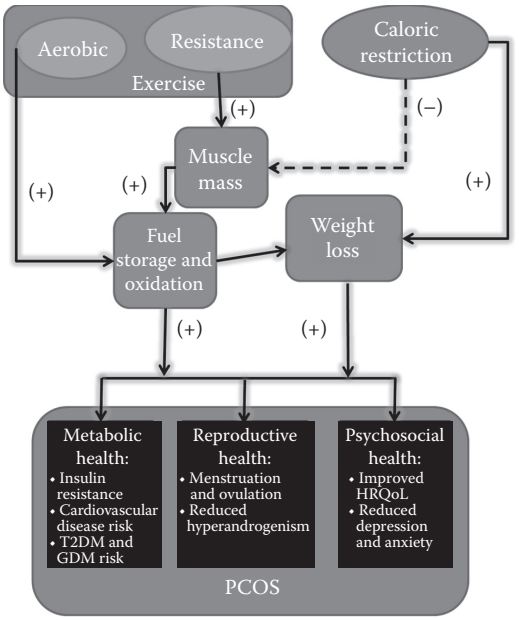

FIGURE 2.3 Conceptual model of the contributions of exercise to weight loss and health outcomes in polycystic ovary syndrome (PCOS), demonstrating that both aerobic and resistance exercise contribute to reduction of body weight. More specifically, the model shows the important contribution of resistance exercise in offsetting muscle mass loss that occurs under caloric restriction used for rapid and effective weight loss in PCOS. GDM, gestational diabetes mellitus; HRQoL, health-related quality of life; T2DM, type 2 diabetes mellitus.

image distress [118], and insulin sensitivity [36,37,117,119] after 6 months of walking [116,118] or 3 months of supervised high- and moderate-intensity aerobic exercise [36,37,117,119]. Conversely, they also reported no changes in body composition [36,37,117], fasting insulin [116], reproductive hormones [36,37,116,117], and lipid profiles [36,37,116]. Three aerobic exercise only randomized controlled trials [45,47,120], three comparing aerobic exercise with acupuncture [46,121,122] and control groups, and one nonrandomized controlled trial [123] showed improvements following exercise in fitness [45,47,120,121,123], weight [46], body composition (waist circumference [47,120], waist-to-hip ratio, and BMI [46,47,120,123]), insulin resistance [47,120,123], lipid profiles [120], blood pressure and health-related quality of life [122], and menstrual cyclicity [47,121] after 3–6 months of a supervised aerobic exercise program. Conversely, they also reported no changes in lipids, glucose [46,47,123], blood pressure [45,46,123], reproductive hormonal profile [46,47,120,121], hirsutism [46,120,121], weight and waist circumference [45], or fasting insulin [45,46].

One study compared the effects of 24 weeks of aerobic exercise (30 minutes cycling, 3 times per week) with a hypocaloric, high-protein diet in an unrandomized, controlled study of 40 obese women with PCOS. Although both treatments reduced body weight, waist circumference, fasting insulin, and insulin resistance and improved menstrual cyclicity, ovulation, and the reproductive hormone profile, greater improvements in waist circumference, SHBG, FAI, and insulin levels were reported with exercise, despite greater weight loss in the dieting participants [124]. This suggests that exercise training may offer greater benefits for improving insulin resistance and reproductive hormone levels compared with diet induced weight loss.

Studies have also compared the effect of diet alone or combined with exercise (Table 2.4). Five uncontrolled studies with combined diet and exercise showed improvements in weight [125–129], waist circumference [125,127–129], hormonal profile [127,128,130], insulin resistance [127,128,130],

TABLE 2.4
Studies Investigating the Effect of Exercise Training and Diet in Women with PCOS

Study	Participants Enrolled (Participants Completed)	Interventions	Duration	Intervention Outcomes in Women with PCOS
		Uncontrolled Studies		
Aubuchon et al. [125]	37 (32) overweight/obese women with PCOS	Nutrition classes and supervised exercise sessions (resistance and moderate-intensity aerobic training 60 minutes/session, goal of 200–300 minutes/week)	14 weeks	↓ Weight, BMI, WC, hip circumference 6 pregnancies (46% who desired)
Thomson et al. [127]	57 overweight/obese women with PCOS	High-protein energy-restricted diet (5–6000 kJ/day (1–1400 kcal/day), 30% protein, 40% carbohydrate, 30% fat) with and without exercise (walking/jogging 5 times/week, 25–45 minutes, 60–80% HR max or walking/jogging 3 times/week, and 5 resistance exercises 2 times/week)	10 weeks	↑ Heart rate recovery, SHBG ↓ Weight, WC, BP, glucose, insulin, HOMA-IR, testosterone, FAI
Vosnakis et al. [130] Panidis et al. [128]	61 overweight/obese women with PCOS and 20 controls 101 women with PCOS and 29 controls	Energy-restricted diet (basal metabolic rate 600 kcal/day (2520 kJ/day), 50% carbohydrate, 30% fat, 20% protein), physical exercise (3 days/week for 1 hour moderate intensity aerobic exercise), and Orlistat (120 mg before each meal)	24 weeks	↑ SHBG, LH ↓ BMI, WHR, FAI, insulin resistance (fasting glucose, insulin, HOMA-IR, QUICKI) – Ovarian volume and follicles, FSH, D4A, prolactin ↑ Anti-Müllerian hormone – Testosterone, DHEA-S, 17α-OHP ↓ Weight, WC, BP, cholesterol, LDL, triglycerides, testosterone, DHEA-S – HDL ↑ 17α-OHP
Mahoney [126]	12 infertile overweight/obese women with PCOS	Lifestyle modification through individualized counseling sessions Advised to engage in low-impact aerobic exercise for 3–5 days/week for at least 30–60 minutes and resistance exercise 2–3 times/week and healthy eating	12 weeks	↓ Weight 2/4 amenorrheic women reported spontaneous menstrual cycles

		Controlled Studies			
Palomba et al. [124]	40 obese anovulatory infertile patients with PCOS	Hypocaloric high-protein diet (800 kcal deficit/day [3360 kJ/day], 35% protein, 45% carbohydrate, and 20% fat) vs. aerobic exercise program (3 times/week in 30-minute cycle at 60–70% $\dot{V}o_2max$)	24 weeks	↓Weight (greater in diet group) ↓ WC, insulin, testosterone, FAI (all greater in exercise group) – Glucose ↑SHBG, menses frequency, ovulation rate (all greater in exercise group) Improved menstrual cycles and fertility	
		Randomised Controlled Studies			
Bruner et al. [131]	12 women with PCOS	Nutritional counseling (long-term nutritional strategies) vs. nutritional counseling and combined aerobic resistance exercise (3 times/week of 30 minutes of moderate-intensity aerobic exercise and 12 resistance exercises)	12 weeks	↓ Fasting insulin, WC ↓ Body fat (exercise only) – SHBG, testosterone, FAI	
Thomson et al. [132]	94 (52) overweight/obese women with PCOS	High-protein energy restricted diet (5–6000 kJ/day (1190–1430 kcal/day), 30% protein, 40% carbohydrate, 30% fat) vs. diet and aerobic exercise (walking/jogging 5 times/week, 25–45 minutes, 60–80% HR max) vs. diet and combined aerobic-resistance exercise (walking/jogging 3 times/week, 25–45 minutes, 60–80% HR max, 5 resistance exercises 2 times/week)	20 weeks	↓ Weight	↓ WC, BP, lipids, glucose, insulin, HOMA-IR, testosterone, FAI, fat mass (greater in exercise groups) ↑SHBG 49% improved menstrual cyclicity and ovulation
Thomson et al. [133]	94 (49) overweight/obese women				↓ Depression scores ↑ Health-related quality of life

Source: Adapted from Thomson RL et al. *Obes Rev*. 2011;12(5):e202–e210.

Note: 17α-OHP, 17α-hydroxyprogesterone; BMI, body mass index; BP, blood pressure; D4A, D4-androstenedione; DHEA-S, dehydropiandrosterone sulfate; FAI, free androgen index; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HR, heart rate; LDL, low-density lipoprotein cholesterol; LH, luteinizing hormone; QUICKI, quantitative insulin sensitivity check index; SHBG, sex hormone-binding globulin; $\dot{V}o_2max$, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; –, unchanged; ↓, decrease; ↑, increase.

aerobic fitness [129], AMH [130], and lipids [128]. Some of these studies also found improvements in reproductive function; 2 out of 4 previously amenorrheic women reported spontaneous menstrual cycles during a 12-week study [126]; 6 women became pregnant during a 14-week study or within 3 months of completion, which represented 46% of women desiring pregnancy [125]; and 3 of 14 women had regular menses during a 16-week study and 3 changed from polycystic to normal ovaries [129]. However, others found no changes in ovarian volume [128–130] and follicles [128,130]. Randomized control trials comparing diet only with diet and exercise programs have shown similar reductions in fasting insulin levels, waist circumference [131,132], weight, insulin resistance, blood pressure, hormonal profile, lipids, and glucose and improvements in ovulation and menstrual cyclicity [132], depression, and quality of life [133]. The addition of exercise to diet led to greater improvements in body composition, with greater reductions in fat mass [131,132] and preservation of lean tissue [132], and it has been shown that the preservation of lean mass assists in maintaining resting metabolic rate and long-term weight objectives [134–136]. Exercise training during weight loss may have important implications for long-term maintenance of weight loss in PCOS; however, longer term studies are required to confirm this. Overall, exercise studies have shown improvements in menstrual cyclicity and/or ovulation in approximately 50% of women with PCOS, comparable to that observed with other weight loss interventions. Improvements in insulin and hormonal profile appear to play important mediating roles for improving reproductive function, although this is not exclusively the case.

Based on the limited data available, exercise appears to have beneficial effects, either alone or in combination with caloric restriction, where it has been shown to improve aerobic fitness, body composition, fasting insulin, insulin resistance, menstrual cyclicity, ovulation, self-esteem, quality of life scores, and depression. Although lifestyle modification including regular exercise appears to be an effective strategy for the management of overweight women with PCOS, methodological limitations (small samples sizes, lack of appropriate control groups and assessments of habitual physical activity, nonrandomization to treatments, and/or short study durations) in the current research limits the generalizability of the findings, making it difficult to draw definitive conclusions and make specific recommendations. Further research with rigorous study designs is needed to determine specific exercise guidelines alone or in combination with specific dietary interventions (necessary exercise dose, type, intensity, and frequency) that will provide the greatest benefit for these women. The evidence-based guidelines for PCOS management recommend exercise participation of at least 150 minutes per week to all women with PCOS, especially those with a BMI greater than 25 kg/m^2, given the metabolic risks of PCOS and the long-term metabolic benefits of exercise [31]. Of this, 90 minutes per week should be aerobic activity at moderate to high intensity (60–90% of maximum heart rate) to optimize clinical outcomes [31].

2.4.7 Barriers to Diet and Exercise in PCOS

Women with PCOS face a range of barriers when attempting to implement lifestyle modifications, with some being typical of the general population and others being unique to women with the condition. We have previously noted in a Cochrane review of lifestyle management of PCOS that high dropout rates are noted in the majority of studies, with up to 45% of women not completing clinical lifestyle interventions [38]. This is higher than in studies of individuals without PCOS (e.g., up to 38% over 1–4 months for PCOS [51,54] compared to up to 9% over 4 months in women without PCOS [137–139]). The reasons for these poor retention rates are unclear. However, anecdotally, women with PCOS are considered a population with specific barriers to weight management. Young women have high perceived time pressure due to work and family commitments, which can be a fundamental barrier to healthy eating and physical activity [140–142]. This may result in the development of a perception that the recommended behavioral changes to achieve optimum lifestyle habits are not feasible [143].

2.4.7.1 Abnormalities in Energy Expenditure or Appetite Regulation

Barriers to weight management may mechanistically work either through increasing susceptibility to weight gain or resistance to weight loss. It is as yet unclear whether this is due to physiological or environmental causes. Physiological barriers to weight management may include insulin resistance and hyperinsulinemia, which are common in PCOS. These may predispose women with PCOS to gain weight, supported by insulin therapy inducing weight gain [144] through anabolic effects of insulin and changes in energy expenditure, glycosuria, and food intake [145–150]. However, a range of studies have shown elevated insulin secretion, hyperinsulinemia, or insulin resistance (using surrogate fasting or postprandial or clamp measures of insulin resistance) is associated with greater, similar, or lesser weight gain in free-living observational studies [151]. Androgen excess may favor abdominal fat deposition [152], which could in addition contribute to insulin resistance in PCOS. Altered energy expenditure or appetite regulation has also been reported in PCOS, including reduced resting energy expenditure and the thermic effect of food [153,154] or impaired regulation of gut hormones such as ghrelin or cholecystokinin [155,156]. However, this is not consistently reported [91,151,157,158].

2.4.7.2 Self-Efficacy Concerns

Additional barriers to a healthy lifestyle, specifically physical activity, may include self-efficacy concerns. An individual's belief in his or her ability to participate successfully in physical activity has long been recognized as an important factor in physical activity participation [159]. For women with PCOS, a lack of self-efficacy could stem from any number of experiences. Other chronically diseased populations indicate that many symptoms of their condition and associated comorbidities reduce their belief in their ability to engage successfully in physical activity [160], which may be similar for women with PCOS. Older populations often report poor health, pain, or fear of pain reduces their participation in physical activity [160–162]. This may be similar for women with PCOS who may believe they are unable to participate in physical activity without causing further pain, or not be able to participate for sufficient time at the intensity required. Women with PCOS who also have problems with weight may report similar efficacy-related concerns to other overweight populations who feel that they are "too fat," not fit enough, or do not have the right body type for successful engagement in physical activity [163,164]. It has been found that individuals with comorbid chronic diseases lack the knowledge and ability to incorporate self-care strategies into their management plan for chronic diseases [165]. In a similar way, women with PCOS who are participating in physical activity for the first time, or are returning to physical activity after a long period of inactivity, may lack the knowledge required to participate and may not understand the amount of time and intensity required to achieve positive health outcomes. Women in this position may be overwhelmed by the amount of information available to them, and unable to distinguish evidence-driven recommendations.

To improve women's belief in their ability to do more physical activity, health professionals can assist by providing concise and high-quality information. To overcome the worry associated with participation, health professionals may recommend that women with PCOS consult an accredited exercise physiologist to receive practical and individualized training. Non-weight-bearing exercise options may also be particularly useful for obese women with joint pain or injuries. Options include stationary or recumbent cycling and water-based exercises, such as swimming, walking in water, and water aerobics where the buoyancy of the water takes much of the weight off the joints.

Regardless of whether a woman consults an exercise physiologist, women should be encouraged to engage in activities that they find enjoyable and achievable. Encouraging women to exercise with friends or family may also alleviate some of the concerns women may feel about their ability to be physically active. A 6-month group treatment program conducted in Adelaide, Australia found beneficial improvements even after only 5% weight loss (4.3 kg), with 92% of previously anovulatory women ovulating within 4 months, and 85% conceived within 12 months of finishing

(46% spontaneous pregnancies) [166]. The group program encouraged group support and cohesion and emphasized gradual dietary changes in conjunction with regular exercise, with an emphasis on doing something positive about their lifestyle and health, not on fertility. The program included 1 hour of seminars per week covering a wide range of weight-related topics, including diet and nutrition, abnormal eating behavior, and endocrine effects of obesity and one formal exercise session per week (1 hour of low-impact aerobics and stretching and increasing to walk/jog regimen and stair climbing) and were encouraged a minimum of two more sessions per week.

2.4.7.3 Motivational Concerns

For any person trying to be more active, motivation to do so is a strong factor in determining success. A strong personal interest in physical activity is conducive to physical activity, whereas being driven solely by a doctor's instructions or pressure from external parties is unlikely to produce ongoing behavioral change [167,168]. Motivation to be physically active may stem from any number of sources: doctor's advice, a desire to be fit or in shape, a value of health or physical challenge. Whatever the driver, a woman is likely to be more successful if the reason for exercising is personally important or aligned with other meaningful goals (such as quality family time or healthy aging, etc.) [169]. Physical activity motivation is also likely to be determined by a woman's perception of the value of the activity compared to the cost of performing that activity [170]. Some women may feel that physical activity is a positive health behavior, but also have concerns about any number of factors such as body image, ability, or the financial and time investment required. When the negative concerns outweigh the positive motivation to engage in exercise, the woman is unlikely to adhere long-term to any physical activity program [170]. Further, some women who have previously exercised in the past may perceive the effort required to achieve health benefits as too great. In the case of weight loss, a common goal for women with PCOS, a significant amount of effort is required to lose weight and maintain the weight loss [104]. Women who have previously attempted physical activity may feel that achievement of their health and fitness goals is too difficult and unlikely.

Productive motivation is likely to stem from a personal desire to be more active. Women should be encouraged and assisted to consider the real costs and benefits of physical activity. Health professionals can help women to understand the links between physical activity participation and their important goals in life. Health professionals can also manage women's expectations and provide a realistic timeline for desired health outcomes. Exercise physiologists can also be useful to assist women to track their progress and focus on achievements made by the women on the path to overall goal achievement. In the case of weight loss, exercise physiologists can ensure women recognize other fitness outcomes (such as strength and cardiovascular fitness) as they move toward their weight loss goals.

Simple advice can also be useful. Women should be encouraged to participate in activities that they find enjoyable or challenging. Social activities or increases in incidental physical activity can also be ways to engage women in a more active lifestyle.

2.5 CONCLUSION

There is a strong evidence base associating obesity and a worsening of the clinical presentation of PCOS. The importance of lifestyle management for treating PCOS is also internationally recognized. Weight management (weight loss, prevention of weight gain, or maintenance of a reduced weight) through lifestyle interventions should be first-line therapy in lean and overweight women with PCOS. There is currently no evidence that any specific dietary composition is beneficial, and healthy food choices consistent with general population recommendations, with individualization based on personal preferences providing the dietary approach is nutritionally adequate and safe, are currently recommended. Although women with PCOS do not consistently show worsened dietary intake or physical activity levels compared to women without PCOS, future recommendations and research should focus on additional factors including appropriate portion size, sedentary activity,

and the effects of vitamin D supplementation. Consideration of potential physiological and behavioral barriers to optimal dietary and physical activity is also warranted in the clinical management of PCOS.

2.6 KEY POINTS SUMMARY

1. PCOS is the most common female hormonal condition and the number one cause of anovulatory infertility. The health consequences of PCOS are significant and diverse and include reproductive dysfunction (anovulatory infertility, menstrual disturbance, increased risk of endometrial polyps and cancer), metabolic disturbance (insulin resistance and non-insulin-dependent diabetes mellitus, adverse lipid profile and associated increased cardiovascular disease), and psychological disability (depression, anxiety, sexual difficulties).
2. Although the exact underlying pathophysiological mechanisms behind PCOS are not fully understood, insulin resistance appears to be a common feature in both lean and obese individuals with PCOS. The resulting hyperinsulinemia increases ovarian androgen production, which in turn inhibits ovulation and also exacerbates the laying down of central "android" fat deposits. Android obesity further potentiates insulin resistance, creating a positive feedback loop that further exacerbates PCOS phenotype severity. As such, the prevention of weight gain in women with a genetic predisposition to insulin resistance, and therefore PCOS, is a critical therapeutic strategy in management of PCOS.
3. Several studies have investigated differences in nutritional intake between PCOS and non-PCOS cohorts that may account for the initiation or maintenance of PCOS. Androgens have been reported to increase an individual's cravings for carbohydrate, possibly helping explain why some early studies reported that women with PCOS have a tendency to consume more high-GI foods (white bread, fried potatoes) than women without PCOS. However, since the advent of consumer education programs linking PCOS with insulin resistance and high-GI foods, the majority of women with PCOS do not consume a diet high in carbohydrate. More recent large-scale dietary studies have in fact shown that the diet of women with PCOS is slightly better than that of women without PCOS, with the PCOS group consuming less high-GI and saturated fat food, and a better vitamin/micronutrient intake.
4. Research suggests that women with PCOS are predisposed to weight gain because of an unfavorable energy balance. First, lean women with PCOS have been reported to consume less total energy/calories than their weight-matched non-PCOS controls, suggesting that women with PCOS have a lower metabolic rate of energy consumption. Second, large diet surveys have identified that although the composition of diet (percentage protein, fat, and carbohydrate) does not differ between PCOS and non-PCOS subjects, women with PCOS do consume more calories per day. These two factors combined will result in a net positive energy balance that favors the development of obesity and eventual insulin resistance.
5. The micronutrient vitamin D may play a role in PCOS etiology. Several studies have identified that PCOS women have reduced serum vitamin D levels compared to the non-PCOS population, with the degree of vitamin D deficiency being correlated with an increase in insulin resistance. This observation makes good physiological sense as the promoter for the insulin receptor gene contains a vitamin D response element, with vitamin D exposure significantly increasing the insulin receptor's function. Although good quality randomized controlled trials are lacking, several uncontrolled studies have reported that vitamin D supplementation in vitamin D-deficient PCOS patients can reduce insulin resistance, normalize reproductive hormones and ovulation, and result in an improved rate of natural conception. Furthermore, because vitamin D deficiency has been linked with adverse obstetric complications (preterm labor, preeclampsia, and gestational diabetes), all women

with PCOS found to be vitamin D-deficient (serum levels <50 nmol) should receive supplemental therapy.

6. The composition of the ideal diet to facilitate weight loss and normalize reproductive function in PCOS has been a matter of a significant amount of research. Although some studies have suggested that a low GI index diet is best for normalization of insulin resistance and reproductive function, the current consensus view supported by research is that a simple reduction in calorie intake in the setting of a healthy balanced diet (high in fruits, nuts, and vegetables while low in saturated fat and simple sugars) is most important, rather than a specific low-GI or high-protein diet that historically has been advocated by some practitioners.
7. The combination of diet with exercise ("lifestyle management") has been shown to maximize weight loss, while normalizing insulin resistance, reproductive hormones, and improving menstrual cyclicity, compared to either dieting or exercise alone, and therefore is the preferred treatment approach in PCOS. Exercise helps maintain lean muscle mass during dieting, increasing resting metabolic rate and improving insulin sensitivity. Although studies have not shown a diminished exercise performance in women with PCOS, they have shown that on average women with PCOS spend 30 minutes more per day on sedentary activities (e.g., watching television) than non-PCOS subjects. Current recommendations suggest that women with PCOS should aim to participate in at least 150 minutes per week of exercise, with at least 90 minutes of this being moderate to high intensity (60–90% of maximal heart rate).
8. Women with PCOS appear to experience motivational difficulties with implementing lifestyle changes, with dropout rates in lifestyle modification programs being up to 4-fold higher in PCOS than in non-PCOS subjects. The reasons for this are unclear, but may include disturbed energy balance signaling (satiety, hunger adipokines), a self-perceived lack of skills to exercise, or a low self-esteem impaired motivation. Research suggests that encouraging women to adopt lifestyle modification along with family and friends, or other women with PCOS, may help improve motivation and long-term adherence to lifestyle modification goals. A loss of as little as 5% of body mass can result in normalization of reproductive function.

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3 Macronutrient Intake, Fertility, and Pregnancy Outcome

Tarek El-Toukhy and Amira Osman

CONTENTS

3.1 INTRODUCTION: OBESITY AND REPRODUCTION

Obesity is a worldwide epidemic [1]. According to the World Health Organization (WHO), a body mass index (BMI), measured in kg/m^2, of 18.5–24.9 is considered normal; a BMI of 25–29.9 is considered overweight; and 30 or above is considered obese [2]. In many Western countries, more than half of women of reproductive age are either overweight or obese, and an increasing number of these women are seeking fertility treatment [3]. There is also evidence of lack of awareness of the reproductive sequelae of increased BMI [4].

Obesity has numerous health consequences, including type 2 diabetes mellitus, hypertension, cardiovascular disease, and increased risk of certain types of cancer [5]. Recent evidence suggests reproductive health is also detrimentally influenced by increased BMI [6]. Compared with women

of normal BMI, women with raised BMI have a 3-fold greater risk of infertility; lower ovulation, implantation, and spontaneous conception rates; and higher miscarriage rate due to endocrine, paracrine, psychological, as well as social factors [7,8].

The effects of raised BMI manifest at various levels of the reproductive process. Successful implantation and pregnancy depends on a delicate interaction between the embryo and the endometrium. The impact of raised BMI on fertility could be mediated through alteration of oocyte and embryo quality, endometrial receptivity, or both [6,9–13].

3.2 RAISED BMI AND IMPAIRED OVULATION

Obesity is closely linked with impaired ovulation and polycystic ovary syndrome (PCOS) [8]. Approximately 50% of women with PCOS are obese. Excess adipose tissue is associated with insulin resistance and hyperandrogenemia, leading to hypothalamic–pituitary dysfunction and anovulation. Obesity is thus considered an independent risk factor for PCOS and its metabolic features. Adipokines may serve as an endocrine link between obesity, anovulation, and PCOS [14]. For more details, see Chapters 1 and 2.

3.3 RAISED BMI AND OOCYTE QUALITY

Mammalian oocytes remain arrested in an immature stage (prophase I) until the preovulatory luteinizing hormone (LH) surge induces progression to the metaphase II stage and full maturation. Suboptimal conditions during the oocyte maturation process can interfere with its developmental potential and competence [15]. Experimental animal studies have reported slower *in vitro* oocyte development and maturation and increased apoptosis in cumulus and granulosa cells in association with obesity [16–19].

In humans, several studies have demonstrated poorer oocyte quality and maturity in overweight and obese women [20–23]. Multiple factors are thought to mediate the effect of raised BMI on oocyte quality. Aberrations in endocrine and paracrine factors associated with raised BMI could negatively influence folliculogenesis and oocyte development [24].

3.3.1 Endocrine Factors

Elevated androgen level is closely linked to obesity [25,26]. Hyperandrogenemia can retard oocyte maturation and development [24] and has been associated with recurrent miscarriage. Tesarik and Mendosa [27] have demonstrated that elevated androgen level could impair the intracellular oscillations in calcium ions, which are important in oocyte cytoplasmic metabolism and maturation. In addition, a high androgen level reduces aromatase enzyme activity, thus limiting the intraovarian conversion of androgen to estrogen, which is known to promote oocyte maturation [28,29]. Hyperandrogenemia may also impair cumulus cell–oocyte signaling, which is required for germinal vesicle breakdown and formation of a haploid metaphase II (mature) oocyte [29]. Microarray analysis has demonstrated that the transcription of several genes necessary for oocyte maturation and chromosome alignment, and segregation could also be influenced by the raised androgen level common in obese women [30].

Increased insulin resistance is associated with obesity and can influence oocyte quality. The relationship between insulin resistance and oocyte quality is mediated through multiple factors, including reduction in hepatic production of steroid hormone binding globulin (SHBG) and consequent hyperandrogenemia, increased intraovarian androgen secretion via stimulation of 17-α-hydroxylase activity in theca cells, and increased insulin-like growth factor I (IGF-I) and LH secretion [29,31]. Hyperinsulinemia could also alter the expression of genes necessary for completing meiosis and alter serum IGF levels, which are involved in folliculogenesis and oocyte proliferation and differentiation [32–34]. Furthermore, recent evidence suggests that increased insulin resistance and

hyperglycemia associated with obesity could lead to oocyte mitochondrial dysfunction, resulting in accumulation of reactive oxygen species, poor oocyte development and maturation, and granulose cell apoptosis and lipotoxicity [18,35–38]. Thus, increased oxidative stress could be a potential additional mechanism by which obesity affects oocyte quality [35,39].

Adipose tissue is now considered to be a potent endocrine organ. Leptin, a protein hormone produced primarily by adipose cells, correlates positively with BMI [25]. Leptin receptors have been identified in oocytes and preimplantation embryos [40–43]. Higher leptin levels could reduce oocyte quality via intensifying the degree of insulin resistance associated with raised BMI [38,44,45]. Leptin also impairs steroidogenesis in granulose cells, which in turn disrupts folliculogenesis and oocyte developmental competence [41].

Adiponectin, a protein hormone secreted from adipose tissue, plays an important role in regulation of lipid metabolism and neuroendocrine functions, including ovarian function [46]. Serum levels of adiponectin are inversely related to BMI. Low adiponectin levels linked to obesity can intensify insulin resistance and hyperandrogenism and interfere with granulose and cumulus cell function and apoptosis [47].

Lastly, lower SHBG and increased peripheral aromatization of androgens to estrogens associated with obesity result in hypothalamic–pituitary–ovarian axis dysfunction and LH hypersecretion, which could impair oocyte quality via disruption in the control of meiosis and ovulation [24,48].

3.3.2 Paracrine Factors

Adipokines, the cytokines produced by adipose tissue, could alter oocyte quality via inducing a proinflammatory environment through increasing C-reactive protein, plasminogen activator inhibitor 1 (PAI-1), interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-α) levels [49–51]. For example, high intrafollicular TNF-α level has been shown to negatively influence oocyte quality by reducing the percentage of oocytes reaching metaphase II stage and increasing the frequency of abnormal microfilament distribution and chromosomal alignment, and defective meiotic spindle formation [52,53]. These observations raise the possibility that adipokines could impair oocyte quality through increased intrafollicular oxidative stress.

3.4 RAISED BMI AND EMBRYO QUALITY

Increased BMI has been associated with poorer preimplantation embryo quality in many, but not all, studies [10,54]. In addition to its effect on oocyte development, raised BMI could further impair embryo quality through endocrine and paracrine factors. Hyperandrogenemia induces an abnormal calcium influx response to oocyte fertilization by the sperm, thus impairing embryonic development [27]. Increased insulin level and LH hypersecretion have been shown to disrupt cumulus cell–oocyte signaling, increase cellular apoptosis, and hence impair embryonic development to the blastocyst stage. High leptin concentration could also decrease the preimplantation embryo cleavage rate and blastocyst formation. Altered IGF-I and IGF-II levels and their binding proteins (IGFBPs), as a result of hyperisulinemia, are thought to influence negatively embryo development, early embryonic cleavage and blastocyst formation [24,55,56]. Glucose-induced mitochondrial dysfunction [55] and epigenetic modifications [57] have also been proposed as additional underlying mechanisms for poor embryo quality associated with obesity.

3.5 RAISED BMI AND ENDOMETRIAL RECEPTIVITY

Recent evidence points toward a detrimental effect of raised BMI on the endometrium [58]. In addition to luteal phase insufficiency, many studies have used the oocyte donation model to remove the effect of oocyte quality on fertility treatment outcome [58–61]. Bellver and colleagues reported a lower ongoing pregnancy rate and higher miscarriage rate in obese oocyte

recipients compared to those of normal BMI, underscoring the impact of obesity on endometrial receptivity [58].

Two other study deigns have been used to evaluate the influence of raised BMI on endometrial receptivity by controlling for oocyte or embryo quality. DeUgarte and colleagues studied the outcome of assisted conception cycles in which embryos from healthy donors were transferred into fertile surrogates [62]. Raised BMI of the surrogate was associated with a reduction in the implantation and pregnancy rates, indicating an independent effect of BMI on endometrial receptivity.

Rittenberg and colleagues used an elective single blastocyst transfer model to control for embryo quality in *in vitro* fertilization (IVF) cycles [63]. The increased risk of miscarriage demonstrated after fresh and frozen embryo transfer in overweight and obese patients reflected the effect of raised BMI on endometrial function. The study also suggested a dose-dependent relationship between the risk of miscarriage and increasing BMI. Such a phenomena awaits confirmation in future studies [63].

It is worth highlighting that the link between raised BMI and poor embryo implantation is complex and not yet conclusive. A recent systematic review and meta-analysis of donor oocyte recipients [64] found no significant association between recipient obesity and implantation, pregnancy, or live birth rate. On the other hand, a large retrospective cohort analysis of 9587 first cycles of oocyte donation from normal weight donors reported a significant association between recipient obesity and reduced implantation, pregnancy, and live birth rates [65]. Well-controlled and adequately powered studies are still needed to clarify the correlation between high BMI and endometrial receptivity.

3.6 PATHOPHYSIOLOGY OF ENDOMETRIAL IMPAIRMENT ASSOCIATED WITH RAISED BMI

3.6.1 Endocrine Dysfunction

Abnormal insulin and androgen secretion associated with obesity inhibit endometrial secretion of glycodelin *in vitro* [66]. Glycodelin, a progesterone-regulated glycoprotein, is involved in inhibition of the endometrial immune response to the implanting embryo [67]. Thus, a reduction in its secretion could predispose to defective implantation and miscarriage [33,68,69].

Similarly, alterations in serum leptin level associated with raised BMI may impair trophoblastic invasion, leading to defective implantation via interference with the normal synthesis and secretion of fetal fibronectin and the matrix metalloproteinases MMP2 and MMP9 [70,71]. Furthermore, higher secretion of estrogen associated with obesity predisposes to abnormal endometrial thickness and endometrial polyp formation, which could lead to abnormal implantation [6].

3.6.2 Cytokine Dysfunction

Leukemia inhibitory factor (LIF) is an interleukin closely related to implantation and placentation [72]. Raised BMI is thought to be associated with a low level of LIF and increased expression of TNF-α receptors in the endometrium, thus predisposing to defective implantation and miscarriage [73,74]. Likewise, alteration in the level of PAI-1 produced by adipose cells could impair the process of trophoblastic invasion and placental blood flow, suggesting that this factor could mediate the detrimental effect of obesity on the risk of miscarriage [75]. In addition, BMI correlates with endometrial concentration of the inflammatory markers IL-6 and TNF-α, both of which have been associated with impaired implantation and increased risk of miscarriage [53,72].

3.7 RAISED BMI AND IVF OUTCOME

Numerous studies have evaluated the impact of raised BMI on the outcome of assisted conception treatment [9,76]. Overweight and obese women require higher doses of injectable gonadotropins

for ovarian stimulation, tend to produce fewer oocytes, and have a higher cycle cancellation rate. Ferlitsch and colleagues reported that for each unit increase of BMI, the probability of pregnancy after IVF could decrease by 2.2–4.3% [77]. A recent systematic review of published literature summarized the results of 33 studies [78]. Only studies that had reported their results in accordance with the WHO classification of BMI categories were included in the review. Meta-analysis of the study results demonstrated lower clinical pregnancy and live birth rates and higher miscarriage rate associated with raised BMI [78]. This review allowed separate evaluation of the effect of being overweight or obese on IVF outcome. Compared with women who had a normal BMI, being overweight decreased the live birth rate per IVF cycle by 9% (risk ratio [RR] = 0.91, 95% confidence interval [CI]: 0.85–0.98, $P = 0.01$) and increased the miscarriage rate by 23% (RR = 1.23, 95% CI: 1.12–1.34, $P < 0.001$), whereas being obese decreased the live birth rate per IVF cycle by 20% (RR = 0.80, 95% CI: 0.71–0.90, $P = 0.0002$) and increased the miscarriage rate by 43% (RR = 1.43, 95% CI: 1.22–1.67, $P < 0.0001$). Thus, the results of this study confirmed the trend toward a poorer reproductive outcome with rising BMI, which was previously suggested [10]. The study results therefore should empower primary care physicians as well as fertility specialists to provide patients with clear advice regarding the effect of raised BMI on the outcome of assisted conception treatment.

3.8 EFFECT OF WEIGHT LOSS ON FERTILITY

Female fertility is sensitive to alterations in body weight [79]. Available data suggest that as little as 5% weight loss could result in improvement in endocrine parameters, resumption of regular menstrual cycles, increased frequency of ovulation, and improved conception rates [8]. Chavarro and colleagues demonstrated that among overweight and obese women, weight loss before assisted conception treatment was associated with a significantly increased yield of mature oocytes [80]. Furthermore, a higher pregnancy rate is achieved in IVF patients who reduce their weight before treatment [81]. Ferlitsch and colleagues reported that for each unit reduction in BMI, the odds of achieving a pregnancy after IVF could improve by 19% [77]. Likewise, Musella and colleagues reported improvement in fecundity after weight loss following bariatric surgery. Weight loss and BMI achieved were the only significant predictors of pregnancy [82]. Infertile women can be motivated to address lifestyle modifications that could enhance their fertility [83]. Therefore, interventions aimed at gradual, rather than rapid [84], weight loss should be encouraged in overweight and obese women of reproductive age, and access to integrated and effective weight management programs must be facilitated to enable these women to achieve optimal preconceptional weight and restore their full reproductive potential.

3.9 RAISED BMI AND OBSTETRIC AND NEONATAL OUTCOMES

Obesity has been associated with many adverse outcomes in relation to pregnancy, delivery, and the postpartum period. It has also been linked to multiple fetal and neonatal complications.

3.9.1 Maternal Complications

3.9.1.1 Thromboembolism

Venous thromboembolism in considered one of the leading causes of maternal mortality during pregnancy. Normal pregnancy on its own is a known risk factor for thromboembolism [85]. Increasing levels of coagulation factors VII, VIII, and XI as well as fibrinogen level and a significant decrease in the activation of fibrinolysis are well documented during pregnancy [86]. Other factors adding to the risk of thrombosis during pregnancy are the associated venous stasis and pressure on the inferior vena cava caused by the gravid uterus.

Obesity has been associated with thrombotic disease independently. Adipocytes secrete inflammatory cytokines whereby macrophages are attracted to and accumulate in adipose tissue. A state of chronic low-grade inflammation is then initiated, leading to further increase in the secretion of systemic cytokines such as TNF-α and interleukins [87]. Consequently, platelet activation and thrombin generation are enhanced, which results in a prothrombotic state. Studies have also found an association between increasing levels of PAI-1 and obesity. Plasminogen activator factor converts plasminogen into plasmin, which promotes fibrinolysis. The increasing levels of PAI-1 observed in obesity inhibit this process [88], resulting in increased risk of thrombosis. A study using the UK Obstetric Surveillance System data demonstrated that obesity is one of the major risk factors for developing pulmonary embolism, with an adjusted odds ratio (aOR) of 2.65, 95% CI: 1.09–6.45 [89].

Therefore, it is has been recommended to evaluate the BMI among other factors such as previous history of deep vein thrombosis and thrombophilia screening result, when scoring pregnancy with regard to the need for requiring thromboprophylaxis antenatally as well as in the postpartum period. The dose and duration of the antithrombotic agent will depend on other associated risk factors including age, postpartum hemorrhage, and emergency cesarean delivery [90].

3.9.1.2 Preeclampsia

The risk of hypertensive disorders in pregnancy, including preeclamptic toxemia (PET), is increased in obese women. A systematic review that evaluated 1.4 million women concluded that each increase in pre-pregnancy BMI of 5–7 kg/m^2 doubles the risk of preeclampsia [91]. Another study in support of these findings showed that not only obese women with a BMI above 30 kg/m^2 have a higher risk of developing PET (OR = 2.59, 95% CI: 2.87–3.01), but also that a higher than average weight gain among obese women during pregnancy further increases the incidence of preeclampsia to more than 4-fold [92].

Various mechanisms have been proposed to explain the relationship between obesity and PET. Insulin resistance is commonly found in obese women and has been associated with increased risk of PET. Insulin resistance can activate the sympathetic nervous system, which in turn can lead to increase in blood pressure [93]. The associated hyperinsulinemia and hypertriglyceridemia can result in endothelial damage and may alter prostaglandin regulation, leading to arteriolar constriction [94]. It has been suggested that inflammatory mediators such as C-reactive protein, interleukins, and TNF-α may also contribute to the pathogenesis of preeclampsia in obese women [95,96]. Such inflammatory and metabolic derangement observed in obese women can lead to impairment in the microvascular function. An added factor is the obstructive sleep apnea that is often associated with obesity. Disordered breathing during sleep has been linked with increasing blood pressure [97].

3.9.1.3 Gestational Diabetes Mellitus

Maternal obesity is one of the major risk factors for developing gestational diabetes mellitus (GDM). Developing diabetes during pregnancy has maternal, fetal, as well as neonatal detrimental effects, such as fetal macrosomia, large for gestational age, increasing risk of stillbirth, neonatal hypoglycemia, and a higher risk of developing type 2 diabetes later in life. Early diagnosis and controlling blood sugar levels significantly improve maternal and fetal outcome. Therefore, the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom has recommended early screening for women who are at risk of developing gestational diabetes. Risk factors include previous history of GDM or fetal macrosomia, family history of diabetes, racial origin, and most of all a BMI above 30 kg/m^2 [98]. The metabolic disturbance associated with obesity, particularly hyperglycemia, insulin resistance, and hyperinsulinemia, predispose to the development of diabetes in pregnancy. Hyperglycemia at a lesser degree than overt GDM has proven to be associated with adverse obstetric and neonatal outcome. This is evident by the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, which included a cohort of 25,000 women. It showed a continuous positive association between maternal glucose concentrations and adverse outcome such as birth weight above the 90th percentile, neonatal hypoglycemia, and primary cesarean section [99].

Another study demonstrated that obesity could independently result in birth weight above the 90th percentile with an OR of 1.73 (95% CI: 1.50–2.00) and that GDM has a similar effect with an OR of 2.19 (95% CI: 1.93–2.47). A combination of obesity and GDM further increases this risk (OR = 3.62, 95% CI: 3.04–4.32) [92].

3.9.1.4 Preterm Labor

Preterm labor (PTL), defined as labor at less than 37 weeks of gestation, has been associated with increasing pre-pregnancy maternal BMI. A large systematic review and meta-analysis conducted by Tolroni and colleagues concluded that maternal obesity was associated with a higher risk of preterm birth below 32 weeks of gestation. Further studies supported these findings [100]. A prospective cohort study conducted by the Norwegian Institute of Public Health demonstrated that women with grade I, II, and III obesity had an increased risk of preterm delivery with an adjusted OR of 1.50 (95% CI: 1.35–1.68, $P < 0.001$), 1.51 (95% CI: 1.24–1.84, $P < 0.001$), and 2.00 (95% CI: 1.48–2.71, $P < 0.001$) respectively [101].

Possible mechanisms that could explain the relationship between maternal obesity and PTL include the development of subclinical chorioamnionitis, which is not uncommon in obese women and is a well-known precursor for PTL or preterm premature rupture of membrane. Adipokines (such as interleukins and TNF-α) secreted by fat cells contribute to the pathogenesis of systemic inflammation [102]. The resulting cytokines weaken the membranes, ripen the cervix, and enhance myometrial contractions, mostly through activation and release of prostaglandins and metalloproteases [103].

Another hypothesis that may explain the relationship between obesity and PTL is the higher prevalence of genitourinary bacterial infection in these women, which is a predisposing factor for subclinical chorioamnionitis [104]. In addition, maternal obesity during pregnancy is commonly associated with medical conditions (e.g., PET, diabetes) that may also lead to spontaneous or induced PTL.

Paradoxically, few studies have reported an association between postdate delivery and obesity [105–107]. The exact explanation for this association remains unclear. Multiple mechanisms have been proposed for the initiation of parturition at term. The rising production of corticotropin-releasing hormone (CRH) by the placenta is one of the factors for parturition. It stimulates the release of maternal and fetal pituitary corticotropin as well as maternal and fetal adrenal cortisol and dehydroepiandrosterone (DHEAS), which further increases the production of CRH. The increasing cortisol levels lead to fetal lung maturation. Consequently, surfactant proteins and phospholipids produced activate the inflammatory pathways in the amnion, which in turn results in myometrial activation. The rising levels of DHEAS that is produced by the fetal adrenal gland are metabolized by the placenta into estrogens [108]. It has been suggested that obese women have lower circulating cortisol levels during pregnancy compared to those of normal weight, which could reduce placental CRH production and thus influence the timing of delivery. Furthermore, the increased estradiol level in adipose tissue may reduce the circulating maternal estrogen, altering the estrogen-to-progesterone ratio, which is normally increased in pregnancy.

3.9.1.5 Intrapartum Complications

Maternal obesity is known to be a significant risk for higher rates of induction of labor, oxytocin use, instrumental delivery, and cesarean section [109,110]. Failure to progress during labor, particularly during the first stage, has been correlated with poor myometrial contractility in obese women. The high cholesterol level and low-density lipoprotein associated with obesity may alter myometrial membrane structure, which in turn could hinder the transport of calcium ions into the myometrial cytoplasm required for the contraction–relaxation cycle of the muscle. A recent study reported a reduced frequency and amplitude of contractions recorded in myometrial stripes taken from obese women at the time of elective cesarean section when compared to those from women with normal BMI [111]. Furthermore, it has been demonstrated that leptin hormone has an inhibitory effect on

the pregnant myometrium by reducing both the amplitude and frequency of myometrial contractility *in vitro* [112]. It has also been suggested that the suppression of myometrial contractility associated with obesity is related mainly to dysfunctional labor and larger fetal size [106].

Shoulder dystocia is an intrapartum emergency that could have serious perinatal morbidity and mortality as well as maternal complications. A BMI greater than 30 kg/m^2 is one of the recognized factors that predispose to shoulder dystocia [113]. Other factors include macrosomia, diabetes mellitus, induction of labor, and prolonged second stage of labor, which are not uncommonly associated with obesity.

Furthermore, high BMI, particularly trunk obesity, represents a challenge to obstetricians during intrapartum fetal monitoring. Difficult monitoring of fetal heart rate and uterine contractions is frequently experienced with obese women, and the use of fetal scalp electrodes to achieve clear cardiotocography monitoring is often required.

3.9.2 Fetal Complications

3.9.2.1 Stillbirths

Several studies have evaluated the association between obesity and stillbirth. Those studies have suggested there is an increase in the risk of stillbirth in pregnant obese women when compared to women with normal BMI [114–116]. A systematic review and meta-analysis that included nine studies supported these findings and concluded that the risk of stillbirth among obese pregnant women is almost doubled when compared to pregnant women with a normal BMI (OR = 1.47, 95% CI: 1.08–1.94 for overweight, and OR = 2.07, 95% CI: 1.59–2.74 for obese women) [117].

Hyperlipidemia and hyperinsulinemia are commonly associated with obesity. They reduce prostacyclin secretion and induce thromboxane production, which increases the risk of placental thrombosis and reduced placental perfusion. This can lead to fetoplacental dysfunction and contribute to stillbirth [118]. One study suggested that the apnea–hypoxia episodes that could be associated with extended snoring in pregnant obese women could reduce the oxygenation to the fetus, which in turn can increase the risk of stillbirth [119]. Although the adverse medical complications associated with obesity are known factors that could increase the risk for stillbirth independently, often the cause for stillbirth remains unexplained, particularly at term. This is likely to be due to the close monitoring and relatively earlier intervention for obese pregnant women who are complicated with medical conditions such as diabetes and hypertensive disorders.

3.9.2.2 Fetal Congenital Complications

Maternal pre-pregnancy obesity has been associated with increasing risk of birth defects, such as cleft palate, diaphragmatic hernia, hydrocephalus, hypoplastic left heart syndrome, pulmonary valve atresia and stenosis, pyloric stenosis, rectal and large intestinal atresia or stenosis, transposition of great arteries, and ventricular septal defects [107,120,121]. The exact mechanism for the observed increased risk of birth defects is not fully understood. A possible explanation is the significant insulin resistance that is commonly found in obese women, possibly an extension of preexisting diabetes, which is a well-known risk factor for increasing the risk of developing a congenital abnormality.

Neural tube defect (NTD) is a recognized risk associated with maternal obesity. This was shown in the systematic review of Rasmussen and colleagues, who reported a 1.7-fold increase in the incidence of NTD in obese women and a 3-fold increase in morbidly obese women [122]. Possible explanations for these findings include the impairment of glucose metabolism and hyperinsulinemia associated with obesity. Furthermore, folic acid intake has been related to a lower risk of NTD. Obese women were found to have increased requirements for folic acid to maintain a serum folate level similar to that of women who have a normal BMI [123]. As a result, obese women are advised to have a higher dose of folic acid (5 mg) preconceptionally [124].

Ultrasound in obstetrics is routinely used for pregnancy dating and for detecting fetal viability and fetal anomalies. Adipose tissue and scarring tissue may affect the image quality and demonstrate limitations to the study despite the advanced technologies in the ultrasound machines. The risk of missing a congenital abnormality is higher in obese women, resulting in a higher probability of a live birth affected with a congenital anomaly undiagnosed during pregnancy, compared to normal weight women. This is due to the presence of a fat layer that can make visualization of the fetus during ultrasound examination more challenging and results in difficult fetal assessment [125]. Wolfe and colleagues concluded in their study that there was a reduction in visualization by an average of 14.5% during the second and third trimester scans in women with a BMI above the 90th percentile. This was most marked when examining the fetal heart, spine, and umbilical cord [126].

3.9.2.3 Large for Gestational Age and Childhood Obesity

A growing body of evidence suggests the presence of an association between maternal obesity and fetal birth weight as well as obesity in adult life [127]. Studies demonstrating the adverse metabolic effect of maternal obesity on the offspring have been well described in animals. For example, injecting insulin in rats during the peripartum period resulted in hyperphagia, and the subsequent maternal as well as offspring hyperinsulinemia can cause altered innervation in the hypothalamus [128]. Consequently, abnormal glucose tolerance and obesity were observed later in life.

In humans, a large study conducted in Sweden concluded that large for gestational age (LGA) births are associated with increased maternal BMI [127]. A recent systematic review and meta-analysis suggested that pre-pregnancy obesity increased the risk of LGA (OR = 2.08; 95% CI: 1.95–2.23), macrosomia (OR = 3.23; 95% CI: 2.39–4.37) and subsequent childhood obesity (OR = 3.06; 95% CI: 2.68–3.49) [129]. This can be explained by maternal over nutrition which leads to insulin resistance and hyperglycemia and subsequently fetal hyperinsulinemia. Insulin is known to have a growth hormone like action that results in fetal overgrowth.

Another recently published systematic review included 20 studies and concluded that high birth weight babies (>4000 g) have a higher risk of developing obesity later in life (OR = 2.07; 95% CI: 1.91–2.24) [130]. Furthermore, it has been suggested that weight gain during the first few weeks of life was found to be associated with an increased risk of childhood obesity [131].

The mechanism that links the relationship between maternal obesity and development of childhood and adolescent obesity is not entirely understood. The hypothesis of "fetal or early origins of adult disease," originally described by Barker and colleagues, may explain this relationship [132]. It was suggested that environmental factors, particularly nutrition, are associated with adverse health outcomes in adult life, especially in relation to cardiovascular disease, obesity, and metabolic disorder [133]. The idea of fetal programming describes the effect of a suboptimal intrauterine environment, which may act as a stimulus during the sensitive period in early fetal development and could affect long-term function [134]. McMillen and colleagues concluded that not only fetal undernutrition has an association with the development of adult disease, but also that overnutrition may result in neuroendocrine responses that could affect subsequent metabolic health later in life, such as permanent changes in pancreatic islet cells, hypothalamus, and adipose cells [135]. A large prospective cohort study that examined the association between maternal weight and offspring BMI included 146,894 individuals from 136,050 families. It concluded that offspring BMI later in life is related to maternal weight and is influenced by intrauterine environment in overweight and obese women [136].

3.9.2.4 The Effect of Being Underweight on Fertility and Obstetric Outcome

A critical level of body weight is required for initiation of menarche and maintenance of a regular menstrual cycle [137]. A body weight-to-fat ratio of 3:1 is generally considered to be a prerequisite for initiation of menarche [138]. This relationship is thought to ensure that pregnancy and lactation occur only in favorable health and energy availability conditions. A weight loss of 10–15% of normal weight for height could cause disruption of the menstrual cycle.

Being underweight is known to be associated with multiple reproductive consequences, including hypothalamic–pituitary–ovarian axis dysfunction leading to low follicle-stimulating hormone and LH secretion, hypoestrogenemia, anovulation, amenorrhea, and reduced sex drive [139]. This is invariably associated with higher circulating cortisol level, reduced fertility, and longer time to conceive [140].

It has been postulated that low leptin level associated with reduced body fat suppresses hypothalamic secretion of gonadotropin-releasing hormone and disrupts the normal LH pulsatility, via increased secretion of neuropeptide Y, higher dopamine concentration, and lower serotonin secretion in the hypothalamic nuclei [141,142].

The hypoestrogenic state commonly associated with low BMI is exaggerated further by the lack of peripheral aromatization of androgen to estrogen due to loss of body fat. As a result, reproductive organs such as the uterus decrease in size and volume. More seriously, loss of bone density and osteoporosis as well as cardiac dysfunction and electrolyte imbalance are recognized complications of low BMI. Therefore, attention needs to be paid to early dietary advice and psychological support to restore fertility and prevent the negative physical sequelae of low BMI [143].

Recent data suggest that underweight women are nearly twice as likely to be infertile (OR = 1.86, 95% CI: 1.62–2.13, $P < 0.001$) compared to those with a normal BMI [144]. Furthermore, Pinborg and colleagues studied the effect of low BMI on IVF outcome in 487 consecutive patients undergoing 1417 IVF/intracytoplasmic sperm injection (ICSI) cycles [145]. Being underweight (BMI <18.5) was associated with significantly fewer embryos available for transfer ($P = 0.03$) compared to patients with normal BMI. However, a similar effect of low BMI on the overall chance of pregnancy after IVF treatment has not been clearly demonstrated [146–149].

Veleva and colleagues studied 3330 first pregnancy cycles after fresh and frozen-thawed embryo transfer. Using a multivariate logistic regression model, the study could demonstrate that being underweight significantly increased the risk of miscarriage after fresh and frozen-thawed transfers [38]. Likewise, Doherty and colleagues studied the effect of being underweight (BMI <18.5 kg/m^2) in 331 underweight pregnant women recruited between 16 and 18 weeks of gestation. The authors reported a significantly increased risk of fetal growth restriction in this group compared to 1982 women with normal weight (BMI = 18.5–25 kg/m^2) [150].

Schieve and colleagues studied 3511 mothers who had their weight measured pre-pregnancy and between 14 and 28 weeks of gestation. The authors concluded that low weight gain during pregnancy, particularly in women who were underweight before pregnancy, significantly increased the odds of preterm delivery by more than six times (OR = 6.7, 95% CI: 1.1–40.6) [151].

More recently, Salihu and colleagues studied the risk of preterm birth in singleton pregnancies in relation to their pre-pregnancy BMI. The authors reported that underweight mothers with a pre-pregnancy BMI of 18.5 kg/m^2 or less were significantly more likely to experience a preterm delivery [152]. The risk of preterm delivery intensified with progressive reduction in pre-pregnancy BMI and was higher in conjunction with a modest weight gain during pregnancy.

3.10 KEY POINTS SUMMARY

1. In many Western countries, more than half of women of reproductive age are either overweight or obese, placing them at significantly higher risks of infertility and obstetric complications, while also potentially adversely affecting the health of their children.
2. Obesity is linked with a 3-fold increased risk of infertility and a reduction in successful pregnancy even when IVF technology is used to treat infertility. The underlying causes of this infertility are multiple and include impaired ovulation, reduced oocyte and subsequent embryo quality, increased risk of defective implantation and miscarriage, plus sexual difficulties related to poor body image.
3. The process of producing a mature good quality oocyte appears to be impaired by obesity in a number of ways. First, increased peripheral aromatization of androgens to estrogen

in adipose tissue can result in hypothalamic–pituitary (HP) dysfunction, impairing HP drive for ovulation. Second, obesity-related insulin resistance results in high intrafollicular levels of insulin and related insulin-like growth factors, which in turn alter the normal balance of steroid hormone production and cumulus–oocyte paracrine regulation of oocyte meiotic development, resulting in aberrant oocyte development and increased rates of oocyte immaturity. High levels of leptin, produced by adipose tissue, have also been suggested to interfere with follicular steroidogenesis and oocyte developmental potential. Third, insulin resistance and hyperglycemia, together with an increase in proinflammatory cytokines present in the follicles of obese individuals, results in oocyte mitochondrial dysfunction and the generation of reactive oxygen species that damage oocyte developmental potential. All of these pathological processes combine to result in the clinical outcome of increased risk of anovulation, oocyte immaturity, and reduced mature oocyte quality. This may explain, at least in part, the observation that during IVF treatment, obese women on average require higher dosages of gonadotropins for ovarian stimulation and tend to produce fewer oocytes.

4. Although some investigators have linked obesity with impaired preimplantation embryo quality, this has not been confirmed by others. However, because obesity is correlated with diminished oocyte quality, and an altered uterine milieu (proinflammatory, increased IGF growth factors, hyperglycemia), it is probable that obesity may adversely affect embryo development.
5. The observation of reduced pregnancy rates during IVF, and an increase in the risk of miscarriage after natural and IVF-assisted conception, point to the possibility of obesity adversely affecting implantation/placental development. Potential mechanisms for this adverse response include immune-mediated attack on the embryo/fetus (increase in endometrial proinflammatory cytokines, reduced immunosuppressive glycodelin production), placental thrombosis initiated by obesity related prothrombotic state (elevated PAI-1), or impaired endometrial development (polyps, endometrial hyperplasia) which are more commonly seen in obese women. One meta-analysis [63] suggests that being overweight or obese reduces a woman's chances of live birth by 9% and 20% if her own oocytes are used during the IVF cycle, with this reduction in live birth being mediated primarily by an increase in miscarriage (20% and 43% increase, respectively). However, when oocytes from healthy donors are transferred into obese women, another recent meta-analysis [64] reported no adverse effect of recipient obesity on implantation and miscarriage. This report implies that the well-established reduced implantation rates and increase in miscarriage associated with obesity are mediated primarily by a negative impact on oocyte/embryo quality, rather than later implantation and placental development.
6. Obesity has been conclusively associated with many adverse obstetric outcomes including an increased risk of maternal thromboembolism, pregnancy-related hypertension (including preeclampsia), gestational diabetes, preterm labor, and stillbirth. Intrapartum difficulties related to obesity include an increased need for delivery by caesarean delivery caused by impaired myometrial contraction in labor, plus cephalopelvic disproportion and a wish to avoid predicted shoulder dystocia related to fetal macrosomia, which is more commonly seen in association with obese mothers. Obstetric hemorrhage is more difficult to manage in obese women because of the difficulty in gaining intravenous access, and anesthesia is complicated by difficulty inserting epidural/spinal anesthetics because of obscured spinal bony landmarks created by maternal adiposity. Furthermore, general anesthetic is made more complicated by difficult endotracheal intubation and an increased risk of gastric reflux in an obese obstetric patient.
7. Fetal congenital abnormalities such as NTDs, cleft palate, diaphragmatic hernia, cardiac anomalies (hypoplastic left heart, ventricular septal defects, pulmonary stenosis, transposition of great vessels), and gastrointestinal abnormalities (pyloric stenosis, intestinal atresia)

are all more commonly seen in obese women. The underlying causes of these increased risks are not fully understood but potential mechanisms include hyperglycemia in the early embryogenesis phase of pregnancy in obese women undiagnosed with diabetes, plus a relative deficiency of folate leading to babies with NTDs in women with high BMI. Furthermore, maternal obesity reduces the accuracy of ultrasound, making identification of serious fetal abnormalities in routine mid-trimester morphology scans less likely, in turn limiting the parents' ability to make a decision regarding termination of an abnormal pregnancy.

8. Weight loss through diet, exercise, or bariatric surgery has been shown to improve reproductive function, with a loss of as little as 5% causing a resumption in ovulation and regular menstrual cycles, improved conception rates, and a reduction in miscarriage. Optimal pre-pregnancy management of an obese woman should include advice to slowly reduce weight by diet and exercise, elective screening for undiagnosed diabetes (oral glucose tolerance test), use of high-dose perinatal folate (5 mg) supplements to minimize the risk of conceiving a child with NTD, and highlighting the role of limiting weight gain during pregnancy to minimize adverse obstetric outcomes.
9. A critical level of body fat is required for both the initiation of menarche and maintenance of a regular menstrual cycle. A low BMI is associated with anovulation, amenorrhea, low ovarian steroid hormone production, and an associated increased risk of impaired libido and osteoporosis. Underweight women are twice as likely to be infertile as their normal weight counterparts, and have been reported to experience an increased risk of miscarriage after natural or assisted conception. Obstetric risks associated with underweight include fetal growth restriction and preterm delivery. Therefore, women with a BMI below 18.5 should be encouraged to attain a normal body weight before attempting conception.

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4 The Role of Micronutrients in the Prevention of Congenital Anomalies in the Fetus and Optimization of Pregnancy Outcomes

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CONTENTS

4.1 INTRODUCTION

Nutrition plays an essential role in the reproductive health of women of childbearing age. Reproductive health generally covers the period beginning in adolescence with the onset of puberty and continuing throughout pregnancy and lactation until menopause. During this fertile period, maternal diet is recognized as one of the foremost factors with a major role in the health of the mother and developing fetus. The importance of proper nutrition during pregnancy, with the increased metabolic demands on the mother attendant on a growing fetus, cannot be stressed enough. A mother requires all essential micronutrients to boost her immune system to help protect her from infections and also to improve the quality of breast milk, during lactation, for the growth and development of her infant. If the intake of essential micronutrients is inadequate during pregnancy, a deficit may develop in the fetus, which affects both the fetus and the mother [1].

Micronutrient deficiencies usually result from inadequate or low dietary intake. Nutritional and dietary surveys conducted throughout the world have confirmed that women of reproductive age are deficient in multiple micronutrients, rather than a single micronutrient [2]. These surveys have confirmed that women of reproductive age have a low intake of iron [3], vitamin A [3–7], riboflavin [3], and calcium [3]. Women also suffer from iodine [8], zinc, and copper deficiencies [9–11]. According to Caulfield et al. [11], 82% of pregnant women globally have inadequate intake of zinc. Similarly, studies have also reported suboptimal levels of vitamin B_6 and B_{12} in lactating women [10,12]. Riboflavin deficiency, on the other hand, is more prevalent in The Gambia and other parts of Africa, China, Indonesia, and India [3]. In India, B_{12} deficiency is common because of the strict limitation on animal products. Folate deficiency has been reported in Burma, India, West Africa [13], and Kenya [14], and is a frequent complication of protein energy malnutrition in both West and Southern Africa [15–17].

With these deficiencies, the consequences can be detrimental and can lead to intrauterine growth restriction (IUGR), preterm birth, and maternal and infant morbidity and mortality. Infants merit added concern because they need extra nutrients to maintain optimal growth and development. These effects are more prominent among women and children of low- and middle-income countries, where women often enter pregnancy undernourished and their increasing demands for micronutrients are underserved. In women, anemia is an intractable concern, and a majority of women manifest iron-deficiency anemia and folate-deficiency megaloblastic anemia, both of which may be preventable with regular prenatal iron and folate supplements [18]. Among others, a low vitamin B_{12} status during pregnancy may be associated with adverse pregnancy outcomes, including preeclampsia, premature delivery, and low birth weight infants [19].

It is very important for pregnant mothers to provide a source of nourishment and gaseous exchange to the developing fetus. This not only supports embryonic and fetal growth but also prepares and strengthens the body of a woman for childbirth and for the later demands of lactation. Further, an adequate diet protects women from infections and also safeguards the implantation and survival of conceptus [20,21]. Proper and adequate diet also ensures the development and establishment of a healthy placenta and its circulatory system, which is crucial for the development of the embryo and growth of the fetus.

Poor nutrition during pregnancy is linked to an increased risk of developing physical birth defects as well as neurological disorders in children born with low birth weight (<2500 g) and a short gestational period (<37 weeks), leading to a greater susceptibility to disease processes later in life. The greatest benefit of an adequate nutritional status during pregnancy is the avoidance of low birth weight, which affects the immediate survival of the infant and the long-term health status and development of the child. Maternal nutritional status is implicated in programming growth, development, and function of the major fetal organ systems, with prenatal growth directly sensitive to maternal dietary intake [22].

This chapter discusses key micronutrients and their role in the prevention of congenital anomalies in the fetus and optimization of pregnancy outcomes.

4.2 ROLE OF SPECIFIC DIETARY MICRONUTRIENTS

Malnutrition, defined as an inadequate intake of a well-balanced diet, may refer to undernutrition or overnutrition. In low- and middle-income countries, undernutrition is a serious concern, and in vulnerable populations such as the elderly, infants, and pregnant women, increased demands on dietary intake may eventually lead to nutritional deficiencies. Pregnancy and lactation place high demands on a woman's body, and adequate nutrition, paying attention to micronutrient needs for both the mother and the growing fetus, must be accomplished. A poor-quality diet, especially one that is lacking in micronutrients, may increase the risk of low birth weight infants, prematurity, and also birth defects. Because the body cannot synthesize micronutrients, these must be provided in the diet. Micronutrients regulate the cellular osmolality that ensures the volume and viability of blood

TABLE 4.1
Metabolic Functions of Micronutrients

Micronutrient	Role in the Periconceptional Period
Folate	• DNA synthesis, methylation, cell division, fetal growth
Iron	• Oxygen carrier in red blood cells as part of hemoglobin; hematopoiesis; metabolism
Antioxidants	• Homeostatic balance between anti- and pro-oxidants to prevent oxidative damage and allow for proper gene expression • Contribute to the immunity
Vitamin B_{12}	• Cofactor of methionine synthase (required for methylation of homocysteine)
Vitamin B_6	• Coenzyme in heme, sphingolipids, and neurotransmitter synthesis; gluconeogenesis; glycogenolysis; involved in gene expression
Vitamin A	• Growth factor for epithelial cells; gene transcription
Zinc	• Cofactor for metalloenzymes; gene expression; contributes to immunity; cell signaling
Copper	• Important antioxidant expressed in both maternal and fetal tissues • A cofactor for enzymes involved in metabolism, angiogenesis, antioxidant function, especially superoxide dismutase

and cells. They also mediate and serve as cofactors for enzymes, participating in electron transfer, signal transduction, and transcription. The metabolic functions of micronutrients and the required dosage are summarized in Tables 4.1 and 4.2, respectively. Recommended daily intakes are given in Table 4.3. In this chapter we review only those micronutrients that optimize a pregnancy or are commonly implicated in fetal malformations.

4.2.1 Folic Acid

Folic acid is a synthetic derivative of folate and is often used in supplements and fortified food. Folate occurs naturally contained in legumes such as lentils and kidney beans, grains, green leafy vegetables, and some citrus fruits and juices.

Folic acid and folate derivatives play a role in DNA methylation, cell division, and tissue growth. The bioavailability of folate in the body is determined by 5-methyltetrahydrofolate (5-MTHF). Folate is transported and stored in the body in the form of 5-methyltetrahydrofolate (5-MTHF). The synthetic form of folate—folic acid—is first converted in the mucosa cell into dihydrofolate (DHF) and tetrahydrofolate (THF) and then transported to the liver, where it is converted into 10-formyl-THF and 5,10-methylene-THF. Methyltetrahydrofolate (MTHFR) then catalyzes the transformation of 5,10-methylene-THF into 5-MTHF [23]. This 5-MTHF is then used in methylation of homocysteine to produce methionine (an essential amino acid). Therefore, in folate deficiency the methylation cycle slows down, and with that plasma total homocysteine (tHcy) concentration in the body increases, which leads to hyperhomocysteinemia (HHCY). This condition is sometimes inherited as genetic polymorphisms of MTHFR or acquired as a result of low dietary intake of folate and/or vitamin B_6/B_{12} [24].

Increased maternal homocysteine concentration in early pregnancy may negatively impact placental development [25]. Studies have shown that elevated homocysteine concentration in the body induces oxidative and cytotoxic stress, leading to endothelial cell impairment [26]. Therefore, homocysteine is thought to be related to early placentation and may affect subsequent fetal growth [27]. In folate deficiency, homocysteine accumulates in the serum and is found to be associated with placental vasculopathy, leading to preterm birth [28,29]. It is also associated with an increased risk of maternal cardiovascular disease, late pregnancy complications such as preeclampsia, and recurrent miscarriages [30–34]. Similarly, the high homocysteine concentration is also responsible for megaloblastic changes in bone marrow and rapidly dividing cells. Therefore, maternal folic acid supplementation

TABLE 4.2
Micronutrient Supplementation and Its Impact on Improved Fetal and Pregnancy Outcomes

Micronutrient	Fetal and Pregnancy Outcomes
Folic acid	Periconceptional folic acid supplementation • Incidence of NTDs (OR = 0.28, 95% CI: 0.15, 0.53) • Recurrence of NTDs (OR = 0.32, 95% CI: 0.17, 0.60) Folic acid supplementation during pregnancy • Incidence of megaloblastic anemia (RR = 0.21, 95% CI: 0.11, 0.38) • Mean birth weight (MD = 135.75, 95% CI: 47.85, 223.68) Folic acid fortification • NTDs (RR = 0.55, 95% CI: 0.46, 0.63)
Iron	Daily supplementation of iron during pregnancy • Incidence of anemia (RR = 0.31, 95% CI: 0.22, 0.44) • Incidence of iron-deficiency anemia (RR = 0.44, 95% CI: 0.28, 0.68) • Incidence of low birth weight (RR = 0.80, 95% CI: 0.71, 0.90) • Incidence of low birth weight (RR = 0.81, 95% CI: 0.68, 0.97) • Birth weight (MD = 30.81, 95% CI: 5.94, 55.68) Intermittent supplementation of iron during pregnancy • Hemoglobin concentrations (RR = 0.48, 95% CI: 0.35, 0.67) • Side effects (RR = 0.56, 95% CI: 0.37, 0.84) • Iron-deficiency anemia (RR = 0.71, 95% CI: 0.08, 6.63)
Antioxidant	Preeclampsia (RR = 0.73, 95% CI: 0.51, 1.06) Preterm birth (before 37 weeks) (RR = 1.10, 95% CI: 0.99, 1.22) Infant death (RR = 1.12, 95% CI: 0.81–1.53)
Vitamin B_6	Mean birth weights (MD = –0.23 kg, 95% CI: –0.42, –0.04)
Vitamin A	Stillbirth (RR = 1.41, 95% CI: 0.57, 3.47) Maternal anemia (RR = 0.86, 95% CI: 0.68, 1.09) Preterm birth (RR = 0.39, 95% CI: 0.08, 1.93) Neonatal mortality (RR = 0.65, 95% CI: 0.32–1.31) Neonatal anemia (RR = 0.75, 95% CI: 0.38–1.51) Low birth weight infants (RR = 0.67, 95% CI: 0.47, 0.96) Maternal anemia (RR = 0.64, 95% CI: 0.43, 0.94) Maternal clinical infection (RR = 0.37, 95% CI: 0.18, 0.77)
Zinc	Preterm birth risk (RR = 0.86, 95% CI: 0.76, 0.97)
Iodine	New cases of cretinism (RR = 0.27, 95% CI: 0.12, 0.60)
Calcium	Preeclampsia (RR = 0.45, 95% CI: 0.31, 0.65) Preterm birth (RR = 0.76, 95% CI: 0.60, 0.97) Stillbirths (RR = 0.81, 95% CI: 0.63, 1.03) Perinatal mortality (RR = 0.86, 95% CI: 0.70, 1.07) Developing countries • Preeclampsia (RR = 0.41, 95% CI: 0.24, 0.69) • Preterm birth (RR = 0.88, 95% CI: 0.78, 0.99)
Omega-3 fatty acids	Birth weight (MD = 42.2 g, 95% CI: 14.8, 69.7) Preterm delivery (<34 weeks) (RR = 0.74, 95% CI: 0.58, 0.94) (RR = 0.69, 95% CI: 0.49, 0.99) Child attention (SMD = 0.50, 95% CI: 0.24, 0.77)
Vitamin D	Preeclampsia (RR = 0.67, 95% CI: 0.33, 1.35) Birth weight <2500 g (RR = 0.48, 95% CI: 0.23, 1.01)

Note: CI, confidence interval; MD, mean difference; NTD, neural tube defects; OR, odds ratio; RR, relative risk.

TABLE 4.3
Recommended Daily Intake of Micronutrients

Supplement	Recommended Daily Intake Level	Maximum Daily Intake
Folate	Females older than 13 years: 400–600 mcg Pregnancy (all ages): 400–600 mcg Breastfeeding females (all ages): 500 mcg For the prevention of pregnancy complications, 0.25–5 mg of folic acid taken by mouth daily for 12–24 weeks For anemia caused by folate deficiency, 1–5 mg taken by mouth daily until recovery	People 19 years and older (including pregnant or breastfeeding women): 1000 mcg
Vitamin A	For women: 700 mcg (2300 IU) Pregnant women 19 years old and older: 770 mcg (2600 IU) Lactating women 19 years old and older: 1300 mcg (4300 IU)	Insufficient evidence
Vitamin B_6	Women aged 51 and older: 1.3 mg For anemia, 25 mg of vitamin B_6 taken by mouth with multivitamins	The maximum daily intake of vitamin B_6 in adults and pregnant or breastfeeding women older than age 18 is 100 mg
Vitamin B_{12}	Pregnant females: 2.6 mcg Breastfeeding females: 2.8 mcg For prevention of anemia, the following doses taken by mouth: 2–10 mcg of vitamin B_{12} daily combined with iron and/or folic acid for up to 16 weeks; 100 mcg of vitamin B_{12} every other week plus daily folic acid and/or iron for up to 12 weeks	Insufficient evidence
Iron	Adolescents and women: 10–15 mg per day Pregnant females: 30 mg per day Lactating females: 15 mg per day	Upper intake levels that are tolerable for women older than 19 years of age as well as lactating mothers older than 19 years of age are 45 mg per day
Zinc	Women 19 years and older: 8 mg Pregnant females 14–18 years of age: 12 mg daily Pregnant women 19 years old and older: 11 mg Lactating females 14–18 years of age: 14 mg daily Lactating women 19 years and older: 12 mg daily	The tolerable upper level for zinc for adults 19 and older is 40 mg daily

during critical periods of organogenesis is associated with reduced incidence of neural tube defects (NTDs), congenital heart defects, obstructive urinary tract anomalies, limb anomalies, and orofacial clefts in the newborn [35]. NTD often leads to severe disability or even death [36].

A recent Cochrane review by De-Regil et al. [37] reported a beneficial effect on NTDs of daily periconceptional (i.e., before pregnancy and in the first 2 months of pregnancy) folic acid supplementation in women with a previous history of NTD-affected pregnancy. The risk of a neonate with NTD in the group that received supplementation was reduced significantly (odds ratio [OR] = 0.28, 95% confidence interval [CI]: 0.15, 0.53), as well as the risk of recurrence (OR = 0.32, 95% CI: 0.17, 0.60) (Table 4.2). Another review that assessed the impact of folic acid supplementation during pregnancy found a significant impact on improving mean birth weight (mean difference [MD] = 135.75 g, 95% CI: 47.85, 223.68 g) and reduction in the incidence of megaloblastic anemia (risk ratio [RR] = 0.21, 95% CI: 0.11, 0.38) [38].

While supplementation is one way of ensuring the intake of folic acid, fortification of food is another successful approach. A review of observational studies on folic acid–fortified foods found a similar reduction in NTDs by 45% (95% CI: 37, 54%) [39].

Folate is essential for embryonic, fetal, and infant growth and development, and deficiency in early pregnancy can disrupt neural tube formation. Folate supplementation showed a statistically significant reduction in the prevalence of NTDs. Therefore, to reduce NTDs effectively, the World Health Organization (WHO) recommends 400 mcg of folic acid for all women trying to conceive until 12 weeks of pregnancy [40]. Several countries have also adopted folic acid fortification, and these countries achieved a significant increase in folate intake and a significant decline in the prevalence of NTDs and spina bifida [41].

Though folic acid deficiency tends to increase the risk of spontaneous abortion (OR = 1.47, 95% CI: 1.01, 2.14), some studies controversially also propose the idea that folic acid supplementation may increase the risk of, or shift the timing of, spontaneous abortion; however, there is limited evidence in support of this statement at the present time [42]. Safety issues with folate supplementation are also a major concern, and may lead to many possible complications, some of which include decreased zinc absorption, increased susceptibility to malaria, and difficulty in identifying cobalamin deficiency because of the "masking effect" of folate on cobalamin. Both folate and vitamin B_{12} (cobalamin) have an intricate synergy in the methylation of homocysteine, and thus detecting a deficiency in one while the other is deficient is problematic [43].

4.2.2 Iron

Iron, an essential mineral, is required by the body for normal functioning of enzymes, acting as a cofactor in various vital reactions, as well as functioning in the delivery of oxygen to cells. Sources include liver, egg yolks, dark red meat, as well as plant sources, which are harder for the body to absorb and include prunes, lima beans, soybeans, kidney beans, broccoli, spinach, and other foods. Iron deficiency is the most common nutrient deficiency that is prevalent worldwide, and it often affects children and women of reproductive age. Iron deficiency starts with low reserve iron stores and then leads to anemia, which is characterized by low hemoglobin concentration in serum. Iron deficiencies can result in a wide variety of adverse outcomes including fatigue, impaired thermoregulation, immune dysfunction, gastrointestinal disturbances, and neurocognitive impairment. Anemia in pregnant women reduces infant growth and increases the risk of adverse pregnancy outcome [44]. Neonates acquire iron sources from the mother—which the mother reserves from cessation of the menstrual cycle during pregnancy and obtains some from dietary intake. During pregnancy, the fetus requires extra iron for the formation of the placenta and the mother requires iron for expansion of maternal red cell mass and to cope with blood loss during delivery [45]. However, women often become pregnant when they are already suffering from iron deficiency or lack adequate iron reserves.

Iron also plays a key role in the production of myelin in the developing brain of the fetus [46]. Animal studies have already suggested that severe iron deficiency is associated with alteration in motor activity [47]. Clinical literature from human studies suggests that in children, iron deficiency early in life is also associated with cognitive deficits [48], and children with severe iron deficiency are found to be irritable and apathetic and have poor appetite. It has also been suggested that the condition affects academic performance [49]. Hence, iron therapy acts by replenishing the iron stores in the body. Because iron is essential for neurotransmitters, it may improve psychomotor development and cognitive function in iron-deficient anemic children [50].

The major determinant of iron deficiency is low dietary intake of iron, which is further confounded by several factors such as limited access to food because of family income, or medical conditions such as bleeding or inflammatory diseases. In low- and middle-income countries, poverty limits dietary diversity and affordability of animal sources of iron, that is, meat. High consumption of plant sources also interferes with bioavailability of iron in body. Phytic acid, one of

the substances found in some plant foods, binds with nonheme iron and lowers iron absorption in the body. Further, parasitic infections such as malaria, hookworm, whipworm, and schistosomiasis, which are more prevalent in low- and middle-income countries, cause or further exacerbate anemia, especially when the infection is moderate to heavy, and when women are coinfected with multiple parasites. The agricultural revolution, which restricted or shifted the population's choice from animal sources to plant-based diets, is contributing to iron deficiency. Regular menstrual blood loss puts women at risk, with heavy loss a significant risk factor. Other factors include use of an intrauterine device and high parity [51].

Iron–folic acid deficiency translates into 115,000 maternal deaths each year [52]. Anemia is a predominant risk factor of obstetric hemorrhage. Therefore, correction of anemia with iron supplements improves the iron stores and enables a woman to withstand antepartum and postpartum hemorrhage, which is a major cause of maternal mortality [53].

A recent review by Imdad and Bhutta [54] reported 69% and 66% reduction in incidence of anemia (RR = 0.31, 95% CI: 0.22, 0.44) and iron-deficiency anemia (RR = 0.44, 95% CI: 0.28, 0.68) respectively at term when iron is supplemented on a daily basis. The review also found a beneficial impact on reducing the incidence of low birth weight (RR = 0.80, 95% CI: 0.71, 0.90). Another review of 60 trials involving 27,402 women [55] also showed a significant effect of daily iron supplementation on reducing the incidence of low birth weight infants (<2500 g) (RR = 0.81, 95% CI: 0.68, 0.97) and improvement in birth weight by 30.81 g (MD = 30.81 g, 95% CI: 5.94, 55.68 g) (Table 4.2).

Although daily supplementation of iron has shown beneficial effects, issues with compliance and potential side effects have also raised concerns in the recent past. Therefore intermittent oral iron therapy has been proposed as an alternative. A review [56] that examined the effect of intermittent supplementation of iron found a lower risk of high hemoglobin concentrations (>130 g/L) during the second or third trimester of pregnancy (RR = 0.48, 95% CI: 0.35, 0.67). Women receiving intermittent iron supplements also had fewer side effects (RR = 0.56, 95% CI: 0.37, 0.84) than those receiving daily supplements. The debate over whether intermittent or daily iron and folic acid supplementation has a greater effect on reducing iron-deficiency anemia was suggested to be insignificant (RR = 0.71, 95% CI: 0.08, 6.63).

Anemia is a common condition among pregnant women in low- and middle-income countries. Although daily preventive iron supplementation during pregnancy showed reductions in the incidence of anemia in mothers and low birth weight in neonates, intermittent supplementation ensured compliance and had lower associated side effects. Accordingly, intermittent oral iron therapy therefore can be proposed as an alternative.

4.2.3 Antioxidants

Antioxidants, which include copper, zinc, manganese, and vitamins C and E, are important cofactors in various reactions in the body, especially during pregnancy. Its deficiency mediates oxidative stress, which may lead to adverse pregnancy outcomes including preeclampsia and fetal growth restriction. Various hypotheses have been put forward to support supplementation with antioxidants that might delay or reverse the process that is seen with a decreased supply of antioxidant micronutrients. Evidence supports that glutathione peroxidase, an enzyme that protects from oxidative damage, requires antioxidants as its cofactor, and is seen to be decreased in cases of preeclampsia and miscarriage [3]. In high-income countries, preeclampsia during pregnancy is a leading cause of maternal and perinatal mortality and morbidity [57]; however, preeclampsia along with other hypertensive disorders is responsible for a large proportion of maternal and neonatal deaths each year in low-income countries.

Although a clear pathophysiology exists regarding oxidative stress at the molecular level and the occurrence of preeclampsia, none of the reviews on women supplemented with antioxidants showed any beneficial effect on maternal and other fetal/perinatal outcomes [58–61]. A recent Cochrane review that looked at 10 trials comprising 6533 women showed no significant difference between

antioxidant and control groups for the relative risk of preeclampsia (RR = 0.73, 95% CI: 0.51, 1.06), preterm birth (before 37 weeks) (RR = 1.10, 95% CI: 0.99, 1.22), or infant death (RR = 1.12, 95% CI: 0.81, 1.53) [58] (Table 4.2). Although the review did not find favorable maternal and fetal outcomes, it stressed pooling the data with ongoing trials to reach to conclusion. On the other hand, a cohort study of 1231 gravid women reported a positive association of plasma concentrations of vitamin E (α-tocopherol) with increased fetal growth, decreased risk of small-for-gestational-age births, and an increased risk of large-for-gestational-age births [62].

Antioxidants might be important for the prevention of preeclampsia; however, the evidence for the efficacy of antioxidants efficacy in preventing preeclampsia and other fetal and perinatal outcomes has not been established yet.

4.2.4 Vitamin B_{12} (Cobalamin)

Vitamin B_{12} deficiency is prevalent among poor people who cannot afford an animal-rich diet with any regularity or among those who prefer a vegetarian diet based on personal preference or religious grounds [63]. Vegan diets have very low cobalamin content; however, this greatly depends on whether people consume dairy products or eggs or maintain a strict vegetarian diet. Vitamin B_{12} (cobalamin) is one of a group of complex molecules produced in nature only by microorganisms. Because of bacterial symbiosis, it is possible for humans to receive vitamin B_{12} solely from the diet [64].

Vitamin B_{12} is needed as a cofactor for two important enzymes catalyzing methylation reactions in the body. During the development of an embryo, DNA modulates gene expression, cell differentiation, and the formation of organs. An inadequate vitamin B_{12} status during pregnancy causes elevated homocysteine levels, which may be associated with adverse pregnancy outcomes, including preeclampsia, premature delivery, low birth weight (<2500 g), very low birth weight (<1500 g), NTDs, and stillbirth [30].

Evidence suggests that vitamin B_{12} deficiency may also be linked to an increased risk of IUGR, abnormal fetal brain development, cleft palate, and metabolic syndromes [65]. A study conducted on rats that were supplemented with a omega-3 fatty acid and vitamin B_{12}-enriched diet showed a positive effect on synaptic plasticity and cognition, which may lead to a decrease in neurocognitive disorders [66]. Folate and vitamin B_{12}, among other micronutrients, play a critical role as cofactors in DNA methylation and epigenetic processes, dysfunctions of which may lead to an increased risk for neurodevelopmental disorders in the offspring. Further, this study demonstrates that a maternal diet with an imbalance in micronutrients such as folic acid and vitamin B_{12} can affect proper gene expression of neurotrophins and their signaling molecules and adversely affect the brain of the offspring [67]. Moreover, unbalanced vitamin B_{12} and total folate intakes during pregnancy may have adverse outcomes such as an increased risk of small-for-gestational-age infants [68].

4.2.5 Vitamin B_6

Vitamin B_6, found naturally in foods such as poultry, fish, starchy vegetables, and non-citrus fruits, has an average daily recommended intake of 1.9–2.0 mg for pregnant and lactating women [69]. Vitamin B_6 is often prescribed to treat nausea and vomiting during pregnancy, and appears to be more effective in reducing the severity of nausea [70]. Vitamin B_6 has a major role in metabolic processes in the body that include nervous system development and its functioning. It is essentially required for the synthesis of neurotransmitters, polyamines, and histamine, as well as the removal of ammonia from the body [71].

A Cochrane review of five trials (1646 women) [72] did not show any impact of vitamin B_6 on maternal, fetal, or perinatal outcomes and urged future trials to assess its impact on congenital malformation, neurological development, and other pregnancy and maternal outcomes. A small trial in this review showed low mean birth weights with vitamin B_6 supplementation (MD = –0.23 kg, 95% CI: –0.42, –0.04 kg, n = 33) (Table 4.2).

4.2.6 Vitamin A

In its natural form, vitamin A is available in different configurations. In the form of retinol or retinyl-esters, vitamin A can be obtained from animal sources and is abundant in liver and fish liver oil. Vitamin A as provitamin A-carotenoids can be obtained from plant sources, with yellow and green leafy vegetables having the highest concentrations [73]. Retinol is its endogenous metabolite and retinoic acid (RA), a vitamin A derivative, is a morphogenetically active compound [74].

Vitamin A deficiency is more prevalent in low- and middle-income countries because of poverty and poor dietary intake. Along with poor diet, infections also make this population more prone to deficiency. Vitamin A deficiency affects body stores and leaves the individual more susceptible to infection. This is further evident by an increased documentation of xerophthalmia after an outbreak of measles [75]. It has been suggested that in vitamin A deficiency, proliferation of T cells is impaired as they require retinoids for normal functioning. Thus the suppression of T cells results in weak immune functioning.

The fetus starts to accumulate vitamin A during the third trimester of pregnancy, and needs several months of sufficient intake after birth to build up a sufficient hepatic store for adequate fetal consumption. During this time, if the vitamin A supplementation is not adequate in the maternal diet then newborns often suffer from night blindness [76]. Moreover, for the healthy growth of an infant, maternal vitamin A concentration in blood is very important. Breast milk also contains vitamin A and is influenced by immediate and last-trimester serum vitamin A status of women [77]. Colostrum and early milk are highly rich in vitamin A, and inadequate levels of vitamin A in milk may not meet the physiologic needs of a newborn during the first weeks [78–81].

Vitamin A plays several important metabolic and regulatory functions in the human body. Retinol is required for production of the visual pigment rhodopsin; retinol and its derivatives are responsible for gene expression and cell proliferation and differentiation; its metabolites regulate immune functions and activate macrophages. It is also necessary for alveolar formation and lung development and also maintains hematopoiesis by stimulating the differentiation of stem cells into red blood cells [82]. The recommended dietary allowance for vitamin A during pregnancy is 1000 retinol equivalents/day (i.e., 3300 IU as retinol or 5000 IU of vitamin A) [83]. Currently, the WHO recommends routine vitamin A supplementation during pregnancy or at any time during lactation in areas with endemic vitamin A deficiency (where night blindness occurs) [84].

Compliance with safe dosage limits is very important for vitamin A, as a few derivatives of vitamin A such as retinoids (but not carotenoids) can be teratogenic [85–88]. A synthetic form of retinoid can cause congenital fetal anomalies [89,90]. Evidence suggests that retinoids play a role in the development of cephalic neural cells and closure of the neural tube [90–95]. Every 1 in 57 infants who were born to women who took supplements of more than 10,000 IU of preformed vitamin A had a risk of malformation [96].

A Cochrane review that studied more than 100 trials and reports on vitamin A supplementation (with other micronutrients) did not find any effect on the risk of stillbirth (RR = 1.41, 95% CI: 0.57, 3.47), maternal anemia (RR = 0.86, 95% CI: 0.68, 1.09), preterm birth (RR = 0.39, 95% CI: 0.08, 1.93), neonatal mortality (RR = 0.65, 95% CI: 0.32, 1.31), or neonatal anemia (RR = 0.75, 95% CI: 0.38, 1.51). There were fewer low birth weight infants in the supplemented groups (RR = 0.67, 95% CI: 0.47, 0.96) [97]. However, a single trial showed significant reduction in maternal night blindness (RR = 0.70, 95% CI: 0.60, 0.82). The review reported that in vitamin A-deficient populations and HIV-positive women, vitamin A supplementation reduces maternal anemia (RR = 0.64, 95% CI: 0.43, 0.94) and maternal clinical infection (RR = 0.37, 95% CI: 0.18, 0.77). Also, administration of vitamin A together with other micronutrients led to fewer low birth weight infants (<2.5 kg) in the supplemented group (RR = 0.67, CI: 0.47, 0.96) (Table 4.2).

During pregnancy, vitamin A is required for fetal growth, fetal reserves, and for maternal functions. It boosts immunity and improves anemia in women and reduces the risk of low birth weight infants. In a vitamin A-deficient population, supplementation with vitamin A has shown a protective

yet insignificant effect on maternal deaths from infections (RR = 0.78, 95% CI: 0.39, 1.58) [98]. Vitamin A also plays a vital role in production of red blood cells, and experimental trials have shown improved hemoglobin levels when supplementation is combined with iron (12.78 g/L, 95% CI: 10.86, 14.70), where one-third of the response was attributable to vitamin A (3.68 g/L, 95% CI: 2.03, 5.33 g/L) and two-thirds to iron (7.71 g/L, 95% CI: 5.97, 9.45 g/L) [99].

4.2.7 Zinc

Zinc, an essential micronutrient, occurs naturally in animal and plant sources including lean red meat, whole grain cereals, rolled oats, nuts, seeds, brown rice, tofu, and legumes, making up a vital part of a regular and balanced daily diet. Unrefined grains provide a high concentration of zinc because zinc is present predominantly in the outer layer of grains. Green leafy vegetables and fruits also provide high amounts of zinc because of their high water content [9]. Zinc deficiencies are starker in women from lower income countries because they may not be able to afford meat and other dietary sources of zinc to the same extent as women from higher income countries can; this, along with various factors such as health education among women and nutritional labels on foods such as cereals, all play a major role in determining intake of essential micronutrients.

Zinc plays an active role in nutrient metabolism, cell division, protein synthesis, and growth. It does this by binding with proteins, predominantly albumin, α_2-macroglobulin, and transferrin, to allow them to be transported to cells; however, only free zinc, or zinc that is not bound to protein, is biologically active [100]. Zinc regulates α_2-macroglobulin by altering its structure, which increases its interaction with cytokines and proteases, leading to changes in immune function [100]. Zinc also plays an oxidative role through binding with proteins and by occupying the binding sites for iron and copper in lipids, proteins, and DNA [101]. Animal studies showed evidence of increased fetal deaths due to spontaneous abortions or multiple congenital anomalies because of a zinc deficiency [102]. Zinc deficiencies can contribute to multiple organ malformations as a result of anomalous synthesis of nucleic acids and protein, tubulin polymerization, impaired cellular growth, and morphogenesis, leading to chromosomal defects and excessive lipid peroxidation of cellular membranes. Because of its intrinsic requirement at the molecular level, maternal zinc deficiency as suggested by numerous animal studies has long-term effects on the growth, immunity, and metabolic status of the surviving offspring [103,104]. For example, maternal zinc depletion had a direct detrimental effect on the offspring's immune response that was manifested across three generations [105].

Human studies suggested that women with acrodermatitis enteropathica, an inherited defect that interferes with zinc absorption, are at a greater risk for fetal losses and congenital malformations [106]. Even in the absence of acrodermatitis entheropathica, some women who had low maternal zinc levels experienced poor pregnancy outcomes, such as preterm birth, prolonged labor, postpartum hemorrhage, small-for-gestational-age infants, intrauterine growth restriction, or reduced birth weight [107–109]. Therefore, an adequate intake and supplementation with zinc is especially important for pregnant women and the developing fetus. These deficiencies can lead to adverse pregnancy outcomes such as prolonged labor, atonic postpartum hemorrhage, preeclampsia, preterm labor, and post-term pregnancies, although these have not yet been proven.

A review that included 20 trials reported that zinc supplementation in pregnant women showed significant reduction in preterm birth (RR = 0.86, 95% CI: 0.76, 0.97), with no significant effect on the reduction of numbers of infants with low birth weight [110] (Table 4.2). Because micronutrient deficiencies may compound the effects of HIV infection, many reviews have studied the effects of micronutrient supplementation, especially zinc, during pregnancy and evaluated the effects on reducing morbidity and mortality in women with HIV infection. A study in pregnant Tanzanian women suggested no significant clinical benefits after zinc supplementation [111].

4.2.8 Iodine

Iodine is an important building block of the hormones that are produced by the thyroid gland. Thyroid hormones, and therefore iodine, are essential for life. Because thyroid hormones govern the metabolic rate, which increases during pregnancy, the demand for iodine increases to maintain free thyroxin. During the first trimester, the demand for iodine increases as its excretion in urine increases [112]. WHO recommendations for iodine intake during pregnancy and lactation have recently increased from 200 to 250 mg/day, and the WHO has also suggested that a median urinary iodine concentration (UIC) of 150 to 250 mg/L indicates adequate iodine intake [113]. In countries where less than 90% of the population uses iodized salt, supplementation is advised, particularly during pregnancy (150 mg/day), to cope with the increased demand [114].

Iodine deficiency during pregnancy has a direct negative effect on the neurological development of the fetus. In iodine-deficient endemic areas, women experience hypothyroxinemia during early gestation [115]. Thyroid hormone is required for the maturation of the human brain, with important inputs in the myelination cycle and neuronal migration of the brain during fetal development; hypothyroidism during fetal life may cause irreversible damage to the neurological system. The disruption and deficiency develop into cretinism, which is manifested as severe mental retardation along with multiorgan involvement exhibiting as squints, deafness, mutism, and motor spasticity.

Low iodine intake in pregnancy is also associated with poor pregnancy outcomes such as miscarriage and small-for-gestational-age infants [113]. Because fetal brain development starts early in life and thyroxin plays a vital role in it, an adequate iodine intake, especially within the first trimester, is therefore important [116]. A study has shown that pregnant women with UICs below 50 mg/L during the third trimester or higher levels of thyroid-stimulating hormone were more likely to have a small-for-gestational-age infant and a lower mean birth weight infant compared to women with UICs between 100 and 149 mg/L (OR = 0.15, 95% CI: 0.03, 0.76] [117]. Intelligence quotients (IQs) were also found to be lower in children whose mothers had UICs below 50 mg/L [118].

A recent meta-analysis of controlled studies in moderate to severely iodine-deficient areas suggested that iodine supplementation before or during early pregnancy prevented new cases of cretinism (RR = 0.27, 95% CI: 0.12, 0.60) [119]. Similar findings were also reported by a very recent review [120]. The review by Zimmermann also reported an increase in birth weight (3.8–6.3%), decrease in infant mortality (20–60%), and increase in developmental scores in young children (10–20%) [119] (Table 4.2). Reviews have also estimated a reduction in IQ of 12–13.5 points in iodine-deficient populations. Therefore, salt iodization in areas with endemic deficiency is the most effective approach [119].

4.2.9 Calcium

Calcium plays an essential role in blood coagulation, nerve conduction and transmission, cell membrane and muscle function, and skeletal development. Because of its major role in the development of the skeleton, the demand for calcium increases during pregnancy as a result of an increase in intestinal adsorption and mobilization from bones to meet the requirements of the developing fetus [121]. A deficiency of calcium during pregnancy increases the risk for preeclampsia, and may induce IUGR.

A recent meta-analysis [122] showed a reduction in cases of preeclampsia with calcium supplementation (RR = 0.45, 95% CI: 0.31, 0.65); however, the effect was greater for women with low baseline calcium intake (RR = 0.36, 95% CI: 0.20, 0.65) and in those selected as being at high risk (RR = 0.22, 95% CI: 0.12, 0.42). The risk of preterm birth was also reduced with supplementation (RR = 0.76, 95% CI: 0.60, 0.97) and among those at high risk (RR = 0.45, 95% CI: 0.24, 0.83). Maternal mortality and serious morbidity also declined by 20% with calcium supplementation

(RR = 0.80, 95% CI: 0.65, 0.97). One study included in this review also assessed the later blood pressure in children whose mothers were given calcium supplementation and found a significant reduction in blood pressure readings (RR = 0.59, 95% CI: 0.39, 0.91) [123] (Table 4.2).

Similar findings were also reported from a review that assessed the impact of calcium supplementation only to women from low- and middle-income countries, where deficiency is more prevalent [124]. The review reported lower incidence of preeclampsia (RR = 0.41, 95% CI: 0.24, 0.69), preterm birth (RR = 0.88, 95% CI: 0.78, 0.99). However, the impact on stillbirths and perinatal mortality was found to be insignificant (RR = 0.81, 95% CI: 0.63, 1.03) and (RR = 0.86, 95% CI: 0.70, 1.07) respectively [125].

4.2.10 Omega-3 Fatty Acids

The literature has suggested potential links between the intake of marine food and pregnancy outcomes. Deficiencies or low intakes of marine food are often compensated by supplementation with marine/fish oils. Although the affordability of marine food is lower among people living in low- and middle-income countries, the rates of supplementation are higher among women in high-income countries. The *n*-3 long-chain polyunsaturated fatty acids (LCPUFAs) eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) are precursors of the 3-series prostaglandins, which act as modulators of inflammatory and vascular effects, and can be supplied by marine oils [126]. However, with limited evidence from human studies, a final word is yet to be established on the subject. Because vasoconstriction and endothelial damage impose a risk for preeclampsia and gestational hypertension, it is likely that marine oil fatty acids may play an active role in preventing or decreasing the incidence of these mechanisms. Prostaglandins have a delaying effect on birth by delaying the initiation of labor and cervical ripening and by relaxing the myometrium by increasing the production of prostacyclins (PGI_2 and PGI_3), which is hypothesized to also be an effect of marine oils. Decreased maternal intake of these essential fatty acids also affects the development of nervous system tissue in the growing fetus [127]. Therefore women are advised to take these supplements not only during pregnancy but also during the lactation period, when breast milk serves as the biggest source of these essential fatty acids to a child. Infants require these essential fatty acids for growth and maturation of the brain and retina [128,129].

A meta-analysis of randomized controlled trials showed that omega-3 supplementation during pregnancy increases birth weight (MD = 42.2 g, 95% CI: 14.8, 69.7 g) and leads to a 26% lower risk of early preterm delivery (<34 weeks) (RR = 0.74, 95% CI: 0.58, 0.94) [130]. A Cochrane review of six trials also reported higher mean gestation in women who were given fish oil (weighted mean difference [WMD], 2.55 days, 95% CI: 1.03, 4.07 days) and they had a lower risk for preterm birth (<34 weeks) (RR = 0.69, 95% CI: 0.49, 0.99). Birth weight was also higher in infants born to women who were given supplementation (WMD = 47 g, 95% CI: 1, 93 g) [131] (Table 4.2).

Omega-3 fatty acids when supplemented during lactation were found to have no impact on children's neurodevelopment in terms of language development (standard mean difference [SMD] = –0.14, 95% CI: –0.49, 0.20), intelligence or problem-solving ability (SMD = –0.22, 95% CI: –0.23, 0.66), psychomotor development (SMD = 0.34, 95% CI: –0.11, 0.78), and motor development (SMD = 0.08, 95% CI: –0.13, 0.29). The impact was significant for child attention (SMD = 0.50, 95% CI: 0.24, 0.77) [132].

4.2.11 Vitamin D

Vitamin D, a fat-soluble vitamin, is obtained mainly from sunlight [133]. It is also naturally present in fish liver oils, fish, egg yolk, and liver. Vitamin D plays an important role in various bodily functions such as immunity, cell differentiation, and bone growth, and also has anti-inflammatory roles. Vitamin D is essential for calcium homeostasis and in reducing the risk of chronic diseases. There are two physiologically active forms of vitamin D: D_2 (ergocalciferol), which is synthesized

by plants, and D_3 (cholecalciferol), which is formed in the skin from 7-dehydrocholecalciferol after exposure to ultraviolet B (UVB) radiation from the sun [134]. Vitamin D in supplements is found as either vitamin D_2 or D_3. D_3 metabolites have a higher affinity for vitamin D binding proteins; in addition, vitamin D_3 may be more effective than vitamin D_2 in raising serum concentrations of vitamin D and maintaining those levels for a longer time [135,136]. To ensure sufficient circulating levels, adequate vitamin D intake is necessary because of its short half-life. Both D_2 and D_3 forms share a similar metabolic fate, with both entering the circulation from the gut or skin, whereby they are transported to the liver and hydroxylated to 25-hydroxyvitamin D (25(OH)D), the major circulating form of vitamin D. Vitamin D deficiency is variable in adolescents and in countries where women cover themselves for religious or personal reasons.

There is a unique relationship between vitamin D and calcium. Parathyroid hormone is responsible for raising the serum calcium concentration through bone resorption, while another hormone, calcitriol, inhibits parathyroid hormone and thus allows an increase of serum calcium concentration from sources other than bone. Renal and intestinal absorption of calcium and phosphorus is augmented in the presence of calcitriol. Maternal vitamin D deficiency in pregnancy has been associated with an increased risk of preeclampsia [137,138], gestational diabetes mellitus [139,140], and preterm birth (<37 weeks of gestation) [141,142], and it influences fetal bone development and children's growth [137,143] and boosts the immune system [144,145].

A Cochrane review on maternal vitamin D supplementation during pregnancy reported a nonsignificant reduction in preeclampsia (RR = 0.67, 95% CI: 0.33, 1.35) and a lower likelihood for an infant with a birth weight below 2500 g (RR = 0.48, 95% CI: 0.23, 1.01) [146] (Table 4.2).

4.2.12 Other Micronutrients

Several other micronutrients play a direct role or act as cofactors at the molecular level that may have a beneficial effect on maternal and fetal outcomes. Their roles have not been reviewed in detail in human studies. One such micronutrient is copper, which is an essential cofactor of several enzymatic reactions involved with metabolism, angiogenesis, oxygen transport, and particularly antioxidant function. Copper levels rise during pregnancy and the effects of severe copper deficiency may be disastrous, including reproductive failure and early embryonic death, whereas mild or moderate deficiency does not have such serious effects on the fetus [147].

4.3 CONCLUSION

A completely balanced and healthy diet during pregnancy has a well-established role in preventing adverse birth outcomes such as low birth weight infants; however, evidence now supports the role of micronutrient supplementation in reducing the incidence of congenital anomalies or maternal morbidities. Given the conclusive data on the roles of the various micronutrients such as antioxidants and folate on the growing embryo and its differentiation, a link between the prevention of defects during organogenesis and proper supplementation can be made, albeit with further trials that explore the effects of such supplementation on different parameters. Further research is needed to investigate the required amounts of each micronutrient as well as toxicities that may be a result of excessive intake.

Because it is recommended that some micronutrients, such as iron and folate, be taken before conception (preconception period), all women of reproductive age must be educated to have an adequate intake. Anemic women of reproductive age must be given supplements if dietary modifications cannot be made, given that iron plays a vital role in behavioral and neural development, and its deficiency has an irreversible effect [148].

Nutritional care must begin at an early stage, with special attention paid to the periconceptional period and the first two trimesters, where a major part of organogenesis occurs, to prevent serious fetal defects, adverse birth outcomes, and poor developmental milestones later on in the child.

4.4 KEY POINTS SUMMARY

1. Deficiencies in micronutrients such as iron, folate, vitamin A, calcium, iodine, and zinc are common among women of reproductive age, potentially adversely impacting their pregnancies and the health of their children. Micronutrients play important roles in the function of enzymes and cofactors involved in the production of energy, DNA, and signal transduction, as well as maintenance of viability of cells in the body. Therefore it is not surprising that deficiencies may adversely affect pregnancy outcomes. Micronutrient deficiencies are significantly more common in low- to medium-income countries, although they are also seen in women from high-income countries. The heightened maternal metabolic demands for micronutrients during pregnancy, together with the growing child's increased needs, further compound any micronutrient deficiency that may have existed before pregnancy.
2. Iron deficiency is the most prevalent micronutrient deficiency found worldwide. A diet low in red meat, poor absorption of iron (parasitic infections, high consumption of cereal crops that interfere with iron absorption), or excessive loss of iron (heavy menstruation, iron loss from high parity) all can lead to iron deficiency. As iron plays an important role in the production of red blood cells, plus the function of many enzymes, the clinical effects of iron deficiency are widespread and include fatigue, anemia, immune dysfunction, and gastrointestinal and neurocognitive impairment. Iron is essential for normal growth and brain development in the fetus, with iron deficiency in pregnancy resulting in an increased risk of intrauterine growth restriction (IUGR) and cognitive deficit. The use of iron supplements in pregnancy has been shown to reduce the incidence of anemia and IUGR and improve women's resilience to postpartum hemorrhage, thereby reducing maternal deaths.
3. Folic acid and folate derivatives play a role in DNA methylation, cell division, and tissue growth. Folate plays an important role in the methylation of homocysteine to methionine (an essential amino acid), with a deficiency of homocysteine leading to a rise in homocysteine levels that then induce oxidative stress and inflammation. The resulting endothelial damage may impair placental perfusion, leading to an increased risk of miscarriage and preeclampsia. Folic acid supplementation, or fortification of food, can result in a reduction in homocysteine levels and significant decline in the incidence of neural tube defects, congenital heart defects, orofacial clefts, and limb and obstructive urinary anomalies. For this reason, the WHO advocates that all women trying to conceive should take 400 mcg of folic acid until 12 weeks of pregnancy.
4. Vitamin B_{12} deficiency is prevalent among poor women who cannot afford an animal-rich diet or who prefer a vegetarian diet for personal or religious reasons. Vitamin B_{12} plays an important role in reducing homocysteine levels, as well as playing a positive role in DNA methylation. Inadequate vitamin B_{12} status during pregnancy is associated with adverse outcomes such as preeclampsia, prematurity, and low birth weight, as well as abnormal fetal brain development and cleft palate. For these reasons, vegetarians and vegans should take vitamin B_{12} supplements during pregnancy.
5. Vitamin A, through its metabolites retinol and retinoic acid, plays an important role in gene expression and cell proliferation and differentiation. In low-income countries vitamin A deficiency is common, leading to night blindness in the mother and child, impaired immune function predisposing to infection, and impaired red blood cell formation (anemia). Low vitamin A levels may interfere with normal fetal lung and neural development. Excessive consumption of preformed vitamin A (>10,000 IU) or synthetic vitamin A analogues such as those used to treat acne may actually be teratogenic and should be avoided during pregnancy. The WHO recommends routine vitamin A supplementation (5000 IU/day) during pregnancy and breastfeeding in all areas where vitamin A deficiency/night blindness is prevalent. Some evidence suggests that such supplements can reduce maternal infection and anemia rates, while also reducing maternal mortality and night blindness and fetal growth restriction.

6. Zinc deficiencies are relatively common in lower income countries where access to red meat may be limited. Zinc plays an important antioxidant role, as well as aiding immune function. Zinc deficiency has been linked with fetal growth restriction, preterm delivery, and poor uterine contractions (prolonged labor and increased risk of postpartum hemorrhage). Zinc supplementation in pregnancy has been shown to produce a significant reduction in preterm birth.
7. Iodine plays an important role in the fetal and maternal production of thyroid hormone. As thyroid hormone is important for fetal brain development, minor deficiencies in iodine resulting in subclinical hypothyroidism have been linked with a reduction in the child's IQ of up to 13 points, with more serious deficiencies causing severe mental retardation (cretinism). Many countries fortify salt with iodine to prevent deficiency. In countries where iodized salt use is not prevalent, the WHO suggests supplementation with 150 mg/day.
8. Calcium deficiency is more common in women from low- and middle-income countries with limited dairy intake. Calcium demands in pregnancy increase because of the need for calcium to build the fetal skeleton. This increased demand can exacerbate maternal calcium deficiency and lead to an increase in risk of preeclampsia and possibly growth restriction. Calcium supplements have been shown to reduce the incidence of preeclampsia, especially in women who had low calcium intake at baseline.
9. Vitamin D, a vitamin produced primarily by the skin in response to sun exposure, also appears to play a beneficial role in pregnancy. Vitamin D deficiency has been linked with an increased risk of preeclampsia, gestational diabetes, and preterm birth, while also influencing fetal immune and bone development. Vitamin D supplementation has been reported to reduce the risk of preeclampsia and low birth weight.
10. Antioxidants such as vitamins C and E, copper, zinc, and manganese may all play a role in helping prevent pregnancy complications such as preeclampsia that are characterized by a state of oxidative stress. Some studies have observed trends suggesting that antioxidant supplements may reduce preeclampsia and IUGR, although the evidence for this effect is not conclusive. Vitamin B_6 supplements have been shown to have some beneficial effects in treating hyperemesis gravidarum ("morning sickness"). Omega-3 fatty acids, as found in fish and fish oil supplements, have been identified as having anti-inflammatory and positive vascular effects, potentially reducing preeclampsia and preterm delivery risks. The use of omega-3 supplements in pregnancy has been reported to result in significant decreases in preterm delivery and low birth weight. Omega-3 fatty acids may also assist in the development of a healthy fetal nervous system.

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5 A Review of the Pivotal Studies Supporting the Use of Periconceptional Multivitamins in the Prevention of Congenital Abnormalities

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CONTENTS

5.1 INTRODUCTION

"The goal of preventive nutrition is to maximize the years of HEALTHY life expectancy so that individuals can enjoy their lives and be competent in performing the activities for daily living independently" [1]. Previously, malnourishment and different dietary deficiencies represented the major nutrition-related problems for human health and most intervention strategies were directed toward their management. Recent attention has focused on the major role of an optimal diet in health maintenance and prevention of cardiovascular disorders, cancers, and other conditions.

As the beginning of life is conception, followed by the very sensitive period of fetal development, dietary deficiencies associated with risk of fertility, pregnancy complications, birth defects, preterm

birth, and other adverse effects, in addition to nutritional exposure *in utero*, have long-term effects for the quality of life [2].

In general, original research papers have concentrated on the presentation of the main objectives of the study and reported only the major results. This was the case in the publication of the results of the Hungarian Randomized Controlled Trial (HRCT) for testing the efficacy of a micronutrient combination containing 0.8 mg of folic acid in the prevention of first occurrence of neural tube defects (NTDs). However, nutrition has several other associations with human fertility and reproductive function and it would be useful to understand better their complex relationship. In our view, the HRCT resulted in—beyond the main objective, that is, prevention of NTDs—some important secondary findings, which we summarize in this chapter.

5.2 PREVIOUS INTERVENTION TRIALS

In 1964, Hibbard [3] reported a 3.0% rate of structural birth defects (we use the term congenital abnormalities [CAs]) in infants of folate-deficient mothers whereas the rate of CAs was 1.6% in appropriate controls. Later Hibbard and Smithells [4] showed a relationship between human embryopathy and a deficiency of folate metabolism. Finally, Smithells and co-workers [5] focused their studies on NTDs and demonstrated the role of vitamin deficiencies in the etiology of CAs in this group.

NTDs correlate with maternal socioeconomic status [6]; thus Smithells hypothesized that undernutrition could be the common factor in the origin of NTDs. His group therefore tested the effect of diet supplemented with a multivitamin containing 0.36 mg of folic acid in the first intervention trial [7]. Women who had given birth to one or more previous infants with NTDs were supplemented with this multivitamin during the periconception period while controls were recruited among similar women, that is, with previous infants with NTDs who were already pregnant but had not taken vitamin supplements. The results of this intervention study were published separately for the Yorkshire region of the United Kingdom [8] and Northern Ireland [9], and they found 91% and 83% reduction in NTD recurrence, respectively.

However, their results were not accepted by some experts because of possible selection bias in their nonrandomized controlled trial (RCT). Thus the Medical Research Council (MRC) in the United Kingdom [10] organized a multicenter RCT (43% of the participants came from Hungary). There were four supplementation groups: folic acid (4 mg), other vitamins (A, 4000 U; B1, 1.5 mg; B2, 1.5 mg; B6, 1.0 mg; folic acid, 4.0 mg; C, 40 mg; D, 400 U; and nicotinamide, 15.0 mg), folic acid + other vitamins and minerals (iron [dried ferrous sulphate 120 mg] and calcium [di-calcium phosphate 240 mg]) as control. The MRC Vitamin Study found that a pharmacological dose (4 mg) of folic acid alone can reduce NTD recurrence by 71% (0.8% vs. 4.3%; 0.29, 0.12–0.71).

5.3 THE HUNGARIAN PERICONCEPTION SERVICE

The Hungarian Periconception Service (HPS) was established in 1984 [11,12]. The concept of HPS is a new health infrastructure for prospective parents to achieve their goal to have healthy babies, that is, to reduce the occurrence of adverse pregnancy outcomes, mainly preterm births and CAs as the two major factors in infant mortality and disabilities, by counseling, examination, and medical intervention. Thus HPS is a free of charge health service with three criteria for participation: (1) The participant is not infertile (defined as no conception after more than 12 months of sexual activity without contraception); (2) the participant is not currently pregnant (pregnant women are referred to regional prenatal care clinic); and (3) participation is voluntary. The HPS is part of primary healthcare based on provision of services by qualified and trained midwives who are able to select couples who may require secondary care performed by specialists.

The three main steps of the HPS are summarized in Figure 5.1. The third step explains that we prefer to use the term periconception care/service in Hungary instead of the well-established term preconception care because pregnant women visit prenatal clinics after 6–12 gestational weeks, at

I. *Reproductive health check-up*. This is a preconception screening of reproductive risk factors at the first visit.

II. *The 3-month preparation for conception*. The explanation is simple: the beginning of life is at conception, and the major events of life such as conceptus's sex, health, and/or many diseases are determined at that time; thus it is necessary to prepare for conception. The start of the 3-month preparation for conception is also at the first visit.

The evaluation of the available results of recent investigations and preparation of couples for the optimal conception is the task of the second visit. If more examinations are needed and/or the results of treatments are not sufficient, there are more (further second type meetings) visits.

III. *To achieve "optimal" conception and to provide a better protection of early pregnancy*. The task of the third visit is to confirm the pregnancy, while the fourth visit is the so-called "farewell" because pregnant women are referred with the discharge summary of the HPS to prenatal care clinics. In addition, pregnant women are asked to inform us on the results of their delivery and the health condition of their newborn infants, and to send us a photo of their babies.

FIGURE 5.1 Three steps of the Hungarian Periconception Service according to the visits.

which time the embryo is more or less "ready"; thus the most sensitive and vulnerable period of fetal development is omitted from the medical health system. HPS therefore includes 1–3 months before conception and 1–3 months after conception.

5.4 PREPARATION OF THE HRCT

We were informed of the results of Smithells' studies and Hungary took part in the MRC Vitamin Study; thus we wished to incorporate the periconceptional multivitamin supplementation into the second step of the HRCT. Accordingly, a micronutrient combination contained 12 vitamins: 6000 IU of vitamin A (from the end of 1989, 4000 IU), 0.6 mg of B_1, 1.8 mg of B_2, 19.0 mg of nicotinamide, 2.6 mg of B_6, 10.0 mg of calcium pantothene (B_5), 0.2 mg of biotin (B_7), 4.0 μg of B_{12}, 100.0 mg of C, 500 IU of D, 15.0 mg of E, and 0.8 mg of folic acid; four minerals: 125.0 mg of calcium, 125.0 mg of phosphorus, 100.0 mg of magnesium, and 100.0 mg of iron; and three trace elements: 1.0 mg of copper, 1.0 mg of manganese, and 7.5 mg of zinc. Half of the participants received this micronutrient combination (multivitamin group), while the other half of participants received placebo-like trace element combination (trace element group) randomly. (The Hungarian ethical committee did not allow use of a true placebo.) These women used these supplements for at least 1 month before conception and at least 2 months after conception, that is, during the periconception period.

There were two major questions of the HRCT after publication of the two aforementioned "recurrence" trials [8–10]:

1. Does a folic acid-containing multivitamin reduce the risk of *first* occurrence of NTDs? About 95% of women who have a fetus or infant with NTD had no previous NTD-affected pregnancies; thus the prevention of the first occurrence of NTDs would be a real public health success. However, we have to consider two crucial aspects of this question:
   a. At the evaluation of NTD cases it is necessary to differentiate the so-called isolated and multiple-syndromic cases [13]. Isolated NTDs are not associated with other CAs except NTD-related CAs such as secondary hydrocephalus and clubfoot, whereas syndromic NTDs have a concurrence of one or more other CAs in the same person. Syndromic NTDs are caused by chromosomal aberrations (e.g., trisomy 13), mutant major genes (e.g., Meckel–Gruber syndrome with autosomal recessive inheritance), and teratogens (e.g., valproate). However, it is worth mentioning that syndromic NTD cases represent only a minor part of all NTD cases (about 10%); thus the isolated manifestation is characteristic for NTDs.

      Most isolated NTD cases have a multifactorial origin, that is, polygenic predisposition with interaction by external agents that can trigger or suppress this genetic

predisposition. The polygenic predisposition is confirmed by the recurrence risk, which is 10-fold higher in the first-degree relatives of patients with NTD than the first occurrence of NTDs in a given population. On the other hand, the importance of environmental agents is indicated by the socioeconomic correlation with NTDs (the occurrence of NTDs is much lower in the highest class than in the lowest class) and their obvious geographical differences (0.21 per 1000 in Bogota, Colombia and 10.5 per 1000 in North China) [13].

b. The brain and spinal cord develop from the neural tube, which is formed by dorsal folding of the neural plate after the 15th postconception day. The fusion of this folding proceeds in cranial and caudal directions and in humans is normally completed at 21–26 postconception days in the cranial pole and 23–28 days in the caudal pole, respectively. These periods therefore correspond to the critical period of cranial pole defect—anencephaly—and caudal pole defect—spina bifida. However, it is necessary to consider that at present in clinical practice gestational age is calculated from the first day of the last menstrual period; therefore it is necessary to add 14 days to the postconception/fetal age to estimate the gestational age. Thus the critical period of anencephaly is between the 35th and 40th gestational days, while for spina bifida this period is between the 37th and 42nd gestational days [13,14]. Occipital encephalocele is part of the NTD spectrum but with a different pathogenesis [13].

2. The pharmacological dose (4 mg) of folic acid used in the MRC Vitamin Study may have some adverse effects. In 1990 the Institute of Medicine, US National Academy, Washington, DC [15] declared that it is necessary to differentiate the physiological dose of folic acid (<1 mg daily) for preventive purposes in healthy persons including pregnant women and the pharmacological dose of folic acid (>1 mg daily) for the treatment of patients. At present we cannot exclude possible adverse events after the use of high doses of folic acid; the major concern is the masking effect of a high dose of folic acid in patients with pernicious anemia [16]. Thus, the upper tolerable dose of folate–folic acid for healthy persons including pregnant women is 1 mg, and in the HRCT the efficacy of a multivitamin containing 0.8 mg folic acid was tested.

   The great majority of the women in the HRCT were healthy and not malnourished. The usual daily intake of folate is about 0.16–0.20 mg/day in Hungary [17], and this level of consumption is not significantly higher in other countries [18].

The natural polyglutamate folate was discovered by Lucy Wills in 1931 [19], and she recommended using the term vitamin 11 as a "twin" of vitamin B_{12}. Later the monoglutamate form of this vitamin, as folic acid, was produced [20]. However, folate is an umbrella term that encompasses all substituted/unsubstituted, oxidized/reduced, and mono-/polyglutamate forms of pteroyl-L-glutamic acid including the synthetic form, folic acid. The latter is called vitamin B_9 in France and vitamin B_{11} in the Netherlands and Hungary. From a medical aspect, the differentiation of dietary folate and synthetic folic acid is useful.

Humans cannot produce folate. The major dietary sources of folates are fresh and frozen green leafy vegetables, citrus fruits and juices, liver, wheat bread, and legumes such as bean. Thus the requirement for this water-soluble vitamin is supplied partly by dietary intake of folates and partly by the use of synthetic folic acid, and for estimation of the required dose it is necessary to add the quantities of these two forms of the vitamin.

5.5 PRIMARY RESULTS OF THE HRCT

The HRCT was initiated in February 1984; the randomization was stopped at the end of April 1992 and the evaluation of pregnancy outcomes of 7905 participants was closed at the end of April 1993. The results of the HRCT were published [21–23]: NTDs did not occur in 2391 offspring of the

multivitamin group, whereas 6 NTDs were found in 2471 offspring of trace element group ($P = 0.01$; risk ratio [RR] with 95% confidence interval [CI]: 0.07, 0.01–0.13). Thus, the HRCT demonstrated first that a multivitamin containing 0.8 mg of folic acid prevented about 90% of the first occurrences of NTDs.

The total dataset of the HRCT was invited and stored in the US NIH Science Data Archive [24].

For ethical reasons, the HRCT could not be continued; thus a cohort controlled trial (CCT) was designed to collect more data regarding the preventive effect of this multivitamin for NTD and mainly other CAs [25]. All participants in the HPS were supplied with the multivitamin used in HRCT while women for the unsupplemented cohort were recruited at the 14th week of pregnancy from the regional prenatal care clinics and they were each matched to a pregnant woman of the supplemented cohort. The protective effect of this multivitamin for the reduction of NTDs was confirmed in these 3056 "pairs" (1 vs. 9; odds ratio [OR] with 95% CI: 0.11, 0.01–0.91). However, the occurrence of syndromic NTDs was not reduced either in the RCT or in the CCT [26].

Later the results of only one intervention trial were reported. The efficacy of 0.4 mg of folic acid was shown for the prevention of a first occurrence of NTDs in a Chinese–US study [27]. There was a 79% reduction in the risk of NTDs in areas with high rates of NTDs (6.5 per 1000), whereas this reduction was 41% in areas with low rates of NTDs (0.8 per 1000).

5.6 SECONDARY RESULTS OF THE HRCT

5.6.1 Fertility

During preconceptional multivitamin supplementation, menstrual cycles became more regular, that is, the variance was lower [28]. The mean length of the menstrual cycle before multivitamin supplementation was 29.26 ± 2.98 days, whereas after it was 29.00 ± 2.77 days. Thus, multivitamin supplementation may have a beneficial effect for women with irregular menstrual cycles.

The time interval between the onset of sexual activity for conception and the achievement of conception can be measured, and it was slightly but significantly shorter in the multivitamin group (3.8 ± 3.2 vs. 4.0 ± 3.3 months). This difference resulted in a 7% higher rate of conceptions within 1 year in women who were supplemented with the multivitamin preconceptionally compared with those who were not supplemented [29].

There was no difference in the sexual activity (measured by a rate of weekly sexual intercourse) of couples between the multivitamin and the trace element groups in the preconceptional period [30].

The rate of multiple births was 41% higher after periconceptional multivitamin supplementation [31]. The rate of multiple births in Hungary as a whole was 2.2% during the study period. In the multivitamin group, 2421 mothers had 2468 births, 93 of which (3.8%) were multiple births (90 twins, 3 triplets). In the trace element group, 2346 women had 2378 births, and among them 64 (2.7%) were twins. The difference was significant ($P = 0.03$), but this relative increase of 40.7% in the rate of multiple births resulted in an absolute increase of multiple births of only 1.1%. The higher rate of twin conceptions could not be explained by maternal factors (age, parity) or by a higher rate of infertility drug use [32], and at that time *in vitro* fertilization was not used in Hungary. This finding was confirmed later in the United States [33] and Sweden [34], but not in China [35]. However, the prevalence of twins is lowest in people of the Oriental race including the Chinese population, and Chinese people have an extremely high occurrence of the TT genotype of methylenetetrahydrofolate reductase (*MTHFR*) gene (55%) [36], which is associated with a very low dizygotic twin rate [37]. These conflicting data stimulated us to check the large population-based material of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980–1996. There was a somewhat higher incidence of multiple pregnancies/births both after periconceptional high doses (mainly 6 mg) of folic acid and of folic acid (0.1–1.0 mg) containing multivitamin supplementation in 38,151 controls without CA [38]. A systematic review of the recent literature from July 1994 to

July 2006 resulted in the conclusion: "Overall…there is possible evidence for a relationship between periconceptional folic acid intake and increased twinning" [39].

5.6.2 The Effect for Pregnant Women

A significantly lower rate of severe (treated) morning sickness, that is, nausea and vomiting, in early pregnancy occurred after periconceptional multivitamin supplementation (3.0% vs. 6.6% in the trace element group) [40].

There was no difference in maternal weight gain between the multivitamin and trace element groups after periconceptional use [41]. The mean body weight was 57.1 ± 7.7, 57.7 ± 8.1, and 58.9 ± 8.4 kg before conception, at the time of pregnancy confirmation, and the 12th gestational week in women with multivitamin supplementation, respectively. These figures were 57.3 ± 7.3, 58.1 ± 7.8, and 58.8 ± 8.0 kg in the mothers with trace element supplementation. In Hungary this possible side effect caused the major concern among women.

All other possible side effects of the multivitamin used in the HRCT were monitored continuously. Patients with pernicious anemia have not been recorded among these reproductive-aged women. There was no case with multivitamin-related side effects of epilepsy during the periconception period [42]. However, a 22-year-old epileptic woman was treated continuously with carbamazepine. After the end of multivitamin intake in the 12th gestational week she used another folic acid (1 mg) containing a multivitamin from the 20th week, and she had repeated status epilepticus after this new supplementation in parallel with the manifestation of symptoms of systemic lupus erythematosus. Her pregnancy ended in the delivery of a stillborn fetus in the 39th gestational week [42]. Autoimmune disease of epileptic pregnant women could damage the blood–brain barrier, and the pharmacological dose (≥1 mg) of folic acid may trigger a cluster of seizures.

Of 2793 pregnant women supplemented with the multivitamin used in the HRCT, two (0.07%) had severe allergic exanthema and discontinued the use of the multivitamin (however, both had a history of drug-induced allergic diseases). Among all other possible side effects, constipation (1.8% vs. 0.8%) and diarrhea (1.4% vs. 0.4%) were reported somewhat frequently after multivitamin supplementation compared to the trace element group in the preconception period [41].

5.6.3 Effect for Pregnancy Outcomes

The distribution of pregnancy outcomes is shown in Table 5.1. Four types of fetal demise were differentiated in pregnancies with singleton fetuses: (1) chemical pregnancies (positive pregnancy test without clinical symptoms of pregnancy later), (2) ectopic pregnancies, (3) miscarriages (including the so-called missed abortions or blighted ova), and (4) late fetal death (stillbirths) after the 28th gestational week [43].

There was no significant difference in the rate of different fetal demise types between the multivitamin and trace element supplemented groups of pregnant women. However, the total figure was higher in the multivitamin group ($\chi^2 = 4.82$, $P = 0.03$), explained mainly by the somewhat higher rate of miscarriage. This finding was in agreement with a lower sex ratio (i.e., proportion of boys) in the multivitamin-supplemented group of pregnant women compared to the well-known 51% male predominance in the trace element group. Thus, a small change in the pattern of prenatal selection cannot be excluded. This finding was confirmed in the United States [44] but not in China [45]. In our opinion the slightly higher rate of fetal death cannot be explained by terathanasia [46], that is, higher prenatal selection of male fetuses with NTDs, but—if it is not due to chance—by the higher proportion of multiple conceptions in the multivitamin group [47] because twin conceptions are associated with a mild increase of fetal death.

The evaluation of live births did not show a difference in gestational age at birth and birth weight or in the rate of preterm birth and low birth weight newborns in the groups of mothers with multivitamin or trace element supplementation in the periconception period [43]. However, it is

TABLE 5.1
Pregnancy Outcomes of Pregnant Women with Multivitamin and Placebo-Like Trace Element Supplementation in the Hungarian Randomized Controlled Trial

	Multivitamin (n = 2793)		Placebo-Like Trace Element (n = 2660)	
Pregnancy Outcomes	**No.**	**%**	**No.**	**%**
	Fetal Death			
Chemical pregnancy	55	2.0	40	1.5
Ectopic pregnancy	7	0.2	4	0.2
Miscarriage	301	10.8	251	9.4
Stillbirth (late fetal death)	11	0.4	9	0.3
Total	374	13.4	304	11.4
	Pregnancy Termination			
First trimester	6	0.2	6	0.2
Second trimester[a]	3	0.1	13	0.5
	Live Births			
Preterm birth	178	7.5	166	7.2
Low birth weight	101	4.3	81	3.5
Males	1181	49.9	1196	51.9
Quantitative Variables	Mean	SD	Mean	SD
Gestational age (weeks)	39.6	1.7	39.6	1.6
Birth weight (g)	3291	488	3288	478

Note: These comparisons did result in significant difference therefore P values were not mentioned.
[a] Due to elective termination pregnancy after the diagnosis of severe fetal defect.

worth mentioning that birth weight and gestational age have major progress in the second and third trimester of pregnancy.

5.6.4 Other Congenital Abnormalities

The major unexpected finding of the HRCT was a significant reduction in the total rate of informative offspring with CAs after periconceptional multivitamin supplementation [48]. The rate of CAs was 20.6 per 1000 informative offspring in the multivitamin and 40.6 per 1000 in the trace element group (RR = 0.53, 95% CI: 0.35–0.70). After the exclusion 6 NTD cases, the difference in the rate of CAs between the two study groups remained very highly significant ($P < 0.0001$). In conclusion, periconceptional multivitamin supplementation reduced not only the occurrence of NTDs but also the rate of some other major CAs, particularly congenital heart defects and CAs of the urinary tract [23,49,50].

Of 2471 children born to mothers with multivitamin supplementation, 10 (0.40%) had congenital heart defects, whereas of 2391 children who had mothers with trace element supplementation, 20 (0.83%) were affected with congenital heart defects. The difference was significant (RR with 95% CI: 0.42, 0.19–0.98), particularly in the two subgroups of ventricular septal defect and conotruncal defects (3 vs. 10, RR = 0.29, 95% CI: 0.09–0.97) [23,49,50].

There was a significant reduction in the CAs of the urinary tract (2/2471 vs. 9/2391, RR with 95% CI: 0.21, 0.05–0.95) [23,49,50]. There was some reduction in the prevalence at birth of congenital limb deficiencies (1/2471 vs. 5/2391, RR with 95% CI: 0.19, 0.03–1.18), but it did not reach the level of statistical significance [23,49,50].

On the other hand, the HRCT did not find reduction in two frequent types of isolated orofacial clefts (OFCs): cleft lip ± palate and cleft palate with birth prevalence 1.0/1000 and 0.5/1000 in Hungary. In 1982 Tolarova reported a protective effect of a multivitamin and folic acid (10 mg) during the periconception period against the recurrence of cleft lips [51]. However, the reduction of cleft lip ± palate and cleft palate was not confirmed after the use of a multivitamin containing a low dose (0.8 mg) of folic acid in the Hungarian RCT [23,49,50].

For ethical reasons, the HRCT could not be continued; thus the major objective the Hungarian Cohort Controlled Trial (CCT) was to confirm or reject the preventive effect of periconceptional folic acid-containing multivitamin supplementation for the reduction of congenital heart defects, urinary tract defects, and limb deficiencies [25]. The cohort of multivitamin supplemented pregnant women was recruited in the HPS compared to the cohort of matched unsupplemented pregnant women recruited at the prenatal care clinics.

The Hungarian CCT confirmed the significant reduction in the prevalence at birth of congenital heart defects. Of 3056 informative offspring, pairs were evaluated in the cohort of supplemented and unsupplemented matched mothers. The occurrence of congenital heart defects (31 vs. 50) was significantly reduced (OR with 95% CI: 0.60, 0.38–0.96), explained mainly by the lower occurrence of ventricular septal defect (5 vs. 19) (OR = 0.26, 95% CI: 0.09–0.72) in the supplemented cohort [25].

The rate of the urinary tract defects was not significantly reduced in the Hungarian CCT (14 vs. 19 cases; OR with 95% CI: 0.71, 0.33–1.50). However, the stenosis of the pelvic–ureteric junction (2 vs. 13) showed a significant difference (OR = 0.19, 95% CI: 0.04–0.86) within the group of obstructive CAs (10 vs. 19) [25]. Later Li et al. [52] and Werler et al. [53] also found a significant reduction in the rate of urinary tract CAs after multivitamin use in the first trimester of pregnancy.

Cases of limb deficiencies again showed a decreasing trend in the supplemented group compared to the unsupplemented group (1 vs. 3; OR with 95% CI: 0.33, 0.01–3.71) in the Hungarian CCT, but did not reach a level of significance owing to the small number of children [25]. One US study showed a significant reduction of congenital limb deficiencies after multivitamin supplementation [54], while two others found a reduction (RR = 0.50 and 0.64) but because of the too wide confidence interval, the differences were not significant [53,55].

The rate of children with isolated cleft lip ± palate and cleft palate also did not show any reduction in the CCT [25]. However, a significant reduction was seen in the prevalence at birth of these two types of OFCs in the data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities [56] after the use of a high dose (in general 6 mg) of folic acid alone. Only one type of folic acid tablets was available during the study period, and Hungarian obstetricians recommend in general two tablets, that is, 6 mg of folic acid for all women in the early phase of pregnancy after the first visit in the prenatal care. Thus a dose-dependent preventive effect of folic acid for OFC cannot be excluded. The observational studies resulted in controversial results, as the findings of Shaw et al. [57] and of Hayes et al. [58] showed.

In our opinion, the possible prevention of congenital heart defect (CHD) by folic acid-containing multivitamins would be extremely important because CHD represents the most frequent and severe group of CAs.

The population-based observational Atlanta studies [59–61] showed that the use of periconceptional folic acid-containing multivitamins reduced the risk of CHD by 24% (OR with 95% CI: 0.76, 0.60–97). When evaluating the association with specific types of CHD, these data also suggested that the association was strongest for ventricular septal defect and some conotruncal defects. Meta-analysis of both case-control studies (OR with 95% CI: 0.78, 0.67–0.92) and cohort or RCT (OR with 95% CI: 0.61, 0.40–0.92) confirmed the preventive effect of folic acid-containing multivitamins in the reduction of CHD [62].

Folic acid antagonist drugs, which inhibit dihydrofolate reductase that is required for DNA synthesis, increased the risk of CHD in the children of pregnant women [63]. However, the most important argument for the role of folic acid in the pathogenesis of CHD was that the risk of CHD after the use folic acid antagonists without concomitant use of multivitamins was 7.7 (95% CI: 2.8–21.7)

whereas this risk was only 1.5 (95% CI: 0.6–3.8) after the parallel use of folic acid antagonist drugs and multivitamins [63].

On the other hand, an association was also found between higher plasma homocysteine level due to *MTHFR* gene polymorphism and higher risk of CHD [64]. The recent meta-analysis of 29 studies showed that both infant and maternal *MTHFR* C677T polymorphisms may contribute to the risk of CHD [65].

Thus the available data support that folic acid or folic acid-containing multivitamins are essential for normal fetal cardiac development during early embryogenesis, and these supplements during the periconception period or in very early pregnancy reduce the risk for CHD.

In our opinion, it would be necessary to incorporate CHD in our public health action of NTD prevention with periconceptional folic acid-containing multivitamin or folic acid supplementation. We support this with four arguments:

1. The birth prevalence of CHD was estimated as 8 per 1000 in the United States [66], but a detailed personal examination of all newborn infants in a Hungarian cohort showed a rate of 10.2 per 1000 in Hungary [67]. The total (birth and fetal) prevalence of NTDs is 2.8 per 1000 in Hungary [68].
2. Available findings indicate about 40% efficacy of folic acid-containing multivitamins in the reduction of CHD, though obviously the efficacy of these dietary supplements in NTDs is much better.
3. However, we have to consider the absolute numbers. The efficacy of folic acid in the prevention of NTDs is about 70%; thus theoretically the Hungarian population-based rate of NTDs is reduced to 0.84 per 1000, and this reduction is equal to 195 cases per 100,000 births. However, it may be possible to reduce the birth prevalence rate of cases with CHD to 6.1 per 1000 on the basis of 40% efficacy of folic acid; thus the preventable absolute number of CHD cases is 408 for 100,000 births. Of course, these estimations depend on the population's baseline prevalence rates of CHD and NTDs.
4. CHD accounts for a third or more of infant deaths [69], though fortunately the life expectancy of patients with CHD increased significantly as a result of effective surgical management [70]. However, the cost of these medical interventions requires a very considerable financial investment by society.

In conclusion, there is no doubt that the majority of NTDs are preventable by folic acid-containing multivitamins, but we also have growing evidence for the prevention of a certain proportion of CHD, the most common CA group, by folic acid-containing multivitamins.

5.6 5 Postnatal Development

Children born to mothers with multivitamin and trace element supplementation in the HRCT were followed after birth. In the first study 1876 and 1837 children born to a mother with multivitamin or trace element supplementation were examined between postnatal 8th and 12th months, respectively [71]. There was no difference in mortality and the occurrence of serious or chronic diseases with one exception. Atopic dermatitis was diagnosed in 15 children born to mothers with multivitamin supplementation whereas this figure was 4 in the trace element group ($P = 0.012$). The multivitamin-supplemented mothers also had a higher number of infants with asthma (4 vs. 1) and wheezy bronchitis (7 vs. 3). If these three disorders, which are closely related, are combined, the difference (26 vs. 8) was highly significant ($P = 0.0022$). Postnatal somatic (body weight, body length, head circumference) and mental (measured by three tests) development did not show any significant difference between the multivitamin and trace element groups.

In the second study 6-year-old children were examined [72]: 147 children born to mothers with multivitamin supplementation and 142 children who had mothers with trace element

supplementation. There were no difference in the rate and distribution of disorders including allergies; thus the previously found higher incidence of atopic dermatitis, asthma, and wheezy bronchitis (45; 30.6% vs. 48; 33.8%, $P = 0.56$) was not found. There was only one pathological condition, otitis media, which occurred more frequently in the children of mothers with multivitamin supplementation (32; 18.2%) than in the children of mothers with trace element supplementation (19; 11.9%) ($P = 0.05$). The detailed ophthalmological and audiological examination did not find a difference between the two study groups. In addition, there was no difference in their anthropometric development. Six-year-old children born to mothers with multivitamin supplementation had somewhat but not significantly higher intelligence quotient (IQ; 103.0 ± 17.5) than children whose mothers had trace element supplementation (100.7 ± 20.3). The proportion of children with IQ 115 or higher was 49 (33.3%) in the multivitamin and 39 (27.5%) in the trace element group.

Thus, the previously found higher rate of worrying, fussiness, and fearfulness in girls born to mothers who previously had NTD-affected infants and were supplemented in the next pregnancy with a multivitamin during the periconception period [73] was not confirmed. However, it is necessary to stress that our pregnant women used a folic acid-containing multivitamin until the 12th gestational week, and the main part of brain development is in the second, but mainly in the third trimester of pregnancy. Mental development of children born to mothers with folic acid or multivitamin supplementation until the end of pregnancy therefore would be worth studying.

Recent studies have suggested that the use of prenatal folic acid supplements around the time of conception was associated with a lower risk of autistic disorder [74]. In addition, the role of genetic polymorphism of folate metabolism and vitamin B_6 was shown in the etiology of idiopathic intellectual disability, that is, deficiency as a risk factor and supplementation as a preventive effect [75].

The use of folic acid or folic acid-containing multivitamins in pregnant women was associated with a lower risk of childhood tumors [76], particularly neuroblastoma [77,78], primitive neuroectodermal brain tumors [79], astrocytic glioma [80], and acute lymphoblastic leukemia [81]. The carcinogenic effect of folate deficiency is known but recent studies suggested a similar carcinogenic effect of high doses of folic acid.

However, the effect of folic acid supplementation on overall and site-specific cancer incidence during the randomized trials on the basis of meta-analysis of data on 50,000 individuals was not confirmed [82].

5.7 SOME DEBATED QUESTIONS

5.7.1 Is the Consumption of Folate- and Other Vitamin-Rich Diets Enough?

As mentioned previously, humans cannot produce folate; the major dietary sources of folates are fresh and frozen green leafy vegetables, citrus fruits and juices, liver, wheat bread, and legumes such as beans. Thus the requirement for this water-soluble vitamin is supplied by dietary intake of folates. The preconception period is an appropriate time to change the dietary habit and to improve the lifestyle of prospective parents, particularly good compliance among mothers, as they want to do their best to have a healthy baby. Thus it is important to advise all women to have a folate- and other vitamin-rich diet from the preconception period onwards.

However, the usual daily intake of folate is about 0.20 mg/day in European countries [18], and the necessary folate/folic acid consumption is estimated as 0.66–0.80 mg [83–85]; thus it is difficult to imagine about a 3.5-fold increase in folate intake every day in anticipation of conception, which would require the consumption of 500 g of raw spinach, 900 g of boiled spinach, or 900 g of raw broccoli [86], that is, about 15 servings of broccoli each day. Furthermore, a part of dietary folate is lost through cooking and processing. Finally, an extreme increase in the consumption of extra folate from natural food is relatively ineffective at increasing folate status in humans [87].

In conclusion, a diet rich in folate is important in general and for the prevention of NTDs and CHD, but folate alone cannot completely neutralize the genetic predisposition for these CAs.

5.7.2 Is a Multivitamin or Folic Acid Better?

A dilemma is the choice between folic acid and folic acid-containing multivitamins [88]. The use of multivitamins containing folic acid and other B vitamins in the Hungarian RCT [21–24] and CCT [25] and in the study of Smithells' team [7–9] showed a higher efficacy (about 90%) in the reduction of NTDs than did the MRC Vitamin Study [10] using a high dose (4.0 mg) of folic acid alone (71%) and Chinese–US study [27] using a low dose (0.4 mg) of folic acid (41–79%). The usual argument against the use of other vitamins is that the supplementation with "other vitamins" in the MRC Vitamin Study [10] did not result in a significant reduction in recurrent NTDs. However, it is worth mentioning that there was a 40% reduction (2.6% vs. 4.3%) in the recurrent NTDs near the level of significance after supplementation with other vitamins.

Another argument for the use of folic acid-containing multivitamins is that this supplementation may be effective for the reduction of CHD as well, but there are very limited data concerning a similar preventive effect of folic acid alone [89,90].

Finally, hyperhomocysteinemia plays a role in the origin of at least some part of NTDs and CHD. Obviously folate/folic acid is a key factor in homocysteine metabolism, but vitamins B_{12}, B_2, and B_6 also have a role in the "detoxication" of homocysteine. The combined effect of these four vitamins in multivitamins may explain its higher efficacy compared to the effect of folic acid alone in the reduction of NTDs and CHD. The terms cobalamin (vitamin B_{12}), pyridoxine (vitamin B_6), and riboflavin (vitamin B_2) are not well known among pregnant women; therefore we use the term "fetal protective vitamins" in the HPS [12].

In conclusion, folic acid-containing multivitamins seem to be more effective in the primary prevention of NTDs and CHD, though obviously the use of folic acid alone is simpler and less expensive.

5.7.3 What Is the Optimal Dose of Folic Acid?

To tell the truth, we do not know. Based on the HRCT and some observational studies, the CDC in 1992 recommended that "all women of childbearing age who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or other NTD" [91], and this recommendation was subsequently followed in several countries. However, at the time of this recommendation there was no scientific evidence for the efficacy of 0.4 mg of folic acid. The efficacy of this dose was confirmed only in 1999 by the Chinese–US trial [27]. McPartlin et al. [82] estimated that the optimal daily intake of folate/folic acid in the periconception period is about 0.66 mg per day for the prevention of NTDs. Daly et al. [83] demonstrated that the lowest risk of having a child with NTD was related to a red blood cell folate concentration of 906 nmol/L or more. However, practically 8–12 weeks are needed to reach this level after the previously recommended 0.4 mg of folic acid supplementation. The use of 0.8 mg of folic acid resulted in the necessary level red blood cell folate concentrations at 4.2 ± 3.5 weeks [85].

Thus, the intake of folate/folic acid recommended for a woman of childbearing age who is sexually active is 0.70 mg daily [92]. In our opinion, 1.0 mg of folate/folic acid daily seems to be optimal for all pre-pregnant and pregnant women, via the consumption of 0.2–0.3 mg of folate through diet and supplementation with 0.7–0.8 mg folic acid [93] 2–3 months before until 3 months after conception to reduce most occurrences of NTDs and a certain proportion of CHD.

5.8 CONCLUSIONS

The results of the Hungarian Randomized Control Trial, supported by subsequent trial results, have proven that a periconception multivitamin supplement containing 0.8 mg of folate has the ability to significantly reduce primary NTDs and congenital cardiac and urological abnormalities, while

possibly also reducing limb reduction defects. Periconceptional multivitamins may also improve childhood intellectual development, while reducing the rate of autism and childhood cancers (brain, leukemia). Current evidence strongly advocates that all women contemplating pregnancy should take periconceptional multivitamins for at least 1 month before conception and 2 months into the pregnancy.

5.9 KEY POINTS SUMMARY

1. A possible link between maternal nutrition and the development of fetal congenital abnormalities was first suggested by Hibbard [3] when he observed a doubling in the risk of neural tube defects (NTDs) in the infants of folate-deficient mothers. This environmental predisposition to congenital abnormalities was also supported by the observation of significantly increased risk of NTDs in infants born to women of low socioeconomic background, a group often characterized by suboptimal nutrition.
2. Early non-randomized studies suggested that low-dose folate supplements (0.36 mg/day) could significantly reduce the risk of developing NTDs in infants born to mothers who previously had given birth to an infant with an NTD (secondary prevention). A follow-up secondary prevention randomized control study (MRC RCT) confirmed that a daily dose of 4 mg of folate was capable of reducing the rate of reoccurrence of NTDs by 71%. Interestingly, the MRC trial also reported a 40% reduction in NTDs with the use of a non-folate-containing multivitamin supplement. Although this result did not quite reach statistical significance, it did suggest the possibility that vitamins other than folate may help prevent NTDs.
3. After the publication of the MRC RCT in 1991, it became standard practice to use pharmacological dosages of folate (4 mg and above) to prevent secondary NTDs. However, as 95% of women who conceive a child with an NTD have no history of a previous NTD-affected pregnancy, the ability of periconceptional folate supplementation to prevent primary cases of NTDs was of greater public health importance. This question was addressed by the Hungarian Randomized Controlled Trial (HRCT), the largest RCT ever conducted in the field of reproductive nutrition.
4. The HRCT enrolled more than 5000 women contemplating pregnancy in an RCT comparing reproductive outcomes in women administered a multivitamin supplement containing 0.8 mg of folate, 11 other vitamins (B_1, B_2, B_5, B_6, B_7, B_{12} and vitamins C, D, and E), minerals (Ca, P, Mg, Fe), and three trace elements (Cu, Mn, Zn) or just trace elements alone (the "placebo" comparator). Women were asked to take these supplements for at least 1 month before conception and for at least 2 months after conception, covering the major organogenesis period of pregnancy. The main findings of this pivotal study were as follows:
   a. The folate-containing multivitamin supplement produced a 90% reduction in first occurrences of NTDs.
   b. The overall rate of congenital abnormalities in the folate-containing multivitamin–supplemented group declined by 50% compared to those mothers taking a trace element supplement (20.6 vs. 40.6 congenital abnormalities per 1000 children, $P < 0.0001$). Although some of this difference was attributed to differences in NTD incidence, there was still a significant reduction in non-NTD-related congenital abnormalities in the folate-containing multivitamin group. Specifically, congenital heart defect (primarily ventricular septal defects) and urinary tract abnormalities were significantly reduced. Furthermore, there was a trend in reduction in congenital limb defects in the folate-containing multivitamin supplemented arm of the HRCT, a finding that did reach statistical significance in a later follow-up US study.

   c. Women allocated to the folate-containing multivitamin had a statistically higher chance of conceiving in the next 12 months (7%), a higher risk of twins (41% higher), and a more regular menstrual cycle than women allocated to the trace element arm of the study. These findings suggest that a folate-containing multivitamin may affect ovulation.
   d. Nausea and vomiting ("morning sickness") was significantly less common in women receiving the folate-containing multivitamin, but adverse side effects such as gastrointestinal upset (constipation, diarrhea) and rash were also slightly more common.
   e. Follow-up studies since the publication of the HRCT have confirmed the ability of a folate-containing multivitamin to reduce the incidence of NTDs, congenital heart defect, and limb defects, with a possible though not yet conclusively proven reduction in orofacial clefts, autism, and intellectual disability plus childhood malignancy (brain and leukemia).
5. The biological mechanisms behind the ability of folate and B group vitamins to reduce NTDs and congenital heart defects such as ventricular septal defects are likely to be centered on the role of these vitamins in DNA and homocysteine metabolism. Folate and vitamins B_2 (riboflavin), B_6 (pyridoxine), and B_{12} (cobalamin) all are involved in recycling homocysteine to produce methyl groups for DNA methylation. An excess of homocysteine, and a resulting impairment in DNA methylation, may result in epigenetic changes that interfere with normal neural tube closure and cardiac development. All of these B group vitamins are capable of enhancing the reprocessing of homocysteine and generation of methyl groups for DNA methylation. This fact, together with the findings of the HRCT and the observed trend in reduction of NTDs in the multivitamin only (no folate) arm of the MRC study, suggest that a combination of folate and multivitamins such as B_2, B_6, and B_{12} is superior in preventing NTDs and cardiac anomalies than folate alone.
6. Although the US Centers for Disease and Prevention has suggested a dose of 0.4 mg of folate per day as being sufficient to prevent NTDs, there is evidence that a more optimal dosage of folate supplementation may be 0.8 mg per day based on the following:
   a. Studies suggest that the lowest risk of NTDs occurs when daily intake of folate is sufficient to produce a red blood cell folate concentration >900 nmol/L. Although the average woman consumes only 0.20 mg of folate per day in her diet, it is estimated that it takes as long as 8–12 weeks of supplementation with 0.4 mg of folate per day to achieve these protective levels, yet only 4 weeks to achieve this protective effect at a higher dose of 0.8 mg per day. Most women are unlikely to be willing to wait 3 months before attempting conception, placing them at elevated risk of having a child with a NTD if the lower 0.4 mg daily supplement is used.
   b. The HRCT using 0.8 mg of folate in combination with multivitamins produced a superior rate of reduction in NTDs than that reported in a similar Chinese primary prevention RCT of 0.4 mg of folate.
   c. Although public health directives to fortify wheat-based products with folate have helped reduce NTDs, the use of targeted periconceptional folate supplements results in higher body folate levels than can be practically achieved with food fortification or the voluntary ingestion of high-folate foods (green leafy vegetables). As such, a supplement with 0.8 mg of folate per day is advocated.
7. A periconception multivitamin supplement containing 0.8 mg of folic acid, together with other vitamins and minerals, taken for at least 1 month before conception and for at least the first 2 months of pregnancy, is effective in reducing the incidence of primary NTDs and congenital cardiac and urological abnormalities. Although not yet conclusively established, there is some supportive evidence that this type of supplement may also reduce congenital limb defects, childhood cancers, and "morning sickness" in pregnancy.

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6 Maternal Nutrition and Its Influence on the Health of the Next Generation

The "Developmental Origins Hypothesis"

Hitomi Okubo, Siân M. Robinson, and Keith M. Godfrey

CONTENTS

6.1 INTRODUCTION

The importance of maternal nutrition during pregnancy and lactation in determining short-term health of offspring (e.g., lower risk of neonatal mortality and morbidity) has been well established. Over the past two decades epidemiological studies have demonstrated that small size at birth and during infancy is associated with an increased risk of developing coronary heart disease [1], type 2 diabetes [2], metabolic syndrome [3], and osteoporosis [4] in later life. It is now recognized that overnutrition during early development also influences susceptibility to develop obesity and type 2

diabetes. These associations have led to the "developmental origins of health and disease" hypothesis [5], and provided new evidence of the importance of maternal nutrition for optimal growth and development for lifelong health of the offspring.

The relationships between early life nutrition and disease risk in later life observed in epidemiological studies have been supported by experimental studies using a variety of animal models. These have shown that manipulation of nutrition in the period extending from conception to infancy can result in permanent changes in body structure, function, and metabolism in the offspring. The associations may reflect "developmental plastic responses," whereby the developing fetus adapts to nutritional and hormonal signals from the mother *in utero* and adjusts its developmental trajectory to produce a phenotype that is matched to the predicted postnatal environment [6]. When there is a mismatch between the anticipated and the actual environment experienced in later life, disease risk increases [7,8]. The developmental responses include epigenetic processes that play a central role in regulating tissue-specific gene expression; alterations in these processes can induce persisting changes in gene expression and metabolism in the offspring throughout his or her life [9]. Furthermore, such long-term consequences of adverse nutritional conditions during early development may not be limited to one generation, but may lead to poor health in the succeeding generations [10,11]. Adopting a life course approach, which takes into account intergenerational effects, is therefore needed to understand the influence of maternal nutrition on an offspring's disease risk in later life. In addition, a better understanding of the mechanisms that link early life nutritional influences with long-term effects will enable more rapid development of interventions to prevent chronic disease in the future.

In this chapter, we provide an overview of research into the influence of maternal nutrition on the health of future generations, consider evolutionary and developmental aspects, and discuss the potential mechanism of epigenetic regulation linking maternal nutrition with the offspring's later metabolic phenotype.

6.2 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

6.2.1 Origins of the Hypothesis: Links between Nutrition in Early Life and Lifelong Health

The "fetal," or subsequently "developmental," origins of adult disease hypothesis, originally championed by researchers in Southampton, United Kingdom, stated that environmental factors, particularly variations in nutrition in early life, can have permanent effects on physiology and function that determine risks of cardiovascular and metabolic disease in later life [12]. Before this hypothesis was articulated, some associations between early life events and later disease risk had been described. In 1934, Kermack et al. demonstrated that death rates from all causes in the United Kingdom and Sweden fell between 1751 and 1930, and they concluded that this was the result of better childhood living conditions during this period [13]. In the early 1970s, Dörner and his group proposed that the conditions before and soon after birth were related to later risks of arteriosclerosis and obesity, and that gestational diabetes presented a risk for subsequent diabetes mellitus [14–17]. Dörner's work was recognized in East German public health measures to support longer maternity leave and promote breastfeeding [18]. In 1977, Forsdahl found a geographical correlation within Norway between coronary heart disease (CHD) mortality in 1964–1967 and infant mortality rates some 70 years earlier (1896–1925). Forsdahl suggested that poverty may act through a nutritional deficit to result in a lifelong vulnerability to a more affluent adult lifestyle [19]. Finally, in 1985, Wadsworth et al. reported in a study of the 36-year-old members of a UK national birth cohort that birth weight was related to blood pressure [20], and in the same year, a Finnish study linked poverty in childhood with increased risk of ischemic heart disease, myocardial infarction, and coronary death [21].

However, the studies by Barker and Osmond in the United Kingdom were key in the development of our understanding of early life effects on adult disease, and shifted the focus back to prenatal rather than childhood events. In 1986, Barker and Osmond noted that the geographical distribution

of mortality rates from CHD and stroke in 1968–1978 was closely related to rates of infant mortality in 1921–1925 [22,23]. In these studies they were able to subdivide deaths occurring in the first year of life into those occurring in the first month (neonatal) and those occurring in the rest of infancy (post-neonatal) [23] (Table 6.1). Stroke mortality was linked to neonatal mortality but not to post-neonatal mortality, whereas mortality from CHD related to both neonatal and post-neonatal mortality rates. These data suggested that the origins of cardiovascular disease might be earlier in the life course than childhood—and that events in fetal life were important in the etiology of adult cardiovascular disease. In their subsequent study, the discovery of birth records dating from 1911–1948 in the county of Hertfordshire made it possible to show the first direct evidence that lower birth weight and weight at 1 year were associated with an increased risk of death from CHD and stroke [24]. The associations were linear and graded across the range of birth weight, with no evidence that they were limited only to low birth weight individuals. It has since been shown that lower birth weight is also associated with a higher rate of adult hypertension [25], type 2 diabetes [2,26], metabolic syndrome [27], chronic obstructive pulmonary disease [28], and osteoporosis [29].

Since then, these associations have been reproduced in a range of studies across the world. They cannot be explained by variations in gestational age at birth, or by differences in adult lifestyle [30]. These findings have also been supported by experimental data in a variety of animal models [31]. The evidence from both human and experimental studies accumulated over the past two decades has now led to a new branch of scientific research: Developmental Origins of Health and Disease (DOHaD) [5].

TABLE 6.1
Death Rates from Stroke, Ischemic Heart Disease, and Chronic Bronchitis (SMRs, Ages 35–74, Both Sexes, 1968–1978) in the 212 Areas of England and Wales Grouped by Neonatal and Post-Neonatal Mortality (1911–1925)

		Post-Neonatal Mortality					
		(Low)	1	2	3	4	5 (High)
			Stroke				
Neonatal mortality	1 (Low)		85	81	79	78	79
	2		86	90	98	74	76
	3		102	100	104	104	104
	4		—	108	110	115	117
	5 (High)		124	—	121	123	117
			Coronary Heart Disease				
Neonatal mortality	1 (Low)		84	89	91	88	98
	2		85	93	95	88	91
	3		86	94	99	106	113
	4		—	98	109	111	115
	5 (High)		83	—	114	119	116
			Chronic Bronchitis				
Neonatal mortality	1 (Low)		67	78	106	115	161
	2		64	84	85	104	126
	3		69	65	89	88	151
	4		—	91	99	120	142
	5 (High)		41	—	108	123	144

Source: Reproduced from Barker DJ, Osmond C, Law CM. *J Epidemiol Community Health*., 43:237–240, 1989.

6.2.2 Developmental Programming

The work of Barker and colleagues led to the hypothesis that the roots of cardiovascular disease and other chronic diseases lay partly in the effects of poor diets of mothers that resulted in undernutrition of their children during critical periods of development in fetal and early postnatal life [32]. In 1992, Hales and Barker provided a mechanistic explanation of relationship between poor fetal and infant growth and the subsequent development of type 2 diabetes and metabolic syndrome by proposing the "thrifty phenotype" hypothesis [2], which was placed in contradistinction to the prior "thrifty genotype" hypothesis [33]. Neel had argued that a predisposition to diabetes might result from "thrifty genes" that were selected for during evolution to promote fat storage during periods of famine, to maximize possibility of survival; such populations with "thrifty genes" are now at risk in a world of abundant nutrition [34]. On the contrary, the "thrifty phenotype" hypothesis by Hales and Barker proposed a very different etiological model of adult insulin resistance and type 2 diabetes, suggesting that maladaptive responses occurred as a result of alterations in physiology induced by undernutrition *in utero* [2,35]. The hypothesis suggests that the nutritionally deprived fetus facing threats to its survival becomes "thrifty" and adapts to an inadequate intrauterine nutritional environment in several ways: restriction of its growth, prioritization of brain growth at the expense of other organs (e.g., muscle, pancreas, and liver) and altered secretion and sensitivity to the fetal growth hormones (such as insulin and insulin-like growth factor [IGF]-I). Developing organs and physiological systems may be permanently changed in response to the reduced availability of nutrients [2,35]. These acute adaptations, although advantageous for short-term survival, may be detrimental for health in later life if nutrition is more abundant in the postnatal environment than in the prenatal environment. The concept of the "thrifty phenotype," based on embryonic and fetal adaptive responses to a suboptimal intrauterine nutritional environment, which have permanent adverse consequences, is consistent with the biological phenomenon known as "programming," championed by Lucas in 1991. This was defined as a process whereby a stimulus or insult acting at a "critical" or "sensitive" period of development results in permanent change in the structure and function of the organism [36].

Central to the concept of developmental programming was the proposal that variations in the nutritional environment were responsible for permanent changes in physiology and function. The growth of a fetus and its functional characteristics are certainly influenced by its genes, but there is strong evidence that the intrauterine environment has a greater effect on fetal growth and may therefore underlie later chronic disease risk. According to the classic studies that analyzed the birth weight of relatives, Penrose concluded that 62% of the variation in birth weight could be attributed to the intrauterine environment, 20% to maternal genes, and 18% to fetal genes [37]. In broad support of this thesis, the study of babies born after ovum donation showed that although their birth weights were strongly related to the weight of the recipient mother, they were unrelated to the weight of the woman who donated the egg [38]. Consistent with this proposition, there is now a growing body of evidence from animal models that genome regulation can be modified by differences in the nutritional environment that determine nutrient availability to the offspring during prenatal and neonatal periods [39–41]. It has been now recognized that ontogenetic development involves "developmental plasticity," which implies that a single genotype can result in a range of phenotypes (e.g., different form or structure, physiological state, or behavior) in response to environmental conditions during development [6]. Although the term "programming" has been used extensively, it implies a predetermined trajectory of development insulated from any subsequent epigenetic or environmental input [42]. Therefore, it has been argued that environmental induction of "developmental plasticity" would be more appropriate than the term "programming" [43].

6.2.3 Mismatch and Chronic Disease

Developmental plasticity attempts to "tune" gene expression to produce a phenotype best suited to the predicted later environment [8]. When the resulting phenotype is matched to its environment,

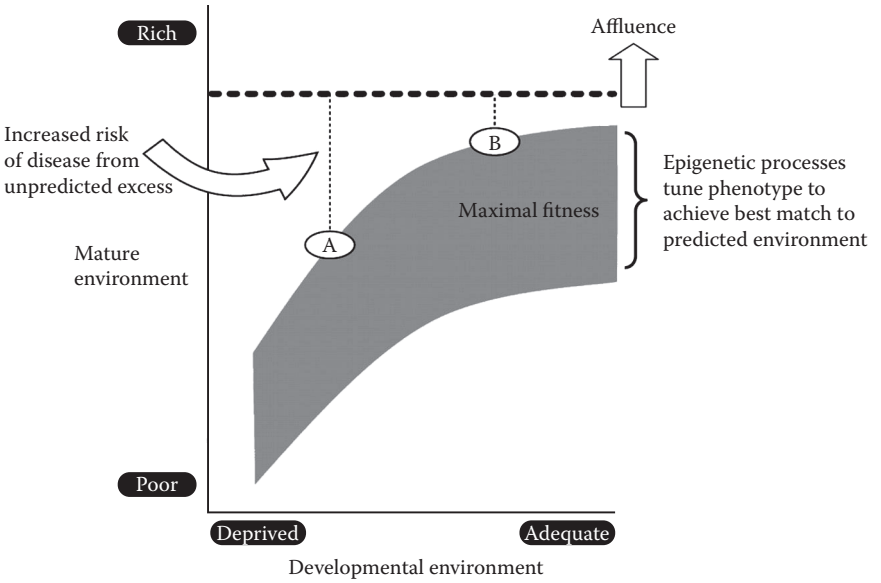

FIGURE 6.1 The mismatch concept emphasizes that the degree of disparity between the environment experienced during development and that experienced later influences the risk of disease. (Derived from Godfrey KM et al. *Pediatr Res*., 61:5R–10R, 2007.)

the organism will remain healthy. When there is a mismatch, the individual's ability to respond to environmental challenges may be inadequate and risk of disease increases. Thus, the degree of the mismatch determines the individual's susceptibility to chronic disease [7].

The degree of mismatch can, by definition, be increased by either poorer environmental conditions during development or richer conditions later, or both [7]. For example, an unbalanced maternal diet, body composition, or disease can perturb the former; the rapid increase in energy-dense foods and reduced physical activity levels associated with a Western lifestyle will increase the degree of mismatch via the latter (Figure 6.1). Such changes are of considerable importance in developing societies going through rapid socioeconomic transitions.

6.3 THE INFLUENCE OF MATERNAL NUTRITION ON HEALTH OF THE NEXT GENERATION

The supply of nutrients to the fetus is a major influence that regulates its growth. The fetus is entirely dependent on its mother; its nutrient supply depends on her diet, body composition and size, her nutrient stores, and transport of nutrients to the placenta and transfer across it [44]. Sections 6.3.1–6.3.4 describe what is known about the effects of maternal nutrition, including body composition and diet, on health of offspring by providing evidence from animal models and human studies.

6.3.1 Maternal Nutrition before Pregnancy

Animal models have provided evidence of causal links between maternal nutrition before pregnancy and cardiometabolic risk factors in the offspring. For example, maternal undernutrition during the period of conception and implantation has been shown to cause hypertension and cardiovascular dysfunction in the offspring [45–48]. Emerging data from animal studies suggest that overnutrition may also be important, with long-term effects on offspring body composition [49]. In comparison, little is known about maternal influences, acting before pregnancy, on offspring health in humans.

The importance of periconceptional nutrition has been demonstrated by the reduction in neural tube defects with preconception folate supplementation [50]. A recent systematic review of observational studies concluded that preconceptional and periconceptional intake of vitamin and mineral supplements is associated with reduced risks of preterm deliveries and of offspring with low birth weight and/or are small-for-gestational age (SGA) [51].

Maternal pre-pregnancy weight and body mass index (BMI) have been used as markers of maternal nutritional status before conception and related to cardiovascular disease risk in the offspring. One study found an association between maternal obesity before pregnancy and the risk of metabolic syndrome in children aged 6–11 years [52]. Using the modified criteria of the National Cholesterol Education Program, children who were born to obese mothers (pre-pregnancy BMI > 27.3 kg/m^2) had a 2-fold increase in risk of developing the metabolic syndrome (hazard ratio [HR] = 1.81, 95% confidence interval [CI]: 1.03, 3.19, $P = 0.039$) compared with the offspring of mothers whose weight was in the recommended range. One of the most consistent patterns of association is of high maternal weight before pregnancy, with a greater risk of adiposity in the offspring and with higher levels of a range of cardiometabolic risk factors [53–55].

6.3.2 Maternal Undernutrition and Poor Diet Quality during Pregnancy

In animal models, dietary manipulation in pregnancy can result in small offspring that display shortened life span [56,57], obesity [58,59], hypertension [60,61], diabetes [61], and alteration in the hypothalamic–pituitary–adrenal (HPA) axis [45,62]. One of the best-studied and most characterized animal models of nutritional induction of an altered metabolic phenotype is feeding rats a protein-restricted (PR) diet from conception throughout pregnancy [31,49]. Offspring from PR dams show a number of features of human cardiometabolic disease including hypertension [63], increased fat deposition and altered feeding behavior [64,65], impaired glucose homeostasis, dyslipidemia [66], vascular dysfunction [67], impaired immunity [68], and increased susceptibility to oxidative stress [69]. The phenotype of the offspring, however, does vary according to the exact composition of diet [40]. This indicates that even small variations in maternal diet can affect the risk of disease in later life [49].

In humans, there are fewer observational studies in which variations in maternal nutrition can be definitively linked to the later health of the offspring. Important findings regarding the role of maternal diet during pregnancy on subsequent disease susceptibility have come from follow-up studies of children who were conceived and born during periods of famine. One of the most well-known of these is the Dutch Hunger Winter, when a period of extreme food shortage in the western Netherlands occurred during the last 5–6 months of World War II (November 1944–May 1945) [70,71]. The mean caloric rations during the famine were as low as 400–800 kcal/day. In a follow-up of 2414 men and women born around the time of the famine, the influence of maternal undernutrition during pregnancy on long-term health effects of offspring have been gradually elucidated.

One of the important observations from the Dutch Hunger Winter Study is that intrauterine exposures that have long-lasting consequences for adult health do not necessarily result in altered birth weight. Women exposed to the famine during mid- to late pregnancy had babies with significantly reduced birth weight. However, babies born to mothers who were exposed only during early gestation were of normal birth weight, but they grew up to have higher rates of obesity than those born before and after the war, and higher than those exposed during mid- to late pregnancy [71]. This finding suggests that although reduced birth weight is the most easily measured proxy for intrauterine undernutrition, it is not necessarily a good indicator of prenatal exposures that lead to adult disease.

A related observation from the Dutch Hunger Winter Study has been the importance of timing in the programming of adult disease. Compared to findings in those not exposed to the famine, exposure during early gestation was associated with a preference for fatty foods, a more atherogenic

lipid profile, increased risk for coronary heart disease, disturbed blood coagulation, obesity (women only), increased stress responsiveness, and increased risk of breast cancer (women only). In comparison, exposure to famine in mid-gestation was associated with obstructive airways disease and microalbuminuria [70,71]. These studies provide valuable insights into the importance of the timing of nutritional constraints during pregnancy in determining the future risk of disease.

The analyses from the study of the Chinese Famine (1959–1961) have provided further evidence of the long-term consequences of famine exposure during early life. In contrast to the Dutch famine (which was a period of acute starvation of a well-nourished population) [70,71], the Chinese famine was a period of chronic starvation principally affecting an impoverished rural population. In comparison with non-exposed subjects, men and women who had been exposed to the famine during fetal life had a markedly higher risk of developing metabolic syndrome in adulthood (odds ratio [OR] = 3.13, 95% CI: 1.24, 7.89) [72]. Further research revealed that in severely affected famine areas, adults exposed to famine *in utero* had significantly higher systolic blood pressure (Δ = 2.2 mm Hg, 95% CI: 1.3–3.0), diastolic blood pressure (Δ = 0.9 mm Hg, 95% CI: 0.3–1.5) and a marginally higher risk of hypertension (OR = 1.88, 95% CI: 1.00–3.53) when compared to those who were not exposed to famine [73]. Increased risk of hyperglycemia was observed in famine-exposed subjects in severely affected areas compared with non-exposed subjects (OR = 3.92, 95% CI: 1.64–9.39). One of the important findings from the studies of the Chinese Famine was that these associations were more pronounced if the adults followed a Western dietary pattern, characterized by high consumption of meat, eggs, dairy, sugary beverages, and edible oils and low intake of vegetables, or were overweight (BMI ≥24 kg/m^2) in later life [72–74]. This finding is consistent with the hypothesis that it is the "mismatch" between the early and later nutritional environments that is particularly important in determining the risk of metabolic disease in later life [42,75].

Evidence suggesting that maternal diet quality during pregnancy has long-term implications for the offspring is now starting to emerge. Follow-up studies of mother–offspring cohorts have, for example, shown that a poor quality "imprudent" maternal dietary pattern during pregnancy is associated with lower childhood bone mineral content at age 9 years [76]. Moreover, an unbalanced high-protein, low-carbohydrate diet during pregnancy has been linked with raised adult blood pressure [77] and heightened adult stress responses in the adult offspring [78].

6.3.3 Maternal Vitamin D Insufficiency

In many populations vitamin D insufficiency is highly prevalent during pregnancy, and there is now increasing evidence that this can have long-term effects on bone health and body composition in the offspring. A recent systematic review identified eight observational studies that had examined maternal vitamin D status in relation to offspring bone mass; although no significant associations were found in three studies, five demonstrated a significant positive relationship between maternal vitamin D status and offspring bone outcomes (which included whole body; lumbar, femoral, and tibial bone mineral content [BMC]; and whole body and lumbar spine bone mineral density [BMD]) [79]. Review of observational studies has indicated a possible association between maternal vitamin D repletion and a reduced incidence of preeclampsia, gestational diabetes, and primary cesarean section [79], and some support for this has come from a randomized controlled trial of vitamin supplementation in pregnancy [80]. There is also evidence that lower vitamin D status in pregnancy may have important effects on offspring adiposity; in a prospective cohort study, lower maternal 25-hydroxyvitamin D (25OHD) status in late pregnancy was associated with lower fat mass assessed by dual-energy x-ray absorptiometry (DXA) at birth but with higher fat mass assessed at age 6 years [81]. In contrast, no consistent associations have been found between low late pregnancy serum 25OHD levels and offspring allergic and respiratory outcomes [82]. Definitive evidence on the role of maternal vitamin D status in relation to offspring health must await a number of randomized controlled trials that are currently in progress.

6.3.4 Maternal Overnutrition during Pregnancy

The "thrifty phenotype" hypothesis proposed by Hales and Barker is a widely accepted hypothesis to explain the adverse effects of inadequate nutrition in early life on adult disease risk [2,35]. Although epidemiological research initially focused on lower birth weight as a proxy for developmental programming effects, at the highest birth weight the risk of cardiovascular disease also increases, resulting in a U- or J-shaped relationship [83]. There is now growing awareness that overnutrition, as well as undernutrition, in early life may result in an offspring phenotype that is more susceptible to the cardiometabolic disease.

Maternal gestational weight gain (GWG) has received attention as a marker of the nutritional status of the mother during pregnancy. Maternal GWG is positively associated with offspring body mass index in childhood [84–88], adolescence [89], and adulthood [90]. Recent studies have shown that exceeding the recommended GWG range, as defined by the US Institute of Medicine (IOM) in 2009, has an adverse impact on the risk of childhood overweight and adiposity [88,91]. However, suboptimal GWG has also been shown to be linked to greater offspring adiposity [92]. According to data from mother–offspring pairs from a UK prospective cohort study, women who gained more weight than recommended (Institute of Medicine) during pregnancy had offspring who were more likely to have greater adiposity, higher levels of systolic blood pressure, C-reactive protein, and interleukin-6 levels and lower high density lipoprotein (HDL)-cholesterol and apolipoprotein A1 levels at 9 years of age [53]. GWG from mid-pregnancy (14–36 weeks) was positively and linearly associated with adverse lipid and inflammatory profiles in offspring, largely because of the association of GWG with offspring adiposity [53]. A further study of 12,775 children (aged 2–9 years) from eight European countries [87] also showed that indices of glucose metabolism, namely blood glucose, serum insulin, and homeostatic model assessment of insulin resistance (HOMA-IR) index, and systolic blood pressure increased significantly across tertiles of GWG, but these differences were no longer evident after adjustment for current child BMI. These studies suggest that higher maternal GWG (especially excessive weight gain) is likely to influence offspring metabolic status in childhood, mainly through its effects on adiposity.

Gestational diabetes mellitus is now an established risk factor for later diabetes in the offspring. GDM and maternal gestational hyperglycemia are associated with oversupply of glucose and other nutrients to the fetus. Diabetic mothers are not only hyperglycemic, but also have elevated circulating lipids and amino acids. The fetal pancreas and liver are stimulated to secrete increased insulin and IGFs, both of which are growth-promoting hormones in the fetus, predisposing to macrosomia. Freinkel suggested that this could cause obesity and diabetes in later life ("fuel-mediated teratogenesis") [93]. Consistent with his suggestion, children of GDM mothers are more likely to be overweight or obese, and tend to have greater central adiposity [52,94–97]. Reduced insulin sensitivity and/or impaired glucose tolerance, high blood pressure, and dyslipidemia are also more common among children born to women with GDM [94–96], suggesting intergenerational transmission of increased disease risk.

6.4 POTENTIAL MECHANISMS LINKING MATERNAL NUTRITION WITH HEALTH OF THE NEXT GENERATION

A variety of animal models, including both large (e.g., sheep) and small (e.g., rats and mice) animals, have been used to address the biological mechanisms underlying the developmental influences on later health. The multiple mechanisms by which maternal nutrition during a period of developmental plasticity induces permanent changes in the offspring are now beginning to receive considerable scientific interest and to be better understood. This understanding will enable the development of preventive and intervention strategies to combat the burden of chronic disease. Sections 6.4.1–6.4.4 describe potential mechanisms underlying the relationship between maternal nutrition and health of the next generation.

6.4.1 The Role of the Placenta

The fetal nutrient supply is influenced by the mother's diet, nutritional stores, and metabolism and by the placenta's ability to transfer nutrients from mother to fetus [98]. The primary functions of the placenta are to provide an immunological barrier between fetus and mother; mediate the transfer of respiratory gases, water, ions, and nutrients; and produce and secrete a vast array of hormones, cytokines, and signaling molecules [99]. The functional ability of the placenta is reflected in its size and shape: small infants generally have small placentas but, in some circumstances, an undernourished infant can expand the placental surface to extract more nutrients from the mother [100,101]. Studies from the Helsinki Birth Cohort showed that CHD among men was associated with the shape of the placental surface at birth, as well as with the ratio of placental size to birth size [102]. This provides support for the proposition that adult cardiovascular and metabolic disease originate via developmental plasticity and adaptations arising from failure of the maternal-placental nutrient supply to match fetal requirements [100,103]. It emphasizes the role of the placenta in developmental programming [99,103].

The mechanisms through which programming can occur are less well understood, but it is clear that multiple placental dysfunctions, whether aberrant signaling, altered protein expression, mitochondrial dysfunction, or vascular structure, produce different effects [99]. A lack of a normal increase in maternal placental blood flow, leading to "placental insufficiency," probably represents the most common underlying cause of intrauterine growth restriction (IUGR). In IUGR pregnancies, the increased placental vascular resistance subjects the fetal heart to increased work load, which is suggested to result in an adaptation that may be advantageous in the short-term perspective, but could contribute to cardiovascular disease postnatally [104]. Furthermore, some IUGR fetuses are hypoxic *in utero* [105], which can lead to a reduction in the number of cardiomyocytes and make the heart more sensitive to hypoxic insults later in life [106]. Thus the thicker placental exchange barrier (a primary cause of fetal hypoxia in IUGR) and the increased placental vascular resistance in IUGR may represent alterations in placental structure that are directly involved in the fetal programming of cardiovascular disease [104].

The placenta seems to function as a "nutrient sensor" regulating the transfer of nutrients to the fetus according to the mother's ability to deliver nutrients [99]. The placenta responds to maternal nutritional cues, resulting in down-regulation of placental nutrient transporters in response to maternal undernutrition or restricted *in utero* placental blood flow. Therefore, fetal nutrient availability becomes limited and fetal growth decreases. In addition to nutritional deficits as associated with growth restriction, the placenta is sensitive to the "overnourished" status. These observations suggested that changes in placental nutrient transport capacity directly contribute to, or cause, alterations in fetal growth rather than being a consequence thereof. Fetal growth is critically dependent on placental nutrient transport; hence factors that regulate placental nutrient transport will influence fetal growth. Maternal levels of metabolic hormones, such as insulin, IGF-I, and leptin are influenced by the mother's nutritional states and convey critical information to the placenta with respect to the ability of the maternal supply line to allocate nutrients to fetal growth. Jansson et al. also suggested that the mammalian target of rapamycin (mTOR) plays a critical role in placental nutrient sensing [99]. They showed that placental mTOR stimulates the activity of the placental System A and System L amino acid transporters, which transport essential amino acids to the fetus, and that placental mTOR activity is decreased in IUGR, suggesting that mTOR signaling links maternal nutrient availability to fetal growth by modulating the flux of amino acids across the placenta.

Maternal nutrition is also likely to have an important effect on epigenetic mechanisms within the placenta [107]; epigenetic control of placental gene expression and function is an additional potential "nutrient sensor" mechanism. Maternal environmental influences before or at the time of conception may alter the methylation status of trophoblast genes, which could result in a permanent change in placental structure and function [108]. Furthermore, changes in placental transport of folate, vitamin B_{12}, and choline could alter DNA methylation in the fetus through changes in the availability of methyl groups.

6.4.2 Disruption of Organs and Tissues

Adverse environmental factors acting during critical periods of development for organs and tissues have the potential to disturb the processes of cell proliferation and differentiation [109]. The timing of the differentiation and proliferation phases is distinct for each tissue and organ; for example, the development of the heart occurs very early in gestation, while the kidney is one of the later organs to fully develop. It is clear that these periods of development will be sensitive to the nutritional environment *in utero* and it is also apparent that the same insult at different stages of development could have different consequences. If an insult were to occur during an organ's differentiation phase then it would be expected that the organ would be of normal size but would have an altered profile of cells. Although insults during the proliferative phase would result in a normal profile of cells, the organ may be smaller, with a reduced total number of cells. Disruption to organ development during either of these phases could have a significant impact on subsequent alterations in gene expression and physiological function.

In the kidney, for example, maternal dietary imbalance may lead to developmentally induced deviations from the optimal ratio of body mass to nephron number. A reduction in nephron number has been observed in offspring of sheep exposed to reduced nutrition during early to mid-gestation [110,111]. A relative deficiency in the number of nephrons is thought to create an increased risk of inadequate renal function and hypertension in later life [112] and, ultimately, a predisposition to renal failure and a potentially reduced life span. Similarly, in the pancreas, a low-protein diet in the rat has been shown to reduce total pancreatic weight, islet cell mass, and the relative contribution of β-cell lineages during development [113,114]. Such structural changes within the pancreas may contribute to the subsequent impaired glucose homeostasis observed [115]. Maternal undernutrition during the critical proliferative period for muscle fiber development has also been shown to affect the numbers of secondary muscle fibers in the young offspring of a variety of species, including rat, guinea pigs, sheep, and pigs [116].

6.4.3 Disruption of the Endocrine System

Endocrine changes during pregnancy not only can alter cellular responses, but may also change homeostatic regulation. Of particular interest is the effect of early nutritional stress on steroid hormonal influences and the HPA axis.

A large body of evidence suggests that an increase in circulating glucocorticoids may play a role in programming during early development. Glucocorticoids are steroid hormones with a wide range of postnatal functions including regulation of stress responses, immune function, and glucose metabolism. During fetal development, they have additional functions including the promotion of tissue maturation and function. There is a range of studies demonstrating that exposure of the fetus to excess glucocorticoids at critical periods of development results in fetal growth retardation, impaired renal development, hypertension, glucose intolerance, and insulin resistance in offspring. Normally, fetal glucocorticoid levels are much lower than maternal levels owing to the placental barrier by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity, which converts glucocorticoids to their inactive form [47]. In animal models, there is strong evidence for programming by glucocorticoids. Many studies have reported decreased birth weights and abnormal levels of plasma HPA-axis hormones in rats prenatally exposed to synthetic glucocorticoids or inhibition of 11β-HSD2, with increased blood pressure and glucose intolerance in adulthood [63,117]. Consistent with these findings, hypertension in rats whose mothers were fed a low-protein diet during pregnancy was shown to be preventable by chemical blockade of maternal glucocorticoid synthesis [118].

Alterations of the fetal HPA axis and sympathoadrenal responses are likely to be an important mechanism by which developmental exposures affect the subsequent responses of the offspring to stressful challenges. Lower birth weight has been linked with increased fasting cortisol concentrations in later adult life [119]. Moreover, in a study of girls aged 7–9 years, whose antenatal growth

was restricted, alteration of adrenocortical activity has been demonstrated [120,121]. Similar gender differences in HPA responses have been reported in animals. Given the known associations between small alterations in adrenocortical activity and features of the metabolic syndrome, these effects may have important health implications. The maternal influences underlying developmental effects on HPA and sympathoadrenal responsiveness remain to be defined, but evidence indicates that both maternal diet and stress in pregnancy may be important [78,122].

6.4.4 Epigenetic Processes

There is growing evidence that epigenetic mechanisms are responsible for tissue-specific gene expression during differentiation and that these mechanisms underlie the processes of developmental plasticity. The term "epigenetics" has been defined as the study of heritable changes in genome function that occur without alterations to the DNA sequence [123]. The major epigenetic processes are DNA methylation, histone modification, and microRNA, which are responsible for regulating the intensity and the timing of expression of specific genes. To date, most studies on the effect of early life nutrition on the epigenetic regulation of genes have focused on DNA methylation [124]. Regulated DNA methylation occurs mostly at a cytosine immediately 5′ to a guanine (CpG sites). CpG dinucleotides are not randomly distributed throughout the genome but are clustered as the 5′ ends of genes/promoters in regions known as CpG islands. When the CpGs in such islands are unmethylated, gene transcription proceeds normally but when some or all of the CpGs become methylated, the genes are switched off [125,126]. Such processes are involved not only in cell differentiation and parental genomic imprinting, but also in developmental plasticity through which the environment in early life can affect the developmental trajectory, with long-term effects on gene expression and phenotypic outcome [127].

There is a growing body of evidence from animal studies to suggest that the diet of the mother can influence the epigenetic status and phenotype of the offspring. In rats, a PR diet during pregnancy induced hypomethylation of the glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor alpha (PPARα) promoters in the livers of juvenile and adult offspring, which was accompanied by increased mRNA expression of these genes [128,129]. This was the first evidence that moderate changes in macronutrient intake during pregnancy can alter the epigenome. The PR diet-exposed offspring also exhibited changes in histone acetylation of the GR promoter, which would further facilitate transcription [130]. Hypomethylation of the proximal promoter of the type 1b angiotensin receptor (AT1b) has also been observed in the adrenal glands of PR-exposed rat offspring, associated with a persistent up-regulation of AT1b gene expression that may contribute to the development of hypertension [131]. Hypomethylation has also been observed in sheep that were exposed to a diet deficient in methyl donors (i.e., vitamin B_{12}, folate, and methionine) during the periconceptional period [132]. These sheep exhibited insulin resistance and elevated blood pressure in postnatal life. Restricted landmark genome scanning showed that 4% of 1400 CpG islands in the fetal liver were differentially methylated in a way that may cause overexpression of certain genes [132]. These data provide evidence that clinically relevant reductions in specific dietary inputs to the methionine/folate cycle during the periconceptional period can lead to widespread epigenetic alterations to DNA methylation in offspring. Recent studies have also provided evidence that maternal diet in humans has a long-term effect on DNA methylation in the offspring. Individuals who were exposed during a periconceptional period to famine during the Dutch Hunger Winter in 1944–1945 had a small reduction in DNA methylation in the differentially methylated region (DMR) of the imprinted insulin-like growth factor II gene (*IGF2*, a key factor in human growth and development and maternally imprinted) in adulthood compared with their unexposed, same-sex siblings [133]. The association was specific for periconceptional exposure, highlighting the importance of very early mammalian development as a critical period for establishing and maintaining epigenetic marks.

Although epigenetic processes operating in early development have been implicated in perinatal growth and later body composition, until recently there has been little direct evidence for this

proposition in humans. Using Sequenom MassARRAY we found that the methylation status varied greatly at 31 of 68 CpG sites 5′ from five candidate non-imprinted genes in umbilical cord tissue DNA from healthy neonates [134]. Of these 31 CpGs with variable methylation, 7 had significant associations with the child's adiposity age 9 years. Further analyses found that greater methylation of a single CpG within the retinoid X receptor alpha (RXRA) promoter measured in umbilical cord was robustly associated with greater adiposity in later childhood. The child's mean fat mass rose from 4.8 kg (17.3% body fat) in the lowest quarter of RXRA methylation to 6.6 kg (21.3% body fat) in the highest quarter of the distribution. Regression analyses, which took account of the child's gender, showed that neonatal epigenetic marks explained more than 25% of the variance in childhood adiposity. These findings were replicated in a second independent cohort and provide the first evidence in humans that early-life nutritional environment can cause epigenetic changes with important effects on offspring body composition [134].

In an experimental rat model in which the pregnant dam was fed a low-protein diet, the induction of an altered phenotype (hypertension and endothelial function) and expression changes were prevented by supplementing the diet with folic acid or glycine [135–137]. Hypomethylation of the hepatic GR and PPARα promoters was also prevented by the addition of 5-fold more folic acid to the PR diet [128]. This adds to other evidence that one-carbon metabolism may play a central role in the induction of altered phenotypes by maternal dietary alterations [124]. It has now become apparent that the period of epigenetic plasticity may extend beyond the early intrauterine period: Burdge et al. have shown that increasing folic acid intake in juvenile-pubertal period in rats whose mothers were fed protein sufficient or restricted diets during pregnancy altered their phenotype and epigenotype [138]. Together this suggests that induced changes in phenotype can be reversed by nutritional interventions during pregnancy or early postnatal life.

6.5 INTERGENERATIONAL EFFECTS OF MATERNAL NUTRITION

Programming in early life is one mechanism whereby there is transgenerational (mother–offspring) transmission of disease risk. When a pregnant mother is undernourished, then her fetus (first filial [F_1] generation) and its germ cells (future F_2 generation) are both directly exposed to these adverse conditions *in utero*. Prenatal undernutrition during critical periods of organ development, including the placenta, of the F1 offspring may have detrimental effects on their reproductive outcome, such that the F2 offspring are developmentally compromised as well [139]. The mechanisms underlying the effects of intergenerational programming are largely unknown and likely to involve a complex interplay among environmental, metabolic, and epigenetic factors [140].

There is some evidence indicating transmission of programming effects into succeeding generations from a number of rodent studies. Feeding rats a PR diet during gestation resulted in higher blood pressure and endothelial dysfunction not only in the offspring, but also in the grand-offspring [141]. A low-protein diet during pregnancy led to insulin resistance in the adult male and female F2 offspring [142,143]. There is evidence suggesting that glucose metabolism in the F3 generation is also affected by F0 undernutrition [144]. On the contrary, little is known about intergenerational effects of maternal undernutrition during pregnancy on the health of succeeding generations in humans. Very limited evidence suggesting intergenerational effects in humans has come from the study of the Dutch Hunger Winter 1944–1945. It revealed an increase in neonatal adiposity among the grand-progeny of women who were pregnant during the famine [145]. In addition, recent subsequent findings showed that prenatal exposure of men (F1) to poor nutrition increased their offspring's BMI, regardless of the sex of the offspring (F2), whereas no such effect was found among offspring of prenatally exposed women. This suggests that the mechanism involved in intergenerational transmission of disease may differ in men and women. It may be due to differences in physiological and epigenetic contributions to men and women in the production of the next generation [11]. These results warrant further follow-up of the health of the F2 generation (grandchildren) to elucidate intergenerational effects of prenatal undernutrition. Current evidence does nonetheless

suggest that public health strategies to improve maternal nutrition before and during gestation will benefit generations to come.

6.6 NUTRITION OF YOUNG WOMEN

There have been positive changes in the diet of adults in developed populations over recent decades, but there remain substantial inequalities in diet and nutrition [146]. The current dietary patterns of older children and young adults are a cause for considerable concern, not only in the prevalence of rising obesity, but also coupled with growing evidence of low micronutrient intakes and status in women of reproductive age. In addition, there are strong social patterning influences on diet, so that poor diets are more common in disadvantaged groups in the population [147,148].

Thus, for many young women, not only could their current patterns of diet impact on their own nutritional status, but they may also affect their ability to meet the nutrient needs of future pregnancies. Furthermore, the dietary patterns of young mothers are a major influence on the way that they feed their children [149], suggesting that inequalities in diet and nutrition will persist in the next generation. In the context of the long-term consequences of developmental effects of poor nutrition, inequalities in early nutrition may be expected to translate into differences in risk of adult disease in the future. Intervention strategies to lower this risk will require a very clear understanding of the influence of current variations in diet and nutrition on growth and development in early life.

6.7 THE IMPORTANCE OF LIFE COURSE APPROACH FOR PREVENTIVE MEDICINE

It is now clear that associations between development in early life and later health outcomes do not simply reflect genetic influences. Rather, the findings indicate that interactions between the genetic influences and the early life environment determine metabolic profile, susceptibility to adverse environmental challenges, and disease in later life. Such concepts are fundamental to current life course approaches to the prevention and treatment of chronic non-communicable diseases (NCDs) (Figure 6.2) [150]. Risk increases throughout the life course as a result of declining plasticity and the resulting accumulated effects of inadequate responses to challenges. However, although the greatest risk

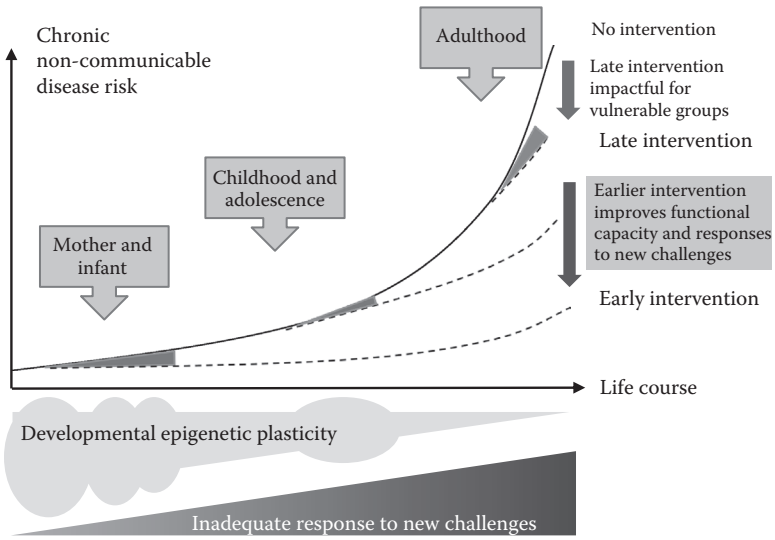

FIGURE 6.2 Life course strategies for the prevention and treatment of non-communicable diseases. (Derived from Godfrey KM et al. *Trends Endocrinol Metab*., 21:199–205, 2010.)

occurs in adult life, the trajectory is set much earlier, being influenced by factors such as the mother's diet and body composition before and during pregnancy, and fetal, infant, and childhood nutrition and development. Adopting a life course perspective allows identification of phenotype and markers of risk early, with the possibility of nutritional and other lifestyle interventions. Timely, relatively modest interventions in early life have the potential to have a large effect on disease risk later, although later interventions still remain important for vulnerable groups [150]. Early life preventive measures require a long-term investment, but are more likely to be effective than population screening programs that identify the early stages of disease or treatments initiated after the disease is manifest.

6.8 CONCLUSION

A substantial body of evidence from experimental and epidemiological studies suggests an important influence of maternal nutrition on the later risk of chronic diseases in the offspring. The effects of nutrition in early life on later metabolic condition may track from childhood to adulthood and amplify with age. In addition, the long-term consequences of adverse conditions during early development may not be limited to one generation, but may lead to poor health in the generations to follow [10]. Although animal studies provide multiple biologically plausible mechanisms for nutritional programming in early life, many questions remain unresolved. A better understanding of the programming mechanisms will enable the development of early life interventions to prevent cardiovascular disease, metabolic syndrome, and type 2 diabetes in the future. Central to preventive strategies will be efforts to improve the nutrition of young women before and during pregnancy, which will be an important part of preventing chronic disease in future generations.

6.9 KEY POINTS SUMMARY

1. Extensive epidemiological studies in humans, backed up by animal experimental models, point to the ability of under- or overnutrition during pregnancy to affect not only birth weight but also, more importantly, adult health outcomes in the next generation. These observations are generally referred to as the "developmental origins of heath and disease hypothesis."
2. Low birth weight has been shown to be associated with a higher risk of hypertension, type 2 diabetes, metabolic syndrome, chronic obstructive pulmonary disease and osteoporosis in later adult life, with all of these associations remaining significant even after controlling for adult lifestyle risk factors. A key cause for low birth weight is inadequate nutrition during pregnancy. Maternal famine conditions (Dutch Hunger Winter of World War II and the Chinese Famine of 1959–1961) have conclusively proven that fetal nutritional deprivation increases the child's risk of adverse health outcomes in later adult life.
3. The "thrifty phenotype" hypothesis has been proposed as a way of explaining one of the links between fetal undernutrition and subsequent adult disease. This hypothesis suggests that a fetus adapts to undernutrition by developing a thrifty phenotype, shunting scarce nutrients to vital organs such as the brain, at the expense of less vital organs such as the pancreas, muscle, and liver. Although these changes are positive adaptations in times of low nutrient delivery, if these "programming" changes persist into adult life when nutrients are abundant, they can become maladaptive and lead to adult diseases. It is believed that the degree of mismatch between *in utero* nutrition and nutritional status during adulthood determines the individual's susceptibility to chronic disease.
4. The biological mechanisms behind fetal "programming" of adult disease are still a matter of intense research interest. However, animal and human data suggest the following processes may play a role:
   a. Poor nutrition leads to a small inadequately functioning placenta with increased vascular resistance. This subjects the fetal heart to an increased work load, which may contribute to cardiovascular disease later in life.

b. Animal studies have shown that nutritional deprivation in early to mid-gestation results in a reduced number of nephrons in the fetal kidneys, increasing the risk of renal impairment and hypertension in later adult life.
c. Animal studies have reported that a low-protein diet in pregnancy results in a reduction in pancreatic and islet cell mass, the endocrine tissue responsible for the production of insulin. These changes may then contribute to a higher risk of diabetes later in life.
d. The placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) protects the fetus from high maternal corticosteroid levels by acting as an enzymatic barrier to placental corticosteroid transfer. During times of undernutrition, the activity of this enzyme in the placenta is reduced, exposing the fetus to high maternal corticosteroid levels, which may prime heightened stress responses and impair renal development, increasing the chances of later hypertension while also producing insulin resistance and glucose intolerance.
e. During pregnancy a limited availability of micronutrients such as folate, vitamin B_{12} and methionine that are involved in DNA methylation may result in epigenetic changes in the offspring's DNA that can persist into adult life. For example, animal studies have linked protein restriction during pregnancy, with epigenetic alterations in the promoter sequences for genes encoding the glucocorticoid and angiotensin receptor, with both likely to alter the offspring's propensity to develop hypertension and diabetes. An inadequate maternal diet of B group vitamins involved in DNA methylation has been linked with insulin resistance and hypertension in postnatal life. Finally, human studies have now linked maternal undernutrition with epigenetic changes in genes encoding IGF-II and the retinoid X receptor alpha (RXRA)—epigenetic change in the RXRA gene promoter at birth has been linked with obesity risk in later childhood.

5. The adverse effect of maternal undernutrition appears to extend beyond the first (F1) generation and may also include the following (F2 or grandchildren) or succeeding generations, at least in animal models. The F2 generation of children whose parents were exposed to undernutrition *in utero* are also at increased risk of developing hypertension and diabetes in adult life. Although the mechanisms behind this are unknown, it is speculated to involve epigenetic changes to the F2 generation's parents' gametes while exposed to poor nutrition *in utero*.
6. Overnutrition in pregnancy may also have long-term health consequences on adult health of the offspring. Children born to obese mothers, or to mothers with an excessive weight gain in pregnancy, are at increased risk of developing obesity, metabolic syndrome, and cardiovascular disease in adult life.
7. Given the long-term consequences of adverse nutritional conditions (both under- and overnutrition) during early development to the resulting adult health of the child, and possibly even the next generation, a life course approach to health needs to be taken as the ideal form of preventative medicine. Efforts to promote optimal nutrition among young women before and during pregnancy are a promising strategy in preventing chronic disease in future generations.

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7 Chinese Herbal Supplements and the Treatment of Female Infertility

Karin Ried and Clare Pyers

CONTENTS

7.1 INTRODUCTION

Fertility problems are encountered by about 15% of couples in Western countries [1]. Impaired fecundity, or the impaired ability to become pregnant or to carry an infant to term, affected about 6.7 million (10.9%) of women in the United States between 2006 and 2010 [1].

Although 80% of infertility might be related to conditions such as endometriosis or polycystic ovary syndrome (PCOS), 20% are "unexplained" in the Western Medicine (WM) model [2]. However, diagnosis of a specific disease/condition and subsequent treatment with surgery, drugs,

in vitro fertilization (IVF) or other assisted reproductive technologies (ARTs) does not always result in a viable pregnancy and live birth. In 2011, for example, more than 170,000 ART cycles were recorded in the United States, and of these 29% resulted in live births [3].

Moreover, ART treatment is costly for both the government and individuals; in 2011, costs for one IVF cycle were between US$10,000 and US$15,000, and individual couples' out-of-pocket expenses were on average US$5300 and up to US$19,000 for the first IVF cycle, and on average US$7000 for subsequent cycles [4].

Holistic approaches to infertility management, such as Traditional Chinese Medicine (TCM), might address some of the needs of women experiencing infertility that are not met in the WM approach [5,6].

In the last decade, herbal medicines, including Chinese herbal medicines, have been used for fertility by a small proportion of women in Western countries, for example, 5% of those surveyed at an infertility clinic in South Australia, 10% in the United Kingdom, or 18% in the United States [7–10].

A previous meta-analysis of eight randomized controlled trials (RCTs) reported a doubling in the pregnancy rate in subfertile women using Chinese Herbal Medicine (CHM) within a 4-month treatment period compared with WM drug therapy [11].

Section 7.2 outlines the principles of TCM diagnosis and treatment and illustrates in particular the usefulness of a thorough assessment of the menstrual cycle as a fertility indicator. Assessment of menstrual health during the female reproductive years is embedded in standard TCM pattern diagnosis, including also pulse and tongue diagnosis, and assessment of general physical and emotional well-being [12,13]. Section 7.3 outlines TCM treatment principles and approaches. Section 7.4 discusses the safety of CHM for fertility and during pregnancy and provides a summary of herbs contraindicated or to be used with caution during pregnancy. In Section 7.5, we update our meta-analysis on the effect of CHM on female infertility and pregnancy rates using data of 40 RCTs and involving 4247 women. In addition, we summarize the effects of CHM therapy on ovulation rates and other fertility indicators. Section 7.6 introduces principles of Chinese dietetics and nutritional therapy that can enrich fertility treatment.

7.2 TCM PATTERN DIAGNOSIS AND THE MENSTRUAL CYCLE

The language used in Chinese medicine predates WM; in the text that follows we provide some translation and insight into the way that Chinese medicine describes various fertility-related conditions.

When employing Chinese herbal medicines for the treatment of infertility, practitioners use a mix of WM diagnosis alongside signs and symptoms experienced during the menstrual cycle to determine the Chinese medicine diagnosis. Using a Chinese medicine diagnostic system enables the practitioner to use the established knowledge of the subtleties of single herb actions and herbal formula combinations for the treatment of gynecological, fertility, and obstetric-related conditions that have a written history in Chinese medicine of almost 1000 years.

TCM pattern diagnosis refers to whole body systems such as meridians and involves the Kidney, Liver (Blood), Spleen, Heart, and Lung systems, excess or deficiency patterns, and heat or cold patterns.* The fundamental treatment principle in TCM is to treat imbalance and restore balance within the body [12–16].

By taking into account the individual symptoms experienced by a patient the practitioner arrives at a specific "pattern" diagnosis that accounts for the patient's symptoms. Common TCM patterns identified in women with infertility include Kidney Jing deficiency; Kidney Yin or Yang deficiency; Spleen Qi deficiency; Liver Blood deficiency; Liver Qi stagnation; Blood stasis; Heat, Cold, or Dampness; and Heart Fire or Heart Yin or Blood deficiency due to long-term stress. These TCM patterns are associated with observable manifestations and symptoms, including pulse and tongue diagnosis and menstrual cycle characteristics (Table 7.1).

* TCM terms, such as Kidney, Liver, Spleen, are capped to distinguish the meaning in Chinese medicine from the Western meaning.

TABLE 7.1
Common Traditional Chinese Medicine (TCM) Patterns in Infertility and Associated Manifestations

TCM Pattern	Basal Body Temperature	Quality of Menses					Tongue	Pulse	Other Symptoms	Western Medicine Condition
		Color	Clots	Flow	Frequency	Pain				
Kidney Jing deficiency	Long follicular phase, low-temperature luteal phase; unstable; monophasic, little fertile mucus	Pink, watery	No	Scanty	Delayed	Lower back	Pale	Weak, thready	Low energy/poor stamina, frequent urination, dark under eyes	Resistant ovary disease, ovarian failure, advanced ovarian age, amenorrhea
Kidney Yin deficiency	Short follicular phase, with higher temperature (>36.5°C)	Bright red	No	Scanty	Irregular, shortened	Lower back, knees	Red with little coat, cracked	Thin, floating, rapid	Insomnia, dry throat/skin/hair	PMS, amenorrhea, mild polycystic ovary syndrome (PCOS), premature menopause
Kidney Yang deficiency	Long follicular phase, low temperature (≤36.2°C); short luteal phase with low temperature	Pale, light pink	Sometimes	Scanty	Irregular, delayed	Lower back, knees, legs	Pale, swollen	Deep, slow, weak	Pale complexion, fatigue, loose stools, feeling cold, low energy, low libido	Amenorrhea, insufficient progesterone
Spleen Qi deficiency	Slow-rise luteal phase (≥3 days, not usual 1–2 days); short luteal phase	Pink, watery	No	Scanty	Short	Mid-cycle pain	Swollen with white fur	Weak, slippery	Pale face, poor appetite, listlessness, loose stools	Amenorrhea, fibroids

(*Continued*)

TABLE 7.1 (CONTINUED)
Common Traditional Chinese Medicine (TCM) Patterns in Infertility and Associated Manifestations

TCM Pattern	Basal Body Temperature	Quality of Menses					Tongue	Pulse	Other Symptoms	Western Medicine Condition
		Color	Clots	Flow	Frequency	Pain				
Liver Blood deficiency	Long follicular phase	Dark red, sometimes dry or brown	No	Scanty	Delayed	Mild lower abdominal, pressure relieves pain	Pale with little fur	Weak, deep, choppy	Dizziness, sallow complexion, fatigue, muscle spasm, brittle nails	Dysmenorrhea, amenorrhea, PCOS
Liver Qi stagnation	Slow-rise unstable luteal phase	Dark red/ purple	Yes	Scanty	Irregular	Abdominal cramps	Dark red with purple spots	Deep wiry/ stringlike or choppy	Mood swings, irritability, headaches, breast distension	PMS, dysmenorrhea, irregular menstruation, amenorrhea
Heat, Kidney Yin deficiency	Follicular phase too short and temperature too high	Dark red/ purple	Often	Heavy	Irregular, mid-cycle bleeding	Thick itchy leukorrhea	Red, peeled (sides, tip) with yellow coating	Thin, rapid, wiry	Red cheeks, hot palms/soles, hot flushes, insomnia, agitation, weight loss, constipation, thirst, nosebleed	May be associated with hyperactive thyroid, essential hypertension, menopausal symptoms
Liver or Heart Fire	Unstable follicular + luteal phase, sawtooth or saddle pattern	Dark red	Sometimes	Thick, excessive	Shortened, intermittent bleeding	Abdominal	Red tip or edges	Rapid	Irritability, thirst, restless sleep, headache, red complexion	Metrorrhagia/ midcycle bleeding, PMS
Damp-Heat	Monophasic, little fertile mucus	Dark red	No	Scanty, sticky thick, often malodorous	Irregular	Lower abdomen, loins, bloating	Red with thick, greasy yellow fur	Slippery, full, rapid	Dysbiosis in digestive system, no appetite, nausea	Infection, inflammation, endometritis, pelvic inflammatory disease

Phlegm-Damp, Cold-Damp	Long follicular phase, Monophasic temperature chart	Dark	Yes	Scanty	Delayed	Abdominal distension, pain	Pale with white fur	Deep, fine, slow	Cold limbs, poor appetite, overweight, dull-pale complexion, congested throat, heaviness	Fallopian tube blockage, PCOS, ovarian cysts, amenorrhea, dysmenorrhea
Blood Stasis and Dampness	Slow fall of temperature at start of cycle	Dark purple	Yes	Heavy	Irregular	Lower abdominal	Purple swollen sticky coating	Wiry, slippery	Low energy, feeling cold, emotional stress, anxiety	Endometriosis, ovarian cysts, fibroids
Cold Uterus	Low temperature	Dark brown spotting	Sometimes	Scanty or heavy	Irregular	Painful periods	Pale, or purplish	Deep, weak, slow, wiry	Cold limbs, loose stools, frequent urination	Recurrent miscarriage
Heart Yin deficiency	Short unstable follicular phase, high temperature	Bright red	No	Scanty	Short, irregular	Possible mid-cycle pain	Red tip	Rapid, thin or thready	Palpitations, hot feet at night, anxiety, insomnia, urination at night	Anovulation, stress, amenorrhea
Heart Blood deficiency	Long follicular phase, low temperature, possibly non-biphasic	Dark red, sometimes dry or brown	Sometimes	Scanty	Long, irregular	Possible mid-cycle pain, dull pain in lower abdomen relieved by warmth or pressure	Pale	Normal-slow, weak	Palpitations, low energy, cold hands, easy to cry/tearful, insomnia, poor memory	May be associated with hypoactive thyroid, anovulation, amenorrhea

Source: Ried K, Stuart K. *Complement Ther Med.*, 19:319–331, 2011; Lyttleton J. *Treatment of infertility with Chinese medicine*. London: Churchill Livingston; 2004; Noll AA, Wilms S. *Chinese medicine in fertility disorders*. Stuttgart: Thieme; 2010.

TCM therapy is based on the individual's underlying TCM pattern diagnosis. Different diseases may receive one treatment (*tong bing yi zhi*) and one disease may receive different treatments (*yi bing tong zhi*) [12–16].

Conditions associated with infertility, such as PCOS, endometriosis, idiopathic infertility, recurrent miscarriage, or unexplained stillbirth, may have similar underlying TCM pattern (e.g., Kidney deficiency Heat), and vice versa, one disease/condition might be caused by a different underlying TCM pattern (e.g., PCOS might be due to Kidney Yang deficiency, Blood stasis, or Phlegm-Dampness).

Herbal formulas are tailored to target the patient's unique combination of symptoms rather than only accounting for the diagnosed medical condition. The focus of the treatment is not on removing the pathology but rather on "restoring normal." Herbal formulas are adjusted by adding and removing individual herbs, and also by altering the dosage of particular herbs to focus the effect of the formula toward particular symptoms. In this way, side effects are minimized, effectiveness is increased, and the herbal formula creates an environment within the body in which normal physiology can be restored.

PCOS, for example, may be caused by an underlying TCM pattern of Kidney Yang deficiency, Blood stasis, or Phlegm-Dampness. Each TCM pattern has characteristic signs and symptoms (Table 7.1). For example, Kidney Yang deficiency often manifests with a deep, slow, weak pulse, pale and swollen tongue, and scanty pale menstruation that is often irregular (Table 7.1).

Endometriosis, on the other hand, is mainly a manifestation of the TCM pattern Blood stasis. However, Blood stasis (the branch problem) may have been caused by different underlying "root problems," for example, Qi stagnation, Kidney Jing deficiency, accumulation of Cold, or Kidney Yang deficiency. Depending on the individual's combination of TCM pattern decoctions of herbal mixtures are formulated.

Multiple TCM patterns may be present in an individual, and the skill and experience of a TCM practitioner will identify the best treatment approach. TCM therapy simultaneously addresses the most prominent pattern ("branch problem"), and the underlying long-term pattern ("root problem"), by not only identifying the current disharmony within the body but also looking to remedy the process that allowed the disharmony to develop in the first place. The practitioner will assess how the patient responds to treatment and adjust the prescribed herbal formulas over time.

7.2.1 The Menstrual Cycle

A thorough assessment of the women's menstrual history, embedded in standard TCM pattern diagnosis in female infertility, provides a visible window into the woman's (in-) fertility status.

Zhu Dan-Xi (1281–1358 A.D.), one of the great masters of internal medicine during the Jin-Yuan dynasties, reasoned: "In order to cure infertility, one must adjust menstruation" [17,18].

A balanced regular menstrual cycle provides a physiological environment conducive to conception, implantation, and maintenance of a viable pregnancy. The quality of the environment in the body is considered as important as the quality of eggs, sperm, and embryo. TCM practitioners often refer to the environment, including the endometrium, as the "soil" and eggs/embryo as the "seed" (Chinese proverb: "Cultivate the soil before planting the seed") [12–16]. Any irregularities in the menstrual cycle and general well-being, often seen in conditions associated with infertility, are fundamental factors that can be optimized before conception is attempted [12,15,19].

The combination of the basal body temperature (BBT) curve, menstrual flow, color of the menstrual blood and clot formation, mucus changes, and any associated pain or distension are directly related to TCM pattern diagnosis and therefore therapy (Table 7.1) [11–13].

Charting of the BBT taken orally with a digital thermometer shortly after awakening in the morning can track underlying imbalances and response to treatment. Figure 7.1 illustrates three examples of common TCM patterns in infertility (Figure 7.1b–d) compared to the BBT pattern of a balanced fertile menstrual cycle (Figure 7.1a).

As treatment with CHM progresses, a woman should expect her menstrual cycle symptoms will change and come closer toward balanced physiology. Table 7.2 provides a checklist of indicators of

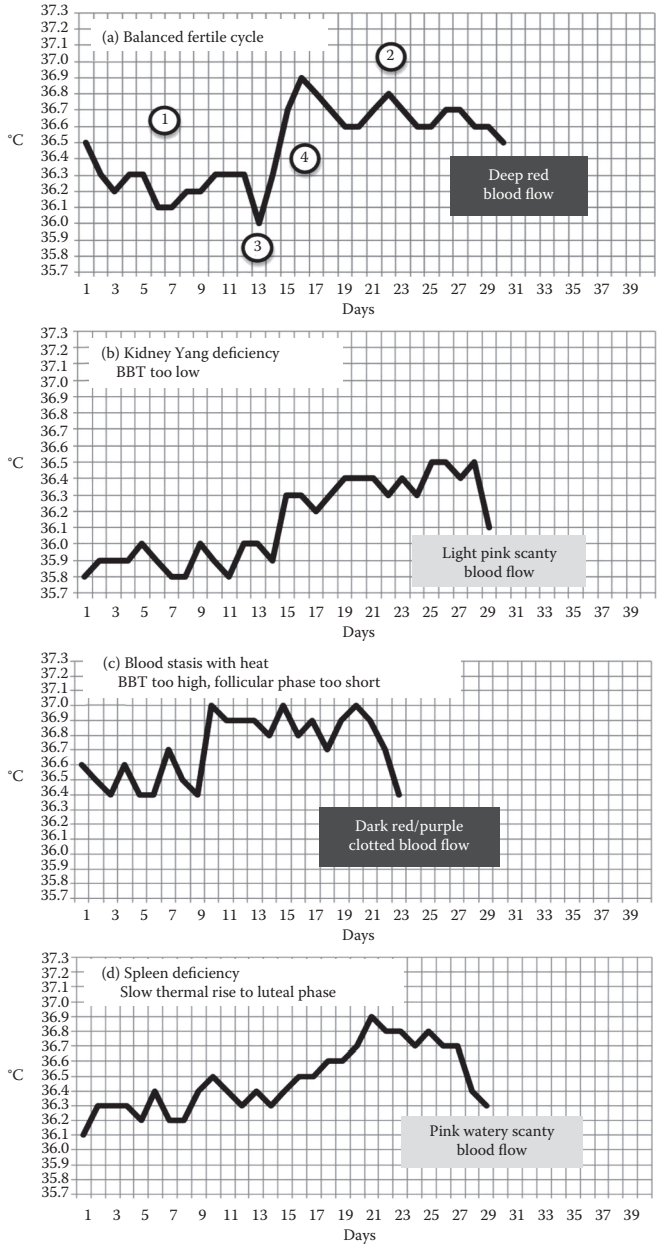

FIGURE 7.1 (a) Basal body temperature (BBT) of a balanced fertile menstrual cycle. (1) Follicular phase, (2) luteal phase, (3) ovulation, (4) thermal shift. Part (b) illustrates a low BBT, linked with light pink scanty blood flow, as well as a pale swollen tongue and a deep, slow, weak pulse, which is associated with the TCM pattern of Kidney Yang deficiency. Other common symptoms associated with Kidney Yang deficiency are pale complexion, fatigue, loose stools, feeling cold, low energy, and low libido. Part (c) illustrates a high BBT and short follicular phase, linked with dark purple and often clotted blood, as well as a purple tongue or colored sublingual veins, and a fast and choppy pulse, which is associated with Blood stasis and Heat. Other common symptoms associated with the TCM pattern of Blood Stasis with Heat are headaches, agitation, constipation, and thirst. Part (d) illustrates a slow thermal rise to the luteal phase spreading over more than 2 days, linked with pink watery scanty blood flow, as well as a swollen tongue with white fur, and a weak and slippery pulse, which is associated with the TCM pattern of Spleen deficiency. Other common symptoms associated with Spleen deficiency are a pale complexion, poor appetite, listlessness, and loose stools.

TABLE 7.2
Checklist of Indicators of a Balanced Fertile Menstrual Cycle

A Fertile Balanced Menstrual Cycle Is Ideally

- Regular and between 28 and 31 days long with menstruation lasting 4–5 days.
  - Full menstrual flow starts immediately (not spotting).
  - Menstrual flow is constant, without stopping and starting.
  - Menstrual flow is not too heavy (flooding) or too light.
  - Normal menstrual bleeding requires changing of sanitary products every 2–4 hours on heavy flow days.
- The blood is
  - neither too runny nor too sticky;
  - deep red in color, almost as if caused by a cut
  - without clots; and
  - without pain or spotting experienced at any part of the cycle.
- The basal body temperature (BBT) should not deviate by more than 0.1°C in each phase,
  - with the BBT in the follicular phase hovering around 36.2°C–36.5°C and
  - rising up to 36.7°C–37.0°C in the luteal phase.
- Ovulation should occur around day 12–14
  - with a distinct thermal shift of 0.3°C–0.5°C in 1–2 days.
- Cervical mucus is
  - nonfertile when runny or cloudy and
  - fertile when stretchy, clear, and slippery, like egg white around ovulation.

Examples of Infertile/Imbalanced Menstrual Cycles Are

- Dark purple colored clotted menstrual blood linked with heavy flow and long cycles
- Pink watery scanty flow of blood
- A slow rise of temperature/extended thermal shift of more than 2 days from follicular to luteal phase
- Absence of fertile mucus
- An unstable BBT pattern in either the follicular or luteal phase
- A menstrual cycle 25–26 days in length

Source: Lyttleton J. *Treatment of infertility with Chinese medicine*. London: Churchill Livingston; 2004; Noll AA, Wilms S. *Chinese medicine in fertility disorders*. Stuttgart: Thieme; 2010; Billings EL, Westmore A. *The Billings method*, 9th ed. Melbourne, Australia: Anne O'Donovan Publishing; 2011.

a balanced fertile menstrual cycle, described by TCM fertility experts and the Billings ovulation method [12,13,20].

The BBT should hover around 36.2°C–36.5°C in the first part of the cycle, also known as the follicular phase, and between 36.7°C and 37.0°C in the second part of the cycle, known as the luteal phase. A balanced fertile cycle is 28–31 days long, with ovulation occurring on day 12–14 indicated by a thermal shift of 0.3°C–0.5°C within 1–2 days (Table 7.2; Figure 7.1a). The menstrual blood should have a deep red color, as if caused by a cut, with no clots, and bleeding should last for 4–5 days. Menstrual flow should be moderate and constant with no brown spotting. In a balanced menstrual cycle no pain or discomfort is felt during the cycle, before or during menstruation. A fertile cervical mucus discharge, observed around ovulation, is stretchy and transparent like egg white, while infertile cervical mucus is more runny and cloudy.

The color and flow of the menstrual blood are directly observable by the woman. Whereas pale watery scant flow is indicative of deficiencies (Kidney Jing, Kidney Yang, Spleen Qi), dark red/purple and sometimes clotted blood is indicative of stagnation (Blood, Liver Qi). Color and flow also indicate the quality of the endometrium; pale scant menstrual blood relates to thin endometrium, brown colored discharge is old endometrial lining, and dark purple clotted blood is indicative of thickened stagnated endometrium and its shedding is often associated with pain.

TABLE 7.3
The Cervical Score

	Score			
Parameter	**0**	**1**	**2**	**3**
Amount of mucus	None	Scant	Dribble	Abundant
Spinnbarkeit	None	Slight, mucus thread ~5–6 cm	Moderate, mucus thread ~10–12 cm	Pronounced, mucus thread ~20–25 cm
Ferning[a]	None	Linear, few spots, no side branches	Partial, in parts of slide, side branches	Complete, full on whole slide
Cervix	Closed		Partially open	Fully open

Source: Insler V et al. *Int J Gynaecol Obstet.*, 10:223–228, 1972.

[a] Requires microscope (100×).

In addition to the mucus characteristics described previously, assessment of the cervical score, a semiquantitative, reproducible method for monitoring mucus changes, may be a useful addition to assessing fertility status without hormonal blood tests [21]. The four-point cervical score summarizes three characteristics of the mucus on a four-point Likert scale: the amount, the stretchiness (Spinnbarkeit), and ferning, as well as the cervix on a three-point Likert scale (Table 7.3) [21]. Fertility hormone concentrations directly correlate with the cervical score. The sum of the scores gives the total score, with a score of 10–12 relating to maximal fertility, a score of 7–9 to moderate fertility, 4–6 to slight fertility, and 0–3 to infertility.

Unexplained infertility is a diagnosis that can often be well treated with TCM. Common patterns that present with unexplained infertility are accumulation of Cold, and Blood deficiency. Unexplained fertility that occurs with no obvious pathology in the woman in most cases can be linked with several menstrual cycle irregularities (Figure 7.1, Tables 7.1 and 7.2). Bringing the menstrual cycle back toward normal will restore fertility and allow pregnancy to occur (provided there is not also unexplained fertility in the male partner).

7.3 CHM FOR INFERTILITY AND DURING PREGNANCY

Following from Section 7.2, where we described the Chinese medicine approach to the diagnosis of infertility, here we focus on the treatment approach and the requirements for safe and effective use of CHM.

CHM has a written history dating back to 2800 B.C., with gynecology, fertility, and obstetrics classified as separate areas of specialization since the publication of *Shi Chan Lung* (Treatise on Ten Obstetric Problems) by Yang Zijian in 1078 A.D. [22]. Chinese medicine theory dictates the use of herbal formulas rather than single herbs to focus the effects of the formula and to harmonize any harsh or undesirable effects. There are many rules regarding forbidden combinations, along with lists of herbs that counteract one another. For a formula to be both safe and effective, these principles of herb combining must be adhered to.

The Chinese medicine diagnostic framework allows a practitioner to have a sound understanding of the patient's condition, as well as the types of herbs that are going to be either useful or potential harmful. Without Chinese medicine training, it may be difficult to understand the range of possible effects of a Chinese herbal formula, a point especially poignant given the rising popularity of Chinese medicine for treatment of infertility, the perceived safety of natural medicines, and the general public's ease of access to Chinese herbs. For these reasons patients with an interest in pursuing CHM should seek expert advice rather than pursuing their own treatment.

7.3.1 The Importance of TCM Treatment Principles

Usually, a Chinese herbal formula contains at least four or more herbs. Within the prescription, each herb has a precise role. The "emperor" or "chief" is the principal or main therapeutic herb; the "minister" or "deputy" herbs support the function of the principal herb; the "assistants" support the overall action of the formula; the "messenger" or "courier" herbs direct to a particular meridian/organ system; and the "helper" or "harmonizer" auxiliary and/or correcting herbs can counter any toxic effects of the major ingredients.

Depending on the diagnosis of the underlying TCM pattern a herbal formula is mixed with a selection of herbs that support the transformation of the identified disharmony into balance; for example, if a "heat" pattern is evident, herbs of a cooling nature are included in the treatment formula, or vice versa, if a "cold" pattern is evident, warming herbs are included in the formula. Likewise, giving heating herbs to a patient with an existing heat pattern can exacerbate the problem rather than alleviating symptoms. An understanding of both the patient as well as the nature of the herbal formula is paramount to avoid causing harm.

A common Chinese medicine diagnosis for problems with fertility is Kidney essence deficiency, also referred to as Kidney Jing deficiency or Kidney Jing vacuity.

The TCM organ Kidney is the central reservoir of the energies that are significant for maintaining life and reproduction. An individual's Kidney Jing is dependent on his or her genetic constitution (prenatal Kidney Jing), and the individual's lifestyle (postnatal Kidney Jing). The natural depletion of postnatal Kidney Jing (Kidney Jing is depleted with age) can be slowed down by avoiding overexertion, excess tension, mental and physical stress, and by nourishing the body with a supportive diet and a balance of rest and activity. If Kidney Jing deficiency is the main problem causing infertility, a formula with tonifying herbs, in particular Qi and Blood tonifying herbs, may build the basis of a treatment. Herbs that will deplete the body of Qi and Blood are avoided so that the treatment can be both safe and effective for the patient [12–16].

7.3.2 Examples of TCM Herbal Formulas

Table 7.4 provides some examples of herbal formulas used to treat conditions associated with infertility and their underlying TCM pattern. Chinese herbal medicines are usually categorized according to their main characteristics or actions: herbs that tonify (strengthen), herbs that warm, herbs that cool, herbs that move, herbs that transform, and herbs that expel. Categories of herbs that are found in a CHM *Materia Medica* include herbs that tonify Yang, tonify Yin, tonify Blood, and tonify Qi and herbs that warm and expel Cold, warm and transform Phlegm-Cold, and warm and release the Exterior (Table 7.5).

For example, PCOS can often be associated with Kidney Yang deficiency, Blood stasis, Phlegm-Dampness, and/or Liver-Qi stagnation. As discussed in Section 7.2, PCOS can be "caused by" different TCM patterns. A correct diagnosis is important to ensure an effective treatment is prescribed.

For instance, if PCOS is caused by an underlying Kidney Yang and Spleen Qi deficiency, the treatment with a herbal formula will consist mainly of Yang tonifying herbs, as well as other supporting herbs (Table 7.4). On the other hand, if PCOS is associated with an underlying Blood Qi and Kidney Jing deficiency, the herbal treatment formula would consist of mainly Blood tonifying herbs, as well as other supporting herbs (Table 7.4). An incorrect diagnosis of PCOS due to Damp-Heat would result in the patient being prescribed herbs that are cooling and drying, and this would further exacerbate the underlying Yang deficiency symptoms and could worsen the patient's condition.

There are many herbs with similar main properties, strengths, and other supporting actions, giving rise to the many different herbal mixtures/formulas available for certain conditions and their associated TCM pattern, which may be confusing for the lay person. However, it is important to understand that a good functional herbal formula is based on the individual's underlying TCM

TABLE 7.4
Examples of Herbal Formulas for Conditions Associated with Female Infertility

WM Condition	TCM Pattern	Formula	Pinyin Name	Latin Name	Main Action	Reference
PCOS	Kidney Yang and Spleen Qi def		*Tu si zi*	*Semen Cuscuta chinensis*	Tonifies Yang	Hua et al. [23]
			Yin yang huo	*Herba Epimedium grandiflorum*	Tonifies Yang	
			Du zhong	*Cortex Eucommia ulmoides*	Tonifies Yang	
			Tao ren	*Semen Prunus persica*	Invigorates Blood, remove stasis	
			Chuan xiong	*Rhizoma Ligusticum chuanxiong*	Invigorates Blood, removes stasis	
			Yi yi ren	*Semen Coicis lachyrma-jobi*	Regulates water, drains dampness	
			Che qian zi	*Semen Plantaginis*	Regulates water, drains dampness	
			Dang gui	*Radix Angelica sinensis*	Tonifies Blood	
PCOS	Blood Qi and Kidney Jing def		*Gou qi zi*	*Fructus Lycium chinensis*	Tonifies Blood	Shao et al. [24]
			Dang gui	*Radix Angelica sinensis*	Tonifies Blood	
			Bai shao	*Radix Paeonia lactiflora alba*	Tonifies Blood	
			Shu di huang	*Radix Rehmannia glutinosa*	Tonifies Blood	
			Bu gu zhi	*Fructus Psoralea corylifolia*	Tonifies Yang	
			Tu si zi	*Semen Cuscuta chinensis*	Tonifies Yang	
			Nu zhen zi	*Fructus Ligustrum lucidum*	Tonifies Yin	
			Han lian cao	*Herba Eclipta prostrate*	Tonifies Yin	
			Chuan xiong	*Rhizoma Ligusticum chuanxiong*	Invigorates Blood, removes stasis	
			Zi shi ying	*Fluoritum*	Anchors, calms, settles spirit	

(*Continued*)

TABLE 7.4 (CONTINUED)
Examples of Herbal Formulas for Conditions Associated with Female Infertility

WM Condition	TCM Pattern	Formula	Pinyin Name	Latin Name	Main Action	Reference
Endometriosis	Blood stasis due to Liver Qi stagnation		*Chuan xiong*	*Rhizoma Ligusticum chuanxiong*	Invigorates Blood, removes stasis	Zhang [25]
			Dan shen	*Radix Salvia miltiorrhiza*	Invigorates Blood, removes stasis	
			Chuan niu xi	*Radix Cyathula officinalis*	Invigorates Blood, removes stasis	
			Yan hu suo	*Rhizoma Corydalis yanhusuo*	Invigorates Blood, removes stasis	
			Xue jie	*Resina Sanguis draconis*	Invigorates Blood, removes stasis	
			Mo yao	*Resina Commiphora myrrha*	Invigorates Blood, removes stasis	
			Dang gui	*Radix Angelica sinensis*	Tonifies Blood	
			Chi shao	*Radix Paeonia lactiflora rubra*	Cools Blood	
			Xiang fu	*Rhizoma Cyperus rotundus*	Regulates Qi	
			Gui zhi	*Ramulus Cinnamomum cassia*	Warms, releases the exterior	
Endometriosis	Blood stasis	*Shao Fu Zhu Yu Tang*	*Xiao hui xiang*	*Fructus Foeniculi vulgaris*	Warms interior, expels cold	Case study 4 in Section 7.6
		= Fennel seed and Corydalis combination	*Pao jiang*	*Rhizome Zingiberis preparatum*	Warms interior, expels cold	
		= Drive out stasis from the lower abdomen decoction	*Yan hu suo*	*Rhizome Corydalis*	Invigorates Blood, removes stasis	
			Chuan xiong	*Rhizoma Ligusticum chuanxiong*	Invigorates Blood, removes stasis	
			Mo yao	*Resina Myrrhae*	Invigorates Blood, removes stasis	
			Chi shao	*Radix Paeonia rubrae*	Invigorates Blood, removes stasis	
			Dang gui	*Radix Angelicae sinsensis*	Tonifies Blood	
			Rou gui	*Cortex Cinnamomi*	Tonifies Yang	
			Pu Huang	*Pollen Thyphae*	Stops bleeding	

Blocked fallopian tubes	Blood stasis	*Jiu Teng Zhu Yu* (vine-derived)	*Da xu teng*	*Caulis Sargentodoxa*	Clears Heat, eliminates toxins	Pang et al. [26]
			Ren dong teng	*Caulis Lonicera*	Clears Heat, eliminates toxins	
			Huang teng	*Caulis Fibraureau*	Clears Heat	
			Qing feng teng	*Caulis Sinomenii*	Dispels Wind-damp	
			Luo shi teng	*Caulis Trachelospermi*	Clears Wind-damp	
			Shou wu teng	*Caulis Polygoni multiflori*	Dispels Wind	
			Tong guan teng	*Caulis Mardeniae tenacissimae*	Dispels Phlegm	
			Ji xue teng	*Caulis Spatholobi*	Invigorates Blood, remove stasis	
			Tian ji xue teng	*Caulis Milletiae*		
			Huang qi	*Radix Astragli*	Tonifies Qi	
Anovulation	Blood and Spleen Qi def	*Wen Jing Tang* = Unkeito = Warm the menses decoction	*Bai shao*	*Radix Paeonia lactiflora alba*	Tonifies Blood	Ushiroyama et al. [27]
			Dang gui	*Radix Angelica sinensis*	Tonifies Blood	Case study 2 in Section 7.3
			E zhao	*Gelatinum Corii asini*	Tonifies Blood	
			Gan cao	*Radix Glycyrrhiza uralensis*	Tonifies Blood	
			Ren shen	*Radix Ginseng*	Tonifies Qi	
			Mai men dong	*Tuber Ophiopogonis japonica*	Tonifies Yin	
			Gui zhi	*Ramulus Cinnamomum cassia*	Warms, releases the exterior	
			Sheng jiang	Fresh *Rhizoma zingiberis*	Warms, releases the exterior	
			Wu zhu yu	*Fructus Evodia rutaecarpa*	Warms interior, expels cold	
			Ban xia	*Rhizome Pinellia temata*	Transforms Phlegm-Cold	
			Mu dan pi	*Cortex Radicis moutan*	Cools Blood	
			Chuan xiong	*Rhizoma Ligusticum chuanxiong*	Invigorates Blood, remove stasis	

(*Continued*)

TABLE 7.4 (CONTINUED)
Examples of Herbal Formulas for Conditions Associated with Female Infertility

WM Condition	TCM Pattern	Formula	Pinyin Name	Latin Name	Main Action	Reference
Unexplained infertility	Blood and Yin def	*Gui Shao Di Huang Tang* (follicular phase) = Angelica peony and rehmannia decoction	*Bai shao*	*Radix Paeonia lactiflora alba*	Tonifies Blood	Case study 1 in Section 7.3
			Dang gui	*Radix Angelica sinensis*	Tonifies Blood	
			Shu di huang	*Radix Rehmannia glutinosa*	Tonifies Blood	
			Shan yao	*Radix Dioscoreae opposite*	Tonifies Qi	
			Fu ling	*Sclerotium Poria cocos*	Regulates water, drains dampness	
			Ze xie	*Rhizoma Alismantis orientalis*	Regulates water, drains dampness	
			Mu dan pi	*Cortex Radicis moutan*	Cools Blood	
			Shan zhu yu	*Fructus Corni officinalis*	Astringes	
		You Gui Wan (luteal phase) = Restore the right kidney pill	*Dang gui*	*Radix Angelica sinensis*	Tonifies Blood	Case study 1 in Section 7.3
			Shu di huang	*Radix Rehmannia glutinosa*	Tonifies Blood	
			Gou qi zi	*Fructus Lycium chinenses*	Tonifies Blood	
			Tu si zi	*Semen Cuscuta chinensis*	Tonifies Yang	
			Du zhong	*Cortex Eucommia ulmoides*	Tonifies Yang	
			Lu jiao jiao	*Gelatinum Cornu cervi*	Tonifies Yang	
			Shan yao	*Radix Dioscerae*	Tonifies Qi	
			Rou gui	*Cortex Cinnamomi preparata*	Warms interior, expels cold	
			Zhi fu zi	*Radix Lateralis aconite preperata*	Warms interior, expels cold	
			Shan zhu yu	*Fructus Corni officinalis*	Astringes	

Note: Herbs in formulas are sorted by their actions. *Caulis*, stem; *Cortex*, bark; def, deficiency; *Fructus*, fruit; PCOS, polycystic ovary syndrome; *Radix*, root; *Resina*, resin; *Rhizoma*, rootstalk; TCM, Traditional Chinese Medicine; WM, Western Medicine.

TABLE 7.5
Glossary of Traditional Chinese Medicine (TCM) Terms

	General Terms
Blood (Xue)	Part of the Organ/Fluid system in TCM. Although it refers to the blood, as understood in Western Medicine, Blood is interconnected with other organ systems, for example, in TCM, Blood is the material form of Qi; it nourishes the body as the substance of Qi.
Damp	Dampness in TCM is a pathogenic condition involving accumulation of fluid in the tissues of the body, manifesting in a feeling of heaviness and swelling, such as in edema.
Phlegm	Accumulated Body Fluids may amass in the creation of Phlegm, for example, Lung congestion, lumps under the skin, numbness, gall or bladder stones.
Qi	Life force, energy
Stagnation/Stasis	Lack of movement, often associated with pain. In TCM, one distinguishes between Blood stasis and (Liver-) Qi stagnation. Stagnant Qi manifests as pain and distention and is dull, crampy, or colicky. Long-term Qi stagnation can give rise to Blood stasis. Blood stasis is associated with stabbing, sharp, fixed pain, and clots in the menstrual discharge.
Yin/Yang	The qualities of Yin are cooling, fluid, nourishing, material. The qualities of Yang are warming, active, expanding, moving. Yin and Yang should be in a balanced relationship.
Wind	Indicates its exterior origin, can manifest as Wind-Cold or Wind-Heat pattern, for example, Wind-Cold: sinusitis with runny clears mucus; Wind-Heat: sinusitis with fever and thick yellow mucus.
	Eight Guiding Principles of TCM Therapy
Hot/Cold	Overall energy of the patient. Cold conditions may be characterized by slow metabolism and chills, cold hands and feet, while hot conditions may have symptoms such as a fast metabolism, higher body temperature, and feelings of heat within the body.
Interior/Exterior	Location of the imbalance/disharmony. Exterior conditions are usually short lasting and are caused by germs entering the body. Interior conditions may result from emotional reason or exterior pathogens that have penetrated the inside of the body and affect the internal organs. Diseases caused by an exterior pathogen may begin in the exterior, but in time they may affect the interior, and would be treated as Interior regardless of their etiology.
Deficiency/Excess	This principle is used to describe the strength of an illness. A deficient condition is the lack of blood, energy, heat, or fluid. An excess condition is where the body has too much of something. Deficient conditions are usually chronic whereas excess conditions are acute.
Yin/Yang	These are the generalization of all of the preceding principles and conditions are categorized according to the dominance of the Yin or the Yang. In general, Yin is cold and represents the solid organs, while Yang energy is hot and represents the hollow organs.
	Eight Common Therapeutic Methods
Diaphoretic	Dispersion of pathogens from the body's surface
Emetic	Expelling toxic substances via the mouth
Purgative	Relieving the bowels
Regulating	Building the body's resistance to pathogens by controlling body functions
Warming	Eliminating cold and boosting Yang
Heat-removing	Diminishing fever and quenching bodily thirst
Tonifying	Nourishing and boosting Qi or life energy
Resolving	Invigorate, disperse, move—elimination of accumulated and stagnated Qi, blood, phlegm, retained food and fluids that have hardened into lumps

pattern and consists of herbs that have the properties to restore balance and resolve the diagnosed imbalance and dysfunction.

Likewise, endometriosis is associated with the TCM pattern of Blood stasis, which has manifested as a result of other long-term underlying problems, for example, Blood stasis due to Liver Qi stagnation, Blood stasis due to Kidney Yang deficiency, or Blood stasis due to

accumulation of cold. Herbal formulas to resolve Blood stasis contain herbs that invigorate (move) blood and remove stasis, and are combined with herbs addressing the additional associated underlying TCM pattern.

If endometriosis is associated with Blood stasis due to Liver Qi stagnation, for example, the herbal formula would consist mainly of herbs with Blood moving properties supported by Qi regulating herbs. In Table 7.4, the formula given to a sample of patients with endometriosis associated with Blood stasis and Liver Qi stagnation consists of 60% of herbs whose main actions are to "invigorate blood and remove stasis."

Further examples are listed in Table 7.4, for example, the effective formula *Jiu Teng Zhu Yu* used to resolve fallopian tube blockage (later in the chapter see also Table 7.8: CHM vs. WM effective rate 78% vs. 32%) [26] as well as a popular formula, *Wen Jing Tang* or *Un Kei To* or "warm the menses decoction," often given to patients with anovulatory problems, for example, luteal phase defect.

Long-term treatment with CHM may be approached using different strategies. Some TCM practitioners resort to "periodic therapy" by using different herbal formulas during different parts of the menstrual cycle (the follicular, ovulation, luteal, and menstrual phases); other TCM practitioners prescribe a particular herbal formula that addresses the underlying TCM patterns for an extended time, adjusting the formula on a 2-weekly, monthly, or 3-monthly basis depending on the individual's response to treatment and the formula's main properties.

For example, switching to an ovulation-stimulating herbal formula (*Wen Jing Tang*, also known as *7*, Table 7.4) for 8 weeks in a subgroup of 60 Japanese women with PCOS resulted in a marked increase in their ovulation rate (59%) compared to a subgroup of women who did not switch the formula (7%) (risk ratio [RR] = 8.0, 95% confidence interval [CI]: 2.03–31.48, $P = 0.0036$) [27]. Clinical studies demonstrated that the *Wen Jing Tang* formula had definite endocrinological effects on follicle-stimulating hormone, luteinizing hormone, and estradiol favorable for ovulation [28]. Other trials have linked Chinese herbal medicines to improvement of insulin levels, a key problem in women with PCOS, but pregnancy rates were not assessed in these studies [29].

The following case studies illustrate principles of TCM diagnosis and treatment with CHM, and highlight menstrual cycle changes over time, indicating improvements in fertility status.

7.3.3 Case Study 1

A 32-year-old woman presented with a diagnosis of unexplained primary infertility. She and her husband had stopped using birth control 3 years earlier, testing indicated no abnormal results, and they were referred to IVF by their doctor. She sought CHM to see if they could conceive naturally. She had a history of anxiety, insomnia, and dry skin, and had regular periods with scant bleeding for 3 days. She also noticed her hair was dry and brittle in the past 6 months. She experienced dizziness from time to time, and when run down would be susceptible to breakouts of urticaria. Her pulse was weak and thready, and her tongue was pale with a red tip.

Medical diagnosis: Unexplained primary infertility
Chinese medicine diagnosis: Blood and Yin deficiency

She was given herbs to nourish Blood and Yin and to calm the spirit. She was given a modified version of *Gui Shao Di Huang Tang* during her follicular phase, and *You Gui Wan* to take during her luteal phase (details of formulas can be found in Table 7.4). After the first month, her sleep and mood improved, and her skin was less dry. Her formula was modified slightly over the next 5 months, during which time her periods lengthened to 5 days and the amount of menstrual blood increased. She went on to conceive in the 6th month of taking Chinese herbal medicines, and gave birth to a healthy baby girl at 41 weeks of gestation.

7.3.4 Case Study 2

A 39-year-old woman presented with secondary infertility for the previous 18 months. She had two children from previous pregnancies with no difficulty conceiving. She experienced uncomplicated deliveries for both children with use of ice on the perineum post-delivery. Her menstrual cycle was regular every 28 days, blood was dark red with some clots, no pain. Other symptoms included cold abdomen, cold hands and feet (Reynaud's phenomenon diagnosed), fatigue, pale complexion.

BBT: 35.8 in follicular phase, 36.3 luteal phase
Tongue: Pale
Pulse: Weak, slow
Medical diagnosis: Unexplained infertility (IVF recommended by GP)
Chinese medicine diagnosis: Cold in uterus leading to infertility

The patient was treated with both acupuncture and CHM, with herbs that warm the uterus and remove internal cold. The guiding formula was *Wen Jing Tang* (Table 7.4), which was prescribed in granule form and adjusted periodically according to improvement in her presenting symptoms. Food therapy was prescribed, with advice including avoidance of all cold-temperature food and drinks and to use more spices in cooking. Lifestyle advice including keeping feet warm at all times and not putting anything cold near or on her body. During the course of treatment her hands and feet became warmer, there was no more clotting in the menstrual blood, her complexion improved, and her energy levels were higher. Her BBT eventually rose to 36.3 in the follicular phase and 36.8 in the luteal phase. She conceived during the 6th month of treatment and went on to give birth to a healthy baby girl weighing 3.4 kg at 40 weeks of gestation.

7.4 SAFETY OF CHINESE HERBAL MEDICINES DURING PREGNANCY

Of several thousands of TCM herbs and substances described in comprehensive texts such as the *Encyclopedia of Chinese Materia Medica*—for example, the 1977 *Zhong Yao Da Ci Dian* has 5767 entries [30]—a small number of herbs and substances are regarded as toxic if taken in higher dosages or for inappropriate conditions [31]. Chinese medicines have been used in humans for several millennia, and documentation on safety and toxicity exists for at least 3000 years [31]. However, these data on toxicity are often not available in English, and modern toxicity studies on single herbs or chemical compounds are not necessarily relevant or comparable [31].

Here we list herbs and substances that are contraindicated during pregnancy or should be employed with caution under the guidance of an experienced TCM practitioner, as compiled by experienced TCM practitioners who specialized in infertility and extracted details from historical TCM texts containing traditional knowledge acquired over thousands of years (Table 7.5) [12,13,32–34]. Standard Chinese *Materia Medica* including the most common Chinese herbal medicines contain a section on each herb's safety in pregnancy, as well as information on potential herb–drug interactions [34,35].

A large proportion of the contraindicated herbs during pregnancy fall into the TCM category of "herbs that are invigorating blood and removing stasis" (Tables 7.5 and 7.6). Although biomedically an embryo/fetus can be seen as a foreign body, in TCM it can be viewed as a manifestation of "stasis"; the blood moving action of herbs therefore is directed toward removing stasis and consequently could compromise the pregnancy or cause miscarriage. In addition, herbs with a strong descending action are also avoided because they can interfere with the woman's ability to "hold" the embryo/fetus.

In addition, common safety concerns of CHM intake during pregnancy include the self-prescription of herbal medicines.

TABLE 7.6
Safety of Chinese Herbal Medicines

Properties/Characteristics	Pinyin Name	Latin Name	English Name
		A1. Herbs Contraindicated during Pregnancy	
Invigorate Blood, Remove Stasis	*Chuan niu xi*	*Radix Cyathulae*	Cyathula root
	E zhu	*Rhizoma Curcumae zedoriae*	Curcuma zeodaria rhizome (not turmeric)
	Hong hua	*Flos Carthami tinctorii*	Safflower
	Hu zhang	*Rhizoma Arisaematis*	Bushy knotweed root
	Ji xue teng	*Caulis Spatholobi*	Milletia root and vine
	Lu lu tong	*Fructis Liquidambris*	Sweetgum fruit
	San leng	*Rhizoma Sparganii*	Bur reed rhizome
	Tao ren	*Semen Persicae*	Peach kernel
	Yan hu suo	*Rhizoma Corydalis yanhusuo*	Corydalis rhizome
	Yi mu cao	*Herba Leonuri heterophylli*	Chinese motherwort
	Yue ji hua	*Flos et fructus Rosae*	Chinese tea rose flower
Harsh Expellants	*Ba dou*	*Fructus Crotonis*	Croton seed
	Gan sui	*Radix Euphorbia kansui*	Gansui root
	Shang lu	*Radix Phytolaccae*	Pokeberry root
Purgatives	*Qian niu zi*	*Semen Pharbitidis*	Morning glory seed
	Lu hui	*Aloe*	Dried aloe leaf juice
	Da huang	*Radix et rhizoma Rhei*	Rhubarb
	Yuan hua	*Flos Daphnes genkwa*	Genkwa flower
Expel Parasites	*E wei*	*Asafoetida*	Dried gum of ferula root
	Guan zhong	*Rhizoma Guanzhong*	Basket fern (dryopteris) root
Clear Heat, Eliminate Toxins	*Ma chi xian*	*Herba Portulacae oleracae*	Purslane herb
	She gan	*Rhizoma Belamcandae*	Blackberry lily rhizome
Extinguish Wind, Stop Tremors	*Bai ji li*	*Fructus Tribulus*	Tribulus fruit/bindii/caltrop/puncturevine
Stop Bleeding	*Da ji*	*Radix Euphorbiae seu knoxie*	Japanese thistle
Warm Interior, Expel Cold	*Fu zi*	*Radix Aconiti*	Aconite root
	Rou gui	*Cortex Cinnamomi*	Cinnamon
Regulate Water, Remove Dampness	*Che qian zi*	*Semen Plantaginis*	Plantago/plantain seed
	Qu mai	*Herba Dianthi*	Lilac pink herb
Cooling, Transform Phlegm-Heat	*Tian hua fen*	*Radix Trichosanthis*	Chinese cucumber root
Warming, Transform Phlegm-Cold	*Zao jiao*	*Fructus Gleditsae sinensis*	Chinese honeylocust (soap thorn) fruit
		A2. Substances Contraindicated during Pregnancy	
Applied Externally	*Ban mao*	*Mylabris/cantharidin* (used to remove warts)	Blister beetle
	Chan su	*Secretio bufonis*	Toad skin venom
	Liu huang	Sulfur	Sulfur
	Ma qian zi	*Semen Strychnotis*	Nux vomica seed
	Qing fen	*Calomelas*	Mercurous chloride

TABLE 7.6 (CONTINUED)
Safety of Chinese Herbal Medicines

Properties/Characteristics	Pinyin Name	Latin Name	English Name
		A2. Substances Contraindicated during Pregnancy	
	Zhang nao	*Camphora*	Camphor
	Zuan ming fen	*Natrii sulphas exciccatus*	Sodium sulfate
Move Blood, Remove Stasis	*Di (tu) bie chong*	*Eupolyphagae seu opisthoplatiae*	Cockroach
	Meng chong	*Tabenus bivittatus*	Horsefly
	Mo yao	*Myrrha*	Myrrh tree resin
	Ru xiang	*Gummi olibanum*	Frankincense resin
	Shui zhi	*Hirudo seu whitmanae*	Leech
Purgative	*Mang xiao*	*Mirabilitum; Magnesia sulfaricum; Natrum sulfaricum*	Magnesium/sodium sulfate
Clear Heat, Eliminate Toxins	*Niu huang*	*Calculus bovis*	Cow gallstone
Open the Orifices	*She xiang*	*Secretio Moschus moschiferi*	Musk deer secretion
Extinguish Wind, Calm the Liver	*Wu gong*	*Scolopendra subspinipes*	Centipede
Tonify Yin	*Bie jia*	*Carapax trionyei*	Chinese soft turtle shell
	Gui ban	*Testudines plastrum*	Freshwater turtle shell
Tonify Yang	*Hai long*	*Hailong*	Pipe fish
	Hai ma	*Hippocampus*	Seahorse
	Xiong huang	*Realgar*	Arsenic sulfide
		B1. Herbs to Be Used with Caution during Pregnancy	
Move Blood, Remove Stasis	*Huai niu xi*	*Radix Achyranthis bidentatae*	Achyranthes root
	Su mu	*Lignum Sappan*	Sappan wood
	Wang bu liu xing	*Semen Vaccariae segetalis*	Vaccaria seed
	Xue jie	*Sanguis draconis*	Dragon's blood resin
	Yu jin	*Radix Curcumae*	Curcuma root/turmeric
	Ze lan	*Herba Lycobi lucidi*	Bugleweed
Purgatives	*Fan xie ye*	*Folium Sennae*	Senna leaf
Moist Laxative	*Yu li ren*	*Semen Pruni*	Bush cherry kernel
Expel Parasites	*Chang shan*	*Radix Dichorae febrifugae*	Dichroa root
	Bing lang	*Semen Arecae*	Betel nut
Clear Heat, Eliminate Toxins	*Lou lu*	*Radix Rhapontici seu echinops*	Echinops root
	Ban xia	*Rhizoma Pinellia*	Chinese dittany root
Clear Heat, Dry Dampness	*Huang lian*	*Rhizoma Coptis*	Chinese goldthread rhizome
Stop Bleeding	*Pu huang*	*Pollen Typhae*	Catail pollen
	San qi	*Radix Pseudoginseng*	Pseudoginseng root
Warm Interior, Expel Cold	*Chuan jiao*	*Fructus Zanthoxyli bungeani*	Szechuan pepper fruit
	Gan jiang	*Rhizoma Zingiberis officinalis*	Dried ginger rhizome
	Xiao hui xiang	*Fructus Foeniculi*	Fennel fruit

(*Continued*)

TABLE 7.6 (CONTINUED)
Safety of Chinese Herbal Medicines

Properties/Characteristics	Pinyin Name	Latin Name	English Name
		B1. Herbs to Be Used with Caution during Pregnancy	
Warming, Transform Phlegm-Cold	*Bai fu zi*	*Rhizoma Gigantei*	Typhonium rhizome
	Tian nan xing	*Rhizoma Arisaematis*	Jack in the pulpit, Himalayan cobra lily
Dispel Wind-Dampness	*Cang er zi*	*Fructus Xanthii*	Cocklebur fruit
Regulate Water, Drain Dampness	*Dong kui zi*	*Semen Abutiloni seu malvae*	Musk mallow seed
	Mu tong	*Caulis Mutong*	Akebia vine
	Tong cao	*Medulla Tetrapanacis papyriferi*	Rice paper pith
	Yi yi ren	*Semen Coicis lachrymal-jobi*	Job's tears
Transform and Dissolve Dampness	*Hou po*	*Cortex Magnolia officialis*	Magnolia bark
Drain Fire	*Dan zhu ye*	*Herba Lopatheri*	Lopatherum herb
	Jue ming zi	*Semen Cassiae*	Foetid cassia seed
Warming, Release the Exterior	*Ma huang*	*Herba Ephedra*	Ephedra herb
	Xin yi hua	*Flos Magnolia*	Magnolia flower
Astringe	*Bai guo*	*Semen Gingko*	Gingko nut
Regulate Qi	*Da fu pi*	*Arecae pericarpium*	Betel husk
	Zhi ke	*Fructus Citri seu ponciri*	Bitter orange peel
	Zhi shi	*Fructus Citri seu ponciru immaturis*	Immature fruit of bitter orange
Tonify Yang	*Bu gu zhi*	*Fructus psoraleae*	Psoralea fruit
Tonify Blood	*Dang gui*	*Radix Angelica sinensis*	Chinese angelica root
Nourish Heart, Calm Spirit	*He huan pi*	*Cortex Albizzia*	Mimosa tree bark
	Suan zao ren	*Semen Zizyphus spinosae*	Sour jujube seed
	Yuan zhi	*Radix Polygalae*	Chinese senega root
Relieve Food Stagnation	*Shen qu* contains: *Qing hao, Xing ren, Cang er zi, Chi xiao dou, Bai zhu*	*Massa Fermenta medicata;* contains: *Artemisiae annuae, Semen Armeniacae, Fructus Xanthii, Semen Phaseoli, Rhizoma Atractylodis macrocephalae*	Medicated leaven: mix of herbs in wheat flower
		B2. Substances to Be Used with Caution in Pregnancy	
Open the Orifices	*Bing pian*	*Borneolum*	Borneol resin from blumea balsamifera
Calm the Spirit	*Dai zhe shi*	*Haematitum*	Hematite mineral
	Hua shi	*talcum*	Talcum mineral
External Application	*Su he xiang*	*Styrax liquidis*	Rose maloes resin
Extinguish Wind, Stop Tremor	*Di long*	*Pheretima*	Earthworm

Source: Lyttleton J. *Treatment of infertility with Chinese medicine*. London: Churchill Livingston; 2004; Noll AA, Wilms S. *Chinese medicine in fertility disorders*. Stuttgart: Thieme; 2010; Maclean W. *Clinical handbook of Chinese herbs: Desk reference.* Sydney: Pangolin Press; 2011; Chen JK, Chen TT. *Chinese medicinal herbology and pharmacology*. City of Industry, CA: Art of Medicine Press; 2004; Hempen CH, Fischer T. *A materia medica for Chinese medicine*, 2nd ed. Philadelphia: Churchill Livingstone Elsevier; 2007.

Note: *Caulis*, stem; *Cortex*, bark; *Flos*, flower; *Fructus*, fruit; *Radix*, root; *Resina*, resin; *Rhizoma*, rootstalk.

A large cohort study of pregnant women in Taiwan involving 14,551 live births (1984–1987) investigating herbal medicine use during the first trimester and incidence of major congenital malformations found an increased risk of malformations with two preparations [36]. Taking the 13-herb formula *An Tai Yin* in the first trimester was associated with an increased likelihood of congenital malformations of the musculoskeletal and connective tissues (adjusted odds ratio [OR] = 1.61, 95% CI: 1.10–2.36) and eye (adjusted OR = 7.30, 95% CI: 1.47, 36.18). Taking the single herb *huang lian* or *coptis* rhizome (Chinese goldthread) in the first trimester was associated with an increased likelihood of congenital malformations of the nervous system (adjusted OR = 8.62, 95% CI: 2.54–29.24).

The herbal formula *An Tai Yin* is very popular in Taiwan, taken by 11.4% of the surveyed population.

Traditional wisdom in Taiwan is that *An Tai Yin* prevents spontaneous abortion and helps pregnant women with a smooth delivery. Hence the most appropriate time to use *An Tai Yin* is in the third trimester, which was not the case in this study. The cohort study also revealed that 95% of women using *An Tai Yin* did self-prescribe and did not consult a TCM practitioner [36]. *An Tai Yin* contains 13 herbs, including *hou po* (magnolia bark), *zhi ke* (bitter orange peel), and *dang gui* (Chinese angelica root), which are listed in Table 7.6 under "Herbs to Be Used with Caution during Pregnancy" and therefore would be prescribed only in very specific circumstances.

The second preparation associated with congenital malformation, if used in the first trimester of pregnancy, was *huang lian* (*coptis* rhizome), taken by 1.5% of the women studied [36]. In Taiwan, *huang lian* is seen as a herb to improve the appearance of skin, and it is believed that taking this herb in pregnancy would help the newborn and the pregnant woman herself to have beautiful skin.

Interestingly, *huang lian* contains berberine, with the latter being found useful to improve IVF pregnancies in women with PCOS [37,38]. The therapeutic effect of berberine was found to be more pronounced achieving more live births with fewer side effects than the drug metformin [37,38].

Importantly, however, individual herbs used in Chinese medicine are rarely prescribed on their own. The principles of formulating a proper CHM prescription should be kept in mind, especially when interpreting studies that look at the effects of single herbal medicines. The outcome of recent animal and *in vitro* studies expressed safety concerns of single Chinese herbal medicines when used in early pregnancy [39–41]. The two animal studies administered single herbal extracts in high concentrations to pregnant mice and evaluated pregnancy outcomes, finding increased maternal and perinatal mortality, maternal weight gain, decreased embryo growth, and increased fetal resorption and skeletal malformation. However, some of the herbal extracts tested in one study provided better outcomes than in the control group [39].

Although results from these animal studies may raise concerns about the use of CHM around pregnancy, these studies also reinforce the importance of properly prescribed medicines in safe dosages by an experienced TCM practitioner, even with the use of herbs that are considered safe for use in pregnancy. Safe doses of herbs are cited in herbal medicine texts and are well known to experienced TCM practitioners. TCM practitioners are aware of selected herbs contraindicated or to be used cautiously during pregnancy. Herbs to use with caution are typically avoided in all but severe cases where their use may be beneficial, for example, threatened miscarriage due to subchorionic hematoma.

Finally, a few case studies have reported on potential safety issues with Chinese herbal medicines taken around pregnancy [42]. The alcohol in an alcoholic herbal tincture taken by a 29-year-old Chinese woman during pregnancy was linked to her baby being diagnosed with fetal alcohol syndrome [43]. A 32-year-old woman taking the single herb *dong quai* (Chinese angelica root) postpartum developed hypertension, as did her 3-week-old son who was breastfed [44]. Potential hepatotoxicity of a herbal formula (*Shou Wu Pian*) containing *he shou wu* (*Polygonum multiflorum*) was reported in a case study [45]; however, *Materia Medica*, the Chinese herbal encyclopedia, lists this particular herb as being allowed during pregnancy [34]. *He shou wu* (*Polygonum multiflorum*) is known to cause hepatotoxicity in susceptible individuals, with several brands of manufactured pills explicitly stating this warning on their packaging. This potential hepatotoxic effect is not limited to pregnant women.

7.5 META-ANALYSES OF THE EFFECT OF TRADITIONAL CHINESE HERBAL MEDICINE VERSUS WESTERN MEDICINE IN THE MANAGEMENT OF INFERTILITY

Here, we have updated our previous meta-analysis [11] of eight RCTs and 1005 women on the effect of with CHM on female infertility with a further 32 trials described in recently published three meta-analyses [46–48] and one RCT by Pang et al. [26].

7.5.1 Methods

Participants were women of reproductive age with primary or secondary infertility. Infertility may have been associated with PCOS, anovulation, endometriosis, amenorrhea, fallopian tube blockage, or unexplained infertility.

CHM treatment was defined as treatment with Chinese herbs according to TCM pattern diagnosis. We included studies that used CHM alone or in combination with WM in the form of drugs or surgery. The control group in RCTs received WM treatment only. We excluded studies using acupuncture alone or TCM therapy in combination with ARTs.

The primary outcome was clinical pregnancy. We also summarized reported ovulation rates, basal body temperature pattern, endometrial thickness, cervical mucus score, pain, adnexal mass reduction in patients with endometriosis, and restoration of tubal patency in women with blocked fallopian tubes.

In addition, we report on the effect of CHM therapy on the continuation of pregnancy in women with threatened miscarriage [49].

We conducted an analysis of trials of sufficient quality using the Cochrane Review methodology and the Review Manager program [50].

7.5.2 Characteristics of Included Studies

Characteristics of studies included in this meta-analysis were extracted from published reviews and trials [11,26,46–48] and are summarized in Table 7.7, including 8 trials [23–25,51–55] from the meta-analysis by Ried and Stuart [11]; 13 trials [56–68] from the meta-analysis by See et al. [46]; 15 different trials [69–82] from the meta-analysis by Tan et al. [47]; and 4 trials [83–86] from the meta-analysis by Zhang et al. [48].

In summary, 40 trials involving 4247 women were included in our meta-analysis on pregnancy rates. Only one trial, by Xia et al. [66] was reported in two reviews, by See et al. [46] and Tan et al. [47], but included only once in our meta-analysis.

The majority of trials involved women with anovulation ($n = 24$) and PCOS ($n = 8$), while two trials studied women with endometriosis, one with immunological infertility and one with blocked fallopian tubes (Table 7.8). About half of the trials compared CHM only with WM treatment ($n = 21$), 40% used CHM plus clomiphene (CC) versus CC alone, and four trials combined CHM with other WM treatment and were compared to WM treatment. In the control groups, two-thirds of the trials used CC as the only WM drug therapy, seven trials combined CC with another WM treatment, and five trials used other drug therapy or WM treatment options (Table 7.8). The intervention in most trials was given between 3 and 6 months in most trials.

Included women were of reproductive age (18–45 years), with a mean of about 30 years [11,26,46–48]. Two meta-analyses [11,48] and the trial by Pang et al. [26] reported on the duration of infertility experienced by participating women before their involvement in a trial, ranging on average between 3 and 7 years.

TABLE 7.7
Characteristics of Trials Included in the Meta-Analysis on the Effect of CHM on Female Infertility Compared with WM

Study	Infertility Problem	CHM Treatment	Control WM Treatment	Duration (Months)
In Ried and Stuart [11] (review)				
Hua et al. [23]	PCOS	8-herb formula	CC	6
Wu et al. [51]	Endometriosis	2-herb formula	Gestrinone	3
Lin [52]	PCOS	Periodic CHM + Acu + CC	CC, HCG	3–6
Xia and Guo [53]	PCOS	16-herb formula (2 months) + CC (1 month)	CC (1 mth)	1–3
Chen et al. [54]	Immunological	8-herb formula	Prednisolone, Vit E	1–3
Shao et al. [24]	PCOS, anovulation	10-herb formula (15 days) + CC (5 days)	CC (5 days)	1–3 weeks
Ren [55]	PCOS	7-herb formula + CC	CC, Tamoxifen	2–3
Zhang [25]	Endometriosis	4 diff formulas containing 10–17 herbs by underlying TCM pattern	Danazol	ng
In See et al. [46] (review)	All: anovulation, incl. due to PCOS		All: CC	All: 3–6
Tang [56]		14-herb formula + CC		
Cui [57]		20-herb formula + CC		
Huang and Dan [58]		10-herb formula + CC		
Hu [59]		31-herb formula + CC		
Wu [60]		12-herb formula + CC		
Liu [61]		20-herb formula + CC		
Ma et al. [62]		12-herb formula + CC		
Li et al. [63]		20-herb formula + CC		
Fan et al. [64]		15-herb formula + CC		
Wang and Zhan [65]		9-herb formula + CC		
Xia et al. [66][a]		10-herb formula + CC		
Li and Zhang [67]		26-herb formula + CC		
Qiu et al. [68]		7-herb formula + CC		
In Tan et al. [47] (review)	All: anovulation			
Yin and Zuo [69]		Periodic CHM	CC	6–9
Luo et al. [70]		Periodic CHM	CC	3
Xu [71]		Periodic CHM	CC	3
Liu [72]		Periodic CHM	CC	3
Cui [73]		Mixed herb formula	CC	6
Qiu and Wang [74]		12-herb formula	CC	3
Chu and Wang [75]		Mixed herb formula	CC	3
Huang et al. [76]		Mixed herb formula	CC	6
Pang and Zhao [77]		Mixed herb formula	CC	ng
Huang [78]		Mixed herb formula	CC	6
Yin et al. [79]		Periodic herbs	CC	3–6
Huang [80]		Mixed herb formula	CC	6
Xia et al. [66][a]		Mixed herb formula	CC	3
Qiu et al. [81]		11-herb formula, Qi tonifying	CC, diethylestrol, medroxyprogesterone	3–6
Fu [82]		Mixed herb formula	CC, HCG	1

(*Continued*)

TABLE 7.7 (CONTINUED)
Characteristics of Trials Included in the Meta-Analysis on the Effect of CHM on Female Infertility Compared with WM

Study	Infertility Problem	CHM Treatment	Control WM Treatment	Duration (Months)
In Zhang et al. [48] (review)	All: PCOS			
Li et al. [83]		Group 1: CHM only: 6 + 7-herb formulas; Group 2: CHM + CC	CC	6
Ye et al. [84]		Periodic CHM + LOD	CC + LOD	6
Ma et al. [85]		Periodic CHM + Diane-35 + CC, HCG	Diane-35 + CC, HCG	6
Liang et al. [86]		14-herb formula + FA + HMG, HCG	FA + HMG, HCG	6
Pang et al. [26] (single RCT)	Blocked fallopian tubes	10-herb formula (oral)	Intrauterine infusion of 3 drugs 3 times	3

Note: CC, clomiphene; CHM, Chinese Herbal Medicine; Diane-35, ethinylestradiol cyproterone acetate; FA, follicle aspiration; HCG, human chorionic gonadotropin; HMG, human menopausal gonadotropin; LOD, laparoscopic drilling; ng, not given; PCOS, polycystic ovary syndrome; WM, Western Medicine. Periodic CHM, formula changes according to phase in menstrual cycle. Pang et al. [26]: Three meds: dexamethasone sodium phosphate (corticosteroid), chymotrypsin (digestive enzyme), gentamicin sulfate (antibiotic).

[a] Same study included in See et al. [46] and Tan et al. [47] reviews.

7.5.3 Meta-Analysis of RCTs

7.5.3.1 Effect of CHM on Pregnancy Rates

Our meta-analysis of 40 RCTs with more than 4200 women revealed a 1.74 higher probability of achieving a pregnancy with CHM therapy than with WM therapy alone (RR = 1.74, 95% CI: 1.56–1.94, $P < 0.0001$) in women with infertility. Mean pregnancy rates in the CHM group were 60% compared with 33% in the WM group (Figure 7.2).

This result is in line with our previous meta-analysis of 8 RCTs [11]. In fact, each of the 4 published meta-analyses comprising different trials, different infertility problems, and different CHM formulas reported similar pregnancy rates (RR range = 1.50–1.93; Figure 7.2) [11,26,46–48].

The greatest difference with CHM compared to WM treatment was found in the Pang et al. trial, which achieved a 3-fold higher probability of pregnancy in women with fallopian tube blockage (as confirmed by hysterosalpingography), using a 10-herb vine-derived herbal oral formula (*Jiu Teng Zhu Yu*) over 3 months compared to intrauterine injections of a cocktail of three drugs (RR = 2.95, 95% CI: 1.59–5.45, $P < 0.0001$; Table 7.8) [26]. The herbal formula was selected to address the underlying TCM pattern of Blood stasis and because of its anti-inflammatory properties (Table 7.4). Pregnancy rates were boosted to 52% in the CHM group compared to 18% in the WM group, and tubal patency was restored in 78% of women in the CHM group compared to 32% in the WM group, with 2 out of 10 women suffering an ectopic pregnancy in the WM group and none in the CHM group (Table 7.8) [26].

7.5.3.2 Effect of CHM on Fertility Indicators

In addition to pregnancy rates, a number of trials assessed the effect of CHM on other fertility indicators compared to WM treatment. About half of the trials ($n = 19$) involving 2059 women reported on ovulation rates. A meta-analysis of these trials revealed a 18% increased chance of improved ovulation with CHM compared to standard WM therapy in women with previously anovulatory cycles (RR = 1.18, 95% CI: 1.12–1.25, $P < 0.001$) (Table 7.8).

TABLE 7.8
Summary of Effects of Chinese Herbal Medicine (CHM) Compared with Western Medicine (WM) Treatment on Fertility Indicators

Outcome	Study ID	Infertility Problem	No. of Studies	No. of Women	CHM *n*/*N*	Control *n*/*N*	Treatment	RR	95% CI	*P*
Ovulation rate	All studies		19	2059	958/1205 (80%)	573/854 (67%)		1.18	1.12–1.25	<0.001
	Ye et al. [84]	PCOS	1	30	17/20	8/10	CHM: 10-herb formula WM: CC Time: 6 months	1.42	0.19–10.49	0.73
	See et al. [46]	Anovulation	6	604	288/341	166/263	CHM: diff formulas + CC WM: CC Time: 1–4 months	1.34	1.21–1.48	<0.001
	Tan et al. [47]	Anovulation	11 + 1	1425	653/844	399/581	CHM: diff formulas WM: CC ± other drugs Time: 3–6 months	1.13	1.05–1.20	<0.001
Basal body temp biphasic	See et al. [46]	Anovulation	4	315	ng	ng	As above	1.14	1.00–1.29	0.05
	Tan et al. [47]	Anovulation	3 + 1	650	242/373	175/277	As above	1.03	0.91–1.15	0.66
Endometrial lining >6 mm	See et al. [46]	Anovulation	2	138	ng	ng	As above	1.78	1.22–2.60	0.003
Cervical mucus score	Huang et al. [76]	Anovulation	1	130	59/86 (69%)	16/44 (36%)	CHM: herb formula WM: CC Time: 6 months	1.89	1.24–2.86	0.003

(*Continued*)

TABLE 7.8 (CONTINUED)
Summary of Effects of Chinese Herbal Medicine (CHM) Compared with Western Medicine (WM) Treatment on Fertility Indicators

Outcome	Study ID	Infertility Problem	No. of Studies	No. of Women	CHM *n*/*N*	Control *n*/*N*	Treatment	RR	95% CI	*P*
Pain reduction	Wu et al. [51,87]	Endometriosis	2	153	44/46 (96%)	46/49 (94%)	CHM: oral or oral + enema WM: gestrinone	1.02	0.93–1.12	0.07
					24/40 (60%)	2/18 (11%)	WM: danazol Time: 3 months	5.40	1.43–20.44	0.01
Adnexal mass reduction	Wu et al. [87]	Endometriosis	1	48	28/33 (85%)	8/15 (53%)	CHM: oral or oral + enema WM: danazol Time: 3 months	1.59	0.97–2.61	0.07
Effective elimination of tubal obstruction	Pang et al. [26]	Blocked fallopian tubes	1	115	45/58 (78%)	18/57 (32%); incl. 2 ectopic pregn.	CHM: 10-herb formula (oral) WM: intrauterine infusion of 3 drugs, 3 times Time: 3 months	2.46	1.64–3.69	<0.001
Continuing pregnancy >28 weeks gestation	Li et al. [49]	Problem in pregnancy: threatened miscarriage	5	553	287/304 (94%)	181/249 (73%)	CHM: diff formulas WM: Vit E, folic acid, progesterone ($n = 4$)[a], and/or HCG ($n = 2$)	1.28	1.18–1.38	<0.001

Note: CC, clomiphene; diff, different; HCG, human chorionic gonadotropin; ng, not given; Vit, vitamin; WM, Western Medicine. Pang et al. [26]: Three drugs: dexamethasone sodium phosphate (corticosteroid), chymotrypsin (digestive enzyme), gentamicin sulfate (antibiotic).

[a] One study used in the WM treatment group Salbutamol (β_2 antagonist) and $MgSO_4$ in addition to Vit E, folic acid, progesterone.

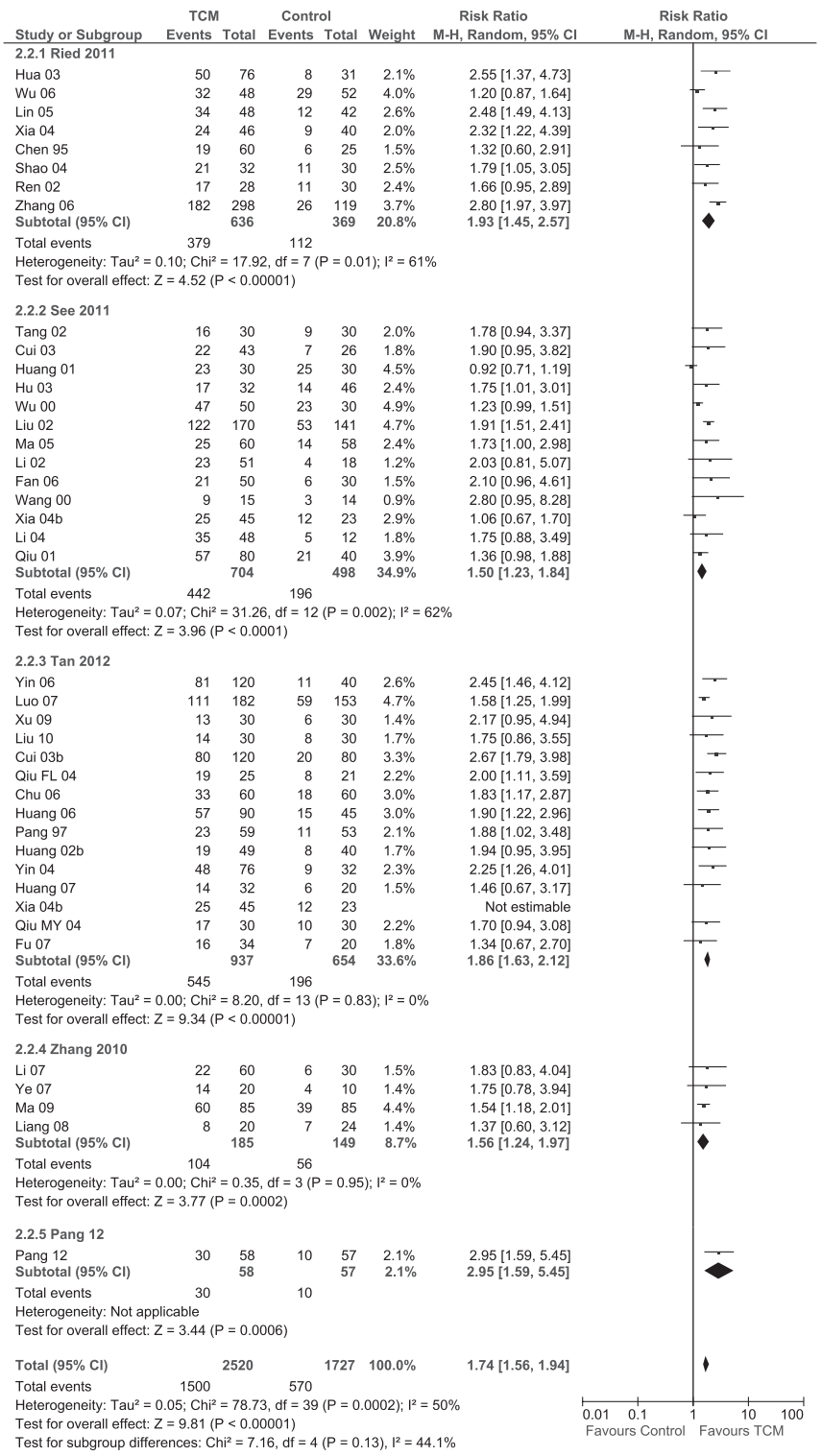

FIGURE 7.2 Meta-analysis of randomized controlled trials investigating pregnancy rates of infertile women treated with Chinese Herbal Medicine compared with Western Medicine fertility medications. CI, confidence interval; ES, effect size; *n*, number of pregnancies; *N*, number of women; TCM, Traditional Chinese Medicine.

Table 7.8 summarizes other outcomes assessed in some of the trials, including restoration of biphasic basal body temperature, reestablishment of thickness of the endometrial lining of more than 6 mm, and increase in cervical mucus score in anovulatory women with ovulation problems, all indicators of an environment conducive to a viable pregnancy. Treatment with CHM achieved a more favorable environment for pregnancy than WM treatment (Table 7.8). Reduction in pain and adnexal mass was assessed in two trials of women with endometriosis. Compared to the WM treatment with danazol, women reported less pain, and a reduction of adnexal mass was observed when patients were treated with CHM, while the WM drug gestrione had similarly favorable outcomes compared with CHM (Table 7.8).

7.5.3.3 Effect of CHM on Threatened Miscarriage

A Cochrane review by Li et al. [49] investigating the effect of CHM on threatened miscarriage in pregnant women using CHM compared to WM drug therapy found a 28% increase in the continuation of pregnancy beyond 28 weeks of gestation (RR = 1.28, 95% CI: 1.18–1.38, $P < 0.001$, $n = 5$ trials, $n = 553$ women) (Table 7.8). Continuing pregnancy rates improved to 94% with CHM compared to 73% using WM. As the review by Li et al. [86] looked into the effect of CHM *during* pregnancy, it was not included in our meta-analysis of the effect of CHM on infertility. However, the review illustrated that CHM therapy can be beneficial beyond conception, and may contribute to improving viable pregnancy and live birth [49].

7.5.4 Meta-Analysis of Cohort Studies

Our previous review on the effect of CHM on pregnancy rates in women experiencing infertility also included a meta-analysis of cohort studies [11]. The pooled pregnancy rate of seven high quality cohort studies included in the adjusted meta-analysis involving 616 women treated with CHM therapy was 49% (95% CI: 45–53, $P < 0.001$).

The adjusted likelihood of achieving a pregnancy with CHM therapy ranging between 1 and 12 months was 2.3 higher (95% CI: 1.6–3.4, $P < 0.0001$) compared with the pregnancy rate of 30.4% in a cohort of women ($n = 7439$) undergoing IVF in Australia in 1 year (2008/2009) [11,88].

A recently published cohort study testing an immune-stimulating Chinese herbal formula for 1 month reported a 1.9-fold increase in improved embryo quality in the early blastocyst stage (good quality: 18.7% ± 16.2 improved to 36.1% ± 27.1, $P < 0.01$) and a 1.4-fold increase in the late blastocyst stage (good quality: 14.8% ± 11.2 improved to 21.1% ± 23.1%, $P < 0.05$), leading to pregnancy in 33% of cases [89].

7.6 AN INTRODUCTION TO CHINESE DIETETICS AND NUTRITIONAL THERAPIES

7.6.1 History of Nutrition and Dietetics in the East and West

In Chinese culture, there is a focus on evaluating food in terms of Yin and Yang balance that has continued for the past two millennia. Some foods are considered Yin, tending toward cold and dampness, while others are more Yang, with a hot and dry nature. For example, the Chinese classify crab or squid as Yin foods. Eating too much would make the body more Yin, so to prevent any imbalance, a pinch of the herb purple perilla (*zi su ye*) is added to the dish. Just like fennel, this is a warm and pungent herb, and assists with preventing potential stomach cramps and diarrhea that could come from indulging in a Yin food. A glass of wine would also have a warming effect [90].

In Western terminology, "a healthy diet" usually means one that is rich in the right nutrients, vitamins, minerals, good fats, with little sugar and containing fiber, somewhat independent of the individual's energy demands in balance with the seasons. Historically though, in the days of Hippocrates in ancient Greece, food was classified similarly to herbs in terms of their heating

and drying qualities. As late as the 17th century the English herbalist Nicholas Culpeper could assume that his readers understood why it was important to eat fennel with fish [91]. Fish, as we might not be aware now, has a nature that is cold and damp, so too much of it may cause a chill in the stomach. This can be prevented by neutralizing the coldness and dampness of the fish with a hot, dry herb, such as fennel. Other traditional food combinations have been long established with similar intentions of harmonizing the diet; lamb with rosemary is another example.

Separate from the knowledge of traditional diets and food combining practices, we still have a body of evidence around healthy eating in modern times and how that might affect fertility. A large cohort study based on the Nurse's Health Study following 17,544 women and 25,217 pregnancies over 8 years found that a fertility-promoting diet needed to contain high amounts of monounsaturated fats and less *trans* fats, higher amounts of vegetable protein and less animal protein, low glycemic index carbohydrates, high-fat dairy, and multivitamin and iron supplements [92].

7.6.2 TCM Nutritional Approach

In TCM, a diet rich in foods that support the Kidney Jing is important for fertility and for ovarian function and egg quality. Women presenting to a TCM clinic with a diagnosis of poor ovarian reserve or poor-quality eggs will often be recommended a Kidney Jing nourishing diet in conjunction with their herbal medicine treatment. Good Kidney Jing strengthening foods are chicken, duck and fish eggs, oysters, bone marrow, milk, seeds and nuts, ghee (clarified butter), oats, artichoke, nettles, seaweed, and microalgae such as *spirulina* and *chlorella*. Small amounts of meat, fish, and internal organs such as liver (organically sourced or otherwise pure in quality) are also supportive of Kidney Jing [13,90]. Diets rich in large quantities of vegetables, low in preservatives and processed foods, with herbs used in the diet and medicinally, supplement any gaps in nutrition. Iron and folic acid supplements, for example, can be replaced by a combination of foods and herbs that optimizes iron absorption and the availability of folic acid in the diet. Not only does this involve adding densely nutritious foods into the diet, but also removing foods that might impair the absorption or utilization of nutrients. Foods that can impair the function of Kidney Jing include highly stimulating foods and substances such as coffee, chili, black pepper, dry ginger, sugar, and many food additives and preservatives.

Generally, foods as well as herbal medicines are characterized by one of five tastes. Spicy and sweet tastes have a Yang or warming character, and bitter, sour, and salty have a Yin or cooling character. Additional food and herb characteristics are bland (Yang) and astringent (Yin).

The preparation of food also influences its properties. Yang-increasing methods of preparation include grilling, roasting, baking, and prolonged braising, whereas Yin-increasing methods of food preparation include steaming, blanching, boiling in water, and cooking vegetables in their own juices [13,90].

A common TCM pattern in infertility is Kidney Yang deficiency. Recommended foods to strengthen Kidney Yang should be warming in nature, including—but not exclusive to—sweet-warm spices such as garlic, ginger, cinnamon, rosemary, and anise; boiled, grilled or fried red and darker colored meats (venison, lamb, goat, duck), root vegetables, sulfur-containing vegetables (onion, leek, cabbage, broccoli) and red-colored fruit (cherries, raspberries, raisins), as well as food supportive of Kidney Jing [13,90]. In Kidney Yang deficiency, foods to be avoided include cool and cold foods such as raw foods, salads and fruit, excess dairy products, sugar, alcohol, excess acrid spices, and excess coffee (whereas a small amount of coffee, about one or two cups, is warming and therapeutic) [90].

Another common TCM pattern in infertility is Phlegm-Dampness. In TCM, phlegm pathology extends beyond the Western definition of phlegm in the form of sputum/blockage in the respiratory system (sinusitis, bronchitis, pneumonia) and is categorized as either substantial or insubstantial phlegm. Substantial phlegm manifests physically in the body, and in addition to the phlegm produced in the respiratory tract it can appear as nodules (lipoma, adenoma, ganglia, goiter, cysts) or in its hardened form as stones (gallstones, kidney stones). Insubstantial phlegm does not manifest

physically; instead it pools in the channels of the body and obstructs Qi flow, manifesting as foggy or clouded thinking or a general feeling of heaviness, sluggishness, and numbness.

In a fertility context, phlegm can be part of the diagnosis of conditions such as blocked fallopian tubes, PCOS, anovulation, and amenorrhea. Both substantial and insubstantial phlegm are associated with impaired function of the Spleen Qi organ system. Nutritional therapy consists of recommending foods to strengthen the Spleen, and to avoid phlegm-producing foods such as sugary sweets and drinks, very oily and fatty foods, excess of dairy products, bananas, as well as Yin foods such as raw foods; salads; and cold types of fruits, for example, citrus fruits.

Other recommended foods are dependent on the nature of phlegm, which is differentiated according to the accompanying symptoms. Phlegm-Heat will present with symptoms such as yellow sputum, red tongue with yellow coating, dry mouth; Phlegm-Cold can manifest with white viscous sputum, lack of appetite, and oppression of the chest and epigastrum. In Phlegm-Heat, cooling foods are recommended, for example, those of bitter-cool, salty-cool, or sometimes sweet-cool nature (endive hearts, dandelion, soy milk, mung beans, radish, pears, grapes, carp, salt, seaweed), whereas in Phlegm-Cold, warming foods are recommended, for example, bitter-warm or spicy-warm nature (pepper, garlic, ginger, cardamom, leeks, cherries) [90].

The following case studies illustrate the influence of diet and lifestyle on fertility.

7.6.3 Case Study 3

A 41-year-old woman sought TCM for IVF support. She had undertaken a previous IVF cycle that resulted in six fertilized embryos; she had three prior transfers of single embryos that successfully implanted but that resulted in miscarriage at approximately 6–7 weeks of gestation. She had been using progesterone pessaries.

She was slightly overweight (body mass index [BMI] ~26) despite eating a "healthy diet" of salads and lean meats. She complained of fatigue, cold hands and feet, and had a cold abdomen and cold buttocks. She often felt bloated, suggesting a sluggish digestion, and was prone to constipation. Her menstrual cycle was irregular, with a cycle length of 30–48 days and heavy bleeding on day 2. Her BBT was very low, featuring a saddle pattern with 35.1°C in the follicular phase and an average of 35.6°C in the luteal phase. Her pulse was soggy and slippery. Her tongue showed blue sublinguals and a red body with teeth marks.

Medical diagnosis: Recurrent miscarriage (due to age 40+)
Chinese Medicine diagnosis: Kidney Yang deficiency, internal Cold

She was given acupuncture and CHM for 2 months before the next IVF transfer. In addition, she was advised not to have bare feet on cold floors at home (even during summer), eat more cooked foods, add spices to cooking, include more good fats in the diet, and avoid eating salads and cold/raw foods. Her energy improved, and her BBT rose to 36.4°C in the follicular phase and 37.0°C in the luteal phase. Her pulse became soft, and her tongue became pink without flabbiness or distended sublingual veins. Her next IVF egg transfer was successful and continued to full term. She gave birth to a healthy boy via scheduled cesarean section at 38 weeks.

Two years later she had the remaining embryos transferred without success. She had a further egg collection at age 44 and conceived on her third embryo transfer, giving birth to another healthy boy via scheduled cesarean section at 38 weeks.

7.6.4 Case Study 4

A 36 year-old woman presented with primary infertility and endometriosis. The couple had tried to conceive for 2 years before being referred to IVF. Within a further 18 months, she underwent more than eight egg collections and numerous embryo transfers, unsuccessfully. She underwent a dilation

and curette procedure three times. On presentation at the TCM clinic, her pulse was choppy, and her tongue was purple with a red tip.

Medical diagnosis: Endometriosis
Chinese medicine diagnosis: Blood stagnation

The TCM practitioner advised her to make some dietary changes, specifically to avoid sugar and gluten, grains, and processed foods, and to increase her protein and fat consumption, including nuts, avocado, fish, and chicken, and to use coconut oil in cooking. She was given a modified version of the herbal formula *Shao Fu Zhu Yu Tang*. After the patient made the dietary changes and took herbal medicine for 1 month, she conceived naturally in her next menstrual cycle.

7.7 CONCLUSIONS

Our meta-analysis of 40 RCTs involving more than 4200 women suggests CHM taken over 3–6 months is more effective in the treatment of female infertility than WM drug treatment, achieving on average a 60% pregnancy rate compared to 33% with WM. Trials included women with PCOS, endometriosis, anovulation, fallopian tube blockage, or unexplained infertility.

Fertility indicators such as ovulation rates, cervical mucus score, biphasic basal body temperature, and appropriate thickness of the endometrial lining were positively influenced by CHM therapy, creating an environment conducive for a viable pregnancy.

Here, we summarize common TCM patterns in infertility and their associated manifestations, including pulse, tongue, and menstrual cycle characteristics, forming the basis of TCM pattern diagnosis. Common TCM patterns in infertility include Kidney Jing deficiency, Kidney Yin or Yang deficiency, Heart Fire, Heart Yin or Heart Blood deficiency, Spleen deficiency, Liver Blood deficiency, Liver Qi stagnation, Blood stasis, Heat, Cold, or Dampness.

Observation of the menstrual cycle, including basal body temperature, color, and flow of the menstrual blood and clot formation, mucus changes, and any associated pain or distension, provide a window into the woman's fertility status. In this chapter, we included a checklist summarizing the signs and symptoms associated with a balanced fertile menstrual cycle, a useful tool for women as well as practitioners to gauge fertility status and response to treatment.

TCM pattern diagnosis guides the practitioner toward a treatment with appropriate herbal formulas. A CHM formula usually contains an average of 10–15 herbs, each of which is characterized by a main action/property, and includes principal, supporting, directing and correcting herbs. In this chapter we provided some examples of herbal formulas addressing conditions associated with infertility. In addition, we listed herbs contraindicated or to be used with caution during pregnancy, and discussed safety measures regarding Chinese herbal medicines.

An introduction to nutrition through Chinese medicine eyes provides some insights into dietetic principles, similar to TCM treatment principles, that can be used to enrich CHM therapy and improve fertility.

7.8 KEY POINTS SUMMARY

1. Traditional Chinese Medicine (TCM) treatment of infertility and pregnancy complications such as miscarriage dates back more than a thousand years, with a large body of evidence supporting its safety and efficacy. Although TCM treatment of infertility has always been popular in Asia, recent surveys suggest that as many as one in five Western women experiencing infertility are now using TCM therapy.
2. TCM diagnosis refers to whole body systems such as meridians and involves the Kidney, Liver (Blood), Spleen, Heart and Lung systems, with excess or deficiency, heat or cold patterns in each. The fundamental treatment principle in TCM is to treat imbalance and

restore balance within these systems. Common TCM patterns identified in female infertility include Kidney Jing deficiency; Kidney Yin or Yang deficiency; Spleen Qi deficiency; Liver Blood deficiency; Liver Qi stagnation; Blood stasis; Heat, Cold, or Dampness; Heart fire; or Heart deficiency. It is important to note that in TCM different pathologies such as polycystic ovary syndrome (PCOS), endometriosis, recurrent miscarriage, and stillbirth may all have a similar or identical underlying TCM pattern (e.g., Kidney Heat), while one condition may have multiple underlying TCM patterns (e.g., PCOS might be due to Kidney Yang deficiency, Blood stasis, or Phlegm Dampness). As such TCM does not use one herbal therapy for one infertility diagnosis, but rather tailors the treatment to the individual patient's TCM defined imbalance.

3. TCM practitioners use a combination of symptoms and signs experienced during the menstrual cycle to determine the underlying Chinese medical diagnosis. As early as the 13th century it was recognized by Chinese practitioners that to cure infertility, one must adjust abnormal menstruation. Therefore TCM practitioners take a detailed history of menstrual characteristics (flow, clotting, color, pain) as indicators of particular pathologies, together with an assessment of basal body temperature and cervical mucus changes during the menstrual cycle. The infertility diagnosis may also be aided by examination of abnormalities in the circulatory pulse (e.g., weak or slow) and tongue (pale, red, and inflamed). For example, infertility caused by a Kidney Yang deficiency manifests itself with a deep, slow weak pulse, pale and swollen tongue, and scanty pale menstruation that is often irregular.
4. Herbal formulas used in TCM are tailored to target the patient's unique combination of symptoms rather than accounting for just the diagnosed medical condition (infertility). Generally a TCM herbal formula will contain at least four or more herbs, with each herb having a balanced role in concert with its co-partners. TCM formulations consist of the principal or main therapeutic herb ("emperor herb"), a supportive herb of the principal herb ("minister" or "deputy" herb), and a series of "assistant" herbs that support the overall action of the formula, together with "harmonizer" herbs that may counter any toxic effects of the main ingredients. A complete list of TCM herbal combinations used in the management of female infertility is contained in Table 7.6.
5. A meta-analysis of 40 RCTs with more than 4200 women has shown that TCM herbal therapy results in a significant increase in the probability of achieving pregnancy compared to traditional Western medicine alone (RR = 1.74, 95% CI: 1.56–1.94). TCM has been shown in trials to be efficacious in assisting ovulation, restoring tubal patency, reducing pain and aiding conception in the setting of endometriosis, improving embryo quality during IVF, and reducing miscarriage risk.
6. Like all medications, TCM herbal remedies have the potential to be harmful to women and their unborn children if used inappropriately. For this reason it is imperative that women do not self-medicate with TCM, but rather seek an expert opinion from a TCM practitioner. A list of TCM herbs contraindicated in those trying to conceive or who are pregnant is contained in Table 7.7.
7. In Chinese culture food is also evaluated in terms of Yin and Yang balance, with some foods considered Yin (cold, dampness), while others are more Yang (hot, dry). A balance of Yin and Yang foods is likely to protect the individual from illness. For example, seafood such as crab or squid is considered cold (Yin), so to prevent imbalance they should be consumed with a hot (Yang) food such as wine or the herb fennel.
8. In TCM, a diet rich in foods that support Kidney Jing is felt to be important for assisting fertility, especially improving oocyte health. Foods to boost Kidney Jing include chicken, duck, fish, oysters, eggs, nuts, seeds, milk, artichoke, oats, nettles, and seaweed. In addition, foods that can deplete Kidney Jing such as coffee, chili, black pepper, dry ginger, artificial preservatives, and sugar should be avoided where possible.

9. Finally, the correction of Kidney Yang deficiency with foods such as garlic, cinnamon, rosemary, onion, leek, cabbage, red fruits (cherries, raspberries and raisins), plus dark meats (venison, duck, goat) may also assist fertility.

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8 Nutritional and Dietary Interventions for Menopause

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CONTENTS

8.1 INTRODUCTION

Information from the World Health Organization suggests that by the year 2025 the number of people older than the age of 65 will be more than 800 million (approximately 10% of the world population) [1]. Of these 800 million people, the majority (>60%) will be women [1]. It has been suggested further that by the year 2030 there will be more than 60 million postmenopausal women in the United States alone and approximately 1.2 billion postmenopausal women worldwide [1–3]. Menopause is defined as the permanent cessation of menstruation (>12 months) resulting from the loss of ovarian follicular activity, and is a natural process of aging [4]. The median age at menopause in North America is 50.5–51.4 years; in Europe the age at menopause ranges from 50.1 to 52.8 years; in Latin American countries the age at menopause varies widely from 43.8 to 53 years depending on lifestyle; and in Asia it varies from 42.1 to 49.5 years [2,3]. As the median age at menopause in North America is 51 years, and the current life expectancy is approximately 80 years, most women will spend almost one third of their life post-menopause.

The prevalence of vasomotor symptoms also varies widely depending on the geographical area, and these symptoms range from 74% of women in Europe to 36–50% in North America, 45–69% in Latin America, and 22–63% in Asia [3]. Considering the large geographic variation in average age at

onset of menopause, plus the variation in symptom severity, it is possible that culturally associated differences in diet may play a role. This topic is discussed first, followed by a discussion of how diet and nutrition can influence disease processes that become more prevalent in the postmenopausal phase of women's life (cardiovascular disease, cancer, cognitive impairment).

8.2 THE ROLE OF NUTRITION AND LIFESTYLE FACTORS INFLUENCING THE ONSET OF MENOPAUSE

As previously mentioned, the median age at menopause varies significantly in various ethnic groups and countries, and may be linked to diet and lifestyle factors [2,3]. The reasons for these differences are pleotropic and include tobacco use, poor nutrition, and low socioeconomic status, which are all found to be associated with an earlier age at menopause [4–7].

The data for oral contraceptives (OC) use are conflicted, and no conclusion can be made [7]. Furthermore, the effects of diet and body mass index (BMI) on the age at menopause also appear to be important factors.

Investigations on the impact of dietary factors and nutrition on the onset of menopause are few, although the influence of the nutritional status of women on their age at menopause makes perfectly sound scientific sense. Much of the work on diet and menopause comes from studies in Japan [8,9]. One cross-sectional study involving 4186 Japanese women found that increased intake of fat, cholesterol, and coffee were significantly associated with an older age at menopause after controlling for age, total energy, parity, menarche age, and relative weight (odds ratios [ORs] for the highest amounts of fat, cholesterol, and coffee intakes were 0.78, 0.79, and 0.70, respectively, $P < 0.05$) [8]. Interestingly, postmenopausal women who entered menopause at a later age had a higher calcium intake than those who experienced menopause at early age [8]. In a follow-up study, these authors also showed that a high intake of green and yellow vegetables was inversely related to the age at menopause [9]. In a more recent study, these same authors investigated the impact of dietary fat and physical exercise on the age at menopause in 3115 Japanese women [10]. The results of this investigation confirmed their previous study, and found that high levels of physical activity and a high intake of polyunsaturated fat were significantly associated with a younger age at menopause. However, total fat, other types of fat, dietary fiber, soy isoflavones, and alcohol were not associated with the onset of menopause [10].

The influence of reproductive and dietary factors on the age at menopause was investigated as part of a follow-up to the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort in Heidelberg [11]. The study included 1009 menopausal women who were compared with 3798 nonmenopausal women. Data on reproductive history and diet were obtained to identify factors impacting the age at natural menopause. The results of this study demonstrated that increasing age at first full-term pregnancy and delayed beginning of regular menses were associated with a later onset of natural menopause. Smokers had a younger age at menopause [11]. High carbohydrate consumption and high intake of vegetable, fiber, and cereal products lowered the age at natural menopause. Women with higher intake of total fat, protein and meat increased the age at menopause [11]. Another study, involving 12,676 women ages 45–69, investigated socioeconomic, reproductive, and behavioral factors and the age at menopause in three urban populations in Central and Eastern Europe [12]. Using multivariate analyses, these researchers found that higher education, using vitamin and mineral supplements, and the use of oral contraceptives were associated with a later age at menopause, whereas smoking, abstaining from alcohol, and low physical activity were associated with earlier menopause. These factors and associations of age at menopause were fairly similar to that reported in other female populations [12].

A population-based prospective Shanghai Women's Health Study, involving 33,054 subjects, investigated the impact of dietary, lifestyle, and reproductive factors on age at natural menopause and reproductive span in Chinese women [13]. Dietary intake at baseline was assessed by food-frequency questionnaire. The results of this study showed that early menarche, younger age at first

live birth, older age at last live birth, longer duration of breastfeeding, and higher parity were associated with longer reproductive years. Higher BMI at age 20, mid-life weight gain, and leisure-time physical activity during adolescence and adulthood predicted later menopause and longer reproductive span ($P < 0.01$ for all). Total intake of calories, fruits, and protein was positively associated with later menopause ($P < 0.05$ for all) except for carbohydrates. Furthermore, long-term tea consumption predicted longer reproductive span ($P = 0.03$). Vegetable, fat, soy, and fiber intakes did not significantly affect reproductive span or age at menopause. Smoking was inversely associated with both early age at menopause and shorter reproductive span ($P < 0.01$) [13].

In a cross-sectional clinical trial involving 157 Turkish women aged 45–60, Aydin and colleagues investigated the association between various diet and lifestyle factors and age at menopause [14]. Using multivariate analyses, these researchers found that an early age at menopause was associated with low levels of lifetime sun exposure ($P \leq 0.0001$), heavy physical activity ($P = 0.0043$), calcium supplement use ($P = 0.0076$), diagnosis of hypertension ($P = 0.0093$), and extended use of oral contraceptives ($P = 0.0487$). Interestingly, for women who could recall if their mother's menopausal age was <50 or ≥50, sun exposure ($P < 0.0001$) was a better predictor of age at natural menopause than maternal menopausal age ($P = 0.0019$) [14].

In one cross-sectional study, higher alcohol and meat intake was related to older age at menopause. When compared with women who ate meat, vegetarians tended to have lower estrogen levels and an earlier onset of menopause [7]. A follow-up randomized prospective survey involving 1227 women (aged 47–51) used proportional odds regression analysis to identify factors that were independently predictive of age at menopause [15]. The results of this study showed significant univariate associations between menopausal status and age ($P < 0.001$), age at maternal menopause ($P = 0.006$), alcohol consumption ($P = 0.005$), and socioeconomic status ($P = 0.03$). Maternal age at menopause and alcohol consumption were significantly correlated with estradiol levels ($P = 0.02$ for both). Thus, moderate consumption of alcohol was associated with older age at menopause [15].

Overall, the age at natural menopause is dependent on a variety of factors including genetic, environmental, socioeconomic, reproduction, diet, and lifestyle [16]. Age at menopause is reduced by nulliparity, long-term vegetarian diet, and smoking, whereas a high fat intake, cholesterol, and caffeine increases it [16]. Age at menopause is an important risk factor for long-term morbidity and mortality, as delayed menopause is associated with increased risk of endometrial and breast cancer, and menopause at an earlier age enhances the risk for cardiovascular diseases and osteoporosis [16]. Although the correlation between diet and age at menopause has not been well investigated, studies to date suggest that high intake of total calories, fruits, and proteins increases the age at menopause, whereas a high polyunsaturated fat intake reduces it. The role of other dietary factors such as soy, total fat, saturated fat, red meat, and dietary fiber remains controversial. Lifestyle factors such as smoking and vigorous exercise are associated with early menopause, while moderate alcohol consumption delays it [16].

8.3 THE ROLE OF NUTRITION AND DIETARY SUPPLEMENTS IN THE MANAGEMENT OF THE ACUTE SYMPTOMS OF MENOPAUSE

In terms of menopausal symptoms, different ethnic and cultural groups have different clusters of symptoms and intensities of vasomotor symptoms [1–6]. For example, in North America, approximately 60–80% of women complain of vasomotor symptoms, and approximately 50% of these women report having night sweats as well [1–3,17]. A 2010 study suggested that vasomotor symptoms were higher in women of African American descent (46.5%) and Latinas (49%), as compared with Chinese-American women (29%) [3]. In comparison, the prevalence of vasomotor symptoms in Asia varies widely depending on cultural and ethnic background (there are >50 ethnic groups), with the prevalence reported at 10.2–38.5% [3]. For example, vasomotor symptoms are more frequently reported in Taiwan than on mainland China or in Hong Kong [5,6,18,19]. In Taiwan, approximately 38% of women report hot flushes and 18% report night sweats [5,19] whereas only 17.5% of women

in Southern China and 10–23% of women in Hong Kong report having hot flushes [6,18]. Although vasomotor symptoms of Asian women are less severe than those of US women, Asian women tend to have more vasomotor symptoms during the perimenopausal transition, as well as more back pain, headaches, and insomnia [5,6,18–21].

8.3.1 Impact of Soy Isoflavones on Menopausal Symptoms

A 2013 cross-sectional population-based study involving 5634 women (aged 45–58) evaluated the association between self-reported vasomotor symptom (VMS) and race/ethnicity in the United States, and then further correlated these differences with the ingestion of dietary soy isoflavones (genistein + daidzein) [22]. Of the 5634 subjects, 82.1% reported hot flashes ever and 73.1% reported night sweats ever, while 48.8% and 38.6% reported recent hot flashes or night sweats, respectively. As compared with Caucasian women, Chinese, Japanese, Vietnamese, other Asian (each $P < 0.001$), and Filipino ($P < 0.01$) women reported fewer hot flashes [22]. Asian women also reported less recent VMS ($P < 0.001$). Hispanic women were also less likely to report hot flashes ($P < 0.05$) or night sweats ($P < 0.001$). African American women more commonly reported hot flashes ever ($P < 0.05$) and recent VMS ($P < 0.05$). The study also showed that Chinese, Japanese, Vietnamese, Filipino, and other Asian women had a higher estimated dietary isoflavone intake than did other racial/ethnic groups; however, they also had a lower BMI than other groups [22]. The authors concluded that there was little evidence to support a variation in VMS due to soy isoflavone intake; however, the overall isoflavone intake was low and it is possible that higher levels of exposure are required to see an effect [22].

The lack of effect of isoflavones on VMS has also been confirmed in a systematic review and meta-analysis of the clinical randomized controlled trials (RCTs) on the subject [23]. This Cochrane review included 43 RCTs involving a total of 4364 women. Only five trials provided suitable data for pooling in a meta-analysis. From these studies no significant effect was demonstrated on the incidence of VMS in women using a red clover extract as compared with placebo. Four studies suggested that extracts with high concentrations of genistein (>30 mg) reduced the frequency of hot flushes. Other studies that used dietary soy, soy extracts, and other types of phytoestrogens found that these treatments reduced the frequency and severity of hot flushes and night sweats when compared with placebo, but most of these studies were small with a high risk of bias [23]. There was no study that showed data indicating that these treatments caused estrogenic effects when used for up to two years. Thus, the study concluded that to date there is no concrete evidence to support the use of phytoestrogen supplements for reducing the frequency or severity of hot flushes and night sweats in perimenopausal or postmenopausal women [23].

8.3.2 Impact of Black Cohosh on Menopausal Symptoms

Beyond soy isoflavones, the most commonly used dietary supplement for the treatment of VMS is black cohosh [24–28]. In the most recent Cochrane systematic review, 16 clinical RCTs involving 2027 women were evaluated for safety and efficacy of black cohosh (*Actaea racemosa*) for treating menopausal symptoms [28]. The clinical trials included comparisons with placebo, hormone therapy, red clover, and the selective serotonin reuptake inhibitor fluoxetine. Outcomes measured in these trials included vasomotor symptoms, vulvo-vaginal symptoms, menopausal symptom scores, and adverse effects. In the RCTs comparing black cohosh with placebo there was no significant difference in the frequency of hot flushes or in menopausal symptom scores. When black cohosh extracts were compared with conjugated estrogens (HRT), the HRT significantly reduced VMS and menopausal symptom scores as compared with black cohosh. Similarly, the evidence supporting the safety of black cohosh was inconclusive owing to poor reporting [28]. Interestingly, this systematic review and meta-analysis has been challenged, and a recently published reanalysis of all appropriate placebo-controlled clinical trials data suggests that there is a standardized mean difference of 0.385

in favor of black cohosh ($P < 0.0001$) [29,30]. However, regardless of the outcomes for efficacy, the safety of black cohosh has been a topic of significant controversy owing to the potential association between black cohosh and many cases of hepatotoxicity [31].

In 2008, regulatory agencies in Australia, Canada, and the European Union released statements regarding the "potential association" between black cohosh and hepatotoxicity [31]. This topic have been extensively reviewed; however, proving a direct causality for black cohosh and hepatotoxicity is difficult, as has been pointed out by various groups, and the outcomes of these reviews depend on the scale used to determine causality [31–34]. Using a more general scale such as the Naranjo scale, showing causality is possible [31–33], but when using a more liver-specific scale such as the Council for International Organizations of Medical Sciences (CIOMS, also known as the Roussel Uclaf Causality Assessment Method [RUCAM]) scale with modifications to review the case reports, causality is unlikely or excluded [34,35]. However, the authors of this study used a modified CIOMS scale that had not been previously validated [36]. The European Medicines Agency (EMEA) has stated that modifications of the established CIOMS scale were not validated, and the procedures for the assessment of possible hepatotoxicity were not feasible using this modified scale because nearly all cases would be rated "not assessable" according the proposed modifications (EMEA). Similarly, the conclusions of "unlikely or excluded" causality were not supported by evaluations from several other organizations, although they used different causality analysis approaches. Thus, the safety and efficacy of black cohosh for the treatment of VMS associated with menopause are inconclusive [36].

8.3.3 Use of Herbal Medicines for the Emotional Symptoms of Menopause

Psychological symptoms, including anxiety, depression, irritability, and nervousness, are commonly observed in menopausal women. Psychological symptoms are similar in Asian women as those observed in U.S. women and there is a significant decrease in the reported quality of life of postmenopausal women living in China [3,19]. Interestingly, data from Central America, specifically the Q'eqchi Maya of Guatemala, indicate that these women enter menopause at an average age of 46 [37]. These women report that anxiety (excessive worry) is one of their common complaints [37]. In semistructured interviews the most common menopausal symptoms experienced by Q'eqchi women were headache (87.5%), excessive worry (87.5%), and muscular pains (87.5%). Heart palpitations, depression and irritability were also commonly associated with menopause [38]. These women do seek treatments for menopausal symptoms, and use a variety of medicinal plants to treat these symptoms [37,38]. From a total of 47 medicinal plants used to treat women's reproductive health by the Q'eqchi, 12 plants were used to treat nervous conditions and VMS. For example, the rhizome of sarsaparilla (*Smilax domingensis*) or ginger (*Zingiber officinale*) is taken as a tea to ease hot flashes, while a tea of the leaves and flowers of hibiscus (*Hibiscus rosa-sinensis*) is used to treat nervous conditions. In addition, hot teas of chamomile (*Matriarca* sp.) and valerian (*Valeriana officinalis*) are used to relieve nervous complaints and insomnia [37,38].

Other herbal medicines used to treat the psychological symptoms of menopause include St. John's wort and soy isoflavones. In a 2009 study, equol, which is a metabolite of the soy isoflavone daidzein, was shown to have antidepressant and anxiolytic effects [39]. One randomized, double-blind, placebo-controlled trial assessed the effects of equol on menopausal symptoms including depression and anxiety [39]. This study involved 134 Japanese women (aged 40–59 years) who were randomly assigned to three groups: placebo ($n = 44$), 10 mg of equol per day (EQ-1; $n = 44$), and 10 mg of equol three times per day (EQ-3; $n = 46$) for 12 weeks. Other dietary isoflavone ingestion was limited to 20 mg/day. Participants completed menopausal symptom and Profile of Mood States questionnaires at baseline and after treatment. The results showed that efficacy was based on equol producer status (34.3% of women). The anxiety scores of equol producers were lower than those of nonproducers ($P < 0.05$). A significant reduction in premenopausal and perimenopausal/postmenopausal symptom scores were observed for anxiety, somatic, and total scores. After the EQ-3 treatment, equol nonproducers showed significant decreases from baseline in all menopausal

symptom scores except depression ($P < 0.01$). Compared with placebo, the EQ-3 group showed significant decreases in depression scores ($P < 0.05$), as well as significant decreases in Tension-Anxiety ($P < 0.05$), Depression-Dejection ($P < 0.05$), and Fatigue ($P < 0.01$) and increases in Vigor ($P < 0.05$) of the Profile of Mood States. These data suggest that for some women equol may be used to treat anxiety and depression associated with menopause [39].

In one double-blind, clinical RCT the combination of black cohosh and St. John's wort was tested to evaluate its efficacy in menopausal women with pronounced psychological symptoms [40]. The results of this study showed a reduction in the Menopause Rating Scale by 50% in the treatment group and 19.6% in the placebo group. The Hamilton Depression Rating Scale decreased by 41.8% in the treatment group and 12.7% in the placebo group, suggesting that the combination product was more effective in treating menopausal symptoms with a significant psychological component. There were no relevant group differences regarding adverse events, laboratory values, or tolerability [40].

Overall, data from published studies indicate that a significant percentage of the female population worldwide, regardless of ethnicity or culture, exhibit menopausal symptoms including anxiety, pain, hot flushes, night sweats, irritability, depression, and sleep disorders, which can lead to significant disruptions in the course of daily life and the quality of life. These symptoms lead women to seek medical attention and drug treatment; however, a better approach may be to emphasize diet and nutritional recommendations that may reduce some of the acute symptoms associated with the menopausal transition.

8.4 DIET AND THE PREVENTION OF POSTMENOPAUSAL CHRONIC DISEASES

8.4.1 Diet, Menopause, and Cardiovascular Disease

Beyond vasomotor symptoms, the postmenopausal period is accompanied by an increased incidence of more serious chronic diseases such as osteoporosis, dementia, cancers, and cardiovascular disease [1–3]. Cardiovascular disease (CVD) is the leading cause of mortality in women worldwide, and the risk of CVD in women increases dramatically after menopause [41–43]. Higher blood pressure, increased cholesterol levels, and BMI, as well as an increased risk of subclinical CVD, have been reported among women with hot flashes [44–48]. Published studies have suggested that diets having low intakes of saturated and *trans* fatty acids and higher intakes of unsaturated fatty acids, dietary fiber, and vegetables reduce the risk of some types of CVD, particularly coronary heart disease (CHD) [49–53]. Data generated from the Women's Health Initiative (WHI) Dietary Modification (DM) Trial, a clinical RCT of a low-fat (20% of energy) diet that included increased intakes of vegetables, fruit, and grains in postmenopausal women, indicated that reductions in saturated and *trans* fatty acids, as well increased fruit and vegetable consumption, lowered the risk of CHD, but overall, the trial was not conclusive [49]. A more recent study extended this work to measure whether a diet index that expanded beyond the total fat goals of the WHI-DM trial and beyond CHD risk and stroke reduced the risk of CVD and further assessed whether a diet with lower intakes of saturated fat, trans fat, and dietary cholesterol and higher intakes of fruit and vegetables and grains is associated with incidence of CVD and heart failure (HF) [54]. In this study, postmenopausal women (ages 50–79 years old at baseline) in the highest quintile of the DM score had a modest, yet significant, 12% reduction in risk of incident CVD. However, the risk of HF was not significantly lowered in women in the highest DMI quintile [54]. Because other dietary ingredients are known to contribute to CVD risk, these investigators also further incorporated a more conventional index, the Alternate Healthy Eating Index (AHEI), to measure dietary components that were not assessed by the DM score [54]. These included nut and soy protein intake, ratio of white to red meat, alcohol intake, and the ratio of polyunsaturated to saturated fats. Women having scores in the highest quintile of the AHEI were associated with a 23% lower risk of incident CVD and a 30% lower risk of HF [54]. The stronger association obtained with the AHEI indicates that there are food components in the AHEI that are not included in the DM that are important predictors of CVD

and HF risk. Thus, these data suggest that sound nutritional diets may potentially lower the risk of incident HF by lowering systolic blood pressure as well as lowering the risk of interim myocardial infarction and/or hypertension [54]. Taken together, this suggests that diet quality, or nutrient density, as well as dietary total and saturated fat are important risk predictors for incident for CVD and HF in postmenopausal women.

8.4.2 Diet and Supplements to Improve Carotid Artery Compliance Post-Menopause

Another clinically important change that occurs post-menopause is a reduction in the compliance of large elastic arteries within the cardiothoracic region, which contributes to a number of adverse age-associated changes, including increased aortic impedance, left ventricular hypertrophy, and reduced cardiovagal sensitivity [55]. Cardiovascular aging in women is delayed and usually occurs at a slower rate than in men during the premenopausal years; however, women quickly catch up with men during the postmenopausal period [56]. Possible mechanisms responsible for the acceleration in cardiovascular aging post-menopause are not entirely clear but may be due to the increase in oxidative stress. Markers of oxidative stress are higher and endogenous antioxidant defenses tend to be lower in many postmenopausal women when compared with premenopausal women [55]. One clinical trial assessed whether oxidative stress contributes to a reduction in large elastic arterial compliance in postmenopausal women [55]. Carotid artery compliance was measured during acute intravenous infusions of saline (baseline control), or supraphysiological doses of ascorbic acid in premenopausal ($n = 10$) and estrogen-deficient postmenopausal ($n = 21$) healthy sedentary women. Carotid artery compliance was 56% lower in postmenopausal women as compared with premenopausal women during baseline control ($P < 0.0001$). Ascorbic acid infusions increased carotid artery compliance by 26% in postmenopausal women, but had no effect in premenopausal women [55]. These results suggest that oxidative stress may be an important mechanism contributing to the reduced large elastic artery compliance of sedentary, estrogen-deficient postmenopausal women. This study suggested that the administration of antioxidants such as vitamin C may be useful in reversing arterial stiffness and thus reducing cardiovascular risk [55]. Interestingly, this same group of investigators also demonstrated that habitual exercise could improve atrial compliance as much as vitamin C but that the combination of the two was not synergistic or additive [57].

Another dietary or nutritional supplement product that may reduce CVD risk factors and in menopause is flaxseed [58]. Numerous clinical trials have reported on the positive effects of large doses of flaxseed (40–50 g per day) in postmenopausal women, with reductions in total cholesterol and low-density lipoprotein (LDL)-cholesterol and a reduction in inflammatory markers such as C-reactive protein (Table 8.1). Thus, the reduction of cardiovascular risk in postmenopausal women by flaxseed appears to be due to its ability to improve lipid profiles [58]. In animal studies, flaxseed supplementation of animal chow reduced body weight and LDL-cholesterol, increased high-density lipoprotein (HDL)-cholesterol, and reduced the thickness of the aorta, indicating that it may be used as a preventative measure in reducing modifiable risk factors for cardiovascular disease [59–61]. One animal study performed in ovariectomized golden Syrian hamsters found that animals treated with flaxseed had increased bile acid synthesis, and thus this may be one of the mechanism by which flaxseed exerts its hypocholesterolemic effects [62].

Other interventions for the reduction of CVD risk include fish oils and omega-3 fatty acids and the Mediterranean diet [63,64]. Some central features of a traditional Mediterranean diet are the use of olive oil as the principal component of fat; relatively high consumption of fruit, vegetables, fish, whole grains, legumes, and nuts; low or moderate consumption of meat and dairy; and moderate alcohol consumption with meals [63,64]. One recent Cochrane review of the clinical trials, involving 52,044 subjects showed reductions in total cholesterol and LDL, suggesting that limited data suggest some small reduction in CVD from following a Mediterranean diet [63]. The second large review of a cohort of 40,011 men and women ages 20–70 that stricter adherence to a Mediterranean style diet was inversely associated with total CVD and more strongly so with fatal

TABLE 8.1
Results from Clinical Trials Investigating the Effects of Flaxseed on Cardiovascular Risk Factors

Reference	Results	Study Details	Treatments
Arjmandi et al. [65]	↓ Total cholesterol in both treatment groups ↓ LDL cholesterol with flaxseed alone	Hypercholesterolemia PM women only DB, CR study	Four slices of bread and three muffins daily containing 38 g/day of flaxseed or sunflower seeds (n = 23)
Bloeden et al. [66]	↓ LDL cholesterol with flaxseed at 5 weeks but not sustained at 10 weeks ↓ HDL cholesterol with flaxseed only in men Flaxseed improves insulin sensitivity, no effect on markers of Inflammation	Hypercholesterolemia American adults (PM women and men) SB, PC controlled study 10 weeks duration	Low-fat and low-cholesterol diet Breads and muffins containing 40 g/day of ground flaxseed (men = 16; women = 14) or matched wheat bran control (men = 15; women = 17)
Coulman et al. [67]	No effect on plasma lipids, antioxidant markers (total antioxidant activity, LDL cholesterol or protein oxidation)	12-week treatment PM women (n = 16) >10 years post-menopause CR design 4-week treatment and 4-week washout	Food bars containing: Flaxseed (25 g) Sesame seeds (25 g) Flaxseed + sesame seeds (12.5 g each)
Dodin et al. [68]	↓ Total and HDL cholesterol with flaxseed Mild reduction in body weight and blood pressure	French Canadian women, 5 years post- menopause DB, PC design 12-month treatment	Two slices of bread plus powder daily containing (40 g/day total) Ground flaxseed (21,071 µg total lignans daily n = 85) or wheat germ (196 µg total lignans daily; n = 94)

Dodin et al. [69]	Modest ↓ apolipoprotein B and A-1 No effect on LDL, glucose, insulin, fibrinogen, and C-reactive protein	French Canadian women 5 years post-menopause DB, PC study 12-month treatment	Two slices of bread plus powder containing (total of 40 g/day) daily: Ground flaxseed (21,071 μg total lignans daily; $n = 85$) or wheat germ (196 μg total lignan daily; $n = 94$)
Jenkins et al. [70]	↓ Total and LDL–cholesterol apolipoprotein B and A-1 by flaxseed	Hyperlipidemia (men = 22; postmenopausal women = 7) DB, CR design	Four muffins daily containing 50 mg of partially defatted flaxseed meal and white flour wheat bran flour + whole meal flour
Lucas et al. [71]	↓ Total cholesterol and non-HDL cholesterol	DB, CR design 6-week treatment and 2-week washout SB, PC study 3-month treatment	40 g daily of ground flaxseed ($n = 20$) or wheat-based control ($n = 16$)
Patade et al. [72]	↓ Total and LDL cholesterol in both flaxseed groups No changes in C-reactive protein or hematological parameters	Mildly to moderately hypercholesterolemic Native American women	Daily consumption of control (two slices of white bread + two muffins; $n = 9$) or 35 g of flaxseed (two slices of bread + two muffins + 2 tbsp. flax powder; $n = 17$) 35 g of flaxseed + 8 g of oat bran soluble fiber (two slices of bread + two muffins + 2 tbsp. flax powder; $n = 16$)
Simbalista et al. [73]	No significant difference in lipid profile or menopausal symptoms with 25 g of flaxseed as compared with the control	Brazilian women 1–10 years post-menopause DB, PC study 12-week treatment	Two slices of bread daily containing flaxseed (25 g/day; 46 mg/day of lignan $n = 20$) or wheat bran <1 mg of lignin; $n = 18$)

Note: CR, crossover design; DB, double blind; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PC, placebo-controlled; PM, postmenopausal; SB, single blind.

CVD. Inverse associations were found for composite CVD, myocardial infarction, stroke, and pulmonary embolism. Alternate exclusion of components of the Mediterranean diet showed that the ingestion of moderate amounts of alcohol with meals contributed most to the inverse association between Mediterranean diet and CVD [64].

Since the 1970s, research on fish oil and the omega-3 fatty acids has received much attention due to the observation that Eskimos have a lowered CVD risk due to their high ingestion of fish [74]. Since then many investigations have shown that omega-3 fatty acids have anti-inflammatory, antiatherogenic, and antiarrhythmic effects, thereby explaining the mechanisms by which fish oil and omega-3s reduce cardiovascular risk. In a review of 11 double-blind, clinical RCTs involving 15,348 patients with a history of CVD, long-term use of high-dose omega-3s (1–6 g/day for 1–5 years) had no effect for all-cause mortality, but was statistically significant for reducing cardiac death (–32%), sudden death (–33%), and myocardial infarction (25%) [74].

8.5 NUTRITION AND DIETARY SUPPLEMENTS FOR POSTMENOPAUSAL OSTEOPOROSIS

Along with CVD, osteoporosis is a significant global public health problem that poses substantial economic burden on society and the families of patients who suffer from related fractures and have reduced functional independence [75]. Fractures occur most frequently at the hip and spine but can also affect the wrist. Vertebral fractures can also cause serious morbidity, including chronic pain and disability that lead to dependence. A recent review published in 2011 concluded that the highest hip fracture rates are currently in northern Europe and the United States, while Asian countries such as Kuwait, Iran, China, and Hong Kong show intermediate hip fracture rates [75]. However, because the demographics of world populations are changing, with more elderly living in developing countries, and it is estimated that by 2050 half of hip fractures will occur in Asia [75]. Fracture risk increases exponentially with age and bone loss accelerates after the onset of menopause in women. The primary determinants of age-related bone turnover include declining estrogen levels, poor nutrition, decreasing physical activity, and lack of sunshine (reducing vitamin D levels) [75].

8.5.1 Impact of Body Mass Index on Osteoporosis

The scientific studies suggest that BMI may have a positive or negative impact on osteoporosis [76,77]. In a 2014 study of 3296 overweight adults in the United States aged 50 or older, every unit increase in BMI was associated with a statistically significant increase of 0.0082 g/cm ($P < 0.001$) in bone mineral density (BMD) [76]. This positive association of BMI and BMD did not differ by age, sex, or race. A 10-unit increase in BMI would result in altering a person's osteoporotic BMD level to a normal BMD level; thus this study showed a protective effect between obesity and osteoporosis [76,77]. A number of mechanisms including the weight-bearing effect of excess soft tissue on the skeleton and the association of fat mass with the secretion of hormones (i.e., estrogens, leptin, and adiponectin) from the adipocyte and from other organs such as the gut (i.e., ghrelin, which stimulates growth hormone) and the pancreas (including insulin and amylin) explain the increase in BMD. Furthermore, the distribution of body fat influences circulating hormones and may also alter bone mass independently of obesity, in which visceral fat is associated with both higher bone mass and levels of estradiol [77].

Considering the large percentage of older adults who fall into the category of obese, if higher BMI has a significant impact on osteoporosis reduction, then the United States should have lower rates of osteoporosis. However, this is not the case. This may be explained by a recent study that suggests that aging may actually shift the composition of bone marrow by increasing adipocytes and osteoclast activities and decreasing osteoblast activity, thereby causing osteoporosis [78]. Adipocytes secret proinflammatory cytokines, and thereby may actually increase the risk of osteoporosis, not decrease it [78]. Because visceral fat is detrimental to bone mass, obese individuals with higher percentage of

extra visceral fat may not be protected from osteoporosis by their extra fat, even though they have a high BMI. Measurements of body composition may be more helpful in understanding how high BMIs are associated with bone mass and my explain some of the inconsistent findings in the literature. Thus, there are two conflicting aspects of obesity on bone mass, the first one being a positive weight-bearing of a heavier load on BMD and the second the detrimental aspects associated with fat mass [76–78]. Thus, in terms of high BMI and osteoporosis, the scientific and medical literature is still conflicted.

This being said, low BMI is much more of a problem in aging, particularly in women who are widowed, smoke, drink alcohol, and have a poor diet [78–81]. In the population-based Tromsø Study recently published in Norway, BMD was measured in 2580 women and 2084 men aged 30–80 years for a 2001–2002 survey, and then repeated in 1401 women and 1113 men for a 2007–2008 survey [80]. The results of this study show that from 40 years to 80 years of age, BMD loss rates ranged between 4% at the total hip and 14% at femoral neck for nonsmoking, physically active men with a BMI of 30 kg/m^2 to approximately 30% at both femoral sites in heavy smoking, physically inactive men with a BMI value of 18 kg/m^2 [80]. These results were worse in women, in whom the BMD loss rates were greater than 30%, with a lower BMI value of 18 kg/m^2. Thus, the impact of the BMI was high for BMD, especially in the oldest age groups. For example, an 80-year-old man with a moderate BMI to slightly overweight, non-smoker with 4 hours of vigorous physical activity per week through his whole adult life would have 1–2 standard deviations higher BMD levels as compared with a low-BMI, inactive smoker [80]. Thus, in terms of BMI, it is recommended that normal or slightly higher but not obese BMI be maintained to prevent loss of BMD.

8.5.2 Impact of Alcohol on Osteoporosis

Bone mass peaks at the age of 35, at which women lose BMD at a slow pace. However, after menopause BMD loss increases rapidly [82]. There is some suggestion that moderate alcohol consumption (one drink for women or one or two drinks for men with a meal) may protect bone, while chronic heavy drinking during young adult years can significantly reduce bone quality and increase the risk of osteoporosis [82]. Alcohol's effect on bone mass cannot be reversed even after cessation of alcohol consumption. Alcohol is also a calorie-rich drink that impacts physical activity, physical stability, appetite, and fat metabolism [83,84]. One study investigated the association between alcohol consumption and components of body composition including bone, fat, and lean tissue [84]. Higher alcohol intake was associated with higher central adiposity and reduced bone quality in men [84].

8.5.3 Impact of Calcium and Vitamin D on Osteoporosis

Proper nutrition is one of several important modifiable factors for optimal bone health and prevention of osteoporosis, especially in women with a familial history of the disease. Indeed, the role of calcium and vitamin D for improving BMD and reducing fracture risk is well known. But other studies have also shown that diets high in fruits and vegetables have beneficial effects on bone mineral status and that nutrients and vitamins, including vitamin K, vitamin C, phosphorus, potassium, magnesium, protein, and sodium, are important for the maintenance of optimal bone health [85,86]. Calcium is important for bone health throughout a life span, while vitamin D is necessary for calcium absorption in the intestines and thus essential for maintaining calcium homeostasis [85]. The ability of the skin synthesize vitamin D decreases with age, causing problems with bone formation and also muscle strength. Osteoporosis is prevalent in older postmenopausal women, and thus it is important for women to understand that they must manage this problem with a well-balanced diet, adequate calcium and vitamin D intake, adequate exercise, smoking cessation, avoidance of excessive alcohol intake, and prevention of falls. It is well known that a low calcium intake and poor vitamin D status are key factors in increasing the risk of osteoporosis and fractures [86]. Thus, supplementation of both calcium and vitamin D is critical for the prevention and treatment of osteoporosis and osteoporotic fractures, and 1200–1500 mg of calcium per day and at least 400–800

IU/day of vitamin D (the dose that was used in the fracture prevention studies to date) have been recommended [86]. Treatment improves BMD and prevents falls [86]. Where other pharmacologic therapy is required, bisphosphonates, selective estrogen-receptor modulators, parathyroid hormone, estrogens, and calcitonin are commonly used.

8.5.4 Impact of Vitamins and Antioxidants on Osteoporosis

Beyond calcium and vitamin D, other foods, vitamins, and dietary supplements have been investigated for their effect on estrogen-dependent bone loss. Many studies have shown that ingestion of vitamin B complex and vitamins C, E, and K all correlated positively with improved BMD at multiple skeletal sites and/or were associated with reduced risk of fracture, independent of BMD [87]. These nutrients may impact bone strength in a variety of different ways, not just by affecting bone density [87]. For example, one of the modifiable risk factors for osteoporosis is the elevated homocysteine (HC) levels. Increased plasma HC concentrations are caused by a defect in the metabolism of intracellular HC, which is caused by genetic factors or nutritional deficiencies. Vitamin B, including B_2 (riboflavin), B_6 (pyridoxine), B_{11} (folate), and B_{12} (cobalamin), serve as cofactors for the enzymes involved in HC metabolism, and a lack of these vitamins can increase HC levels, thereby increasing the risk of osteoporosis [87]. In addition, vitamin C is necessary for the hydroxylation of lysine and proline, which are involved in the formation of stable collagen triple helixes and therefore normal bone development. Other studies suggest that vitamin C stimulates alkaline phosphatase activity and is required for the formation of type I collagen matrix as well as for the expression of osteoblastic markers and mineralization [87]. In rodent models, a diet low in vitamin C resulted in reduced femur calcium and hydroxyproline contents, low collagen formation, low femoral bone density, and abnormal cartilage growth morphology of the proximal tibial metaphysis. Antioxidants such as vitamin C and E vitamin E have antiosteoporotic activities and improved the trabecular structural and cellular properties in different animal models subjected to ovariectomy, orchidectomy, thyroidectomy, and oxidative stress. There is a link between increased oxidative stress and reduced BMD in humans. In one study, vitamin E supplementation prevented osteoclast formation and bone loss in elderly men by reducing RANKL inductions in osteoblasts and c-Fos expression in osteoclast precursors [87]. Another observational study involving 533 postmenopausal women showed that the length of use of vitamins C and E and other antioxidants negatively correlated with serum C-telopeptide, a marker of bone resorption, thus suggesting that antioxidant vitamins may suppress bone resorption in humans [87]. In a Swedish mammography cohort, low ingestion of vitamins C and E increased the risk of hip fracture in smokers [88]. Furthermore, a large case-control study that included data on hip fractures in more than 1000 male and female patients aged 50 years or older found an inverse dose–response association between intake of vitamin E and risk of hip fracture. This association was found in current and ex-smokers but not in never-smokers [89].

In addition to other vitamins, vitamin K, present in leafy green vegetables, may have a protective effect on age-related bone loss [90]. This activity appears to be mediated through the vitamin-K-dependent γ-carboxylation of osteocalcin, a protein in bone that is a marker for bone metabolism. Reviews of the vitamin K studies have shown that low circulating levels and/or low dietary intake of vitamin K are associated with low bone density and with increased fracture risk in humans [90]. Thus, overall a healthy diet rich in fruits and vegetables and adequate nutrition are essential for preserving bone mass and preventing osteoporosis.

8.5.5 Impact of Soy and Isoflavones on Osteoporosis

Soy protein, milk, and isoflavones, as well as green tea, have been tested for their effects on bone mineral density in postmenopausal women [91–93]. However, conflicting data for soybean proteins and isoflavones have been reported in RCTs, with only 3 of 15 studies showing any effect on BMD in postmenopausal women (Table 8.2).

TABLE 8.2
Clinical Trials for Soy and Soy Isoflavones and Bone Mineral Density (BMD)

Reference	Study Details	Isoflavone Treatments	Results	BMD Results	
				Spine	Hip/Neck
Alekel et al. [94]	RBC *n* = 224 postmenopausal women Age 46–65 USA	Three groups: 80 mg/day or 120 mg/day of soy isoflavones or placebo; 3 years	No difference between placebo and the two soy treatment groups over time	NS	NS/ 120 mg/day better than placebo at femoral neck
Arjmandi et al. [95]	RBC *n* = 87 Postmenopausal women Age <65 years Italy	Two groups: 60 mg/day of soy isoflavones or placebo; 1 year	No difference between isoflavones and placebo	NS	NS
Brink et al. [96]	RBC *n* = 237 Early Postmenopausal Women Italy, France and The Netherlands	Two groups: 110 mg/day of soy isoflavones or placebo; 1 year	No significant difference between treatment and placebo	NS	NS
Chen et al. [97]	RBC *n* = 203 Early postmenopausal women Age 48–62 years Hong Kong	Three groups: 40 mg/day or 80 mg/day of soy isoflavones, or placebo; 1 year	No significance difference between placebo and the two soy treatment groups over time	NS	NS
Kenny et al. [98]	RBC *n* = 131 Postmenopausal women Age >60 years USA	Four groups: Soy protein + 105 mg of isoflavones, soy protein + placebo, control protein + 105 mg of isoflavones, control protein + placebo	No significant difference between groups	NS	NS
Kreijkamp-Kaspers et al. [99]	Randomized study *n* = 202 Postmenopausal women Age 60–75 years Holland	Two groups: 25.6 g of soy + 99 mg of isoflavones or milk protein as a powder; 1 year	No significant difference between groups	NS	NS

(*Continued*)

TABLE 8.2 (CONTINUED)
Clinical Trials for Soy and Soy Isoflavones and Bone Mineral Density (BMD)

				BMD Results	
Reference	**Study Details**	**Isoflavone Treatments**	**Results**	**Spine**	**Hip/Neck**
Levis et al. [100]	RBC *n* = 248 Postmenopausal women Age 45–65 USA	Two groups: 200 mg/day of soy isoflavones or placebo; 2 years	No significant difference between groups	NS	NS
Lydeking-Olsen et al. [101]	RBC *n* = 107 Postmenopausal women Age <75 years Denmark	Four groups: Soymilk + 76 mg isoflavones, progesterone, combination, or placebo; 2 years	Significant treatment effects for soymilk	Treatment significant	NS
Marini et al. [102]	RBC *n* = 389 Postmenopausal women Age 49–67 Italy	Two groups: Genistein 54 mg or placebo; 2 years	Significant treatment effects for genistein	Treatment significant	Treatment better than placebo
Morabito et al. [103]	RBC *n* = 90 Women Age 47–57 Italy	Three groups HRT genistein 54 mg/day or placebo; 1 year	Significant treatment effects for genistein	Treatment significant	Treatment significant

Newton et al. [104]	RBC *n* = 99 men and 16 women Age 50–80 years USA	Two groups: Soy isoflavones 83 mg/day or control group (3 mg isoflavones); 1 year	No significant difference between groups	NS	NS
Tai et al. [105]	R, DB, PC *n* = 431 Postmenopausal women Age 45–65 Taiwan	Two groups: 300 mg/day of isoflavones (aglycone equivalents) (172.5 mg of genistein + 127.5 mg of daidzein) or placebo plus 625 calcium and 125 IU vitamin D; 2 years	No significant difference between groups	NS	NS
Vupadhyaula et al. [106]	R, DB *n* = 203 postmenopausal women Age 55–70 USA	Three groups: Soy protein, no isoflavones, milk protein; control—soy protein + 90 mg isoflavones; 2 years	No significant difference between groups	NS	NS
Wong et al. [107]	R, DB, PC *n* = 403 Postmenopausal women Age 45–65 USA	Three groups: 80 mg of isoflavones, 120 mg/day of isoflavones, or placebo; 2 years	BMD for total body (120 mg/day) significantly better than placebo NS for 80 mg	NS	NS

Note: DB, double blind; NS, not significant; PC, placebo controlled; R, randomized; RBC, randomized, blinded, and controlled.

Interestingly, dried plums (aka prunes) have also been investigated for their effect on BMD in postmenopausal women [108,109]. In the first study, researchers determined if the addition of dried plums to the diets of postmenopausal women positively influenced markers of bone turnover [37]. Fifty-eight postmenopausal women were randomly assigned to ingest either 100 g of dried plums or placebo. The results of this study showed that in comparison with corresponding baseline values, ingestion of dried plums significantly increased serum levels of insulin-like growth factor-I (IGF-I) and bone-specific alkaline phosphatase (BSAP) activity, both of which are associated with greater rates of bone formation. Serum and urinary markers of bone resorption, however, were not affected [109]. In a follow-up study, the extent to which dried plum reverses bone loss in osteopenic postmenopausal women (n = 236) was investigated [108]. Qualified participants (n = 160) were randomly assigned to one of the two treatment groups: dried plum (100 g/day) or dried apple (negative control). The women also received supplements of 500 mg Ca plus 400 IU (10 mcg) of vitamin D daily. BMD of lumbar spine, forearm, hip, and whole body was assessed at baseline and at the end of the study using dual-energy x-ray absorptiometry [108]. The results of the study showed that ingestion of dried plums significantly increased BMD of ulna and spine in comparison with dried apple. In comparison with corresponding baseline values, only dried plum significantly decreased serum levels of bone turnover markers including BSAP and tartrate-resistant acid phosphatase-5b. These results suggest that dried plum (prunes) may be helpful for in improving BMD in postmenopausal women, in part due to suppressing the rate of bone turnover [108].

8.5.6 Nutritional Supplements for Cognitive Decline in Menopause

The transition into menopause has been associated with an increase in forgetfulness and an increased risk of dementia [110–113]. Some of these associations have come from observed gender differences in Alzheimer incidence and prevalence and the fact that women with Alzheimer's disease have disproportionate difficulty with specific cognitive tasks, suggesting a possible relation between sex hormones and Alzheimer's disease [110]. Over the past 20 years, many clinical studies have examined the association between the use of estrogen-containing hormone therapy (HT) and the risk of developing Alzheimer's disease. Before the WHI [112–114], meta-analyses linked HT to reductions in Alzheimer risk of approximately 40% [111]. The neuroprotective effects of estrogens have been consistently demonstrated *in vitro* and *in vivo*; however, findings from human studies have been inconsistent. Analysis of these studies has suggested that estrogens may have positive or detrimental effects on the brain depending on the type of menopause (natural vs. surgical) and the age at the time of treatment [110]. Some studies have shown protective effects of estrogens in women who were treated early post-menopause (ages 50–60 years); however, the WHI showed that women (aged 65–79 years) who started an estrogen or combination progesterone therapy had an increased risk of dementia and cognitive decline regardless of the type of menopause [113]. The WHI involved a large observational cohort and two parallel clinical trials that were stratified by hysterectomy status and used a partial factorial design [113]. There were three randomized interventions: low-fat diet, hormone therapy (conjugated estrogens with or without medroxyprogesterone acetate, depending on hysterectomy status), and calcium plus vitamin D dietary supplements [112,113]. Participants in the dual trials were relatively healthy community-dwelling postmenopausal women aged 50–79 years at baseline (mean age 63 years). In a supplementary study (WHI-Initiative Memory [IM] study) was a double-blind controlled trial among women in the WHI hormone therapy trials who were at least 65 years of age [114]. The objective of the WHI-IM was to determine the incidence of "all-cause dementia," and women who scored below screening cut points underwent further neuropsychological and diagnostic testing. Mild cognitive impairment was a secondary outcome. In the studies, 108 cases of incident dementia were reported, of which 50% were diagnosed as Alzheimer's disease, but results for Alzheimer's disease were not reported separately. The dementia rate was increased in women allocated to active treatment [112–114]. The hazard ratio was approximately doubled for women in the estrogen-plus-progestogen group and increased by about half for women in the

estrogen-alone group [114]. The difference was significant in the E + P group but not in the E alone group [112,113]. Since this study, several other clinical trials have shown no effect of estrogens alone on cognitive function in older women [110–111].

Although older women may not benefit from HT for cognition, there are differences in the cognitive response to HT depending on women's age or timing of HT initiation [112]. It has been suggested that HT administered at or around the time of menopause may elicit improved cognition, while HT initiated ≥5 years after menopause has no effect. Some studies have shown a positive association between early initiation of HT and cognition, improved memory, and hippocampal function [110–114]. However, support for this "critical window theory" is limited, and more recent studies suggest no effect of time since menopause on HT therapy and cognition [110–114].

Owing to the increased health risks associated with long-term HT use, menopausal women are now looking for alternative therapies to alleviate symptoms such as memory loss and cognitive decline. These alternatives include soy, red clover, isoflavones, *Ginkgo biloba*, black cohosh, and *Gingko*/ginseng combinations [115]. One recent systematic review of the clinical trials for these botanical dietary supplements and memory has recently been published [115]. For the review, the authors included all randomized, placebo-controlled trials that lasted for at least 6 weeks, and used validated measurements to determine performance at memory and cognitive function tests. A total of 12 RCTs were included involving a total of 971 postmenopausal women (aged 46–76). Of all of the supplements tested, only soy showed some improvements in short- and long-term memory, but no effects on cognition tasks, and several large studies showed no effect [115]. In addition, most of the studies suffered from methodological flaws that may have impacted the study outcomes [115]. In the recent Women's Health Across the Nation Phytoestrogen Study [116], longitudinal analysis of data from various ethnic groups found that Asian participants ingested 10–25 times the amounts of coumestrol and isoflavones than did non-Asian participants. During the late perimenopause and postmenopausal phases, Asian women with the highest isoflavone intakes did better on processing speed, but during early perimenopause and post-menopause, high-isoflavone Asian consumers performed worse on verbal memory. The highest isoflavone consumers in non-Asians had lower verbal memory scores during early perimenopause. A verbal memory benefit of higher dietary lignan (flaxseed being one example of a food containing lignans) consumption was apparent only during the late perimenopausal phase, when women from all ethnic/racial groups who were in the highest percentage intake showed a slight advantage [116].

Interestingly, one meta-analysis of RCTs was performed to determine if multivitamins could be used efficaciously to improve cognition [117]. Meta-analysis was performed on those cognitive tests that were used across the largest number of studies. Randomized, placebo-controlled trials were included if they reported on the chronic effects (≥1 month) of oral multivitamin supplementation on any valid cognitive outcome, and 10 RCTs were included in review (n = 3200). Meta-analysis indicated that multivitamins were effective in improving immediate free recall memory, but not delayed free recall memory or verbal fluency. The study concluded that multivitamins were found to enhance immediate free recall memory but no other cognitive domains [117]. Other systematic reviews of vitamin B_6, vitamin B_{12}, folic acid, and vitamin B_{12} or omega 3-fatty acids have failed to find direct evidence of the effect of these nutritional supplements on cognition function [118–122].

8.6 DIETARY PATTERNS AND CANCER

Breast cancer is one of the most commonly diagnosed cancers among women in Western countries, although advances in early detection and treatment have led to high 10-year survival rates [123]. Recommended lifestyle and dietary changes for reducing breast cancer risk include maintaining a healthy lifestyle by reducing alcohol consumption, following a low-fat diet enriched with fruits and vegetables, exercising, and reducing weight if obese or maintaining a normal weight [124]. Breast self-examinations, clinical breast examinations, and screening mammography are all important for the early detection of breast cancer. The preemptive use of antiestrogenic drugs such as tamoxifen

or raloxifene should be reserved only for those women at an increased risk for the development of breast cancer, as there is a significant increased risk of cardiovascular events and hypertriglyceridemia in women using tamoxifen [124]. In terms of phytochemicals from fruits and vegetables, preclinical and clinical studies have suggested that their daily consumption reduces the risk of several cancers by inducing detoxifying and antioxidant enzymes, by regulating inflammatory/proliferative signaling pathways, and by inducing apoptosis [125].

It is estimated that as much as 20% of all cancers are associated with obesity or excessive weight, especially in postmenopausal women [126–128]. Obesity has been associated with chronic low-grade inflammation in the adipose tissue, liver, and heart that is accompanied by increased oxidative stress [126]. This excessive oxidative stress leads to an increase in the expression of proto-oncogenes, which in turn leads to the enhance transcription of pro-inflammatory and cell-cycle regulatory genes that lead to cancer. Chronic inflammation can enhance the initiation and progression of cancer [126]. Weight loss and a diet high in fruits and vegetables have been shown to reduce inflammation in obese patients and greatly suppress inflammation and cancer-related gene expression [126]. These data suggest that obesity induces a state of inflammation and that diet-induced weight loss reduces this inflammatory state, thereby lowering cancer risk. It has been suggested that with simple modification of the US diet, maintaining normal body weight, and performing regular physical activity, as much as 30–40% of cases of cancer could be prevented [127]. Just increasing the servings of fruits and vegetables daily may prevent as much as 20% or more of all cases of cancer and prevent 200,000 cancer-related deaths annually [126]. The recommendations from the American Cancer Society for cancer prevention are to eat a healthy diet that is low in red meat, with five to seven servings of fruits and vegetables per day and no more than one alcoholic drink (indicating a limit) per day for women, get sufficient exercise, avoid tobacco, and maintain a healthy weight throughout the life span, including menopausal women.

Although the studies of cancer prevention have strongly suggested that a healthy diet can play a role in reducing cancer risk, observational studies have indicated that there is not a strong independent role for dietary pattern after the prognosis of breast cancer. A 2009 analysis of the Women's Intervention Nutrition Study (WINS) and the Women's Health Eating and Living (WHEL) study, two large RCTs, examined the diet versus cancer question in postmenopausal women [123,129]. Both studies significantly reduced energy from fat and the WHEL study introduced large increases in vegetables, fruit, and fiber. WINS examined postmenopausal women only and reported no significant improvement in prognosis for women in the intervention group. The WHEL study reported a lack of association between diet and cancer prognosis. However, a secondary analysis suggested that the dietary intervention reduced distal recurrences among the subgroup without hot flashes at baseline [123]. The report concluded that there is no convincing evidence that changing dietary pattern after breast cancer diagnosis will improve prognosis for most women with early stage breast cancer. However, it may be important for some subgroups, but this would require further study [123].

8.7 KEY POINTS SUMMARY

1. The median age of Western women at the onset of menopause is 51 years, meaning that based on current life expectancy these women spend nearly a third of their lives postmenopause. Menopause is associated with the potential for significant debilitating symptoms (hot flashes, anxiety, depression, muscular aches, sexual difficulties, and headaches), plus a sharp rise in significant health problems such as loss of bone mass, cardiovascular disease (CVD), and cancer. Given the large proportion of the female population experiencing menopause, and its significant implications on their health, it is important to understand nutritional influences that may modify both the age at onset of the menopause and positively influence symptoms and diseases associated with the menopause.
2. A wide variation in the median age at onset of menopause exists between different ethnic populations, suggesting the possibility that lifestyle factors such as diet may influence the

onset of menopause. The median age at menopause in Caucasian populations is 51 years, but is only between 42 and 49 years for Asian women. Although some aspects of studies investigating the role of diet and onset of menopause are contradictory, the majority of studies suggest that a diet high in fat and protein delays the onset of menopause, even when controlling for body mass index (BMI). Furthermore, a moderate consumption of alcohol also delays menopause. Conversely, a vegetarian diet, or the intake of large amounts of vegetables, fiber, and cereal products has been linked with an earlier age at menopause. High BMI at 20 years of age, mid-life weight gain, and socioeconomic status plus education are all positively correlated with age at onset of menopause, while exercise intensity and smoking status are negatively correlated with age at onset of the menopause.

3. The nature and severity of menopausal symptoms varies among different ethnic populations, also raising the possibility of dietary influences. Vasomotor symptoms (hot flashes) are more common among Caucasians, less frequent among Latinos and African Americans, and least common among Asian women. One proposed explanation for this observation is that Asian women consume the largest amount of estrogenic soy isoflavones in their diet. Although isoflavone compounds such as red clover extract are marketed for the treatment of hot flashes, randomized controlled trials (RCTs) to date do not shown any evidence of a positive effect.
4. Many different dietary supplements are suggested as effective treatments for menopausal symptoms, with varying degrees of evidence supporting their use. Black cohosh is commonly used, yet the majority of RCTs do not show it to reduce hot flashes, and some evidence suggests that it may actually be harmful, causing liver damage. The combination of black cohosh and St. John's wort has been shown to reduce psychological symptoms associated with menopause in one RCT. Furthermore, another RCT using the isoflavone daidzein derivative equol found that equol was an effective treatment for reducing menopause-related depression and anxiety symptoms. Finally, traditional therapies for the psychological symptoms of menopause include sarsaparilla, ginger, hibiscus, chamomile, and valerian extract, yet the scientific evidence for their utility is incomplete at present.
5. Epidemiological studies show that women have lower rates of CVD than men, yet these differences narrow post-menopause, suggesting a role for ovarian hormones in the prevention of atherosclerosis. Oxidative stress is one potential mechanism for these observations, as the levels of oxidative stress rapidly increase after menopause, while infusion of antioxidant compounds such as vitamin C has been shown to improve cardiovascular function (improved arterial compliance) in postmenopausal women. This may help explain why a diet high in antioxidants such as fruits and vegetables may lower CVD in postmenopausal women. Furthermore, as both inflammation and adverse lipid profiles (high cholesterol/low-density lipoprotein [LDL], low high-density lipoprotein [HDL]) accelerate atherosclerosis, diets with anti-inflammatory activity (Mediterranean diet high in fish, olive oil, whole grains, and nuts) or that improve lipid profiles (omega-3 fish oils, flaxseed) have been shown to reduce CVD. Therefore, to minimize the increased risk of CVD postmenopause, women should maintain a healthy BMI and reduce their intake of fat, while increasing their intake of fish, fruits and vegetables, whole grains, legumes, and nuts.
6. Bone mineral density peaks in early adulthood, but then rapidly declines from the onset of menopause, reflecting the important role that estrogen plays in bone homeostasis. In general high BMI protects bone density (increased estrogen production by fat tissue, stimulation of bone formation by physical sheer forces), while a BMI below 18 kg/m^2 is associated with an increase in the incidence of low bone density (osteoporosis). Because osteoporosis can lead to painful, debilitating, and possibly even fatal fractures of the hip, spine, and other bones, it is important to understand how diet influences bone density in postmenopausal women. Moderate alcohol (one drink per day) and a diet rich in calcium, vitamin C and E, vitamin K, phosphorus, magnesium, and protein all positively influence

bone density, while heavy alcohol consumption reduces bone density. Therefore a diet rich in fruits, vegetables, and dairy products benefits bone density. The mechanisms behind this protective effect include minerals assisting the development of bone matrix (calcium, phosphorus), the production and cross linking of bone collagen (vitamin C), a reduction in harmful homocysteine levels (vitamins B_2, B_6, B_{12}, and folate), and antioxidant action (vitamins C and E suppress bone reabsorption). Vitamin D, the "sunshine vitamin," is predominantly produced in the skin and plays a vital role in calcium absorption and maintenance of bone density. As women get older, they generally reduce their sun exposure, and in addition their skin becomes less efficient at making vitamin D, resulting in many postmenopausal women becoming vitamin D deficient. Therefore it is generally recommended that women with low bone density should receive supplementation with 1200 mg of calcium and 800 IU of vitamin D per day to aid bone development. Dried plums (prunes) have also been shown in an RCT to reduce bone resorption while increasing bone density. The majority of studies do not support the ability of isoflavones (soy, red clover) to reduce the rate of loss of bone density in menopause.

7. Cognitive decline (poor memory, slow cognition, and dementia) becomes more common during menopause, with evidence that estrogen deprivation plays a significant role in the early perimenopausal phase of cognitive decline. The ingestion of high levels of estrogenic isoflavones (either as a supplement or a high soy diet) in early menopause has been shown in some trials to improve cognitive performance (improved memory, processing speed), but has no long-term benefits on the incidence of dementia. Multivitamin supplements may also boost short-term memory function, but they also have no long-term benefits. Other menopausal supplements such as *Gingko biloba*, black cohosh, ginseng, and fish oil have not been shown to offer any significant benefit in terms of reducing menopausal cognitive decline.
8. The incidence of cancer increases significantly in postmenopausal women, making cancer prevention a primary concern for this group. Epidemiological studies suggest that the inflammatory and oxidative stress environment created by obesity and poor diet plays a significant role in initiating the onset of cancer. The American Cancer Society suggests that eating a healthy diet (five to seven servings of fruits and vegetables per day, low intake of red meat, no more than one alcoholic drink per day), exercising regularly, avoiding tobacco, and maintaining a healthy weight throughout life will significantly reduce a woman's chances of getting cancer.

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9 The Effect of Obesity on Male Reproductive Function

Nathalie Sermondade, Charlotte Dupont, Celine Faure, and Rachel Levy

CONTENTS

9.1 INTRODUCTION

Infertility affects about 15% of couples who seek to attain a pregnancy. Many factors related to lifestyle and environment (drugs, alcohol, tobacco, pollution, heat exposure, diet, physical activity) are likely to influence female or male fertility. Among those factors, a recent and growing interest has been placed on weight and nutritional status, particularly as an increasing prevalence of overweight/obesity is observed among individuals of reproductive age. However, although there is a rather good consensus and abundant documentation on the impact of obesity on female reproductive function, the literature on male reproductive function is much younger and controversial. An association between weight and male reproductive function could be reversible, at least partially, through diet and/or exercise.

9.2 OBESITY AND ITS EFFECT ON MALE FERTILITY

Avicenna was probably the first to refer to a link between male obesity and male infertility in his "*The Canon of Medicine*" (Ibn Sina's Al-Qānūn fī al-ţibb, late 10th century). However, this association was largely ignored until the recent "epidemic" of obesity worldwide. The number of studies evaluating the impact of obesity on male fertility has since then multiplied, although they are still a source of controversy.

9.2.1 Results of Epidemiological Studies

Three large-scale epidemiological studies have examined the relationship between body mass index (BMI) and male fertility.

The first publication [1] reported a study of secondary data extracted from *The Agricultural Health Study*, which analyzed 52,395 men handling pesticides ("pesticide applicators certified") in North America, as well as their 32,347 spouses. Among them, 1329 couples met the inclusion criteria, including BMI data available for both partners. Infertility was defined as a wish to conceive for more than 12 months during the previous 4 years, whatever the outcome was (pregnancy or not). A link between male BMI and the couple's infertility was highlighted (odds ratio [OR] = 1.12, 95% confidence interval [CI]: 1.01–1.25) after adjustment for male and female age, female BMI, tobacco and alcohol use, and exposure to pesticides. There was a dose–response relationship, with a plateau for BMI higher than 32. Although this study could be criticized (a small fraction of the initial population included, only one BMI taken into account during a 4-year study, high incidence of infertility, not fit for modulating the effects of exposure to pesticides in BMI), it had the merit of exposing the potential link between obesity and male infertility.

The following publication reported data extracted from *The Danish National Birth Cohort* interrogating 100,000 pregnant women. A total of 49,957 couples were included because BMI of father and time to conceive were available, without any female infertility factor [2]. Subfertility was defined as a time to conceive longer than 12 months. After adjustment for maternal BMI and age, an increase of subfertility was demonstrated for both overweight men (OR = 1.15, 95% CI: 1.09–1.22) and obese men (OR = 1.49, 95% CI: 1.34–1.64). The main limitation of this study is that it considered only couples who attained a birth, without taking into account those who did not conceive or whose pregnancy ended in a miscarriage. In addition, there was a period sometimes exceeding 2 years between conception and BMI collection. Nevertheless, a clear dose–response relationship was confirmed between male BMI and subfertility in large size population.

The most recent publication was a secondary analysis of *The Norwegian Mother and Child cohort study*, which examined women during their second trimester of pregnancy, allowing the inclusion of 26,303 couples [3]. Infertility was defined as a time to conceive longer than 12 months and results were adjusted for ages, maternal BMI, smoking, frequency of sexual intercourse, and female infertility factors. Infertility was significantly increased in couples with an overweight or obese man (OR = 1.20, 95% CI: 1.04–1.38 and OR = 1.36, 95% CI: 1.13–1.63, respectively), with a plateau beyond a BMI of 35 kg/m². Again, there is a bias in the selection of couples who conceived,

as well as the use of reported BMI. However, the results suggested a link between obesity and infertility, regardless of sexual dysfunction.

In total, these three publications, although not without bias, found a dose–response relationship between BMI and male subfertility and a plateau effect beyond a BMI of 32–35 kg/m².

9.2.2 Studies Conducted among Infertile Couples

Studies on couples treated for their infertility generally conclude that male fertility is impaired in the case of obesity.

In a population of 72 infertile couples, Magnusdottir et al. showed an incidence of obesity three times higher in the "male factor infertility" group (concentration and abnormal sperm mobility) compared to "female factor infertility" or "idiopathic infertility" groups [4].

For Hanafy et al., average body weight was significantly higher in 50 infertile men presenting oligozoospermia compared to 30 fertile men with normozoospermia. There was a similar trend for BMI, without reaching significance [5].

For 210 men, male partners from infertile couples, Zorn et al. found a significantly increased BMI in case of nonobstructive azoospermia compared to men with normal sperm counts ($P = 0.02$). In contrast, average BMI of men with oligozoospermia was not significantly different from controls [6].

According to Pauli et al., out of 87 fertile or infertile men, men with a history of paternity have a significantly decreased BMI compared to men who never conceived (28.0 against 31.6 respectively, $P = 0.04$) [7]. This difference was confirmed when the presence of obesity was assessed by measuring skinfold in the arm (24.7 mm against 34.1 mm, respectively, $P = 0.02$).

Although mostly suggesting a link between obesity and male infertility, these studies with bias realized on small size populations do not allow a formal conclusion.

9.2.3 Impact of Male Obesity on Assisted Reproductive Technology Results

Few studies have evaluated the impact of BMI on male "assisted" fertility, with publications concerning the results of *in vitro* fertilization (IVF), without or with intracytoplasmic sperm injection (ICSI) (Table 9.1).

TABLE 9.1
Summary of Publications Evaluating the Impact of Male Obesity on *In Vitro* Fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI) Outcomes

		Male BMI ≥25 kg/m²	
Author	**Cycles (*n*)**	**IVF**	**ICSI**
Keltz et al. [8]	282	Decreased clinical pregnancy rate	No impact on clinical pregnancy rate
Kupka et al. [9]	706, 360	Best implantation rates in IVF or ICSI were observed with the combination of an obese man and a normal-weight woman	
Bakos et al. [10]	305	No impact on early embryo development Decreased pregnancy rate Decreased live birth rate	
Colaci et al. [11]	172	No impact on embryo development, embryo quality, pregnancy, and delivery rates	Decreased live birth rate
Braga et al. [12]	250		No impact on fertilization, implantation, pregnancy and miscarriage
Anifandis et al. [13]	301	Negative impact on embryo quality and pregnancy rates	
Petersen et al. [14]	1906	Decreased live birth rate	No significant effect

Animal models suggest that paternal obesity may impair preimplantation embryo development and implantation. In a mouse model, embryos from males presenting a high-fat diet-induced obesity (but from normal weight females) had slowed kinetics of cell cleavage with a decrease of *in vitro* blastoformation rate compared to embryos derived from normal-weight males (27.0% vs. 56.6%). Blastocysts obtained had a reduced total number of cells in their trophectoderm and inner cell mass due to excessive apoptosis. Their implantation rate was decreased compared to control blastocysts (73.3% vs. 86.7%) [15]. In a recent murine model of IVF, male obesity (with the woman being of normal weight) was associated with a significant delay in early preimplantation embryo development (syngamy was delayed for 15 min, and the second cleavage 60 min) and a delay of fetal development of 0.25 days at birth compared to normal weight controls [16].

Few publications have analyzed the influence of male BMI on outcome of IVF. The influence of male BMI on pregnancy rates was first assessed during 290 cycles of IVF/ICSI [8]. Without taking into account the main confounding factors, a decrease in clinical pregnancy rate after IVF/ICSI was detected if the male partner presented overweight or obesity compared to normal weight (35.9 and 36.1% vs. 53.2%, respectively, $P = 0.04$). In addition, after adjustment for female age and BMI, number of transferred embryos and sperm concentration, a high male BMI was negatively associated with clinical pregnancy in IVF (OR = 0.21, 95% CI: 0.07–0.69, $P = 0.01$), but not in ICSI (OR = 0.75, 95% CI: 0.38–1.49, $P = 0.41$). These findings suggest that ICSI could circumvent a sperm dysfunction associated with obesity, remaining to be characterized. However, this study describes a small size population, and disturbingly, no effect of female BMI on the outcome of IVF/ICSI was highlighted.

The second publication studied the outcome of IVF/ICSI based on male and female BMI over 700,000 cycles [9]. Clinical pregnancy rate (defined as the presence of a gestational sac with or without fetal cardiac activity) was optimal when the woman was of normal weight and the man obese. Despite a very large size population, this publication suffered from the lack of consideration of confounding factors (including age, rank of attempt, tobacco), and was not interested in the intermediate outcomes of IVF (fertilization rate, embryo quality) or the clinical outcome of pregnancy (miscarriage, ectopic pregnancy, childbirth).

The third study reported a link between male BMI and blastocyst embryo formation rate, pregnancy, and birth rates after IVF/ICSI (live birth rate per attempt of 41.3%, 26.4%, 22.6%, and 12.1% in groups of men with normal BMI, overweight, obesity, and morbid obesity, respectively, $P < 0.05$) [10]. However, the study involved only 305 couples and did not clearly include the main confounding factors such as maternal BMI, partners' age, or rank attempt.

These results have not been confirmed by Braga et al. in 250 IVF with ICSI [12]. Indeed, no correlation was found between male BMI and fertilization or implantation rates. The chances of pregnancy and the risk of miscarriage were not influenced by male BMI. However, again, the sample size seems quite small and confounding factors concerning female conditions were not taken into account.

A recent study, but concerning only 172 cycles of IVF/ICSI in 114 couples, reported a decrease in the live birth rate per cycle and per transfer after ICSI in the case of an overweight or obese male partner [11]. This difference was not significant in IVF or for all cycles of IVF and ICSI combined. In this study, confounding factors were carefully considered, including female BMI. However, as the ICSI subgroup concerned only 55 couples and 91 cycles, the findings should be interpreted with caution.

Recently, 301 couples were categorized according to their BMI during an IVF procedure [13]. No association was observed between male BMI and sperm parameters. However, regardless of the female BMI, embryo quality was significantly better and clinical pregnancy rates were significantly higher in couples in whom the man had a normal BMI compared to those who were overweight/obese.

Finally, according to the Danish national registry studying 12,566 women and their partners corresponding to 25,191 IVF/ICSI cycles, increasing female and male BMI, independently or combined,

may influence birth rates after IVF [14]. However, BMI showed no significant effect on the chance of a live birth after ICSI.

In total, epidemiological studies suggest the existence of an association between BMI and male infertility. Although controversial, some studies also suggest possible deleterious effects of obesity on embryo development, blastoformation potential, and fetal development.

9.3 OBESITY AND ITS EFFECT ON MALE REPRODUCTIVE HORMONES

9.3.1 Hypogonadotropic Hyperestrogenic Hypogonadism

The common hormonal profile in obese men showed a decrease in serum testosterone levels (total and free) and gonadotropins (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) associated with an increase in circulating levels of estrogens (usually estradiol and estrone). This hypogonadotropic hyperestrogenic hypogonadism is partly due to aromatization of androgens to estrogens in peripheral tissues [17,18], especially in white adipose tissue, where aromatase cytochrome P450 is highly expressed. Estrogens exert a negative feedback on the pituitary and hypothalamus, which leads to an alteration of testicular function with decreased synthesis of testosterone. However, the decrease in the testosterone/estradiol ratio is deleterious to spermatogenesis and can be associated with infertility [19].

In addition, a decrease in serum levels of sex hormone binding globulin (SHBG) is often observed in obesity, particularly mediated by a state of insulin resistance that inhibits hepatic synthesis of globulins [20]. Although this decrease in SHBG reduces the testosterone fall (by increasing the active free fraction), it also contributes to amplification of the negative feedback control of estradiol, thereby maintaining hypogonadism. However, it also seems to be a direct effect of insulin on the production of testosterone, as testosterone was negatively correlated with circulating insulin levels, even after adjustment for levels of SHBG [21].

The literature is rich in observational studies evaluating the hormonal balance in overweight and obese men, with more than 20 publications available. In a recent systematic review on the subject, a negative association between BMI and serum testosterone (total and free) and between BMI and SHBG was highly plausible [22], as well as a negative effect of BMI on inhibin B, a marker of Sertoli cell function. In contrast, this systematic review revealed that the majority of population studies did not find any relationship between BMI and estradiol, although such a relationship was classically admitted because of the physiopathology of the hypogonadotropic hyperestrogenic hypogonadism.

9.3.2 Impairment of the Hypothalamic–Pituitary Axis

In addition, in cases of obesity, there is an impairment of the hypothalamic–pituitary axis that is not related to hyperestrogenemia. Increased endorphins, well described in the case of obesity [23], are involved in a decreased production of gonadotropin-releasing hormone (GnRH) and a decrease in the amplitude of LH pulses [24]. This alteration would emphasize the central depression of testosterone described in the previous paragraph [25].

9.3.3 Adipose Tissue as an Endocrine Organ

Long considered a simple storage tissue and energy reserve, white adipose tissue is now recognized for its own endocrine function. It synthesizes and secretes a variety of molecules called adipokines, such as leptin, adiponectin, and resistin, as well as cytokines and chemokines. These factors have a major role in the development of complications related to obesity. Ghrelin, synthesized by the stomach, works in concert with adipokines. In addition to their role in energy balance, these hormones are involved in male reproductive functions.

9.3.3.1 Leptin

Leptin, a product of the *Ob* gene, is synthesized and secreted mainly by the adipocytes of white adipose tissue. This hormone is involved in the regulation of food intake and energy expenditure, in particular by reducing appetite. Obese individuals generally exhibit high circulating levels of leptin because of a resistance to the effects of this hormone.

Leptin also plays an essential role in reproductive functions at both central and local levels.

Leptin has a stimulatory action on the secretion of GnRH by the hypothalamus, by regulating the arcuate nucleus neurons that emit projections to GnRH neurons. The neuropeptide Y (NPY)/agouti-related protein (AgRP) and proopiomelanocortin (POMC) neurons are respectively stimulated and inhibited by leptin. Leptin also indirectly regulates the secretion of gonadotropins by modulating the secretion of Kiss 1 by kisspeptin neurons, also present in the arcuate nucleus [26,27].

Leptin can cross the blood–testis barrier and acts directly on testicular function: leptin receptors are present on Leydig cells, Sertoli cells, germ cells, and mature spermatozoa membranes [28–30]. On rats, leptin significantly reduced the secretion of testosterone by the testis [31]. Leptin is present in seminal plasma, but its involvement in sperm motility, capacitation, and acrosome reaction remains controversial [29].

In *ob/ob* mice presenting leptin deficiency, an altered reproductive system, reduced gonadotropins levels, and a lack of sexual maturation are observed [26]. Increased apoptosis in the germ cell and overexpression of pro-apoptotic genes in seminiferous tubules alter spermatogenesis processes [32].

Leptin appears to play a critical role in regulating the impact of obesity on male reproductive functions. In a study evaluating sperm parameters and leptin in 122 obese men, serum leptin levels were higher in obese infertile men than in obese fertile men [28]. There was also a negative correlation between leptin levels and sperm concentration and mobility. Moreover, despite methodological weaknesses (including the failure to take into account BMI), a study suggested that infertile men presenting with oligozoospermia would show higher leptin levels than fertile men with normozoospermia [5]. Some have suggested that leptin participates in the reduction of androgen levels in the case of male obesity [33]. However, after adjustment for BMI, Zorn et al. found a negative correlation between serum leptin levels and inhibin B, total testosterone, and SHBG, suggesting a link between leptin and testicular function independent of gonadotropins [6]. In contrast, they could not find any correlation between leptin and sperm parameters.

Overproduction of leptin leads to leptin resistance in the hypothalamic–pituitary axis, and contributes to hypogonadotropic hypogonadism. Several mechanisms may be involved in this resistance such as leptin transport saturation across the blood–brain barrier, altered signal transduction, and degradation of the receptor [33]. The blood–testis barrier is, however, not affected by leptin resistance: consequently, leptin excess worsens testosterone deficiency with a local action [27]. Central and peripheral actions of leptin would both contribute to spermatogenesis impairment.

9.3.3.2 Ghrelin

Ghrelin is a peptide mainly secreted by the fundus of the stomach. It displays an appetite stimulant action through the activation of the NPY/AgRP neurons located in the arcuate nucleus and through inhibition of *Kiss-1* gene expression [34].

Ghrelin levels are lower in obese patients, but no resistance to this hormone seems to exist. However, in obese men, the postprandial decrease in ghrelin is attenuated, thus leading to lack of satiety [35,36].

With regard to reproductive functions, ghrelin has central and gonadal local actions.

At the hypothalamic level, ghrelin receptors (growth hormone secretagogue [GHS] receptor) are highly expressed. Ghrelin inhibits GnRH secretion, and consequently FSH and LH secretion. Ghrelin also has a direct impact at the pituitary level, where it inhibits the secretion of LH induced by GnRH [30].

At the peripheral level, ghrelin has a direct effect on the gonads. Receptors (GHS-R1a) are present in mature Leydig cells, Sertoli cells, and germ cells [30]. Ghrelin mainly inhibits testosterone secretion.

9.3.3.3 Adiponectin and Resistin

Adiponectin and resistin are adipokines secreted by white adipose tissue. Adiponectin is involved in lipid and carbohydrate metabolism. This hormone is insulin sensitive; it acts by decreasing hepatic glucose production and increasing the action of insulin in the liver. Adiponectin is inversely correlated with the risk of diabetes, regardless of BMI [37,38]. Although produced by adipose tissue, it is inversely correlated with the amount of visceral fat, possibly due to a negative feedback. Resistin tends to induce a state of insulin resistance. Nevertheless, mechanisms of action and involved receptors are so far poorly described.

Adiponectin and resistin have an impact on reproductive functions both centrally and locally.

At the central level, adiponectin was found in the hypothalamus and pituitary. It binds to AdipoR1 and AdipoR2 receptors located mainly in muscle and liver, but also in th hypothalamus and the pituitary gland [38,39]. It inhibits the secretion of GnRH, via the inhibition of the *Kiss-1* gene. At the pituitary level, adiponectin secretion inhibits LH secretion [38]. Resistin is expressed in the hypothalamus and the pituitary gland. Its role at the central level is still poorly known, but it would affect the expression of adiponectin receptors [38].

Adiponectin and resistin are also present in the testes. They can be locally produced or come from the general circulation. In a rat model, adiponectin is detectable in Leydig cells, receptors present in the seminiferous tubules [40], and resistin is localized in Leydig and Sertoli cells. *In vitro* studies in rodents have shown that adiponectin inhibits testosterone production, whereas resistin would tend to increase it [40,41].

Leptin, ghrelin, adiponectin, and resistin, involved in energy metabolism, also modulate male reproductive functions through their effects on the hypothalamic–pituitary axis or directly on the testes. Plasma concentrations of these hormones are disrupted in cases of overweight and obesity. They therefore presumably play an important role in altered reproductive functions observed in men with increased BMI.

9.4 OBESITY AND ITS EFFECT ON SPERM QUALITY

Semen analysis is an easily accessible marker of male fertility. Many studies therefore have focused on the impact of male BMI on conventional sperm parameters. Some recent data have also demonstrated an impact of overweight and obesity in humans on nonconventional sperm parameters, assessing DNA fragmentation or fertilizing ability.

9.4.1 Impact on Conventional Sperm Parameters

9.4.1.1 Observational Studies

Studies evaluating the impact of weight and/or BMI in the general population have increased from the 2010s especially in the male partners of infertile couples (Table 9.2).

It should be noted that, among these studies, very few have assessed the potential impact of underweight on sperm parameters, usually because of small sample sizes. The few studies found conflicting results: lack of significant difference for some [42–45] and altered sperm parameters for others (decreased semen volume [46], sperm concentration [47,48], total sperm count [49,50], sperm mobility [48,51], and morphology [46,52]). Interpretation of the results is complicated further by the inclusion of different thresholds for the definition of underweight (BMI <20 kg/m² for some and BMI <18.5 kg/m² for others).

Overall, altered sperm parameters are reported in case of male obesity, and sometimes in case of overweight. These deleterious effects mainly concern sperm concentration and/or count, but some studies also found impaired sperm motility and morphology.

Interpretation of results and comparisons between studies may be difficult because of several factors. First, the populations studied are highly heterogeneous: general population, men of infertile

TABLE 9.2
Summary of Publications Evaluating the Association between Body Mass Index (BMI) and Semen Parameters

	Significant Association between BMI and Sperm Parameters			No Significant Association between BMI and Sperm Parameters
	Sperm Concentration and/or Count	Sperm Motility	Sperm Morphology	
Men from the general population	Jensen et al. [47] (*n* = 1558) Sekhavat and Moein [48] (*n* = 852) Stewart et al. [49] (*n* = 223) Egwurugwu et al. [53] (*n* = 120) Robeva et al. [54] (*n* = 42)	Sekhavat and Moein [48] (*n* = 852) La Vignera et al. [52] (*n* = 150) Robeva et al. [54] (*n* = 42)	La Vignera et al. [52] (*n* = 150)	Aggerholm et al. [42] (*n* = 1989) Li et al. [43] (*n* = 1346) Qin et al. [45] (*n* = 990) Ramlau-Hansen et al. [55] (*n* = 259) Eskenazi et al. [56] (*n* = 97)
Men from infertile couples	Paasch et al. [50] (*n* = 2157) Kort et al. [57] (*n* = 520)[a] Chavarro et al. [58] (*n* = 483) Koloszar et al. [59]; Fejes et al. [60,61] (*n* = 473) Hammoud et al. [62] (*n* = 390) Braga et al. [12] (*n* = 250) Tunc et al. [63] (*n* = 81) Magnusdottir et al. [4] (*n* = 72)	Martini et al. [51] (*n* = 794) Fariello et al. [64] (*n* = 305) Braga et al. [12] (*n* = 250)	Shayeb et al. [46] (*n* = 2035) MacDonald et al. [65] (*n* = 511)	Duits et al. [44] (*n* = 1401) Relwani et al. [66] (*n* = 530) Eskandar et al. [67] (*n* = 500) Nicopoulou et al. [68] (*n* = 349) Lotti et al. [69] (*n* = 222) Keltz et al. [8] (*n* = 185) Vujkovic et al. [70]; Hammiche et al. [71] (*n* = 175) Rybar et al. [72] (*n* = 153) Wegner et al. [73] (*n* = 107) Pauli et al. [7] (*n* = 87)

[a] Kort et al. observed a significant decrease of number of normal-motile sperm cells in overweight or obese infertile men.

couples, men or couples treated with IVF/ICSI. Sample sizes are very variable, ranging from low numbers to high numbers ($n = 42$ to $n = 2157$). Moreover, studied parameters are very heterogeneous, consistent with those described by the World Health Organization (WHO), such as concentration or motility, but sometimes total motile sperm count or motile sperm of normal morphology count are used. Finally, statistical tools used also vary: means or medians comparisons, correlation, regression, and comparisons of patients' numbers.

9.4.1.2 Meta-Analysis

In 2010, a first review with meta-analysis was published, focusing on the studies published until February 2009 [22]. It collected 13 publications of interest, corresponding to 6793 men. Among them, the authors ultimately retained only 5 studies with the requirements for the meta-analysis, corresponding to 4853 men [42,45,47,59,61]. No effect of weight on sperm parameters was observed. However, beyond the small number of publications included compared to those available, this meta-analysis is also questionable with regard to statistical analysis: The studies did not all use the same classes of BMI, some results were expressed as mean ± standard deviation and others as

median (25th–75th percentiles), and the primary endpoint varied across studies (sperm concentration and/or total sperm count). Hence we believe that the conclusions of this article may have a limited impact.

In this context, we conducted a systematic literature review to assess the impact of BMI on sperm parameters, especially on total sperm count, reflecting the efficiency of spermatogenesis. Owing to methodological and statistical difficulties (heterogeneity of expression patterns of results and statistical methods used in the different articles), we proposed a collaborative meta-analysis in which all the authors of the relevant studies agreed to participate, providing standardized data. Data were stratified according to total sperm count as normozoospermia ($\geq 40 \times 10^6$ sperm per ejaculate), oligozoospermia ($<40 \times 10^6$ but >0 sperm per ejaculate), and azoospermia (no spermatozoa), as recommended by WHO [74]. The prevalence of men presenting abnormal total sperm count was compared across the different BMI categories [75]. The literature search identified 10,400 articles. Of the 44 selected articles, data from 20 studies (corresponding to 25 publications) were sent to us [4,6,8,12,42–47,51,52,55,56,58–61,63,66,67,69,71,76,77]. The meta-analysis therefore included a total of 13,077 men, representing many countries, on all continents except Africa. Recruitment included men from the general population or partners of infertile couples. Overweight men were at increased risk for oligozoospermia (OR = 1.11, 95% CI: 1.00–1.23) or azoospermia (OR = 1.27, 95% CI: 0.97–1.66) compared with normal BMI men. Similarly, obese and morbidly obese men were at risk of oligozoospermia (OR = 1.30, 95% CI: 1.05–1.61 and OR = 2.03, 95% CI: 1.56–2.65) or azoospermia (OR = 1.73, 95% CI: 1.07–2.79 and OR = 7.31, 95% CI: 2.98–17.94) compared to men with normal BMI [78]. Considering azoospermia and oligozoospermia as a single group, an association following a J-curve was found between BMI and the risk of developing abnormal sperm count. Indeed, compared to normal-weight men, the ORs (95% CI) for abnormal total sperm count were 1.15 (0.93–1.43) for underweight men (nonsignificant), 1.11 (1.01–1.21) for overweight men, 1.28 (1.06–1.55) for obese men, and 2.04 (1.59–2.62) for morbidly obese men [79]. When considered as continuous variables, no association between BMI and sperm concentration or total sperm count could be demonstrated, in agreement with the conclusions of the previous meta-analysis [22]. However, owing to a highly dispersed and non-normal distribution of values, the alternative approach stratifying data into categories seems more appropriate. Finally, referring to the well-known curves analyzing the relationship between BMI and morbidity/mortality, our results strongly suggest an association between BMI and sperm production.

9.4.2 Impact on Other Sperm Parameters

Although conventional semen analysis remains the gold standard for the exploration of male infertility, its relevance may be discussed because of moderate sensitivity and specificity. Specialized sperm tests have been proposed, including the assessment of chromatin content integrity in the sperm nucleus. Yet, a key role of oxidative stress, systemic and also local, is strongly suggested as supporting the relationship between obesity and sperm alterations. Therefore, research teams had a growing interest in evaluating the impact of male BMI on these specialized analyses.

9.4.2.1 Impact on DNA Integrity of Sperm Cells

Different techniques are identified in the recent *WHO Laboratory Manual for the Examination and Processing of Human Sperm*, 5th edition (2010).

Although the literature remains controversial, sperm DNA fragmentation assessment has been identified by some authors as a relevant marker of male infertility, affecting spontaneous pregnancy rate and assisted reproductive technology outcomes [80–83]. It has also been linked to an increased risk of spontaneous IVF/ICSI miscarriages, confirmed by a recent meta-analysis [84].

The techniques assessing sperm DNA fragmentation evaluate the primary structure of DNA. We can distinguish the following:

- Terminal uridine nick end labeling (TUNEL) assay: incorporation of labeled nucleotides to the 3′-OH ends of DNA fragments with terminal deoxynucleotide transferase.
- Sperm chromatin dispersion (SCD) or halosperm assay: acid agarose gel migration without denaturation.
- Comet assay: alkaline denaturation followed by migration in gel electrophoresis with migration of the DNA fragments according to their molecular weight and forming a comet tail around fragmented sperm.
- Acridine orange (sperm chromatin structure assay [SCSA]): testing the resistance of sperm DNA to denaturation in acid medium, and allowing the determination of both level of DNA fragmentation by establishing a fragmentation index (DNA fragmentation index [DFI]) and the degree of nuclear maturity by the high stability DNA (HSD) index.

Increased sperm DNA fragmentation in the case of high BMI has been reported [52,57,58,64], but this association remains controversial (Table 9.3).

Kort et al. reported an increased sperm DNA fragmentation rate determined by SCSA in overweight and obese men [57]. Chavarro et al. and Farriello et al., using the Comet assay method, and La Vignera et al., using the TUNEL assay, observed higher sperm DNA damages in obese but not in overweight men [52,58,64]. Then Tunc et al., Rybar et al., and Hammiche et al. did not find any significant association between BMI and sperm DNA integrity [63,71,72], but these studies involved small population sizes.

Recently, in a 3-year multicenter study (n = 331 men) using the TUNEL assay, we observed an increased risk of sperm DNA damage in obese but not in overweight men [85]. This increased risk was confirmed after adjustment for age and tobacco use, adjustments that are usually lacking in previous published studies, although these factors are known to increase sperm DNA fragmentation. These sperm nuclear alterations, affecting reproductive functions both *in vivo* and *in vitro*, would contribute to impaired fertility in obese patients. They may also influence the outcome of pregnancies, considering the relationship between the high sperm DNA fragmentation and increased risk of spontaneous miscarriage [84,86].

Decondensation of sperm chromatin is an alteration of the tertiary structure of DNA, corresponding to a defect in the histone–protamine conversion. It is of interest in clinical practice; according to some authors, it could be a predictive marker of fertilization failure during IVF [87,88]. La Vignera et al. found a significant increase of sperm chromatin decondensation (assessed by propidium iodide flow cytometry) in overweight or obese men compared to normal-weight men (n = 150

TABLE 9.3
Summary of Key Publications Evaluating the Link between Body Mass Index (BMI) and DNA Fragmentation of Sperm

Author	N	Technique	Observations
Kort et al. [57]	520	SCSA	Higher sperm DNA fragmentation rate in overweight and obese men compared to men with normal BMI
Chavarro et al. [58]	413	Comet	Higher sperm DNA fragmentation rate in obese men compared to men with normal BMI
Fariello et al. [64]	305	Comet	
La Vignera et al. [52]	150	TUNEL	
Dupont et al. [85]	331	TUNEL	
Tunc et al. [63]	81	TUNEL	No association between BMI and DNA fragmentation
Rybar et al. [72]	153	SCSA	
Hammiche et al. [71]	175	SCSA	

Note: SCSA, sperm chromatin structure assay; TUNEL, terminal uridine nick end labeling.

men) [52]. Neither Martini et al. (n = 794 men, aniline blue assay) nor Rybar et al. (n = 153 men, aniline blue and chromomycin A3 toluidine assays) confirmed these results [51,72], suggesting that overweight and obese men have normal and similar nuclear maturity compared to normal-weight men. Regarding sperm chromatin structure assessed using acridine orange, Zorn et al. did not find any significant difference in the percentage of spermatozoa denatured across BMI classes (n = 142 men) [76].

Thus, although a number of these studies have very small sample sizes, a deleterious impact of overweight/obesity on sperm DNA fragmentation seems highly plausible. Further studies are needed to reach conclusions about other alterations of chromatin structure.

9.4.2.2 Impact on Mitochondrial Membrane Potential and Apoptosis of Spermatozoa

Some authors were also interested in other specialized semen analysis parameters, such as apoptosis markers. Three recent publications concluded there were alterations of mitochondrial function in sperm cells of obese men (n = 597 men in total) [52,64,76]. Obese men would also present a significant decrease in vitality and a significant increase in externalization of phosphatidylserine [52,76]. Together these results suggest that obesity is associated with an increase in apoptotic processes of sperm cells.

9.4.2.3 Impact on Male Genital Tract

A significant decrease of α-glucosidase in seminal fluid was detected in overweight and obese men [51]. This enzyme secreted into the epididymal fluid modulates sperm maturation in the epididymis, including motility acquisition. This result suggests impaired epididymal function in case of obesity, which may explain reduced sperm motility. As the secretion of α-glucosidase is androgen dependent, hypogonadism observed in obese men may be responsible for this mechanism.

9.4.3 Impact on Fertilization Ability

Conventional quantitative sperm parameters are not sufficient to predict sperm fertilization ability. Data obtained in animal models suggest that male BMI could alter sperm function and fertilization ability. In mice, a high-calorie and high-fat diet induces significant decreases of sperm capacitation and binding to the zona pellucida of oocytes, resulting in significantly lower fertilization rates [89]. In humans, one publication assessed the ability of sperm from 107 men of infertile couples to bind to hyaluronic acid. This test has been proposed as a good proxy of sperm maturity and competence for attachment to the zona pellucida of the oocyte. A significant negative correlation between BMI and hyaluronic acid binding scores was observed [73], suggesting a reduced ability of binding to the zona pellucida for sperm of overweight and obese men. However, opinions on this test remain controversial, because it provides only an indirect assessment of sperm binding ability. Recently, in a selected population of men from couples diagnosed with primary idiopathic or mild male factor infertilities, we investigated the impact of overweight and obesity on sperm binding ability assessed by the zona-binding test (ZB test) (n = 316) [90]. Within the limits of our protocol, we did not find any significant association between BMI and binding ability of sperm to oocyte. This could be related to the selected population of men with idiopathic infertility or could suggest that obesity-related alterations of sperm function may be related other mechanisms, such as abnormalities of capacitation or other steps of gamete interaction (including acrosome reaction).

9.4.4 Physiopathological Mechanisms

Mechanisms possibly involved in the alterations of male reproductive functions in the case of overweight or obesity are numerous and remain poorly characterized. Hormonal changes related to obesity (see Section 9.3) seem to play an important but not exclusive role in the physiopathology.

9.4.4.1 Oxidative Stress

Oxidative stress likely plays a key role in the impact of overweight and obesity on male reproductive functions. A low level of reactive oxygen species (ROS) is necessary for sperm fertilization ability and gamete interaction [91]. However, an imbalance, due to either an excessive production of ROS and/or a decrease in sequestration, leads to an excess of oxidative stress in semen. Peroxidation lesions impair sperm function (decreased motility, imbalance of plasmic membrane) and chromatin integrity (abnormal DNA condensation and/or fragmentation) (reviewed in Refs. [92,93]). Oxidative stress is involved in many chronic diseases, including obesity, due to a state of systemic oxidative stress [94]. In the mouse model, the production of hydrogen peroxide is increased in white adipose tissue of KKAy obese mice, but not in other tissues, suggesting that adipose tissue is a major site of ROS production [95]. In humans, antioxidant enzyme activities and lipid peroxidation are respectively negatively and positively correlated with BMI [96].

9.4.4.2 Inflammation

Proinflammatory cytokines—interleukin (IL) 1a, IL6, tumor necrosis factor alpha (TNFα), Activin-a—are necessary for spermatogenesis [97]. Obesity is associated with a state of chronic immune activation, due to the production proinflammatory cytokines by adipose tissue. The balance of proinflammatory cytokines may then be challenged, leading to a negative impact on testicular functions. Indeed, proinflammatory cytokines increase insulin resistance, which is known to affect male reproductive functions. These cytokines also alter steroidogenesis and may have a direct effect on the seminiferous epithelium, thus compromising spermatogenesis [98]. In addition, IL6 and TNFα stimulate the production of ROS by leukocytes [99], which directly affects spermatogenesis. This inflammatory condition would extend to the male genital tract, causing an increase in macrophage activity and oxidative stress in the semen [63].

9.4.4.3 Increased Scrotal Temperature

An increase in scrotal temperature may be responsible for disruption of spermatogenesis [100,101]. Obesity is frequently associated with changes in lifestyle, such as a decrease in physical activity, which could increase local testicular temperature [4]. In addition, there is an accumulation of fat in hips and abdomen, or in the scrotum [102], which also plays a role in the increase of testicular temperature, thus contributing to altered sperm production.

Recent publications suggest that male overweight or obesity is associated with an increased risk of impaired spermatogenesis. In particular, male obesity was linked to a higher risk of oligozoospermia and increased sperm DNA fragmentation. Male BMI should be taken into account in case of infertility, even if conventional semen parameters remain normal, because it does not exclude other sperm qualitative alterations. Physiopathological mechanisms involved in the impact of overweight and obesity on male reproductive functions are multiple and intricate.

9.5 STUDIES EXAMINING THE LINK BETWEEN WEIGHT LOSS AND SPERM QUALITY

Obesity is a potentially reversible health risk and one can ask whether weight loss could lead to a reversal of sperm alterations and/or improvement of fertility. The effects of weight loss on female reproductive functions are particularly well studied and almost exclusively positive [103]. Conversely, few data evaluating the effect of weight loss on male reproductive functions are available.

9.5.1 Weight Loss through Diet and/or Exercise

9.5.1.1 Effects on Hypogonadism and Quality of Sexual Life

Although initially controversial [104], most publications agree in concluding there is an improvement of hypogonadism in the case of weight loss.

In 1981, a normalization of the evaluated parameters (estrone, estradiol, total and free testosterone binding capacity to SHBG) was shown in 24 obese men with moderate obesity, who had lost an average of 19.5 kg during an 8-week program [105]. Strain et al. confirmed the reversibility of the hypogonadotropic hypogonadism in case of weight loss in 11 obese men after a loss of 26–129 kg [106]. The testosterone and SHBG increase was proportional to the weight loss, but was observed in the absence of a decrease in hyperestrogenemia, suggesting a functional modification of the hypothalamic–pituitary axis. In nine men with morbid obesity, a significant increase in androgens, but not SHBG, was also found after weight loss [107]. A randomized study in 38 obese men confirmed the increase in testosterone (total and free) and SHBG after a loss of 21 kg on average during a 10-week low-calorie diet [108]. However, the authors suggest that SHBG may decrease again during the weight stabilization phase. In 58 obese men with metabolic syndrome, a similar hypogonadism improvement was reported after a hypocaloric diet [109]. In a recent cohort study, Håkonsen et al. also demonstrated a significant increase in testosterone and SHBG in the case of weight loss through diet and physical activity [110].

Finally, a prospective randomized study showed that obese men who had lost at least 10% of their weight had a significant improvement in erectile function compared to obese men who did not lose weight [111].

Thus most publications highlight that weight loss in obese men is associated with an improvement of hypogonadism, probably also associated with an improvement of quality of sexual life.

9.5.1.2 Effects on Fertility and Semen Parameters

The literature evaluating the effects of weight loss on sperm parameters is particularly poor, as only two publications are available, one in an animal model and one in humans.

In mice, Palmer et al. evaluated the reversibility of obesity and of its effects on sperm in the case of weight loss through diet and/or exercise [112]. Male obesity was associated with reduced sperm motility and morphology, increased nuclear damages, and oxidative stress. Diet and/or exercise significantly improved motility and sperm morphology. Alone or in combination, they also reduced DNA fragmentation, ROS, and mitochondrial membrane potential, and increased sperm binding to the zona pellucida.

In humans, a Danish cohort study following 43 obese men (BMI 33–61) during a 14-week weight loss program was published [110]. The median weight loss was 15% of initial body weight (range 3.5–25.4%), corresponding to a median weight loss of 22 kg (range 4–39 kg). This weight loss was associated with a significant increase in ejaculate volume ($P = 0.04$) and total sperm count ($P = 0.02$). The group presenting the largest weight loss (17.2–25.4% of initial weight) also had a significant increase in normal sperm morphology evaluated according to Kruger criteria (+4%, 95% CI: 1–7%). The authors did not observe any significant change in sperm DNA fragmentation index (assessed by SCSA) after weight loss. However, it is currently not possible to say that the observed improvement was due to weight reduction itself, rather than lifestyle changes. In addition, no data concerning the fertility of these men were available. Finally, although the study size is quite small owing to a low participation rate (41%), this study emphasizes the potential beneficial effects of weight loss on sperm parameters.

Recently, our team reported the cases of six overweight or obese male partners of couples presenting idiopathic infertility [113]. These men also had a documented excess of abdominal adipose tissue. They agreed to have personal lifestyle management during 3–8 months, after which they all lost weight (a mean of 3.9% of weight loss, corresponding to a mean of 1.5 points of BMI), but also reduced their waist circumference (mean 8 cm) and lost visceral fat (measured by impedance). Conventional semen parameters were broadly unchanged, but a significant decrease in sperm DNA fragmentation rate was observed for all men. In addition, all the partners of these men achieved clinical pregnancy (spontaneous for two of them and after intrauterine insemination for the other four), followed by the birth of a healthy child. Although they involved a limited number of patients, these results seem to underline the potential benefits of lifestyle coaching for obese men with excess

of visceral fat, not only for their health in general, but also in the context of infertility. This work also emphasizes the benefits obtained with moderate weight loss, but associated with a significant change in body composition. Further cohort studies are needed to confirm these results and understand these effects better.

Sperm abnormalities associated with obesity may be reversible through the combined approach of diet and exercise, including when complete normalization of weight is not obtained, suggesting that changes in lifestyle could improve the prognosis of fertility of obese men.

9.5.2 Weight Loss through Bariatric Surgery

In cases of morbid obesity, or severe obesity with comorbidity, the use of bariatric surgery may be considered. Surgical techniques can be restrictive (adjustable gastric band or sleeve gastrectomy) or both restrictive and malabsorptive (gastric bypass).

Bariatric surgery has proven to be effective on cardiometabolic comorbidities and mortality [114,115]. It also effectively protects against the onset of type 2 diabetes in obese subjects [116], suggesting its importance as a preventive treatment of metabolic diseases. However, it should be noted that it can cause severe nutritional deficits or deficiencies. Micronutrients and vitamins supplementation is highly recommended, especially in case of malabsorptive surgical technique.

9.5.2.1 Effects on Hypogonadism and Quality of Sexual Life

Although published studies concern only small numbers of patients (maximum 22), and a relatively short follow-up (up to 2 years), a correction of the hormonal profile of men who underwent bariatric surgery is currently accepted.

Bastounis et al. found an increase in testosterone and SHBG and a decrease in estradiolemia in 19 obese men after vertical gastroplasty [117]. The same normalization of abnormally low inhibin B levels was observed after vertical gastroplasty [118]. In a cohort of 64 men with morbid obesity followed for 2 years, Hammoud et al. reported an increase in total and free testosterone associated with a decrease in estradiol in 22 men who underwent gastric bypass compared to nonoperated males [119]. These results are also observed in a recent prospective randomized study comparing 10 obese men undergoing bariatric surgery to 10 obese men without surgery [120].

In addition to its effect on the regulation of food intake, ghrelin is likely involved in reproductive functions in humans, as indicated by the presence of testicular receptors. *In vitro* studies have shown that ghrelin inhibits the secretion of testosterone [121]. The decrease in ghrelin levels expected after certain bariatric surgery techniques could contribute to the correction of hypogonadism by a direct action on androgen synthesis.

Several authors have described the positive impact of bariatric surgery on erectile dysfunction and sexual quality of life [119,120]. A recent review of the literature suggested that the quality of sexual life improves more after weight loss through bariatric surgery than after nonsurgical weight loss [122].

9.5.2.2 Effects on Fertility and Semen Parameters

Although more than 300,000 bariatric surgeries are performed each year around the world, and involve younger patients, the impact of this surgery on the reproductive functions and sperm quality remains poorly understood (Table 9.4).

The first study addressing the issue of the impact of surgery on sperm quality was a clinical case [123]. It reported a series of six previously fertile patients who underwent a gastric bypass. Sixteen months after surgery, the authors recorded a secondary azoospermia on those six men, controlled several times over a period of 12–15 months. Testicular biopsies were performed and showed a complete meiotic arrest. The authors suggested that surgery would have induced malabsorption of certain nutrients essential for spermatogenesis. This case therefore raises potential concerns about the possible consequences of bariatric surgery on sperm parameters. Then, we reported the cases

TABLE 9.4
Summary of Publications Evaluating the Impact of Bariatric Surgery on Semen Parameters

Author	Population Studied	N	Surgical Technique	Main Results
Di Frega et al. [123]	Men from previously fertile couples, then becoming infertile	6	Bypass	Secondary azoospermia (meiosis arrest observed on testicular biopsy). No reversibility 3 years after the surgery.
Sermondade et al. [124]	Men from infertile couples	3	Bypass (two cases), sleeve gastrectomy (one case)	Major defects of semen parameters. Reversibility after 2 years (1 case), tendency after 15 months (1 case).
Lazaros et al. [125]	Men from infertile couples	2	Bypass	Major defects of semen parameters. No reversibility 1218 months after the surgery.
Reis et al. [126]	Men of unknown fertility	10	Bypass	No significant change of sperm parameters 4 and 24 months after the surgery.

of three men from infertile couples, in whom we observed a severe worsening of sperm parameters in the months following bariatric surgery, resulting in extreme oligoasthenoteratozoospermia [124]. This may seem paradoxical, as weight loss should eliminate the deleterious effects of obesity on spermatogenesis. Several hypotheses may be raised: hormonal changes related to a relative status of undernutrition; the negative impact of persistent organic pollutants (POPs), contained in the adipocytes of abdominal visceral fat and massively released during severe weight loss phase [127]; and the negative impact of chronic nutritional deficiencies secondary to surgery. However, this alteration was reversible 2 years after the surgery in one of the cases. In addition, the three partners of these men achieved clinical pregnancy with birth after ICSI, suggesting that the observed quantitative sperm abnormalities could not have been associated with qualitative abnormalities (not evaluated in this study because of the drastic decrease in sperm concentrations). This result was confirmed by another case report of two patients from infertile couples [125], observing a significant change in sperm parameters in a period of 12–18 months after surgery. In contrast, in a case-control study involving 20 patients with unknown fertility, including 10 men undergoing gastric bypass, Reis et al. did not observe any secondary alteration of sperm parameters 4 and 24 months after surgery [126]. The different recruitment modes of these studies (infertility with preexisting abnormalities in sperm for the first publications, obesity with unknown past fertility) may suggest that the impact of bariatric surgery on sperm parameters could depend on the populations studied and on the initial reproductive abilities.

Few data are available regarding the effects of bariatric surgery on male reproductive functions. It could contribute to an improvement or correction of hypogonadism and sexual dysfunction related to obesity. However, further longitudinal prospective studies and randomized trials are required to evaluate the impact of these surgeries on sperm parameters and male fertility.

9.6 KEY POINTS SUMMARY

1. Large epidemiological studies of more than 77,000 couples from the general community suggest a significantly higher risk of infertility or delayed conception when the male partner is overweight or obese.
2. The majority of reports studying infertile patients suggest that male obesity is at least partially responsible for male subfertility by reducing the overall sperm concentration, with only a few studies linking obesity with impaired sperm motility or morphology.
3. Although animal studies suggest that male obesity may impede oocyte fertilization, the majority of human studies do not confirm this link. Fertilization rates with routine

insemination (*in vitro* fertilization [IVF]) or sperm microinjection (intracytoplasmic sperm injection [ICSI]) do not appear to be impaired when using sperm from obese males, nor is sperm-zona binding impaired in this standardized *in vitro* model of fertilization potential.

4. The majority of clinical studies have shown a significant relationship between increasing male body mass index (BMI) and sperm DNA fragmentation. As good paternal DNA integrity is paramount to the development of good-quality embryos, this observation may explain why male obesity has been linked with a reduction in good-quality blastocyst embryo development and implantation potential in the IVF setting. The link among male obesity, sperm DNA damage, and impaired embryo development is also seen in animal studies.
5. Male obesity has been conclusively linked with impaired hypothalamic–pituitary (HP) function (reduced luteinizing hormone and follicle-stimulating hormone drive), as well as impaired testicular function with reduced production of testosterone by Leydig cells and inhibin B by the Sertoli cells, both likely to result in impaired sperm production. The mechanisms behind these changes are likely to be multiple and include negative feedback on the HP axis by increased cerebral opioids and estrogen created by the peripheral aromatization conversion of androgens to estrogen in fat tissue, together with the direct inhibitory effect of adipokines on the testis. Obesity is recognized to be characterized by a systemic state of low-grade inflammation, resulting in the release of cytokines that directly inhibit testosterone production while also activating the immune system to create oxidative stress related impairment of sperm production and function.
6. Reduction in weight in obese men through a combination of diet and exercise does result in improvements in testosterone levels and improvements in sperm concentration. Although no randomized controlled trial has proven that weight reduction does result in a statistically significant improvement in fertility, published and unpublished case series suggest this is certainly true. As such, weight loss through supervised diet and exercise should be advocated as a first-line fertility treatment in overweight men attempting to become fathers, especially given its additional general health benefits.
7. Very limited work has been conducted examining the effect of underweight status on male fertility, but the data currently available do not suggest any material negative effect, at least in terms of sperm parameters.

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10 Nutrition and Sperm Function

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CONTENTS

10.1 INTRODUCTION

Infertility is a common disease affecting one in six couples during their reproductive lifetime [1]. Male factors (abnormalities in semen quality parameters) are identified in as many as 58% of the couples evaluated for infertility [2]. Two meta-analyses have documented a drastic decrease in semen quality parameters in Western populations during the 20th century [3,4]. In trying to explain this downward trend, most of the attention has focused on the role of environmental contaminants, particularly environmental estrogens [5] and anti-androgens [6]. Although there are data suggesting associations of some environmental contaminants such as plasticizers (phthalates) with lower semen quality parameters [7,8], much of the decline in semen quality parameters remains unexplained. Other factors coinciding with the decline may also explain this trend. For example, the obesity epidemic [9–11], increased sedentary activity [10], lower physical activity [12,13], increased paternal age [14], and secular changes in diet quality [15] are equally likely potential explanations for this downward trend. For instance, the average diet in the United States today has higher total calories, refined grains, added sugars, meat, and added fats compared to diets in the 1950s [15]. However, most of these alternative hypotheses have, until recently, received little attention.

In this chapter we review the current literature of the role of nutritional factors on clinical markers of semen quality parameters (sperm counts, motility, and morphology) and other measures of sperm function, such as markers of sperm DNA integrity. Most of the existing literature linking nutritional factors to male reproductive function are studies examining the relation between diet

and semen quality parameters, which, although far from perfect proxies for fertility [16–20], shed light on the role nutrition has on male reproductive function. Because the role of obesity is covered elsewhere, we focus our discussion of the contribution of diet to semen quality parameters above and beyond its contribution to energy balance. We also touch on the relation between physical activity and sperm function. To facilitate the discussion, we first address dietary factors generally held as purported reproductive toxicants, namely alcohol and caffeine. Next, we discuss the impact of dietary factors suspected to be vehicles for environmental estrogens on semen quality. We then turn our attention to dietary factors affecting the availability of substrates necessary for spermatogenesis and those that may protect sperm against oxidative damage. This is followed by a discussion of the current evidence on vitamin D and spermatogenesis and a summary of the data on how overall dietary patterns, as opposed to individual foods and nutrients, affect semen quality. Last, we review the literature on the relation between physical activity and semen quality parameters. We close the chapter with a brief discussion on some of the most important limitations of the current literature and suggestions on how to address these in future studies.

10.2 ARE CAFFEINE AND ALCOHOL REALLY THAT BAD?

Caffeine and alcohol have been some of the most extensively studied aspects of diet as potential determinants of semen quality parameters. Contrary to common perception, there is ample evidence that caffeine intake is not related to poor semen quality parameters. If anything, a few studies suggest that caffeine intake is associated with a higher percentage of motile sperm [21,22]. In 2011, a meta-analysis combining data from 1256 men across 57 studies found no evidence of an association between caffeine intake and sperm concentration, motility, or morphology [23]. A study among 2554 young Danish men and other studies not included in the meta-analysis also found no relation between caffeine intake and semen quality parameters [23–27]. Studies of the effect of caffeine on DNA damage in sperm are inconsistent. A cross-sectional study of 179 men—half of them healthy donors and half with impaired fertility—found no association between coffee consumption and semen quality or concentration of sperm DNA adducts (which are negatively correlated with sperm motility and concentration in infertile men) [28], suggesting that there is no link between caffeine intake and DNA damage in sperm [29]. On the other hand, a cross-sectional study of 80 men without known infertility explored the effect of caffeine on DNA damage and found that high caffeine consumption (>308 mg/day, equivalent to three cups per day) was associated with a higher frequency of sperm with DNA damage measured under neutral conditions (thought to represent double-stranded DNA breaks) but no increased DNA damage measured under alkaline conditions (thought to represent single-stranded DNA breaks) [30]. However, percent tail DNA (the marker of DNA damage used in this study) under neutral conditions did not correlate with sperm concentration, total sperm count, motility, or progressive motility [29,30].

The literature on caffeinated (energy and soft) drinks and semen quality parameters is scarce [24,27,31]. Among young healthy men in Denmark, compared to non-cola beverage consumers, those who consumed more than 1 L/day of cola had significantly lower total sperm count and sperm concentration; this association was not explained by caffeine intake [27]. Other studies examining the association between caffeine and semen parameters do not report the association between caffeinated (energy and soft) drinks and semen quality parameters [24,31].

Although semen quality parameters have been related to spontaneous pregnancy, they have limited predictive value on fertility [16,32–34]. A better marker of fertility is time to pregnancy, which has been examined in relation to caffeine in three studies. Whereas Jensen and colleagues observed that the male partner's caffeine intake was related to a lower probability of conception (relation was confined to nonsmokers) [35], two studies did not find any association [36,37]. Cola consumption in relation to fecundability has been reported in one study, which did not find any association [36].

Although the literature on the relation between alcohol intake and male reproductive function suggests that alcohol impairs testicular function in general and spermatogenesis in particular,

interpretation of this literature is generally oversimplified. The negative impact of alcohol on semen parameters is dose dependent [38] and appears to occur only at very high quantities and among alcoholics. A study comparing 66 alcoholic men (who were nonsmokers and non-drug users) who consumed a minimum of 180 mL of alcohol per day for a minimum of five days per week in the past year to a control group of men who had never consumed alcohol, found that sperm count, percentage of rapid progressively motile sperm, percentage of live sperm, and percentage of morphologically normal sperm were significantly lower among alcoholics than among the control group [39]. In addition, the percentages of slow progressively motile sperm, nonprogressively motile sperm, immotile sperm, dead sperm, head-defective, neck-defective, and tail defective sperm were all significantly higher compared to controls [39].

Biologically, chronic alcoholism seems to affect the reproductive hormone axis detrimentally at various levels [39,40]. At the level of the hypothalamus, ethanol blocks the secretion of gonadotropin-releasing hormone, leading to lower concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn decrease spermatogenesis [41]. At the level of the pituitary, ethanol blocks both LH synthesis and secretion [41]. At the level of the testes, ethanol toxicity leads to a reduction in testosterone levels [41], an increase in transferrin and mRNA expression in Sertoli cells involved in spermatogenesis [42], and an increase in histologic abnormalities of the testes and degeneration of sperm cells [40]. Alcohol has secondary effects on the hormone axis through liver damage. Alcoholics have decreased liver function, which, among other things, decreases the production of sex hormone-binding globulin (SHBG), which in turn affects the proportion of bound and free steroid hormone levels [43].

In contrast to the effects of alcoholism on male fertility, an extensive body of literature suggests that moderate consumption of alcohol is not related to semen quality parameters or fertility. In a meta-analysis [23] evaluating the association between alcohol and semen parameters that included data from 6465 men from 57 studies, the only consistent finding was an inverse association between alcohol consumption and ejaculate volume. There was no association, however, with sperm concentration, total count, motility, or morphology [23]. Some studies have even suggested that alcohol intake may be related to better semen quality parameters. A prospective multicenter study found a slight increase in the percentage of normal sperm with moderate alcohol consumption compared to high consumption or no consumption [44].

In concordance with the findings on alcohol and semen quality parameters, most studies evaluating the male partner's alcohol consumption in relation to a couple's time to pregnancy have not found evidence of a negative effect of alcohol [36,37,45,46]. The few studies that found a deleterious effect of alcohol consumption on a couple's time to pregnancy were restricted to heavy drinkers. For example, among 2112 pregnant women in the United Kingdom whose time to pregnancy was retrospectively examined, compared to male partners who did not consume alcohol, heavy alcohol consumption (>20 units/week) was related to 2-fold longer time to pregnancy, but moderate consumption (≤20 units/week) was not associated with decreased fecundability [47].

10.3 DIET AS A VEHICLE FOR ENVIRONMENTAL ESTROGENS

10.3.1 Soy-Derived Products

Recent evidence suggests that exogenous pro-estrogenic or anti-androgenic dietary exposures (sources include dairy, meat, and soy-derived products) may affect spermatogenesis [48,49]. Soy beans and soy-derived products contain isoflavones, which are weakly estrogenic plant-derived polyphenolic compounds [50–55] that bind to estrogen receptors on cell membranes [56]. Studies have found isoflavones to be associated with male reproductive disorders among rats [57] and to exert nongenomic negative effects on sperm capacitation and acrosome reaction [58]. The literature on soy or soy-derived products and male fertility is scarce and inconsistent. A study among 14 men found that compared to pre-supplementation status, semen quality parameters and reproductive

hormone levels were unchanged after supplementation with 40 mg/day of isoflavones for 2 months [59]. Another study found isoflavone intake was positively related to sperm count and motility and inversely related to sperm DNA damage among 10 fertile control men and 48 men with abnormal semen parameters [60]. Conversely, among 99 male partners of couples seeking fertility treatment, intake of soy foods was negatively associated with sperm concentration; this association was restricted to overweight and obese men [61]. In agreement with these findings, a 2013 study of 609 idiopathic infertile men and 469 fertile controls in China observed that urinary levels of isoflavones were related to lower sperm concentration, total count and motility, and higher odds of idiopathic male infertility [62]. Not only is this last study important because it is larger than all the previous studies combined, but also because it addresses one of the major arguments offered against a potentially detrimental role of soy on fertility: that Asian diets include high amounts of phytoestrogens from soy foods without any apparent deleterious effects on fertility. In a population-based study among couples desiring pregnancy, higher concentrations of urinary lignans (enterolactone and enterodiol) among females but not males were associated with a shorter time to pregnancy [63]. Other measured urinary phytoestrogens in the male partner were not related to time to pregnancy [63].

As research in this field develops, future work should focus on male fertility markers other than semen quality parameters and among populations with a high intake of soy.

10.3.2 Dairy under Modern Farming

Meat and dairy have been hypothesized to be vehicles for environmental estrogens under certain modern dairy farming [64] and livestock production practices [65]. Commercial milk is obtained mostly from pregnant cows in the United States and other countries [64,66], and there is concern that pregnancy hormones in milk [67,68] could have reproductive effects in milk drinkers. In fact, milk and milk products contain measurable amounts of estrogen and other pregnancy hormones [67,68] and account for 60–80% of estrogen intake from food in Western countries with low soy intake [69]. Rodent models have been inconsistent in identifying reproductive or estrogenic effects of milk intake [66,70–72]. In boys, intake of milk and other dairy foods has been associated with higher circulating levels of prepubertal growth hormone, insulin-like growth factor-I (IGF-I), and ratio of IGF-I to insulin-like growth factor binding protein 3 [73], increased excretion of estrone, estriol, estradiol, and pregnanediol [74], and higher incidence of teenage acne [75]. In men, dairy food intake has been related to lower circulating levels of LH, follicle-stimulating hormone FSH, and testosterone [74]. Literature on the relation between dairy food intake and semen quality parameters is inconsistent. Although some studies have suggested that dairy is a possible contributing factor for lower semen parameters [64,76–79], this theory has not been corroborated in many studies [76,77,80].

In a case-control study comparing dietary habits of oligoasthenoteratospermic versus normospermic fertility clinic patients in Spain, Mendiola et al. observed that cases had higher intakes of full-fat dairy products (yogurt, whole milk, cheese, and semi-skimmed milk) and lower intakes of skimmed milk than controls [76]. In a different case-control study of asthenozoospermic men in Iran, the odds of asthenozoospermia were marginally higher with greater intake of total dairy products (P-trend = 0.06) and significantly lower with greater intake of skim milk (P-trend = 0.02) [77]. In a longitudinal cohort study (n = 155) among men attending a fertility clinic in Boston, Massachusetts, we found that intake of low-fat dairy products, specifically low-fat milk, was associated with higher sperm concentration and percent motile sperm [79]. We also observed that cheese intake was related to lower sperm concentration among past or current smokers [79]. In a cross-sectional study of physically active young men, we observed that intake of full-fat dairy foods, specifically cheese, was inversely related to progressive sperm motility and normal sperm morphology [78]. A cross-sectional study among men attending a fertility clinic in the Netherlands, however, found that dairy intake was unrelated to semen parameters [80]. Given the paucity of

literature on this topic, it is important that this relation is examined further, ideally in randomized controlled trials (RCT) and prospective studies exploring the biological mechanisms explaining these associations.

10.3.3 Meat under Modern Livestock Production Practices

Anabolic sex steroids—a combination of estrogen, progesterone, testosterone, and any of three synthetic hormones (zeranol, melengestrol acetate, and trenbolone acetate)—are administered 60–90 days before slaughter to meat cattle for growth promotion in the United States and other countries [65,81]. These hormones are administered either as an ear implant (in which the hormones are spread through circulation) or in the feed [82]. Hormonal residues are present in meat products [81,83], and there is concern of reproductive consequences [68,82,84] of meat consumption in places where this practice takes place.

Data are split on the relation between meat intake and semen parameters. High beef consumption among women during pregnancy has been associated with lower sperm concentration among male offspring 30 years later [82]. In a cross-sectional study among young college men, we found that processed meat intake was associated with lower total sperm count and total progressive motile count [85]. Another cross-sectional study observed that intake of processed red meats was approximately 31% higher among oligoasthenoteratospermic men than among controls but did not find any difference in unprocessed red meat intake between these groups [76]. Eslamian and colleagues reported that the odds of asthenozoospermia were 2.03 [1.7–2.4] higher among men in the third tertile of processed red meat intake as compared to those in the first tertile of intake but red meat intake was not associated with the odds of asthenozoospermia [77]. Vujkovic and colleagues found that intake of meat products was unrelated to semen quality parameters [80]. In a longitudinal study in the United States among men attending a fertility clinic, we found that processed meat intake was inversely related to sperm morphology [86].

It should be pointed out that the studies by Mendiola and Vujkovic were conducted within the European Union after the ban on steroid hormones went into effect [81,87], suggesting that the similar findings in Mendiola's study and the US studies are probably not due to hormonal residues but rather may be a consequence of other factors such as saturated fat (discussed in Section 10.4.1) or preserving agents. Future work in the United States and other countries where the practice of hormone administration to cattle still exists is desirable.

10.4 SPERM MEMBRANES, SPERM DNA, AND DIET

Although highly specialized, sperm are relatively simple cells and two of its key components, its cell membrane and its DNA, could be heavily influenced by nutritional inputs. As sperm mature there is a dramatic increase in the proportion of unsaturated fatty acids, particularly docosahexaenoic acid (DHA), in their membranes [88] because of both local metabolism and dietary input [89–93]. Studies investigating sperm fatty acid composition with semen quality parameters in humans suggest a role of fatty acid metabolism on male fertility. For instance, sperm membrane DHA content has been associated with higher sperm motility [89,94–98], sperm normal morphology [98], and sperm concentration [94,97,98], though some studies have found opposite relations [99]. Likewise, lower concentrations of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in sperm membranes has been related with better semen quality parameters [94–98,100].

10.4.1 Polyunsaturated Fatty Acids

Some studies in humans have addressed the relation between intake of specific fats and semen quality parameters. Two trials investigated the effect of supplementation with long-chain omega-3 polyunsaturated fatty acids (PUFAs) on semen quality parameters. In the first trial, among

28 asthenospermic men, DHA supplementation during 3 months did not improve sperm motility, but decreased 20:4*n*-6 (arachidonic acid) in sperm phospholipids, and decreased 22:4*n*-6 (adrenic acid) levels in both seminal plasma and sperm phospholipids [101]. In the second trial, however, long-chain omega-3 fatty acid (DHA + eicosapentaenoic acid [EPA]) supplementation among 211 men led to higher total sperm count, sperm concentration, and the percentages of motile and morphologically normal sperm [102]. In addition, sperm DHA and EPA concentrations were higher in the supplementation group compared to the placebo group.

Similar results have been found in observational studies among fertility patients [103]. A longitudinal study in the United States among men attending a fertility clinic found that omega-3 fatty acid intake was associated with higher normal sperm morphology [86]. A case-control study found that infertile men had lower blood serum concentrations of both omega-3 and omega-6 fatty acids than fertile men [104].

Walnuts, which contain large amounts of plant omega-3 fatty acids (alpha-linolenic acid), have been related to higher sperm parameters. In an RCT of young healthy men consuming a typical Western-style diet, men randomized to walnut supplementation of 75 g/day for 12 weeks had improvements in sperm vitality, motility, and morphology compared to controls [105]. These improvements were accompanied by increases in blood serum of omega-6 and omega-3 [105]. Recent evidence suggests that the omega-6 to omega-3 ratio is associated with semen quality parameters [106]. In a case-control study among 160 men, infertile men had a higher omega-6 to omega-3 ratio in both serum and sperm [104]. In an RCT, asthenozoospermic men supplemented with DHA had a higher ratio of omega-3 to omega-6 in both blood serum and seminal plasma compared to controls [101]. A study among male rodents found that those fed a higher ratio of omega-3 to omega-6 had higher semen parameters (sperm concentration, motility, and morphology) and litter sizes than those fed a low omega-3 to omega-6 ratio [107].

10.4.2 Saturated Fat

Conversely, saturated fat intake was inversely related to sperm concentration and total sperm counts among fertility patients [103] and among healthy young men [108]. Intake of saturated fat is unrelated to sperm or seminal plasma levels of saturated fats [103]; thus it is unlikely that these observed associations are due to a relation between sperm fatty acid levels and sperm counts. Instead, saturated fat intake may have an indirect effect on spermatogenesis by, for example, influencing circulating cholesterol levels, as suggested by rodent models [109]. Similar to other dietary factors, it is not known whether any of the observed relations with semen parameters translates into modifications in reproductive success.

10.4.3 *Trans* Fatty Acids

Concentrations of *trans* fatty acids in sperm reflect *trans* fat intake, because they cannot be synthesized endogenously. Concentrations of *trans* fatty acids in sperm among rodents are related to decreased spermatogenesis and increased testicular degeneration when *trans* fats are added to rodents' diets [110–112]. *Trans* fats have been associated with significantly lower sperm concentrations among fertility patients [100] and lower total sperm counts among healthy young men [113]. These results are comparable to findings from rodent models. Rodents fed hydrogenated oils instead of nonhydrogenated vegetable oils not only accumulated *trans* fats in the testis [110,114], but also had decreased serum testosterone levels, sperm count, motility and normal morphology, and fertility [110–112].

10.4.4 Folic Acid

Dietary inputs are also important for maintaining sperm DNA production. In particular, the literature strongly suggests a key role of folic acid metabolism in spermatogenesis and male fertility. During folic acid metabolism, purines and thymidine are produced and subsequently used in DNA

synthesis [115,116], which is essential for spermatogenesis. One-carbon metabolism (Figure 10.1) not only takes place in the testes [117–119], but animal models also suggest that genetic [120] or pharmacologic [121–123] disruption of this metabolic pathway has detrimental consequence on spermatogenesis. In humans, genetic variation (such as the methylenetetrahydrofolate reductase [MTHFR] enzyme; see Figure 10.1) has been related to semen quality parameters. MTHFR reduces 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate and then produces *S*-adenosylmethionine, a methyl donor, which is important for DNA and protein methylation [124]. The C677C → T genetic polymorphism leads to lower enzymatic activity and accumulation of homocysteine in the heterozygote (*CT*) and homozygote (*TT*) [124]. In 2007, a meta-analysis on the association between *MTHFR C677T* and male factor infertility reported pooled odds ratios (ORs) of 1.39 (95% confidence interval [CI] for male factor infertility: 1.15–1.69) for *TT* homozygotes and 1.23 (95% CI: 1.08–1.41) for *T* allele carriers [124]. In addition, a large study conducted in Korea reported an association between homozygocity for the variant G allele in *MTR* A2756G and nonobstructive azoospermia (OR = 4.63, 95% CI: 1.40–15.31) as well as an association between being a carrier (OR = 1.75, 95% CI: 1.07–2.86) or homozygote (OR = 2.96, 95% CI: = 1.51–5.82) for the variant G allele in *MTRR A66G* and oligoasthenoteratospermia [125].

Evidence suggests that folic acid intake affects sperm production. One trial on folic acid supplementation of 10 mg per day for 30 days found that despite sharp increases in blood and seminal plasma folate levels, no changes were observed with semen quality parameters [126]. In a different trial, however, folic acid supplementation of 15 mg per day for 90 days led to a 53% increase in sperm concentration and a doubling in the proportion of motile sperm [127]. Likewise, in an RCT of folate (5 mg per day for 182 days), zinc, folate + zinc, or placebo, subfertile men assigned to the folate + zinc arm had an 74% increase in total normal sperm count compared to pre-intervention values and a 41% increase when compared to post-intervention values in the placebo arm that did not reach statistical significance [128]. Dietary folates also seem to have an impact on semen quality parameters. In a case-control study among fertility patients in Spain, men in the highest tertile of folate intake had an 87% lower odds of oligoteratospermia than men in the lowest tertile of intake [129].

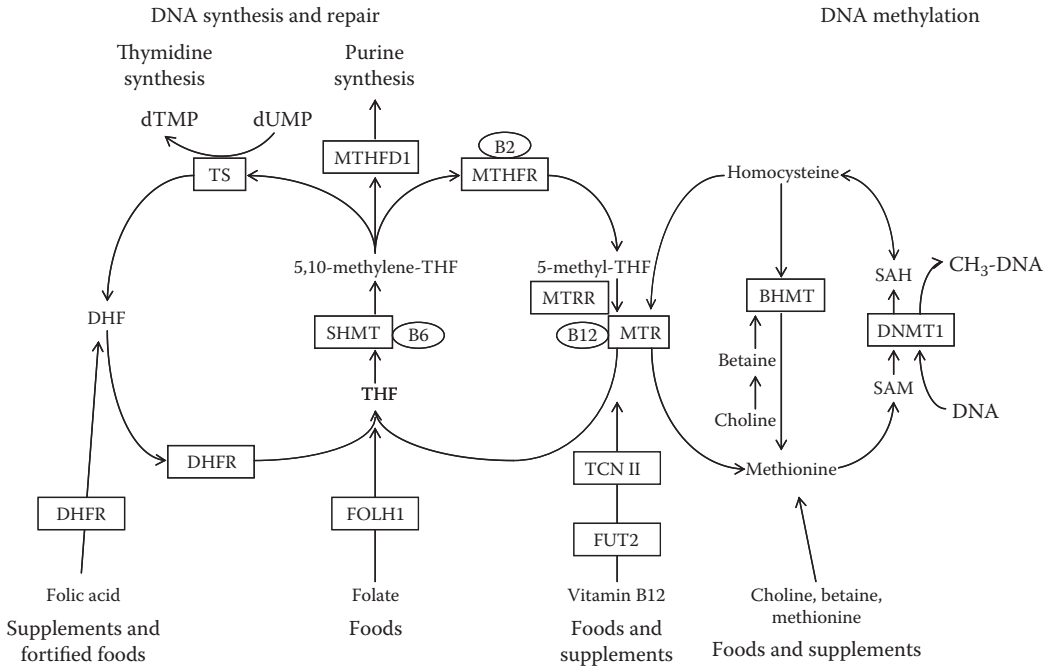

FIGURE 10.1 One-carbon metabolism emphasizing its relationship with DNA synthesis and methylation.

Similarly, two cross-sectional studies found a positive correlation between seminal plasma folate and vitamin B_{12} and sperm concentration [130,131]. Among men who had previously fathered a pregnancy and have sperm counts above 20×10^6/mL, seminal plasma folate is inversely related to sperm DNA fragmentation [132]. Also, folate intake has been related to a lower frequency of sperm aneuploidy [133]. Moreover, in rodent models, folate-deficient diets result in differential sperm DNA methylation at sites associated with cancer and chronic human diseases, decreased pregnancy rates, increased post-implantation embryo loss, and increased gross anatomical abnormalities in their offspring [134].

10.5 ANTIOXIDANTS AND MALE FERTILITY

Sperm are particularly susceptible to oxidative damage owing to several factors. First, even though increasing the proportion of PUFAs in their cell membranes is a normal event in sperm maturation that may confer reproductive advantage, PUFAs are highly vulnerable to oxidation by reactive oxygen species (ROS). In fact, dietary supplementation with PUFA-rich oils (compared to MUFA- and SFA-rich oils) in rats increased DNA fragmentation, depleted testicular levels of antioxidant vitamins, and generated high concentrations of ROS [135]. ROS, in turn, may result in a cascade of lipid peroxidation that drastically affects the functional integrity of sperm [136]. In addition, because sperm lose almost all of their cytoplasm during spermatogenesis [137], these cells have essentially no intracellular defense mechanisms against oxidative damage, forcing them to rely on enzymatic and nonenzymatic antioxidants in seminal plasma [136]. Dietary intake of antioxidant vitamins may therefore be associated with greater male reproductive potential by protecting sperm from oxidative damage.

There is a vast literature on the role of antioxidants on male fertility, mostly based on RCTs [128,138–171]. A meta-analysis of RCTs published in 2011 assessed the effects of antioxidant supplementation in subfertile men on semen quality parameters and, particularly, on clinical pregnancy and live birth rates [172]. Among 214 couples from three trials [141,142,144], compared to controls, couples where men were randomized to antioxidants had higher live births rates [172]. Likewise, among 964 couples based on 15 trials [138,142,144,147,152–156,159,161,162,164,166,169], the meta-analysis found evidence of a statistically significant higher pregnancy rate in couples where men were randomized to antioxidants [172]. The meta-analysis also found evidence of a significant benefit of antioxidant supplementation on sperm motility after 6 months of treatment (10 trials, 963 men) but not after 3 months (10 trials, 514 men) or 9 months (3 trials, 332 men) of treatment, as well as an increase in sperm concentration after 6 months (6 trials, 825 men) and 9 months (3 trials, 332 men) of treatment but not after 3 months of treatment (7 trials, 320 men) [172].

Although this meta-analysis of RCTs presents robust evidence, there are still many gaps in the literature to clarify the role of antioxidants in the management of couples seeking fertility treatment. In fact, the authors rated the quality of the evidence from this meta-analysis as "very low" and labeled their results as inconclusive [172]. Some of the limitations of the meta-analysis include the very broad definition of antioxidants, which led to the inclusion of trials of nutrients that are not typically consider antioxidants, such as trials of marine omega-3 PUFAs [101,102] or that could influence male reproductive function through other mechanisms completely unrelated to preventing oxidative damage such as by influencing sperm DNA production [128], as discussed in Section 10.3. Second, the number, type, and dose of antioxidants varied greatly across studies. There were no two trials that compared the exact intervention among all the trials included in the meta-analysis [172]. In other words, although pooled together in a single analysis, these were all trials of different interventions. Consequently it is challenging to attribute the beneficial effects observed to any one nutrient or combination of nutrients and it is nearly impossible to identify the minimal doses of individual antioxidant nutrients that could be reasonably expected to have a clinical impact. Another major concern, especially among trials reporting live birth or clinical pregnancy outcomes, is that dropout rates were relatively high and tended to be higher in the control group, raising concerns about the possibility of selection bias.

Some of the gaps from the clinical trial literature on antioxidants and semen parameters can be filled by observational studies. Although few, they have documented dose–response relations between intakes of vitamins C, E, and carotenoids with higher sperm concentration, motility, or morphology [173–175] and reduced risk of oligoasthenoteratospermia [129], suggesting that these nutrients may explain some of the beneficial effects observed in the trials. However, there is clearly more research needed to identify which antioxidants or combinations of antioxidants have positive effects on male fertility.

10.6 VITAMIN D AND MALE REPRODUCTIVE FUNCTION

The role of vitamin D in reproduction has been the source of increased attention [176]. The vitamin D receptor (VDR) present in human testis, epididymis, seminal vesicles, and spermatozoa [177,178] induces nongenomic effects on spermatogenesis. Vitamin D as 1,25-hydroxyvitamin D ($1,25(OH)_2D$) promotes intracellular calcium [179] through channels in the sperm tail such as CatSper and voltage-dependent calcium channels [180,181]. Calcium influx across these channels [182,183] activates sperm motility [179,184]. A *VDR* knockout male rodent model revealed decreased sperm motility and sperm counts and increased LH and FSH levels [185]. In a different study, males fed a vitamin D-deficient diet had smaller corresponding litter sizes [186].

Several cross-sectional studies in men older than 50 years of age have found a positive association between serum vitamin D and testosterone. They have also been observed to have concordant seasonal fluctuations [187,188]. In an RCT among 31 vitamin D-deficient men (mean 25(OH)D = 29.7 nmol/L, mean age = 49 years), supplementation of 83 μg vitamin D per day for 1 year significantly increased total testosterone levels by 25% [189]. There were no changes in testosterone levels in the placebo group [189]. A cross-sectional study among 2299 men (mean age of 62 years old) routinely referred for coronary angiography found a positive association between vitamin D (25(OH)D) and testosterone [188]. In a different cross-sectional study among 1362 men (mean age 66 years old), Nimptsch et al. observed that vitamin D (25(OH)D) and testosterone were positively associated at levels lower than 75–85 nmol/L [190]. This association, however, was present only among men older than 70 years of age (P trend = 0.002), but not among men younger than 70 years old (P trend = 0.53) [190]. Furthermore, this association reached a plateau above 85 nmol/L. Among 3369 men in a cross-sectional multi-European countries study, vitamin D was weakly associated with testosterone levels [191]. A potential explanation for the age-related association is that it may be mediated by SHBG and calcium and phosphate homeostasis [187]. With age, SHBG levels increase while vitamin D and testosterone decrease [187]. In addition, vitamin D is associated with SHBG in young but not older men [187]. The relationship between vitamin D, testosterone, and other sex hormones deserves further investigation.

An increasing number of epidemiologic studies have examined the association between vitamin D and semen quality parameters. They, however, provide opposing results. Two studies reported that circulating vitamin D levels were positively related to semen parameters [184,192], yet another found an inverse U-shaped association [193], and a fourth found no association [194]. In a cross-sectional study among 300 men (mean age of 19 years) from the general population in Denmark, those with serum vitamin D (25(OH)D) greater than 75 nmol/L had 13% higher percent motile sperm and 33% higher morphologically normal sperm as compared to those with serum vitamin D less than 25 nmol/L [184]. A cross-sectional study among men aged 20–40 years found that serum vitamin D (25(OH)D) was positively associated with sperm motility and morphology among both a group of 195 fertile men and a group of 314 oligoasthenoteratospermic or normospermic infertile men [192]. In a cross-sectional study among 147 men (mean age 29 years) from the general population in Utah, men with serum vitamin D (25(OH)D) between 50 and 125 nmol/L had 16% higher motile sperm, 36% higher morphologically normal sperm, and 109% higher total sperm count as compared to those with serum vitamin D less than 50 nmol/L [193]. In addition, there was evidence of an inverse U-shaped association with total progressive motile sperm count; men with serum vitamin D (25(OH)D) between 50 and 125 nmol/L had 118% higher total progressive motile sperm count than

those with serum vitamin D less than 50 nmol/L, and 89% higher total progressive motile sperm count than those with serum vitamin D 125 nmol/L or greater [193]. Finally, a cross-sectional study among men from the general population did not find evidence of an association between serum vitamin D and semen parameters [194]. All of the studies to date have been cross-sectional, thereby limiting causal inference. In addition, no studies have evaluated whether male vitamin D status relates to fertility in couples trying to conceive naturally or to pregnancy and live birth rates among couples undergoing infertility treatment. An RCT of vitamin D supplementation and semen quality parameters is underway as of the writing of this chapter [195]. This study will shed more light on the strength and direction of the association between vitamin D and semen quality.

10.7 DIETARY PATTERNS, FOOD GROUPS, AND SEMEN QUALITY PARAMETERS

Two studies have investigated whether overall dietary patterns were associated with semen quality parameters. In a cross-sectional study among fertility patients in the Netherlands, two diet patterns were identified: the "health conscious" pattern (characterized by high intake of fruits, vegetables, fish and seafood, whole grains, and legumes and low intake of mayonnaise, meat products, refined grains, and desserts) and the "traditional Dutch" pattern (characterized by high intake of potatoes, meat products, whole grains, margarine, and mayonnaise and low intake of alcohol, nonalcoholic drinks, breakfast cereals, fruits, soup, and desserts) [80]. The "health conscious" pattern was associated with lower sperm DNA fragmentation while the "traditional Dutch" pattern was associated with higher sperm concentration [80]. When individual foods were investigated, higher intakes of fruits and vegetables were related to lower sperm DNA fragmentation and higher sperm motility [80]. In a cross-sectional study among young healthy men in the United States, the same statistical technique was employed to identify dietary patterns and related these to semen parameters [196]. In this study, the "prudent" dietary pattern, characterized by high intake of fruits, vegetables, legumes, and whole grains, was positively associated with progressive sperm motility. No one food group appeared to explain this positive association [196]. Although the patterns identified in the two studies are clearly different, there is some consistency in the relation of fruit-containing patterns. Other studies should examine this relation and whether overall dietary patterns have an impact on a couple's fertility.

Another two studies examined food groups in relation to semen quality parameters. In a case-control study comparing dietary habits of oligoasthenoteratospermic versus normospermic fertility clinic patients in Spain, Mendiola and colleagues observed that cases had higher intake of full-fat dairy, processed meat products, and potatoes; and lower intake of skimmed milk, fruits, vegetables, and shellfish than controls [76]. In a different case-control study among asthenozoospermic men in Iran, the odds of asthenozoospermia were marginally higher with increasing intake of total dairy, processed meat products, and sweets and significantly lower with increasing intake of skim milk, fish, poultry, fruits, and vegetables [77].

The main limitation of these studies is that, by design, dietary assessment was concurrent with outcome assessment and therefore it is difficult to attribute causality of the observed associations. Second, although three out of the four studies were carried out among infertility patients, it is not possible to generalize these results to men from the general population as fertility patients may have changed their behavior as a result of having difficulties conceiving. Third, because the Mendiola et al. study dichotomized semen quality as poor versus normal, it is not possible to identify which of the individual semen parameters (or if multiple parameters) was driving the association; thus it is not possible to compare these results with the other studies.

10.8 PHYSICAL ACTIVITY AND SEMEN QUALITY PARAMETERS

Physical activity has been associated with many health benefits; however, the relation between exercise and male fertility is unclear. This is likely due to differences in type, range, and intensity of

physical activity across studies. Most studies have examined this relation among endurance runners and cyclists. Prolonged strenuous exercise has been associated with reduced semen parameters and reproductive [12,197–204] hormones. Specifically, reductions in sperm motility [197,205,206], morphology [197,205], concentration [197], and volume [205] and in total [200,201,207] and free testosterone [200]. In addition, variations in hormone levels across seasonal training periods have been observed. For example, among high-endurance cyclists, testosterone levels decreased 2 weeks into the competition [207] as compared to pre-competition levels.

Prolonged strenuous physical activity is related to oxidative stress [208]. The effect of strenuous exercise on sperm morphology may, however, be reversed through antioxidant supplementation [209]. Regular moderate exercise, on the other hand, has been shown to reduce markers of oxidative stress [210] and to suppress testicular aging among male mice engaging in lifelong running [211]. Aged lifelong runner mice exhibited increased testosterone levels, preserved testicular cellular composition and integrity, and decreased levels of lipid peroxidation and oxidative stress in the testes compared to sedentary mice [211].

Lower intensity physical activity, on the other hand, has been associated with higher semen parameters in some studies [13,197,212] but not in others [12]. Among men attending a fertility clinic, no deleterious effect of moderate exercise on semen quality was found [12]. However, men who biked more than 5 hours per week had lower sperm concentration. This association was probably due to a mechanical effect caused by the pressure of the scrotum on the bicycle saddle. In a population of healthy young men, higher moderate to vigorous physical activity was associated with higher sperm concentration and total sperm count [13]. These results may not be generalizable to the general population given that these men were, on average, significantly more active than men in the general population. In a different study, compared to sedentary men, physically active men practicing 2–4 h/week of endurance activities, except bicycling, for at least three different days of the week for more than a year had higher percent motile sperm and morphologically normal sperm [212].

Overall, endurance or high-intensity athletic training, when the body is in negative energy balance, is related to lower semen parameters; however, recent evidence suggests that moderate levels of physical activity may be beneficial [13,212].

10.9 EPIDEMIOLOGICAL STUDY DESIGNS IN SEMEN QUALITY STUDIES

Most semen quality studies are cross-sectional, that is, exposures and semen parameters are assessed concurrently and typically involve men presenting to fertility clinics for evaluation and treatment. Although this study design is extremely convenient and facilitates the study of potential determinants of semen quality parameters in clinical settings, there are also features that make them particularly problematic.

A major problem of cross-sectional studies (and of case-control studies that are not nested within a prospective cohort study) is the particular weakness of this design for differentiating between causal and noncausal relations. In these studies it is not always possible to adequately identify the temporal relation between the purported risk factor and semen quality parameters. Even when clear temporal relations can be established, the risk factor of interest is usually retrospectively recalled, which can introduce error owing to inaccurate recall. The second common design characteristic, drawing on men presenting to fertility clinics as research subjects, presents additional challenges. Men attending fertility clinics may have changed their behavior (including potential risk factors such as diet, alcohol use, drug use, etc.) as a result of having difficulties conceiving. This increases the chance of reverse causation further complicating causal inference. In addition, results of studies among fertility patients may not be generalizable to men in the general population because infertile couples who eventually receive treatment are known to differ significantly from infertile couples who do not receive treatment [213,214]. Although semen parameters have been related to spontaneous pregnancy, they are not good at discriminating men who will eventually father a pregnancy (natural or as a result of treatment) from those who will not [16–20].

RCTs evaluating semen quality parameters as an outcome are rare and have so far been used to evaluate, for the most part, effects of specific nutrients or supplements [128,172,215–217]. Nevertheless, RCTs are not well suited to evaluate exposures that cannot be randomized due to logistic or ethical issues (e.g., smoking, obesity).

Prospective cohort studies with standardized assessment of a variety of potential confounders might be the best study design if RCTs are not feasible. Ideally, these studies would not only evaluate repeated semen samples over time, but also couple fecundity. This approach has been successfully implemented among couples trying to conceive naturally [218] and among couples presenting for fertility treatment [219]. Prospective cohort studies could be used as a cost-effective tool to assess multiple factors contributing to male fertility, which could be screened and evaluated in terms of feasibility to test in RCTs.

10.10 SUMMARY

In summary, research on diet and sperm function is an emerging area of investigation. The literature suggests that a few dietary factors may be associated with semen quality parameters. For example, a healthy diet pattern and intakes of low-fat dairy, unsaturated fatty acids, and folic acid present consistent associations with higher semen quality parameters, whereas processed red meat, high-fat dairy, saturated and *trans* fat, excessive alcohol, and soy intake seem to have deleterious associations with semen quality parameters. There are still many areas of controversy such as vitamin D and antioxidants. Multiple RCTs of the effect of antioxidants on semen parameters have been conducted suggesting a positive effect on semen quality parameters. However, effects on live births or clinical pregnancies among couples seeking fertility care are inconclusive. Similarly, data from animal studies strongly suggest a role of vitamin D on fertility but human data have been highly inconsistent and equivocal and its clinical significance is currently unknown. RCTs of these factors are needed. Finally, prospective studies with comprehensive dietary assessments and rigorous assessments of potential confounders, evaluating the role of men's diet on a couple's fertility are essential to understand better how diet affects men's fertility.

10.11 KEY POINTS SUMMARY

1. Several studies suggest that semen quality in Western populations has been declining over the last few decades. Although environmental pollutants such as plasticizers (phthalates) may be one of the causes for this decline, changes in diet may be another possible cause.
2. Consumption of certain meats (fatty red meat cuts, processed meats) and full-fat dairy products (whole milk and cheese) have been linked with a reduction in semen quality parameters, particularly impaired sperm morphology and motility. The precise mechanisms for this adverse effect on sperm production are uncertain, but may include a negative effect of saturated fat, hormone residues contained in milk and meat, and possibly preserving agents in processed meats.
3. Several studies have suggested that a diet containing high amounts of fruit and vegetables may assist in maintaining optimal sperm function. This may be mediated primarily by their high content of beneficial antioxidants and folate.
4. Studies have reported positive correlations between dietary antioxidant intake, specifically vitamins C, E, and carotenoids, and male fertility. A recent meta-analysis of 15 trials using various different antioxidant supplements concluded that antioxidants may have the capacity to improve sperm concentration and motility, decrease DNA damage, and boost pregnancy rates.
5. Folate, a vitamin found in abundance in leafy green vegetables, has also been linked with improved sperm concentration, motility, and a reduction in sperm DNA fragmentation and genetic aneuploidy.

6. Serum vitamin D levels have been positively correlated with sperm motility, morphology, and concentration in many, although not all studies. However, no randomized controlled trial evidence currently exists supporting the role of vitamin D supplementation in boosting sperm quality or pregnancy rates.
7. The majority of studies have shown no link between coffee intake and sperm quality, with only one study linking high coffee consumption (>308 mg/day or five brewed cups) with increased DNA damage.
8. Moderate alcohol intake has not been linked with any impairment in sperm function, with some studies actually suggesting a small beneficial effect from moderate consumption compared to no consumption. High alcohol intake (>20 standard units week) has been linked with impaired sperm function, altered male reproductive hormone profile (increased estrogen, reduced testosterone), and significantly reduced fecundity.
9. Soy products contain isoflavones with weak estrogenic activity that may theoretically impair spermatogenesis. Although not all studies on the topic are consistent, some reports, including a large study among Asian men, suggest that high consumption of soy isoflavones is related to lower sperm concentration.
10. Studies linking nutrition with male reproductive health are difficult to conduct for a number of reasons. First, the majority of studies use semen parameters as a proxy for male fertility, despite its limited predictive ability. Second, most studies examining the link between sperm health and nutrition have been conducted among infertile men, who may not be representative of the larger fertile population and who may have made recent dietary changes in an attempt to boost their own fertility, thereby biasing these studies' results. Finally, cross-sectional studies typical of this area of investigation are extremely weak at identifying causal relationships because it is not always possible to adequately identify the temporal relationship between the purported risk factor and semen quality.
11. Based on the existing evidence, a diet designed to boost sperm health would contain large amounts of fruit and vegetables, fish, and walnuts (high in omega-3 fatty acids) and a moderate amount of alcohol, while minimizing intake of processed and red meats, full-fat dairy (whole milk and cheese), hydrogenated oils (a source of trans fats), and soy products. In addition, regular moderate to vigorous exercise, as opposed to strenuous exercise such as high-intensity athletic training, seems to be beneficial. Finally, some evidence supports the use of antioxidant, omega-3, and vitamin D supplements to boost sperm quality and/or testosterone production.

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11 Nutritional Supplementation for the Treatment of Male Infertility

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CONTENTS

11.1 INTRODUCTION

11.1.1 Male Infertility

Infertility is a common healthcare problem affecting nearly 15% of couples. Although previous studies have focused on female factors as the primary cause, current research indicates that about 25% of cases are exclusively caused by a male factor, and nearly 30–40% of all infertility cases have direct contributions from male factor insufficiency [1]. Several research groups have also noted a steady decrease in sperm quality over the past 50 years, further contributing to male factor infertility [2,3].

11.1.2 Reactive Oxygen Species and Male Infertility

The etiology of male infertility is often varied and may include environmental, endocrine, and genetic factors. One significant cause of male factor deficiency is the generation of reactive oxygen species (ROS), which can damage sperm DNA, membranes, and proteins. If not properly neutralized, ROS can lead to impaired motility, cytoskeleton damage, disruption of membrane fluidity, and even sperm apoptosis [4]. ROS include oxygen-containing compounds that have one or more unpaired electrons (free radicals) as well as non-radical oxygen compounds. The most frequently produced free radical is the superoxide radical O_2^-, but other common free radicals include $HClO^-$ and OH^-. Ozone and hydrogen peroxide (H_2O_2) are examples of non-radical ROS [5].

Numerous sources of ROS exist. The two most prevalent sources are leukocytes and abnormal sperm [6,7]. Leukocytes (particularly macrophages and neutrophils) produce ROS such as O_2^- and H_2O_2 as part of the natural host immune defense against pathogens. Neutrophils also produce $HOCl^-$ through the action of oxygen-dependent myeloperoxidase. Abnormal sperm may retain more cytoplasm than developmentally normal ones, leading to increased ROS production [5]. NADPH oxidase in the sperm membrane produces ROS, and the concentration of this enzyme may increase with abnormal cytoplasmic retention [6]. Along with this, diaphorase (a mitochondrial NADH-dependent oxidoreductase) may also increase sperm ROS production [8]. Lastly, H_2O_2 generated by superoxide dismutase (SOD) may temporarily inactivate glucose-6-phosphate dehydrogenase, which decreases the amount of NADPH available to neutralize ROS [5].

Under normal physiological conditions, the amount of ROS produced does not exceed the rate at which the sperm are able to neutralize these compounds. The production of ROS is imperative for cellular functions in a limited capacity. O_2^- may serve as a metabolite for signal transduction within the sperm [9,10]. A natural overproduction of ROS also occurs during sperm hyperactivation, capacitation, and fertilization [6,9,11,12]. Significantly elevated levels of ROS, however, may cause 30–80% of all male infertility cases [10]. Specific risk factors for the development of ROS

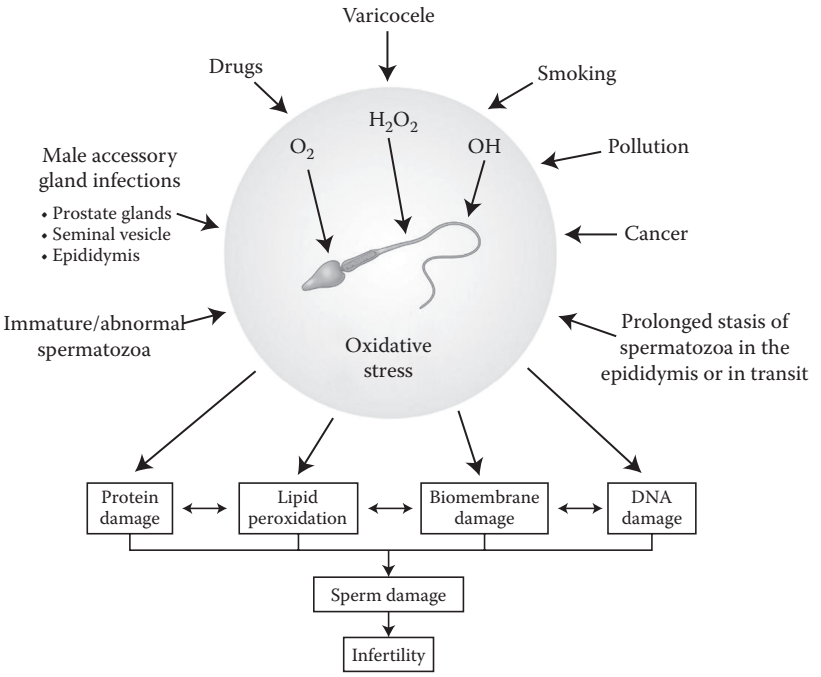

FIGURE 11.1 Causes, mechanisms, and effects of oxidative stress on male infertility.

are diverse, and common examples include lifestyle (obesity, stress, alcohol, tobacco), environment (heat, pollution), infection (acute, chronic), and inflammation [13]. Figure 11.1 illustrates the effect of oxidative stress on male fertility.

11.1.3 Oxidative Stress

When the generation of ROS surpasses the sperm capacity to eliminate the free radicals and reactive metabolites that are generated, oxidative stress (OS) occurs. Figure 11.1 illustrates the mechanism of oxidative stress. OS can lead to a decrease in semen quality, resulting in a pathological decline in several sperm parameters as a result of free radical damage. For example, it may lead to a decrease in sperm motility as a result of reduced ATP production or damaged axonemal proteins [14,15]. The ability of sperm to undergo capacitation and induce fertilization may also decrease owing to reduced membrane fluidity, increased membrane peroxidation, and loss of membrane ATP [16]. OS can also lead to a loss of membrane integrity as well as DNA damage and apoptosis [4,7,17].

The functional morphology of sperm increases its susceptibility to OS and subsequent oxidative damage. Sperm are vulnerable to oxidative damage owing to their abundance of polyunsaturated fatty acids (PUFAs), which are at a high risk for lipid peroxidation. In addition, sperm maturation leads to a reduction in cytoplasmic content, limiting the amount of enzymes and antioxidants available to counter ROS production and repair ROS-induced nuclear and mitochondrial DNA damage [5].

11.2 ANTIOXIDANTS

Semen naturally contains antioxidants to counter ROS. Antioxidants are molecules that scavenge free radicals and prevent deleterious cell damage due to chain reactions involving unpaired elections. Sperm contain two broad categories of antioxidants: enzymatic antioxidants, including SOD, catalase, and glutathione peroxidase [18–20], and nonenzymatic antioxidants including vitamin C, vitamin E, glutathione, amino acids, albumin, carnitines, carotenoids, flavenoids, and prostasomes [13]. The nonenzymatic antioxidants can be further divided into synthetic and dietary antioxidants.

Dietary antioxidants can be found in natural foods. These foods often contain multiple antioxidants and other enzymatic cofactors that may act synergistically within the body to eliminate ROS. In contrast, synthetic antioxidants are manufactured dietary supplements. For this reason, isolated supplementation with a synthetic antioxidant may limit the synergistic effect commonly observed with dietary antioxidants, and therefore, these compounds may offer suboptimal protection against ROS in certain cases [21,22]. The effective use of synthetic antioxidants may require combination supplementation to achieve a significant ROS-scavenging ability [23].

Because of the prominent role OS plays in male infertility and the promising ability of dietary and synthetic antioxidants to neutralize ROS, significant research has been performed to assess their efficacy. A substantial number of these studies have reported diminished ROS levels on supplementation and improved semen parameters. However, other trials have found that many of the same antioxidants had little or no effect on ROS and/or semen quality.

Sections 11.2.1–11.2.10 review the most common antioxidants, together with their recommended dosing and possible value in improving sperm function and male fertility potential. Table 11.1 shows the effects of antioxidants on male fertility.

11.2.1 Vitamins A, C, and E

Vitamins are metabolic cofactors needed in a variety of biochemical processes for synthesis of essential nutrients. Vitamins A, C, and E have been heavily researched in the last two decades and possess antioxidant activities. Vitamin A is lipid soluble and necessary for visual acuity, and it may also act as a growth factor [24]. Vitamin A also helps maintain the mucous membranes of the genitourinary tract, gastrointestinal tract, eye, and skin [25]. The mechanism of action as an

TABLE 11.1
Effects of Antioxidants on Male Fertility

Study	Antioxidant	Effects on Semen Quality	Reproductive Outcomes
Scott et al. [26]	Vitamin A (with selenium, vitamins C and E)	Increased motility	N/A
Ehrenkranz [27]	Vitamin C (with vitamin E)	No change	No change
Palamanda and Kehrer [28]	Vitamin C (with vitamin E)	No change	No change
Kessopoulou et al. [29]	Vitamin C	No change	No change
Thiele et al. [30]	Vitamin C	Increased normal morphology	N/A
Baker et al. [31]	Vitamin C (with vitamin E)	No change	No change
Rolf et al. [32]	Vitamin C (with vitamin E)	No change	No change
Kessopoulou et al. [29]	Vitamin E	No change	No change
Suleiman et al. [33]	Vitamin E	Decreased MDA, increased motility	Improved pregnancy rate
Geva et al. [34]	Vitamin E	No change	No change
Keskes-Ammar et al. [35]	Vitamin E	Increased motility	No change
Menchini-Fabris et al. [36]	Carnitine	Increased motility with increased supplementation	N/A
Bornman et al. [37]	Carnitine	Increased motility with increased supplementation	N/A
Moncada et al. [38]	Carnitine	Increased progressive motility	N/A
Costa et al. [39]	Carnitine	Improved count, motility, and morphology	N/A
Vitali et al. [40]	Carnitine	Increased motility	N/A
Lenzi [41]	Carnitine	Increased concentration and motility	N/A
Cavallini et al. [42]	Carnitine	Increased concentration, motility, and morphology	N/A
De Rosa et al. [43]	Carnitine	Increased motility, count, and membrane integrity	Increased cervical mucus penetration
Balercia et al. [44]	Carnitine	Increased motility velocity	No change
Sigman et al. [45]	Carnitine	No change	N/A
Zhou et al. [46]	Carnitine	Increased motility	Improved pregnancy rates
Balercia et al. [47]	CoQ_{10}	Increased motility	Improved pregnancy rates
Balercia et al. [48]	CoQ_{10}	Increased motility	No change
Mancini et al. [49]	CoQ_{10}	No change	No change

Lewin and Lavon [50]	CoQ_{10}	No change	Increased IVF/ICSI success rates
Safarinejad [51]	CoQ_{10}	Increased concentration, morphology, and motility	N/A
Safarinejad et al. [52]	CoQ_{10}	Increased count, motility, morphology, acrosome reaction efficiency. Increased inhibin B and FSH	N/A
Nadjarzadeh et al. [53]	CoQ_{10}	Increased morphology, concentration, and activity of catalase and SOD	N/A
Gupta and Kumar [54]	Lycopene	Increased motility, morphology, and concentration	Improved pregnancy rates
Mendiola et al. [55]	Lycopene	Decreased semen quality with decreased supplementation	N/A
Vicari et al. [56]	NSAIDs	Increased motility and viability, decreased ROS	Improved pregnancy rates
Cavallini et al. [42]	NSAIDs	Increased concentration, motility, and morphology	Improved pregnancy rates
Srivastava and Agarwal [57]	Arginine	Improved motility	N/A
Landau et al. [58]	Folic acid	No effect	N/A
Wong et al. [59]	Folic acid	Improved sperm concentration, no improvement in sperm motility or morphology	N/A
Ebisch et al. [60]	Folic acid	Decrease in folic acid in seminal plasma caused by increase in DNA fragility and sperm DNA damage	N/A
Tremellen et al. [61]	Folic acid	N/A	Improved pregnancy rate
Imhof et al. [62]	Folic acid	No effect	N/A
Lenzi et al. [63–65]	GSH	Increased sperm motility and morphology	N/A
Kodama et al. [12]	GSH	Increased sperm concentration	N/A
Atig et al. [66]	GSH	Maintained good sperm quality and motility	N/A
Oeda et al. [67]	NAC	Preserved sperm motility	N/A
Erkkila et al. [68]	NAC	Improved germ cell survival	N/A
Comhaire et al. [24]	NAC	Decreased ROS. No effect on semen motility and morphology	N/A

(*Continued*)

TABLE 11.1 (Continued)
Effects of Antioxidants on Male Fertility

Study	Antioxidant	Effects on Semen Quality	Reproductive Outcomes
Ciftci et al. [69]	NAC	Increased sperm volume, concentration, motility and viscosity	N/A
Safarinejad and Safarinejad [70]	NAC	Improved sperm motility and morphology	N/A
Noack-Fuller et al. [71]	Selenium	Preserved normal sperm morphology	N/A
Scott et al. [26]	Selenium	Sperm concentration unchanged, increased sperm motility	N/A
Tremellen et al. [61]	Selenium	N/A	Improved pregnancy rates
Safarinejad and Safarinejad [70]	Selenium	Improved sperm motility and morphology	N/A
Wong et al. [59]	Zinc	Improved concentration and sperm count	N/A
Colagar et al. [72]	Zinc	Improved sperm parameters, increased seminal antioxidant capacity, and reduced oxidative stress	N/A
Atig et al. [66]	Zinc	Fertile men have greater concentration of zinc than infertile men. Decreased amount of zinc correlated with poor sperm production, concentration, and motility	N/A
Tremellen et al. [61]	Menevit	No effect	Increased pregnancy rates
Tunc and Tremellen [73]	Menevit	No change in concentration, motility, or morphology. Increased DNA integrity and protamine packaging	N/A
Omu et al. [74]	Vitamin E, zinc	Increased motility	Increased fertilization capabilities

Note: CoQ_{10}, coenzyme Q_{10}; FSH, follicle-stimulating hormone; GSH, glutathione; IVF/ICSI, *in vitro* fertilization/intracytoplasmic sperm injection; MDA, malondialdehyde; NAC, *N*-acetylcysteine; NSAIDs, nonsteroidal anti-inflammatory drugs; ROS, reactive oxygen species; SOD, superoxide dismutase.

antioxidant is not yet well understood, but studies have shown that it can reduce ROS levels and improve semen parameters [24,26]. Studies on vitamin A as a monotherapy have yet to be performed, and therefore, its efficacy as a sole agent remains unknown [25].

Vitamin A has been studied in combination with other antioxidants including zinc, *N*-acetylcysteine (NAC), selenium, and vitamins C and E. Scott et al. reported a 30% increase in motility after a 3-month regimen of vitamin A supplementation combined with vitamins C and E and selenium [26]. Galatioto et al. found seminal Xuid analysis showed that the median value of sperm count was 14.42 (11.75–15.45) millions/mL before treatment and 32.58 (18.75–35.25) millions/mL after antioxidant treatment ($P = 0.009$) [75]. Over-supplementation (above 10,000 IU/day) is associated with decreased visual acuity, skin dryness, hepatotoxicity, gastrointestinal (GI) distress, and other side effects [76]. Vitamin A is found in many foods including meat, dairy products, eggs, fish, fruits, and vegetables such as carrots and leafy greens [25].

Vitamin C is a water-soluble antioxidant that is available in semen at a concentration 10 times higher than that in in serum [77]. It functions in the generation of biomolecules such as collagen, proteoglycans, and other essential intercellular proteins [69]. Vitamin C also prevents peroxyl attack by lipoproteins, sustains vitamin E in its reduced form, and defends against oxidative DNA damage [77,78]. Many studies have been performed to assess the efficacy of vitamin C as a supplement and its ability to combat ROS. A study of 42 patients by Thiele et al. showed that vitamin C levels were positively correlated with the number of morphologically normal sperm in a specimen [30]. Most studies combined vitamin C with vitamin E and various other antioxidants [79,80]. Although some studies have reported a possible synergistic effect when vitamins C and E are used together [31], most have shown no efficacy. For instance, a randomized, placebo-controlled, double-blind study in which subjects received 1000 mg of vitamin C and 800 mg of vitamin E for 56 days showed that the regimen had no effect on sperm parameters or pregnancy rates [32]. Studies assessing vitamin E as a monotherapy also found that it had no significant effects [27–29,32]. Over-supplementation with vitamin C has been associated with ROS generation and kidney stones [81,82]. The most common sources of vitamin C are fruits and vegetables [25].

Vitamin E is the most studied vitamin antioxidant. This lipid-soluble vitamin directly blocks lipid peroxidation in the sperm membrane by neutralizing ROS-generated free radicals. Vitamin E may also block ROS formation by diminishing leukocyte attraction via its anti-inflammatory actions [83]. Lastly, vitamin E is similarly thought to reduce ROS by aiding the activity of other important antioxidants in the body [27,28]. Studies contain conflicting data on the efficacy of oral vitamin E supplementation in reducing ROS levels in semen. However, two randomized controlled studies showed that it improved semen parameters. Suleiman et al. reported an increase in sperm motility and a 21% increase in pregnancy rates and decreased malondialdehyde (MDA) levels in asthenozoospermic men after supplementation with vitamin E [33]. In another double-blind randomized, placebo, crossover controlled trial by Kessopulou et al., a 3-month vitamin E supplementation regimen increased the efficiency of sperm zona binding [29]. In addition to these studies, Geva et al. also showed decreased MDA levels and increased fertilization rates in a prospective study analyzing the effects of a 3-month, 200 mg/day supplementation regimen [34].

The current allowable intake of vitamin E for men is 15 mg/day, with possible side effects beginning at 400 IU [82]. Common sources of vitamin E include fruits, vegetables and vegetable oil, eggs, meat, poultry, grains, and wheat germ [25]. Because it can inhibit platelet aggregation, it is contraindicated in patients with hemorrhagic illnesses or those currently taking anticoagulants [84]. Side effects associated with supplementation include GI distress, rashes, blurry vision, headache, fatigue, and muscle weakness [85].

11.2.2 Carnitines

Carnitines, which include both L-carnitine and L-acetyl carnitine, are important water-soluble antioxidants that generate metabolic energy by shuttling long-chain fatty acids across mitochondrial

membranes to initiate β-oxidation. This process generates reducing equivalents in the form of NADH and $FADH_2$ as well as the two-carbon molecule acetyl-CoA which can then be further oxidized in the tricarboxylic acid (TCA) cycle to generate cellular energy. Carnitines provide the principle energy source necessary for sperm motility [86]. They also play a role in protecting the phospholipids of the mitochondrial membrane by preventing the oxidation of fatty acids located within it [41,87]. Carnitines are synthesized in the liver, transported to the epididymal epithelium, and carried into the lumen of the epididymis, which is an important site of sperm development [88]. As antioxidants, they play a role in preventing membrane and DNA damage from free radicals and subsequent apoptosis of the cells [41,42,63,89]. Supplementation with carnitines has also been shown to increase both sperm concentration and motility [36,37]. The effects of carnitines on sperm parameters are thought to occur in the epididymis after the sperm have left the testes [41,63].

For L-carnitine or combined carnitine supplementation, many studies have reported increased motility and/or concentration with supplementation [38–43,46,47]. For example, Vitali et al. studied subjects on a 3-month, 3 g/day supplementation with L-carnitine and found that it increased motility in 78% of patients along with sperm density [40]. Other studies, however, indicated no improvement in semen parameters with L-carnitine supplementation [39,45].

The most prominent sources of carnitines are red meat, but they also may be found in poultry, fish, dairy products, fruits, and vegetables [90]. Supplementation above 4 g/day is associated with GI distress, foul-smelling body odor, and possibly seizures [85].

11.2.3 Coenzyme Q_{10}

Coenzyme Q_{10} (CoQ_{10}) is an antioxidant involved in many biochemical reactions but most commonly in those related to metabolism and the electron transport chain (ETC). Within the ETC, this lipid-soluble molecule transfers electrons from complexes I and II to complex III. Because of the role it plays in mitochondria, it is located primarily in the midpiece of the sperm. As an antioxidant, the active form, ubiquinol, acts as a free radical scavenger for lipoproteins and membrane lipids [83]. This antioxidant may have a natural protective function because its concentration increases with ROS sperm damage [49].

A study by Safarinejad et al. analyzed supplementation with CoQ_{10} in 228 men with idiopathic infertility by dividing them equally into two groups: one received 200 mg of ubiquinol/day and the other received a placebo. This study reported a significant increase in sperm count (9.8%), motility (4.5%), and morphology (1.8%) and an improvement in the efficiency of the acrosome reaction. The authors also reported a significant increase in levels of inhibin B and follicle-stimulating hormone (FSH), indicating that CoQ_{10} had a positive effect on both semen and Sertoli cells [51]. Most recently, Nadjarzadeh et al. showed that supplementation with CoQ_{10} increased catalase and SOD activity and improved sperm concentration and morphology [53]. Several other studies have reported similar positive effects on motility, count, morphology, and pregnancy rates, but whether these improvements are clinically significant remains to be established [44,47,48,50,70]. Mancini et al. found that CoQ_{10} supplementation did little to improve sperm motility and quality in subfertile men with varicocele [91].

Although no Recommended Daily Allowance (RDA) has been established, the optimal dose is thought to be 200–300 mg/day [25]. Common sources of CoQ_{10} include fish, organ meats (heart, kidney, and liver), nuts, soybeans, grains, and vegetables [85]. Over-supplementation has been associated with skin rashes, decreased appetite headache, and GI distress [25].

11.2.4 Lycopene

Lycopene is a natural antioxidant that is part of the carotenoid family. This compound scavenges free radicals and prevents cellular membrane and protein damage. It plays a role in immune reactions,

signaling in gap junctions, modulation of cell growth, and regulation of gene expression [92]. Of interest is the fact that lycopene is thought to have the greatest ROS-quenching rate of all the antioxidants [93]. Lycopene levels are high in semen and the testes. Infertile men are noted to have lower levels than fertile men [54].

Several studies have been performed to assess the efficacy of lycopene on semen quality. Gupta and Kumar found that lycopene significantly increased sperm count, motility, and normal morphology. In addition, in the patients who had a sperm count of at least 5 million/mL, supplementation also increased conception rates [54]. A study by Mendiola et al. compared the dietary intake of 30 men with poor semen quality to that of 31 normozoospermic men. In this study, semen quality improved with lycopene supplementation [55].

Lycopene is found in fruits (tomatoes, grapefruits, and watermelons) and vegetables [54]. As of yet, there are only very mild observed side effects (GI distress and skin color change) with gross over-supplementation [25].

11.2.5 Arginine

Arginine is a semi-essential amino acid that is a precursor to nitric oxide and spermine. It is necessary for spermatogenesis and sperm motility and plays a crucial role in cell division, wound healing, immune function, and hormone production [94]. Men who have arginine-deficient diets may suffer from low sperm count and have increased levels of nonmotile sperm. In a recent study by Srivastava and Agarwal, arginine improved motility and metabolism in sperm through the nitric oxide pathway [57]. However, the lack of a large number of randomized controlled studies exploring the efficacy of arginine makes it difficult to gauge its effect on male fertility [95,96].

Owing to lack of human data, there is no current RDA for arginine. Sources of arginine include animal and plant products such as dairy, turkey, beef, pork, seeds, soybeans, and nuts [96]. Commonly observed side effects associated with supplementation with arginine are GI distress, renal insufficiency, electrolyte imbalance, hypotension, and increased bleeding risk [95].

11.2.6 Folic Acid

Folic acid is a derivative of the water-soluble vitamin B_9 and is essential for the synthesis of purines and thymidine. It also plays a role in many other important cellular functions such as the transfer of one-carbon molecules and promoting proper spermatogenesis, although the underlying mechanism for this process is currently unknown [59,62]. Folic acid also acts as a free radical scavenger to prevent damage to lipids and proteins [86]. Infertile men may have lower concentrations of folic acid than fertile men [97].

Several studies have noted that folic acid supplementation improves sperm quality. A study by Wong et al. showed an increase in sperm concentration despite a lack of improvement in sperm motility or morphology [59]. Ebisch et al. showed that a decrease in folic acid concentration in seminal plasma correlated with an increase in sperm DNA damage [60]. These findings underscore the role folic acid plays in DNA synthesis and protein methylation reactions [98]. Despite these positive results, several other studies have reported that folic acid supplementation is ineffective in improving sperm quality. Originally, a study by Landau et al. reported that folic acid alone failed to improve sperm concentration in subfertile men [58]. A more recent study by Imhof et al. showed that supplementation with folic acid in fertile and subfertile men did not improve sperm parameters [62].

Sources of folic acid include green, leafy vegetables, beans, citrus fruits, and avocados. Oversupplementation with folic acid is associated with GI distress, rash, sleep disturbances, confusion, increase in seizure frequency, allergic reaction, and increased risk for myocardial infarction [99–101].

11.2.7 Glutathione

Glutathione (GSH) is an essential antioxidant that is synthesized in the liver and transported into the epididymis. It alleviates OS and helps maintain exogenous antioxidants in their active state. It is the most abundant reducing agent in the body, making it vital to the intracellular defense against ROS [86]. Its sulfydryl group directly neutralizes superoxide anions and other free radicals and therefore protects proteins and nucleic acids against oxidative damage [26,86]. Many studies have shown that GSH supplementation has a positive effect on semen parameters. Others have found that it increases sperm motility, morphology, counts, and forward progression as well as prevents DNA fragmentation [63–65,102]. Recently, Atig et al. studied the altered antioxidant status of the seminal plasma in infertile men and found that GSH helps maintain good sperm quality and motility. These authors showed that infertile men may have lower GSH levels than fertile men and that insufficient amounts of GSH can lead to abnormal sperm motility. In addition, their results suggested that GSH may enhance fertility by reducing lipid peroxidation [66].

GSH is found in fresh meat, fruits, and vegetables. The maximum RDA is 3 g/day [86], and a deficiency of GSH results in an unstable sperm midpiece, leading to defective morphology and motility [103].

11.2.8 *N*-Acetylcysteine

N-Acetylcysteine (NAC) is a nontoxic derivative of L-cysteine. It is a precursor of GSH and promotes its production to assist in neutralizing ROS [104,105]. It can also interact directly with oxidants, thiols, and hydroxyl radicals to remove free radicals and reduce oxidative stress in seminal plasma [69]. Many studies have shown that it has a beneficial effect on semen parameters. An *in vitro* study by Erkkila et al. showed that NAC improved germ cell survival [68]. Several studies have reported increased sperm motility, concentration, volume, and viscosity. Additional improvements included decreased ROS levels, increased efficiency of the acrosome reaction, and reduced oxidation of the sperm DNA [24,67,69,75]. In contrast, another study found that NAC supplementation did not improve semen parameters even though its seminal plasma concentration increased [51].

Sources of NAC include poultry, yogurt, egg yolks, oats, onions, and other vegetables. The limited side effects associated with supplementation are GI distress, rash, fever, headache, drowsiness, hypotension, and hepatic toxicity [106].

11.2.9 Selenium

Selenium is an essential micronutrient necessary for normal male reproductive function, testicular development, spermatogenesis, and spermatozoa motility and function [107]. It is a required cofactor for the reduction of antioxidant enzymes. It maintains sperm structural integrity by protecting against oxidative DNA damage. However, the exact mechanism of its action remains unknown [5,86]. Many studies have shown a positive correlation between increased selenium levels and increased sperm concentration, motility, and normal morphology [26,70,71,108].

Selenium is commonly found in soil, plants, and meat. The current recommended daily dose of selenium is 55–400 mcg [86]. A selenium deficiency is linked to decreased motility, breakage at the midpiece, and sperm head morphologic abnormalities. On the other hand, over-supplementation is associated with GI distress, nail changes, fatigue, and irritability [109]. Excessive intake has also been linked to serious side effects such as liver cirrhosis, pulmonary edema, and even death [83].

11.2.10 Zinc

Zinc is an essential micromineral that is an important cofactor for metalloenzymes, and it plays a role in DNA transcription and protein synthesis [86]. Zinc SOD is involved in DNA repair [96]

and also helps increase the concentration of GSH, both of which limit the damaging effects of ROS [86]. Many studies have reported that zinc supplementation improves sperm parameters such as concentration, progressive motility, and sperm integrity as well as pregnancy rates. In an *in vivo* study, men treated with 66 mg of zinc daily for 6 months experienced improved sperm concentration and count [59]. Omu et al. conducted a prospective study that showed zinc therapy increased sperm parameters and antioxidant capacity and reduced sperm DNA fragmentation and apoptotic markers. Omu's study also showed that fertile men have a greater concentration of zinc than infertile men and that a decrease in zinc concentration leads to an increase in OS and loss of sperm integrity [74]. A study by Colagar et al. showed improved sperm parameters, an increase in seminal antioxidant capacity, and a reduction in oxidative stress with zinc supplementation [72]. These results suggest that zinc may be useful in reducing OS and thus helping prevent sperm membrane and DNA damage [86].

A study by Atig et al. showed that fertile men have a greater concentration of zinc in their seminal plasma than infertile men. The researchers also found that the infertile men had an increased ROS level, which is associated with increased abnormal sperm parameters. They reported a positive correlation between decreased zinc levels and poor sperm production and concentration as well as motility [66]. Zinc supplementation has been shown to improve sperm parameters such as concentration, progressive motility, sperm integrity, and pregnancy rates.

Zinc is found naturally in soil, plants (such as wheat and seeds), beef products, oysters, and liver. Zinc deficiency has been associated with abnormal flagella and deformed midpiece [86]. The limited amount of experimental data for humans prevents the establishment of an RDA [110]. Side effects that may be a consequence of zinc supplementation above 200 mg/day include GI distress, rash, headache, loss of appetite, and dehydration [85]. More severe side effects such as anemia, low copper levels, impaired iron function, reduced immune function, and decreased high-density lipoprotein (HDL) levels are associated with supplementation above 450 mg/day [111].

11.2.11 Combination Antioxidant Therapy

Many research studies have focused on the use of combination therapy to determine whether there is a beneficial synergism between individual components. Vitamin E has been studied in several other combinations with different antioxidants. Keskes-Ammar conducted an *in vivo* study in which men were given a mixture of vitamin E and selenium daily for 6 months. Sperm motility was the only sperm parameter that improved [35]. A study by Omu et al. showed that combination supplementation with vitamins C and E and zinc improved motility and fertilizing capacity [74]. Wong et al. showed that combined supplementation with zinc and folic acid improved sperm concentration and sperm count [59]. Other examples include combination therapies with selenium and vitamins A, C, and E [26]; NAC with vitamins [75]; as well as nonsteroidal anti-inflammatory drugs (NSAIDs) combined with carnitines [42,56] as discussed previously in this chapter. One study showed that the combination of NAC and selenium improved sperm count, motility, and morphology [70].

One important emerging combination supplement is Menevit, which is a synthetic compound composed of several common antioxidants. It includes vitamin C, vitamin E, zinc, folic acid, lycopene, garlic oil, and selenium. This supplement was designed so that the different components would perform specific functions to reduce ROS levels synergistically and increase semen parameters and sperm quality. To date, two studies have been performed to analyze the efficacy of Menevit. In the first one, Tremellen et al. studied 60 couples with known male factor infertility. They found that Menevit supplementation increased *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) pregnancy rates better than placebo [61]. In the second study, Tunc et al. assessed the antioxidant therapy in 50 infertile male patients for 3 months. In that study, Menevit reduced ROS levels and apoptosis in sperm cells while improving DNA integrity and protamine packaging [73]. Neither of these studies found any improvements in sperm count, motility, or morphology [61,73].

11.3 HERBAL THERAPY FOR MALE INFERTILITY

Herbal therapy, along with other complementary and alternative therapies, is used for medicinal purposes in the treatment of infertility in up to 18% of cases in the United States [112]. It has been known to regulate systemic dysfunctions naturally and may affect levels of luteinizing hormone (LH), FSH, and testosterone in humans. In an *in vivo* animal study by Abdillahi and Van Staden, aqueous stem extracts of *Bulbine natalensis* were shown to increase blood testosterone concentrations in animals, thereby modifying their sexual function and behavior (especially effective for rats that have hypotestosteronemia) [113]. In a rodent study by Wang et al., 8 weeks of supplementation with the *Wuziyanzong* herbal pill lead to improvements in sperm quality. These improvements included such parameters as sperm density and viability and mitochondria in rodents with oligoasthenozoospermia [114]. In the *in vitro* study of Peng et al., human spermatozoa were incubated with *Tu Si Zi*, which enhanced spermatic motility [115]. In another *in vitro* study, the combination of six herbal extracts incubated with infertile male semen resulted in an increase in viability, number of progressive motile sperm, curvilinear velocity, average path velocity, and lateral head displacement [116].

Clinical observations of married couples showed that the herb *Sheng Jing Zhongzi Tang* improved spermatic density, motility, percentage of normal spermatid morphology, and pregnancy rates [117]. This herb was also associated with a decrease in the amount of antisperm antibodies in the semen [118]. In an investigation of *Withania somnifera* effects on semen, the herb effectively reduced various oxidants, decreased OS, and increased antioxidant levels. This herbal treatment also returned levels of testosterone, LH, FSH, and prolactin back to normal in infertile men [119]. In another study, 219 men with varicocele-associated infertility were treated for 2 months with a daily dose of 60 mg of Escin, an extract of *Aesculus hippocastanum* seed. The herb was shown to target OS, resulting in an increase in sperm motility, density, and normal sperm morphology. In addition, Escin significantly improved the severity of varicocele [120]. These results were similar to those of an older study in which infertile men treated with *Bu-Zhong-yi-qi-tang* for 3 months experienced an increase in sperm concentration and motility [121]. Another herb, *Rou Cong Rong*, was shown to promote the sperm generating functions of the testes as well as improve the microenvironment of epididymis.

TABLE 11.2
Herbal Therapy for Male Infertility

Study	Compound	Outcome
Peng et al. [115]	*Tu Si Zi*	Improved sperm motility
Liu et al. [116]	Mixed herbal (6 components)	Increased viability, number of motile and progressive sperm, curvilinear velocity, average path velocity, and lateral head displacement
Furuya et al. [121]	*Bu-Zhong-yi-qi-tang*	Increased concentration and motility
Sun and Bao [122]	*Yikang Tang*	Improved sperm parameters and pregnancy rates
Tijani et al. [123]	*Manix*	Improved sperm quality
Ahmad et al. [119]	*Withania somnifera*	Decreased oxidative stress and increased levels of antioxidants
Fang et al. [120]	*Escin*	Increased sperm motility, sperm density, and normal sperm morphology
Yang et al. [109]	*Sheng Jing Zhongzi Tang*	Improved spermatic density, motility, percentage of normal spermatid morphology, and pregnancy rates
Yang et al. [109]	*Rou Cong Rong*	Increased sperm generation in testes
Abdillahi and van Staden [113]	Mixed herbal	Increased serum testosterone levels
Wang et al. [114]	*Wuziyanzong* herbal pill	Increased sperm density and improved sperm viability

In a 6-month study, men with idiopathic oligozoospermia treated with Manix, a combination of 11 herbs with honey and sugar, experienced a significant improvement in sperm count, motility and density, and serum testosterone levels. The only sperm parameter that did not improve was semen volume [123]. Another study conducted on 100 infertile men utilized an herbal combination called *Yikang Tang*. The therapy increased sperm parameters and pregnancy rates while decreasing the sperm agglutination rate [122]. Other combinations of herbs taken by infertile men have also been shown to significantly reduce sperm disomy [124].

In vitro studies and clinical trials have continuously found that the use of herbal treatment can improve OS-induced male infertility [113]. Herbal treatments may become a more important factor in treating male infertility in the future, and they have been shown to significantly increase sperm density and sperm viability [114]. Most research indicates that these supplements have little effect on vital signs, blood counts, and liver and kidney function [120], which may make them a safer option than some currently prescribed medications [125]. Herbal treatments may also offer a better and safer method to restore sex hormones in infertile men [119]. Unfortunately, owing to a lack of studies, the data surrounding their efficacy is very limited. More research on herbal treatments should be explored to further evaluate their effects and possible clinical applications and their potential side effects. Table 11.2 shows herbal therapy for male infertility.

11.4 THE ROLE OF NUTRITIONAL SUPPLEMENTATION IN THE TREATMENT OF CHRONIC PROSTATITIS

Chronic prostatitis is just one of the many causes of OS-induced male infertility. We have included it in this chapter because it is a rising problem. It affects up to 14% of men of reproductive age and is difficult to treat. This condition, defined as inflammation of the prostate gland with genitourinary pain but without the presence of bacteria, is associated with increased ROS levels and sperm damage [126]. Kullisaar et al. reported a positive correlation between chronic prostatitis and seminal OS [127]. The most common symptoms involve the lower urinary tract and often include genitourinary pain, dysuria, and altered frequency, although a host of other symptoms have also been linked to this condition [128]. The National Institutes of Health (NIH) International Prostatitis Collaborative Network divides prostatitis into four categories: (1) acute bacterial prostatitis, (2) chronic bacterial prostatitis, (3) chronic prostatitis or chronic pelvic pain syndrome (CPPS), and (4) asymptomatic prostatitis [129]. Of particular importance is category III (genitourinary pain without bacteria present), which is the most prevalent among the four categories [129,130]. The causes of CPPS may be such conditions as an unidentified bacterial infection (*Ureaplasma* and *Mycoplasma*), autoimmune or inflammatory reactions (including ROS, inflammatory cytokines, and white blood cells), and other causes such as pelvic muscle spasms and anti-inflammatory drug supplementation [131–133].

Much of the current therapy for chronic prostatitis involves a multidisciplinary approach involving dietary supplementation, acupuncture, and physical therapy. Several antioxidants have been investigated as possible treatments, but few have been researched in great detail. In one study, a 3-week regimen of lycopene decreased prostate-specific antigen (PSA) levels [134]. Lycopene supplementation has also been associated with decreased interleukin-6 (IL-6) production and therefore decreased inflammation [135]. This anti-inflammatory effect may act synergistically with other agents such as zinc, selenium, ellagic acid (EA), and epigallocatechin-3-gallate (EGCG) [116,136]. Vicari et al. described carnitines as a possible therapy for chronic prostatitis owing to their ability to reduce ROS and oxidative damage and improve semen parameters [56].

In addition to the aforementioned dietary supplements, several compounds have also been investigated as possible treatments for chronic prostatitis. One study examined supplementation with pollen extract (Cernilton) in patients with CPPS. Supplementation increased the patient-reported quality of life, decreased pain, and generally improved patients' overall well-being [128]. The use of quercetin, a flavinoid, has also been explored. Shoskes conducted a preliminary prospective,

TABLE 11.3
Nutritional Supplements for the Treatment of Chronic Prostatitis

Study	Supplement	Outcome
Shoskes et al. [137]	Quercetin	Improved NIH symptom score
Kaplan et al. [138]	Saw Palmetto	No long-term improvement in prostate parameters
Herzog et al. [135]	Lycopene	Decreased IL-6 and inflammation
Wagenlehner et al. [128]	Cernilton	Increased quality of life, decreased pain, and improvement of overall condition of chronic prostatitis
Shoskes et al. [139]	Quercetin	Improved pain, urinary symptoms, and quality of life
Morgia et al. [126]	Profluss (*Stylidium repens*, selenium, and lycopene)	Decreased CPPS symptoms
Lombardo et al. [134]	Lycopene	Decreased PSA

Note: CPPS, chronic pelvic pain syndrome; IL-6, interleukin-6; PSA, prostate-specific antigen.

double-blind, placebo-controlled trial in which men with CPPS received 500 mg of quercetin twice daily for 1 month. Mean NIH chronic prostatitis symptom scores decreased (from 21 to 13), and 67% of the patients experienced an improvement in symptoms [137]. Another study by Shoskes et al. showed that 84% of patients with chronic prostatitis experienced an improvement in their symptoms after supplementation with quercetin for 26 weeks [139].

Another common nutritional supplement is saw palmetto. Kaplan et al. indicated that supplementation with saw palmetto did not have any long-term efficacy in relieving the symptoms of chronic prostatitis [138]. Another researched nutritional supplement is profluss. Morgia et al. showed that treatment with profluss (composed of *Stylidium repens*, selenium, and lycopene) effectively improved symptoms associated with CPPS [126]. Although some trials showed improvements with supplementations, more studies with larger, more defined cohorts are needed to draw meaningful conclusions and to determine the clinical efficacy of these supplements in the management of chronic prostatitis. Table 11.3 shows nutritional supplements for the treatment of chronic prostatitis.

11.5 CONCLUSION

Owing to the growing prevalence of infertility around the world, much research is being devoted to understanding its underlying causes and in devising more effective treatments. Many current therapies for treating OS-induced male infertility are not fully reliable and are expensive. Owing to the ever-growing cost of medical care, alternative therapies represent a possible way to minimize costs and improve pregnancy rates. A systematic review of the literature indicates that a wide variety of supplementation is available for the treatment of OS. Theoretically, supplementation with antioxidants should provide a valuable solution to OS-related sperm damage. Although many experiments and trials have been performed, the efficacy of many of these antioxidants has not been proven and, as a result, acceptable daily allowances have yet to be established. Many antioxidants decrease ROS levels and increase sperm motility, morphology, and count with supplementation. An increase in pregnancy rates is also often observed. Many trials, however, report conflicting data for the same antioxidant, indicating that larger trials and more well-defined studies are needed to fully uncover the hidden mechanisms that may be present. Furthermore, even when an antioxidant is positively correlated with sperm quality, it is often difficult to infer with a high degree of confidence whether the effect is clinically relevant. In general, little research has been performed to find alternative,

nonantioxidant treatments and herbal supplements for the treatment of OS-induced male infertility, as these generally exist outside of current mainstream medical care. Both of these treatment strategies, however, are becoming more prevalent and may one day represent the preferred treatment option. One of the most important and fundamental issues is finding the normalized cutoff values that define OS and subsequent sperm damage. This has yet to be elucidated partially because current methods for measuring ROS are expensive and unreliable.

In conclusion, studies investigating antioxidants and other nutritional supplements often suffer from a lack of standardization, making it difficult to quantify the results of a given study. For the efficacy of these supplements to be determined, larger trials with high-quality controls and randomization must be performed to establish clinically relevant guidelines for supplementation.

11.6 KEY POINTS SUMMARY

1. Infertility affects 15% of couples, with impairment in sperm function being the primary underlying cause in up to 40% of cases.
2. Although the causes for sperm dysfunction in male infertility are many and varied, oxidative stress related damage to sperm appears to be a contributing factor in up to 80% of cases. Oxidative stress occurs when the production of reactive oxygen species (ROS) by leukocytes or sperm themselves exceeds the neutralizing capacity of antioxidants contained in the sperm or seminal plasma, resulting in damage to the sperm. This oxidative damage impairs fertility by reducing sperm motility (direct ROS damage to the sperm tail or mitochondrial energy source for sperm propulsion), by damaging the sperm acrosomal membrane leading to impaired fertilization capacity, or by initiating paternal DNA fragmentation.
3. The use of nutrition supplements with antioxidant capacity to prevent ROS-mediated damage to sperm function holds promise as an effective treatment of male infertility for several reasons. First, nutrients such as minerals (zinc, selenium), vitamins (vitamin C, folate), and phytochemicals (lycopene), all with powerful antioxidant capacity, have been reported to be present in lower concentrations in the serum or seminal plasma of men experiencing infertility. Therefore, supplements of these nutrients may reverse these relative deficiencies, bolstering natural antioxidant defenses and in turn improve sperm quality. Second, several studies have shown that antioxidants such as vitamin C, vitamin E, and selenium do have the capacity to reduce oxidative damage to the sperm membrane and DNA, which theoretically should improve sperm functionality.
4. Although the theoretical basis for antioxidant supplementation to boost sperm function and assist male fertility is strong, the quality of studies proving this point is poor for several reasons. First, the majority of studies are not placebo controlled, making firm therapeutic conclusions impossible. Second, very few studies have fertility (live birth) as the primary outcome, with the majority reporting only changes in sperm parameters. Although an antioxidant nutritional supplement may produce a statistically significant improvement in sperm concentration or motility, it is not always possible to extrapolate that this will translate into improvements in natural fertility given the limited diagnostic sensitivity of routine semen analysis for natural fertility. Finally, although dozens of studies have been conducted examining the effects of antioxidant herbal or nutritional supplements on sperm quality or fertility, each has tended to use a different combination of antioxidants at various dosages, making firm conclusions by comparison of studies impossible.
5. No universally accepted antioxidant nutritional supplement for the treatment of male infertility has been agreed on. However, scientific principles would suggest that a combinational approach using antioxidants with different modes of action would have the best chances of success. For example, the use of a combination of vitamin C and vitamin E is likely to be useful, as vitamin C potentiates the antioxidant effect of vitamin E by keeping it in its active

reduced form. The nutrients zinc and selenium are likely to be useful for their direct antioxidant effect and because they play an important role in protamine packaging of sperm DNA, protecting it from ROS-mediated attack. Carnitine and coenzyme Q_{10} both play important roles in sperm mitochondrial energy production, above and beyond their antioxidant effects. Therefore, these two agents are likely to boost sperm motility and fertility potential. Finally, the group B vitamin folate has been shown to boost sperm quality, possibly due to its antioxidant effects and its important role in DNA synthesis. Although the results of clinical studies are conflicting, all of the above nutrients have been shown to boost sperm quality, as have nutrients such as lycopene, glutathione, *N*-acetylcysteine, and antioxidant herbs such as *Withania somnifera* and *Aesculus hippocastanum*.

6. Future placebo-controlled studies with clinically important end points (sperm DNA damage, live birth) will need to be conducted before firm conclusions can be drawn on the benefits of antioxidant nutritional supplements for the treatment of male fertility. However, as antioxidants are generally inexpensive and carry minimal side effects, a combination antioxidant therapy appears to be a reasonable treatment for optimizing male fertility potential.

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12 The Role of Nutrition in Male Sexual Function

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CONTENTS

12.1 OBESITY AND TESTOSTERONE

Testosterone production in men is regulated by the hypothalamic–pituitary–testicular axis. Pulsatile hypothalamic secretion of gonadotropin-releasing hormone (GnRH) leads to release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary, which stimulate testosterone production by Leydig cells in the testis [1]. Sex hormone-binding globulin (SHBG), as the main carrier protein of testosterone in the circulation, is a major determinant of total testosterone level, and is reduced in obese insulin-resistant states [2]. In adipose tissue, testosterone is converted to estradiol by the enzyme aromatase, leading to reduced circulating testosterone. Obesity, characterized by excessive accumulation of adipose tissue, is defined by the World Health Organization as body mass index (BMI) exceeding 30 kg/m^2 (though ethnic-specific cutoffs vary according to cardiovascular risk) [3]. Abdominal obesity, defined by increased waist circumference (WC), also has ethnic and sex-specific thresholds [3].

Both increased BMI and abdominal obesity are inversely associated with circulating testosterone in large epidemiological studies in diverse populations. In 3144 men aged 45–84 years in the Multi-Ethnic Study of Atherosclerosis (MESA), which examined the progression of subclinical atherosclerosis in four racial/ethnic groups (White, Chinese, Black, and Hispanic) within the United States, serum testosterone and SHBG were inversely associated with waist/hip ratio, after adjusting for age, race, and cardiovascular risk factors, at baseline and on follow-up over a median follow-up period of approximately 2 years [4]. Low total testosterone (<300 ng/dL or 10.4 nmol/L) was found in 52% of 2162 obese men aged ≥45 years in the Hypogonadism in Males (HIM) study in the United States [5], with obesity doubling the risk of hypogonadism in this population. In the Men Androgens Inflammation Lifestyle Environment and Stress (MAILES) Study, the annual decline in serum total testosterone levels of approximately 1% in Australian men over 5 years was predominantly related to becoming obese, while a decrease in WC or BMI was associated with an increase in testosterone [6].

SHBG-adjusted calculated (based on Vermeulen's formula) free testosterone, which correlates well with serum free testosterone as measured with the gold standard of equilibrium dialysis [7], is reduced in obesity and type 2 diabetes mellitus [8]. Significantly, in men aged 40–79 in the European Male Aging Study (EMAS), weight gain ≥10% from baseline was associated with reductions in testosterone and SHBG, and a similar degree of weight loss with increases in total and free testosterone, SHBG and LH, over a mean follow-up period of approximately 4.5 years when compared with the men whose weight remained stable [9]. The dose–response relationship between the reciprocal changes in BMI and reproductive hormones indicates the sensitivity of the hypothalamic–pituitary–testicular axis to changes in adiposity, underscoring the importance of weight management in attenuating or reversing the apparent testosterone decline with aging.

12.1.1 Mechanisms of Low Testosterone Level in Obese Men

Testosterone levels are lowered in obese and insulin-resistant states via several mechanisms, including aromatization to estradiol, disturbances of hypothalamic GnRH and pituitary FSH and LH secretion, reduced testosterone synthesis in testicular Leydig cells, and reduced production of SHBG [1,2]. These are discussed in subsequent sections (Figure 12.1).

12.1.1.1 Disruption of Hypothalamic–Pituitary–Testicular Axis

Obesity impairs hypothalamic and pituitary function (Figure 12.1). Estradiol, formed from aromatization of testosterone in adipose tissue, binds to hypothalamic receptors to reduce GnRH pulse frequency and hence LH secretion from the pituitary, thus reducing testosterone synthesis in Leydig cells [10]. Decreased GnRH also leads to reduced FSH secretion, which down-regulates LH receptors on Leydig cells, thus blunting responsiveness to LH [1]. GnRH and LH pulse frequency and amplitude were found to be significantly attenuated in severely obese men, inhibiting stimulation of testosterone synthesis in testicular Leydig cells and leading to reduced circulating testosterone [1]. Accordingly, treatment with

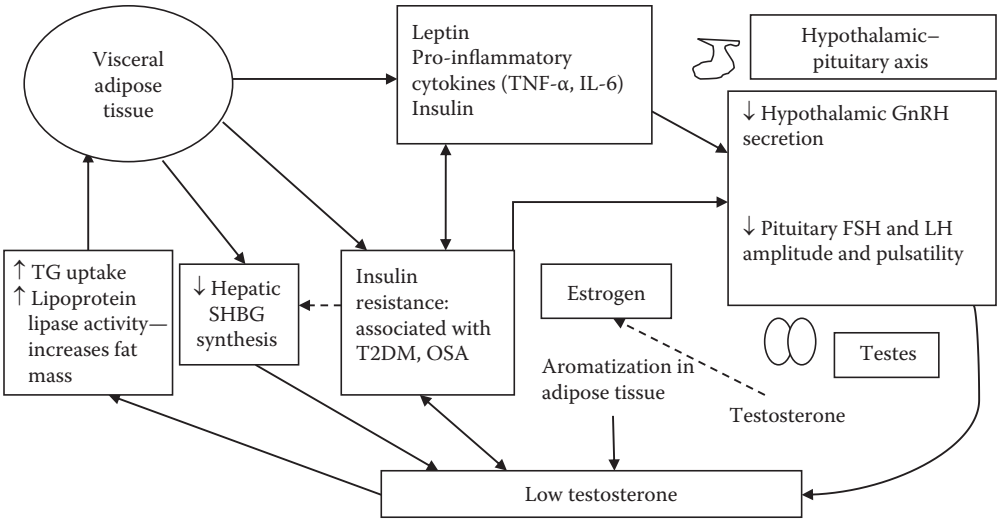

FIGURE 12.1 The relationship between obesity and testosterone production. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IL-6, interleukin-6; LH, luteinizing hormone; OSA, obstructive sleep apnea; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; TG, triglyceride; TNF-α, tumor necrosis factor-alpha.

aromatase inhibitors such as letrozole normalized total and free testosterone in obese men with subnormal testosterone levels [11], while the anti-estrogen clomiphene citrate increased testosterone in men with secondary hypogonadism [12]. Estradiol directly suppresses the neuronal response to kisspeptin, a neuropeptide hormone that stimulates GnRH secretion and thus LH pulsatility [13].

Glucose clamp studies in obese insulin-resistant men demonstrated that insulin sensitivity correlated with serum testosterone level across a full spectrum of glucose tolerance, with hypogonadal men being twice as insulin resistant as their eugonadal counterparts [14]. Although the precise mechanisms by which insulin resistance disrupts the hypothalamic–pituitary–testicular axis in humans have not been elucidated, neuron-specific insulin receptor knockout mice were found to have LH levels that were 60–90% lower than in their wild-type counterparts because of impaired insulin receptor signaling in the hypothalamus, which normalized with GnRH administration [15], implying that insulin facilitates GnRH release and may be a mechanism by which insulin resistance mediates reduction in testosterone production.

Leptin, a hormone secreted by adipocytes that increases with weight and fat mass is the best hormonal predictor of lower androgen levels in obese men, and correlates with total and free testosterone even after controlling for SHBG, LH, and estradiol [16]. Elevated leptin levels in obesity lead to chronic leptin resistance, which suppresses normal hypothalamic GnRH secretion [17], while leptin also directly alters the function of hypothalamic *Kiss1* gene expression to reduce synthesis of kisspeptin [18].

Macrophages and inflammatory cells in adipose tissue secretes pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which impair insulin signaling, resulting in compensatory hyperinsulinemia that suppresses GnRH and LH secretion [19].

12.1.1.2 Impaired Leydig Cell Function

In addition to interference with GnRH and LH, circulating and/or locally produced hormones, growth factors, and cytokines from adipose tissue also reduce testosterone synthesis by direct effects on Leydig cell steroidogenesis. Excessive circulating leptin binds to leptin receptors on Leydig cells to inhibit the stimulatory effects of LH on Leydig cells [17], and blocks intracellular conversion of the steroid precursor 17-hydroxyprogesterone to testosterone. Increased local cortisol production in abdominal adipose tissue directly suppresses testicular Leydig cell steroidogenesis [19].

Insulin resistance, and possibly hyperinsulinemia, is also associated with reduced Leydig cell testosterone synthesis as demonstrated by lower human chorionic gonadotropin-stimulated testosterone secretion with more severe insulin resistance in men who had been deprived of endogenous testosterone by use of GnRH antagonists [20]. Although the underlying mechanisms remain unclear in humans, in mouse models, injected insulin directly binds to insulin receptors on Leydig cells leading to increased expression of the *DAX-1* gene (which directly inhibits testosterone synthesis without affecting FSH and LH production), while knockout of the *DAX-1* gene reversed the effects of insulin administration [21]. Furthermore, obesity and type 2 diabetes mellitus induced by a high-fat diet in mice induced greater expression of testicular *DAX-1* in comparison with mice fed a normal diet [21], suggesting that insulin resistance and endogenous hyperinsulinemia may also reduce Leydig cell steroidogenesis through similar mechanisms in obese and diabetic men.

12.1.1.3 Reduced SHBG

The association between obesity and low SHBG is well established, and the reduction of SHBG contributes significantly to the reduction in circulating total testosterone level in obese men [2,8,19]. As SHBG binds testosterone with high affinity, reduction in circulating SHBG decreases circulating testosterone and therefore delivery of testosterone to peripheral tissues, while in adipose tissue decreased SHBG may increase the availability of free testosterone for aromatization to estradiol [2]. Low SHBG levels were the main determinant of a 4-fold higher prevalence of low testosterone levels in type 2 diabetic, insulin-resistant men, compared with men with type 1 diabetes [8]. Weight and body composition, in particular the ratio of fat to lean mass, are major determinants of hepatic SHBG production [2,21].

Visceral obesity reduces hepatic levels of the nuclear transcription factor hepatocyte nuclear factor-4-alpha (HNF-4α), leading to reduced SHBG synthesis [22]. Accordingly, low SHBG is a sensitive biomarker for reduced hepatic HNF-4α activity and therefore the metabolic syndrome, which is characterized by visceral obesity and insulin resistance. Insulin resistance is associated with low SHBG levels [2] and SHBG levels fall acutely during hyperglycemic-euglycemic clamp studies [20], though the relationship between SHBG and insulin resistance is unclear: insulin administration inhibits hepatocyte SHBG production *in vitro* and pharmacological inhibition of insulin secretion or insulin-sensitizing therapies raises SHBG concentration, but there are little data on the association of clearance of SHBG with insulin *in vivo* [22]. The effects of hyperinsulinemia on SHBG are difficult to separate from the suppressive effects of coexisting derangements associated with obesity and hyperglycemia on hepatic HNF-4α activity, particularly increased lipid production in the liver [22].

Although low testosterone and SHBG are both associated with the constellation of cardiovascular risk factors which define the metabolic syndrome (abdominal obesity, insulin resistance, dyslipidemia, and hypertension), low SHBG concentration is more strongly associated with insulin resistance and development of type 2 diabetes than is low total or free testosterone. As such, the relationship between obesity and circulating testosterone may be confounded by the independent association of low SHBG with obesity, insulin resistance, and type 2 diabetes [2]. Moreover, biochemical testosterone deficiency does not always reflect a true hypogonadal state, as circulating testosterone is influenced not only by SHBG but also by circadian rhythms, pulsatile secretion, use of concomitant medications, and measurement variations [19], which further complicate the association of hypoandrogenism with obesity, metabolic syndrome, and diabetes.

12.1.2 The Obesity–Hypogonadism Cycle

The relationship between obesity and hypogonadism is complex and bidirectional, as obesity is associated with low testosterone and SHBG, while hypogonadism and decreased SHBG can also promote visceral fat accumulation with resulting insulin resistance [23], leading to a vicious cycle wherein states of excess adiposity and low testosterone perpetuate each other. As discussed in previous sections, increased adipose tissue is associated with indirect (disruption of normal pituitary FSH and LH secretion) and direct inhibition of testosterone synthesis in testicular Leydig cells, decreased hepatic

SHBG production, and aromatization of testosterone to estradiol in adipocytes, which all decrease circulating testosterone. Testosterone deficiency in turn shifts development of stem cells from that of muscle to fat [24]. Further fat accumulation is mediated by lack of inhibition of the enzyme lipoprotein lipase due to testosterone deficiency, which leads to reduced lipolysis and increased free fatty acid (FFA) uptake and triglyceride storage in adipocytes [19]. This is shown in Figure 12.1.

Evidence from animal studies supports the responsiveness of adipose tissue to androgen status. Greater numbers of fat cells and fat mass develop in mice lacking androgen receptors [25], and increased accumulation of visceral fat in mice with deletion of androgen receptors that are fed a high-fat diet compared to similar mice given normal feed, suggesting that low testosterone may augment the effects of excessive caloric intake [26].

Because adipose tissue is relatively metabolically inactive compared to muscle, the increased proportion of fat relative to muscle mass results in a reduction in energy expenditure, resulting in further accumulation of excess calories as fat. In skeletal muscle, low testosterone levels and insulin resistance were associated with mitochondrial dysfunction, leading to further decreased energy expenditure which is independent of the reduction in muscle mass seen with testosterone deficiency [14]. Evidence for the association of low testosterone levels with insulin resistance was seen in castrated mice, in which impaired insulin receptor signaling was reversed by testosterone administration [27]. Insulin resistance and testosterone deficiency may therefore also be associated with each other, independent of the degree of adiposity.

12.2 DIET AND TESTOSTERONE

Differences in dietary intake of macronutrients have been associated with variations in testosterone and SHBG levels in humans, though the mechanisms by which macronutrients affect testosterone and SHBG synthesis and binding have not been fully elucidated. Animal studies have provided additional, but limited, information on these mechanisms (Table 12.1).

TABLE 12.1
Effects of Macronutrients on Testosterone and SHBG Levels

Macronutrient	Association with Testosterone Level	Association with SHBG Level
High fat (humans)	↑ [28,29] /↓ [30]	—
Low fat (humans)	↓ [31,32]	↓ [31]
Saturated fat (humans)	↑ [31,32]	↓ [33–35]
MUFA (rats)	↑ [36,37]	↑ [36,37]
PUFA (rats)		
• Omega-3 (ALA, EPA, DHA)	↓ [36]	—
• Omega-6 (CLA, AA, DPA)	↑ [36]	↑ [36]
Protein (humans)		
• Total	↓ [28,34,38,39]	↓ [34]
• Lean meat	↓ [32]/0[a] [40]	↑ [40]
• Soy	↓ [32]/0[b] [40]	↑ [40]
Carbohydrate (humans)		
• Total	↑ [38,39]	↑ [39]
• Fiber	↓ [39]	↑ [39,41]
• Acute glucose load	↓ [42]	0

Note: AA, arachidonic acid; ALA, α-linolenic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentanoic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SHGB, sex hormone binding globulin.

[a] Increased testosterone/estradiol ratio.

[b] Decreased testosterone/estradiol ratio.

12.2.1 Macronutrients and Testosterone Level

12.2.1.1 Dietary Fat and Fiber

Variations in the dietary fat content, and the types of fat, acutely affect testosterone synthesis. In young athletic men, significant positive correlations were observed between testosterone level and percentage energy intake from fat [28], while higher saturated fat intake was inversely associated with SHBG in older men in a population study (though this association was attenuated or no longer significant after controlling for age and BMI and/or adiposity) [33,34]. British men who had a BMI above 30 kg/m^2 had 45% lower SHBG values, and saturated fat intake correlated inversely with plasma SHBG levels [35]. However, in the same population, irrespective of the type of diet, men who had a BMI above 30 kg/m^2 with reduced SHBG had 30% lower testosterone levels and 5% lower free testosterone levels, which suggests an independent impact of BMI [35], Another study found that in men consuming a vegan diet with higher fiber intake, free testosterone levels were 3% lower and total testosterone levels were 7% higher in vegans, in association with higher levels of SHBG, after statistical adjustment for BMI, age, and alcohol intake [41].

Normal-weight healthy men fed a fat-rich (86% of calories) meal after having been on a high-fat (64% of calories) diet for 8 weeks demonstrated reduction in total and free testosterone concentrations by approximately 20% which remained low 8 h after the meal [30]. In contrast, total and free testosterone and SHBG decreased by approximately 15% after 8 weeks on a low-fat high-fiber diet in men previously consuming high amounts of fat [31], and total testosterone and free androgen index decreased more after a low-fat (20% of calories) mixed meal containing tofu or lean meat compared to a high-fat (54%) meal [32]. In a crossover study in which 43 healthy men consumed a low-fat and high-fiber diet for 10 weeks followed by a high-fat and low-fiber diet for 10 weeks, mean plasma concentrations of total testosterone were higher on the high-fat and low-fiber diet [29]. These apparently conflicting results may be due to varying degrees of energy restriction, changes in adiposity and insulin resistance, as well as the effects of caloric content and possibly fat subtypes (animal or plant-derived, saturated, monounsaturated fat [MUFA], and polyunsaturated fat [PUFA]). There are no studies in humans that have directly compared the effects of fat subtypes in isocaloric diets on testosterone synthesis in the absence of caloric restriction.

Studies in animal models have investigated the effects of MUFA and PUFA on testosterone binding and synthesis. In rats, MUFA increases free testosterone by decreasing binding to SHBG, while the omega-3 (α-linolenic acid [ALA], eicosapentanoic acid [EPA], docosahexaenoic acid [DHA]) PUFAs reduce circulating testosterone and inhibit the enzyme 5α-reductase, which converts testosterone to the active dihydrotestosterone [36]. On the other hand, omega-6 (γ-linolenic, linoleic, arachidonic, docosapentaenoic) PUFAs increased basal and LH-stimulated testosterone synthesis in rat Leydig cells [36], possibly by altering the phospholipid composition of testicular plasma membranes, which influenced membrane-mediated unmasking of gonadotropin receptor-mediated action in testicular tissue. Olive oil, which is high in MUFA, a major component of the Mediterranean diet, was shown in rats to increase the activity of the enzymes 3-β-hydroxysteroid dehydrogenase (3-β-HSD) and 17-β-HSD which are involved in testicular Leydig cell testosterone synthesis, and consequently testosterone levels, in comparison to isocaloric diets containing soybean, coconut, or grapeseed oil [37]. However, the long-term effects of fat consumption are difficult to separate from higher caloric intake and changes in weight, adiposity, and insulin resistance, even in animal studies. No clear association between dietary fat and androgen status has therefore been established.

12.2.1.2 Protein and Carbohydrates

Differences in proportions of other macronutrients may also affect testosterone and SHBG levels. In healthy young men, significant inverse correlations were found between serum testosterone and percent energy intake from protein and the protein-to-carbohydrate ratio [28]. Testosterone levels correlated inversely with total protein intake, and were positively associated with carbohydrate intake, without any impact of adiposity measures, in Greek men [38]. In a study of normal-weight

men fed isocaloric diets with the same fat content for 10 days, percentage of energy intake from protein and the protein-to-carbohydrate ratio were inversely associated with total testosterone level, and testosterone and SHBG increased by approximately 30% more in the high-carbohydrate (70% of calories) group compared to the high-protein (44% of calories) group [39]. Healthy men of different ages placed on a 4-week diet that used tofu (soyabean product) as the main protein source decreased serum testosterone/estradiol ratio by a greater degree compared to a diet using lean meat to provide an equivalent amount of protein in a crossover study [40], suggesting that the source of protein may also influence androgen levels.

The heterogeneous nature of the study populations (in terms of age, BMI, and insulin resistance status), and that of the type of protein and carbohydrates used in the different diets studied (animal or vegetable protein; simple or complex carbohydrate, fiber content) of macronutrient, present difficulties with evaluating the effects of acute changes in diet composition on testosterone and SHBG. Glucose loads significantly decreased total and free testosterone in men aged 19–74 with varying degrees of glucose tolerance [42], suggesting that acute changes in insulin production and resistance influence testosterone levels independent of the effects of other nutrients. Moreover, as with fat consumption, long-term changes in protein and carbohydrate intake may impact testosterone metabolism by indirect effects on weight and visceral adiposity.

12.2.2 Possible Roles of Micronutrients in Testosterone Synthesis and Sexual Function

Zinc, a trace metal, is involved in the regulation of LH and FSH secretion, testicular growth and development, testosterone synthesis in Leydig cells, and conversion of androstenedione to testosterone in peripheral tissues [43]. Zinc deficiency is associated with significantly reduced numbers of androgen receptors, inhibition of testosterone binding to androgen receptors, decreased testosterone secretion, and conversion to the active form dihydrotestosterone (DHT) by 5α-reductase [44]. Zinc also inhibits reactive oxygen species (ROS) formation and lipid peroxidation, and thereby improves endothelial dysfunction [43]. In rats, zinc deficiency decreases the concentration of active metabolites of testosterone, because of inhibition of hepatic 5α-reductase activity (which converts testosterone to DHT) and conversion of DHT into the weak androgen 3α-diol [44]. In healthy young men, dietary zinc restriction for 20 weeks was associated with reduction in serum testosterone by approximately 75%, while zinc supplementation at a dose of 459 μmol elemental zinc daily (recommended daily allowance [RDA] in the United States being 229.5 μmol) for 6 months doubled testosterone levels in zinc-deficient healthy elderly men [43]. SHBG and weight did not change significantly during this study. In contrast, zinc supplementation had no significant effect on serum testosterone levels and the metabolism of testosterone in subjects who were consuming a zinc-sufficient diet [45]. Zinc sufficiency is therefore important to maintain normal androgen status and erectile function, but the benefits of supplementation in zinc-replete men are uncertain.

Selenium, a trace element present in high concentrations in wheat, poultry, fish, and Brazil nuts, is necessary for normal growth and reproduction, with numerous reports implicating selenium deficiency in reproductive disorders [46]. Dietary selenium is incorporated into glutathione peroxidases (GPxs), which scavenge hydrogen peroxide and lipid peroxides and reduce oxidative damage to lipids, lipoproteins, and DNA, and maintain membrane integrity; testicular tissue contains a high concentration of GPx [46]. The testosterone level in selenium-depleted rats was lower than that of adequately supplied controls, and the stimulation of testosterone secretion by administration of GnRH or LH resulted in a significantly less marked rise in the serum concentration of testosterone [47]. In offspring of the selenium-deficient rats, testicular mass was lower compared to the previous generation, and sperm count and motility were decreased. These alterations were reversible and spermatogenesis was restored by feeding a selenium-adequate diet. However, there are no randomized controlled trials (RCTs) in men with defined selenium deficiency and hypogonadism.

Vitamin D (VD) is largely derived from conversion of 7-dehydrocholesterol to inactive vitamin D (cholecalciferol) by sunlight, with dietary sources mainly through fortification of dietary products or use of oral VD supplements. Cholecalciferol is hydroxylated in the liver and kidney to form active 1,25-cholecalciferol ($1,25(OH)_2D_3$) that binds and activates the VD receptor (VDR), mediates genomic effects in the nucleus through binding to VD response elements (VDRE) in target genes, and regulates genetic transcription [48]. VD is likely to be involved in testosterone synthesis: VDR have been found on animal and human Leydig cells; VD metabolizing enzymes co-localize in human Leydig cells; and transcriptional changes occur in these genes in response to VD deficiency [48]. VDR knockout mice demonstrated decreased serum estrogen and testosterone, and elevated FSH and LH, compared with wild-type animals [49]. Vitamin D levels have been positively associated with total testosterone in population studies [50,51]. However, the relationship between vitamin D and testosterone levels may be confounded by the presence of obesity; comorbidities such as metabolic syndrome, diabetes, or cardiovascular disease; and aging, which decrease both testosterone and vitamin D. The positive associations between serum vitamin D ($25\text{-}(OH)D_3$) and testosterone/free androgen index (FAI) were reported mainly in men older than 40 years of age [50,51], with median BMI above 25 kg/m^2 and the presence of comorbidities, and disappeared after correction for confounders such as age and BMI [51], whereas in younger healthy men, serum $25\text{-}(OH)D_3$ levels were not associated with androgen levels but correlated with SHBG [50]. In a small RCT, serum testosterone increased by a mean of 2.7 nmol/L in overweight men (mean age 48 years) with vitamin D deficiency after average daily intake of 83 mcg of vitamin D (equivalent to approximately 3300 IU, where the RDA is 600–800 IU for adults with normal vitamin D levels) daily for 1 year to raise vitamin D levels into the adequate range (above 50 nmol/L), compared to no significant change in men receiving placebo daily [52]; however, these men had enrolled in a study primarily designed to evaluate effects of weight loss. Although vitamin D is important for regulation of gonadal steroid production, there is insufficient evidence that vitamin D supplementation stimulates testosterone synthesis in the presence of normal vitamin D status. No studies thus far have examined the effect of vitamin D repletion on erectile function or libido.

The possible roles of micronutrients in testosterone synthesis and sexual function are listed in Table 12.2.

TABLE 12.2
The Role of Micronutrients in Testosterone Synthesis and Sexual Function

Micronutrient	Effects of Deficiency	Effects of Supplementation
Zinc	Increased ROS formation and lipid peroxidation [43] Reduced numbers of androgen receptors with decreased binding of testosterone [43] Decreased testosterone secretion, conversion to active DHT and active metabolites of testosterone [44]	Doubled testosterone levels in zinc-deficient elderly men [43] No effect on serum testosterone and testosterone metabolism in zinc-replete men [45]
Selenium	Increased ROS formation and lipid peroxidation [46] Smaller testicular mass [46,47] Lower testosterone level and response to GnRH or LH stimulation [46,47]	Restored testicular mass and spermatogenesis in selenium-deficient rats [47] Protected against testicular atrophy, hypogonadism, ED, and defective spermatogenesis induced by oxidative stress in rats [53]
Vitamin D	Decreased aromatase expression and estrogen production [48] Associated with lower testosterone level in men who are older, overweight, and/or have metabolic comorbidities [50,51]	Increased serum testosterone in conjunction with weight loss in overweight vitamin D–deficient men [52]

Note: DHT, dihydrotestosterone; ED, erectile dysfunction; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; ROS, reactive oxygen species.

12.2.3 Alcohol, Testosterone, and SHBG

Alcohol abuse reduces testosterone and SHBG levels, as a result of inhibition of hypothalamic GnRH and pituitary LH production and testicular Leydig cell function, and indirectly by hepatic dysfunction and cirrhosis leading to increased estradiol production. The evidence for the effects of smaller amounts of alcohol on testosterone and SHBG is inconclusive. Men in the National Health and Nutrition Examination Survey who consumed one or more drinks per day had lower SHBG than men who drank less frequently while total and free testosterone tended to increase with number of drinks per day [54], and there was an inverse association between SHBG and alcohol intake in a study of male vegans and omnivores [41]. However, no associations were observed between alcohol intake and androgen levels in middle-aged men in the European Prospective Investigation into Cancer and Nutrition study [55] and the Multiple Risk Factor Intervention Trial [56]. Acutely, moderate (40 g/week) of alcohol consumption for 3 weeks decreased testosterone levels by approximately 7% in a diet-controlled crossover study in middle-aged men [57], but a lower amount of alcohol (0.5 g/kg) increased testosterone levels as a result of a change in the redox state in the liver in healthy men in a crossover study [58]. Moreover, the optimal amount and frequency of alcohol consumption for normal testosterone levels are influenced by numerous factors including BMI, age, diet, and comorbidities and are difficult to separate from the effects of these confounders.

12.3 OBESITY AND ERECTILE DYSFUNCTION

Erectile dysfunction (ED), the impairment of ability to achieve or maintain an erection long enough to engage in sexual intercourse, was present in 40% of obese men with both BMI ≥ 30 kg/m^2 and/or abdominal obesity (WC ≥ 102 cm) in the European Male Aging Study (EMAS) [59]. Obesity doubled the risk of incident ED in men aged 40–70 years in the Massachusetts Male Aging Study, independent of age, serum testosterone, depression, and metabolic comorbidities such as hypertension [60], and in the National Health and Nutrition Examination Survey (NHANES), which included 2536 men aged 20 and older [61]. The EMAS also found strong associations between hypogonadism (defined as decreased frequency of morning erections and sexual thoughts and erectile dysfunction in combination with total testosterone levels below 11 nmol/L and free testosterone < 220 pmol/L) and obesity, particularly abdominal obesity, in men aged 40–79 years, with a more than 6-fold increased risk of hypogonadism in men with WC above 102 cm [62].

The prevalence of ED increases from 25% in normal-weight men to 30% in overweight and 40% in obese men in the EMAS study [59]. In the NHANES survey, diabetes increased the risk of ED by 2.7 times compared to non-diabetic men, and ED was found in 50% of diabetic men [51]. The issue of obesity-related ED is of broader significance than its sexual consequences: ED is a predictor of coronary artery disease (CAD) and a marker of subclinical cardiovascular and peripheral vascular disease, and is associated with increased mortality [63].

12.3.1 Mechanisms of ED in Obesity

Following sexual stimulation, release of nitric oxide (NO) in penile nerves facilitates the vasodilation of cavernosal arteries and arterioles and increases blood flow to the corpus cavernosum, which stimulates release of endothelial NO, resulting in smooth muscle relaxation and expansion of cavernosal sinusoids, which leads to penile engorgement and trapping of blood in the penis by veno-occlusion, leading to an erection [63]. ED results from impairments in these processes, and multiple interrelated factors contribute to ED in obese men (Figure 12.2). In particular, low testosterone, endothelial dysfunction, inflammation, and oxidative stress, which are associated with ED, are more prevalent in obesity. These are discussed in Sections 12.3.1.1–12.3.1.3.

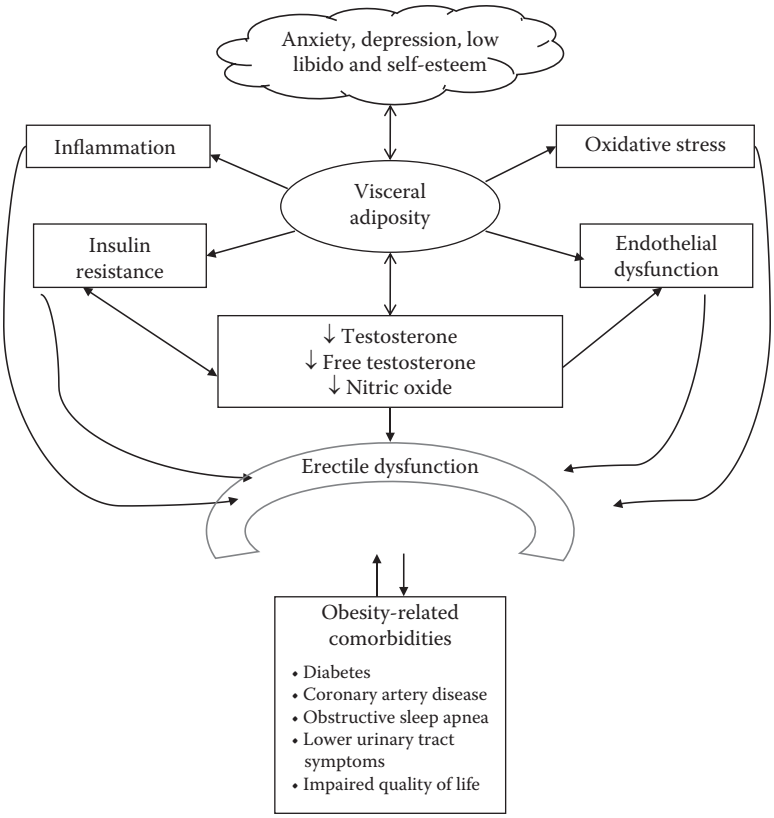

FIGURE 12.2 Mechanisms of erectile dysfunction in obese men.

12.3.1.1 Low Testosterone

Arteriogenic (vasculogenic) ED is characterized by reduced penile blood flow with impaired vasodilatory response to intracavernosal prostaglandin injection as a result of hemodynamic dysfunction and chronic exposure of erectile tissue to ischemia and hypoxia, which is associated with obesity, insulin resistance, and hypoandrogenism as evidenced by a low testosterone/estradiol ratio [64]. Testosterone regulates neural and endothelial NO synthesis, which is necessary for the normal erectile response, and maintains normal cellular architecture of the corpus cavernosum growth and differentiation of vascular smooth muscle [63]. Low testosterone impairs these processes and leads to further fat accumulation and exacerbates insulin resistance, inflammation, and endothelial dysfunction in a vicious cycle (as discussed in preceding sections), contributing to ED [63]. Accordingly, in men aged 40–79 in the EMAS study, poor morning erections, low sexual desire, and ED were significantly associated with decreased total testosterone (8–13 nmol/L) and free testosterone (160–280 pmol/L) levels [65]. Below a threshold level of testosterone, erectile function has been found to decline in a dose–response fashion [66], underscoring the contribution of low circulating testosterone to ED. The mechanisms of testosterone deficiency in obese men were discussed in the preceding section.

12.3.1.2 Inflammation and Endothelial Dysfunction

Visceral adipose tissue produces pro-inflammatory cytokines, particularly TNF-α and interleukins, which enhance expression of adhesion molecules in both the endothelium and in vascular

smooth muscle cells and facilitate monocyte adhesion and migration into the vascular wall [67]. ED and endothelial dysfunction in obese men therefore have in common a low-grade inflammatory state [68]. Endothelial dysfunction has been significantly associated with a higher organic contribution to ED (as assessed by high Structured Interview on Erectile Dysfunction scale 1 score) in obese men, as evidenced by impaired peak systolic velocity penile Doppler ultrasound at baseline and response to intra-cavernosal prostaglandin injection [64]. Endothelium-dependent and -independent vasodilation was impaired in men with vasculogenic ED [68], and markers of endothelial activation (such as endothelin-1 and P-selectin) and adipocytokines (such as TNF-α, IL-6, plasminogen activator inhibitor-1, endothelin-1, and vascular endothelial growth factor), which mediate endothelial dysfunction, vascular inflammation, and atherosclerosis, are elevated in men with ED with or without CAD [67], suggesting that ED adds an incremental inflammatory and endothelial-prothrombotic activation in addition to that induced by CAD. Improvements in erectile function are associated with increased endothelial function response and may be mediated by greater cavernosal smooth muscle relaxation in response to a reduction in visceral adiposity and inflammation, which consequently increase NO bioavailability [63].

12.3.1.3 Oxidative Stress

Oxidative stress occurs when cells are exposed to excessive levels of ROS as a result of an excess of pro-oxidants that overcome the protective mechanisms conferred by antioxidants and enzymes that prevent and repair oxidative damage or limit its spread, and is induced by ischemia and hypoxia [69]. Obesity is associated with increased levels of markers of systemic oxidative stress, as well as seminal ROS, which indicate testicular oxidative stress [70]. The interaction between endothelial-derived NO and ROS is an important pathophysiological mechanism underlying ED. ROS are formed during regular metabolism, and include superoxide (SOD), hydrogen peroxide, hypochlorous acid, and peroxynitrite, which are formed in the vascular endothelium, platelets, and leukocytes [69]. NO synthesized by endothelial NO synthase (eNOS) interacts with SOD to form peroxynitrite which inactivate SOD dismutase and thus reduces removal of SOD. Peroxynitrite reacts with more NO to perpetuate a vicious circle that decreases the bioavailability of NO [59]. Peroxynitrite and SOD also increase the incidence of apoptosis in the endothelium, leading to denudation of endothelium and further reduction of available NO. In obesity, elevated levels of fatty acids produced in adipocytes activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which leads to oxidative stress; this increases circulating adipocytokines, such as plasminogen activator inhibitor-1 and IL-6, which promote inflammation and endothelial dysfunction [70]. In animal models, activation of NADPH oxidase in the penis results in eNOS uncoupling, which is the decrease in the abundance of the active dimeric form of eNOS leading to a switch in eNOS activity from an NO to a predominantly SOD production [71,72]. eNOS uncoupling therefore further worsens oxidative stress and endothelial dysfunction, perpetuating the vicious circle that leads to ED.

12.3.1.4 Obesity-Related Comorbidities

Obesity-related comorbidities, in particular obstructive sleep apnea (OSA), also adversely affect erectile function [73]. OSA is an independent correlate of ED and sexual dysfunction [74]. The pathophysiology of sexual dysfunction in obesity-associated OSA is likely multifactorial and affected by several disease-related factors, including insulin resistance, inflammation, endothelial dysfunction, sleep fragmentation, hypoxia, and intermittent desaturations [74]. Intermittent hypoxic events and sleep fragmentation limit spontaneous nocturnal erections, while OSA in obese men perpetuates the cycle, as sleep fragmentation and hypoxia disrupt the hypothalamic–pituitary–testicular axis, leading to decreased LH amplitude and pulsatility [75]. Obesity also reduces libido and enjoyment of sexual activity, and is associated with depression, anxiety, and lower quality of life [73]. These psychological issues compound the sexual difficulties caused by organic contributors to ED.

12.4 EFFECTS OF DIET COMPONENTS ON FACTORS UNDERLYING ED

12.4.1 Effects of Diet Composition on Endothelial Dysfunction, Inflammation, and Oxidative Stress

A high-fat (>30% of calories) diet increased the risk of incident ED in the Massachusetts Male Aging Study [76]. The effects of high-fat and high-cholesterol diets on erectile function have been demonstrated in animal models. Mice fed a high-fat diet to induce hypercholesterolaemia developed ED that was associated with endothelial dysfunction, inflammation, and elevated markers of oxidative stress [71], and rabbits fed a high-cholesterol (1.5% of calories) diet for 12 weeks developed ED in association with endothelial dysfunction and hypercholesterolemia [72]. Compared to pigs that had been fed a high-fat diet and put on a sedentary regimen, pigs that were on a low-fat diet had increased penile NO bioavailability due to preservation of eNOS in its active, dimeric form and limited eNOS interaction with its main negative protein regulator caveolin-1, leading to reduced oxidative stress and maintenance of endothelial and erectile function [77]. It is therefore possible that endothelial dysfunction, inflammation, and increased oxidative stress similarly contribute to ED in men on diets containing higher saturated fat and cholesterol (see Table 12.3).

Endothelial dysfunction (as measured by elevated plasma levels of cellular adhesion molecules such as E-selectin and soluble vascular cell adhesion molecule 1 [sVCAM-1]) and systemic inflammation (raised plasma C-reactive protein [CRP] and IL-6) were associated with higher intake of red and processed meats, sweets, desserts, French fries, and refined grains in the Nurses' Health Study [78]. This association remained significant even after adjustment for BMI. In particular, increasing dietary intake of omega-3 PUFA found in fatty fish oils (DHA, EPA) and plant-derived ALA, especially relative to that of omega-6 fatty acids, may prevent or reduce systemic inflammation, especially in obese adults with the metabolic syndrome [79]. Omega-3 fatty acids decrease expression of sVCAM-1 on the vascular endothelium and therefore leukocyte rolling and adhesion to the endothelium, with DHA in particular being shown to improve endothelium-dependent vasodilation [80].

Dietary lipids and carbohydrates are either targets of oxidative modification after absorption or are present in a pro-oxidant form in the diet. Plasma levels of markers of oxidative stress increased after consumption of a meal rich in lipids and/or carbohydrates [81]. Prolonged postprandial elevations in triglyceride-rich lipoproteins, associated with decreased chylomicron clearance, result in greater susceptibility to oxidation [82]. Elevated postprandial glucose levels increase oxidative stress and cellular damage by direct toxic effects on the vascular endothelium, ischemia-reperfusion processes, and apoptosis in dorsal root ganglion neurons [83].

12.4.2 The Mediterranean Diet and Erectile Function

The Mediterranean diet, which is low (<10%) in saturated fat (from animal sources) with relatively higher MUFA (10–15%) and PUFA (5–8%) (e.g., olive oil, whole grains, and nuts), rich in nutrients with favorable anti-inflammatory properties, and low in pro-inflammatory nutrients (e.g., sugar-sweetened soft drinks, refined grains, and processed meat), was represented more in men without ED compared to men with ED [84]. In sexually active type 2 diabetic men aged 35–70, there was a significant increase across tertiles of adherence to Mediterranean diet, and men with the highest score of adherence were more likely to have a lower prevalence of global ED and severe ED (as measured by the International Index of Erectile Function 5-item Questionnaire [IIEF-5] score), compared with low adherers [84]. The intake of fruits and nuts, and the ratio of MUFA to saturated lipids, were higher in men without ED. As with lowered testosterone, in men with ED the effects of obesity resulting from increased intake of energy-dense foods are difficult to separate from the effects of dietary macronutrient composition. The Mediterranean diet also reduced serum concentrations of CRP, IL-6, IL-7, and IL-18 and improved insulin resistance and endothelial function in

TABLE 12.3
Effects of Different Diets on ED, and Changes in ED and Other Factors after Diet-Induced Weight Loss

Diet	Effect on ED	Other Effects
High-fat (>30% of calories)	Develops in mice [71] Develops in pigs [77] Higher incidence in men [76]	Hypercholesterolemia, oxidative stress, inflammation, endothelial dysfunction Increased eNOS uncoupling; reduced NO bioavailability Hypertension, low testosterone
High-cholesterol	Develops in rabbits [72]	Hypercholesterolemia, endothelial dysfunction
Mediterranean • High intake of fruits and vegetables • High MUFA to saturated fat ratio • Daily intake of cereals, legumes, and low-fat dairy products • Moderate intake of fish and nuts • Moderate consumption of wine (one to two glasses per day) • Low consumption of red meat and processed meat	Lower incidence in men [84] Improved in men [85,86]	Lower prevalence of obesity, metabolic syndrome, endothelial dysfunction, and inflammation Weight loss, reduced waist circumference Improved endothelial function Reduced glucose, insulin, LDL cholesterol, TG, and BP; increased HDL cholesterol Reduced inflammatory markers
Low-energy (<1000 kilocalories/day using commercial meal replacements)	Improved in men [87–89]	Weight loss, reduced waist circumference Increased sexual desire Increased total testosterone and SHBG Improved endothelial function Reduced glucose, insulin resistance, LDL, TG Reduced lower urinary tract symptoms
High-protein (300 g of lean meat, poultry, or fish) with reduced-fat (25–30% of calories)	Improved in men [88]	Weight loss, reduced waist circumference Increased sexual desire Increased SHBG Improved endothelial function Reduced glucose, insulin resistance, LDL cholesterol, TG Reduced inflammatory markers Reduced lower urinary tract symptoms
Reduced-fat (25–30% of calories with <10% as saturated fat)	Improved in men [88–90]	Weight loss, reduced waist circumference Increased SHBG Improved endothelial function Reduced glucose, insulin, insulin resistance and BP; increased HDL cholesterol Reduced lower urinary tract symptoms Improved quality of life

Note: BP, blood pressure; ED, erectile dysfunction; eNOS, endothelial nitric oxide synthase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NO, nitric oxide; SHBG, sex hormone–binding globulin; TG, triglycerides.

men and women with the metabolic syndrome [91]. In overweight men, caloric restriction with a Mediterranean-style diet reduced serum insulin and 8-iso-prostaglandin $F_{2\alpha}$ (a biomarker of oxidative stress) [92]. The Mediterranean diet pattern may therefore benefit erectile health by reducing adiposity, insulin resistance, inflammation, and oxidative stress and improving endothelial function.

12.4.3 Alcohol Consumption and Erectile Function

Epidemiological studies suggest a J-shaped relationship between alcohol consumption and risk of ED, with moderate intake of alcohol having a protective effect against ED. A meta-analysis of cross-sectional studies of men in South America, Europe, Asia, and Africa between 1990 and 2006 found an overall protective association of alcohol and ED, with regular heavy intake of alcohol increasing the risk of ED by approximately 25%, and consumption of eight drinks per week significantly reducing the risk of ED, with intake of less alcohol having no effect [93]. In the United States, the Boston Area Community Health survey found that moderate levels of alcohol consumption (one to three drinks per day) were associated with decreased risk of ED in men aged 30–79 [94]. The beneficial effects of the Mediterranean diet, which typically includes one to two drinks daily [84], may be related to alcohol intake in moderation.

12.5 EFFECTS OF WEIGHT LOSS ON TESTOSTERONE AND ERECTILE FUNCTION IN OBESE MEN

Because obesity is a major risk factor for low testosterone levels and sexual dysfunction, weight loss may be expected to improve testosterone levels and erectile function. A meta-analysis of 24 weight loss trials, involving 479 men with a mean follow-up of 38 weeks, found that the percentage change in BMI correlated positively with the magnitude of increase in total testosterone, with greater increases in total testosterone being associated with higher baseline testosterone and BMI [95]. In these studies, FSH and LH levels also rose in association with weight loss, while estradiol levels were significantly decreased. This is consistent with the established mechanisms of lowered testosterone in obesity, in which relative abundance of estrogens produced by aromatization of testosterone in adipose tissue decreases LH secretion due to negative feedback on both the hypothalamus and the pituitary [1], which can be reversed by treatment with an aromatase inhibitor [12] or an anti-estrogen [13]. However, decreased BMI, and not estradiol, was the most important determinant of the rise in testosterone, suggesting that other adiposity-associate factors contributed to the improvement in androgen status. Among the different modalities of weight loss, both caloric restriction and bariatric surgery were associated with a significant increase in plasma total testosterone, with bariatric surgery being significantly more effective, probably reflecting the larger weight loss (30%) with surgery, compared to approximately 10% with diet. SHBG and free testosterone levels also increased in conjunction with weight loss. Low-energy diets, which induced >10% weight loss from baseline, significantly increased SHBG [87,88,96,97] and total [87,88,96,97] as well as free bioavailable [97] testosterone, and normalized total testosterone and bioavailable testosterone levels in 70% and 50% respectively during weight loss and weight maintenance in abdominally obese men with the metabolic syndrome. The increase in SHBG is positively correlated with the degree of diet-induced weight loss [87,88,96,97], independent of diet composition. In comparison to older and diabetic subjects, testosterone increased more in younger nondiabetic men with a greater degree of obesity, likely due to larger weight losses, and possibly because of the attenuating effects of age and insulin resistance/hyperinsulinemia on plasma testosterone levels [4,5].

Weight loss induced by lifestyle intervention has also been shown to improve erectile function. In a meta-analysis of six lifestyle-intervention trials involving 740 men who were followed up 12–104 weeks [98], weight loss of 5–15% (kg) through diet and/or exercise increased IIEF-5 score by an overall weighted mean 2.4 points, consistent with significant improvement in mild ED (IIEF-5 score 17–21 points) and lesser improvement in more advanced ED (IIEF-5 score <17 points), where the minimum clinically important difference in IIEF-5 score varies according to the baseline ED severity (2 points in mild ED and 5 points in moderate ED). The degree of improvement in erectile function may depend on other factors in addition to amount of weight lost. Smaller degrees of weight loss of 5–10% of baseline (approximately 3–15 kg) induced with low-energy [87,88], reduced-fat diets [88–90], or Mediterranean [85,86] diets, with or without increase in physical activity, improved

IIEF-5 score by approximately 3–7 points over periods ranging from 8 weeks [87,88] to 2 years [85,86]. In the studies of Esposito et al. mean IIEF-5 score increased by approximately 3 points in association with weight loss of 15 kg after 2 years of lifestyle intervention (Mediterranean weight-loss diet and an increase in physical activity by approximately 100 minutes/week) in obese men [85], and in abdominally obese (WC > 102 cm) men with the metabolic syndrome who lost a smaller amount of weight [86]. One-third of subjects in the lifestyle intervention group regained normal erectile function (IIEF-5 score 22 or greater) compared with only 5% of the controls who lost 2 kg [85], which was comparable to the proportion (25–33%) of obese men in similar intervention studies who normalized erectile function on low-energy [87,88] or reduced-fat diets [88–90].

In obese diabetic Australian men who underwent dietary modification without change in volume of physical activity, a high-protein, reduced-fat and -carbohydrate diet (including 300 g of lean meat/poultry/fish and three servings of cereals/bread and low-fat dairy foods) prescribed to reduce daily energy intake by 600 kcal increased IIEF-5 score by 3 points at 8 weeks with mean 4% weight loss [88], as did their peers with similar baseline weight who received a low-energy diet that induced approximately double the amount (8%) of weight loss. Men in both groups were subsequently continued on this high-protein diet for a further 44 weeks and enjoyed continued improvement in IIEF-5 score by a further 4 points, despite no further weight loss. Similarly, in obese nondiabetic Asian men, diets utilizing either meal replacements or reduced-fat plans increased IIEF-5 score by approximately 3 points (20% increase from baseline) in association with approximately 5% weight loss after 12 weeks, which was maintained at 40 weeks despite no further weight loss [89]. In the latter study, fat intake decreased by approximately 25% from baseline; although the effects of weight loss are difficult to separate from that of reduction in dietary fat, aiming to decrease fat intake may be an effective strategy for weight management in obese men with ED.

12.6 IMPROVEMENT IN RISK FACTORS FOR LOW TESTOSTERONE AND ED BY DIET-INDUCED WEIGHT LOSS

Improvements in total testosterone level [87,89,90,95,97] and IIEF-5 score loss [85–90,96–98] have been associated with amount of weight lost, as well as decrease in WC, a surrogate of abdominal adiposity [85,87,90,97]. Reduction in adiposity is likely to be the primary factor driving the increase in testosterone, with consequent amelioration of aromatization of testosterone to estrogen, attenuation of LH levels and pulse amplitude and direct inhibition of testosterone synthesis in Leydig cells, which were mediated by insulin resistance, estrogen, leptin, and pro-inflammatory cytokines, as discussed in earlier sections. Accordingly, the increase in testosterone levels after weight loss have been associated with improvement in insulin sensitivity as measured by the quantitative insulin sensitivity check index (QUICKI) [87,88] and homeostasis model assessment (HOMA) [89,90]. Reduction in insulin resistance has also been correlated with increase in SHBG after weight loss in obese men with ED [87,90,96]. Improvements in other cardiovascular risk factors, such as dyslipidemia and hypertension, were also associated with increase in IIEF-5 score in a meta-analysis of studies utilizing lifestyle intervention and statin therapy [98], underscoring the links between ED and cardiovascular disease.

Improvements in risk factors for low testosterone and ED by diet-induced weight loss are summarized in Table 12.3.

12.6.1 Effects of Macronutrient Composition on Reduction in Weight and Adiposity

Alterations in the type and proportion of fat, protein, and carbohydrate have been evaluated in studies of diet-induced weight loss. In the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) study, which examined the effects of weight-loss diets with different fat (20–40%), protein (15–25%), and carbohydrate (35–65%) composition in 811 overweight adults over 2 years, there were no differences in reduction in weight [99] or total, visceral, and hepatic

adiposity [100]. The major determinant of successful weight loss was ability to adhere to calorie restriction, which was better with the high-fat and average-protein diets as these were more similar to the participants' previous diets [99]. The Carbohydrate Ratio Management in European National diets (CARMEN) study had earlier examined the effects of *ad libitum*, low-fat, high-carbohydrate diets on body weight in comparison with a control diet group for 6 months in 400 moderately obese adults, and found that replacement of dietary fat with carbohydrates induced greater losses of weight and fat mass than patients in the control group, concluding that reduction of fat intake results in a modest but significant reduction in body weight and body fatness [101]. In the Diet, Obesity and Genes (Diogenes) study in Europe, a modest increase in protein content to 25% of total calories was more effective than a low-protein (13%) diet for compliance with the intervention and maintenance of weight loss over 26 weeks [102]. Similarly, an increased-protein (30% of calories), reduced-fat (20%) diet was successful for maintaining weight loss of 5–10% over 52 weeks in obese Australian men [88]. It is important for obese men to be able to adhere to a diet in the long term, as successful weight loss is a major determinant of improvement in androgen status [95] and sexual function [98].

12.6.2 Improvements in Inflammation and Endothelial Dysfunction with Diet-Induced Weight Loss

Systemic inflammation and endothelial dysfunction, which are associated with ED, have also improved with diet-induced weight loss [72,85,87–90,101–105]. Weight loss and reduction in total body fat, visceral adiposity, hepatic tissue density, and insulin resistance were associated with decrease in high-sensitivity CRP by a similar extent in all four diets with varying proportions of fat, protein, and carbohydrate in the POUNDS LOST trial [101]. Isocaloric weight-loss diets with increased MUFA (>20% of fat) in a moderate-fat (35–45% of energy) diet or reducing fat intake (20–30% of energy) were similarly effective in lowering the inflammatory markers CRP and IL-6, and improved endothelial cell dysfunction in obese adults over 6 months [102]. The magnitude of reduction in obesity-associated inflammatory markers (such as leptin, CRP, TNF-α, IL-6) is associated with the amount of diet-induced weight loss, with the greatest improvement in systemic inflammation observed after low-energy diets which produced at least 10% weight loss from baseline [103]. Reduction in pro-inflammatory cytokines improves endothelial function by reducing expression of endothelial and vascular adhesion molecules, which facilitate vascular inflammation and atherosclerosis [52,57]. Improvements in endothelial function are also mediated by an increase in NO bioavailability, associated with reductions in abdominal visceral fat [104,105]. Although the effects of varying macronutrient proportions on erectile function in humans have not been extensively studied, evaluation of the effects of diet composition on these factors may offer further insights into how diet modification improves testosterone deficiency and erectile function.

12.6.3 Improvement in Lower Urinary Tract Symptoms, Libido, and Quality of Life with Diet-Induced Weight Loss

In weight loss studies using low-energy [87,88] and reduced-fat [88–90] diets, improvement in ED was associated with reduction in lower urinary tract symptoms (LUTS), which manifest as voiding (incomplete emptying, intermittency, weak stream, straining) and storage (frequency, urgency, nocturia) impairment as a result of prostate and bladder dysfunction. Both LUTS and ED may be the consequence of generalized defects in the regulation of pelvic blood flow caused by impaired NO signaling and autonomic system overactivity, which mediate smooth muscle and endothelial dysfunction and atherosclerosis, leading to impaired penile rigidity, prostatic hyperplasia, and bladder outlet obstruction [106]. Sexual desire was also increased in men who lost weight using low-energy [87–89,96] or reduced-fat diets [88–90]. Improvement in quality of life with weight loss [63,89,90] may also contribute to the improvement in erectile function.

12.7 EVIDENCE FOR DIETARY ANTIOXIDANTS IN IMPROVEMENT OF ED

Although there is a paucity of evidence for the efficacy of antioxidants in improving ED in men, animal studies suggest a role for dietary antioxidants in reducing oxidative stress in the corpus cavernosum, which mediates ED through eNOS uncoupling, vascular dysfunction, and inflammation [59,60]. Pomegranate juice was shown to have the highest antioxidant activity, compared to other beverages that contain antioxidant polyphenols (red wine, blueberry juice, cranberry juice, orange juice, and green tea) [107]. In rabbits with arteriogenic ED, long-term pomegranate juice intake increased intracavernous blood flow and smooth muscle relaxation, and improved erectile function (as measured by increased ratio of mean intracavernosal pressure: mean arterial pressure after stimulation of the cavernosal nerves), reduced erectile tissue levels of oxidative products, and partially normalized mitochondrial and endothelial structural abnormalities associated with oxidative stress [108]. Ellagic acid (EA), one of the five principal polyphenolic components of pomegranate juice, induced relaxation of phenylephrine-contracted rat corpus cavernosum smooth muscle [109]. However, studies in men are limited by small numbers, short duration, and a lack of mechanistic evidence. In a study of 60 sexually active, healthy men aged 21–70 years with mild-to-moderate ED who received 8 oz. of pomegranate juice or placebo for a total of 28 days with crossover after a 2-week washout period, there was a trend toward association between intake of pomegranate juice and improvement of the Global Assessment Questionnaire (GAQ) score, which evaluated perceived improvements in erectile function and sexual ability, but no significant change in IIEF-5 score [110].

L-Arginine is an amino acid that is synthesized in the body, and is also found in plant (nuts, sunflower seeds, beans) and animal (tuna, salmon, shrimp, eggs, whole milk) proteins. Vascular endothelial cells synthesize NO from L-arginine through oxidation of the nitrogen atom in the terminal guanidine group by eNOS [59]. High dose (5 g/day) oral L-arginine administered to men with organic ED for 6 weeks in a randomized placebo-controlled trial improved scores on O'Leary's questionnaire (sexual drive, erectile function, problem assessment, and overall sexual satisfaction) in approximately 30% of subjects, compared to approximately 10% of the control group [93]. The effects of L-arginine on the human corpus cavernosum *in vitro* are mediated by restoration of the endogenous amino acid pool for NO synthesis, and by stimulation of NO-soluble guanylyl cyclase-protein kinase G signaling involving the activation of KCa channels and inhibiting the up-regulated RhoA/Rho-kinase pathway [111]. The addition of sildenafil combined with L-arginine further facilitates smooth muscle and endothelial relaxation in human cavernosal tissue, suggesting a synergistic role of L-arginine in phosphodiesterase-inhibitor treatment [112].

Quercetin, a bioflavonoid found in many plants (e.g., capers, lovage, dill, red onions, watercress, buckwheat, and various berries), has antioxidant properties. In diabetic rats given oral quercetin for 8 weeks, erectile function (as measured by the rise in intracavernous pressure [ICP] after cavernous nerve electrostimulation) improved with higher doses of quercetin [113]. These doses of quercetin also increased SOD dismutase activity, and reduced levels of thiobarbituric acid-reacting substance (TBARS), which are markers of oxidative stress, in cavernosal tissue, while the highest dose of 50 mg/kg ameliorated the diminished eNOS activity induced by diabetes. However, controlled, randomized studies in humans with ED are lacking.

Resveratrol, a plant polyphenolin with antioxidant and anti-inflammatory effects, is found in the skin of red grapes and in other fruits as well as in the roots of Japanese knotweed. The beneficial effects of resveratrol on ED have been demonstrated in animal models. Rats treated with resveratrol demonstrated improvement in diabetes-induced ED as a result of greater NO bioavailability and resistance to oxidative stress [114]. In a separate study, combining resveratrol with the phosphodiesterase (PDE) inhibitor vardenafil had a synergistic effect in increasing NO bioavailability and cavernosal relaxation compared to either alone in diabetic rats [115]. The relaxation of rabbit cavernosal tissue induced by resveratrol was dose dependent, with median effective concentration (EC_{50}) of 0.29 mg/mL, and was associated with increase in blood testosterone concentration of approximately 50% [116]. In rabbits fed a high-cholesterol (2% of calories) diet for 6 weeks, coadministration of resveratrol at

a dose of 4 mg/kg was protective against endothelial dysfunction and atherosclerotic changes, and preserved vasorelaxation responses to acetylcholine in the corpus cavernosum [117]. However, no studies of the effects of resveratrol have been performed in men with ED, and the concentration of resveratrol in red wine is much lower (0.5 mg in 600 mL, approximately two to three glasses) than the experimental doses in animals, reaching only trace serum concentrations in humans [118], which are insufficient to account for the beneficial effects of red wine as a component of the Mediterranean diet.

Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables, such as red carrots, red bell peppers, watermelons, gac and papayas. In Wistar rats, lycopene significantly attenuated cisplatin-induced testicular atrophy and impaired testosterone synthesis caused by oxidant stress and lipid peroxidation [53]. This was associated with suppression of ROS production and increase in SOD dismutase activity and levels of glutathione (an antioxidant that directly removes ROS). In rats with diabetes-associated ED, lycopene administered orally for 8 weeks lowered blood glucose, reduced oxidative stress and up-regulated eNOS expression in association with increase in cavernosal relaxation [119]. There are no studies of the effects of lycopene on ED or testosterone in humans.

Korean red ginseng (*Panax ginseng*) was found in animal studies to induce a dose-dependent relaxation of cavernosal smooth muscle that is mediated by increasing NO bioavailability through ROS-scavenging effects, eNOS induction, and decreasing intracellular calcium [120]. In men with ED (vasculogenic, psychogenic, or mixed), a meta-analysis [121] of RCTs using red ginseng extracts at doses of 1800–3000 mg daily found that ginseng was associated with improvement of erectile function

TABLE 12.4
Effects of Dietary Antioxidants on Sexual Function

Antioxidant	Effect on Sexual Function	Mechanisms
Pomegranate juice (contains polyphenols)	Improved in rabbits [107,108] Relaxed rat cavernosal muscle [109] Improved in men with mild-to-moderate ED [110]	Reverse molecular and ultrastructural abnormalities associated with oxidative stress Increase NO bioavailability Not studied in humans
L-Arginine	Improved in men with organic ED [111] and potentiated effect of PDE inhibitors [112]	Increase NO synthesis Stimulate protein kinase G signaling Inhibit RhoA/Rho-kinase ?Antioxidant
Quercetin	Improved in diabetic rats [113]	Increase NO bioavailability Increase eNOS activity
Resveratrol	Improved in diabetic rats [114,115]. Protected against hypercholesterolemia-mediated ED in rabbits [117]	Increase NO bioavailability Stimulate eNOS Suppresses apoptosis of smooth muscle
Lycopene	Prevented cisplatin-mediated testicular atrophy and low testosterone in rats [53] Improved in diabetic rats [119]	Increase NO bioavailability Up-regulate eNOS expression Improve endothelial dysfunction
Korean ginseng	Improved ED in rabbits [120] Associated with improved ED in men [121]	Increase NO bioavailability Not studied in humans
Vitamin E (α-tocopherol)	Improved in diabetic mice [122] and hypertensive [123] and diabetic [124] rats Improved response to PDE inhibitors in impotent men [125]	Increase NO bioavailability and production Prevent ROS-induced inflammation, vascular and endothelial damage, cell membrane lipid peroxidation Not studied in humans

Note: ED, erectile dysfunction; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PDE, phosphodiesterase; ROS, reactive oxygen species.

(measured by Watts sexual function, structured interview or IIEF-5, and global efficiency questionnaires). However, these trials were limited by inadequate blinding and allocation concealment, possible attrition bias, and small sample size, and were supported by manufacturers of ginseng products [121].

Vitamin E (α-tocopherol) is a fat-soluble antioxidant with membrane-stabilizing properties. In diabetic mice, vitamin E supplementation prevented ROS-induced inflammation and vascular and endothelial damage, and improved insulin sensitivity, which was associated with partial normalization of endothelial relaxation in the corpus cavernosum [122]. Similarly, feeding hypertensive rats α-tocopherol-enriched chow for 8 weeks significantly increased vascular smooth muscle relaxation in the corpus cavernosum by improving neuronal and endothelial function, and increased SOD dismutase activity and decreased TBARS, leading to lower oxidative stress [123]. In another study of diabetic rats, 2% vitamin E increased NO production in penile tissue [124]. Administering 300 mg per day of α-tocopherol for at least 1 month to 89 men who had poor response to PDE inhibitors increased their IIEF-5 scores by an average of approximately 20%, and improved penile rigidity [125], though no objective measures of improvement in erectile function were available.

Various dietary antioxidant components have demonstrated efficacy for improving ED, primarily by increasing NOS bioavailability, thereby improving endothelial function and cavernosal relaxation. However, these studies have largely been conducted in animal models of ED with purified extracts and/or supplements in high doses exceeding that available from food sources. Larger controlled trials of longer duration are needed to evaluate the efficacy of dietary antioxidants in the management of ED in men.

The effects of dietary antioxidants on sexual function are summarized in Table 12.4.

12.8 LOW TESTOSTERONE AND SEXUAL DYSFUNCTION IN UNDERWEIGHT AND ANOREXIC MEN

Just as overweight and obesity increase the risk of hypogonadism and sexual dysfunction, the other extreme of the BMI range is also associated with decreased testosterone synthesis and ED. In particular, anorexia nervosa, a psychiatric disorder in which food intake is restricted to induce a large amount of weight loss leading to underweight (<85% of expected body weight for height), frequently in combination with self-induced vomiting, misuse of laxatives, or excessive exercise, is most prevalent in adolescence, and results in delayed or arrested puberty and growth, metabolic and electrolyte abnormalities, and osteopenia [126]. Although it is estimated that only 5–10% of anorexia sufferers are male, obesity and a history of dieting are more strongly associated with subsequent development of eating disorders in boys compared to girls [127]. In underweight males, low testosterone production results from suppression of hypothalamic GnRH and diminished response of pituitary FSH and LH response to GnRH, leading to hypogonadotrophic hypogonadism and decreased sexual drive and performance [127]. The reduction in GnRH production is thought to be mediated by low leptin production as a result of decreased body fat mass in starvation, and accordingly the restoration of healthy weight is associated with an increased testosterone level and improvement or normalization of sexual function [128,129]. In a study of young men with anorexia nervosa during therapeutically induced weight gain, serum leptin levels were low (below the 5th age centile) at baseline and increased to above the 95th age centile in association with the gains in BMI and fat mass [128]. There were concomitant increases in serum LH and total testosterone levels, and free androgen index (FAI), and positive associations between changes in leptin, testosterone, and FAI. Leptin levels then decreased or plateaued during the period of weight maintenance. In contrast to obese men, the increase in leptin levels during weight regain in anorexia is associated with normalization of the hypothalamic–pituitary–testicular axis and gonadal function [128], suggesting that hyperleptinemia in this context is a metabolic adaptation rather than a pathological process.

Eating disorders are also associated with psychiatric disorders (depression, anxiety, body dysmorphic disorder), difficulty with intimacy and relationships, and higher use of antidepressant

medications and drugs of abuse, which contribute to sexual dysfunction [126]. Although there are no studies correlating increase in testosterone with improvement of sexual function following therapeutic refeeding and weight regain in men, it seems reasonable that, as in women [126], normalization of weight and hormonal abnormalities and management of underlying psychiatric issues would also restore sexual function in anorexic men.

12.9 KEY POINTS SUMMARY

1. Sexual function in the male is governed by endocrine influences such as testosterone, together with psychological inputs such as body image and mood. Because all of these can be adversely affected by obesity, it is not surprising that obese men suffer from a higher incidence of sexual difficulties (erectile dysfunction, reduced sexual desire).
2. Obesity, especially abdominal obesity associated with an increased WC, has been definitively associated with reduced testosterone by several investigators. It is estimated that at least 50% of obese men aged 45 years or older will have suboptimal testosterone levels, with age-associated increases in BMI being the primary cause for the observed drop in testosterone with advancing age.
3. Testosterone levels are lowered in obese men by several interacting mechanisms originating at the level of the hypothalamic–pituitary (HP) axis, the testis, and the liver (sex hormone-binding globulin [SHBG] production). First, increased conversion of testosterone to estrogen by aromatase action in adipose tissue results in negative feedback on the HP axis, reducing luteinizing hormone and follicle-stimulating hormone-driven testosterone production. Elevated levels of insulin, leptin, and inflammatory cytokines have also been implicated in the inhibition of testosterone production. Second, insulin, cortisol, and leptin, all increased in obese men, act directly on the Leydig cells in the testis and inhibit testosterone biosynthesis. Finally, hyperinsulinemia associated with obesity and metabolic syndrome suppresses hepatic production of SHBG, resulting in reduced binding of testosterone to this carrier, which subsequently decreases circulating testosterone and delivery of testosterone to peripheral tissues.
4. The relationship between obesity and male hypogonadism is bidirectional, each exacerbating the other. Low testosterone promotes visceral fat accumulation at the expense of lean muscle mass, while also exacerbating insulin resistance, thereby creating a vicious cycle positive feedback loop.
5. The impact of macronutrient intake on testosterone production is currently uncertain owing to conflicting results of human and animal studies and difficulties controlling for the effects of macronutrient intake from calorie (energy) restriction. However, it appears that a diet high in saturated fats (e.g., animal fats) appears to increase testosterone levels, whereas animal studies suggest that omega-3 polyunsaturated fatty acids (PUFAs; e.g., fish) tend to reduce circulating testosterone. Conversely, omega-6 PUFAs (meat, vegetable oils) and monounsaturated fatty acids (MUFAs) such as those found in olive oil both increase testosterone production. Increasing the percentage of protein in energy intake reduces testosterone levels while increasing carbohydrate intake increases testosterone in healthy men. However, it should be noted that acute high carbohydrate loads in obese glucose-intolerant men does result in a fall in testosterone levels.
6. Inadequate intake of the micronutrients zinc and selenium are associated with hypogonadism, with testosterone levels normalizing on adequate replacement of these micronutrients. Vitamin D deficiency is also associated with hypogonadism in men that can be reversed with dietary supplementation. Light alcohol intake is associated with small increases in testosterone production; however, consumption of 40 g (four standard drinks) or more of alcohol per week results in a drop in testosterone. This fall in testosterone becomes pronounced in alcoholic cirrhosis.

7. Erectile dysfunction (ED) is present in approximately 40% of obese men, with the underlying pathophysiology being multifactorial. First, obesity-related low testosterone impairs normal neural and penile vascular endothelial nitric oxide (NO) synthesis necessary for initiation and maintenance of an erection. Second, obesity-related systemic inflammation and oxidative stress impairs endothelial function and vascular responses. Third, obesity-related hypertension and adverse lipid profiles predispose to atherosclerotic reduction in pelvic arterial blood supply, impeding normal penile vascular erectile responses. Finally, poor personal body image associated with obesity, together with lethargy and negative mood from sleep fragmentation and hypoxia associated with obstructive sleep apnea, all combine to add a psychological element to ED and decreased sexual desire.
8. Both animal and human studies suggest that modification of diet can improve obesity-related sexual dysfunction. A Mediterranean diet (low saturated fat, high MUFA such as olive oil, moderate alcohol intake, abundant fruits and nuts with high antioxidant content, plus fish abundant in anti-inflammatory omega-3 PUFAs) has been shown to reduce the incidence and severity of ED in obese men. Weight loss through either bariatric surgery or diet and exercise has been shown to improve testosterone levels and erectile function. To maintain long-term weight loss and improved sexual function, studies suggest obese men should adopt a diet with increased protein (30% of calories) and reduced fat (20%). Furthermore, human and animal studies have provided some preliminary evidence supporting the use of antioxidant and L-arginine (nitric oxide donor) supplements to boost erectile function.
9. Underweight males also experience hypogonadism, triggered by the relative deficiency of adipose-derived leptin that results in impaired HP axis drive of testosterone production. Restoration of normal body weight will result in normalization of testosterone levels.

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13 The Role of Nutrition in Optimizing Assisted Reproductive Technology Treatment Outcomes

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CONTENTS

13.1 INTRODUCTION

Both female and male fertility is highly susceptible to environmental cues, many of which can have substantial negative effects on reproductive performance. Lifestyle factors such as maternal and paternal age, alcohol intake, cigarette smoking, and the use of illicit drugs can all influence the ability to conceive. In addition, increased body mass index (BMI) can also significantly affect fertility, which is of particular concern because of the significant increase in obesity worldwide (the World Health Organization [WHO] estimates that by 2015 there will be 1.5 billion people in the world who are obese [1]).

Eating a healthy balanced diet and maintaining a BMI within the normal range of 18.5–24.9 kg/m^2 is an essential part of maintaining good overall health and fertility. It has long been demonstrated that a high BMI (>30 kg/m^2) can negatively impact fecundity in both men and women, with obesity being associated with decreased natural conception rates and increased time until conception because of the multifaceted impact that obesity has on male and female fertility [2]. In addition, nutrition is also of vital importance, as many studies have demonstrated that certain vitamins, supplements, and specific food groups can have both positive and negative effects on fertility. It is estimated that one in six couples will require assisted reproductive technologies to conceive, and hence it is of vital importance that the impact of BMI and nutrition on reproductive outcomes as well as long-term offspring health is understood, so that the correct advice can be given by clinicians to achieve optimal assisted reproductive technology outcomes for patients.

13.2 THE IMPACT OF MATERNAL BMI ON FERTILITY AND *IN VITRO* FERTILIZATION OUTCOMES

The increased prevalence of obesity among Western populations is of growing concern in the health community. The WHO estimates that 1.4 billion adults are currently overweight or obese, with the rates of obesity rising in all age groups (including children and both men and women of reproductive age) [3].

It is well established that obesity is associated with a number of health problems including hypertension, type 2 diabetes, and heart disease; however, it is also well documented that obesity can significantly impact various maternal reproductive functions including alterations in secretion of hypothalamic gonadotropins, decreased ovulation rates, increased time to conception, increased miscarriage and stillbirth rates, as well as pregnancy complications such as preeclampsia, gestational diabetes, and fetal abnormalities [4–7]. Obese women are more likely to encounter delivery problems such as preterm delivery, increased risk of emergency caesarean section, as well as postpartum hemorrhage [8], and there are also significant risks to the neonate such as central nervous system abnormalities as well as fetal macrosomia, which in turn has been linked to increased risk of obesity later in life, diabetes, development of metabolic syndrome, and cancer [9–15].

13.3 MATERNAL BMI, OOCYTE, EMBRYO QUALITY, PREGNANCY, AND OFFSPRING OUTCOMES

In addition to affecting natural fertility, maternal BMI can also impact the outcomes of assisted reproductive technology (ART). Multiple studies have demonstrated that obese women require an increased gonadotropin dose for ovarian stimulation during ART [16–19], but even so, fewer oocytes are collected in this group [18–21]. In addition, a recent meta-analysis concluded that raised BMI (>25 kg/m^2) is associated with increased miscarriage rates and decreased live birth rates after *in vitro* fertilization (IVF) treatment [15] when compared to women with a normal BMI (18.9–24.9 kg/m^2); however, embryo quality (as measured by morphology) does not appear to be significantly influenced by BMI (especially in younger patients) [19,22,23]. Therefore, it is likely that obesity is exerting its effect on other aspects of oocyte and embryo quality. In addition, it has also been demonstrated that the increased IVF pregnancy failure rate seen in obese women can be normalized when donor oocytes are used that are obtained from women with a normal BMI, providing further indications that oocyte quality in obese women is compromised and contributes significantly to the decreased pregnancy outcomes seen in these individuals [24].

Because of the effects of obesity on pregnancy and IVF outcome, mouse models have been utilized to assess the impact of obesity on oocyte quality as well as aspects of metabolic homeostasis. Studies have demonstrated that female mice fed a high-fat Western style diet have altered oocyte developmental competence and embryo quality [25]. After 16 weeks on this diet, female mice were significantly heavier and also developed a hyperinsulinemia coupled with increased rates of anovulation compared to mice fed a control diet, and after fertilization had slower cleavage development that was maintained until the blastocyst stage, a result that has also been seen in other studies [26]. Embryo quality was also compromised as blastocysts from a female fed a high-fat diet had alterations to blastocyst differentiation with lower inner cell mass (ICM) cell numbers and higher trophectoderm (TE) resulting in a change to the ICM/TE ratio, with these changes being linked to decreased fetal size in other models [27,28].

13.3.1 Mechanism

The proposed mechanism of how obesity affects oocyte quality is via changes in the reproductive milieu as a result of the excessive consumption of nutrients. It has been demonstrated in both the mouse and the human that obesity is associated with changes in serum and follicular fluid metabolites. In murine models, consumption of a high-fat diet (leading to obesity) resulted in changes to serum concentrations of glucose, insulin, adiponectin, and leptin [29] compared to lean controls and that oviductal fluid leptin levels were also significantly elevated [26]. In human follicular fluid, obesity is also associated with increased levels of insulin, lactate and triglyceride levels [30]. Interestingly, it has been demonstrated in both *in vivo* and *in vitro* models that elevated levels of leptin are associated with impaired blastocyst development [31,32], and in obese women hyperleptinemia is associated with decreased IVF conception rates and increased rates of miscarriage [33]. It is proposed that this altered environment may then impact the developing oocyte, in particular metabolism, thus impeding subsequent embryo development. Live cell dynamic fluorescence imaging has supported this hypothesis by demonstrating that oocytes and zygotes obtained from mice with diet-induced obesity have altered mitochondrial distribution as well as a significant increase (oocytes ↑147% and zygotes ↑74%) in mitochondrial membrane potential (MMP) compared to lean females as well as increased oxidation of pyridine nucleotides and flavoproteins indicative of changes in the cellular redox status [26]. In addition, oocytes and zygotes obtained from obese mice also have increased levels of reactive oxygen species (ROS) and decreased levels of antioxidants (glutathione), indicating an overall increase in cellular oxidative stress [26]. It has also been observed that mitochondrial ultrastructure in both oocytes and surrounding cumulus cells is also different in an obese model, with fewer mitochondrial cristae, indicative of reduced surface area for

electron transport, decreased matrix density, and increased levels of vacuolation [34]. From this it can be concluded that altered mitochondrial structure and function may be one of the mechanisms by which obesity affects oocyte viability and that the increased availability of nutrients (carbohydrates, fatty acids, and leptin) in the reproductive environment may result in an altered flux of energy substrates through mitochondrial metabolic pathways, which in turn is coupled with an increase in oxidative stress. As the oocyte and early embryo lack robust mechanisms to protect against environmental challenges and cannot synthesize antioxidants until later in development, this increased level of ROS may also result in damage to other cellular processes including lipid peroxidation and DNA damage (both genomic and mitochondrial DNA [mtDNA]) [35–37].

In addition to alterations in metabolism, obesity in animal models is also associated with oocyte meiotic aneuploidy. Oocytes from mice fed a high-fat diet have significantly increased rates of meiotic defects, including malformed spindles and chromosome misalignment [34] as well as a higher incidence of aneuploidy. It is proposed that the alterations to mitochondrial metabolism and oxidative state may be responsible for this, as in the human altered mitochondrial membrane potential has been linked to chaotic mosaicism [38]. However, emerging evidence from human studies has not yet shown a link between increased BMI and increased rates of miscarriage due to abnormal karyotype [39,40]. However, these studies have not looked at specific BMI subgroups but rather have assessed normal BMI versus BMI ≥25 kg/m^2; therefore whether the rates of aneuploidy are higher in obese and morbidly obese individuals remains to be determined.

One confounding factor that has emerged from many of these studies is the early indication of a diabetic phenotype. In many of the murine studies the mice have been noted to have altered glucose homeostasis as well as hyperinsulinemia [25,29], with other studies not investigating this aspect of the maternal obesity phenotype. As a result, it is difficult to separate which outcomes on oocyte and embryo quality are due to altered glucose homeostasis and which are due to the presence of increased adiposity/obesity. Studies have also shown that diabetic mice produce oocytes with phenotypes similar to those obtained from the high-fat models. Oocytes obtained from diabetic mice have abnormal mitochondrial structure, uneven mitochondrial distribution, decreased ATP levels, and altered levels of mitochondrial metabolic substrates including citrate, malate, and aspartate [41–43]. In addition, they are also reported to have increased rates of meiotic spindle and chromatin defects [41] and after fertilization have decreased blastocyst development rates [44]. Therefore, more studies are required that assess the impact of obesity in the absence of altered glucose homeostasis to determine what effects are mediated purely by increased adiposity, especially in light of the early evidence that in animal models maternal obesity is linked to growth retardation and brain developmental abnormalities, which also has been noted in human studies linking maternal obesity to an increased risk for neurodevelopmental delay and atypical neurodevelopment in offspring [34,45,46].

Although increased BMI is of great concern, undernutrition and a low BMI can also be problematic. It is understood that women with low amounts of body fat have increased rates of infertility primarily as a result of ovarian dysfunction [47,48] as well as increased rates of preterm birth [49]. In addition, one study has demonstrated that 20% of infertile patients seeking intrauterine insemination had been diagnosed with an eating disorder, suggesting a link between undernutrition and infertility [50]. However, owing to the fact that in our current Western population (in particular those undertaking IVF) obesity is far more prevalent that low BMI, the impact of undernutrition and a low BMI on IVF outcomes is not known.

13.3.2 Can the Effects of Maternal BMI Be Reversed?

Consistent with the negative effect obesity has on fertility, modest weight loss can improve both spontaneous and IVF conception rates [51–53]. In regards to natural fertility, it has been demonstrated that even a small weight loss of 6.5 kg over a 6-month period resulted in 12 out of 13 women resuming normal ovulation patterns and 11 becoming pregnant (5 spontaneously), which was coupled with normalization of testosterone and insulin levels [54]. In a follow-up study using

lifestyle intervention, patients undertaking ART treatment lost an average of 10.2 kg, and 60 out of 67 anovulatory women resumed normal ovulation patterns; of the 52 who achieved a pregnancy, 18 were spontaneous pregnancies. In addition, miscarriage rates decreased from 75% preintervention to 18% after lifestyle changes [55].

Bariatric surgery has also been utilized to sustain weight loss, leading to improvement in fertility. Studies have suggested improvements in natural fertility after bariatric surgery, although it has been noted that many of the studies have been observatory and have not distinguished between ovulatory and anovulatory women [56–58]. In the field of assisted reproduction, very little is known about the impact of bariatric surgery on IVF outcomes, and more research is required before a definitive conclusion can be made as to whether this form of treatment is advised [58].

Although weight loss has been promoted as one of the most effective means of improving fertility in overweight women, it must be noted that quick weight loss using a low-calorie diet can have detrimental effects on ART outcomes. Utilizing a very-low-calorie diet (VLCD), patients undergoing IVF treatment [59] were allocated to one of two treatment groups beginning a VLCD on either day 14 or day 21 of their last menstrual period. For both groups by day 21, all participants were consuming 1914 kJ/day alongside 2 L of water and two cups of vegetables [59]. Dietary intervention stopped on the evening of the oocyte retrieval; therefore both groups had completed a dietary intervention of 4–6 weeks in the peri-implantation period. Although both groups had significant weight loss (2.4–8.8% of body weight) and decreased waist circumference, the concerning observation was the poor IVF outcomes noted. Although the study was canceled due to poor patient retention, and embryology outcomes could not be assessed definitively, it was noted that the three patients who were on the diet for up to 4 weeks all had a failed fertilization. In addition, oocyte numbers were very low across all patients, and those who did achieve fertilization had a very low percentage (average of 30%) compared to what is seen under normal circumstances [59]. This study has therefore raised concerns about crash dieting before and during ART treatment. The developing oocyte and embryo are highly susceptible to the surrounding environment, and the use of a VLCD may induce significant changes to metabolic (ketosis) and hormonal balance, which in turn may have a significant impact on the quality of the developing oocyte and subsequent embryo [60]. Although not a conclusive study, the early evidence from this research suggests that perhaps a more gradual, long-term weight loss regimen before IVF would be a better approach; however, animal data are required to demonstrate the most appropriate weight loss regime to ensure that IVF outcomes are not compromised.

13.4 THE IMPACT OF PATERNAL BMI ON FERTILITY AND IVF OUTCOMES

13.4.1 Male Obesity, Semen Parameters, Embryo Quality, Pregnancy, and Offspring Outcomes

Several studies have investigated the impact of male obesity on the traditional sperm parameters mandated by the WHO, namely, sperm concentration, sperm motility, and sperm morphology [61], as well as ejaculate volume. Other parameters including sperm DNA fragmentation, membrane potential, and sperm chromatic condensation have also been studied.

13.4.1.1 Sperm Concentration

There is some evidence that male obesity reduces sperm concentration, shown in 15 out of 23 recent studies [62–75]; however, others have not shown this relationship [76–81].

13.4.1.2 Sperm Motility

There are many contradicting reports with regard to sperm motility, with some showing an inverse relationship with increased male BMI [63,67–69,72,75,82], whereas others do not [62,64, 65,71,76–81,83].

13.4.1.3 Sperm Morphology

Although some reports suggest an inverse relationship between sperm morphology and male obesity [67–69,71,80,83], others do not confirm this relationship [65,75–79,81,82,84] and it is therefore unclear if male obesity has an impact on this parameter.

The differences observed in the literature are likely due to limitations in the design and populations used in human studies. First, these studies can be confounded by lifestyle factors such as smoking, alcohol consumption, and recreational drug use and co-pathologies. Second, the majority of populations studied are patients seeking infertility treatment, where patient cohorts are frequently biased toward subfertile men. Third, some studies rely on self-reporting of parameters such as BMI, which can lead to underreporting [61].

13.4.1.4 Ejaculate Volume

Overall there appears to be no significant effect of male obesity on ejaculate volume [78,80–82,85–89], suggesting that increased BMI may not have a direct impact on seminal vesicle or prostate secretion capacity.

13.4.1.5 Sperm DNA Damage

Several studies have assessed the effect of male obesity on the percentage of sperm with DNA fragmentation [75,81,90,91]. Two studies assessed DNA fragmentation by the TUNEL (terminal uridine nick end labeling) assay [75,90]; two used the sperm chromatin structure assay [81,92], and some used the Comet assay [91,93]. Overall, all studies indicate a significant increase in the percentage of sperm with DNA fragmentation with increasing male BMI.

13.4.1.6 Sperm Chromatin Condensation

Three studies contained data on the effect of male obesity on the percentage of sperm with decondensed chromatin [81,90,94]. One assessed chromatin condensation by propidium iodide staining and found a statistically significant increase in the percent of spermatozoa with decondensed chromatin in obese men compared with normal weight men [87]; another used aniline blue staining and found a statistically nonsignificant increase [89]; and the third used the sperm chromatin structure assay and found a statistically nonsignificant decrease [81]. Owing to the diversity of methods of used to measure this parameter and the limited number of studies, it is currently unclear if a relationship exists between increased male BMI and decondensed chromatin.

13.4.1.7 Sperm with Low Mitochondrial Membrane Potential

Two studies reported the percent of sperm with low MMP for obese men compared with normal weight men [90,91]. In one study, MMP was assessed by JC-1 staining [87], while in another study, the deposition of diaminobenzidine was used as a measure [82]. La Vignera et al. demonstrated that the percent of sperm in the ejaculate of obese men who had low MMP was significantly increased compared to that in normal weight men [87], and Fariello et al. demonstrated that both the percent of sperm with low MMP and the percent of sperm with no MMP was significantly increased in obese men compared to normal weight men [82].

13.4.1.8 Seminal Plasma Factors

Three papers reported the effects of male obesity on the concentrations of different seminal plasma factors [75,88,94]. Tunc et al. found a statistically significant positive correlation for increasing concentration of neopterin in seminal plasma and increasing male BMI [75]. The authors of the paper concluded that this increased neopterin concentration suggested an enhanced state of macrophage activation in obese men, owing to neopterin being a pterin molecule that is released from macrophages/monocytes on stimulation by pro-inflammatory cytokines, most notably interferon gamma. The study by Lotti et al. showed that being obese correlated with a significantly increased concentration of interleukin-8 (IL-8) in seminal plasma compared with normal weight men [88]. IL-8 is a pro-inflammatory chemokine.

Martini et al. found a statistically significant negative correlation between increasing BMI and the seminal plasma concentration of α-glucosidase as well as a statistically significant positive correlation between male BMI and seminal plasma concentration of fructose [89]. For fructose, there was a seminal plasma concentration 351.6 ± 9.6 mg/mL in obese men compared with a seminal plasma concentration of 333.6 ± 8.1 mg/mL in normal weight men. Martini et al. also investigated the citric acid concentration of seminal plasma, but found no statistically significant correlation with BMI [89].

13.4.1.9 Paternal BMI and *In Vitro* Embryo Development

Couples with an overweight or obese male partner, with a female of normal BMI, have an increased odds ratio (OR) for increased time to conceive compared with couples with normal weight male partners [95,96]. Two studies reported the effects of male partner obesity on embryo development [97,98]. Bakos et al. investigated IVF and intracytoplasmic sperm injection (ICSI) fertilization rates, the percentage of good quality embryos on day 3, the percentage of embryos that had on-time blastocyst development on day 5, and the percentage of embryos that had developed to the expanded blastocysts stage on day 5 [97]. This was done for normal weight and obese men ($N = 63$ and 62, respectively, as well as overweight $n = 148$, and morbidly obese men $n = 32$). Embryo quality was assessed only if fertilization was confirmed by the observation of two pronuclei and two polar bodies the morning after insemination. A statistically significant linear decrease in on-time and expanded blastocyst development was shown with increasing BMI. The other indicators of embryo development did not show statistical significant differences. In the second study by Colaci et al. embryo development was assessed as poor quality, accelerated cleavage, and slow cleavage on day 3 [98]. A total of 149 cycles were investigated; however, no significant differences were found between normal weight and obese men. Therefore, it appears that paternal BMI impacts more on post compaction development and further studies are required to confirm this association.

13.4.1.10 Paternal BMI and Pregnancy Outcomes

Three studies have assessed the effect of male obesity on the OR of couples undergoing ART (IVF or ICSI) achieving a clinical pregnancy [97–99]. Methods measuring clinical pregnancy rate differed between studies. Bakos et al. [97] assessed clinical pregnancy by heartbeat detected per oocyte pick up; Colaci et al. [98] assessed confirmed clinical pregnancy by ultrasound confirmation per embryo transfer cycle; and Keltz et al. [99] assessed intrauterine gestational sac on transvaginal sonogram per cycle. Overall these studies found a significant decrease in clinical pregnancy success rate for obese men compared to normal weight men.

Although a positive association between normal male BMI and pregnancy is not surprising in humans, animal models of obesity have shown that the capacitation status and sperm binding ability of mice fed high-fat diets were impaired compared with controls as well as impaired embryo development and implantation rates [84,100–102]. Taken together the human data as well as the rodent models are highly suggestive of a functional change to the molecular makeup of sperm that impacts directly on both sperm function and on subsequent embryo development [61].

13.4.1.11 Paternal BMI Live Birth from ART

Bakos et al. calculated live birth per oocyte pick up [97]; Colaci et al. calculated live birth per embryo transfer cycle [98]; and Petersen et al. calculated live birth per treatment cycle [103]. Once again, overall it appears that a decrease in live birth success rate was associated with increased paternal obesity.

13.4.1.12 Paternal BMI and Transgenerational Development

The one study that investigated the effect of paternal obesity on offspring characteristics found that paternal obesity significantly altered infant BMI growth curves from birth to 3.5 years compared with normal weight fathers [104]. This effect was still present after adjusting for maternal BMI,

which also had a significant effect on BMI growth curves. Looking only at weight or length, the effect of paternal BMI did not reach significance ($P = 0.08$ in either case). However, when the results were adjusted for maternal BMI the difference in offspring length between obese fathers and normal weight fathers was significant ($P = 0.05$).

Given the limitation of the clinical studies, animal models of paternal obesity have been developed, which have demonstrated significant changes to both the metabolic and reproductive health of subsequent offspring [105,106]. Data from a rat model of diet-induced obesity and reduced glucose tolerance demonstrated that paternal obesity compromised pancreatic function through altered gene transcription and islet cell dysfunction in female offspring [105].

In addition, a mouse model of diet-induced obesity without reduced glucose tolerance showed that paternal obesity compromised both first- and second-generation metabolic and reproductive health, with the female offspring in addition having increased fat mass, demonstrating the first direct evidence of transmission of obesity [106]. Significantly, F1 offspring had compromised gamete health, with increased oxidative stress noted in the sperm of male offspring and changes to oocyte mitochondrial function in female offspring [106]. Taken together these data suggest that paternal obesity at the time of conception has a clear effect on offspring health, implicating the sperm as the mediator for these changes [61].

13.4.2 Mechanism

13.4.2.1 Male Obesity and Altered Hormone Profiles

Examining the effect of obesity on spermatogenesis can be explained by the hypothesis that the hypothalamic–pituitary–gonadal (HPG) axis is deregulated by obesity.

Several studies document that increased male BMI is associated with reduced plasma concentrations of SHBG and therefore testosterone and an associated increased plasma concentration of estrogen [62,65,69,71,75–77,79,96,107]. Decreased testosterone and increased estrogen have long been associated with subfertility and reduced sperm numbers by interfering with the negative feedback loop of the HPG axis [108]. The adhesion of Sertoli cells to the developing germ cells is dependent on testosterone, with decreased testosterone leading to retention and phagocytosis of mature spermatids and therefore reducing sperm counts [109,110]. Other hormones involved in the regulation of Sertoli cell function and spermatogenesis, such as follicle-stimulating hormone (FSH)/luteinizing hormone (LH) ratios and inhibin B and SHBG levels, have all been observed to be decreased in males with increased BMI [111–113]. Studies on mouse knockout models demonstrated both loss of FSH or FSH receptor associated with decreased testis weight and sperm output associated with a reduction in Sertoli cell numbers [114,115]. Therefore, it can be hypothesized that the decreased sperm counts observed in male obesity are at least in part a result of changes to the HPG axis through testosterone and estrogen and likely reduced Sertoli cell function [61].

13.4.2.2 Obesity, Metabolic Syndrome, and Fertility

High circulating levels of insulin are suggested as one possible mechanism for the aforementioned effects, with increased insulin reducing the production of SHBG in the liver and thereby indirectly increasing the amount of active unbound estrogens and testosterone (not bound by SHBG) in the blood stream [116]. Increasing levels of circulating glucose have also been shown to reduce the amount of LH released by the anterior pituitary in sheep [117,118] and therefore could contribute to the impaired HPG axis and altered sperm parameters seen in diabetic and overweight and obese men.

13.4.2.3 Interaction between Adipose Tissue and Hormonal Regulation

It has been suggested that elevated estrogen concentrations may result from an increased conversion of androgens to estrogens by white adipose tissue and therefore contribute to the increased

plasma estrogen levels observed [119–121]. Another key hormone produced by white adipose tissue is leptin, which plays an important role in the regulation of energy regulation [122,123]. Leptin targets receptors in the hypothalamus by counteracting the effects of neuropeptide Y. However, leptin receptors have recently been discovered in ovaries and testes, functioning to regulate the HPG axis [124–128]. Specifically, increased levels of leptin significantly decrease the production of testosterone from Leydig cells [129]. Taken together these findings suggest that elevated leptin levels commonly found in obese males [130] could alter the HPG axis, thus contributing to the decreased testosterone production observed.

13.4.2.4 Effects of Male Obesity on Molecular Aspects of Spermatogenesis

Studies have proposed epigenetic modifications to the sperm through changes to noncoding RNA content and methylation and acetylation status, which are changed in obese men [131,132]. Additional reports suggest that the proteomic profiles of sperm also differ between obese and nonobese men [83]. It is now becoming increasingly accepted that the environment that the founder generation is exposed to impacts the phenotype of subsequent generations, with the term "transgenerational epigenetic inheritance" coined to reflect this phenomenon [133,134]. Rodent models of male diet-induced obesity document impaired metabolic and reproductive phenotypes in F1 [105,106] offspring and therefore suggest a transgenerational effect [100].

13.4.3 Can the Effects of Paternal BMI Be Reversed?

There is some evidence that intake of selenium-enriched probiotics by obese rodents improves both their metabolic health and sperm parameters [135]. Recent studies of diet and exercise interventions in an obese mouse model have determined that sperm function correlates with the metabolic health of the individual [100]. Improvements in metabolic health—the restoration of plasma concentrations of glucose, insulin, and cholesterol to normal levels—result in improvements in sperm motility and morphology, associated with improvements in molecular composition such as reduction in oxidative stress and reduced DNA damage [100]. However, there is little information about the impact of diet/exercise intervention in obese men with regard to semen parameters in humans. One study of 43 obese men placed on a 14-week weight loss regime demonstrated significant improvements in both total sperm count and sperm morphology in men who lost the greatest amounts of weight [136]. However, a recent case report of three patients who underwent bariatric surgery to achieve drastic weight loss demonstrated minimal improvements 2 years after surgery [137]. However, this may have been due to a persistent pattern of nutritional imbalance after surgery. Overall, much research is required to determine the reversibility effects of nutritional/obesity-induced male subfertility.

13.5 SUPPLEMENTS IN THE FEMALE

13.5.1 Fatty Acids

In addition to healthy weight maintenance, there is emerging evidence that nutritional modifications may have an impact on fertility and IVF outcome. Studies have linked macronutrient input to fertility outcomes, with the level of carbohydrate and protein being liked to ovulatory fertility [138]. In addition, the type of fat (*trans* vs. monounsaturated) has also been linked to ovulatory dysfunction [139], and a recent study has found that a Mediterranean diet containing high levels of vegetable oil, fish, and legumes was associated with increased levels of folate and vitamin B_6 levels in blood and follicular fluid and was linked to increased chances of pregnancy after IVF [140,141].

Fatty acid intake has also been assessed in relation to fertility, as it has been suggested that certain fatty acids, such as *n*-3 long-chained polyunsaturated fatty acids (LC-PUFAs) may have a beneficial role in pregnancy [142], with increased levels of fatty acids being present in a Mediterranean diet. In addition, the consumption of LC-PUFAs has been linked to IVF outcomes, with increased levels

of LC-PUFA omega-3 α-linolenic acid and docosahexaenoic acid being linked to improved embryo morphology [143] and serum ratios of PUFAs (increased ratio of linoleic acid [LA]/α-linolenic acid [ALA]) being correlated to an increased chance of pregnancy following IVF. However, a high intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been linked to reduced E_2 response and the number of follicles after ovarian stimulation. In addition, one study in the mouse has shown that very high consumption of omega-3 has been linked to perturbed oocyte and embryo viability. The oocytes obtained from mice fed a diet that was very high in long-chain *n*-3 PUFAs had altered mitochondrial distribution and calcium homeostasis as well as increased level of ROS and after fertilization had decreased development to the blastocyst stage [144]. Therefore, although the evidence for fatty acid consumption may look promising, more studies are required to investigate whether manipulation of dietary macronutrients, such as PUFAs, may be of benefit to women planning to conceive either naturally or via IVF and if excess consumption or altered ratios of fatty acids may have a negative effect.

13.5.2 Micronutrients, Multivitamins

The correct balance of micronutrients is essential for maintaining optimal fertility and preventing fetal structural defects as well as susceptibility to disease later in life, with multivitamin consumption being linked to increased fertility and higher rates of cumulative conceptions in the general population [145,146]. In addition, micronutrient deficiencies are not uncommon in Western society, primarily as a result of the excessive food processing and preservation methods used [147]. Micronutrients such as folate, vitamins B_6 and B_{12}, homocystine, iodine, magnesium, and selenium have all been linked to fertility and IVF outcome; however, evidence-based recommendations are problematic because of methodological and statistical limitations in many of the studies [148].

Micronutrients play vital roles in multiple cellular processes such as DNA synthesis, membrane stabilization, protein synthesis, protection against oxidative stress, catalysis in enzymatic reactions, and nucleic acid metabolism [149], all of which are critical for the developing oocyte. Interestingly it has been demonstrated in animal studies that reduced levels of vitamins A, C, and D are associated with decreased fertility and in humans reduced antioxidant (glutathione peroxidase [GSH-Px]) levels have been found in the follicular fluid of women with unexplained infertility [150–152]; in addition, in a small cohort of patients with celiac disease, decreased serum zinc levels have been linked to infertility. The consumption of multivitamins accordingly has been investigated as a means of improving overall oocyte health in women undergoing IVF treatment. It has been demonstrated that consumption of a multivitamin/mineral supplement can decrease the levels of lipid peroxidation and significantly increase the levels of GSH-Px and vitamin C in follicular fluid [153]. In addition, zinc levels, which were also significantly lower in the control IVF group, were significantly increased in the follicular fluid of women consuming a multivitamin/mineral supplement. Because zinc serves as a cofactor in the metalloenzymes involved in DNA transcription, which is critical during oocyte development, it has been hypothesized that this increase may be important for the developing oocyte as well as for antioxidant defense mechanisms [149].

Multivitamin supplementation has also been used to improve outcomes after ovulation induction. A prospective randomized study showed that the cumulative clinical pregnancy rates were higher in women who consumed an adjuvant multiple micronutrient supplement compared to women who were on folate alone [147]. Therefore, although the evidence for consuming a multivitamin during fertility treatment is not completely conclusive, the demonstrated importance of folate consumption (as discussed in Chapter 4), coupled with the possible benefit of the other micronutrients often found in a multivitamin (vitamin B_6, vitamin C, iodine, selenium, and iron), means that consumption of a multivitamin is routinely recommended for all women undergoing infertility treatment; however, larger studies are required to confirm which factors in multivitamins are of the most benefit and what the optimum formulation should be.

13.5.3 Antioxidants

The paradox of aerobic metabolism is that the generation of energy is coupled with the production of ROS, which are known to damage multiple cellular processes if their levels are not controlled. Although under normal cellular circumstances antioxidant systems regulate the levels of ROS, in some pathologies ROS levels can become dysregulated, leading to oxidative stress within the cell. Strong evidence demonstrates that oxidative stress is a leading contributor to female infertility, with numerous infertility pathologies being associated with increased levels of oxidative stress such as endometriosis, fallopian tube damage, and ovulatory disorders [154–157], with the primary source of ROS being macrophages, parenchymal steroidogenic cells, and endothelial cells [158]. In addition, it has been suggested that controlled ovarian stimulation, as is used in IVF treatment, may stimulate the production of ROS and can further perturb the oxidant–antioxidant balance in the follicle [159,160]. Follicular fluid is rich in antioxidants such as superoxide dismutase (SOD), glutathione (peroxidase, transferase, and reductase), and catalase, which protect the developing oocyte from exposure to ROS and oxidative stress [161]. Studies have evaluated the relationship between follicular fluid total antioxidant capacity (TAC) and ovarian hormone levels and found a positive association between TAC and hormone production within the follicle, in particular estrogen. Therefore, it has been hypothesized that the presence of oxidative stress may affect the local production of hormones from granulosa cells, which in turn may affect ovarian response and oocyte quality [162]. In addition, the level of specific antioxidants can impact the developing oocyte—for example, decreased levels of GSH-Px in follicular fluid, as seen in women who smoke, is linked to decreased fertilization rates after IVF [152]. In addition, the presence of oxidative stress in follicular fluid is also linked to poor oocyte quality, decreased embryo development, and decreased pregnancy rates [151,163–166]. This outcome has also been seen in knockout mice models, with decreases in follicular fluid Cu-Zn-SOD being linked to decreased litter size and number compared to wild-type controls [167,168].

Interestingly, it has also been demonstrated that ROS can be a positive predictor of IVF outcomes. In one study women who became pregnant had significantly higher ROS levels than those who did not become pregnant [169], and therefore it has been proposed that a minimum threshold of oxidative stress may be required for conception to occur [170]. Therefore, it is likely that ROS and antioxidant levels must be in balance, with skewing either way having a possible detrimental effect on IVF outcome [171].

Because of the impact that oxidative stress can have on fertility, it has been proposed that antioxidant therapy may be able to offset these oxidative imbalances and improve oocyte and embryo quality and therefore IVF outcome. Antioxidants come in a variety of forms, for example, zinc, selenium, *N*-acetylcysteine, pentoxyfilline, melatonin, L-arginine, vitamin C (ascorbic acid), vitamin E, and *myo*-inositol, and can be used alone or in combination with or without other vitamins and minerals, which adds a further layer of complexity.

Animal models have been used to investigate the effect of specific antioxidant supplementation directly on oocyte and embryo quality through addition to the culture media. In the bovine model, supplementation of culture media with SOD significantly improved embryo development compared to controls by decreasing the ROS that was induced by a high glucose concentration [172]. In mice, addition of vitamin C and vitamin E to culture media that contained ROS (from exposure of the media to phorbol-12-myristate-13-acetate-activated leukocytes) significantly improved embryo and blastocyst development compared to media without antioxidant supplementation [173]. In addition, mouse follicles exposed to vitamin C in culture media showed increased longevity when compared to those cultured without an antioxidant, likely owing to an increase in tissue inhibitor metalloproteinases [174].

In humans, systemic supplementation with vitamin C has been investigated as an antioxidant in infertile patients and has been shown to improve ovulation rates in women undergoing ovulation induction with clomiphene [175]; in IVF, oral supplementation with vitamin C was reported

to significantly increase pregnancy rates in nonsmoking women compared to those not consuming any supplements [176]. Nonetheless, a recent Cochrane review of 28 trials assessing the impact of antioxidants on IVF outcome has concluded that antioxidant supplementation is not associated with an increase in clinical pregnancy or live birth rate compared to placebo or no treatment, and it was noted that overall the quality of evidence from antioxidant supplement trials is very low because of poor reporting of outcomes and small sample sizes [177]. Therefore, it can be concluded that although there is a substantial rationale for the use of antioxidants during IVF, current data on what type to use and at what dose are lacking and further research needs to be undertaken to assess benefit/harm of antioxidant supplementation for infertile women undergoing IVF treatment.

13.6 SUPPLEMENTS IN THE MALE

13.6.1 Antioxidants

The male reproductive tract, including the epididymal and seminal plasmas, contains a powerful group of enzymatic and nonenzymatic antioxidant molecules that act to protect spermatozoa against oxygen metabolites. The scavenger enzymes SOD, catalase, and GSH-Px in semen are part of the first-line defense against ROS. A recent example of the importance of such enzymes was demonstrated by deletion of GSH-Px5 in male mice, which was found to generate a state of oxidative stress that influenced the incidence of miscarriage and birth defects in mated wild-type female mice, thus demonstrating the protection that this enzyme normally affords [178]. Other important defense antioxidant molecules include ROS scavengers such as vitamins C and E. Several observational studies, involving semen samples obtained from subfertile/infertile men, show low concentrations of these molecules relative to those in semen samples obtained from fertile men [179,180].

The 2011 Cochrane review included 34 trials with 2876 couples in total [181]. Men taking oral antioxidants had an associated statistically significant increase in live birth rate when compared with controls. This result was based on 20 live births from a total of 214 couples in only three studies. Further, there were 96 pregnancies in 15 trials including 964 couples. Antioxidant use was associated with a statistically significant increased pregnancy rate compared to controls [181].

A recent review also gave a detailed account of some 17 studies selected strictly on the basis of randomization, with unselected infertile men as the target population taking oral antioxidants including vitamin C, vitamin E, zinc, selenium, folic acid, carnitine, astaxanthin, and *N*-acetylcysteine [182]. Of the 17 studies, 13 reported improvement in at least one semen variable after a varied regimen of oral antioxidant therapy. The review also found that 6 of 10 studies reported improved pregnancy rates, and all of the studies (7 in total) showed a marked reduction of oxidative stress and/or DNA damage.

For a detailed analysis on the impact of antioxidants on paternal ART outcomes refer to Chapter 10.

13.7 EMERGING SUPPLEMENTS FOR FEMALE INFERTILITY TREATMENT

In Western society the increasing use of IVF is coupled with an increasing use of complementary medicine [183–185]. Studies in Australia have demonstrated that 66% of patients use some form of complementary therapy during IVF treatment, with 29% using remedies other than a multivitamin (such as herbal medicine or mineral supplements) [185]. The supplements consumed are highly variable and include chamomile, Echinacea, and chaste tree berry as well as antioxidants such as vitamins A, C, or E. In addition, other supplements are being promoted heavily on fertility forums such as coenzyme Q_{10} and royal jelly, both of which are deemed to improve fertility and IVF outcome. In addition, patients may be consuming supplements for other medical reasons, such as glucosamine for joint pain management. In addition, there are no data on the possible

interactions between different supplements and also the correct dosage required. Many patients do not inform their treating clinician about which complementary medicines they are using, as they believe them to be "natural" and therefore should not have a negative impact on their IVF outcome. This is concerning, as some supplements may have a negative impact on ART, for example, chaste tree berry (*Vitex agnus castus*) has been demonstrated to have anti-estrogenic and luteotrophic properties [186], and a case report has been linked to disordered serum gonadotropin levels and the onset of mild ovarian hyperstimulation [187]. As a result, there is a crucial need for more research into the effect of complementary medicine on fertility, including IVF outcome, including both animal studies as well as clinical trials, as currently the studies in this area are limited.

13.7.1 Coenzyme Q_{10}

As a result of the evidence linking increasing maternal age to a loss of mitochondrial function (primarily due to accumulation of mitochondrial DNA mutations and base pair deletions), it has been proposed that the consumption of mitochondrial nutrients may assist in reversing some of these age-related perturbations to mitochondrial metabolism [188,189]. In particular, it has been demonstrated that maternal aging is associated with decreased mitochondrial metabolism within the oocyte [190]. Because multiple cellular processes within the oocyte are energy dependent, such as chromosome segregation, it has been suggested that the increased rate of aneuploidy seen in oocytes obtained from women of advanced maternal age may be due to decreased mitochondrial metabolism [191]. In addition, as the ATP content of the oocyte and embryo has been linked to implantation potential as well as correct spindle formation and chromosome distribution, it is reasonable to suggest that supplementation of mitochondrial nutrients may improve mitochondrial metabolism, leading to correct chromosome segregation and increased embryo development and implantation [192].

Coenzyme Q_{10} (CoQ_{10}), or ubiquinone, is a naturally occurring lipid-soluble antioxidant; its main function occurs within the inner mitochondrial membrane, where it is responsible for electron transfer between complex I and complex II to complex III, thereby stimulating oxygen consumption and ATP production [193,194]. In addition, CoQ_{10} also acts as a potent antioxidant and is present in higher levels in cells than other lipid-soluble antioxidants such as vitamin E. Within the cell CoQ_{10} is present mainly in its reduced form (ubiquinol) and is therefore able to oxidize free oxygen radicles [195]. In animal models it has been demonstrated that cellular CoQ_{10} levels either diminish or lose biosynthetic activity in an age- and tissue-dependent manner [196–199] and therefore may impact on mitochondrial function and ATP output as well as oxidative stress. It has been demonstrated that rates of mitochondrial complex I and II oxygen consumption in the brain are significantly lower in aged mice than in young mice and that this effect can be reversed by administering exogenous CoQ_{10} through drinking water [199]. Also a moderate deficiency of CoQ_{10} (as seen in aged tissue) has been linked to an increase in ROS production coupled with increased lipid peroxidation and cell death [200]. Oral administration of CoQ_{10} has been used to improve mitochondrial function in diseases such as hypertension or congestive heart failure [201,202].

In regard to fertility, it has been shown that the levels of enzymes associated with CoQ_{10} production in cumulus cells from aged mice are significantly decreased and that treatment with CoQ_{10} resulted in a significant increase in the number of ovulated oocytes with restored citrate/ATP ratio and increased litter size compared to aged controls [203]. Interestingly, as it has been demonstrated that only approximately 6% of orally administered CoQ_{10} permeates through the gastrointestinal tract and into the blood and that CoQ_{10} uptake in many tissues is low or completely absent unless levels have fallen below a critical threshold [195], this supplement may be of benefit only in the aged population. At this time there have been no published studies investigating the effect of oral supplementation of CoQ_{10} on human oocyte or embryo quality or IVF outcome, although clinical trials are underway (ongoing clinical trial: NCT01048385).

13.7.2 Royal Jelly

Royal jelly is a viscous substance produced by worker honeybees that is composed of a complex array of carbohydrates, lipids, fatty acids, sugars, and proteins [204]. Interestingly, it is the substance that determines honeybee phenotype, as the larvae that consume royal jelly in large quantities develop the queen bee phenotype (as defined by increased body weight and reproductive ability) compared to that of the worker bee, which is much smaller and unable to bear offspring [205]. This difference is induced by epigenetic changes, specifically DNA methylation and histone deacetylation, that occur during early bee development and are mediated by factors found in royal jelly.

Royal jelly has been used to counteract disease states primarily as a result of its anti-inflammatory, immunomodulatory, and antioxidant capacity [206,207]. Using animal models, it has been shown that consumption of royal jelly can offset the effects of chemotherapeutic agents and decrease corresponding ROS levels induced by cisplatin [208] as well as prevent neurodegeneration secondary to spinal cord damage through increased expression of antioxidant genes [209]. In addition, royal jelly also has steroid hormone activity and can alter testosterone and estrogen levels [207,210]. Using an ovariectomized rat model it was shown that royal jelly consumption was associated with increased bone mineral density, similar to what was seen when ovariectomized rats were treated with 17β-estradiol [207]. In addition, in humans it has also been shown that royal jelly has estrogenic activity via interaction and modulation of estrogen receptors (ERα and ERβ) followed by changes to endogenous gene expression, and has therefore been considered for use in menopausal women as an alternative to hormone replacement therapy [210,211].

To date there have been no studies assessing the effects of royal jelly on female fertility in either animal models or humans despite it being promoted as a fertility enhancing supplement. Because of the epigenetic and steroid hormone activities of royal jelly, these studies are important so that clinicians can advise patients on the safety of consuming this product during fertility treatment. It is particularly important to ensure that it does not interfere with stimulation regimes and also does not affect the critical epigenetic events that occur during oocyte and embryo development that can have lifelong consequences for the offspring.

13.7.3 Glucosamine

Glucosamine is a ubiquitous amino sugar that plays a key role in cartilage formation and repair of connective tissue. It is therefore often consumed to assist with joint pain management, and although primarily marketed toward people with osteoarthritis it can also be used by athletes and people of reproductive age who exercise heavily.

Although there are no studies that have assessed the effect of glucosamine consumption on human female fertility, studies in mouse, bovine, and porcine models demonstrated that exposure of oocytes to glucosamine during *in vitro* maturation (IVM) results in significantly decreased blastocyst development after fertilization [212–214]. This perturbation in embryo development was observed in a dose-dependent manner and is believed to be due to up-regulation of hexosamine biosynthesis, resulting in increased levels of *O*-linked glycosylation and altered cellular signaling. Of greater concern, however, is a recent study that has linked glucosamine supplementation during the peri-conception period to altered pregnancy outcomes in the mouse. Utilizing a mouse model it was reported that glucosamine supplementation decreased implantation rate as well as the number of viable fetuses [215]. It was also reported that glucosamine is teratogenic in a mouse model, with the effect of glucosamine supplementation being influenced by maternal age. However, the reason for this is not currently understood and may be due to the changes in body composition and metabolism (including altered glucose homeostasis) that occur with aging and the influence of this on the metabolism and action of glucosamine though the hexosamine biosynthesis pathway [215]. Therefore, although there are no studies available in humans, these preliminary animal data

have caused great concern regarding the consumption of glucosamine while trying to conceive, and therefore its use during fertility treatment should be approached with caution, especially in patients of advanced maternal age.

13.8 EMERGING SUPPLEMENTS FOR MALE INFERTILITY TREATMENT

13.8.1 Royal Jelly

A small number of studies have recently examined the effect of royal jelly on male fertility parameters. One study was designed to investigate the efficacy of treating adult male rats with royal jelly (1 g/kg body weight orally in drinking water) for 1 month on sexual efficiency and glutathione and malondialdehyde testis tissue levels. Treatment of adult male rats with royal jelly produced a significant increase in the weights of testis and body of epididymis, sperm count, testosterone, the percentage of live sperm, and glutathione level and returned to control values, accompanied with a significant decrease in malondialdehyde level and the percentage of sperm abnormalities. The authors conclude that from this study that royal jelly is beneficial in terms of treating male adult rats receiving hydrogen peroxide, especially on sperm count, testosterone level, the percentage of live sperm, and improvement of glutathione and malondialdehyde testis tissue levels [216].

In another study, royal jelly treatments significantly boosted testosterone levels, increased ejaculated volume, increased seminal plasma fructose, improved sperm motility, and reduced abnormal sperm compared to the heat-stressed control animals. It was concluded that royal jelly administration to heat-stressed male rabbits can counteract their "summer infertility" and improve their physiological status [217].

In another study of 83 infertile men, 22 were treated with 100 mg of royal jelly, 21 with 50 mg of royal jelly, 20 with 25 mg of royal jelly, and 20 with pure honey. After 3 months of treatment with royal jelly, sperm active motility, testosterone level, luteinizing hormone level, sluggishly motile sperm, and intercourse per week increased significantly in infertile men, while sperm count and FSH level did not increase significantly [218].

Further randomized controlled trials are required to determine the efficacy of using royal jelly for male fertility.

13.8.2 Glucosamine

Direct evidence in relation to the effects of glucosamine on male fertility is currently lacking. However, there are some *in vitro* studies indicating that glucosamine may have an impact on sperm–oocyte interaction.

In one study [219] the effect of adding *N*-acetylglucosamine (GlcNAc) to bovine IVF medium has been examined. In medium in which sperm and a zona pellucida (ZP) were coincubated with monosaccharides for 5 min, the addition of GlcNAc (5 or 25 mM) significantly reduced the number of sperm that attached to the ZP. Pretreatment of gametes with GlcNAc (5 mM) before coincubation also suppressed sperm–ZP attachment. Addition of GlcNAc (5 or 25 mM) to the medium in which sperm and a ZP were coincubated for 5 h, however, significantly increased the number of sperm binding to and penetrating the ZP in a concentration-related manner. Supplementation of the sperm–oocyte coincubation medium with 5 mM GlcNAc also enhanced the rate of polyspermic fertilization. When the ZPs were removed from the oocytes, GlcNAc did not affect the fertilization rate. Furthermore, incubation of sperm with 5 mM GlcNAc induced sperm membrane destabilization and an acrosome reaction, as evidenced by the hypo-osmotic swelling test and fluorescein isothiocyanate-labeled peanut agglutinin/propidium iodide (FITC-PNA/PI) staining. Finally, GlcNAc suppressed ZP hardening after fertilization, as determined by measuring the time required for pronase to dissolve the ZP. In conclusion, supplementation of IVF medium with GlcNAc has various effects on sperm–oocyte interactions including suppression of initial attachment, induction

of sperm membrane destabilization and acrosome reaction, increase in the number of sperm secondarily bound to and penetrating the ZP, suppression of ZP hardening after sperm–oocyte coincubation, and increase in the rate of polyspermic fertilization [219]. Similar studies conducted in the hamster demonstrated that GlcNAc (1 mM) reduced sperm ability to bind to the zona pellucida [220]. Surprisingly, a spontaneous acrosome reaction was also inhibited by this sugar. IVF assays were also conducted to analyze the participation of GlcNAc in the different steps of sperm–oocyte interaction. When GlcNAc (1 mM) was present during the entire IVF process, a specific and significant inhibition in the proportion of penetrated oocytes (92 ± 8%) was found. Results indicated that GlcNAc inhibition was significantly greater for penetration than for binding [220]. Further studies are required to determine the efficacy of the use of glucosamine on male reproductive function, including randomized controlled trials in humans.

13.9 KEY POINTS SUMMARY

1. One in six couples will require assisted reproductive technologies (ARTs) to help them conceive. As both macronutrient intake (and its related obesity) and consumption of micronutrients (through diet or supplements) have been shown to affect reproductive outcomes in both human studies and relevant animal models, it is important to understand which aspects of nutrition can impact on ART outcomes.
2. Obesity has been shown to negatively impact women's response to gonadotropin (follicle-stimulating hormone [FSH]) stimulation, with obese women's ovaries exhibiting resistance to controlled ovarian hyperstimulation and requiring higher dosages of gonadotropin to produce the same numerical oocyte response compared to lean women. Furthermore, the quality of oocytes collected from obese women appears to be impaired, with some studies suggesting that obese women have reduced implantation rates and higher miscarriage rates than lean women; this reproductive deficit is reversed when oocytes from a lean oocyte donor are used in the obese recipient. Although the exact reason for this reduction in oocyte quality is not fully understood, obesity has been linked with significant metabolic changes in the follicle microenvironment (increased levels of glucose, insulin, lactate, triglycerides, and leptin). It is proposed that this altered environment may negatively impact oocyte developmental competence. Animal studies have shown that obesity alters oocyte mitochondrial energy production and raises reactive oxygen species (ROS) levels, leading to potential oxidative stress, DNA damage, and impaired meiotic spindle formation and resultant oocyte aneuploidy.
3. Several studies have reported that even modest weight loss in obese women can improve the chances of natural conception, plus reduce the risk of miscarriage in both *in vitro* fertilization (IVF) and naturally conceived pregnancies. However, women should be instructed to avoid "crash" very-low-calorie diets around the time of IVF, as these may alter body biochemistry (ketosis) and result in a reduction in IVF response, fertilization rate, and pregnancy rates. Gradual weight loss through a combination of healthy eating and exercise is most likely to optimize IVF outcomes in obese women.
4. Obesity in the male has been shown by the majority of studies to reduce sperm concentration, with inconsistent results linking increased body mass index (BMI) with changes in sperm motility and morphology. Although obesity does not appear to impact male accessory gland function, one study has linked obesity with impaired epididymal function (α-glucosidase assessed). Obesity has been positively correlated with an increase in sperm DNA fragmentation and epigenetic modifications (DNA methylation/acetylation), potentially negatively impacting on embryo development and the health of the next generation.

5. Male obesity appears to negatively affect several steps in the reproductive process, even when controlling for maternal BMI. First, both human and animal studies suggest impaired sperm binding to oocytes with resultant diminished fertilization rates compared to lean controls. Although early cleavage stage embryo development is not impaired in embryos derived from obese fathers, the proportion of embryos that progress to good quality blastocysts is reduced. As a result, several studies have suggested a decrease in clinical pregnancy rates in couples in which the male partner is obese. Finally, obesity-related DNA damage or epigenetic modification has been suggested to have an impact on the health of the next generation. Animal studies have shown that paternal obesity can result in obesity and insulin resistance in female progeny and oxidative stress-related male subfertility in the male offspring.
6. The mechanisms by which obesity impairs male reproductive function include a reduction in testosterone production mediated by a direct negative effect of leptin on Leydig cells, and the negative feedback of increased serum estrogen (from aromatization of androgens to estrogen in fat tissue) and resultant decreased pituitary luteinizing hormone (LH)/FSH drive for sperm and testosterone production. Although animal models have shown that weight reduction through diet and exercise can reverse these reproductive impairments (increase sperm and testosterone production), human studies supporting this observation are scant and contradictory.
7. Surveys suggest that micronutrient supplement use in men and women undergoing infertility treatment is very common. Some of the more commonly used supplements suggested to improve fertility by the lay press and some health practitioners, often with little scientific backing, include chaste tree, antioxidants, coenzyme Q_{10}, and royal jelly. Other supplements often taken for purposes other than fertility by the reproductive age population include the anti-inflammatory/arthritis agent's fish oil and glucosamine.
8. The consumption of chaste tree berry by women undergoing IVF should be discouraged because of its anti-estrogenic and luteotrophic properties. A high intake of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil has been linked with reduced response to IVF stimulation (oocyte number and estrogen levels), plus perturbed oocyte and embryo quality. These observations, plus the fact that fish oil may have anticlotting action, increasing operative bleeding risk, suggests that fish oil should not be consumed during IVF treatment. Antioxidants such as vitamins C and E, zinc, and selenium may be beneficial for ART outcomes, as the presence of oxidative stress in follicular fluid has been linked with poor oocyte and embryo quality and decreased pregnancy rates, while high total antioxidant capacity (TAC) in follicular fluid has been positively correlated with estrogen production. However, despite these observations, a recent meta-analysis of 28 studies has shown no female fertility advantage from the consumption of antioxidant supplements. Coenzyme Q_{10} (CoQ_{10}) is an antioxidant that is known to boost mitochondrial energy production, theoretically assisting correct meiotic separation of the chromosomes in the energy-depleted older oocyte. Although animal studies suggest that CoQ_{10} may increase successful conception in the aged mouse model, no studies have confirmed that this beneficial effect extends to the aged human oocyte. Interestingly, despite a total lack of human or animal studies suggesting a fertility promoting effect of royal jelly, this natural bee supplement is being promoted as an aid to female fertility by many practitioners. Royal jelly has estrogenic and epigenetic effects and therefore its use should be discouraged until studies supporting both its efficacy and safety in aiding female fertility are completed. Finally, the commonly used osteoarthritis supplement glucosamine should be avoided during ART treatment, as it has been shown to reduce blastocyst formation rates and to be teratogenic in animal models.

9. The ingestion of antioxidant nutritional supplements appears to be of some value in improving male fertility. A recent review found that antioxidant supplements improved sperm quality in the majority of studies, reduced oxidative damage and DNA damage in all studies, and resulted in an improvement in pregnancy rates in the majority of studies. Furthermore, a Cochrane meta-analysis observed a statistical improvement in pregnancy rates in women whose partners took antioxidants during IVF treatment. Although animal studies and small nonrandomized human studies suggest royal jelly may boost testosterone production and sperm quality, further randomized controlled trials are needed before advocating this type of nutritional supplement for men. Finally, glucosamine should also be avoided by men hoping to become fathers, as preliminary studies suggest that it may impair sperm–oocyte interaction and fertilization.

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14 Naturopathic Treatments for Infertility and Reproductive Disorders

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CONTENTS

14.1 INTRODUCTION

In this chapter the authors have endeavored to be comprehensive in covering the nutritional, lifestyle, and environmental toxin causes of reproductive system disorders and natural ways to achieve or restore health. This does not mean we expect patients to be supplemented with all the important nutritional factors that are discussed in each condition. Rather, we expect the expert clinician to consider the totality of the patient. A nutritional deficiency rarely manifests only as infertility, for example. Rather, there will almost always be other signs of deficiency that will enable personalized interventions rather than "kitchen sink" generic interventions. The same is true of environmental toxin exposure. A good case history will help reveal the environmental toxin(s) to which an individual patient has been exposed. At times, however, the totality of the toxin exposure is the real problem, rather than any specific toxin. A patient eating only conventionally grown foods with chemical contaminants, or using health and beauty aids with high levels of phthalates and other chemicals or synthetic chemicals for cleaning in the home, will almost certainly sustain a high toxic load. Although some people are fortunate to have a physiology particularly adept at excreting toxins, the patients we see are those who typically are more susceptible to these toxic agents.

14.2 FEMALE INFERTILITY

14.2.1 Overview

A person's fertility is a reflection of his or her general health and well-being. As we are genetically constructed to pass on the best genetic information to the next generation, the most optimal ages for reproduction are believed to be 18–35 years for a female and 16–40 years for a male. Females are believed to contribute to 50% of infertility cases, as either the primary (30%) or combined (20%) factor.

Female infertility is defined by a lack of conception after 12 months of regular, unprotected intercourse (at least twice weekly) with the same male partner and in the absence of male causes. If after 12 months infertility is diagnosed, then intervention and treatment are recommended. It is advisable to initiate earlier intervention and treatment for women older than the age of 35 years. After this time, recommendations are that women receive a comprehensive review and discussion surrounding natural or assisted conception because of age-related concerns.

Causes of female infertility include ovulation disorders (40%), tubal factors (30%), endometriosis (15%), and other (approximately 10%) including uterine/cervical factors (>3%).

14.2.2 Naturopathic Perspective

14.2.2.1 *In Vitro* Fertilization and Naturopathy

Data from The National ART Surveillance System (NASS), an assisted reproductive technologies (ART) data reporting system supported by the Centers for Disease Control and Prevention in the

United States, indicate that ART treatments currently account for 1.2% of total US live births, 16% of US twins, and 38% of US triplet or higher order (triplet/+) live born infants [1]. As such, integrative infertility care is likely for some patients.

To ensure the safety of the patient, integrative infertility treatment should consider the following:

1. Before an *in vitro* fertilization (IVF) cycle, encourage a 3- to 4-month preconception program for both partners to ensure appropriate nutritional status and detoxification for the success of procedure.
2. Investigate thoroughly to eliminate any causative or contributing factors that have or could hinder the success of an IVF cycle.
3. Address and attenuate any preexisting health conditions during the initial three- or four-treatment window.
4. During an IVF cycle, primary health care management should be directed to the fertility specialist with open communication concerning naturopathic prescriptions. Dietary and lifestyle recommendations should be encouraged; however, nutritional and/or herbal medicine supplementation must consider potential negative interactions with IVF medications.
5. Once IVF has concluded and a successful outcome attained, treatment can be modified to support the pregnancy, focusing on miscarriage prevention in the first trimester and then adjusting as the pregnancy continues (by trimester).

Naturopathic treatment cannot address all variables such as genetic factors or overt physical impediments; however, it can attenuate various presentations and factors. In the clinician's initial assessment, the prime objective is to assess fairly and holistically. As such, consideration should be given to the patient's previous paternity, duration of infertility, age of partners, severity of present pathology, and individual factors. For example, if the clinician is presented with a 41-year-old woman who has had no prior paternity, has been infertile for 2 years, and suffers from endometriosis it is crucial to initiate integrative care with an reproductive endocrinology and infertility (REI)/fertility specialist, as ART is likely to be required.

14.2.2.2 Natural Conception

A couple's fertility is generally highest in the first few months of unprotected sex and declines gradually thereafter in the population as a whole. If no conception occurs within the first 3 months, monthly fecundability decreases substantially among those who continue their efforts to conceive [2]. As such, couples who are disposed to conceive naturally are likely to do so in a few short cycles. Educating the couple about timing and ovulation detection is paramount to assist in successful conception.

14.2.3 Treatment

The naturopathic perspective of treating female infertility considers the holistic aspects described in the following subsections.

14.2.3.1 Preconception Care

Naturopathic preconception treatment encourages a preconception program that correlates with the final stages of gamete development (oocytes). Although women possess all of their oocytes at birth, there are limited treatment options available to remedy the primordial stage of development. As the primary follicle development from primordial follicle takes up to 120 days, preconception care treatment is best prescribed within this time period. Following this treatment approach, we can successfully influence additional stages of oocyte development (secondary -> antral -> Graafian). By influencing the oocyte environment during development and the correlating general health of the patient, practitioners are able to positively influence their development and address and attenuate related health concerns.

The principle of the preconception approach is to attenuate preexisting conditions; optimize nutritional repletion; detoxify the body from a dietary, lifestyle, and environmental perspective; and encourage a healthy approach to parenting on all levels. It is imperative to acknowledge that nutritional repletion is required throughout the preconception period and that prescriptions may require higher quantities than typical to optimize the status of the patient. Fertility occurs when a person's individual health is optimal. The body is primed to pass genetic material only when the environment and conditions are at their best. If survival from an evolutionary perspective is compromised, fertility will be hindered. Everything a person eats, drinks, experiences, or is exposed to can and will influence his or her fertility. True naturopathic fertility supports, acknowledges, and considers absolutely all variables for holistic health. Couples should be encouraged to participate in treatment for 3–4 months to properly address all genetic and epigenetic gamete variables.

14.2.3.2 Dietary Recommendations

14.2.3.2.1 General Dietary Approach

Information ascertained from the Nurses' Health Study II [3] has enabled the "fertility diet" to be determined. Key principles of dietary variables correlated with optimal fertility include: Lower intakes of *trans* fatty acids and greater intake of monounsaturated fats [4]; lower intake of animal protein and greater intake of vegetable protein [5]; higher intake of high fiber, low glycemic carbohydrates; preference for high-fat dairy products; higher dietary sources of nonheme iron (vegetarian sources); higher frequency of multivitamin use; more likely to consume coffee, tea, and alcohol; more likely to be physically active (vigorous activity 30 minutes or more daily—but not excessive exercise, as discussed later); less likely to be smokers; less likely to have long menstrual cycles; more likely to be recent users of the oral contraceptive pill; and stable weight control: body mass index (BMI) between 20 and 25 kg/m^2.

Note: In a review of the research, alcohol intake is conflictual. Although the aforementioned study showed a positive correlation, the authors are in no way advocating this practice. There is an inconsistent correlation between alcohol consumption and fertility. It appears to be most problematic in women with slow phase 2 acetylation; however, the authors recommend full cessation for the health of the individual and her future children.

Researchers concluded that these components of a "fertility diet" are conducive with the most successful outcome. Combining these approaches with body weight control and increased physical activity may help prevent the majority of infertility cases due to problems with ovulation.

14.2.3.2.2 Caffeine

Caffeine has been shown to increase time to conception [6–8]; and has been linked to endometriosis and an increased risk of spontaneous abortion [9]. As little as one caffeinated beverage per day is associated with a temporary reduction in conception in a number of studies [10,11]. There is a strong dose-dependent relationship—women who drink less than one cup of coffee per day are twice as likely to conceive compared with moderate coffee drinkers [7]. Coffee has been shown to increase dopamine production, which inhibits the production of prolactin. Because of this effect, it appears that caffeine increases early follicular estrogen levels [12].

14.2.3.2.3 Alcohol

There are strong associations with alcohol intake and miscarriage [13]. Excessive alcohol consumption is associated with hyperprolactinemia [14], and adverse effects on oocyte retrieval and lower chance of subsequent pregnancy in IVF [15]; a minimum of a 50% reduction in conception can be seen with intake as low as one drink per week during a menstrual cycle [16]. In addition, research suggests that alcohol produces negative effects on blastocyst development and implantation and increases estrogen levels, causing subsequent reduction of follicle-stimulating hormone (FSH) secretion, which suppresses folliculogenesis and leads to anovulation and infertility [17].

14.2.3.3 Nutritional Medicine

14.2.3.3.1 L-Arginine

Arginine acts as a precursor to nitric oxide synthesis, which is required for angiogenesis, embryogenesis, fertility (generally), and hormone secretion [18]. In addition, it is required for the replication of cells, making it essential in oocyte development and embryo formation. Studies attesting to its clinical benefit are limited, with one trial showing promising responses; however, study participation was low ($n = 17$) [19].

14.2.3.3.2 L-Carnitine

Similarly to its role in male reproductive function, L-carnitine assists female fertility by playing a vital role in fatty acid metabolism. It is derived from the amino acids lysine and/or methionine and works synergistically with coenzyme Q_{10} (CoQ_{10}). It is essential in the transport of fatty acids into the mitochondria, and deficiency results in a decrease in fatty acid concentrations in the mitochondria and reduced energy production.

14.2.3.3.3 Antioxidants

Oxidative stress has a detrimental effect on female fertility by increasing time to conception, decreasing fertilization rates, decreasing oocyte penetration function and viability, decreasing implantation, and increasing loss of implantation rates [20].

The oocyte has high requirements for regular antioxidant supply to continue to reduce oxidative damage both from current circumstances and historical (or transgenerational) exposure. During conception, the mRNA is responsible for using antioxidant reserves to address any DNA fragmentation or oxidation from both the male's sperm and the oocyte. The very act of conception and the subsequent travel of the embryo through the fallopian tubes before implantation generates free radical damage. As oxidative stress has been shown to hinder optimal fertility [21,22], it is imperative to provide sufficient support for the oocyte to achieve this magnificent feat.

14.2.3.3.4 α-Lipoic Acid

Both lipid and water soluble, α-lipoic acid is a powerful antioxidant found in virtually all cells of the human body that scavenges free radicals implicated in instances of oxidative stress. It assists in the chelation of heavy metals, minimizing the risk of cellular damage, and regenerates other antioxidants including vitamins C and E, CoQ_{10}, and glutathione [23].

14.2.3.3.5 Vitamin A

Vitamin A is a powerful antioxidant that assists in cellular growth and differentiation—qualities that are crucial for embryogenesis [24]—and is required for gene expression and cellular differentiation in organogenesis and embryonic development [25]. It is also required for immunity, regulatory functions, and epithelial tissue integrity and is necessary for the health of the cilia in the fallopian tubes. It has a direct interrelationship with zinc, and its deficiency has been linked with infertility, miscarriage, and the development of cleft palate. It is a cofactor of 3β-dehydrogenase in steroidogenesis (specifically for estrogen production) and deficiencies may result in impaired enzyme activity [26]. In addition, low concentrations of vitamin A are associated with anovulation [27], and plasma levels have been observed to be decreased in women suffering from habitual miscarriage [28].

14.2.3.3.6 Coenzyme Q_{10}

Coenzyme Q_{10}, also known as ubiquinone (ubidecarenone) or ubiquinol, is an endogenous enzyme that is involved in intracellular adenosine triphosphate (ATP) production and serves as a cofactor in oxidative stress respiration for the citric acid cycle and the electron transport chain [29]. It is a fat-soluble antioxidant and free radical scavenger found in every cell in the body and plays a vital role in

all energy-dependent processes. It is required in the maintenance of healthy cell membrane integrity and cell functioning and is therefore specifically important for all new cells in the body and the development of cells in their maturation process. Up to 600 mg/day has been used to improve oocyte quality and improve fertilization rates [30].

14.2.3.3.7 Vitamin C

Vitamin C is the body's primary water-soluble antioxidant. It is protective against free radicals, protects folic acid and vitamin E from oxidation, and has been shown to improve fertility outcomes in women with luteal phase defects [31]. The ovary is considered the primary site of ascorbic acid accumulation, as the mid-cycle change of retention and excretion of ascorbic acid is one of the main markers of ovulation. When reviewing the literature, vitamin C is seen to be crucial for collagen biosynthesis and its positive effect on growth and repair of the ovarian follicle and development of the corpus luteum [32] and has been suggested to assist with luteal steroidogenesis. It is necessary for the maturation of the preovulatory follicle and reduces the risk of preeclampsia when prescribed concurrently with vitamin E. Deficiency increases the risk of miscarriage and spontaneous rupture of membranes.

14.2.3.3.8 Vitamin E

Vitamin E has been shown to maintain the health of the ovaries [33]; it produces positive results for PMS sufferers including relief of affective and physical symptoms [34], relieves benign breast disease [35], and ameliorates menstrual migraine [36], indicating hormonal regulating properties. Low levels have been found in infertile women compared to controls, coupled with lipid peroxidation [37,38].

14.2.3.3.9 Selenium

Selenium is a powerful antioxidant that exhibits marked immune-modulating properties. Supplementation appears to reduce the risk of miscarriage, especially when associated with thyroid autoantibodies [39], specifically TPO antibodies [39–41]. Also called the 21st amino acid, it is essential in thyroid hormone synthesis because several of the enzymes involved are selenoproteins [42]. Selenium also plays a role in the immune system and in the coagulation system [40,43]. Low levels are associated with recurrent miscarriage compared to controls [43], and decreased serum selenium has been observed in women who suffered from miscarriage in the first trimester as well as those who suffered recurrent miscarriage [44].

14.2.3.3.10 Zinc

Zinc is one of the most important minerals for reproductive health (especially in men). It is involved in more than 200 enzyme pathways, including the enzymes required for the production of the nucleic acids in DNA and RNA [45]. It exerts specific antioxidant properties required for the reproductive system and protects oocytes from free radicals and reactive oxygen species (ROS) [46]. It enables reproduction, ovulation, fertilization, and the development of the oocyte [47,48]. Deficiency may result in altered synthesis/secretion of FSH or luteinizing hormone (LH) [49], as it has been reported to have a role in the synthesis, transport, and peripheral action of hormones. Low zinc status is associated with low circulating concentrations of several hormones [50].

Deficiency may produce symptoms such as abnormal ovarian development, increased risk of miscarriage [51], or increased risk of teratogenicity [51]. It is considered to be the single most important nutrient for preconception and pregnancy and is frequently disrupted from the oral contraceptive pill or copper intrauterine device (IUD) usage. In animal trials, low zinc levels are associated with impaired ovulation and an increased number of deteriorated ovocytes [47], whereby supplementation appears to be beneficial. Caution is advised, as excessive intake can be detrimental [52] and dose calculation based on laboratory results is required.

Poor maternal zinc status is association with a number of adverse pregnancy outcomes including low birth weight, premature delivery, labor and delivery complications, and congenital anomalies [53].

In addition, requirements increase during pregnancy, as marginal zinc deficiency has been shown to be prevalent during pregnancy [54]. Monitoring is crucial, as suboptimal levels are associated with growth retardation [54]. In one study, supplementation with 25 mg of zinc from at least 19 weeks of gestation resulted in a significantly larger birth weight [55]. In addition, a meta-analysis of supplementation trials indicated that a 14% reduction in premature delivery was producedamong zinc-supplemented women [56]. Unfortunately, zinc deficiency has become common as a result of modern agricultural methods. The zinc content for most foods has dropped 50% over the period of 1940–1991, and is likely even worse now [57].

14.2.3.3.11 B Group Vitamins

Deficiencies of this group of vitamins has been associated with a number of fertility problems including fetal abnormalities such as neural tube defects [58], neonatal or perinatal death, low birth weight, and miscarriage [59,60]. It is important to consider that alcohol, carbohydrate-rich diets, and the oral contraceptive pill increase the body's requirements for B vitamins.

14.2.3.3.11.1 Vitamin B_1 (Thiamine) Vitamin B_1 has been shown to stabilize the membranes of newly generated neuronal cells during embryogenesis concurrent with slowing cell death (animal model). In addition, it has been shown to be involved in the plasma membrane transformation of uterine epithelial cells during pregnancy [61]. It plays a key role in reproductive function, with deficiency associated with altered cellular differentiation and proliferation and interference with hormonal processes, increasing the risk of miscarriage [62]. In addition, in combination with B_3 and B_6, it has been shown to contribute to the prevention of oral facial cleft [63] when taken preconceptionally.

14.2.3.3.11.2 Vitamin B_2 (Riboflavin) Vitamin B_2 is crucial for energy production, antioxidant defense, and numerous enzyme systems that are reliant on the presence of B vitamins. Insufficient levels can lead to altered estrogen and progesterone levels, often causing irregular menstruation. Supplementation often addresses irregularities such as general menstrual difficulties, irregular menses, migraines, PMS, and infertility [64]. Low dietary intake has also been associated with low birth weight [65] and has been shown to increase the risk of congenital heart defects if insufficient in the periconception period [66].

14.2.3.3.11.3 Vitamin B_6 (Pyridoxine) Vitamin B_6 is required for metabolism of amino acids and lipids, pathways of gluconeogenesis, and synthesis of neurotransmitters during the periconception period [24]. It is required for the synthesis of prostaglandins and also plays a key role in conjunction with vitamins B_9 and B_{12} in their regulation of homocysteine, which when elevated has been linked to infertility and recurrent spontaneous miscarriage [67,68]. In addition, elevated homocysteine concentrations in follicular fluid are also associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction [69], further supporting the B vitamin combination.

It is also beneficial to reduce elevated prolactin levels; assist with luteal phase defects by increasing progesterone secretion; increase the influx of magnesium into the myometrium; improve absorption of zinc; and prevent preeclampsia, toxemia, and infarction of the placenta. Serum levels are often low in hyperemesis gravidarum, and deficiency may be associated with a higher incidence of gestational diabetes. It is a well-known treatment for morning sickness, with a positive Cochrane review [70] promoting its prescription; however, in a more recent Cochrane review [71] this was recanted. Clinical evidence prevails and both authors recommend it highly.

14.2.3.3.11.4 Vitamin B_9 (Folic Acid) Folic acid is required for healthy DNA and RNA synthesis, optimal protein synthesis, and the regulation of gene expression [72]. Deficiency can be corrected with supplementation and is recommended for at least 3 months for any woman planning pregnancy [73]. The primary justification for its preconception prescription is to prevent neural tube defects; however, it is required to be supplemented for the first 28 days of gestation to achieve this task [74].

Supplementation is associated with a decreased incidence of ovulatory infertility [3,75] and has been identified as being important for oocyte quality and maturation [48]. Inadequate folate results in elevated homocysteine and alters DNA methylation, negatively influencing oocyte and early embryo quality [76]. Inadequate B-vitamin status and raised concentrations of homocysteine are also associated with early loss of pregnancy, as evidenced by a systematic review that found that folate deficiency-induced hyperhomocysteinaemia were risk factors for placenta-mediated diseases, such as preeclampsia, spontaneous abortion, and placental abruption [77].

14.2.3.3.11.5 Vitamin B_{12} (Cyanocobalamin) Vitamin B_{12} deficiency has been linked to infertility and recurrent spontaneous miscarriage [78]. Pregnancy has been shown to occur after correction of the deficiency [78]. In addition, it is prudent to acknowledge the ccollaborative role of B_{12} in cases of elevated homocysteine levels.

It is common for women to take folate in isolation and not acknowledge its co-partner in all chemical reactions—vitamin B_{12}. Vitamins B_9 and B_{12} work together to ensure that the replication of DNA and RNA in all cells is uniform and regulated. In one longitudinal study, it was noted that although folate levels decreased slightly during pregnancy and remained decreased for up to 6 weeks post-delivery, vitamin B_{12} progressively declined during pregnancy and reached marginal or deficient levels in some individuals [79]. Clinicians are reminded that supplementation of either B_9 or B_{12} in isolation can cause a rebound anemia of the other nutrient and that pernicious anemia can be masked with supplementation of one in isolation, as evident on red cell indices. Furthermore, low maternal B_{12} levels have been associated with a 3-fold risk of neural tube defects [80], again confirming its combined role with folate.

14.2.3.3.12 Vitamin D

The active form of vitamin D, 1-25-dihydroxyvitamin D_3, has been shown to regulate the transcription and function of genes associated with placental invasion, normal implantation, and angiogenesis [81]. It is a powerful immune modulator, and as such is an effective prescription for those who experience autoimmune conditions that affect fertilization, implantation, and increased miscarriage risk. Maternal vitamin D deficiency has also been suggested as an independent risk factor for preeclampsia, and there are a number of animal studies that highlight that vitamin D deficiency is associated with infertility [82].

14.2.3.3.13 Calcium

Calcium is an important nutrient for conception, embryogenesis, and gestation. It is required for oocyte maturation, fertilization [83], and bone formation of both mother and child and has been shown to exhibit a protective effect against preeclampsia. A Cochrane review highlighted that calcium supplementation appeared to almost halve the risk of preeclampsia [84], which supports the prescription of calcium for all preconception women if dietary intake is inadequate [85].

14.2.3.3.14 Iodine

The maturing oocytes are highly dependent on healthy thyroid hormone levels for optimal reproductive function [86], and deficiency may result in a spectrum of disorders including miscarriage, stillbirth, mental retardation, and cretinism (deafmutism and spasticity) [87,88]. In addition, thyroid autoimmunity is significantly higher among infertile women than among fertile women and increases the miscarriage rate [89]. As such, iodine can assist to supply building blocks for thyroid hormones that are compromised by the autoimmune process.

Adequate dietary intake of iodine preconceptionally is imperative, as it can minimize the risk of thyroid-related disorders and/or mental retardation in the infant that is commonly experienced in deficient mothers. In addition, low iodine levels have been shown to negatively affect the development of the central nervous system in the fetus and can hinder conception because of its role as a nutrient donator for triiodothyronine (T_3) and thyroxine (T_4) to regulate thyroid function (and thus ovulation).

Subclinical hypothyroidism may be associated with ovulatory dysfunction and adverse pregnancy outcomes, and as such it is crucial to assess thyroid (and iodine) status for both males and females if infertile, as correction can reverse or prevent infertility [90]. Repeated miscarriage and stillbirth have been shown to be associated with increasing deficiency, increasing the risk of reproductive failure [91].

As early as 1939, Kemp [92] acknowledged a link between deficiency of iodine and stillbirth of unknown origin. In the late 1970s Potter et al. [93] discussed the decline in stillbirth rates after iodine supplementation, highlighting the positive relationship that ensued between iodine (when taken at the correct dose) and reduced incidence of stillbirth. Current research [94] indicates that that there are still 2000 babies who are stillborn in Australia every year. In spite of advances in technology, the cause is unknown in one third of these deaths.

14.2.3.3.15 Iron

Iron is required for the formation of red blood cells, subsequent transport of oxygen to the tissues via hemoglobin, and nucleic acid metabolism, as well as being involved in numerous enzyme systems within the body [24]. Deficiency is associated with ovulatory infertility [95,96].

14.2.3.3.16 Magnesium

Magnesium is involved in numerous reactions in the body relevant to fertility, including cell signaling and energy production. Females experiencing spontaneous abortion have been observed to have lower plasma magnesium levels than healthy controls [97], and those who are experiencing associated stress with their fertility will benefit from magnesium and B vitamins supplementation. Stress is associated with impaired fertility, including miscarriage and failed pregnancy outcomes in females [98].

14.2.3.3.17 Essential Fatty Acids

Women undergoing preconception care are encouraged to consume a diet rich in essential fatty acids (EFAs) [85], as omega-3 fatty acids are required to maintain the lipid bilayer in all cell membranes and as precursors for prostaglandin synthesis. Specifically in the prevention of miscarriage, omega-3 fatty acids (4 g/day providing 795 mg of docosahexaenoic acid [DHA] and 1190 mg of eicosapentaenoic acid [EPA]) have been found to improve uterine artery blood flow velocity in women with recurrent miscarriage due to impaired uterine perfusion [99].

Omega-3 fatty acid supplementation has been shown to improve endometrial function. *In vitro* studies of decidualized cells have identified a decrease in prostaglandin production by up to 80%, indicating that prescription is beneficial for all women, but most importantly those suffering from endometriosis or those with endometrial insufficiency [100]. In addition, supplementation is believed to assist in reducing spontaneous preterm birth associated with intrauterine inflammation [101].

14.2.3.3.18 Probiotics

Alterations in the microflora of the vagina and subsequent genital and intrauterine infections [102] have been linked to reproductive failure and adverse pregnancy outcomes such as preterm labor, miscarriage, and spontaneous preterm birth [102–104]. In one study during the first half of pregnancy, females with altered vaginal flora of bacterial origin were four times more likely to have a spontaneous preterm birth compared to the overall preterm birth rate [105].

14.2.3.4 Herbal Medicine

Table 14.1 lists herbal medicines for female infertility.

Note: Herbal medicine prescriptions should be closely monitored by the patient's naturopath/herbalist.

TABLE 14.1
Herbal Medicines for Female Infertility

Class	Action	Herbal Medicine Examples
Hormonal modulation	Increase reproductive capacity and support hormone cascades	*Actaea racemosa* (black cohosh) *Asparagus racemosa* (shatavari) *Chamaelirium luteum* (false unicorn root) *Vitex agnus castus* (chaste tree)
Female tonic	Tonify the female reproductive system, support hormone cascades, revitalizes, and enrich—increase libido	*Angelica sinensis* (dang gui) *Asparagus racemosa* (shatavari) *Chamaelirium luteum* (false unicorn root)
Ovulation stimulant	Improve ovulation and menstrual cycle stability	*Paeonia lactiflora* (white peony) *Tribulus terrestris* (tribulus) *Vitex agnus castus* (chaste tree)

14.2.3.5 Lifestyle Recommendations

14.2.3.5.1 Healthy Weight Balance

For optimal conception, women need to ensure that their body fat percentage is between 20% and 25% to support fertility and the subsequent health of their child. Both low and elevated BMI can be problematic. A body fat percentage of 17% can result in anovulation, with some research suggesting that it can take as long as 2 years before regular conception occurs even after body fat correction has taken place [106]. Conversely, obesity can negatively affect conception by reducing ovulation potential; reducing IVF success; and increasing miscarriage risk, risk of birth defects, pregnancy complications, stillbirth, and newborn weight issues [107].

14.2.3.5.2 Environmental Factors

Exposure to environmental toxins such as radiation, heavy metals, and chemicals can cause oxidative stress and damage to DNA with subsequent negative effects on female infertility. Similarly to male infertility and estrogens, environmental exposure risk is problematic. Additional considerations need to review the negative impact of beauty products used by women, such as makeup, fragrances, hair dyes, nail polishes, and others [108]. Most cosmetics contain phthalates for fragrance. These are known endocrine disrupters. One study found that phthalate concentrations are several times higher in infertile compared to fertile couples [109].

Gestational exposure to carcinogens, endocrine disruptors, and other toxins has been shown to affect more than one generation in some cases. Chemicals known to induce phenotypic effects in unexposed generations include alloxan [110], cyclophosphamide [111], orthoaminoasotoluol [112], benzpyrene [113], diethylstilbestrol (DES) [114], and vinclozolin [115]. In addition, phthalates are strongly linked to testicular dygenesis syndrome, with resulting cryptorchidism and infertility in the T_2 generation after the mother's exposure during pregnancy. For more information on this, please see the work by Skakkebaek et al. [116].

14.2.3.5.3 Shift Work and Sleep

Shift work is associated with negative effects on female fertility by impacting menstrual cycle regularity and reproductive function and increases the risk of adverse pregnancy outcome. In one study, 53% of women noted menstrual changes that may impact fertility [117].

14.2.3.5.4 Cigarette Smoking

Cigarette smoking has been shown to decrease ovarian vascularization and reduce oocyte maturation [118]; it is also linked with premature ovarian aging (premature menopause) and an increased

risk of miscarriage [119]. It appears to reduce fertility by having a direct effect on the uterus, oocytes, and embryos by increasing the thickness of the zona pellucida [120].

A Cochrane review found that active and passive smoking reduced fertility and decreased the chance of a healthy live birth in both fertile and infertile populations [121]. In addition, in a number of meta-analyses it has been proven that smokers perform poorly in IVF/ART procedures and that smoking compromises the outcome when compared with nonsmoking counterparts [122,123].

14.2.3.5.5 Marijuana

Cannabinoids have been shown to impair signaling pathways, alter hormonal regulation, and interfere with embryo implantation timing [124]. In addition, they are believed to cause ovulatory abnormalities and disrupt ovarian function [125] and have been shown to cause harmful effects on the developing fetus [126].

14.2.4 Fallopian Tube Scarring

Untreated or inadequately treated infectious pelvic inflammatory disease may result in scarring and subsequent blockage of the fallopian tubes. One study found that for secondary infertility: "Most common causes responsible for infertility were tubal occlusion, endometriosis, peritubal and periovarian adhesions" [127, p. 629]. Author JP found this common in his practice of natural childbirth and used with considerable success a protocol he developed. The protocol involved alternating hot and cold stiz baths one to three times per day; weekly insertion of the vaginal depletion pack developed by Dr. John Bastyr, DC, ND; and, of course, optimizing nutrition and decreasing toxic load in both partners. Within 1 week, the treated women would develop a milky discharge. This would continue for 3–6 months. When the discharge stopped, the blockage appeared to have cleared, according to physical examination (a scarred fallopian tube feels thick and knotty while an open tube is supple and smooth). More than a dozen previously infertile women—whose diagnosis had been confirmed with the dye-enhanced radiology of the time—became pregnant with this protocol. However, a major warning: birth control must be used until the blockage has been cleared. One woman developed an ectopic pregnancy. As the egg is larger than the sperm, this allows fertilization before there is enough patency to allow the fertilized egg to pass through the fallopian tubes into the uterus.

14.3 MALE INFERTILITY

14.3.1 Overview

Infertility affects approximately 7.3 million women and their partners in the United States each year, which equates to approximately 12% of the reproductive-age population [128]. One out of seven couples will experience difficulty conceiving. It affects men and women equally, with male factors responsible for approximately one third of cases, female factors for one third,and joint conception issues for the remaining one third.

Causes of male infertility are many and varied and include primary hypogonadism (30–40%); genetic causes; altered sperm transport (10–20%); secondary hypogonadism (1–2%); and developmental, physiological, immunological, and unknown causes (40–50%).

Numerous epidemiological studies in recent decades have documented a decline in male fertility. Many of these studies propose a link between the deterioration in fertility with growing exposure to environmental toxins such as anti-androgenic pesticides and fungicides (e.g., dichlorodiphenyltrichloroethane [DDT] and vinclozolin), plasticizers (e.g., bisphenol-A and dibutyl phthalate), water disinfection by-products (e.g., dibromoacetic acid), heavy metals (e.g., lead, cadmium, and mercury), and common industrial contaminants in drinking water (e.g., benzene, phenol, and trichloroethylene). From a review of the available literature, it is apparent that a variety of commonly used chemicals, now abundant in the environment, drinking water, and food chain, can have insidious

and long-lasting effects on the male reproductive system. The rising incidence of male infertility warrants a closer look at both preventable causes and potential solutions related to environmental and nutritional factors.

Sperm are produced by repeated divisions of cells in small-coiled tubules within the testes at an average rate of approximately 100 million per day in healthy young men. Each spermatogenesis cycle consists of six stages, and approximately five cycles are required to produce one mature sperm. From the beginning of division of the stem cell to the appearance of mature sperm in the semen it takes between 72 and 76 days. As such, anything that the male experiences during spermatogenesis can affect mature sperm regardless of the man's health at the time of examination. Factors to consider include illness, toxicity, trauma, nutritional status, and environmental exposure, among others.

14.3.2 Naturopathic Perspective

The naturopathic perspective of treating male infertility considers the holistic aspects described in the following subsections.

14.3.2.1 Preconception Care

When treating males for infertility, the optimal approach is to adopt a preconception program for both prospective parents. Preconception treatment adheres to the philosophy that the final stages of gamete production can be modified and influenced. Spermatogenesis takes between 72 and 76 days for full generation of sperm. By influencing the environment to which sperm are exposed and the general well-being of the patient, practitioners are able to positively influence their development and address and attenuate related health concerns.

As mentioned previously, it is always important to acknowledge that the most favorable fertility occurs when individual health is optimal. The body is primed to pass genetic material only when the environment and conditions are at their best. If survival from an evolutionary perspective is compromised, fertility will be hindered. Everything a person eats, drinks, experiences, or is exposed to can and will influence his or her fertility. True naturopathic fertility supports, acknowledges, and considers absolutely all variables for holistic health. Couples should be encouraged to participate in treatment for 3–4 months to properly address all genetic and epigenetic gamete variables.

14.3.2.2 Prevention

Avoid exposure to offending agents that are known to disrupt endocrine function and spermatogenesis:

Water supply: Ensure that the water supply for both the home and workplace is pure, and if required, install water filtration or purchase spring water to prevent exposure to heavy metals and chemicals.

Food supply: Reduce exposure to pesticides and fungicides on fruits and vegetables by consuming organic food produce or using a vegetable wash before consumption.

Reduce exposure to heavy metals: Make conscious decisions and awareness choices to reduce exposure to heavy metals through some vaccines, dental fillings, pharmacological agents, consumer products, certain fish and seafood, and other products.

Clean your home: Use nontoxic alternatives for household pesticide and cleaning products. Scrutinize personal care items to ensure that they are free from phthalates, bisphenol-A, heavy metals, and other harmful chemicals.

Avoid toxins in the kitchen: Limit exposure to plastics used in food preparation and storage. Discard scratched cookware with nonstick surfaces. Store food and beverages in glass instead of plastic containers.

Avoid exposure to toxins in the workplace: Toxins such as lead and fumes are commonly found in the workplace. It is vital that all men wear proper safety equipment and work in a safe environment that adheres to strict codes of practice to protect their workers.

14.3.2.3 Detoxification

General detoxification principles are recommended to support optimal male reproductive health. Detoxification of offending chemicals and heavy metals may involve a variety of therapies including nutritional supplementation, herbal medicines, infrared sauna, chelating agents (if indicated) such as ethylenediaminetetraacetic acid (EDTA, intravenous and oral) and dimercaptosuccinic acid (DMSA, oral), and lifestyle modifications.

14.3.2.4 Repletion

It is crucial to acknowledge and address the nutritional status of the male patient throughout the full spermatogenesis cycle. The concept of nutrient repletion is highly applicable within a fertility context. Optimal fertility is best achieved when prime health is realized. The repletion model indicates that prescriptions are required for a minimum of 3 months to properly address any deficiencies present and to ensure that all nutrients can be used within each required pathway within the body.

14.3.2.5 Holistic Perspective

It is crucial to naturally support and attenuate all coexisting health conditions. As such, treatment should be structured to acknowledge all health concerns that may be present to optimize the general health of the male patient and thus improve fertility outcome.

14.3.3 Treatment

14.3.3.1 Dietary Recommendations

14.3.3.1.1 Wholefood Dietary Principles

It is important to encourage wholefood dietary principles—eating foods as close to their natural state (unprocessed, unadulterated); avoiding chemicals, preservatives, additives, and colorings; encouraging organic food choices; and adopting healthy eating patterns generally. It is important that patients are educated about healthy food choices and encouraged to make optimal food choices that support detoxification and promote the generation of healthy sperm.

14.3.3.1.2 Alcohol Intake

Although a review of the research is inconclusive and limited, it is accepted that excessive alcohol consumption in men is strongly associated with diminished sperm function. Within the naturopathic framework we can deduce that alcohol consumption will impact liver health and thus detoxification; derange endocrinological balance, specifically estrogen pathways; provide high calorie load and contribute to obesity (especially abdominal adiposity); and others. Excessive alcohol consumption also depletes reduced glutathione, one of the key intracellular molecules for protection from environmental toxins and oxidative stress [129]. As such, it is imperative that men refrain from alcohol consumption in the preconception window to enable optimal functioning of the male reproductive system and subsequent positive effects on sperm parameters. In addition, binge drinking and regular heavy drinking must be avoided.

14.3.3.2 Nutritional Medicine

14.3.3.2.1 L-Carnitine

In the epididymis carnitine serves as an energy substrate for spermatozoa, enhancing transport of fatty acids into the mitochondria. It is believed to have protective antioxidant effects and provides energy to the testicles and spermatozoa specifically [130]. In a study involving 124 infertile patients, a direct correlation between semen carnitine content and sperm motility was found [131]. Several studies comparing fertile men to infertile men found that fertile men had a statistically significant larger amount of carnitine in their seminal sample than the infertile men, and that low levels of L-carnitine in the seminal plasma may be a potent marker for infertility [132–134]. In addition, other studies

have demonstrated improvements in sperm health parameters after administration of carnitine in the range of 3–4 g per day [135–140]. After ejaculation, the motility of sperm correlates directly with carnitine content—the higher the carnitine content, the more motile the sperm. Conversely, when carnitine levels are low, sperm development, function, and motility are drastically reduced [141].

14.3.3.2.2 L-Arginine

The amino acid arginine is a precursor in the synthesis of putrescine, spermidine, and spermine, which are thought to be essential in sperm motility. Via its role as a precursor to nitric oxide synthesis, arginine is required for angiogenesis, spermatogenesis, and hormone secretion [18]. A number of studies have shown that arginine can improve sperm count and motility [142–146]. It is required for the replication of cells, making it essential in sperm formation. Nitric oxide synthase uses L-arginine to synthesize nitric oxide, which can protect spermatozoa from lipid peroxidase damage [147].

Arginine supplementation is often, but not always, an effective treatment for male infertility. The critical determinant appears to be the level of oligospermia. If sperm counts are less than 20 million/mL, arginine supplementation is less likely to be of benefit. To be effective, the dosage of L-arginine must be at least 4 g/day for 3 months. In perhaps the most favorable study, 74% of 178 men with low sperm counts had significant improvements in sperm counts and motility after arginine therapy [145].

14.3.3.2.3 Essential Fatty Acids

Fats and oils impact agglutination and cell membrane dynamics. As such, it is important to review specific dietary fat sources to ensure that a positive effect on sperm health is achieved. Excessive consumption of saturated fats combined with inadequate intake of essential fatty acids, especially omega-3, changes the fatty acid composition of the sperm membranes, thus reducing fluidity and interfering with sperm motility.

Unsaturated fatty acids should be increased and *trans* fatty acids, and rancid or oxidized fats must be strictly avoided. The presence of essential fatty acids from unsaturated sources ensures sperm are kept fluid and flexible [148]. This regulates acrosome reaction, sperm–oocyte fusion, and sperm–oocyte fertilization.

Sperm are extremely sensitive to free radicals because they depend so much on the integrity and fluidity of their cell membrane for proper function. Without proper membrane fluidity, enzymes are activated, possibly leading to impaired motility, abnormal structure, loss of viability, and, ultimately, death of the sperm.

The major determinant of membrane fluidity is the concentration of polyunsaturated fatty acids, particularly omega-3 fatty acids such as DHA, which are very susceptible to free radical damage. Sperm have a relative lack of superoxide dismutase and catalase, which can prevent or repair oxidative damage. Adding to this more susceptible state is the fact that sperm generate high quantities of free radicals to help break down barriers to fertilization.

Sperm motility appears to have a direct relationship with sperm membrane DHA levels [149]. In one study, it was noted that excessive omega-6 compared to omega-3 in seminal plasma produced decreased sperm concentration, sperm motility, and sperm morphology among patients with idiopathic oligoasthenoteratozoospermia [150].

14.3.3.2.4 Antioxidants

The generation of ROS and associated links with infertility has been established and extensively studied, with a particular focus on the effects of increased ROS in the serum, semen, and testicular tissues of patients. Alterations in the testicular microenvironment and hemodynamics can increase production of ROS and/or decrease local antioxidant capacity, resulting in generation of excessive oxygen species (OS).

A Cochrane review [151] from 2011 assessed the impact of antioxidants and male subfertility by reviewing 34 trials and 2876 couples. It reached an important conclusion that there is sufficient evidence to warrant antioxidant supplementation in subfertile men to improve the outcome of live

births and pregnancy rates for subfertile couples undergoing ART cycles. In addition, it was noted that antioxidant use was associated with a statistically significant increased pregnancy rate compared to controls; subfertile men have lower levels of antioxidants in their semen compared to fertile men [152]; 1 in 20 males will be affected by subfertility and 3–80% of male factor subfertility is believed to be due to oxidative stress [152]; ROS levels are significantly higher in infertile sperm samples when compared to healthy controls; and ROS causes fertility problems by damaging the sperm membrane, which impacts sperm motility and the ability of spermatozoa to break down the zona pellucida and by altering sperm DNA. Of importance, the authors concluded that no studies reported evidence of harmful side effects of the antioxidant therapy used.

Free radical or oxidative damage to sperm is thought to be responsible for many cases of male infertility, high levels of free radicals being found in the semen of approximately 40% of infertile men [141,153,154]. Sperm are especially susceptible to damage by free radicals as a result of a high membrane concentration of polyunsaturated fatty acids, active generation of free radicals, and a lack of defensive enzymes. All of these factors combine to make the health of the sperm critically dependent on antioxidants. Men exposed to higher levels of free radicals sources are much more likely to have abnormal sperm and sperm counts [141,153,154]. Antioxidants are required to protect sperm against oxidative damage that may alter DNA as well as to instigate cellular repair of the harmful effects of factors such as environmental damage or aging. In the healthy male the seminal plasma is naturally rich in antioxidants to protect from this damage, as sperm are highly susceptible to the effects of ROS.

14.3.3.2.5 α-Lipoic Acid

In addition to its chelating functions, α-lipoic acid is able to regenerate other antioxidants including vitamins C and E, CoQ_{10}, and glutathione [23]. It is a powerful antioxidant for sperm in animal studies [155–157] and protects the sperm against free radical damage [157]. In animal studies it has been shown to improve sperm motility and viability, minimize DNA damage [157], and assist with energy supply to the sperm [157].

14.3.3.2.6 Vitamin A and β-Carotene

Low concentrations of vitamin A are associated with abnormal semen parameters [27] and intake of β-carotene is positively associated with a higher sperm concentration and improved sperm motility [158].

14.3.3.2.7 Vitamin C

Vitamin C (ascorbic acid), a major antioxidant present in extracellular fluid, is present at a high concentration in seminal fluid compared with blood plasma (364 vs. 40 mM) and is present in detectable amounts in sperm [159], where it prevents sperm agglutination and oxidative damage. In infertile men, vitamin C has been found in reduced quantity in the seminal plasma [160,161]. Males with inadequate seminal vitamin C have also been observed to suffer from sperm DNA damage [160], suggesting that that a defect or inadequate intake of vitamin C may initiate ROS to cause breakage and oxidation of sperm DNA.

A marginal deficiency causes oxidative damage to sperm, resulting in reduced sperm motility and viability and leading to infertility and increased damage to the sperm's genetic material [162]. Supplementation leads to improvement in both viability and motility, reduced numbers of abnormal sperm, and reduced sperm agglutination [163–165].

14.3.3.2.8 Vitamin E

Vitamin E supplementation appears to be especially warranted because it is the main antioxidant in various cell membranes, including those surrounding sperm. Free radicals, if left alone, lead to peroxidation of phospholipids in the mitochondria of the sperm, making the sperm immotile.

Vitamin E has been shown to play an essential role in inhibiting free radical damage to the unsaturated fatty acids of the sperm membrane [166] and to enhance the ability of sperm to fertilize

an egg in an IVF setting [167,168]. In addition, it has been shown to protect DNA within the sperm from damage [168] and improve sperm motility [169] and sperm quality [170].

14.3.3.2.9 Coenzyme Q_{10}

Coenzyme Q_{10} is found in seminal fluid and sperm [171], where it assists in optimal sperm motility [172,173]. Deceased levels have been found in the seminal plasma and spermatozoa of males with idiopathic and varicocele-associated asthenospermia [174].

It is concentrated in the head and mid-piece (neck) of the sperm. It is considered to be the most crucial and powerful antioxidant in sperm structure because of its role in mitochondrial energy release. It is believed to promote motility, foster sperm survival, and provide optimal energy to assist the sperm's travel on its journey to fertilize the oocyte.

14.3.3.2.10 Lycopene

Lycopene is found in high concentrations in the testes and seminal plasma, and reduced levels have been demonstrated in men with infertility. Supplementation has been shown to improve concentration, motility, and morphology of sperm [170].

14.3.3.2.11 Selenium

Selenium is a potent antioxidant that is essential for male fertility because of its role in testosterone synthesis, normal sperm maturation, and motility [175,176]. Clinical trials reveal it has the ability to increase sperm motility [177,178] and concentration [178] and assist in the production of healthy spermatozoa [178,179]. It is required structurally, as the sperm capsular selenoprotein is involved in the stability and motility of the mature sperm and also forms part of the glutathione peroxidase antioxidant system, which is paramount for spermatogenesis and protects the sperm against the effects of ROS [44].

In animal studies depletion of mitochondrial glutathione peroxidase has been found to cause impaired sperm quality and severe structural abnormalities in the mid-piece of spermatozoa, leading to infertility [180]. The tail of the sperm relies on adequate selenium status to maintain its "whip-like" action. Without sufficient selenium, sperm are unable to swim in the right direction or often can display marked immotility, thus preventing oocyte location and fusion.

14.3.3.2.12 Zinc

Zinc is found in high concentrations within the prostate and testes, and particularly high amounts are also found in the semen (approximately 2.5 mg of zinc is lost per ejaculate). It is involved in virtually every aspect of male reproduction, including hormone metabolism, spermatogenesis, and sperm motility [181]. Zinc deficiency is associated with decreased testosterone levels and reduced morphology [182]. An adequate amount of zinc ensures proper sperm motility and production, while deficient levels are often found in infertile men with diminished sperm count [72,183–187].

It plays an important role in all human living cells including the transcription of RNA, replication of DNA, as well as synthesis of protein, all of which are crucial for reproduction and fertility. In addition, it protects against free radical damage and ROS that may impair sperm [46]. Deficiency of zinc in males may lead to gonadal dysfunction [181] and has been observed to be associated with idiopathic male infertility [46] and impotence.

When considering sperm structure, zinc has been shown to influence motility and head–neck connection of the sperm [188]; it is important in the stabilization of cell membranes and sperm chromatin [189]. Finally, it has been shown to exert an antimicrobial effect on the seminal plasma, which is a positive application if sperm antibodies or underlying genitourinary infection is present [190].

14.3.3.2.13 Vitamin D_3

Vitamin D_3 has been found in the head and mid-piece (neck) of the sperm and is believed to be involved in protecting DNA with the head and assisting in movement of the sperm. In addition,

low serum levels are correlated with increased intracellular calcium concentration, reduced sperm motility, reduced acrosome reaction in mature spermatozoa, and reduced sperm function [191].

14.3.3.2.14 B Vitamins (Folate [Vitamin B_9] and Cyanocobalamin [Vitamin B_{12}])

The synthesis of RNA and DNA as part of cellular replication requires vitamins B_9 + B_{12}, and deficiency states have been associated with decreased sperm count and motility. Both B_9 and B_{12} facilitate spermatogenesis [192], which is reliant on DNA synthesis [72] for germ cell growth and rapid division of cells. Multiple studies produced similar findings concluding that low levels of B_9 in seminal plasma are associated with increased sperm DNA damage [193], while B_{12} deficiency is strongly associated with reduced sperm motility and count [144]. Studies have administered doses in the range of 1000 to 6000 mcg per day (the average dose being 1500 mcg daily) and have consistently shown improvements in sperm production [185,194].

14.3.3.2.15 Calcium

Calcium has been shown to be involved in the regulation of acrosome reaction, chemotaxis, and sperm motility and metabolism [195]. In addition, it is responsible for enabling hyperactivation swimming (by asymmetric flagellar beating and the development of high-amplitude flagellar waves), which is paramount for fertilization [195].

14.3.3.3 Herbal Medicine

Table 14.2 lists herbal medicines for male infertility.

Note: Herbal medicine prescriptions should be closely monitored by the patient's naturopath/herbalist.

TABLE 14.2
Herbal Medicines for Male Infertility

Class	Action	Herbal Medicine Examples
Male tonic	Increase reproductive capacity, support hormone cascades and subsequent sperm production	*Panax ginseng* (Korean ginseng) *Eleutherococcus senticosus* (Siberian ginseng) *Pygeum africanum* (pygeum) *Mucuna pruriens* (velvet bean)
Sexual tonic	Improve sexual function, increase libido	*Tribulus terrestris* (tribulus) *Panax ginseng* (Korean ginseng) *Turnera diffusa* (damiana)
Adaptogen	Increase vitality, reduce fatigue, restore hypothalamic–pituitary–testicular axis	*Withania somnifera* (withania) *Panax ginseng* (Korean ginseng)
Spermatogenesis	Increase sperm motility	*Astragalus membranaceous* (astragalus) *Panax ginseng* (Korean ginseng)
	Increase sperm count	*Panax ginseng* (Korean ginseng) *Eleutherococcus senticosus* (Siberian ginseng) *Tribulus terrestris* (tribulus)
	Increase seminal fluid	*Pygeum africanum* (pygeum) *Serenoa serrulata* (saw palmetto)
	Improve morphology	*Curcuma longa* (turmeric) *Ganoderma lucidum* (reishi) *Silybum marianum* (St. Mary's thistle) *Gingko biloba* (ginkgo)
	Reduce DNA fragmentation	*Gingko biloba* (ginkgo) *Curcuma longa* (turmeric) *Silybum marianum* (St. Mary's thistle)

14.3.3.4 Lifestyle Recommendations

14.3.3.4.1 Environmental Factors

The environmental impact cannot be underestimated. Industrial growth since the end of World War II has introduced many complex chemicals into the environment that are novel to biological detoxification systems. Some of these molecules are reproductive toxicants, capable of impairing fertility and inducing developmental abnormalities in the embryo, including errors in normal sexual differentiation. The power of reproductive toxicants that target the germ line lies in their capacity to generate damage that can be passed down the generations via genetic or epigenetic means.

As such, it is advisable to discourage exposure in all male fertility patients as much as possible, and in those with suspected heavy exposure further investigations and specialized treatments to chelate and support detoxification are advisable.

14.3.3.4.2 Heavy Metals

Sperm are also particularly susceptible to the damaging effects of heavy metals such as lead, cadmium, arsenic, and mercury [196]. A hair mineral analysis for heavy metals should be performed on all men with reduced sperm counts to rule out heavy metals as a cause.

14.3.3.4.2.1 N-Acetylcysteine When environmental toxin exposure—both metals and persistent organic pollutants—is a primary cause, increasing glutathione production is key to increasing excretion. As the rate-limiting step in the production of glutathione is the availability of cysteine, supplementing with *N*-acetylcysteine (NAC) or whey will increase glutathione levels [197,198]. Nutrients such as NAC also directly chelate toxic metals such as methyl mercury from the body [199].

14.3.3.4.2.2 Estrogen Exposure Increased exposure to environmental estrogens and other environmental pollutants during fetal development, as well as during the reproductive years, is suggested to be a major cause of the tremendous rise in the incidence of disorders of development and function of the male sexual system [196,200,201].

Estrogens have been reported in drinking water [200,201], cow's milk and milk products, and in this environment. Many of the chemicals that we have contaminated our environment with in the past 50 years are weakly estrogenic. Most of these chemicals, such as polychlorinated biphenyls (PCBs), dioxin, and DDT, are resistant to biodegradation and are recycled in our environment until they find a safe haven in our bodies. These toxic chemicals are known to interfere with spermatogenesis, but their effects during sexual development may be more important.

14.3.3.4.3 Obesity

Obese men are known to have lower sperm counts (up to 50%), reduced motility, reduced spermatogenesis, increased DNA fragmentation of sperm, and increased levels of erectile dysfunction. In addition, extra abdominal weight can increase scrotal temperature [202].

14.3.3.4.4 Increased Scrotal Temperature

The mean scrotal temperature of infertile men is significantly higher than that of fertile men, and reduction of temperature can often restore fertility.

14.3.3.4.5 Radiation

Cell and cordless phones emit radiofrequency electromagnetic waves (EMWs) and are implicated in DNA strand breaks [203]. Harmful EMWs may interfere with normal spermatogenesis and result in a significant decrease in sperm quality, morphology, and motility [204–206].

14.3.3.4.6 Cigarette Smoking

Paternal cigarette smoking generates spermatozoa that suffer from high levels of DNA damage, largely as a result of oxidative stress. One of the consequences of this DNA damage is that the children

of such men exhibit an increased incidence of childhood cancer [207]. In addition, decreased sperm counts, decreased sperm motility, increased levels of abnormal sperm [208], increased miscarriage risk [209,210], and reduction in semen quality [211] are also noted.

A Cochrane review found that both active and passive smoking is associated with reduced fertility and decreased chance of a healthy, live birth in *both* fertile and infertile populations. In males cigarette smoking has been observed to impair sperm respiration, affecting their mitochondrial function [212], and a reduction in sperm motility and semen quality [211]. As such, it is strongly recommended that all men cease both passive and active smoking for at least the preconception period and beyond.

14.3.3.4.7 Marijuana

Cannabinoids from marijuana have been found to inhibit mitochondrial respiration of human sperm [213]; reduce testosterone production [124]; decrease sperm motility; reduce sperm morphology; and decrease sperm function, specifically capacitation and acrosome reactions [214].

14.3.3.4.8 Infections and Infertility

Infections in the male genitourinary tract, including infections of the epididymis, seminal vesicles, prostate, bladder, and urethra, are thought to play a major role in many cases of infertility [215]. The exact extent of the role they play is largely unknown because of the lack of suitable diagnostic criteria coupled with the asymptomatic nature of many infections. The presence of antisperm antibodies or high levels of debris in semen sample are considered a good indicator of a chronic infection in the absence of other clinical findings.

14.4 ENDOMETRIOSIS

14.4.1 Overview

Endometriosis is defined as endometrial tissue, composed of endometrial-type glandular tissue and stroma, found outside the uterine cavity [216,217]. It occurs almost exclusively in women in the reproductive years (regresses after menopause or ovariectomy [216]). The exact prevalence of endometriosis is unknown but estimates range from 2% to 10% of women of reproductive age, and up to 50% of infertile women [218,219].

Although women with endometriosis can present with a variety of complaints, recent European Society of Human Reproduction and Embryology (ESHRE) guidelines suggest that clinicians should consider the diagnosis of endometriosis:

1. In the presence of gynecological symptoms such as dysmenorrhea, noncyclical pelvic pain, deep dyspareunia, infertility, and fatigue or
2. In the presence of any of the above in women of reproductive age with nongynecological cyclical symptoms (dyschezia, dysuria, hematuria and rectal bleeding, shoulder pain) [220]

Complications such as adhesion formation, pelvic cysts, endometriomas, ruptured endometriomas, bowel and ureteral obstruction resulting from pelvic adhesions, and peritonitis from bowel perforation should also be considered.

Although endometriosis is a benign disorder, it does exhibit a cellular proliferation, cellular invasion, and neoangiogensis [221]. A growing body of evidence indicates that a combination of genetic, hormonal, immunological, and anatomical factors contributes to the formation and development of the ectopic foci of endometriosis [216].

Etiological risk factors include age [222,223]; social class and race [223,224]; menstrual factors such as early menarche [225,226]; family history [223,227–230]; genetic factors [223,228]; various phase 1 (aryl hydrocarbon receptor, CYPIAI, *N*-acetyltransferase 2) and phase 2 (glutathione *S*-transferases, *N*-acetyltransferase 2) liver detoxification enzymes [228], dioxin exposure such as those found in

tampons (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD] caused by bleached rayon, aluminium, alcohol, and additives) [231,232]; and immune factors such as concurrent autoimmune conditions [223,233–235].

Despite being one of the most frequently encountered gynecological diseases, the pathophysiology of endometriosis remains controversial. Although various theories have been proposed to explain the pathogenesis of endometriosis, no single theory has been found to account for the location of ectopic endometrium in all cases of endometriosis [221]. Pathogenic theories include transplantation theory (retrograde menstruation [236–238], lymphatic dissemination [221], blood vessel dissemination, direct implantation); metaplasia (celomic [239,240] or *in situ* [241]), invasion [242], or induction theory [243]. Other considerations should include peritoneal fluid constituents [244–246]; immunological aberrations [247] (cell mediated [239,248], specifically natural killer cell activity [247,249–251]; humoral mediated immunity [239]; autoimmunity [247]); steroid responsiveness and receptor content [242,252,253] (resistance to progesterone [223,252]); or acquisition of blood supply (vascularization of endometriotic implants [254,255]).

14.4.2 Naturopathic Perspective

The naturopathic approach to endometriosis considers multiple variables in its holistic assessment including the key areas listed in Table 14.3.

TABLE 14.3
Naturopathic Approach to Endometriosis

Consideration	Treatment Objective
Steroid hormone responsiveness and receptor activity	Improve hormone cascades and receptor responsiveness
Endogenous and exogenous exposure to estrogens causing estrogen displacement issues	Avoidance of confounding estrogen interferences (lifestyle, dietary, and other)
Hepato-biliary function, lipid profile, and steroidogenesis	Support hepato-biliary function to support toxin clearance, lipid metabolism, hormone synthesis and metabolism
Immune disruption and consideration of factors that compound autoimmune predisposition or immune hyperreactivity	Immune modulation and reduction of immune triggers
Environmental toxin exposure, toxic burden, and hepato-biliary clearance	Avoidance of environmental toxins and support of elimination
Level of oxidation, pro-oxidant factors in diet and lifestyle, antioxidant status	Antioxidant supplementation, hepato-biliary support
Microflora status of digestive, reproductive, and urinary systems	Microflora recolonization
Digestive health, function; nutrient absorption and assimilation	Digestive support, dietary modifications
Circulatory and lymphatic activity within reproductive organs and surrounding tissues, impediments to circulation throughout the body and blood cleansing through hepato-biliary channels	Lymphatic and circulatory support
Factors that impede menstrual flow and contribute to menstrual retrograde, i.e., sexual intercourse, sanitary items	Lifestyle modification and recommendations
Other	Symptomatic support—decrease inflammation, reduce pain, reduce endometrial tissue displacement

14.4.3 Treatment

14.4.3.1 Dietary Recommendations

14.4.3.1.1 Antioxidants

Antioxidant-rich foods should be encouraged in women with endometriosis. Mexican women [256] were shown to have lower antioxidant status concurrent with endometriosis presentation and diagnosis. Prescriptions to increase antioxidants in the diet improved antioxidant status and reduced symptoms. Translating this recommendation into dietary practice could include vitamin A (1050 mcg retinol equivalents; consumed in vitamin A-rich vegetables, e.g., carrot, broccoli, chard); vitamin C (500 mg; consumed in vitamin C-rich fruits, e.g., guava, oranges); and vitamin E (20 mg; consumed as sunflower seeds and peanuts).

14.4.3.1.2 Fresh Fruits and Vegetables

Fresh fruits and vegetables should be encouraged for all women with endometriosis. In Italian women [257] with endometriosis, a high intake of green vegetables and fresh fruits was observed to protect against endometriosis. This may be due to increased antioxidant consumption, fiber consumption, or other factors. Specifically, brassica vegetables including Brussels sprouts, cabbage, and broccoli should be encouraged because of their content of indole-3-carbinol. Conversely, there was an increased risk in women who consumed high quantities of beef, other red meat, and cold meats such as ham, which has been hypothesized to impact endometriosis owing to its pro-inflammatory nature.

14.4.3.1.3 Essential Fatty Acids

A key dietary goal is to ensure that a patient's essential fatty acid profile is optimal. A large prospective study following 70,709 American nurses enrolled in the US Nurses Health Study followed women for 12 years and assessed the link between dietary fat and risk of endometriosis [258]. Researchers found that although the total amount of fat in the diet did not matter, the type of fat did. Women who ate the highest amount of long-chain omega-3 fatty acids were 22% less likely to be diagnosed with endometriosis and those who ate the most *trans* fats had a 48% increased risk. In addition, the authors noted that a low-fat diet was not necessarily the healthiest and further bolstered the case for eliminating *trans* fats from the food supply. In the study, the highest positive contributor to omega-3 fatty acid intake was mayonnaise and full-fat salad dressing, followed by fatty fish such as tuna, salmon, and mackerel. The major sources of *trans* fats in this study were fried restaurant foods, margarine, and crackers. Saturated fat intake should also be modified. Consumption of palmitic acid, a saturated fat found primarily in animal fat, has also been associated both with an increased risk of endometriosis [258] and with an increased risk of other benign or malignant gynecological conditions [259,260].

14.4.3.1.4 Caffeine

It is important to recommend dietary avoidance of caffeine-containing beverages and foods. A case-control study observed that use of 300 mg/day or more of caffeine was associated with increased prevalence of minimal or mild endometriosis among infertile women [261]. A significant increase in the risk of infertility due to tubal disease or endometriosis was also found for women consuming high amounts of caffeine [262].

14.4.3.1.5 Alcohol

The role of alcohol in endometriosis is controversial [263]; however, it is likely to aggravate symptoms. A moderate intake of alcohol is related to increased levels of circulating estrogens [264] and acts as a diuretic, thus losing valuable nutrients. Adding to the importance of optimal liver function specifically in the endometriosis population, the further burden of alcohol consumption is likely to aggravate the presentation and thus recommendations for avoidance should be encouraged.

14.4.3.2 Nutritional Medicine

14.4.3.2.1 Antioxidants

Oxidative stress has been implicated in the chain of events leading to the development of endometriosis. ROS have been suggested to control and worsen severity and progression of the disease [265]. Women with endometriosis have been observed to have increased oxidative stress [266] and reduced antioxidant reserves when compared to controls [256]. As such, supplementation of specific antioxidants including vitamins A, C, and A; CoQ_{10}; selenium; and zinc is recommended.

14.4.3.2.2 β-Carotene and Vitamin A

β-Carotene displays a number of pertinent functions related to endometriosis including assisting in the normal regulation of cell replication and differentiation in the human endometrium, supporting healthy immune function, and acting as an antioxidant and chemoprotective [267]. Given the links between ovarian endometriosis and an increased incidence of both ovarian cancer and endometrial polyps [268,269], the role of β-carotene to inhibit estrogenic activity of 17β-estradiol and genistein in cancer cells [270] should be considered as a useful treatment.

14.4.3.2.3 Vitamin E

Vitamin E displays potent antioxidant and anti-inflammatory properties [271]. *In vitro* studies reveal vitamin E to have a protective effect on the endometrium, whereby it helps to inhibit proliferation of endometrial cells [272]. Activated macrophages as well as other pro-oxidants cause widespread oxidation in the peritoneal cavity, contributing to the excessive growth of endometrial cells, whereas antioxidants such as vitamin E usually function to offset this. Vitamin E levels have been observed to be low in the lipoproteins of the peritoneal fluid [273]. As such, a deficiency in vitamin E may lead to uncontrolled oxidative damage and promote abnormal endometrial growth.

Vitamin E may also be useful in preventing endometrial adhesions by reducing series 2 prostaglandins and improving elimination and clearance of pelvic debris by white blood cells [274].

14.4.3.2.4 Selenium

Selenium is a powerful antioxidant that carries out its functions through its incorporation into selenoproteins, which play a key role in antioxidant protection, influencing inflammatory and immune responses [275]. Via its action on the liver, selenium also assists with the detoxification of estrogens.

14.4.3.2.5 Zinc

Zinc provides many useful roles for the endometriosis patient. It acts as an antioxidant to assist in mediating oxidative stress and as an anti-inflammatory agent to prevent the generation of inflammatory cytokines [276]. It also supports optimal immune function and assists in healing damaged endometrial tissue.

14.4.3.2.6 Essential Fatty Acids

Essential fatty acids exhibit potent anti-inflammatory properties by regulating prostaglandin metabolism. In one article, *in vitro* studies showed that omega-3 fatty acids suppress the survival of endometrial cells [277]. Unfortunately, specific clinical trials are unavailable to confirm this finding. However, omega-3 fatty acids (1080 mg/day of EPA, 720 mg/day of DHA, and 1.5 mg of vitamin E over 2 months) have been shown to be useful for dysmenorrhea [278], a common symptom experienced in endometriosis.

14.4.3.2.7 Vitamin D_3

Vitamin D plays a key role in the regulation of the immune system. A deficiency of vitamin D can increase inflammatory mediators as the transport protein (D binding protein) transforms into

macrophage activating factor when not used. As such, given that endometriosis has associations with defective immune function and poor major histocompatibility complex antigen receptivity, vitamin D has been proposed to act as a regulatory nutrient/hormone for this function.

In the literature, there are a few interesting studies that correlate this finding in the endometriosis population. One important study showed that women with endometriosis presented with normal-to-high 25-$(OH)D_3$ (25-hydroxyvitamin-D_3) but abnormal 1,25-$(OH)D_3$ (1,25-dihydroxyvitamin-D_3). To convert 25-$(OH)D_3$ to 1,25-$(OH)D_3$ the cholecalciferol has to pass through the liver and then the kidneys for hydroxylation. Given the biochemistry underlying vitamin D absorption, this may relate to questionable phase 1 and phase 2 liver detoxification processing evident in the endometriosis population. Appropriate investigations are thus recommended concurrent with supplementation. When supplementing with vitamin D, ensure adequate balance with vitamins A and K_2.

14.4.3.2.8 Silica

Silica is required for the healthy development and functioning of connective tissue, particularly skin, tendons, and bone. Inflammation commonly experienced in endometriosis can result in adhesion development, contributing to pain and other symptoms. Silica has been shown to assist in reducing scar tissue formation, especially in postsurgical environments in the endometriosis population [279].

14.4.3.2.9 Iron

Because of extensive blood loss and likelihood of menstrual clotting, assessment and treatment of iron deficiency are imperative. Iron deficiency anemia presents with menorrhagia, and as the deficiency worsens, menstrual flow increases, thus exacerbating the endometriosis presentation.

14.4.3.2.10 B Vitamins

B vitamins play an important role in endometriosis, as they participate in numerous essential metabolic functions including energy metabolism, cellular growth, DNA regulation, and neurotransmitter production. The B vitamins as a whole are required for healthy liver detoxification so that estrogen and other substances may be processed and eliminated. Phase 1 detoxification is in part reliant on flavin adenine dinucleotide (part of vitamin B_2) and nicotinamide adenine dinucleotide (part of vitamin B_3); and phase 2 liver detoxification is reliant on vitamins B_6, B_9, and B_{12}. Without adequate intake of these B vitamins normal detoxification processes cannot occur and as such estrogen may be recirculated, thus aggravating the condition. In addition, vitamin B_1 (100 mg/day) has been shown to be effective in treating dysmenorrhea [280], and vitamin B_6 is required for hormone and prostaglandin synthesis.

14.4.3.2.11 Calcium

As calcium is involved in healthy neuromuscular function [87], it should be given in combination with magnesium to help provide relief from spasm and cramps. Calcium deficiency is associated with muscular and premenstrual cramping, and subsequent supplementation has been observed to reduce pain [281].

14.4.3.2.12 Magnesium

A Cochrane review [282] found magnesium to be effective in reducing pain associated with menstrual cramping. Research showed that it needs to be taken for at least 5 months for full benefit to be observed.

14.4.3.2.13 Probiotics

Many endometriosis patients experience abdominal bloating and distension as well as digestive discomfort. These symptoms correlate with hormonal fluctuations during women's cycles and affect nutrient absorption and aggravate symptomatology. The use of probiotics assists in eliminating bad

bacteria that would otherwise be recycled back into the body, contributing to the estrogen excess observed in endometriosis patients.

14.4.3.2.14 Vitamin K

Vitamin K catalyzes the carboxylation of a number of protein factors involved in blood clotting including prothrombin, forming the calcium binding sites on glutamyl side chains in the protein. It also regulates all eight clotting factors (including prothrombin; factors VII, IX, and X; and proteins S and C). Vitamin K is typically provided in sufficient quantities from gastrointestinal tract bacteria and dietary sources, however, in those with proven clotting abnormalities supplementation may be appropriate.

14.4.3.3 Herbal Medicine

Table 14.4 lists herbal medicines for endometriosis.

Note: Herbal medicine prescriptions should be closely monitored by the patient's naturopath/herbalist.

TABLE 14.4
Herbal Medicines for Endometriosis

Class	Action	Herbal Medicine
Analgesic	Symptomatic pain relief	*Anemone pulsatilla* (pasque flower)
Antispasmodic	Symptomatic pain relief	*Chamalerium luteum* (false unicorn root) *Dioscorea villosa* (wild yam) *Viburnum prunifolium* (black haw)
Astringent	Uterine tonification, reduction in menstrual blood loss	*Alchemilla vulgaris* (ladies' mantle) *Cinnamomum* spp. (cinnamon)
Anti-inflammatory	Symptomatic pain relief, reduction in oxidation	*Rehmannia glutinosa* (rehmannia)
Immunomodulator	Modulate immune response	*Andrographis panniculata* (andrographis) *Echinacea* spp. (echinacea) *Rehmannia glutinosa* (rehmannia)
Hormonal modulator	Stabilize hormonal cascades and regulate hormonal secretion. Improve estrogen metabolism, regulate estrogen-to-progesterone ratio	*Asparagus racemosa* (shatavari) *Vitex agnus castus* (chaste tree berry)
Uterine tonic	Reduce endometrial tissue size and impact, improve uterine health	*Anemone pulsatilla* (pasque flower) *Chamalerium luteum* (false unicorn root)
Hepatic, cholagogue	Support hepatic clearance of estrogen metabolites and hormonal synthesis, support clearance of environmental toxins	*Silybum marianum* (St. Mary's thistle) *Andrographis panniculata* (andrographis) *Cynara scolymus* (globe artichoke)
Bitter	Encourage and support gastrointestinal tract microecology	*Gentiana lutea* (gentian) *Chionanthus virginica* (fringe tree) *Cynara scolymus* (globe artichoke)
Lymphatic	Improve pelvic circulation and immunological function	*Calendula officinalis* (calendula) *Cinnamomum* spp. (cinnamon)
Adaptogen, thymoleptic	Support patient emotionally and reduce impact of excessive cortisol production both on immune irregularities, negative impact on hypothalamic–pituitary–adrenal axis and inflammation	*Hypericum perforatum* (St. John's wort) *Withania somnifera* (ashwaganda) *Asparagus racemosa* (shatavari)

14.4.3.4 Lifestyle Recommendations

14.4.3.4.1 Smoking

Some studies have suggested that heavy smokers are at decreased risk of endometriosis [225] likely as a result of the anti-estrogenic effects of smoking; however, any link between smoking and endometriosis risk is limited and controversial [263]. It is, however, important to discourage smoking in patients because of its obvious health risks and pro-oxidant effect.

14.4.3.4.2 Exercise

Regular physical activity might be linked with lower levels of estrogens and reduced endometriosis risk, but data on this issue are limited [263].

14.4.3.4.3 Sanitary Products

Patients should be encouraged to use sanitary pads (or similar) and prevent the use of tampons given the links between TCDD caused by bleached rayon, aluminum, alcohol, and additives and endometriosis [231,232].

14.4.3.4.4 Sexual Intercourse

Patients should be advised to refrain from sexual intercourse during menses owing to potential links with retrograde blood flow. Discussion surrounding use of lubricants and selection of non-phthalate/paraben/bisphenol-A sources should be discussed.

14.4.3.4.5 Environmental Impact

As environmental toxins are linked to endometriosis, a thorough environmental exposure history should be performed to identify possible toxins. Assessment for heavy metals, pesticides, solvents, phthalates, and parabens is beneficial. Every patient should be educated on how to avoid hormone disrupting chemicals in food, water, air, and personal care products.

14.5 MENOPAUSE

14.5.1 Overview

Menopause is technically defined as the last natural menstrual period (so often a retrospective diagnosis). It is the natural state a woman is in after the ovaries have stopped ovulating because of depletion of eggs. The normal age of menopause is between 40 and 55 years, with an average in Western societies of 50–51 years. With the increase in life expectancy, women may spend nearly half their lives in a postmenopausal state and will experience the short-term and long-term effects of menopause. Although universal, menopausal symptoms vary widely between women and across cultures. Some women "sail through it" and some experience severe and debilitating experiences. Natural menopause may be diagnosed after 12 months of amenorrhea a result of loss of follicular ovarian activity and in the absence of another pathological cause [283].

Age will vary according to the individual woman; in 1978 the mean age at menopause in healthy Australian women was 50.4 years [284]; however, this has increased slightly in the last few years, with the average age at menopause at 51 years (± 5 years) [285]. Given the average life expectancy of women, it is likely that they will experience approximately another 27–32 years of postmenopausal life. The World Health Organization estimates that by 2030 there will be around 1.2 billion postmenopausal women, with 47 million women entering life post-menopause every year [285].

Premature menopause (menopause that occurs before the age of 40) affects up to 1% [286] of women, and usually occurs as a result of medical intervention or premature ovarian failure. Caucasian women appear to be more prone to premature ovarian failure compared to other ethnicities including Chinese (0.5%) and Japanese (0.1%) [287] women.

Oopause is defined as the normal cessation of female fertility up to 10 years before the menopause. It occurs in some women after the age of 33 and for most women by 45. It is different from perimenopause, as it reflects failing ovarian function; however, regular menstruation may still be occurring.

If pregnancies are attempted through the oopausal transition, a woman who has had no prior reproductive disturbance will typically experience recurrent miscarriages before developing otherwise unexplained infertility. If she undertakes IVF/ART she typically experiences unexplained implantation failure of apparently satisfactory embryos, and, in turn, a decreased rate of forming blastocysts, defective cleavage, and then failure of fertilization.

Menopause is inevitable for all women; however, a number of risk factors have been linked to earlier onset. These include race and ethnicity [288], genetic factors [289,290], and smoking [291–293]. Other interesting factors include living at higher altitudes, occupation, age at menarche, parity, age at last pregnancy, and prior contraceptive pill use [294].

14.5.2 Naturopathic Perspective

The naturopathic approach to the menopausal transition is dependent on the timing of the consultation, that is, pre-, peri-, or post-menopause. It considers multiple variables in its holistic assessment including the key areas listed in Table 14.5.

14.5.3 Treatment

14.5.3.1 Dietary Recommendations

14.5.3.1.1 Phytoestrogens

It is well known that a diet rich in phytoestrogens has been promoted to assist in the reduction of menopausal symptoms. A 12-week study observed the effects of a diet rich in phytoestrogens as

TABLE 14.5
Naturopathic Approach to Menopause

Consideration	Treatment Objective
To support the body in the transition from using estradiol (ovarian supply) to estrone	Avoidance of confounding estrogen interferences (lifestyle, dietary, and other)
	Support for steroidogenesis from cholesterol (optimize liver function) and address cofactors
	Supplement with phytoestrogens from herbal medicines and dietary sources to support the estrogen loss
Symptomatic support	Symptomatic support as specific for the presentation. May include support for vaginal atrophy, bladder infections, body aches, cognitive changes, hair loss, hot flushes, increased facial hair, irregular bleeding (perimenopause), mood changes, sleep disturbances, or urinary incontinence
Address the presentation and protect for the future	Treatment of any secondary disease processes concurrent with the transition
	Protection against the development of secondary conditions including osteoporosis, breast cancer [295], cardiovascular disease [296], central adiposity, insulin resistance/metabolic syndrome or dyslipidemia, Alzheimer's disease, and others
Premature ovarian failure patients	Acknowledge other variables including autoimmune conditions, genetic disorders, nutritional deficiencies, and other factors

compared to a regular omnivorous diet in women with menopausal complaints [297]. The treatment group showed an increase in serum phytoestrogens, reduction in menopausal symptoms (especially hot flushes and vaginal dryness), and an increase in sex hormone-binding globulin (SHBG).

14.5.3.1.2 Glycine max *(Soybean)*

One of the phytoestrogens, soy isoflavones, is the staple phytoestrogen found in Asian diets. Isoflavones are believed to compete with estrogen for the same receptor sites, thus exerting estrogenic effects [298]. Other isoflavones are found in legumes, lentils, beans such as kidney beans, lima beans, broad beans, and chickpeas; however, the richest source is soybeans, which contain 2–4 mg of isoflavones per gram of protein [299]. It is important to note that supplemental forms lack the protein, lipids, and other phytochemicals found in the whole soybean [299].

14.5.3.1.3 Linum usitatissimum *(Flaxseed)*

Flaxseed is a rich source of two types of phytoestrogens—lignans and isoflavones. A number of studies have reviewed its efficacy for menopausal women with mixed results. One conflicting study assessed the daily consumption of two slices of bread containing 25 g of flaxseed (46 mg of lignans) versus wheat bran (<1 mg of lignans; control) every day for 12 consecutive weeks. Both groups experienced a reduction in the quantity of hot flushes, suggesting that flaxseed-enriched bread was no more effective than control for reducing hot flushes [300]. However, another small pilot study involving 30 women examined the effect of 40 g/day of crushed flaxseed over a 6-week period added to the daily diet [301]. On average the mean decrease in hot flash scores after flaxseed therapy was 57% while the mean reduction in daily hot flash frequency decreased from 7.3 to 3.6 after flaxseed therapy.

From a naturopathic perspective, flaxseed is best consumed fresh from flaxseeds that are freshly crushed and stored appropriately. As a polyunsaturated fatty acid, flaxseed is highly unstable and oxidizes easily when exposed to light or high temperatures. As such, flaxseed when provided in cooked foods such as bread or muffins may not provide substantial health-promoting properties. This is important to note, as most studies use bread sources as their chosen delivery source. Heating of flaxseed at high temperatures (as is the case when it is found in bread or a muffin) results in the flaxseed becoming rancid, particularly when it then sits on the bakery shelf for a number of days.

Daily dietary flaxseed may also help to protect against cardiovascular disease, the risk of which is increased at onset of menopause. Studies have shown that a daily intake of flaxseed (30–40 g/day) has been found to improve lipid profiles in menopausal women [302,303].

14.5.3.1.4 Other Considerations

Given the risk of osteoporosis, women should be discouraged to consume caffeine-containing beverages and alcohol owing to potential loss of bone mineralization.

14.5.3.2 Nutritional Medicine

14.5.3.2.1 Calcium

Menopause leads to bone loss because decreases in estrogen production both increase bone resorption and decrease calcium absorption [304–306]. Annual decreases in bone mass of 3–5% per year frequently occur in the first years of menopause, but the decreases are typically less than 1% per year after age 65 [307]. Increased calcium intakes during menopause do not completely offset this bone loss [308,309].

Many women are unable to obtain the recommended daily intake of calcium through diet alone and thus supplementation may be required. Supplementation with calcium (in combination with vitamin D) has been demonstrated to reduce bone loss in peri- and postmenopausal women while the placebo group actually lost total bone mineral density at a rate of approximately 0.4% per year [310].

Calcium supplementation is undoubtedly the most controversial nutrient for menopausal women. When reviewing the data, the astute clinician needs to review the study designs closely and assess

for forms of calcium prescribed, synergistic cofactors, and other variables that may confound the presentation and result [311–317]. Careful evaluation of the research shows that calcium supplementation is safe and effective for menopausal women if specific guidelines are met. It is important to prescribe bioavailable forms of calcium (citrate or bisglycinate are the most well absorbed and tolerated); to caution women to avoid taking excessive amounts, as they can contribute to hypercalcemia which can lead to renal and cardiovascular complications (menopausal women should consume 1000-1300 mg/day in a combination of dietary and supplemental sources); to prescribe with synergistic cofactors such as magnesium, vitamin D and vitamin K_2; and to avoid interactions with other nutrients such as iron or zinc. In addition, bioavailability is affected by a threshold—absorption of calcium is highest in doses of less than 500 mg/day. As the dose increases, the percentage of absorption decreases [318]. As such, it is pertinent to advise that prescriptions of greater than 500 mg/day be divided over the day in smaller quantities to increase absorption and uptake.

14.5.3.2.2 Vitamin D

Vitamin D is strongly indicated for all menopausal women to support and protect skeletal health. Although calcium supplementation is proven to maintain bone mineral density, concurrent vitamin D prescription provides a synergistic prescription to further prevent osteoporosis. This is achieved by enhancing calcium absorption and bone mineralization [319]; improving muscle health; and stimulating bone maturation, matrix formation, bone remodeling, and osteoclast cell activity [320]. Vitamin D deficiency is very common, but even more so in countries of high latitude with minimal sun exposure or in individuals with dark skin. Vitamin D deficiency is reaching epidemic proportions, and as such it is prudent to screen patients thoroughly and prescribe accordingly.

14.5.3.2.3 Vitamin C

Oxidative stress occurs during menopause and is related to the loss of estrogens that previously exerted a protective and antioxidant action on low-density lipoproteins [21]. Vitamin C has been shown to improve endothelial function in healthy postmenopausal estrogen-deficient women [321] and may also improve bone mineral density in postmenopausal women via its ability to stimulate procollagen, enhance collagen synthesis, and stimulate alkaline phosphatase activity, a marker for osteoblast formation [322].

14.5.3.2.4 Vitamin E

Vitamin E is an antioxidant with an affinity for the female reproductive system. It can provide relief from vaginal dryness (topical and oral prescription) and has been shown to be beneficial in the treatment of hot flushes. After 1 month of administration, study participants showed reduced frequency and severity of hot flushes [323].

14.5.3.2.5 Vitamin K_2

The vitamin K_2 family, also known as menaquinones, are critical for proper utilization of vitamin D and calcium in building bone. The reduction in the consumption of green vegetables and the consumption of dairy products from cows fed corn instead of grass has resulted in a decrease in all forms of vitamin K, especially K_2. Vitamin K_2 is made in humans by gut bacteria and is present in dairy products, especially cheese, from grass-fed cows as well as some fermented products, such as nato. (For a comprehensive discussion of the critical roles of the various forms of vitamin K see Ref. [324].)

14.5.3.2.6 Zinc

Zinc has been shown to support two key functions in menopausal women. There appears to be a regulatory role in estrogen retention, as zinc loss has been shown to be concurrent with estrogen deficiency/decline leading to zinc deficiency [325]. In addition, zinc status is reduced in postmenopausal women with osteoporosis and osteopenia when compared to controls [326].

14.5.3.2.7 Probiotics

The healthy vaginal ecology contains potent lactobacilli strains that assist in protecting the vagina from pathogens. Because of hormonal changes that occur at menopause and the sudden loss of estrogen (estradiol), vaginal atrophy can occur. Concurrent with the atrophy and estrogen loss, menopausal women have been shown to have a reduction in these protective microflora species, which affects the vaginal pH, thereby creates an optimal environment for enterobacteria to colonize and thus causing increased risk of vaginal infection.

Randomized controlled studies assessing the efficacy of probiotic supplementation on menopausal women have produced favorable results. In one study, oral application of lactobacilli (2.5×109 colony-forming units each of *L. rhamnosus* GR-1 and *L. reuteri* RC-14) once daily for 2 weeks resulted in a substantial improvement in the vaginal flora of postmenopausal women, and compared with controls, no bacterial vaginosis [327].

14.5.3.2.8 Essential Fatty Acids

Essential fatty acids have been shown to influence neuronal membranes and modulate neurotransmitter function and the serotonergic system. An essential fatty acid supplement containing a mixture of 400 mg of fish oil (30% EPA and 20% DHA), 100 mg of oil of borage (20% γ-linolenic acid [GLA]), and a mixture of vitamin E (15 mg), policosanols, and lipoic acid (25 mg) taken twice daily exhibited positive results in reducing the incidence of hot flushes in menopausal women [328]. In addition, omega-3 fatty acids are preventative for cardiovascular comorbidities and assist in reducing systemic inflammation and support steroidogenesis for secondary sex hormone production (estrone).

14.5.3.3 Herbal Medicines

Table 14.6 lists herbal medicines for menopause.

Note: Herbal medicine prescriptions should be closely monitored by the patient's naturopath/herbalist.

14.5.3.4 Lifestyle Recommendations

14.5.3.4.1 Weight Stabilization

As a woman approaches her menopausal transition the body often lays down extra adipose tissue in the abdominal region regardless of dietary or lifestyle modifications. This is often seen as a protective mechanism for future hormone release or as an indication of deteriorated systems. For optimal prevention of disease complications it is beneficial to regulate weight gain and support patients to reach and sustain their ideal body weight.

14.5.3.4.2 Exercise

Exercise is a crucial component for successful treatment. It has been shown to

- Decrease bone loss and improve bone mineralization
- Decrease lipid levels
- Improve cardiovascular function
- Improve circulation
- Improve oxygen and nutrient utilization in all tissues
- Improve stress-handling ability
- Increase endurance and energy levels
- Increase self-esteem, mood, and frame of mind
- Lower blood pressure
- Relieve hot flushes

TABLE 14.6
Herbal Medicines for Menopause

Class	Action	Herbal Medicine
Analgesic	Symptomatic pain relief	*Anemone pulsatilla* (pasque flower)
Antispasmodic	Symptomatic pain relief	*Chamalerium luteum* (false unicorn root) *Dioscorea villosa* (wild yam) *Viburnum prunifolium* (black haw)
Astringent	Uterine tonification, reduction in menstrual blood loss	*Alchemilla vulgaris* (ladies' mantle) *Cinnamomum* spp. (cinnamon)
Anti-inflammatory	Symptomatic pain relief, reduction of oxidation	*Rehmannia glutinosa* (rehmannia)
Immunomodulator	Modulate immune response	*Andrographis panniculata* (andrographis) *Echinacea* spp. (echinacea) *Rehmannia glutinosa* (rehmannia)
Hormonal modulator	Stabilize hormonal cascades and regulate hormonal secretion, improve estrogen metabolism, regulate estrogen-to-progesterone ratio	*Asparagus racemosa* (shatavari) *Vitex agnus castus* (chaste tree berry)
Uterine tonic	Reduce endometrial tissue size and impact, improve uterine health	*Anemone pulsatilla* (pasque flower) *Chamalerium luteum* (false unicorn root)
Hepatic, cholagogue	Support hepatic clearance of estrogen metabolites and hormonal synthesis, support clearance of environmental toxins	*Silybum marianum* (St. Mary's thistle) *Andrographis panniculata* (andrographis) *Cynara scolymus* (globe artichoke)
Bitter	Encourage and support gastrointestinal tract microecology	*Gentiana lutea* (gentian) *Chionanthus virginica* (fringe tree) *Cynara scolymus* (globe artichoke)
Lymphatic	Improve pelvic circulation and immunological function	*Calendula officinalis* (calendula) *Cinnamomum* spp. (cinnamon)
Adaptogen, thymoleptic	Support patient emotionally and reduce impact of excessive cortisol production on immune irregularities and negative impact on hypothalamic–pituitary–adrenal (HPA) axis and inflammation	*Hypericum perforatum* (St. John's wort) *Withania somnifera* (ashwaganda) *Asparagus racemosa* (shatavari)
	Symptomatic	
Adaptogen, adrenal restorative	Support patient through transition, HPA axis modulation, reduction in cortisol secretion	*Panax ginseng* (Korean ginseng) *Glycyrrhiza glabra* (licorice) *Asparagus racemosa* (shatavari) *Rehmannia glutinosa* (rehmannia)
Nervine	Support stress recovery	*Melissa officinalis* (lemon balm) *Leonuris cardiaca* (motherwort) *Passiflora incarnata* (passion flower)
Sedative, hypnotic	Assist in regulating circadian rhythm	*Humulus lupulus* (hops) *Lavandula officinalis* (lavender) *Piscidia erythrina* (Jamaican dogwood) *Piper methysticum* (kava)

TABLE 14.6 (CONTINUED)
Herbal Medicines for Menopause

Class	Action	Herbal Medicine
Antidepressant, anxiolytic	Support mood and address anxiety or depression	*Hypericum perforatum* (St. John's wort) *Piper methysticum* (kava) *Panax ginseng* (Korean ginseng)
Emollient, vulnerary	Address mucous membrane dryness	*Calendula officinalis* (calendula) *Althea officinalis* (marshmallow) *Stellaria media* (chickweed) *Urtica dioica folia* (nettle leaf)
Anti-hydrotic	Alleviate hot flushes and associated perspiration	*Cimicafuga racemosa* (black cohosh) *Salvia officinalis* (sage)
Bladder tonic	Improve bladder tonicity	*Crataeva nurvala* (crataeva)
Cognitive enhancer	Improve cognition	*Gingko biloba* (ginkgo) *Rosmarinus officinalis* (rosemary) *Bacopa monniera* (bacopa)
	Endocrine	
Phytoestrogen	Regulate and support hormonal cascades	*Cimicafuga racemosa* (black cohosh) *Humulus lupulus* (hops) *Trifolium pratense* (red clover)
Hypoglycemia	Weight stabilization, prevention of diabetes	*Gymnema sylvestre* (gymnema) *Coleus forskholi* (coleus)
Hormonal modulator	Regulation of hormonal cascades	*Cimicafuga racemosa* (black cohosh) *Dioscorea villosa* (wild yam) *Vitex agnus castus* (chaste tree berry)
	Cardiovascular	
Hypolipidemic	Improve lipid profile and increase steroid hormone synthesis for secondary production	*Cynara scolymus* (globe artichoke) *Curcuma longa* (turmeric) *Allium sativum* (garlic) *Camellia sinensis* (green tea) *Olea europea* (olive leaf)
Hepatic, cholagogue		*Cynara scolymus* (globe artichoke) *Curcuma longa* (turmeric) *Chionanthus virginica* (fringe tree)
Hypotensive	Regulate and support cardiac function	*Leonuris cardiaca* (motherwort) *Crataegus* spp. (hawthorn berry and leaf) *Salvia miltiorrhiza* (dan shen) *Zizyphus spinosa* (zizyphus) *Valeriana officinalis* (valerian)
Cardiac tonic		*Crataegus* spp. (hawthorn berry and leaf) *Leonuris cardiaca* (motherwort) *Astragalus membranaceous* (astragalus) *Gingko biloba* (ginkgo)

(*Continued*)

TABLE 14.6 (CONTINUED)
Herbal Medicines for Menopause

Class	Action	Herbal Medicine
	Skeletal	
Nutritive	Improve bone mineral density	*Urtica dioica folia* (nettle leaf) *Withania somnifera* (ashwaganda)
Phytoestrogen		*Cimicafuga racemosa* (black cohosh) *Humulus lupulus* (hops) *Trifolium pratense* (red clover)
	Immune	
Antioxidant	Reduce oxidation and prevent pro-oxidant pathways	*Silybum marianum* (St. Mary's thistle) *Curcuma longa* (turmeric)
Immune modulation	If required, to stabilize immune response	*Echinacea* spp. (echinacea) *Hemidesmus indicus* (hemidesmus)
Hepatic, cholagogue	Assist with clearance of estrogen metabolites	*Cynara scolymus* (globe artichoke) *Curcuma longa* (turmeric) *Taraxacum officinalis radix* (dandelion root)
	Gastrointestinal	
Bulk laxatives	Regulate bowel transit time, encourage clearance of cholesterol fragments	*Plantago ovate* (psyllium) *Salvia hispanica* (chia) *Ulmus fulva* (slippery elm)
Bitters	Improve nutrient absorption and protein assimilation	*Gentiana lutea* (gentian) *Andrographis panniculata* (andrographis) *Agrimonia eupatoria* (agrimony) *Chionanthus virginica* (fringe tree)

Recommendations should encourage women to "hit the pavement" so as to stimulate osteogenesis (ossification) in the bone and prevent osteoporosis.

14.6 KEY POINTS SUMMARY

1. A person's fertility is a reflection of his or her general health and well-being. Everything a person eats, drinks, experiences or is exposed to can and will influence his or her fertility. Furthermore, it should be emphasized that from an evolutionary perspective the body is primed to pass on genetic material to the next generation only when the environmental and health status of the individual are optimal. True naturopathic fertility support acknowledges and considers absolutely all of these variables for holistic health.
2. Naturopathic fertility support for both men and women is based on the following steps. The first is *prevention* of exposure to environmental toxins in food, water, and the person's general environment. Second, if toxin exposure has already occurred, steps should be taken to allow for *detoxification*. Third, it is crucial to identify nutrient deficiencies, followed with their rectification by improving diet or the use of nutritional supplements (*repletion*). Men and women seeking to become parents should be encouraged to adopt *healthy lifestyle* changes such as maintaining normal weight and a healthy level of exercise. Finally, the use of *naturopathic herbal medicines* to remedy specific problems in the individual's reproductive physiology can be a useful adjunct treatment. If couples are seeking assisted reproductive technologies (ART) care under the direction of a physician, open

communication with the treating fertility specialist is vital, as some herbal medicines and nutrients have potential negative interactions with *in vitro* fertilization treatment.

3. Final stages of oocyte production take approximately 100 days, meaning that any naturopathic treatments will have a lag period of 3+ months before they reach their optimal effectiveness. Women seeking pregnancy should be encouraged to maintain a normal body weight, get plenty of sleep, avoid potential toxins in their environment (use filtered water, organic grown foods, or carefully washed fruit and vegetables to reduce toxin exposure), plus avoid smoking, alcohol, and excessive caffeine intake. Moderate exercise of up to 30 minutes per day is helpful, although exercise of high intensity beyond this may actually harm the chances of conception and increase miscarriage. The key naturopathic principals behind a fertility-promoting diet include the ingestion of abundant fruits and vegetables and a preference for high-fat dairy products, while reducing the intake of red meats and avoiding high glycemic index foods and *trans* fat containing processed foods. Nutritional supplements that may aid female fertility include L-arginine, L-carnitine, antioxidants (α-lipoic acid, vitamins A and C, coenzyme Q_{10}, selenium, and zinc), and B group vitamins (especially folate, but also B_1, B_2, B_6, and vitamin B_{12}). Vitamin D, iodine, calcium, iron, magnesium, and essential fatty acids (omega-3 fish oils) have also been shown to have fertility benefits by aiding ovulation, reducing miscarriage, and generally assisting reproduction. Probiotics may be of benefit in that they can alter the vaginal microflora, reducing the incidence of adverse pregnancy outcomes such as preterm labor. Naturopathic herbal medicines felt to promote female fertility include chaste tree, black cohosh, and tribulus extract.
4. Sperm production takes approximately 70 days, so therefore it is important to optimize lifestyle 3 months before trying for conception to optimize sperm health. Lifestyle modifications known to boost male fertility include maintaining a normal weight, avoiding excessive heat (occupational or baths), minimizing alcohol intake and completely avoiding binge drinking sessions, and not smoking or using marijuana. Avoidance of environmental toxins includes minimization of exposure to electromagnetic radiation from mobile phones, avoidance of exposure to welding and chemical fumes through proper safety equipment and ventilation, plus ingestion of organic or properly washed foods. Chemicals used in the production of nonstick cooking surfaces and lining of food storage tin cans have been shown to interfere with male reproductive function and should be avoided. Oxidative stress is an important pathology seen in a large proportion of infertile men. Therefore the consumption of high amounts of fruits and vegetables, or antioxidant supplements (vitamins A, C, and E, α-lipoic acid, L-carnitine, coenzyme Q_{10}, lycopene, selenium, and zinc) may all be beneficial to male reproductive function. Nutritional supplements containing L-arginine, essential omega-3 fatty acids, vitamin D, folate, and calcium have all been suggested to promote male fertility potential. Finally, naturopathic herbal medicines that may be beneficial include Korean ginseng, tribulus, saw palmetto, and *Ginkgo biloba*.
5. Endometriosis is a condition that is commonly seen in infertile women, and also causes other significant reproductive concerns such as painful periods and sexual difficulties (dyspareunia). Endometriosis is an estrogen-dependent gynecological condition characterized by impaired immune clearance of menstrual fragments that pass up the fallopian tube into the pelvic cavity during menstruation, plus a state of oxidative stress. Therefore, naturopathic treatment of endometriosis relies first on methods to normalize immune function (avoiding environmental toxins, minimizing inflammatory foods such as red or processed meats, using anti-inflammatory omega-3 fatty acid supplements and immune supportive herbal medicines), facilitating the immune system's clearance of retrograde menstrual fragments, while decreasing painful inflammation. Second, techniques to decrease estrogen stimulation of endometriosis include lifestyle modification (avoidance of alcohol, regular exercise) and herbal medicine hormonal modulators such as chaste tree berry. Antioxidants in the form of abundant fresh fruits and vegetables plus dietary supplements

may help prevent endometriosis, while also reducing the adverse effects of existing endometriosis. Finally, pain related to inflammation or endometriosis-related bowel dysfunction can be ameliorated using herbal medicine analgesics (pasque flower), antispasmodics (wild yam, black haw), and probiotics that help relieve painful abdominal bloating.

6. The naturopathic approach to menopause will be dependent on the timing of consultation (pre-, peri- and post-menopause), plus the individual's symptoms. In the perimenopause period estrogen deficiency symptoms such as hot flashes/flushes may be treated using phytoestrogens found naturally in foods such as soy, legumes, lentils, and beans. Flaxseeds are one of the richest natural sources of phytoestrogens and have been shown to be very effective at managing perimenopausal symptoms. The development of anxiety and depression related to the menopause is best treated by a combination of avoidance of alcohol and caffeine, encouraging regular exercise, and herbal medicine support (St. John's wort, ashwaganda). Menopause is associated with an increase in weight, inflammation, and subsequent cardiovascular disease. Encouraging menopausal women to maintain a healthy diet high in antioxidant and anti-inflammatory foods, while regularly exercising, is therefore beneficial. Avoidance of osteoporosis by minimizing alcohol and caffeine intake, engaging in regular weight-bearing exercise, while also taking calcium and vitamin D supplements is encouraged in postmenopausal women.

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Appendix A: Recommended Dietary Intake for Macro- and Micronutrients for Various Countries

Nutrient reference values indicate the daily amount of nutrients required for good health, as well as a safe intake of nutrients. They reflect the levels of intake of essential nutrients considered to be adequate to meet the known nutritional needs of practically all healthy people for prevention of deficiency states. There are a number of terms used across the world to describe this.

- Estimated Average Requirement (EAR)
  A daily nutrient intake level needed to meet the requirements of half the healthy individuals in a particular age, life stage, or gender group.
- Recommended Dietary Intake (RDI), Dietary Reference Intake (DRI), Recommended Dietary Allowance (RDA), and Recommended Intake (RI)
  The average daily intake of nutrients from foods that is sufficient to meet the needs of 97–98% of healthy individuals in a particular life stage, age, or gender group. They include a margin of safety to cover individual differences and are derived from the EAR; therefore they exceed the needs of practically all healthy persons. The terminology differs from country to country.
- Adequate Intake (AI)
  It represents the average daily nutrient intake by a group of apparently healthy people known not to have a deficiency that we can assume is adequate. It is based on observed or experimentally determined approximations or estimates of nutrient intake and is used when an RDI cannot be determined.
- Upper Limit (UL)
  This is the highest average daily intake likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases.

See Tables A.1 through A.22.

TABLE A.1
Australian Tables: Macronutrients

Lifestyle Stage	Protein[a] (g/day) RDI	Protein (g/day) EAR	Fiber (g/day) AI	Water (Food and Liquid) (L/day) AI	Linolenic Acid (g/day) AI	α-Linolenic Acid (g/day) AI	Total LC *n*-3[b] (mg/day) AI
Males							
14–18 years	65	49	28	2.7	12	1.2	125
19–30 years	64	52	30	3.4	13	1.3	160
31–50 years	64	52	30	3.4	13	1.3	160
51–70 years	64	52	30	3.4	13	1.3	160
>70 years	81	65	30	3.4	13	1.3	160
Females							
14–18 years	45	35	22	2.2	8	0.8	70
19–30 years	46	37	25	2.8	8	0.8	90
31–50 years	46	37	25	2.8	8	0.8	90
51–70 years	46	37	25	2.8	8	0.8	90
>70 years	57	46	25	2.8	8	0.8	90
Pregnancy							
<18 years	58	47	25	2.4	10	1	110
19–30 years	60	49	28	3.1	10	1	115
30–50 years	60	49	28	3.1	10	1	115
Lactation							
<18 years	63	51	27	2.9	12	1.2	140
19–30 years	67	54	30	3.5	12	1.2	145
30–50 years	67	54	30	3.5	12	1.2	145

Source: Adapted from Australian Nutrient Reference Values. Available at http://www.nrv.gov.au.

[a] Based on grams of protein per kilogram of body weight for the reference body weight, e.g., for adults 0.8 g/kg body weight for the reference body weight.

[b] Total LC *n*-3 = (DHA22:6 docosahexaenoic fatty acid+EPA20:5 eicosapentaenoic fatty acid+DPA22:5 docosapentaenoic fatty acid).

TABLE A.2
Australian Tables: Micronutrients

Lifestyle Stage	Biotin (µg/day) AI	Choline (mg/day) AI	Choline (mg/day) UL	Folate[a] (µg/day) RDI	Folate[a] (µg/day) AI	Folate[a] (µg/day) UL	Niacin[b] (mg/day) RDI	Niacin[b] (mg/day) AI	Pantothenic Acid (mg/day) AI	Riboflavin (mg/day) RDI	Riboflavin (mg/day) EAR	Thiamin (mg/day) RDI	Thiamin (mg/day) EAR
Males													
14–18 years	30	555	3000	400	330	800	16	12	6.0	1.3	1.1	1.2	1.0
19–30 years	30	555	3500	400	320	1000	16	12	6.0	1.3	1.1	1.2	1.0
31–50 years	30	555	3500	400	320	1000	16	12	6.0	1.3	1.1	1.2	1.0
51–70 years	30	555	3500	400	320	1000	16	12	6.0	1.3	1.1	1.2	1.0
>70 years	30	555	3500	400	320	1000	16	12	6.0	1.3	1.3	1.2	1.0
Females													
14–18 years	25	400	3000	400	330	800	14	11	4.0	1.1	0.9	1.1	0.9
19–30 years	25	425	3500	400	320	1000	14	11	4.0	1.1	0.9	1.1	0.9
31–50 years	25	425	3500	400	320	1000	14	11	4.0	1.1	0.9	1.1	0.9
51–70 years	25	425	3500	400	320	1000	14	11	4.0	1.1	0.9	1.1	0.9
>70 years	25	425	3500	400	320	1000	14	11	4.0	1.1	1.1	1.1	0.9
Pregnancy													
<18 years	30	415	3000	600	520	800	18	14	5.0	1.4	1.2	1.4	1.2
19–30 years	30	440	3500	600	520	1000	18	14	5.0	1.4	1.2	1.4	1.2
30–50 years	30	440	3500	600	520	1000	18	14	5.0	1.4	1.2	1.4	1.2
Lactation													
<18 years	35	525	3000	500	450	800	17	13	6.0	1.6	1.3	1.4	1.2
19–30 years	35	550	3500	500	450	1000	17	13	6.0	1.6	1.3	1.4	1.2
30–50 years	35	550	3500	500	450	1000	17	13	6.0	1.6	1.3	1.4	1.2

Source: Adapted from Australian Nutrient Reference Values. Available at http://www.nrv.gov.au.

[a] As dietary folate equivalents (DFE). 1 DFE = 1 µg of food folate = 0.5 µg of folic acid on an empty stomach = 0.6 µg of folic acid with meals or as a fortified food.

[b] As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan.

TABLE A.3
Australian Data: Micronutrients

Lifestyle Stage	Vitamin B_6 (mg/day) RDI	Vitamin B_6 (mg/day) EAR	Vitamin C (mg/day) RDI	Vitamin C (mg/day) EAR	Vitamin B_{12} (µg/day) RDI	Vitamin B_{12} (µg/day) EAR	Vitamin A[a] (µg/day) RDI	Vitamin A[a] (µg/day) EAR	Vitamin A[a] (µg/day) UL	Vitamin D[b] (µg/day) AI	Vitamin D[b] (µg/day) UL	Vitamin E[c] (mg/day) AI	Vitamin E[c] (mg/day) UL	Vitamin K (µg/day) AI
Males														
14–18 years	1.3	1.0	40	28	2.4	2.0	900	625	2800	5	80	10	250	55
19–30 years	1.3	1.1	45	30	2.4	2.0	900	625	3000	5	80	10	300	70
31–50 years	1.3	1.1	45	30	2.4	2.0	900	625	3000	5	80	10	300	70
51–70 years	1.7	1.4	45	30	2.4	2.0	900	625	3000	10	80	10	300	70
>70 years	1.7	1.4	45	30	2.4	2.0	900	625	3000	15	80	10	300	70
Females														
14–18 years	1.2	1.0	40	28	2.4	2.0	700	485	2800	5	80	8	250	55
19–30 years	1.3	1.1	45	30	2.4	2.0	700	500	3000	5	80	7	300	60
31–50 years	1.3	1.1	45	30	2.4	2.0	700	500	3000	5	80	7	300	60
51–70 years	1.5	1.3	45	30	2.4	2.0	700	500	3000	10	80	7	300	60
>70 years	1.5	1.3	45	30	2.4	2.0	700	500	3000	15	80	7	300	60
Pregnancy														
<18 years	1.9	1.6	55	38	2.6	2.2	700	530	2800	5	80	8	300	60
19–30 years	1.9	1.6	60	40	2.6	2.2	800	550	3000	5	80	7	300	60
30–50 years	1.9	1.6	60	40	2.6	2.2	800	550	3000	5	80	7	300	60
Lactation														
<18 years	2.0	1.7	80	58	2.8	2.4	1100	700	2800	5	80	12	300	60
19–30 years	2.0	1.7	85	60	2.8	2.4	1100	800	3000	5	80	11	300	60
30–50 years	2.0	1.7	85	60	2.8	2.4	1100	800	3000	5	80	11	300	60

Source: Adapted from Australian Nutrient Reference Values. Available at http://www.nrv.gov.au.

[a] 1 µg of retinol equivalents = 1 µg retinol, 1 µg retinol of all-*trans* retinol, 6 µg of all-*trans* β-carotene, 12 µg of all α-carotene, β-cryptoxanthin, and other provitamin A carotinoids. 1 international unit (IU retinol) = 0.3 µg of retinol equivalents.

[b] As cholecalciferol. 1 µg cholecalciferol = 0.2 µg 25(OH)D; 1 International unit (IU) = 0.025 µg of cholecalciferol = 0.005 µg 25(OH)D.

[c] 1 mg of α-tocopherol equivalents = 0.91 *RRR*-α-tocopherol acetate = 0.81 *RRR*-α-tocopherol acid succinate = 0.74 all-rac-DL-α-tocopherol = 0.67 *aa*-rac-DL-α-tocopherol acetate = (0.25–0.40) D-β-tocopherol = 0.1 D-γ-tocopherol = (0.25–0.30) α-tocotrienol.

TABLE A.4
Australian Tables: Micronutrients

Lifestyle Stage	Calcium (mg/day) RDI	Calcium (mg/day) EAR	Calcium (mg/day) UL	Copper (mg/day) AI	Copper (mg/day) UL	Fluoride[a] (mg/day) AI	Fluoride[a] (mg/day) UL	Iron (mg/day) RDI	Iron (mg/day) EAR	Magnesium (mg/day) RDI	Magnesium (mg/day) EAR	Magnesium[b] (mg/day) UL	Manganese (mg/day) AI
Males													
14–18 years	1300	1050	2500	1.5	8	3	10	11	8	410	340	350	3.5
19–30 years	1000	840	2500	1.7	10	4	10	8	6	400	330	350	5.5
31–50 years	1000	840	2500	1.7	10	4	10	8	6	420	350	350	5.5
51–70 years	1000	840	2500	1.7	10	4	10	8	6	420	350	350	5.5
>70 years	1300	1100	2500	1.7	10	4	10	8	6	420	350	350	5.5
Females													
14–18 years	1300	1050	2500	1.1	8	3	10	15	8	360	300	350	3
19–30 years	1000	840	2500	1.2	10	3	10	18	8	310	255	350	5
31–50 years	1000	840	2500	1.2	10	3	10	18	8	320	265	350	5
51–70 years	1300	1100	2500	1.2	10	3	10	8	5	320	265	350	5
>70 years	1300	1100	2500	1.2	10	3	10	8	5	320	265	350	5
Pregnancy													
<18 years	1300	1050	2500	1.2	8	3	10	27	23	400	335	350	5
19–30 years	1000	840	2500	1.3	10	3	10	27	22	350	290	350	5
30–50 years	1000	840	2500	1.3	10	3	10	27	22	360	300	350	5
Lactation													
<18 years	1300	1050	2500	1.4	8	3	10	10	7	360	300	350	5
19–30 years	1000	840	2500	1.5	10	3	10	9	6.5	310	255	350	5
30–50 years	1000	840	2500	1.5	10	3	10	9	6.5	320	265	350	5

Source: Adapted from Australian Nutrient Reference Values. Available at http://www.nrv.gov.au.

[a] 1 mmol of fluoride = 19 mg of fluoride.

[b] Upper limit as a supplement.

TABLE A.5
Australian Tables: Micronutrients

Lifestyle Stage	Phosphorus (mg/day) RDI	Phosphorus (mg/day) EAR	Potassium (mg/day) AI	Copper (mg/day) UL	Sodium (mg/day) AI	Sodium (mg/day) UL	Zinc (mg/day) RDI	Zinc (mg/day) EAR	Zinc (mg/day) UL
Males									
14–18 years	1250	1055	3600	8	460–920	2300	13	11	35
19–30 years	1000	580	3800	10	460–920	2300	14	12	40
31–50 years	1000	580	3800	10	460–920	2300	14	12	40
51–70 years	1000	580	3800	10	460–920	2300	14	12	40
>70 years	1000	580	3800	10	460–920	2300	14	12	40
Females									
14–18 years	1250	1055	2600	8	460–920	2300	7	6	35
19–30 years	1000	580	2800	10	460–920	2300	8	6.5	40
31–50 years	1000	580	2800	10	460–920	2300	8	6.5	40
51–70 years	1000	580	2800	10	460–920	2300	8	6.5	40
>70 years	1000	580	2800	10	460–920	2300	8	6.5	40
Pregnancy									
<18 years	1250	1055	2800	8	460–920	2300	10	8.5	35
19–30 years	1000	580	2800	10	460–920	2300	11	9	40
30–50 years	1000	580	2800	10	460–920	2300	11	9	40
Lactation									
<18 years	1250	1055	3200	8	460–920	2300	11	9	35
19–30 years	1000	580	3200	10	460–920	2300	12	10	40
30–50 years	1000	580	3200	10	460–920	2300	12	10	40

Source: Adapted from Australian Nutrient Reference Values. Available at http://www.nrv.gov.au.

TABLE A.6
Australian Tables: Micronutrients

Lifestyle Stage	Chromium (µg/day) AI	Iodine (µg/day) RDI	Iodine (µg/day) EAR	Iodine (µg/day) UL	Molybdenum (µg/day) RDI	Molybdenum (µg/day) EAR	Molybdenum (µg/day) UL	Selenium (µg/day) RDI	Selenium[a] (µg/day) EAR	Selenium[a] (µg/day) UL
Males										
14–18 years	35	150	95	900	43	33	1700	70	60	400
19–30 years	35	150	100	1100	45	34	2000	70	60	400
31–50 years	35	150	100	1100	45	34	2000	70	60	400
51–70 years	35	150	100	1100	45	34	2000	70	60	400
>70 years	35	150	100	1100	45	34	2000	70	60	400
Females										
14–18 years	25	150	95	900	43	33	1700	60	50	400
19–30 years	25	150	100	1100	45	34	2000	60	50	400
31–50 years	25	150	100	1100	45	34	2000	60	50	400
51–70 years	25	150	100	1100	45	34	2000	60	50	400
>70 years	25	150	100	1100	45	34	2000	60	50	400
Pregnancy										
<18 years	30	220	160	900	50	40	1700	65	55	400
19–30 years	30	220	160	1100	50	40	2000	65	55	400
30–50 years	30	220	160	1100	50	40	2000	65	55	400
Lactation										
<18 years	45	270	190	900	50	35	1700	75	65	400
19–30 years	45	270	190	1100	50	36	2000	75	65	400
30–50 years	45	270	190	1100	50	36	2000	75	65	400

Source: Adapted from Australian Nutrient Reference Values. Available at http://www.nrv.gov.au.

[a] 1 mmol of selenium = 76 mg of selenium.

TABLE A.7
US Tables

Lifestyle Stage	Protein (g/kg/day) EAR	Protein[a] (g/day) RDA	Carbohydrates (g/day) EAR	Carbohydrates (g/day) RDA	Fiber (g/day) AI	Linolenic Acid (g/day) AI	α-Linolenic Acid (g/day) AI	Water[b] AI
Males								
14–18 years	0.73	52	100	130	38	16	1.6	3.3
19–30 years	0.66	56	100	130	38	17	1.6	3.7
31–50 years	0.66	56	100	130	38	17	1.6	3.7
51–70 years	0.66	56	100	130	30	14	1.6	3.7
>70 years	0.66	56	100	130	30	14	1.6	3.7
Females								
14–18 years	0.71	46	100	130	26	11	1.1	2.3
19–30 years	0.66	46	100	130	25	12	1.1	2.7
31–50 years	0.66	46	100	130	25	12	1.1	2.7
51–70 years	0.66	46	100	130	21	11	1.1	2.7
>70 years	0.66	46	100	130	21	11	1.1	2.7
Pregnancy								
<18 years	0.88	71	135	175	28	13	1.4	3
19–30 years	0.88	71	135	175	28	13	1.4	3
30–50 years	0.88	71	135	175	28	13	1.4	3
Lactation								
<18 years	1.05	71	160	210	29	13	1.3	3.8
19–30 years	1.05	71	160	210	29	13	1.3	3.8
30–50 years	1.05	71	160	210	29	13	1.3	3.8

Source: Adapted from the US Department of Agriculture. Dietary Reference Intakes. Available at https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables.

[a] Based on grams of protein per kilogram of body weight for the reference body weight, e.g., for adults 0.8 g/kg body weight for the reference body weight.

[b] Total water includes all water contained in food, beverages, and drinking water.

TABLE A.8
US Tables

Lifestyle Stage	Biotin (µg/day) AI	Choline (mg/day) AI	Choline[a] (mg/day) UL	Folate[b] (µg/day) EAR	Folate[b] (µg/day) RDA	Folate[b] (µg/day) UL	Niacin[c] (mg/day) EAR	Niacin[c] (mg/day) RDA	Niacin[c] (mg/day) UL	Pantothenic Acid (mg/day) AI	Riboflavin (mg/day) RDA	Riboflavin (mg/day) AI	Thiamin (mg/day) EAR	Thiamin (mg/day) RDA
Males														
14–18 years	25	550	3	330	400	800	12	16	30	5	1.1	1.3	1.0	1.2
19–30 years	30	550	3.5	320	400	1000	12	16	35	5	1.1	1.3	1.0	1.2
31–50 years	30	550	3.5	320	400	1000	12	16	35	5	1.1	1.3	1.0	1.2
51–70 years	30	550	3.5	320	400	1000	12	16	35	5	1.1	1.3	1.0	1.2
>70 years	30	550	3.5	320	400	1000	12	16	35	5	1.1	1.3	1.0	1.2
Females														
14–18 years	25	400	3	330	400	800	11	14	30	5	0.9	1	0.9	1
19–30 years	30	425	3.5	320	400	1000	11	14	35	5	0.9	1.1	0.9	1.1
31–50 years	30	425	3.5	320	400	1000	11	14	35	5	0.9	1.1	0.9	1.1
51–70 years	30	425	3.5	320	400	1000	11	14	35	5	0.9	1.1	0.9	1.1
>70 years	30	425	3.5	320	400	1000	11	14	35	5	0.9	1.1	0.9	1.1
Pregnancy														
<18 years	30	450	3	520	600	800	14	18	30	6	1.2	1.4	1.2	1.4
19–30 years	30	450	3.5	520	600	1000	14	18	35	6	1.2	1.4	1.2	1.4
30–50 years	30	450	3.5	520	600	1000	14	18	35	6	1.2	1.4	1.2	1.4
Lactation														
<18 years	35	550	3	450	500	800	13	17	30	7	1.3	1.6	1.2	1.4
19–30 years	35	550	3.5	450	500	1000	13	17	35	7	1.3	1.6	1.2	1.4
30–50 years	35	550	3.5	450	500	1000	13	17	35	7	1.3	1.6	1.2	1.4

Source: Adapted from the US Department of Agriculture. Dietary Reference Intakes. Available at https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables.

[a] Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

[b] As dietary folate equivalents (DFE). 1 DFE = 1 µg of food folate = 0.6 µg of folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a supplement taken on an empty stomach.

[c] As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan.

TABLE A.9
US Tables

Lifestyle Stage	Vitamin C (mg/day) EAR	Vitamin C (mg/day) RDA	Vitamin B_6 (mg/day) EAR	Vitamin B_6 (mg/day) UL	Vitamin B_{12} (μg/day) EAR	Vitamin B_{12} (μg/day) RDA	Vitamin A (μg/day) EAR	Vitamin A[a] (μg/day) RDA	Vitamin D[b,c] (μg/day) AI	Vitamin E[d] (mg/day) EAR	Vitamin E[d] (mg/day) AI	Vitamin K (μg/day) RDA
Males												
14–18 years	63	75	1.1	80	2	2.4	630	900	15	12	15	75
19–30 years	75	90	1.1	100	2	2.4	625	900	15	12	15	120
31–50 years	75	90	1.1	100	2	2.4	625	900	15	12	15	120
51–70 years	75	90	1.4	100	2	2.4	625	900	15	12	15	120
>70 years	75	90	1.4	100	2	2.4	625	900	20	12	15	120
Females												
14–18 years	56	65	1	80	2	2.4	485	700	15	12	15	75
19–30 years	60	75	1.1	100	2	2.4	500	700	15	12	15	90
31–50 years	60	75	1.1	100	2	2.4	500	700	15	12	15	90
51–70 years	60	75	1.3	100	2	2.4	500	700	15	12	15	90
>70 years	60	75	1.3	100	2	2.4	500	700	20	12	15	90
Pregnancy												
<18 years	66	80	1.6	80	2.2	2.6	530	750	15	12	15	75
19–30 years	70	85	1.6	100	2.2	2.6	550	770	15	12	15	90
30–50 years	70	85	1.6	100	2.2	2.6	550	770	15	12	15	90
Lactation												
<18 years	96	115	1.7	80	2.4	2.8	885	1200	15	16	19	75
19–30 years	100	120	1.7	100	2.4	2.8	900	1300	15	16	19	90
30–50 years	100	120	1.7	100	2.4	2.8	900	1300	15	16	19	90

Source: Adapted from the US Department of Agriculture. Dietary Reference Intakes. Available at https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables.

[a] As retinol activity equivalents (RAEs). 1 RAE = 1 μg of retinol, 12 μg of β-carotene, 24 μg of α-carotene, or 24 μg of β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is 2-fold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.

[b] As cholecalciferol. 1 μg of cholecalciferol = 40 IU vitamin D.

[c] Under the assumption of minimal sunlight.

[d] As α-tocopherol. α-Tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the 2*R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the 2*S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements.

TABLE A.10
US Tables

Lifestyle Stage	Potassium (g/day) AI	Sodium (g/day) AI	Calcium (mg/day) EAR	Calcium (mg/day) RDA	Calcium (mg/day) UL	Fluoride (mg/day) AI	Iron (mg/day) EAR	Iron (mg/day) RDA	Iron (mg/day) UL	Magnesium (mg/day) EAR	Magnesium (mg/day) RDA	Magnesium[a] (mg/day) UL
Males												
14–18 years	4.7	1.5	1100	1300	3000	3	7.7	11	45	340	410	350
19–30 years	4.7	1.5	800	1000	2500	4	6	8	45	330	400	350
31–50 years	4.7	1.5	800	1000	2500	4	6	8	45	350	420	350
51–70 years	4.7	1.3	800	1000	2500	4	6	8	45	350	420	350
>70 years	4.7	1.2	1000	1200	2500	4	6	8	45	350	420	350
Females												
14–18 years	4.7	1.5	1100	1300	3000	3	7.9	15	45	300	360	350
19–30 years	4.7	1.5	800	1000	2500	3	8.1	18	45	255	310	350
31–50 years	4.7	1.5	800	1000	2500	3	8.1	18	45	265	320	350
51–70 years	4.7	1.3	1000	1200	2500	3	5	8	45	265	320	350
>70 years	4.7	1.2	1000	1200	2500	3	5	8	45	265	320	350
Pregnancy												
<18 years	4.7	1.5	1000	1300	3000	3	23	27	45	335	400	350
19–30 years	4.7	1.5	800	1000	2500	3	22	27	45	290	350	350
30–50 years	4.7	1.5	800	1000	2500	3	22	27	45	300	360	350
Lactation												
<18 years	5.1	1.5	1000	1300	3000	3	7	10	45	300	360	350
19–30 years	5.1	1.5	800	1000	2500	3	6.5	9	45	255	310	350
30–50 years	5.1	1.5	800	1000	2500	3	6.5	9	45	265	320	350

Source: Adapted from the US Department of Agriculture. Dietary Reference Intakes. Available at https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables.

[a] The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.

TABLE A.11
US Tables

Lifestyle Stage	Manganese (mg/day) AI	Manganese (mg/day) UL	Phosphorus (mg/day) EAR	Phosphorus (mg/day) RDA	Phosphorus (mg/day) UL	Zinc (mg/day) EAR	Zinc (mg/day) UL	Chromium (µg/day) AI	Copper (µg/day) EAR	Copper (µg/day) RDA	Copper (µg/day) UL
Males											
14–18 years	2.2	9	1055	1250	4	8.5	34	35	685	890	8000
19–30 years	2.3	11	580	700	4	9.4	40	35	700	900	10,000
31–50 years	2.3	11	580	700	4	9.4	40	35	700	900	10,000
51–70 years	2.3	11	580	700	4	9.4	40	30	700	900	10,000
>70 years	2.3	11	580	700	3	9.4	40	30	700	900	10,000
Females											
14–18 years	1.6	9	1055	1250	4	7.3	34	24	685	890	8000
19–30 years	1.8	11	580	700	4	6.8	40	25	700	900	10,000
31–50 years	1.8	11	580	700	4	6.8	40	25	700	900	10,000
51–70 years	1.8	11	580	700	4	6.8	40	20	700	900	10,000
>70 years	1.8	11	580	700	3	6.8	40	20	700	900	10,000
Pregnancy											
<18 years	2	9	1055	1250	3.5	10.5	34	29	785	1000	8000
19–30 years	2	11	580	700	3.5	9.5	40	30	800	1000	10,000
30–50 years	2	11	580	700	3.5	9.5	40	30	800	1000	10,000
Lactation											
<18 years	2.6	9	1055	1250	4	10.9	34	44	985	1300	8000
19–30 years	2.6	11	580	700	4	10.4	40	45	1000	1300	10,000
30–50 years	2.6	11	580	700	4	10.4	40	45	1000	1300	10,000

Source: Adapted from the US Department of Agriculture. Dietary Reference Intakes. Available at https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables.

TABLE A.12
US Tables

Lifestyle Stage	Iodine (µg/day) EAR	Iodine (µg/day) RDA	Iodine (µg/day) UL	Molybdenum (µg/day) EAR	Molybdenum (µg/day) RDA	Molybdenum (µg/day) UL	Selenium (µg/day) EAR	Selenium (µg/day) RDA	Selenium (µg/day) UL
Males									
14–18 years	95	150	900	33	43	1700	45	55	400
19–30 years	95	150	1100	34	45	2000	45	55	400
31–50 years	95	150	1100	34	45	2000	45	55	400
51–70 years	95	150	1100	34	45	2000	45	55	400
>70 years	95	150	1100	34	45	2000	45	55	400
Females									
14–18 years	95	150	900	33	43	1700	45	55	400
19–30 years	95	150	1100	34	45	2000	45	55	400
31–50 years	95	150	1100	34	45	2000	45	55	400
51–70 years	95	150	1100	34	45	2000	45	55	400
>70 years	95	150	1100	34	45	2000	45	55	400
Pregnancy									
<18 years	160	220	900	40	50	1700	49	60	400
19–30 years	160	220	1100	40	50	2000	49	60	400
30–50 years	160	220	1100	40	50	2000	49	60	400
Lactation									
<18 years	209	290	900	35	50	1700	59	70	400
19–30 years	209	290	1100	36	50	2000	59	70	400
30–50 years	209	290	1100	36	50	2000	59	70	400

Source: Adapted from the US Department of Agriculture. Dietary Reference Intakes. Available at https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables.

TABLE A.13
European Tables

Lifestyle Stage	Protein (g/day) RDA	Carbohydrates (g/day) EAR	Carbohydrates (g/day) RDA	Fiber (g/day) AI	Linolenic Acid (g/day) RDI/AI[a]	α-Linolenic Acid (g/day) AI	Water (Food and Liquid; L/day) AI
			Males				
14–18 years	52	100	130	38	16[a]	1.6	3.3
19–30 years	56	100	130	38	17[a]	1.6	3.7
31–50 years	56	100	130	38	17[a]	1.6	3.7
51–70 years	56	100	130	30	14[a]	1.6	3.7
>70 years	56	100	130	30	14[a]	1.6	3.7
			Females				
14–18 years	46	100	130	26	11	1.1	2.3
19–30 years	46	100	130	25	12	1.1	2.7
31–50 years	46	100	130	25	12	1.1	2.7
51–70 years	46	100	130	21	11	1.1	2.7
>70 years	46	100	130	21	11	1.1	2.7
			Pregnancy				
<18 years	71	130	175	28	13[a]	1.4	3
19–30 years	71	130	175	28	13[a]	1.4	3
30–50 years	71	130	175	28	13[a]	1.4	3
			Lactation				
<18 years	71	160	210	29	13[a]	1.3	3.8
19–30 years	71	160	210	29	13[a]	1.3	3.8
30–50 years	71	160	210	29	13[a]	1.3	3.8

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

[a] Indicates AI.

TABLE A.14
European Tables

Lifestyle Stage	Biotin (µg/day) AI	Choline (mg/day) AI	Choline (mg/day) UL	Folate (µg/day) EAR	Folate (µg/day) RDA	Folate (µg/day) UL	Niacin (mg/day) EAR	Niacin (mg/day) RDA	Niacin (mg/day) UL	Pantothenic Acid (mg/day) AI	Riboflavin (mg/day) EAR	Riboflavin (mg/day) RDA
Males												
14–18 years	25	550	3000	330	400	1000	12	16	30	5	1.1	1.3
19–30 years	30	550	3500	320	400	1000	12	16	35	5	1.1	1.3
31–50 years	30	550	3500	320	400	1000	12	16	35	5	1.1	1.3
51–70 years	30	550	3500	320	400	1000	12	16	35	5	1.1	1.3
>70 years	30	550	3500	320	400	1000	12	16	35	5	1.1	1.3
Females												
14–18 years	25	400	3000	330	400	1000	11	14	30	5	0.9	1.0
19–30 years	30	425	3500	320	400	1000	11	14	35	5	0.9	1.1
31–50 years	30	425	3500	320	400	1000	11	14	35	5	0.9	1.1
51–70 years	30	425	3500	320	400	1000	11	14	35	5	0.9	1.1
>70 years	30	425	3500	320	400	1000	11	14	35	5	0.9	1.1
Pregnancy												
<18 years	30	450	3500	520	600	1000	14	18	35	6	1.2	1.4
19–30 years	30	450	3500	520	600	1000	14	18	35	6	1.2	1.4
30–50 years	30	450	3500	520	600	1000	14	18	35	6	1.2	1.4
Lactation												
<18 years	35	550	3500	450	500	1000	13	17	35	7	1.3	1.6
19–30 years	35	550	3500	450	500	1000	13	17	35	7	1.3	1.6
30–50 years	35	550	3500	450	500	1000	13	17	35	7	1.3	1.6

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

TABLE A.15
European Tables

Lifestyle Stage	Thiamin (mg/day) EAR	Thiamin (mg/day) RDI	Vitamin B_6 (mg/day) EAR	Vitamin B_6 (mg/day) RDA	Vitamin B_6 (mg/day) UL	Vitamin C (mg/day) EAR	Vitamin C (mg/day) RDA	Vitamin C (mg/day) UL	Vitamin B_{12} (µg/day) EAR	Vitamin B_{12} (µg/day) RDA
Males										
14–18 years	1.1	1.3	1.1	1.3	100	63	75	1800	2	2.4
19–30 years	1.1	1.3	1.1	1.3	100	75	90	2000	2	2.4
31–50 years	1.1	1.3	1.1	1.3	100	75	90	2000	2	2.4
51–70 years	1.1	1.3	1.4	1.7	100	75	90	2000	2	2.4
>70 years	1.1	1.3	1.4	1.7	100	75	90	2000	2	2.4
Females										
14–18 years	0.9	1.0	1.0	1.2	100	56	65	1800	2	2.4
19–30 years	0.9	1.1	1.1	1.3	100	60	75	2000	2	2.4
31–50 years	0.9	1.1	1.1	1.3	100	60	75	2000	2	2.4
51–70 years	0.9	1.1	1.3	1.5	100	60	75	2000	2	2.4
>70 years	0.9	1.1	1.3	1.5	100	60	75	2000	2	2.4
Pregnancy										
<18 years	1.2	1.4	1.6	1.9	100	66	80	1800	2.2	2.6
19–30 years	1.2	1.4	1.6	1.9	100	70	85	2000	2.2	2.6
30–50 years	1.2	1.4	1.6	1.9	100	70	85	2000	2.2	2.6
Lactation										
<18 years	1.2	1.5	1.7	2	100	96	115	1800	2.4	2.8
19–30 years	1.2	1.5	1.7	2	100	100	120	2000	2.4	2.8
30–50 years	1.2	1.5	1.7	2	100	100	120	2000	2.4	2.8

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

TABLE A.16
European Tables

Lifestyle Stage	Vitamin A (μg/day) EAR	Vitamin A (μg/day) RDA	Vitamin A (μg/day) UL	Vitamin D (μg/day) AI	Vitamin D (μg/day) UL	Vitamin E (mg/day) EAR	Vitamin E (mg/day) RDA	Vitamin E (mg/day) UL	Vitamin K (μg/day) AI
Males									
14–18 years	630	900	2800	5	50	12	15	800	75
19–30 years	625	900	3000	5	50	12	15	1000	120
31–50 years	625	900	3000	5	50	12	15	1000	120
51–70 years	625	900	3000	10	50	12	15	1000	120
>70 years	625	900	3000	15	50	12	15	1000	120
Females									
14–18 years	485	700	2800	5	50	12	15	800	75
19–30 years	500	700	3000	5	50	12	15	1000	90
31–50 years	500	700	3000	5	50	12	15	1000	90
51–70 years	500	700	3000	10	50	12	15	1000	90
>70 years	500	700	3000	15	50	12	15	1000	90
Pregnancy									
<18 years	530	750	2800	5	50	12	15	800	75
19–30 years	550	770	3000	5	50	12	15	1000	90
30–50 years	550	770	3000	5	50	12	15	1000	90
Lactation									
<18 years	880	1200	2800	5	50	16	19	800	75
19–30 years	900	1300	3000	5	50	16	19	1000	90
30–50 years	900	1300	3000	5	50	16	19	1000	90

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

TABLE A.17
European Tables

Lifestyle Stage	Potassium (g/day) AI	Sodium (g/day) AI	Calcium (mg/day) AI	Calcium (mg/day) UL	Fluoride (mg/day) AI	Fluoride (mg/day) UL	Iron (mg/day) EAR	Iron (mg/day) RDA	Iron (mg/day) UL	Magnesium (mg/day) EAR	Magnesium (mg/day) RDA	Magnesium (mg/day) UL
Males												
14–18 years	4.7	1.5	1300	2500	3.2	10	7.7	11	45	340	410	350
19–30 years	4.7	1.5	1000	2500	3.8	10	6	8	45	330	400	350
31–50 years	4.7	1.5	1000	2500	3.8	10	6	8	45	330	420	350
51–70 years	4.7	1.3	1200	2500	3.8	10	6	8	45	330	420	350
>70 years	4.7	1.2	1200	2500	3.8	10	6	8	45	330	420	350
Females												
14–18 years	4.7	1.5	1300	2500	2.9	10	7.9	15	45	300	360	350
19–30 years	4.7	1.5	1000	2500	3.1	10	8.1	18	45	255	310	350
31–50 years	4.7	1.5	1000	2500	3.1	10	8.1	18	45	265	320	350
51–70 years	4.7	1.3	1200	2500	3.1	10	5	8	45	265	320	350
>70 years	4.7	1.2	1200	2500	3.1	10	5	8	45	265	320	350
Pregnancy												
<18 years	4.7	1.5	1300	2500	2.9	10	23	27	45	335	400	350
19–30 years	4.7	1.5	1000	2500	3.1	10	22	27	45	290	350	350
30–50 years	4.7	1.5	1000	2500	3.1	10	22	27	45	300	360	350
Lactation												
<18 years	5.1	1.5	1300	2500	2.9	10	7	10	45	300	360	350
19–30 years	5.1	1.5	1000	2500	3.1	10	6.5	9	45	255	310	350
30–50 years	5.1	1.5	1000	2500	3.1	10	6.5	9	45	265	320	350

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

TABLE A.18
European Tables

Lifestyle Stage	Manganese (mg/day) AI	Phosphorus (mg/day) EAR	Phosphorus (mg/day) RDI	Phosphorus (g/day) UL	Zinc (mg/day) EAR	Zinc (mg/day) RDA	Zinc (mg/day) UL	Chromium (µg/day) AI	Copper (µg/day) EAR	Copper (µg/day) RDA
Males										
14–18 years	2.2	1055	1250	4	8.5	11	34	35	685	890
19–30 years	2.3	580	700	4	9.4	11	40	35	700	900
31–50 years	2.3	580	700	4	9.4	11	40	35	700	900
51–70 years	2.3	580	700	4	9.4	11	40	30	700	900
>70 years	2.3	580	700	4	9.4	11	40	30	700	900
Females										
14–18 years	1.6	1055	1250	4	7.3	9	34	24	685	890
19–30 years	1.8	580	700	4	6.8	8	40	25	700	900
31–50 years	1.8	580	700	4	6.8	8	40	25	700	900
51–70 years	1.8	580	700	4	6.8	8	40	20	700	900
>70 years	1.8	580	700	4	6.8	8	40	20	700	900
Pregnancy										
<18 years	2	1055	1250	3.5	10.5	12	34	29	785	1000
19–30 years	2	580	700	3.5	9.5	11	40	30	800	1000
30–50 years	2	580	700	3.5	9.5	11	40	30	800	1000
Lactation										
<18 years	2.6	1055	1250	4	10.9	13	34	44	985	1300
19–30 years	2.6	580	700	4	10.4	12	40	45	1000	1300
30–50 years	2.6	580	700	4	10.4	12	40	45	1000	1300

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

TABLE A.19
European Tables

Lifestyle Stage	Iodine (µg/day) EAR	Iodine (µg/day) RDA	Iodine (µg/day) UL	Molybdenum (µg/day) EAR	Molybdenum (µg/day) RDA	Selenium (µg/day) EAR	Selenium (µg/day) RDA	Selenium (µg/day) UL
				Males				
14–18 years	95	150	900	33	43	45	55	400
19–30 years	95	150	1100	34	45	45	55	400
31–50 years	95	150	1100	34	45	45	55	400
51–70 years	95	150	1100	34	45	45	55	400
>70 years	95	150	1100	34	45	45	55	400
				Females				
14–18 years	95	150	900	33	43	45	55	400
19–30 years	95	150	1100	34	45	45	55	400
31–50 years	95	150	1100	34	45	45	55	400
51–70 years	95	150	1100	34	45	45	55	400
>70 years	95	150	1100	34	45	45	55	400
				Pregnancy				
<18 years	160	220	900	40	50	49	60	400
19–30 years	160	220	1100	40	50	49	60	400
30–50 years	160	220	1100	40	50	49	60	400
				Lactation				
<18 years	209	290	900	35	50	59	70	400
19–30 years	209	290	1100	36	50	59	70	400
30–50 years	209	290	1100	36	50	59	70	400

Source: Adapted from European Dietary Reference Values. Available at http://www.efsa.europa.eu/en/press/news/nda100326.htm.

TABLE A.20
Indian Tables

Lifestyle Stage	Protein (g/day) RDA	Total Visible Fat (g/day) RDA	Folate (μg/day) RDA	Niacin (mg/day) RDA	Riboflavin (mg/day) RDA	Thiamin (mg/day) RDA	Vitamin B_6 (mg/day) RDA	Vitamin C (mg/day) RDA	Vitamin B_{12} (μg/day) RDA	Vitamin A[a] (μg/day) RDA
Males										
14–18 years	54.3–61.5	25	150–200	16–18	1.6–1.8	1.4–1.5	2.0	40	0.2–1.0	600
19–30 years	60	20	200	16–21	1.4–2.1	1.2–1.7	2.0	40	1.0	600
31–50 years	60	20	200	16–21	1.4–2.1	1.2–1.7	2.0	40	1.0	600
51–70 years	60	20	200	16–21	1.4–2.1	1.2–1.7	2.0	40	1.0	600
>70 years	60	20	200	16–21	1.4–2.1	1.2–1.7	2.0	40	1.0	600
Females										
14–18 years	51.9–55.5	25	150–200	14	1.4–1.2	1.2–1.0	2.0	40	0.2–1.0	
19–30 years	55	20	200	12–16	1.1–1.7	1.0–1.4	2.0	40	1.0	600
31–50 years	55	20	200	12–16	1.1–1.7	1.0–1.4	2.0	40	1.0	600
51–70 years	55	20	200	12–16	1.1–1.7	1.0–1.4	2.0	40	1.0	600
>70 years	55	20	200	12–16	1.1–1.7	1.0–1.4	2.0	40	1.0	600
Pregnancy										
<18 years	78	20	500	14–18	1.2–1.6	1.2–1.6	2.5	60	1.2	800
19–30 years	78	20	500	14–18	1.2–1.6	1.2–1.6	2.5	60	1.2	800
30–50 years	78	20	500	14–18	1.2–1.6	1.2–1.6	2.5	60	1.2	800
Lactation										
<18 years	74–68	20	300	16–20	1.3–1.7	1.3–1.7	2.5	80	1.5	950
19–30 years	74–68	20	300	16–20	1.3–1.7	1.3–1.7	2.5	80	1.5	950
30–50 years	74–68	20	300	16–20	1.3–1.7	1.3–1.7	2.5	80	1.5	950

Source: Adapted from Recommended dietary allowances for Indians. Available at http://icmr.nic.in/final/RDA-2010.pdf.

[a] Retinol.

TABLE A.21
Indian Tables

Lifestyle Stage	Calcium (mg/day) RDA	Iron (mg/day) RDA	Magnesium (mg/day) RDA	Potassium (mg/day) RI	Sodium (mg/day) RI	Zinc (mg/day) RDA	Iodine (µg/day) RDR
				Males			
14–18 years	800	32–28	195	3750	2092	12	150
19–30 years	600	17	340	3750	2092	12	150
31–50 years	600	17	340	3750	2092	12	150
51–70 years	600	17	340	3750	2092	12	150
>70 years	600	17	340	3750	2092	12	150
				Females			
14–18 years	800	27–26	235	3225	1902	12	150
19–30 years	600	21	310	3225	1902	10	150
31–50 years	600	21	310	3225	1902	10	150
51–70 years	800	21	310	3225	1902	10	150
>70 years	800	21	310	3225	1902	10	150
				Pregnancy			
<18 years	1200	35	310	3225	1902	12	200
19–30 years	1200	35	310	3225	1902	12	200
30–50 years	1200	35	310	3225	1902	12	200
				Lactation			
<18 years	1200	25	310	3225	1902	12	200
19–30 years	1200	25	310	3225	1902	12	200
30–50 years	1200	25	310	3225	1902	12	200

Source: Adapted from Recommended dietary allowances for Indians. Available at http://icmr.nic.in/final/RDA-2010.pdf.

TABLE A.22
Southeast Asian Tables

Lifestyle Stage	Protein[a] (g/day) RDA	Folate (µg/day) RDA	Niacin (mg/day) RDA	Riboflavin (mg/day) RDA	Thiamin (mg/day) RDA	Iron[b] (mg/day) 75% RDA	Iron[c] (mg/day) 10% RDA	Vitamin C (mg/d) RDA	Vitamin A (µg/day) RDA	Vitamin D (µg/day) RDA	Calcium (mg/day) RDA	Zinc (mg/day) RDA	Iodine (µg/day) RDA	Selenium (mg/day) RDA
Males														
16–18 years	49	400	16	1.3	1.2	25.1	18.8	65	600	5	1000	8.6	150	32
19–30 years	48	400	16	1.3	1.1	18.3	13.7	70	600	5	700	6.5	150	34
31–49 years	48	400	16	1.3	1.1	18.3	13.7	70	600	5	700	6.5	150	34
50–59 years	48	400	16	1.3	1.1	18.3	13.7	70	600	10	1000	6.5	150	34
60–65 years	48	400	16	1.3	1.1	18.3	13.7	70	600	10	1000	6.5	150	34
>65 years		400	16	1.3	1.1	18.3	13.7	70	600	15	1000	6.5	150	33
Females														
16–18 years	40	400	16	1	1.2	41.3	31	65	600	5	1000	6.8	150	26
19–30 years	40	400	14	1.1	1.1	39.2	29.4	70	500	5	700	4.4	150	26
31–49 years	40	400	14	1.1	1.1	39.2	29.4	70	500	5	700	4.4	150	26
50–59 years	40	400	14	1.1	1.1	15.1	11.3	70	500	10	1000	4.4	150	26
60–65 years		400	14	1.1	1.1	15.1	11.3	70	500	10	1000	4.4	150	26
>65 years	40	400	14	1.1	1.1	15.1	11.3	70	600	15	1000	4.4	150	25
Pregnancy														
First trimester	46	600	18	1.4	1.4	15.1	11.3	80	800	5	1000	5.5	200	26
Second trimester	46	600	18	1.4	1.4	15.1	11.3	80	800	5	1000	7	200	28
Third trimester	46	600	18	1.4	1.4	15.1	11.3	80	800	5	1000	10	200	30
Lactation														
First trimester	56	500	17	1.6	1.5	20	15	95	850	5	1000	9.5–8.8	200	35
Second trimester	56	500	17	1.6	1.5	20	15	95	850	5	1000	7.2	200	42

Source: Adapted from Recommended dietary allowances harmonization in Southeast Asia. Available at http://apjcn.nhri.org.tw/server/APJCN/17%20Suppl%202/405.pdf.

[a] High-quality protein.

[b] 75% bioavailability.

[c] 10% bioavailability.

Appendix B: Websites for Key Position Statements on Diet and Fertility

PROFESSIONAL BODIES

American Dietetic Association Foundation
http://www.adaf.org

American Fertility Association
http://www.theafa.org

American Society for Nutrition
http://www.nutrition.org

Australian Nutrition Society
http://www.nsa.asn.au

British Dietetic Association
http://www.bda.uk.com

British Fertility Society
http://www.britishfertilitysociety.org.uk

British Nutrition Foundation
http://www.nutrition.org.uk

Dietitians Association of Australia (DAA)
http://daa.asn.au

European Academy of Nutritional Sciences (EANS)
http://www.iuns.org/affiliated_bodies/european-academy-of-nutritional-sciences-eans

European Federation of the Association of Dietitians (EFAD)
http://www.efad.org

Federation of African Nutrition Societies (FANUS)
http://www.iuns.org/affiliated.../federation-of-african-nutrition-societies-fanus

Federation of Asian Nutrition Societies (FANS)
http://www.fans-asia.org

Federation of European Nutrition Societies (FENS)
http://www.fensnutrition.eu

Fertility Europe
http://www.fertilityeurope.eu

Fertility Society of Australia
http://www.fertilitysociety.com.au

Indian Dietetic Association
http://www.idaindia.com

Indian Fertility Society
http://www.indianfertilitysociety.org

International Nutrition Foundation (INF)
http://www.inffoundation.org

International Union of Nutritional Sciences (IUNS)/Federation of Asian Nutrition Societies (FANS)
http://www.iuns.org/affiliated_bodies/federation-of-asian-nutrition-societies-fans

Nutrition Society of India
http://www.nutritionsocietyindia.org

The Nutrition Society of New Zealand
http://www.nutritionsociety.ac.nz

INTERNATIONAL ORGANIZATIONS

Australasian College of Nutritional and Environmental Medicine (ACNEM)
https://www.acnem.org

World Public Health Nutrition Association (WPHNA)
http://wphna.org

NUTRITION

Nutrition, Fertility, and Human Reproductive Function

One in six couples around the world experience infertility. Before undertaking expensive and intrusive assisted reproductive treatment such as *in vitro* fertilization, many seek advice from their physicians or dietitians on what foods and supplements might enhance their fertility. But health practitioners are often ill equipped to provide dietary recommendations in a scientifically based manner. ***Nutrition, Fertility, and Human Reproductive Function*** provides a comprehensive guide to clinicians on how they can best advise their patients to optimize fertility and reproductive function through optimal nutrition.

Taking a holistic or "whole-of-life" approach, the book reviews the role of nutrition in human fertility and explores its effect on male and female reproductive physiology. Problem-oriented topics are arranged in chapters that each cover a specific clinical topic of interest, allowing easy reference by the practicing clinician. From the female perspective, the book covers the role of nutrition on essential reproductive processes such as ovulation, early embryo development, implantation, and sexual function, together with nutrition's influence on the duration of the reproductive life span. In the male context, it examines the effect of nutrition on hormone and sperm production as well as sexual function. The book also includes information on evidence-based complementary health approaches such as Traditional Chinese Medicine (TCM) and naturopathy.

This book draws on the wide experience of several respected leaders in clinical nutrition who combine research expertise with clinical insight. The information contained herein will enable clinicians to make the best recommendations for their patients for optimizing fertility.

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