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RESEARCH ARTICLE

Association between protein intake, diet quality, and obesity in Australian adults: a comparison of measurement units

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Abstract

Previous investigations on protein associations with diet quality and obesity still have inconclusive findings, possibly due to how protein intake was expressed. This study aimed to compare how different ways of expressing total protein intake may influence its relationships with diet quality and obesity. Usual protein intake was estimated from the 2011–12 Australian National Nutrition and Physical Activity Survey ($n = 7637$ adults, ≥19 years), expressed in grams (g/d), percent energy (%EI), and grams per actual kilogram body weight (g/kgBW/d). Diet quality was assessed using the 2013 Dietary Guidelines Index, and obesity measures included Body Mass Index (BMI) and waist circumference (WC). Sex-stratified multiple linear and logistic regressions were performed and adjusted for potential confounders. Total protein (g/d) was directly associated with diet quality (males, β = 0.15 (95% CI 0.12, 0.19); females, β = 0.25 (0.22, 0.29)), and this association was consistent across units. Protein intake (g/d) was directly associated with BMI (males, β = 0.07% (0.04%, 0.11%); females, β = 0.09% (0.04%, 0.15%)), and WC (males, β = 0.04 (0.01, 0.06); females, β = 0.05 (0.00, 0.09)). While in males, protein as %EI was associated with higher WC, no association was found in females. Adults with higher protein intake (g/d) had higher odds of overweight/obesity (males, OR = 1.01 (1.00, 1.01); females, OR = 1.01 (1.00, 1.01)), and central overweight/obesity (females, OR = 1.01 (1.00, 1.01)), but no significant association with females odds of overweight/obesity when protein was expressed in %EI. In conclusion, protein intake was positively associated with diet quality and obesity, yet these associations were stronger for women. The effect sizes also varied by measurement unit due to the different scales of those units.

Key words: Dietary proteins: Diet quality: Obesity: Unit

Introduction

The global burden of cardiometabolic diseases is increasing and is mainly attributed to modifiable risk factors.[1,2] The global cases of cardiovascular diseases (CVDs) almost doubled from 271 million to 523 million in the 1990–2019 period,[1] while diabetes cases were predicted to increase from 476 million in 2017 to 570.9 million in 2025.[2] About one-third of Australians' disability-adjusted life years were attributed to modifiable risk factors, such as dietary risk factors and high body mass,[3] and the total CVD burden was attributed to several cardiometabolic risk factors including high blood pressure (36%), dietary risk (31%), and overweight/obesity (22%).[4]

Preventing obesity and related diseases requires successful weight management, in which protein plays a significant role. Protein has the most satiating effect compared to carbohydrates and fat, following the large increase of appetite-suppressing hormones (e.g. glucagon-like peptide-1) and the marked decrease of appetite-stimulating hormones (e.g. ghrelin).[5] In addition to its appetite-regulating effects, larger weight loss following high-protein diets is also attributed to the larger

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thermic effect, compared to other macronutrients.[5,6] High-protein diets can also delay obesity by preventing hyperphagia, particularly after high-fat feeding.[7]

Despite inconclusive associations between diet quality indices and obesity,[8] a growing number of studies reported a consistent association between protein intake and diet quality. Higher diet quality as assessed in observational studies using a variety of scores, such as the Healthy Eating Index (HEI), the Dietary Guidelines Index (DGI), and the Mediterranean-Dietary Approaches to Stop Hypertension Diet Intervention for Neurological Delay Index (MIND), were observed among American, Australian, and French adults consuming more protein.[9–15] Protein intake in those studies was expressed in different units, such as gram/day (g/d), per cent of energy intake (%EI), and g/kg body weight (BW)/d, but no single study compared the influence of ways of expressing protein intake on the association.

Each way of expressing protein intake, that is the protein units, may result in different interpretations of adequacy. For example, the current protein intake recommendation for adults is 0.83 g/kgBW/d, with the acceptable BW being based on either actual BW or median weight-for-height.[16] When those references are applied to individuals with obesity consuming adequate grams of protein, they will still be considered to have inadequate protein intake as the ratio between their absolute intake and BW is still lower than the reference value.[17] In fact, this dissimilarity was likely because of their higher actual BW or energy intake rather than absolute protein inadequacy.[17]

The association between protein intake with BMI and other cardiometabolic outcomes also varied across different units. The unit of g/d, %EI, and g/ideal BW/d suggested direct associations, with some inconsistencies across models, but the g/actual kgBW/d unit consistently produced inverse associations, which were considered spurious.[18] Hence, given the lack of consensus on which units should be used in examining protein association with diet quality and obesity, this study aimed to compare how different total protein units are associated with diet quality and obesity.

Methods

Sample and study design

This study was a secondary data analysis from the Australian National Nutrition and Physical Activity Survey (NNPAS) 2011–12, which was conducted across eight states and territories by the Australian Bureau of Statistics (ABS).[19] The survey design was a stratified multistage area of private dwellings with a probability sampling design.[19] The NNPAS included 12153 participants, but this study only focused on adults aged ≥19 years ($n = 9341$). Participants who identified themselves as pregnant and lactating women were excluded accounting for their possibility to consume restricted or unusual diets and potential impact on diet quality and weight. Adult participants with no data on anthropometric or dietary measurement were also excluded, which resulted in 7637 adults being analysed in this study.

Ethics statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethics approval for the ABS in conducting surveys, including the interview component of the NNPAS, was provided through the Census and Statistics Act 1905.[19] Informed consent was sought from all individual participants through the completion of a consent form.[19] All secondary data analyses in this study were conducted using deidentified data, and exemption from ethics review has been approved by the Deakin University Human Research Ethics Committee (DUHREC no. 2023-135).

Dietary assessment

The dietary data of NNPAS was collected through Computer Assisted Personal Interview (CAPI) for the first 24-h recalls by trained interviewers.[19] At least 8 d after CAPI, approximately 65% of overall adult survey respondents participated in the second 24-h recalls through Computer-Assisted Telephone Interview (CATI).[19] The 24-h recall adopted the USDA Automated Multiple 5-Pass Method, which divided the interview into five phases.[19] For each 24-h recall, participants were requested to report foods and beverages, eating occasions, amount, and time of consumption.[19] Further details were also probed, including brand names and preparation methods.[19] Each food and beverage was later coded, followed by the calculations of energy and nutrient intakes referring to the AUSNUT13 food nutrient database that comprises fifty-three nutrients.[20]

Protein and energy intake

Total protein (g) and energy intake (kJ) information was obtained from the dietary intake data of NNPAS, which was estimated from all food and beverage items. Dietary data from both 1- and 2-d 24-h recall was modelled using the Multiple Source Method[21] to estimate usual dietary intake. Total energy, protein, fat, and carbohydrates were modelled separately, with a number of recall days, age, sex, and age–sex interaction terms included in the models. Total protein intake in this study was reported in three measurement units, namely, g/d, g/kgBW/d, and %EI, considering their wide use to evaluate protein intake adequacy and assess protein contribution to total energy intake.

Australian Nutrient Reference Value used the age/gender-standardised BWs from the 1995 National Nutrition Survey,[22] which was similar to the standardised BWs of the US Dietary Reference Intake published prior to 2002.[23] Therefore, protein and other nutrient intake recommendations for Australian adults were based on standard BWs for the 19–30 years age group.[22] However, given that adult BW in most western populations is likely to increase because of increasing body fat,[22] g/kgBW/d in this study was calculated based on participants' actual BWs.

Diet quality

Diet quality was assessed using the DGI to measure adults' compliance with the 2013 Australian Dietary Guidelines.[24–26]

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The DGI consists of seven recommended dietary components (i.e. variety of foods, fruits, vegetables, cereals, meat and other high-protein foods, dairy products, and water) and six discouraged components (i.e. saturated fat, unsaturated fat, added salt, added sugar, discretionary foods, and alcohol).[25,26] Each item is scored between 0 and 10, resulting in 0–130 DGI scores with a higher score suggesting better diet quality.[26]

DGI score in this study was calculated using the disaggregated foods from ABS data, including fruit, vegetable, and protein food groups.[19] All DGI calculations only included non-discretionary foods, except for the discretionary foods component. Food variety was calculated using grams of consumed fruits, vegetables, whole grains, low-fat dairy, and lean meats and alternatives, as described elsewhere.[27] Number of daily servings was directly used to calculate DGI scores of fruits and vegetables, while grains and cereals score was calculated from daily servings of total cereals and whole-grain and refined-grain breads. Meats and alternatives scores were based on red meats, poultry, fish, eggs, nuts, tofu, legumes, and beans consumption, while dairy intake comprised milk, yoghurts, cheese, and alternatives. Total beverages included water, milk and soy beverages, smoothies, juices, low-calorie cordials and soft drinks, tea, and coffee.

The number of discretionary food servings was obtained by dividing energy intake from discretionary foods by 600 kJ.[28] Saturated fat intake score was estimated against lean red meats and poultry (<10% fat), and low-fat milk, while unsaturated fat intake included margarine, seeds, and nuts.[27] Added salt intake score was based on NNPAS questions on whether salt is added during cooking and meals.[25] To obtain the number of servings per day, grams of added sugar and alcohol intake were divided by five and ten, respectively.[20,28] The detailed construction of each DGI score component was provided in Supplementary Material 1.

Anthropometric measurements

BW, height, and waist circumference (WC) measurements were conducted by trained ABS staff during the interview.[19] BW was measured using digital scales (max. 150 kg), and a stadiometer (max. 210 cm) was used for height measurement.[19] A metal tape measure (max. 200 cm) was used to measure WC, and 10% of participants were randomly selected for additional measurement to validate the collected height and WC measurement data.[19] BMI scores were calculated by dividing weight (kg) by squared height $(m)^2$.[19] The overweight/obesity status was determined using BMI and WC as binary variables. Individuals with BMI ≥ 25 kg/m^2 were categorised as having overweight/obesity. Drawing on ABS categories of WC, female individuals with WC ≥ 80 cm or male individuals with WC ≥ 94 cm were classified as having central overweight/obesity.[19,29]

Covariates

Age, country of birth, Socio-economic Indexes for Areas (SEIFA), and physical activity level (PAL) were used as covariates, based on the previous literature.[15,30–33] Age data were reported in years, while country of birth was categorised as (a) Australia; (b) Mainly English-speaking countries; and (c) Other.[19] SEIFA ranked Australia's areas according to relative socioeconomic advantage and disadvantage, where the lower quintile indicated greater disadvantage.[34] Participants' PAL was categorised as meeting and not meeting physical activity guidelines of 150 min and 5 sessions/week.[19] This cut-off was based on Australia's Physical Activity and Sedentary Behaviour Guidelines, which recommend adults to regularly perform moderate-to-vigorous-intensity physical activity and strength training.[35]

Energy misreporting

Energy misreporting was estimated in this study due to previous findings on energy and protein underreporting in self-reported dietary intake.[36] Energy misreporting was examined using the ratio between reported energy intake (rEI) and predicted total energy expenditure (pTEE; rEI:pTEE), as has been done in previous studies.[37,38] pTEE was calculated using the validated equations of the Institute of Medicine, which considered sex, age, height, BW, and physical activity.[39] A low-active PAL was assumed ($1.4 \leq PAL < 1.6$) because the NNPAS did not measure overall physical activity.[37,38] Participants were categorised as plausible reporters, underreporters, or over-reporters of energy intake based on the published calculations for the ±1 SD cutoff for rEI:pTEE, incorporating the coefficient of variation (CV) of rEI, pTEE, and the technical error of measuring total energy expenditure (mTEE).[37,38] Among NNPAS adult participants, the CV_{rEI} for those having 1- and 2-d 24-h recall was 43.2% and 34.5%, respectively. The CV_{pTEE} was 17.6% for NNPAS adult participants with 1-d 24-h recall and 17.7% for those with 2 recall days. The CV_{mTEE} was 8.2%, as estimated by previous research using the doubly labelled water method.[40] This equation resulted in ±1 SD cutoff of 47% and 31% for individuals having 1- and 2-d 24-h recall, respectively.

Statistical analysis

Statistical analyses were performed using Stata v.17, with $P < 0.05$ considered statistically significant for all analyses. All statistical analyses in this study used the benchmarked survey weight to produce estimates for the population (i.e. replicate weights and person-level weights). Descriptive statistics comprising proportions and means with standard deviation were used to examine protein intake data, with differences across protein intake tertiles expressed in g/d were tested using one-way ANOVA and Chi-square test. All statistical analyses were stratified by sex to account for differences in protein requirements between men and women.[24]

The association between protein intake, expressed using the three different measurement units (g, % EI, g/kBW/d), and diet quality was assessed using separate multiple linear regression models. Model 1 was adjusted for age (continuous), country of birth (categorical), SEIFA (categorical), and PAL (categorical), while Model 2 was additionally adjusted for usual non-protein energy intake (continuous). The influence of energy misreporting on the association was examined in Model 3 by additionally adjusting for energy misreporting status (categorical).

Several tests were performed to test if linear regression models satisfy the assumptions of ordinary least squares linear regression. Added-value plots were used to check linear relationships between variables, and models were tested for multicollinearity using variance inflation factor. At this stage, usual fat and carbohydrate intakes were excluded from all models due to the multicollinearity issue (VIF > 5), and BMI outcome was natural-log transformed before stratification to improve the normality of residuals. Jackknife standard errors were estimated in the models to address heteroscedasticity as shown by both graphical (i.e. rvfplot command) and non-graphical (i.e. hettest command) methods.[41]

To investigate protein intake associations with obesity, multiple linear regression was conducted between total protein intake and continuous BMI and WC, and multiple logistic regression was conducted between total protein intake and binary variables of overweight/obesity and central overweight/obesity. Despite the wide use of g/kgBW/d unit in expressing protein intake recommendations,[16] both linear and logistic regressions were only performed using the units of g/d and %EI due to the potentially spurious associations between protein intake expressed in g/kgBW/d, cardiometabolic health, and body composition.[18,42] The first linear regression model for BMI and WC was adjusted for age (continuous), country of birth (categorical), SEIFA (categorical), and PAL (categorical). Model 2 was additionally adjusted for usual non-protein energy intake (kJ, continuous) to address the conditional dependency between protein and other macronutrients in causing obesity and to minimise the composite variable bias due to protein contribution to total energy intake.[43,44] For these reasons, a sensitive analysis adjusting for the usual total energy intake was also conducted and provided in Supplementary Material 2. Model 3 was the same as Model 2 with further adjustment for energy misreporting status (categorical). Using the same adjustments, three multiple logistic regression models were performed with binary variables of BMI and WC as the outcomes.

Results

A total of 7637 individuals were included in this study as summarised in Table 1 (male, $n = 3684$; female, $n = 3953$). There were significant differences in the characteristics of adult Australian males across protein intake tertiles. However, the country of birth and obesity measures of Australian females were not significantly different across tertiles. In both sexes, higher protein intake was associated with higher usual total energy intake, while most energy misreporters were in the lowest tertile of protein intake. Both males and females in the lowest tertile of protein intake had the lowest DGI score, and the detailed component score of DGI was provided in Supplementary Material 3.

Association between protein intake and diet quality

Regression models resulted in statistically significant associations between protein intake expressed in three units and diet quality ($P < 0.001$), as shown in Table 2. Each g/d higher protein intake was associated with 0.15 (95% CI (0.12, 0.19)) unit higher DGI among males, and 0.25 (95% CI (0.22, 0.29)) unit higher DGI among females. Each per cent higher energy intake from protein was associated with 0.94 (95% CI (0.75, 1.13)) and 1.23 (95% CI (1.08, 1.38)) unit higher DGI among males and females, respectively. For each g/kgBW/d higher protein intake among males and females, there was a 7.49 (95% CI (5.19, 9.79)) and 9.67 (95% CI (8.00, 11.35)) unit higher DGI, respectively. In all models and units, the associations were stronger in females than males.

Additional calculations using the average energy intake of Australian adult males in this analysis suggested a comparable increase in DGI using g/d and %EI units. Applying Model 3 to average 86-kg Australian adult males consuming 7939 kJ in a day, each gram higher protein intake is associated with a 0.15 DGI-units higher for males. Since a gram protein being equivalent to 17 kJ, a per cent higher daily energy intake from protein among males (79 kJ) equals to 4.67-g increase in protein, and this increase is correlated to 0.94 higher in DGI-unit. In other words, a g/d higher protein intake equates to 0.20 DGI-units higher among males.

Association between protein intake and obesity

Linear regression models also demonstrated direct associations between protein intake and measures of obesity as shown in Table 3. For each g/d higher protein intake of males and females, BMI was higher by 0.07% (95% CI (0.04%, 0.11%)) and 0.09% (95% CI (0.04%, 0.15%)) kg/m^2, respectively. Each per cent higher energy intake from protein was associated with 0.49% (95% CI (0.28%, 0.71%)) and 0.41% (95% CI (0.15%, 0.68%)) unit higher BMI among males and females, respectively. For each g/d higher protein intake, there was 0.04 cm (95% CI (0.01, 0.06)), and 0.05 cm (95% CI (0.00, 0.09)) higher in males and females WC, respectively. Each per cent higher energy intake from protein was associated with 0.26 cm (95% CI (0.11, 0.41)) higher WC among males only, but there was no association between protein intake expressed in %EI and WC among females. The sensitivity analysis adjusted for total energy intake instead of non-protein energy intake produced comparable coefficients, as provided in Supplementary Material 2.

Multiple logistic regressions suggested direct associations between protein intake and overweight/obesity status as shown in Table 4. Odds ratios (ORs) of overweight/obesity status using BMI categories were comparable between g/d and %EI, and the associations varied across models and sexes. After additional adjustment for nonprotein energy intake and misreporting status, the OR of obesity was higher among those consuming more protein in g/d (males, OR = 1.01, 95% CI (1.00, 1.01); females, OR = 1.01, 95% CI (1.00, 1.01)). The OR was also higher among males and females consuming a larger per cent of energy from protein, but an additional adjustment to misreporting status produced a non-significant association in females.

Multiple logistic regressions also suggested direct associations between protein intake and central overweight/obesity status using WC categories, particularly in females. The OR of central overweight/obesity was higher in females consuming more g/d

Table 1. Descriptive characteristics of adults (N = 7637) by tertiles of total protein intake (g/d)[a]

	Male (3684 (48.2))				Female (3953 (51.8))			
	T1	T2	T3	Pvalue	T1	T2	T3	P value
Demographic characteristics								
Age (year, mean (SD))	50.5 (17.7)	48.7 (16.9)	44.5 (15.8)	<0.001	50.0 (18.4)	50.0 (17.2)	48.3 (16.5)	0.02
Country of birth (*n* (%))				<0.001				0.20
Australia	807 (65.7)	893 (72.7)	856 (69.7)		914 (69.3)	960 (72.8)	963 (73.1)	
Mainly English-speaking countries	174 (14.2)	166 (13.5)	151 (12.3)		167 (12.7)	154 (11.7)	146 (11.1)	
Other	247 (20.1)	169 (13.8)	221 (18.0)		237 (18.0)	204 (15.5)	208 (15.8)	
SEIFA (*n* (%))				0.004				<0.001
Q1 (most disadvantaged)	252 (20.5)	206 (16.8)	198 (16.1)		292 (22.2)	257 (19.5)	219 (16.6)	
Q2	272 (22.1)	240 (19.5)	242 (19.7)		291 (22.1)	252 (19.1)	268 (20.3)	
Q3	233 (19.0)	249 (20.3)	257 (20.9)		253 (19.2)	246 (18.7)	294 (22.3)	
Q4	220 (17.9)	214 (17.4)	249 (20.3)		218 (16.5)	244 (18.5)	207 (15.7)	
Q5 (least disadvantaged)	251 (20.4)	319 (26.0)	282 (23.0)		264 (20.0)	319 (24.2)	329 (25.0)	
Physical activity (*n* (%))				0.006				0.002
Met physical activity guidelines	503 (41.0)	554 (45.1)	580 (47.2)		506 (38.4)	556 (42.2)	596 (45.3)	
Did not meet physical activity guidelines	725 (59.0)	674 (54.9)	648 (52.8)		812 (61.6)	762 (57.8)	721 (54.7)	
BMI (kg/m^2, mean (SD))	28.1 (5.0)	27.9 (4.8)	27.6 (4.5)	0.01	27.5 (6.3)	27.1 (6.0)	27.3 (6.2)	0.28
Obesity status (*n* (%))				0.02				0.46
Overweight/obese[b]	909 (74.0)	878 (71.5)	848 (69.1)		785 (59.6)	755 (57.3)	761 (57.8)	
Not overweight/obese	319 (26.0)	350 (28.5)	380 (30.9)		533 (40.4)	563 (42.7)	556 (42.2)	
WC (cm, mean (SD))	99.3 (13.2)	98.5 (13.0)	97.2 (12.5)	<0.001	89.1 (14.9)	88.2 (14.7)	88.9 (14.6)	0.29
Central obesity status (*n* (%))				<0.001				0.15
Centrally overweight/obese[c]	807 (65.7)	781 (63.6)	685 (55.8)		925 (70.2)	900 (68.3)	945 (71.8)	
Not centrally overweight/obese	421 (34.3)	447 (36.4)	543 (44.2)		393 (29.8)	418 (31.7)	372 (28.2)	
Dietary intake								
Usual energy intake (kJ/d, mean (SD))	6279 (1357)	7791 (1400)	9747 (2251)	<0.001	5142 (1171)	6420 (1162)	7851 (1780)	<0.001
Usual non-protein energy intake (kJ/d, mean (SD))	5283 (1290)	6454 (1378)	7893 (2045)	<0.001	4315 (1112)	5301 (1150)	6338 (1667)	<0.001
Usual protein intake (g/d, mean (SD))	59.7 (9.1)	80.1 (5.3)	111.1 (23.2)	<0.001	49.5 (7.8)	67.0 (4.2)	90.6 (15.6)	<0.001
Usual protein intake (g/kgBW/d, mean (SD))	0.7 (0.2)	1.0 (0.2)	1.3 (0.4)	<0.001	0.7 (0.2)	1.0 (0.2)	1.3 (0.4)	<0.001
Usual protein intake (% of energy, mean (SD))	16.3 (3.2)	17.7 (3.1)	19.5 (3.9)	<0.001	16.6 (3.2)	18.0 (3.2)	19.9 (4.2)	<0.001
Diet quality score (mean (SD))	65.8 (13.6)	68.4 (12.6)	68.4 (13.3)	<0.001	66.1 (12.7)	69.9 (11.9)	71.8 (12.4)	<0.001
Energy misreporting status (*n* (%))				<0.001				<0.001
Plausible reporters[d]	545 (44.4)	966 (78.7)	1022 (83.2)		565 (42.9)	1037 (78.7)	1070 (81.2)	
Misreporters	683 (55.6)	262 (21.3)	206 (16.8)		753 (57.1)	281 (21.3)	247 (18.8)	

BMI, body mass index; WC, waist circumference.
[a]Differences between tertiles for continuous variables were assessed by using analysis of variance. Differences between tertiles for categorical variables were assessed by using Pearson's Chi-square test.
[b]Defined as BMI ≥ 25.
[c]Defined as WC ≥94 cm for males and ≥80 cm for females.
[d]Defined by using 1 SD cutoff for energy intake: energy expenditure between 53% and 147% for individuals with 1 recall day, and between 69% and 131% for individuals with 2 recall days.

of protein (OR = 1.01, 95% CI (1.00, 1.01)), compared to females with less protein intake. The OR of central overweight/obesity was higher among females with a larger per cent of energy from protein (OR = 1.03, 95% CI (1.00, 1.06)), compared to those with a smaller proportion of energy from protein. Protein expressed in both units was positively associated with OR of central obesity in males, but the association was attenuated by adjustment for non-protein energy intake.

Additional calculations using the average energy intake of Australian males in this analysis showed a comparable increase in WC and BMI between protein units. For example, Model 3 showed that each g/d higher protein intake is associated with a 0.04-cm higher WC in males. Referring to 86-kg Australian males consuming 7939 kJ as an example, each per cent increase in energy from protein among males means that their 4.67-g increase in protein is correlated to 0.26-cm higher WC, which translates into approximately 0.06-cm higher WC per gram protein.

Discussion

To our knowledge, this is one of the few studies assessing the associations of protein intake with diet quality and obesity using a nationally representative sample of Australian adults examining the impact of different ways of expressing protein intake. Protein expressed in g/d, g/kgBW/d, and per cent energy showed consistent direct associations with diet quality in males

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Table 2. Associations between protein intake and diet quality of Australian males and females[a]

	g/d			%EI			g/kgBW/d		
	Coeff.	95% CI	P value	Coeff.	95% CI	P value	Coeff.	95% CI	P value
Males									
Model 1	0.04	0.02, 0.06	**<0.001**	1.02	0.85, 1.19	**<0.001**	1.77	0.19, 3.35	**0.03**
Model 2	0.15	0.12, 0.18	**<0.001**	0.94	0.75, 1.13	**<0.001**	7.37	5.05, 9.70	**<0.001**
Model 3	0.15	0.12, 0.19	**<0.001**	0.94	0.75, 1.13	**<0.001**	7.49	5.19, 9.79	**<0.001**
Females									
Model 1	0.11	0.08, 0.14	**<0.001**	1.18	1.06, 1.30	**<0.001**	4.81	3.36, 6.25	**<0.001**
Model 2	0.25	0.22, 0.28	**<0.001**	1.23	1.07, 1.38	**<0.001**	9.57	7.89, 11.25	**<0.001**
Model 3	0.25	0.22, 0.29	**<0.001**	1.23	1.08, 1.38	**<0.001**	9.67	8.00, 11.35	**<0.001**

%EI, percent of energy intake.
[a]Diet quality was measured using the Dietary Guideline Index (range 0–130). Model 1 was adjusted for age, country of birth, socioeconomic status, and physical activity; Model 2 also included non-protein energy intake; and Model 3 also included non-protein energy intake and energy misreporting status.

and females. Protein intake expressed in g/d or %EI also showed direct associations with obesity measures, yet the associations varied between sexes, which may be related to the diversity of protein food sources. While there appears to be a difference in effect sizes in protein associations with diet quality and obesity across measurement units, this is a result of the difference in unit scales. Compared to the absolute protein unit (g/d), relative measurement units (i.e. %EI and g/kgBW/d) require additional information and careful consideration when examining associations with diet quality and obesity.

Association between protein intake and diet quality

Regression models in this study showed the direct association between total protein intake and DGI score among Australian males and females. This finding is in line with previous studies among Australians.[12,13] A study using previous Australia's National Nutrition Survey found a direct correlation between DGI score and protein intake (g/d) among women only, but a significant correlation was observed among both sexes after adjustment for energy intake.[12] A cohort study among Australians aged ≥25 years also showed a direct correlation between total protein intake (%EI) and DGI and Mediterranean-Dietary Approaches to Stop Hypertension Diet Intervention for Neurological Delay Index (MIND) scores.[13] However, no association was observed between protein intake and the Dietary Inflammatory Index score.[13]

Despite the consistent association between protein intake and diet quality, the effect size differed between sexes, and this difference might be due to the impact of plant and animal protein sources on overall dietary quality which may vary between sexes. For example, a previous study found that consuming higher protein diets was correlated with a higher total HEI score among young female American adults, but among males, these high-protein diets were only associated with higher HEI-component scores for dairy, total protein, and total vegetables.[10] In this study, males generally scored higher in protein food components, such as meats, dairy, and alternatives, while females scored higher in fruit and vegetable components. However, some component scores varied within sex-specific tertiles of protein intake, such as males in the second tertile of protein intake scored highest for dairy component score and females in the second tertile scored highest for cereal/grain component score. This variation suggests that different amounts of certain protein food sources might contribute differently to diet quality scores, which warrants further investigation.

This study also found the different effect sizes across units, which are influenced by the scale of measurement units. Both g/d and %EI units produced similar effect sizes in both sexes when accounting for the unit scale, and this is not surprising given the adjustment for energy intake. However, it is important to note that all models in this study assume constant energy intake and contribution of other macronutrients, as done in the previous study.[17] Therefore, the larger difference in effect sizes produced by g/d and %EI units is still possible with a higher intake of energy and other macronutrients.

Expressing protein intake in g/d will enable simple and direct interpretation of protein associations and diet quality, while relative units (i.e. g/kgBW/d and %EI) might require additional information on average energy intake and BW of study participants. Protein association with diet quality can still be captured using the g/kgBW/d unit, yet the results need to be interpreted cautiously as they might not represent the actual protein intake.[18]

Association between protein intake and obesity

Both linear and logistic regressions in this study suggested the direct associations between protein intake and obesity measures, although there were no significant associations between protein with females WC when expressed in %EI. This was consistent with findings on %EI with the majority of previous studies,[42,45–47] although other studies using the same unit found an inverse association[48] or no association with measures of obesity.[49] When protein consumption was expressed in g/d, the associations across most of the obesity measures were only significant after adjustment for energy intake and/or energy misreporting in the models. However, two previous studies reported no association between absolute protein intake (g/d) and obesity even after the adjustment for total energy intake.[17,50]

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Table 3. Associations between protein intake, BMI, and WC of Australian males and females[a]

	Males						Females					
	g/d			%EI			g/d			%EI		
	Coeff.	95% CI	P value	Coeff.	95% CI	P value	Coeff.	95% CI	P value	Coeff.	95% CI	P value
BMI[b]												
Model 1	–0.0000	–0.0003, 0.0003	0.92	0.0061	0.0040, 0.0083	**<0.001**	0.0001	–0.0004, 0.0005	0.82	0.0051	0.0025, 0.0077	**<0.001**
Model 2	0.0007	0.0003, 0.0010	**<0.001**	0.0050	0.0028, 0.0072	**<0.001**	0.0008	0.0003, 0.0013	**0.005**	0.0042	0.0016, 0.0068	**0.002**
Model 3	0.0007	0.0004, 0.0011	**<0.001**	0.0049	0.0028, 0.0071	**<0.001**	0.0009	0.0004, 0.0015	**0.001**	0.0041	0.0015, 0.0068	**0.003**
WC												
Model 1	–0.00	–0.03, 0.02	0.65	0.33	0.18, 0.48	**<0.001**	0.01	–0.02, 0.05	0.37	0.18	–0.01, 0.38	0.06
Model 2	0.03	0.01, 0.06	**0.01**	0.26	0.11, 0.42	**0.001**	0.04	–0.01, 0.08	0.09	0.19	–0.02, 0.40	0.07
Model 3	0.04	0.01, 0.06	**0.004**	0.26	0.11, 0.41	**0.001**	0.05	0.00, 0.09	**0.04**	0.19	–0.02, 0.39	0.08

BMI, body mass index (kg/m^2); WC, waist circumference (cm); %EI, per cent of energy intake.
[a]Model 1 was adjusted for age, country of birth, socioeconomic status, and physical activity; Model 2 also included non-protein energy intake; and Model 3 also included non-protein energy intake and energy misreporting status.
[b]The interpretation of the β-coefficient estimates is 100 × (coefficient), referring to the percentage change for a 1-unit increase in protein intake with all other variables constant.

Table 4. Associations between protein intake and obesity status of Australian males and females[a]

	Males						Females					
	g/d			%EI			g/d			%EI		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Overweight/obesity												
Model 1	1.00	0.99, 1.00	0.52	1.06	1.03, 1.09	**<0.001**	1.00	1.00, 1.00	0.89	1.04	1.01, 1.06	**0.005**
Model 2	1.01	1.00, 1.01	**0.03**	1.04	1.01, 1.08	**0.02**	1.00	1.00, 1.01	0.08	1.03	1.00, 1.05	**0.047**
Model 3	1.01	1.00, 1.01	**0.03**	1.04	1.01, 1.07	**0.02**	1.01	1.00, 1.01	**0.04**	1.03	1.00, 1.05	0.056
Centrally overweight/obesity												
Model 1	1.00	0.99, 1.00	**0.04**	1.03	1.00, 1.06	**0.03**	1.00	1.00, 1.01	0.22	1.03	1.00, 1.05	**0.045**
Model 2	1.00	1.00, 1.00	0.73	1.02	0.99, 1.05	0.30	1.01	1.00, 1.01	0.06	1.03	1.00, 1.06	**0.04**
Model 3	1.00	1.00, 1.01	0.59	1.02	0.99, 1.05	0.30	1.01	1.00, 1.01	**0.03**	1.03	1.00, 1.06	**0.045**

%EI, percent of energy intake; OR, odds ratio; BMI, body mass index; WC, waist circumference.
[a]Overweight/obesity was defined as a BMI ≥ 25. Centrally overweight/obesity was defined as a WC ≥94 cm for males or ≥80 cm for females. Model 1 was adjusted for age, country of birth, socioeconomic status, and physical activity; Model 2 also included non-protein energy intake; and Model 3 also included non-protein energy intake and energy misreporting status.

The varied protein associations with obesity between sexes might be related to the diverse protein food sources consumed by males and females that contribute differently to their energy intake. For example, the direct associations between animal protein intake and BMI in both sexes and WC among males reported in previous studies are somewhat explained by direct associations between animal protein and saturated fat intake, increased BMI, WC, and obesity risks.[42,46,51] On the other hand, previous findings on inverse associations between plant protein intake with obesity measures in both sexes are related to the inverse associations between plant protein with energy, benefiting body composition and weight loss.[42,51] Given that Australian males consumed high proportions of red and processed meats, while Australian females had high proportions of dairy, nuts, and seeds,[52] total protein intake in this study might consist of different proportions of animal and plant protein sources between males and females and may therefore differentially influence associations with higher obesity measures. However, this warrants further investigation into how different food sources influence protein associations with obesity.

The different effect sizes between g/d and %EI units in protein association with obesity are also likely due to the scale of measurement units as those units produced similar effect sizes after accounting for the unit scale. This similarity is expected in isoenergetic models, where the models focus on investigating protein and remain agnostic to the changes of other macronutrients.[18] Given the different influence of macronutrients on obesity or other health outcomes, statistical models are ideally adjusted for all macronutrients.[44] However, the inclusion of macronutrients requires careful consideration, otherwise resulting in nonsensical models.[18] The current study has shown that 'all-components model'[44] is not always possible due to potential multicollinearity among nutrients, and therefore adjustment for non-protein energy intake can be an option to accommodate other macronutrients in examining protein associations in different units with health outcomes.

Strengths and limitations

Strengths of this study include the comparison of different units in influencing protein associations with diet quality and obesity. Another strength is the use of nationally representative data, followed by further estimation of usual dietary intake and stratification by sex. All models assessing diet quality and obesity were also adjusted for usual energy intake and energy misreporting status.

Diet quality in this study was measured using the DGI-2013, which performed well in studies examining diet quality relationships with BMI and health-related quality of life,[25] and predicting risks of CVD and all-cause mortality among Australian adults[26] DGI-2013 accounts for energy intake by considering different cutoffs of food intake between males and females rather than including the ratio of energy intake to energy expenditure in the scoring component, as done in the Australian Total Diet Score (TDS).[53] Also, it is worth noting that many diet quality indices (e.g. DGI, TDS, and HEI) cap their highest scores but do not apply penalties for overeating, which therefore unable to show how far individuals exceed the recommended intake.[8]

Several limitations of this study should be considered. This analysis uses cross-sectional design, which does not allow any statements on causality. This analysis also used the survey data conducted more than 10 years ago, which therefore warrants further analyses when the new survey data are available. Another potential limitation of this study is the 18% missing anthropometric and dietary data which may have introduced sample bias. However, there were no differences between included and excluded individuals in terms of their socio-demographic characteristics (data not shown). It is also worth noting that while all analyses have been adjusted for age, this study did not explore the association across different age groups of adults. Given that older adults need higher protein intake for healthy ageing,[22] further studies may consider age-stratification analyses in examining protein associations with health outcomes. Lastly, this study assumed low PAL due to the limited data available for physical activity measurement, which might lead to residual confounding. Therefore, future studies investigating protein intake may consider advanced methods for PAL measurement.

Future directions/implications

Both absolute (g/d) and relative units (g/kgBW/d and %EI) can give similar results in protein associations with diet quality. However, the use of relative units needs additional information, such as average weight and energy intake, to prevent misleading interpretations of the associations. The use of g/kgBW/d unit in the population with overweight/obesity may also need further examination as the large denominator of the unit (i.e. high BW) may influence protein associations with diet quality.

Further research might specify models with different covariates and stratification in examining protein associations with health outcomes, such as stratifying analyses by age and including a more comprehensive measure of total daily physical activity. Statistical models will also ideally adjust for energy intake and other macronutrients.[18] Adjustments for non-protein energy intake in this study were performed as the increased protein intake was the main interest while remaining agnostic of other macronutrients. This approach differs from previous studies that have adjusted for total energy intake to account for the joint effect between increasing protein and reducing other macronutrient intakes.[43,50,54] However, we found adjustments for either non-protein energy intake or total energy intake produced similar results. Nonetheless, future research should consider how different approaches to energy adjustment may impact the interpretation of results.

The diverse food sources might explain the dissimilar associations in this study, which warrants further investigation. A high proportion of protein intake from animal sources is likely to contribute to high fat and energy intake and therefore increase BMI, WC, and the odds of obesity, while higher plant protein intake, depending on the specific food sources, might also come with lower fat and higher complex carbohydrates that help weight control.[42,51] As protein associations with obesity and diet quality varied between sexes, future studies may also examine whether the consumption of different protein sources by males and females contributes to the varied associations.

Conclusion

In conclusion, the effect size of the direct associations between total protein intake, diet quality, and obesity in males and females are influenced by measurement units. Three protein units consistently showed direct associations with diet quality, but the use of relative units in assessing protein associations with diet quality requires additional information. Both g/d and %EI also produce similar results when examining protein associations with obesity measures. The different associations across sexes are potentially due to diverse protein food sources, while the different effect sizes across protein units are influenced by the scale of measurement units. Adjustment for energy intake with careful consideration is also recommended when examining protein associations with diet quality and obesity. Future research may examine the influence of units on the associations between plant and animal protein with diet quality and obesity, as well as account for the different scales of those units.

Abbreviations

%EI: percent of energy intake; **ABS:** Australian Bureau of Statistics; **AMPM:** Automated Multiple 5-Pass Method; **ANOVA:** Analysis of variance; **BMI:** Body Mass Index; **CAPI:** Computer Assisted Personal Interview; **CATI:** Computer Assisted Telephone Interview; **CV:** Coefficient of variation; **CVD:** Cardiovascular disease; **DGI:** Dietary Guidelines Index; **g/d:** gram/day; **g/kgBW/d:** gram/kilogram body weight/day; **HEI:** Healthy Eating Index; **MIND:** Mediterranean-Dietary Approaches to Stop Hypertension Diet Intervention for Neurological Delay Index;**mTEE:** Measured total energy expenditure; **NNPAS:** National Nutrition and Physical Activity Survey; **OR:** Odds ratio; **PAL:** Physical activity level; **pTEE:** Predicted total energy expenditure; **rEI:** Reported energy intake; **SD:** Standard deviation; **SEIFA:** Socio-economic Indexes for Areas; **VIF:** Variance inflation factor; **WC:** Waist circumference.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.56

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Competing interests

R.M.L. and S.-Y.T. are editors of the Journal of Nutritional Science. H.R.B.A. and S.A.M. have no conflicts of interest to declare.

Authorship

H.R.B.A. was responsible for conceptualisation, methodology, formal analysis and interpretation, and writing the first draft of the manuscript. All co-authors (R.M.L., S-Y.T., and S.A.M.) supervised the research process, as well as contributed to study conceptualisation, development of methodology and statistical models, and interpreted the results. All authors edited and reviewed the final manuscript.

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REVIEW

Barriers to college student food access: a scoping review examining policies, systems, and the environment

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Abstract

College student food insecurity (FI) is a public health concern. Programming and policies to support students have expanded but utilisation is often limited. The aim of this study was to summarise the barriers to accessing college FI programming guided by the social ecological model (SEM) framework. A scoping review of peer-reviewed literature included an electronic search conducted in MEDLINE, ERIC, and PubMed databases, with a secondary search in Google Scholar. Of the 138 articles identified, 18 articles met eligibility criteria and were included. Articles primarily encompassed *organisational* (17/18) level barriers, followed by *individual* (15/18), *relationship* (15/18), *community* (9/18), and *policy* (6/18) levels. *Individual* barriers included seven themes: *Knowledge of Process, Awareness, Limited Time or Schedules, Personal Transportation, Internal Stigma, Perception of Need, and Type of Student.* Four relationship barriers were identified: *External Stigma*, *Comparing Need*, *Limited Availability Causes Negative Perceptions*, and *Staff.* Ten barrier themes comprised the organisational level: *Application Process*, *Operational Process*, *Location*, *Hours of Operation*, *Food Quality*, *Food Quantity*, *Food Desirability or Variety of Food*, *Marketing Materials*, *Awareness of the Program,* and *COVID-19 Restrictions.* Two barrier themes were identified at the *community* level, *Public Transportation* and *Awareness of SNAP*, while one barrier theme, *SNAP Eligibility and Process*, encompassed the *policy* level. Higher education stakeholders should seek to overcome these barriers to the use of food programmes as a means to address the issue of college FI. This review offers recommendations to overcome these barriers at each SEM level.

Key words: **College food security: College students: Environment: Food insecurity: Policy: Systems**

Introduction

Research on college food insecurity (FI) has demonstrated a heightened prevalence among students and reviews have further described the academic, health, and social consequences of experiencing FI while in college.[1,2] To address this, there has been an increased focus on identification of successful initiatives, programmes, and policies that universities can implement to create a culture that supports food security and health equity among students.[3]

Many colleges and universities report a greater availability and increased variety in the type of campus-based food resources to support food insecure students.[4,5] These include food pantries, farmers markets, basic needs centres, and dedicated staff who can assist students with enrolling in federal nutrition assistance programmes.[5] However, the usage of available programming is suggested to be limited among students. Barriers to utilising campus resources have been discussed[6–8] and frequently include individual and operational factors such as social stigma or shame, a student's self-identity, insufficient information on resource use policies, inconvenient availability, and inefficient marketing of available resources to students.

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Beyond campus resources, students may be eligible for food and nutrition assistance programmes such as Supplemental Nutrition Assistance Program (SNAP), the largest component of the social safety net against FI in the United States.[9] However, because of barriers to applying including navigating the daunting and confusing application process and confusion about eligibility requirements, many eligible students do not participate in the programme.[10,11] There have been increased calls for researchers to examine which campus-based programmes are most effective in helping overcome barriers to access and utilisation of nutrition assistance programmes.[11]

To our knowledge, the body of literature does not include a comprehensive review that details the intra- and inter-student barriers students face when accessing resources provided at the campus, community, and/or federal levels. The purpose of this scoping review was to examine the intra- and inter-student barriers college and university students face when accessing existing programming and policies to improve their food and nutrition security. Food and nutrition security was defined in this review as equitable access to adequate quantities of quality food for optimal health. Using the social ecological model (SEM) framework, we examined programmes and policies at the campus, community, and federal government levels.

Methods

The review was conducted in accordance with the JBI methodology for scoping reviews.[12] The PRISMA Extension for Scoping Reviews (PRISMA-ScR) was used to guide the reporting of scoping review methods, process, and results[13](Supplemental Materials).

Eligibility criteria

College students at all levels (undergraduate, graduate, distance, etc) were the target population of this scoping review. College students had to be enrolled at universities or colleges in the United States. We did not exclude specific groups of students (e.g. international, students on an educational visa, etc.). The concept of interest was college student barriers when accessing food assistance programming or resources. The context for programmes included campus, community, and federal programmes intended to support food insecure college students. For the purpose of this scoping review, barriers needed to address programme utilization, thus studies that only identified barriers to food access, which in turn contributed to FI, were excluded. Articles that present the barriers to programming from the viewpoint of college students were included. Articles that included the viewpoints of only faculty, staff, administrators, or other stakeholders were excluded. Articles had to be available in English and published between January 2009 and December 2022. Our search focused on articles published after January 2009 coinciding with the publication of the first manuscript on college FI.[2] Eligible articles included peer-reviewed and grey literature, including theses and dissertations.

Search strategy

A search strategy was designed in consultation with a research librarian to identify peer-reviewed publications relevant to the research question. All search terms from a previous scoping review on college FI initiatives were used[5] and expanded upon to focus on barriers. Search terms for this review included: food insecurit* or food secur*; college or university student; barrier or obstacle or issue or problem or challenge or difficult* or facilitator or motivat* or enabler and intervention* or strateg* or program* or best practice* or direct student support* or student support* or systemic reform* or practice* or protocol*. The comprehensive search occurred using three databases; MEDLINE and ERIC databases through EBSCOhost and PubMed all using similar parameters. These databases were selected based on their inclusion of nutrition and higher education-focused journals. A secondary search occurred in Google Scholar to ensure all relevant articles were included.

Article selection

All identified peer-reviewed and grey literature sources found using selected databases were uploaded to EndNote. Duplicates were removed. The authors used a three-step process to review the articles; title review, abstract review, and full-text review. To ensure inter-rater reliability, the authors met and discussed the application of inclusion and exclusion criteria. The extracted articles were divided into thirds and each author reviewed two thirds of the article titles and abstracts. Authors met to discuss any discrepancies between articles that should be kept or removed during the title and abstract review phases. Disagreements that arose were resolved through discussion, with an additional review by a third author as needed. The remaining articles were divided between the authors for full-text review followed by another meeting to discuss any articles that needed review by the full authorship team. The article selection and screening process is shown in Fig. 1 (PRISMA 2020 Flow diagram).

Data extraction

A standard data extraction template developed by the authors in Google Sheets was used. The authors divided final articles, so each member extracted one-third of the articles. Descriptive information extracted from all articles included the full citation, study aim, sample size, research method (quantitative, qualitative, or mixed-methods), student population, institution description and whether the food programme assessed was an intervention. Additional data were extracted regarding the measures used to assess the barriers, the food resource in question (SNAP, food pantry, etc), the barriers reported, and concluding points. Any disagreements in data extract were discussed and resolved through consensus by the authorship team.

Data synthesis and framework analysis

Extracted data related to barriers were coded by the authors. Each author independently coded the barriers for the articles in

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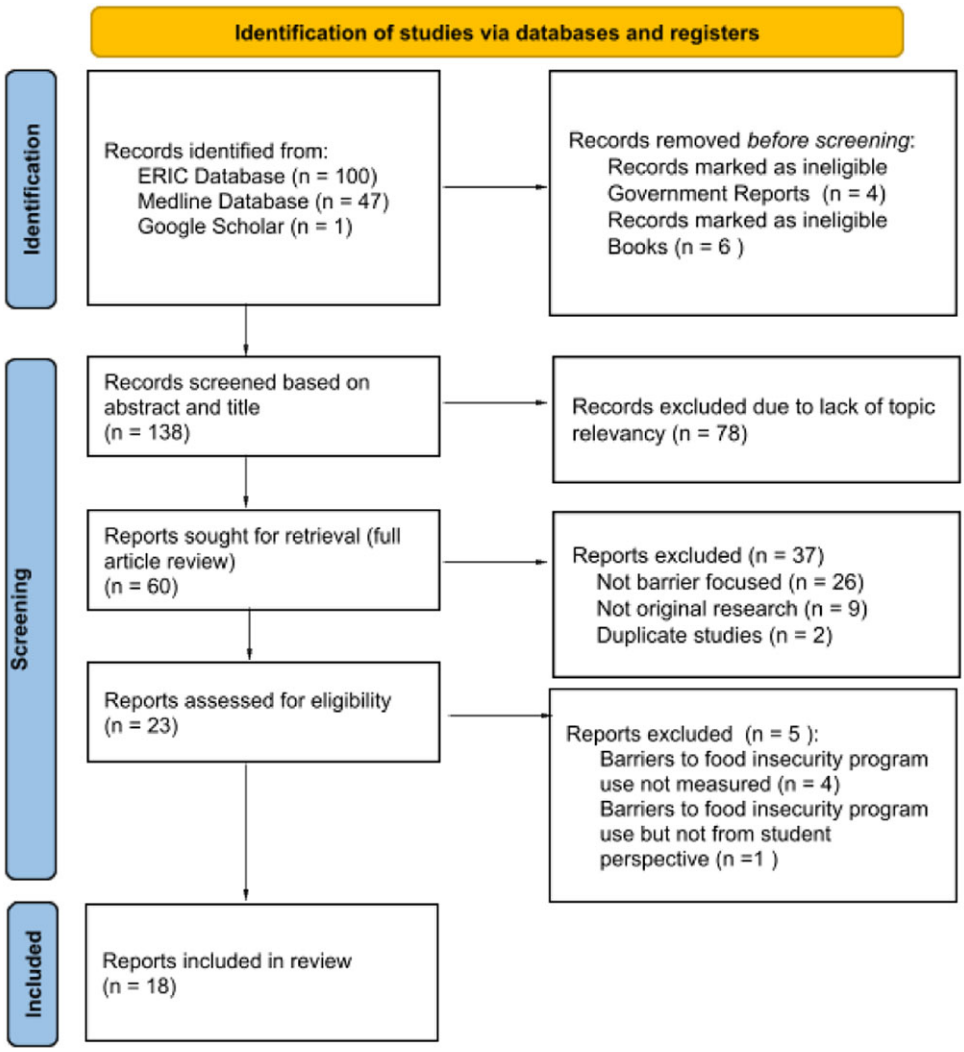

Fig. 1. Prisma flow diagram.

which they extracted data. The authors met to discuss the codes generated and refine the codes. Refined codes were organised by the SEM level the barrier addressed. The SEM levels used in this review align with previous health research.[14] *Individual* or intrapersonal level included all barriers that may relate to one's personal ability to utilise the food resource such as knowledge, attitudes, behaviour, or self-concept. The *relationship* or interpersonal level included barriers that related to the formal and informal social networks that prevent food resource usage such as stigma or competition with other users. *Organisational* level factors related to the food resource's characteristics including location, hours of operation, and marketing as well as the rules and regulations for applying and using the resource. The *community* level included the context of the whole higher education institution and larger community surrounding the institution or food resource such as transportation and outreach. *Policy* level factors focused on the broader state and national policies that prevent food resource usage such as SNAP eligibility restrictions for college students. A final codebook was generated, and one author reviewed all articles using the codebook to confirm all codes had been applied to barriers identified. All authors reviewed the final codes and SEM levels identified and met to discuss until a consensus was present.

Results

A total of 138 unique studies were screened based on abstract and title, and the full text of 60 studies were screened for inclusion in the study (Fig. 1). After review of these studies, a total of 37 studies were excluded for the following reasons: 26 did not focus on students' barriers to accessing FI programming, 9 were not original research and 2 reported the same results as another study that was already included. The remaining 23 articles were reviewed by the study team, and an additional five articles were excluded during full article review for the following reasons (4 did not address barriers to FI programming, and one was not written from the student perspective) a total of 18 articles remained and were included in the final sample.

Selected study characteristics included in the final sample are presented in Table 1. All studies included in the review and applicable to our research question were published in 2020 or

Table 1. Study characteristics

Reference	Study design	Description of study participants (n)	Institutional description
Anderson A. *et al.* 2022[(15)]	Qualitative Semi-structured interviews	Undergraduate and graduate students (n = 30)	Large, public land grant university in the Southeast United States
Beam M. 2020[(16)]	Qualitative Semi-structured interviews	Undergraduate students (n = 8), non-traditional students	Mid-sized, public 4-year doctoral/ research university.
Brito-Silva *et al.* 2022[(17)]	Quantitative Cross-sectional, non-probability, Web-based survey via email	Undergraduate and graduate students (n = 529)	Texas Women's University
Crutchfield *et al.* 2020[(18)]	Qualitative Semi-structured interviews and focus groups	Undergraduate and graduate students (n = 16), social work students	Several campuses within the California State University System
Conrad *et al.* 2022[(19)]	Mixed Methods Cross-sectional, non-probability, Web-based survey via email. Virtual and face-to-face focus groups	Undergraduate students (n = 58, qualitative arm; n = 1159 quantitative arm)	Mississippi State University
El Zein *et al.* 2018[(7)]	Mixed Methods Cross-sectional, non-probability, Web-based survey via email	Undergraduate and graduate students (n = 899)	University of Florida
El Zein *et al.* 2022[(20)]	Qualitative Semi-structured, in-person interviews, and survey	Undergraduate and graduate students (n = 41)	University of Florida
Fortin *et al.* 2021[(21)]	Mixed Methods Focus group and interviews	Undergraduate and graduate students (n = 19, focus group; n = 11 individual interviews)	Midwestern university
Gamba *et al.* 2021[(22)][a]	Mixed Methods Head-to-head crossover trial. In-person interviews	Undergraduate and graduate students (n = 30)	California State University, East Bay
Hege *et al.* 2021[(23)]	Quantitative Cross-sectional, non-probability, Web-based survey via email, social media, or tableling/flyers	Undergraduate and graduate students (n = 1632)	University of Kentucky
Hernandez *et al.* 2021[(24)][a]	Mixed Methods Sequential explanatory design, randomised controlled intervention (phase 1), focus group and photo-elicitation intervention (phase 2)	Undergraduate and graduate students (n = 1000)	Two campuses within a large community college system that operates in the Houston and greater Houston, Texas area
Hiller *et al.* 2021[(25)]	Quantitative Cross-sectional, non-probability, Web-based survey via email	Undergraduate and graduate students (n = 938)	Iowa State University
Kim *et al.* 2022[(26)]	Qualitative Focus groups	Undergraduate and graduate students (n = 21)	Virginia Commonwealth University
Manboard *et al.* 2021[(27)]	Mixed Methods Four-part sequential explanatory design: (1) survey, (2) in-depth interviews, (3) photo and text elicitation; and (4) final survey[b]	Undergraduate and graduate students (n = 18)	Texas State University
Martinez *et al.* 2021[(28)]	Qualitative Semi-structured, focus groups	Undergraduate and graduate students (n = 58)	Five universities in the University of California System (Berkeley, Irvine, Merced, Santa Cruz, and San Francisco).
Richards et al 2023[(29)]	Qualitative Semi-structured, in-person interviews	Undergraduate and graduate students (n = 58)	Three universities in the western United States (Brigham Young University, Oregon State University, and the University of Hawaii-Manoa)
Schinkel et al 2023[(8)]	Mixed Methods Cross-sectional, non-probability, Web-based survey via email; semi-structured virtual focus groups via purposeful convenience sampling	Undergraduate and graduate students, military-connected students (n = 127, survey; n = 8, focus group)	University of Wyoming
Yamashiro *et al.* 2022[(30)]	Qualitative Semi-structured interviews	Undergraduate students (n = 16)	Single campus within the California State University System

[a]Study design involved an intervention.
[b]Manuscript reports on data from the first two phases (initial survey and interviews).

later, except El Zein in 2018. The recency of the literature could be attributable to the more recent focus within the field of understanding intra- and inter-student barriers students experience versus a focus on the prevalence of FI among college students which dominated the early college FI literature starting in 2009. Studies incorporated three research designs,

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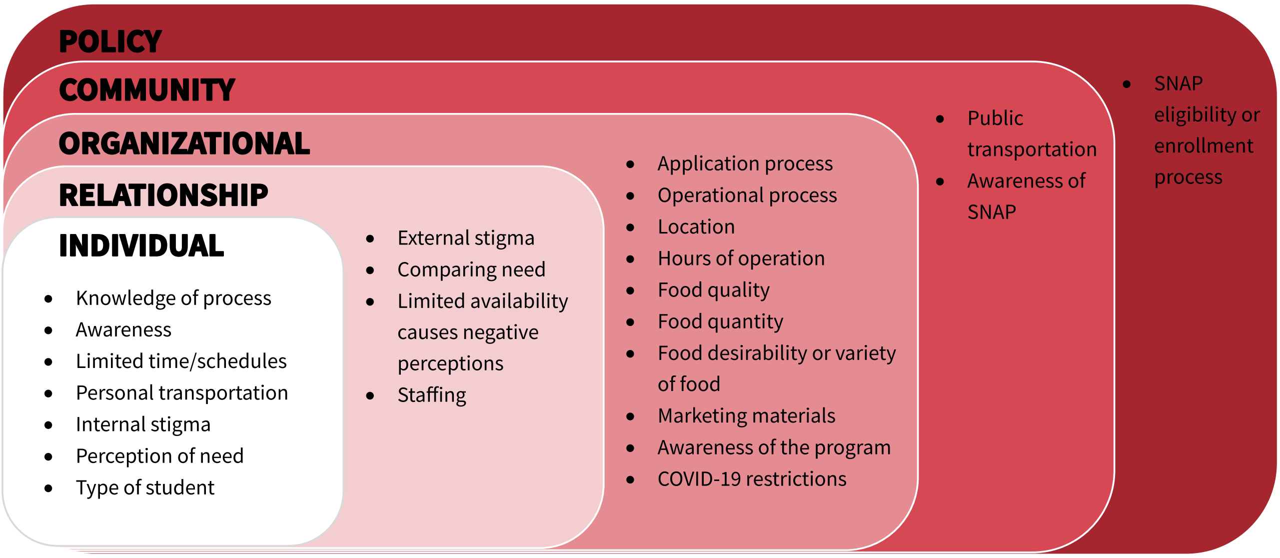

Fig. 2. Socio-ecological model of barriers to college student food access. Abbreviations: SNAP, Supplemental Nutrition Assistance Program.

including qualitative[15,16,18,20,26,28–30] (n = 8), quantitative[17,23,25] (n = 3), and mixed methods designs[7,8,19,21,22,24,27] (n = 7). Two studies included an intervention.[22,24] The sample size across studies ranged from 8 to 1,632. Most studies included both undergraduate and graduate students. Three studies included only undergraduate students.[16,19,30] Two studies focused on a particular segment of students - social work or military connected students.[8,18] Within most studies, data were collected at a single college or university. Four studies collected data from multiple colleges or universities across a region or within a university system.[18,24,28,29] Figure 2 displays the identified barrier themes within studies at each level of the SEM.

Individual

Within the SEM framework, 83% of articles reviewed contained at least one *individual* level barrier that students expressed experienced when accessing FI programming. Seven barrier themes were identified at the *individual* level: *Knowledge of Process, Awareness, Limited Time or Schedules, Personal Transportation, Internal Stigma, Perception of Need,* and *Type of Student*. Of these themes, *Internal Stigma* appeared most frequently, within 11 articles of the 18 articles reviewed.[7,8,15–20,23,27,29] This theme encompassed qualitative or quantitative connections to students feeling internal embarrassment or weakness that prevented them from using an available resource. The *Perception of Need* theme involved students considering it the norm to be hungry in college or downplaying their own FI by believing their situation wasn't severe enough to warrant concern or deem them eligible to receive assistance. This theme was mentioned within eight studies.[8,17–21,27,29] The *Type of Student* theme referred to any mention where a student's perceived or actual characteristics such as living on versus off campus, being an online/distance student, their programme of study, or having international status, impacted their ability to access programmes. This theme was identified in seven of the reviewed articles.[8,17,18,21,27,29,30] For example, a student that is a commuter (i.e. resides off campus) may feel that on-campus services, like a food pantry, are available only to students who live on-campus. The *Knowledge of Process* theme encompasses students being uncertain about how to enrol, access, or utilise available programming and was identified within seven of the reviewed articles.[7,17,20,21,26,27,29] This theme differed from the *Awareness* theme, which was identified in five articles,[7,17,20,26,29] where students were not even aware of the programme/policies available on their campus community to support them in accessing food. The theme of *Personal Transportation* encompassed student's lack of personal transportation prevents them from accessing available programmes. This theme was mentioned within five articles.[16,17,23,24,27] An example of this theme is a student who could visit an on-campus food pantry but may not have a means to get any food back to their place of residence if they do not live near the food pantry. The final individual level theme was *Limited Time or Schedules* which included five studies[7,17,19,23,24] that mentioned student's class, work, or personal schedules limited their opportunities to access available programming. This theme differs from the available hours of programming which was coded as an organisational barrier.

Relationships

At the *relationship* level of the SEM, a student's social circle (i.e. peers), family and campus staff influence the barriers students experienced when accessing FI programming. Four barrier themes were identified: *External Stigma, Comparing Need, Limited Availability Causes Negative Perceptions,* and *Staffing*. A majority of articles (83%) mentioned at least one *relationship* level barrier. The barrier theme of *External Stigma* appeared most frequently of the r*elationship*-level themes, appearing in 12 of the studies.[7,8,15–18,20,21,23,27,29,30] This theme encompassed students feeling embarrassed when seen using available resources/programming by their peers (e.g. seen carrying bags with items from a food pantry). This theme also includes students being hesitant to share about their circumstances or asking for help from peers or staff for fear of being judged. Another common

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barrier at the relationship level was *Comparing Need*, which was mentioned in nine of the reviewed studies.[7,8,15,17–21,27] This theme referred to students not accessing available programming due to feeling that other students needed the programmes more than they do. The *Limited Availability Causes Negative Perceptions* theme occurred in three studies.[20,24,28] This theme encompassed student experiences of limited availability of foods at programming due to perceived programme abuse by peers or feelings of competition between their peers for available food. The final theme within this level of the SEM was *Staffing* which included three studies mentioning this theme.[19,28,30] Studies coded with this theme included reports of students experiencing negative interactions with staff members working or managing the food resource programming which deters student's use of the resource.

Organisational

Organisational level barriers to accessing FI programming were presented in the majority of the articles (94%), and this was the level of the SEM had 10 themes. These barrier themes included *Application Process*, *Operational Process, Location, Hours of Operation, Food Quantity, Food Quality, Food Desirability or Variety, Marketing Materials, Awareness of the Program* and *COVID-19 Restrictions.* These themes can be conceptualised as barriers to programme uptake that are due to the ways students access the programme, components of the programme itself or how students find out about the programme.

The *Application Process* barrier occurred in five of the included studies.[7,17,20,21,29] Application process describes the process to enrol in the programme, that deters the student from accessing the programme. This may include students needing to answer questions to determine their eligibility, not knowing how to enrol, or having difficulties completing the steps necessary to use the programme. One example included students saying that the application process required them to answer embarrassing questions. Additionally, college students may qualify for SNAP, but sometimes their application would be returned saying they did not qualify. The *Operational Process* barrier occurred in five of the studies[7,17,20,22,24] and describes the process of using the programme that deters students from accessing the resource in the future. This may include policies or procedures that require a check in process or having to wait in line. The *Location* barrier was present in seven of the studies,[7,8,17,24,25,27,29] and includes the programme being in a physical location that is inaccessible or inconvenient for students. Oftentimes this had to do with the proximity of the campus pantry to a student's home address. The barrier of *Hours of Operation* occurred in half of the studies (nine)[7,16,17,21,24,25,28–30], and this occurred when the food resource was not aligned with the student's needs making the resource or programme inaccessible or inconvenient for students. Some pantries have limited hours of operation during weekdays, weekends or during semester breaks. Once students access the food programme or resource, another barrier to programme use was *Food Quality. Food Quality* was identified as a barrier to programme use in three studies[17,20,30] and was identified when the food available is of lower quality and not deemed appropriate to consume by students accessing the programme or food resource. This may include the best-by dates or expiration dates being exceeded, fruits or vegetables with bruises, dented cans or food items that are deemed undesirable due to the visual or sensory evaluation. The barrier of *Food Quantity* was present in three studies[20,24,28] and occurred when the food resources or programmes had limited foods that meet the student demand and the overall quantity of certain types of food are limited. This may include a food resource running out of food, or not having many options available. Another barrier with regard to the available food is *Food Desirability or Variety*, which was present in nine of the studies.[15–17,20,22–24,27,28] This may include lack of foods to meet dietary needs, a lack of fresh options, a lack of variety in the types of food available, a lack of culturally appropriate foods, or the overall impression given by a student of not wanting to eat a certain type of food available at the food resource or programme. The barrier of *Marketing Materials* was presented in one study[20] and includes when the marketing materials are unclear or use language that is perceived as negative by students which deters their use of the programme. *Awareness of the Program* was present as a barrier in six of studies[7,15,17,19,20,26] and included outreach to ensure that students are aware that the food resources that exist are not sufficient. The final *organisational* barrier of *COVID-19 Restrictions* was present in one study[27] and described changes to programmes during the pandemic that negatively impacted students' ability to use the programmes.

Community

Community level barriers to accessing FI programming were presented in 44.4% of articles. Two barrier themes were identified at the *community* level: *Awareness of SNAP* and *Public Transportation*. The *Awareness of SNAP* theme encompassed the outreach and efforts to engage students in federal nutrition assistance programmes being limited which prevents students from seeking support beyond campus programming. This theme, identified in six articles,[7,15,21,26,27,29] was labelled at the *community* level due to the higher education institutions role in ensuring college students are aware and connected to broader programming beyond a campus context. The *Public Transportation* theme was mentioned in five articles.[21,23,24,27,30] This theme included the limited availability or structure of public transportation which prevents students from being able to access programming as well as the struggle students face when having to carry food on public transportation. This barrier was noted to be exacerbated during the COVID-19 pandemic as public transportation was deemed unsafe to utilise or access to transportation was restricted.

Policy

Roughly a third of the articles (33.3%) included student barriers at the policy level. These barriers all fell under a singular theme of *SNAP Eligibility or Process.* This theme was noted in six articles and encompassed students being ineligible due to SNAP programme rules, uncertainty about federal nutrition assistance programme eligibility, and the overall enrolment process being confusing or daunting for students.[15,18,21,26,27,29] Although most studies referred to SNAP, it is important to note that one study[27] also identified barriers enrolling in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC).

Discussion

The purpose of this scoping review was to identify barriers college students face across the SEM framework that prevent the utilisation of campus, community, and federal FI resources. To our knowledge, this is the first review to systematically examine the factors that prevent college students from using the resources available to improve their food and nutrition security. The findings of this review can help guide programming improvements at the campus, community, and federal government level to improve student access to programming and address FI among college students.

Addressing barriers at each level of the SEM

Most studies in this review addressed the *organisational* barriers students face when attempting to utilise programming on campus. Many studies described the barriers around the *Application and Organizational Process* to access food resources. University programmes may benefit from adopting best practices as provided by the Indy Hunger Network,[31] which include keeping intake as simple as possible by only collecting necessary information, avoiding client embarrassment, and improving wait times.

An important component of running any programme is ensuring that the programme is evaluated to understand its effectiveness at achieving its goals. An area of future work is to evaluate campus food security programmes to understand how effective they are at addressing college student food and nutrition security.[32] There are many tools available to understand how a food security programme is achieving its goals, at different phases of student (client) engagement[33,34] including *food quality and variety*.[35–38] This work should also include ongoing changes to improve these programmes to better address student needs and decrease student's barriers to programme engagement. This can be difficult to do given limited resources for the evaluation process, however on college campuses it would be meaningful to engage students in this work to fulfil academic requirements.

The student perspective is crucial to include in this line of work, as students are the ones who are accessing these programmes. A community based participatory approach to programme development and evaluation can be especially successful.[39] One single organisational approach will not be the solution to FI for all students, as barriers to food security differ across student groups. This review focused on articles that included information about barriers to food security programmes from the student perspective for this reason. Including student users (those who have experience with food and nutrition insecurity) in the evaluation and development of the entire programme process may remove the majority of the barriers students face. This would include obtaining student feedback about the *organisational* level barriers including the *Application Process*, *Operational Process, Location, Hours of Operation, Food Quantity, Food Quality, Food Desirability or Variety, Marketing Materials, Awareness of the Program*, and *COVID-19 Restrictions. Food Quantity* may always be a concern if the demand for programming exceeds its availability. However, research on barriers to programme use identifies that these programmes are underutilised by students facing food and nutrition security. Some strategies to address this *Food Quantity* barrier may include diversifying funding streams to increase funding to purchase food, partnering with student groups for food drives, and enrolling students in other food programmes they may qualify for, such as SNAP. While operationally, *Location* may be seen as challenging to address due to space being limited on college campuses, creative solutions have been achieved including satellite food pantries,[40] refrigerated smart food lockers,[41–43] mobile food pantries,[44] and delivery services.[45] Additionally, addressing some of these barriers may in turn address other barriers. For example, creating a refrigerated smart locker programme may provide students with access to higher quality and desirable food and the change in location would also address the barrier of hours of operation and awareness of the programme. As these satellite pantry locations are in different areas on campus, the programme is likely to have a greater reach based upon where students typically go for classes and meetings. Once students overcome the *individual* and *relationship* barriers that they face, barriers at the *organisational* level that are inherent in the food security programming offered on campus may limit student's ability to access programming and improve their food security status.

At the *individual* and *relationship* level of the SEM, one of the most impactful ways to reduce barriers college and university students face when accessing existing programming and policies is to reduce stigma. Findings from several studies underscore that many students are either not aware of the severity of their own FI, may downplay it as an inherent aspect of the college experience, or may be too embarrassed to seek out help or assistance.[19,20,29] Findings also suggest that students experience external stigma directed towards them from peers or staff. University culture can reduce the stigma by rejecting cultural and societal narratives that individual weakness or faults are the cause of FI.[3] FI could be framed as an experience that many college students experience and that campus-based resources are available to be accessed. Campus advocacy campaigns have been suggested as an avenue to serve a dual focus of reducing stigma and raising awareness of available campus resources.[11] Raising awareness of available campus resources is particularly important as many students in the studies reviewed experienced individual level barriers stemming from inadequate knowledge about enrolment and eligibility policies and procedures, accessing available programmes, or utilising offered resources. Stigma should also be considered in the context that many FI programmes and initiatives are student-led.[4] These student leaders may need sensitivity training to ensure they possess the skills to deliver programs in a manner that doesn't inadvertently contribute to stigmatisation among their peers. Higher education institutions can employ a combination of outreach,

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education, and dissemination efforts to reduce barriers that students experience when trying to access available resources and programming.

The demanding nature of academic commitments and part-time employment can leave college students with little time for meal planning, preparation, and consumption. This can be even more of a challenge for food insecure students. Logistical challenges at the individual level of the SEM (e.g. transportation, limited time/schedules) were also highlighted within several articles in this review. This suggests that campus-based programming should consider adapting the availability of resources to better suit the needs of students. One emerging solution for this is the use of refrigerated smart food lockers.[43] Similar to the parcel package lockers used by delivery services, smart food lockers pair an online ordering system with a collection of lockers for students to retrieve items discreetly and conveniently from the campus food pantry at locations and on a schedule that work best for the student. Examples of successful food locker programmes can be found at Bunker Hill Community College[42] and Frederick Community College.[41]

While universities and colleges often create their own community, they are also engrained in the larger community context and shared environment. To address barriers at the *community* level it is important to be inclusive of college students as part of the larger community where they most often live and work. Public transportation for college students is not a guarantee; some campuses may lack public transportation altogether[46] while available public transportation at other campuses may still be inaccessible for students due to price.[28,47] In fact, it has been reported that transportation costs can account for almost 11% of a college student's budget.[48] Barriers to transportation for college students have been associated with increased likelihood of experiencing FI as well as contributing to a lack of student success.[49,50] Low- or no-cost public transportation for students provides an opportunity to overcome this barrier. Evidence from the City University of New York (CUNY) and Rio Hondo College support the impact of subsidised public transportation on student retention,[50,51] but more research can help advocates to justify expanded programming in cities across the US. However, this fails to address barriers for students in areas with limited public transportation. Partnership between higher education leaders and local officials is vital to develop a local transportation system that supports college students while simultaneously improving access to public transportation for the whole community. Further, advocates can demand state and federal funding be delegated to expand existing infrastructure, which is suggested to reduce the transportation burden for students.[46]

Despite the notion that college students are part of the larger community, efforts to make them aware of nutrition assistance programmes designed to support food insecure populations locally and federally are often lacking. In particular, this scoping review identified community outreach to engage with college students about SNAP as a barrier to programme utilisation. Efforts to increase student awareness of SNAP and other federal nutrition assistance programmes have trickled upwards in recent years through the use of campus advocacy campaigns[11,52] and state policy requirements,[53] such as Hunger Free Campus initiatives. Yet, a majority of states have failed to require collaboration between higher education institutions and local SNAP offices to connect eligible students with the programme.[53,54] As a result, many eligible students fail to enrol in the programme[55] and college students remain largely unaware of nutrition assistance programmes.[56] Active participation by higher education leadership and other campus stakeholders at community meetings and invitations for community leadership to serve on a campus FI task force may provide an opportunity to forge these relationships.

Even when outreach is available, students remain confused with the process which leads to avoidance in trying to enrol in federal nutrition assistance programmes. Hesitancy can result from the confusing eligibility criteria, the time commitment to navigate the system, and the lack of support to complete the process. A strong relationship between higher education stakeholders and community SNAP agents has been identified as an integral part of successful student enrolment in federal programming.[10] However, lack of consistent guidance for students is present which can increase the frustration students face when attempting to enrol in these programmes.[10] As a result of the 2019 US Government Accountability Office report on college FI, the Food and Nutrition Service revised its webpage on student SNAP eligibility to increase clarity,[55] although it is unclear if this has helped to overcome the barriers students and state SNAP agencies face surrounding eligibility. Faculty and staff on college campuses may also be unaware of how to support student use of these programmes, adding another layer of inconsistent support for students. Thus, it is paramount that local SNAP individual agencies, along with members of the higher education community, be trained on student SNAP exemptions and the enrolment process.[10,11] The disappointment that arises when taking the time to seek assistance but being denied due to technicalities, such as number of hours worked, adds an additional barrier and prevents students from seeking access to federal programming.[57] Advocates have called for revised SNAP eligibility guidelines for college students to eliminate outdated exemptions which could help overcome the *policy* level barrier identified in this scoping review.[57] Ultimately, federal and state policymakers' engagement is necessary to improve the system for college students.

Support for creating a culture of health in higher education

It has been recommended that higher education institutions work to establish a culture of food and nutrition security and that frameworks to guide this cultural shift on campus are of importance. In this review, we utilised the SEM to assess the multi-level barriers that students face. Overcoming the barriers at each level of the SEM will help to address the need to establish a culture of health on campus. However, these results can be applied to existing frameworks used in the college FI literature. Savoie-Roskos and colleagues[3] utilised a justice-based Health Equity Framework[58] to propose changes higher education administrators and stakeholders can make to improve health equity for food insecure college students. This framework identifies four spheres that influence health equity: Relationships and Networks, Systems of Power, Individual Factors, and Physiological Pathways. These four spheres demonstrate the interconnected factors that contribute to health inequities in society including FI on college campuses. The barriers identified in this review align with the individual factors, relationships and networks, and systems of power that must change in higher education institutions to allow all students to access programming to support their basic needs and achieve their degree. Despite student advocates championing change on campus to overcome individual and relationship barriers, addressing systems of power is often a necessary first step to ensure food security is prioritised on campus and resources are allocated to support the removal of barriers for students, including those who have been historically marginalised and excluded from campus resources.

Strengths and limitations

This comprehensive scoping review examined over a decade of research into college FI to determine the intra- and inter-student barriers students face when accessing food security programmes and initiatives. A strength of this review was the examination of barriers at each level of the SEM. This approach has highlighted specific areas at the campus, community, and federal government levels for stakeholders to target. This review was limited to studies published in peer-reviewed journal articles and grey literature. However, information about barriers to FI programming may be available in other forms such as conference proceedings or campus resource documentation. This scoping review searched in three databases to identify articles for this review, as well as a search of an additional database following the review, however it is possible that other databases would also include eligible articles that could have been included in this review. We were specifically interested in student identified barriers to programme and resource use in this review which resulted in exclusion of studies from other stakeholder perspectives. As such, additional barriers, and potentially solutions to the barriers students face, may be identified when considering additional perspectives. Additionally, the evaluation of food and nutrition security programming in college settings is a relatively new, and emerging area of research, and as time passes, we expect a larger body of work to be available about this topic.

Conclusion

Efforts to alleviate college FI are often student driven with support from faculty or staff. Continued awareness among students and faculty can help shift the culture on campus to create an environment that overcomes intra- and interpersonal barriers to FI resource use. However, addressing the systems of power to overcome *organisational*, *community*, and *policy* barriers will require action from higher education administration. College and university executive leadership should look to the barriers identified in this scoping review as a contributing factor to why FI continues to persist on campuses across the country. As universities implement new programmes and initiatives on their campuses, continued evaluation of the barriers that students may experience when accessing these resources is critical to ensure the effectiveness and inclusivity of their endeavours.

Abbreviations

SEM: Social Ecological Model; **SNAP:** Supplemental Nutrition Assistance Program; **FI:** Food insecurity; **CUNY:** City University of New York.

Supplementary material

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Conflict of interest

All authors report no conflicts of interest.

Authorship

All authors contributed to the conceptualisation, development and drafting of this manuscript. All authors provided critical revisions of the manuscript for important intellectual content. All authors have read and approved of the final manuscript.

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RESEARCH ARTICLE

Community-based nutrition education and counselling provided during pregnancy: effects on knowledge and attitude towards iron-folic acid supplementation

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Abstract

Maternal malnutrition is pervasive throughout the world, notably in sub-Saharan Africa, including Ethiopia. This study examined the effect of community-based iron-folic acid supplementation (IFAS) nutrition education on IFAS knowledge and attitude among pregnant women in urban settings in South Ethiopia. A community-based quasi-experimental study was conducted among 198 randomly selected pregnant women attending antenatal care (ANC) (99 intervention and 99 control). We used a multistage sampling technique followed by systematic sampling to select the pregnant women. Pregnant women who participated in the intervention arm received six nutrition education sessions and counselling using a health belief model (HBM), while the control group received only routine ANC services. Baseline and endline data were collected during the ANC and compared. The data was analysed using statistical package for social sciences. Analyses of the effect of the intervention were done using difference-in-difference and generalised estimation equation to allow correlation of repeated observations over time. The results indicated a significant effect of intervention on maternal knowledge towards IFAS; with intervention, group levels increased by 35 percentage points ($P < 0.001$). The odds of being knowledgeable at the endpoint in the intervention group were 2.6 times higher than baseline ($OR = 2.67$, 95% CI 1.88–3.80). There was a significant ($P = 0.001$) change in proportion with a favourable attitude towards IFAS between the two time points. The community-based nutrition education intervention approach has significantly improved maternal knowledge and a favourable attitude towards IFAS among pregnant women. The HBM is effective in improving knowledge and attitude among pregnant women.

Key words: **Adherence: Attitude: IFA supplementation: Knowledge: Nutrition education**

Introduction

Anaemia affects nearly one-third of the world's population, contributing to the high burden of morbidity and mortality worldwide.[1] Women in low- and middle-income countries are the most affected. Anaemia is associated with adverse reproductive outcomes such as preterm birth, low birthweight, impaired child development, and low productivity in adults.[2] Anaemia before, during, and after pregnancy can thus affect the health and well-being of the women, but also of the offspring.[3,4] Despite significant achievements in maternal health-related programmes over the past decade and the attention given to anaemia prevention and treatment as illustrated by its inclusion in the World Health Assembly targets, the prevalence of anaemia has shown little to no reductions over the past decade.[5]

More than 32 million women in pregnancies are anaemic globally. Nearly half (46.3%) of these are in Africa.[6] One of the highest prevalences of anaemia in the world is recorded for the sub-Saharan Africa region, where approximately 57% of pregnant women are anaemic. Likewise, Ethiopia has one of the highest rates of micronutrient deficiencies, including anaemia, making it a serious public health concern.[7,8] Poor

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maternal nutrition prior to and throughout pregnancy is also significantly connected to an increased risk of maternal anaemia, mortality, and unfavourable birth outcomes such as low birthweight and preterm birth, although the mechanism for this link is complex.[9] Despite substantial achievements and hints of progress over the last decade, maternal undernutrition continues to be a major public health concern in Ethiopia.[10,11] In Ethiopia, 41% of pregnant women were anaemic, of which 20% were moderately anaemic, 18% were mildly anaemic and 3% were severely anaemic in 2016.[12] Maternal and child mortality rates also remain high, with 412 maternal deaths per 100,000 live births and 67 child deaths per 1,000 live births.[10] This could be associated with the high prevalence of undernutrition among pregnant women in Ethiopia, ranging from 14.4% to 47.9%.[13,14]

Iron-folic acid supplementation (IFAS) is the main intervention strategy targeting the reduction of anaemia in pregnant women. In Ethiopia, IFAS, as part of routine antenatal care (ANC) services, is the main strategy for preventing maternal mortality due to anaemia.[15] However, the compliance rate remains low and not at the required level to prevent anaemia during pregnancy. For example, according to the Ethiopia Demographic and Health Survey, <11% took an IFAS to the recommended period (≥90); about 12% took 60–89 pills; more than one-third (35.7%) took <60 pills, and around 42.2% did not take any iron tablets during their most recent pregnancy.[16] Several factors contribute to the non-adherence of recommended IFAS during pregnancy, including socio-economic factors, poor knowledge, attitude, ANC utilisation, and perceived side effects of iron-folic acid (IFA) pills.[17–19] This calls for addressing the factors responsible for low adherence to IFAS and developing locally appropriate mechanisms to mitigate them to increase IFAS coverage.

Pregnant women should receive nutrition education and counselling at every ANC visit. In Ethiopia, 62% of pregnant women received at least one ANC visit for their most recent birth, and 66% of these women reported receiving nutritional education and counselling, although the quality and extent of the counselling were unknown.[10] Nutrition education is critical in nutrition behaviour change attempts because it improves participants' nutrition and food literacy. Food literacy encompasses both nutrition literacy and the capacity to apply that knowledge to make sound decisions, whereas nutritional literacy is the set of skills required to comprehend and analyse information about food and its nutrients.[20] In addition, nutrition education interventions that enhance maternal nutritional status are among the most successful mother–and-child health promotion techniques.[21]

Studies show that the use of nutrition education interventions has improved knowledge, awareness, and adherence to micronutrient supplementation, including IFAS.[22,23] For effective intervention, it needs to be locally feasible, simple, and practically implemented through the existing healthcare system. Previous observational studies on maternal nutritional status have been undertaken in Ethiopia, and nutrition interventions are advocated,[24,25] such as counselling on the consumption of nutrient-rich, locally available foods, food and nutrient supplementation (for example, IFA, calcium, and multiple micronutrients), as well as on weight to ensure a healthy weight gain. However, evidence on the effect of HBM-based nutrition education (IFAS) during pregnancy is lacking in the context of low-income countries, including Ethiopia. Therefore, we aimed to assess the effect of nutrition education and counselling on the knowledge and attitude towards IFAS of pregnant women in rural settings in southern Ethiopia.

Methods and materials

Study design, study setting, and participants

A pre-posttest quasi-experimental design consisting of an intervention and control group was employed. Both the control and intervention groups completed baseline and endline testing; the intervention group was the only group that received nutrition education and home-to-home counselling. The study was conducted in Butajira town administration, southern Ethiopia; 135 km from Addis Ababa. The main means of livelihood in the district is rain-fed agriculture, which is characterised by the production of subsistence crops. The study population included all first- and early-second-trimester pregnant women attending ANC in Butajira town. The study was conducted from January to April 2021. The study included pregnant women before 16 weeks of gestation who were permanent residents of the study area. Pregnant women with diabetes mellitus or hypertension (HTN) were not included in the study.

Sample size estimation and techniques

The sample size was determined by using the formula for comparison of two population proportions for the intervention and control groups.

$$n = \frac{(Z_1 + Z_2)^2 \times 2p(1-p)}{(P_2 - P_1)^2}$$

The following assumptions were considered when estimating the required minimum sample sizes: the expected proportion of pregnant women with good knowledge (P1) was 0.77 and P2 was 0.52.[26] We wanted to detect an absolute increase of 25% in the intervention arm at the 5% significance level and 80% power. The calculated sample size was multiplied by 1.5 to adjust for the design effect and a 10% loss to follow-up; the final sample size became 198 (99 pregnant women allocated for each group). Data on births compiled by urban health extension workers was used to estimate the number of pregnant women in each *kebeles*. Butajira town was chosen at random. Butajira town has five *kebeles* (01, 02, 04, and 05). Two *kebeles* (03 and 05) were randomly selected and allocated to the intervention and control groups. Pregnant women residing in 05 received the intervention, whereas those residing in 03 did not receive the nutrition education interventions. Using a probability proportional to size allocation, the sample size was assigned to each cluster. A systematic sampling technique was used to select pregnant women. In the event that a woman missed her interview due to being out of home, the next eligible pregnant woman in the serial number was contacted. The pregnant

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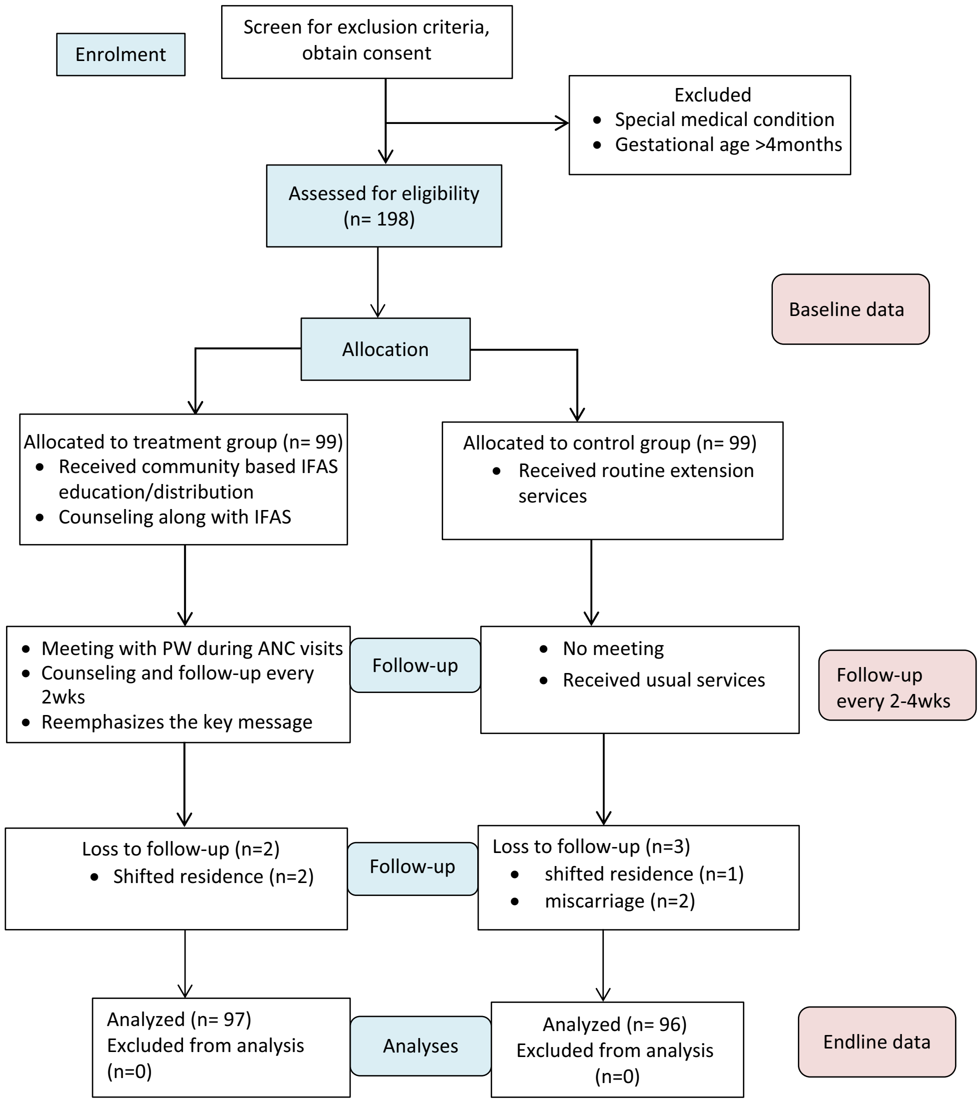

Fig. 1. Flow diagram showing the effect of community-based nutrition education and counselling targeting knowledge and attitude towards IFAS among pregnant women. IFAS, iron-folic acid supplementation.

woman, who had been absent from the interview, was contacted the next day. The gestational age was calculated by asking about the beginning day of the last menstrual period, and the pregnancy was confirmed using a urine human chorionic gonadotropin test (Fig. 1).

The intervention

Pregnant women in the intervention group were organised into three groups of 33 pairs of those living near each other; education sessions were located near participants' homes and held on days when participants had some free time. Nutrition education was delivered in Amharic. An organised work schedule, counselling cards, and nutrition education were provided to the intervention group. The core messages for the lessons were generated utilising the health belief model (HBM).[27,28] It was modified in the recommendation to the Ministry of Health, Ethiopia.[29]

For the intervention and control groups, baseline and endline assessments were collected. Following the gathering of baseline data, intervention groups were placed in groups at a nearby village and received nutrition education regularly, once every two weeks, for a period of three months (six sessions) for 30–45 min per session. Four nurses with Bachelor of Science (BSc) degrees delivered nutrition education, while two Master of Science (MSc) specialists supervised the nutrition education sessions. The core contents of the session were: increasing knowledge about iron-rich food sources, IFAS (how to take the IFAS, when to take it and how to absorb it more, foods rich in iron and folic acid, common side effects and their management; and enhancers/inhibitors of iron/folic absorption), iodised salt, meal frequency, and portion size with increasing gestational age; food groups; taking day rest; reducing heavy workloads; enhancers and inhibitors of iron absorption; increasing utilisation of health services; and interrupting the intergenerational life cycle of malnutrition; increasing pregnant women's perceptions of undernutrition and factors leading to it; poor eating practices causing inadequate dietary intake and disease.

Nutrition education sessions included presentations, discussions, demonstrations, and picture-based exercises. Key messages, realistic activities, and the GALIDRAA (greet, ask, listen, identify, discuss, recommend, agree, and make follow-up appointments) processes were all identified by the trainers as crucial counselling abilities. After the pregnant women were enrolled, reasonable attempts were made to encourage their retention and full follow-up for the duration of the trial by providing them with incentives to reduce missing data. Periodic conversations about compliance with the intervention during routine meetings and home visits by trainers served to retain interest in the study. Moreover, home visits were planned to lessen the strain of follow-up visits among pregnant women.

Outcomes

The primary outcome of this study was the knowledge and attitude towards IFAS supplementation.

Data collection tools and procedures

A pretested interviewer-administered structured questionnaire, including 9 variables on socio-demographic data (age, education level of women, occupation of women, marital status, educational level of husband, occupation of husband, religion, family size and ethnicity), 19 variables on socio-economic data (source of drinking water, kind of toilet facility, household facility (radio, television, mobile phone, table etc.), type of fuel, materials of floor, materials of exterior walls, materials of roof, number of rooms, ownership of agricultural land and its size, ownership of livestock and number, bank or microfinance saving etc.), 9 on obstetric and health-related variables, 9 on IFA supplementation knowledge, 12 Likert scale questions on attitudes, and 3 on reasons for missing the dose of IFAS, was developed and used for data collection. Baseline data on IFA knowledge, attitude, and compliance at the IFA level were collected. To address any potential bias in data collection, training of five research assistants on research ethics and protocol as well as quality data was done at an adjacent *kebele* where the research questionnaires were pretested.

To assess the level of knowledge about IFAS during pregnancy, respondents were asked nine questions: whether they had heard of IFAS or not, the benefits of IFAS; the frequency of use of IFAS; the duration of taking IFAS; the side effects; the management of side effects; the effect of iron or folate deficiency; the signs and symptoms of anaemia; and food sources for iron during pregnancy. A correct answer for each item was scored as '1', and an incorrect answer was scored as '0'. A summation of all the scores for each participant was done, then converted into a percent score. Based on the percent, those who scored above the average value (50%) were considered to have good knowledge, and those who scored below the average value were considered to be less knowledgeable.[30] Attitude towards IFA supplementation by pregnant women was assessed using 12 Likert scale items. A correct answer for each item was recorded as 5, and a negative attitude was scored as '1'. The participants were considered to have a positive attitude if they scored 70% or above and were otherwise unfavourable or negative.[30,31] Though data on intake and compliance with IFAS were collected, the present study mainly focused on knowledge and attitudes towards IFAS.

To check the reliability of the questionnaires used in this study, a test-retest method was used in pretesting, whereby a repeat pretest was conducted after one week, and Cohen's kappa statistic was used to assess the level of agreement of the results from the two pretests. All the questions repeated resulted in a kappa value >0.7; hence, all the questions were retained. To ensure the validity of the questionnaire, it was shared and discussed with experts from the regional health bureau and the field supervisors. The feedback obtained from the experts and the pretesting results were used to refine the questionnaire and improve its quality.

Data management and analysis

The data were entered, cleaned, coded, and analysed using Statistical Package for Social Science version 22.0 software. The Kolmogorov–Smirnov test was used to check the normality of the distribution. The characteristics of respondents were also described in both the intervention and control groups. The wealth index was computed using principal component analysis as a composite indicator of living standards based on ownership of selected household assets, size of agricultural land, number of livestock, materials used for housing construction, ownership of improved water and sanitation facilities, and household possessions.[10] The wealth index values were calculated by summing up the scores of sixteen components. Ultimately, three categories (low, medium, and high) were generated by splitting the wealth index values into three equal classes.

Homogeneity of study groups at baseline was determined by comparing categorical variables of both intervention and control groups using the chi-square test. The analysis of the effect of the intervention was done using a difference-in-difference (DID) (percentage point change) to compare outcomes between intervention and control groups before (baseline) and after (endline) intervention. The intervention effect was measured by the odds ratio and 95% confidence level of the interaction term between study groups (intervention and control) and period of study (baseline and end line) in the multivariate logistic regression model. A $P < 0.05$ was considered statistically significant. Since the same respondents who participated in the baseline were also those who participated in the endline assessment, the analysis considered using a paired analysis with repeated measures instead of treating the respondents in the baseline and endline as independent groups. Therefore, the generalised estimation equation (GEE) was applied in addition to allow correlations of these repeated observations over time since data are collected on the same participants across successive points in time.[32,33]

The ethical statement

Expedited ethical clearance was obtained from Hawassa University Institutional Review Board (Ref. No. IRB/040/13). The purpose of the study was explained in a formal letter to the district administration. Prior to enrolment in the study, informed consent was obtained from each mother.

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Table 1. Baseline characteristics of pregnant women in the intervention and control groups

Variables	IG (n=99)		CG (n=99)		
Age in year	n	%	n	%	P value[a]
15–24	50	50.5	46	46.5	0.774
25–34	46	46.5	45	45.5	
35–44	3	3.0	8	7.9	
Religion					
Orthodox	28	28.3	27	27.3	0.682
Muslim	59	59.6	53	53.5	
Protestant	12	12.1	19	19.2	
Mother education					
No formal education	49	49.5	62	62.6	0.570
Primary education	35	35.4	28	28.3	
Secondary and above	15	15.2	9	9.1	
Occupation					
Housewife	68	68.7	64	64.6	0.981
Merchant	8	8.1	12	12.1	
Government employee	6	6.1	8	8.1	
Self-employee	12	12.1	11	11.1	
Husband occupation					
Merchant	35	35.4	33	33.3	0.984
Government employee	23	23.2	19	19.2	
Self-employee	14	14.1	24	24.3	
Farmer	18	18.2	13	13.1	
Daily labourer	9	9.1	10	10.1	
Family size					
<4	66	66.7	72	72.7	0.151
≥4	33	33.3	27	29.3	
House hold wealth index					
High	26	26.3	30	30.3	0.749
Medium	40	40.4	38	38.4	
Low	33	33.3	31	31.3	
Gravidity					
Primi-gravida	34	34.3	27	27.3	0.280
Multigravida	65	65.7	72	72.7	
Parity					
1	27	42.2	28	38.9	0.569
2–4	30	46.9	38	52.8	
>4	7	10.9	6	8.3	

[a] χ^2 test.

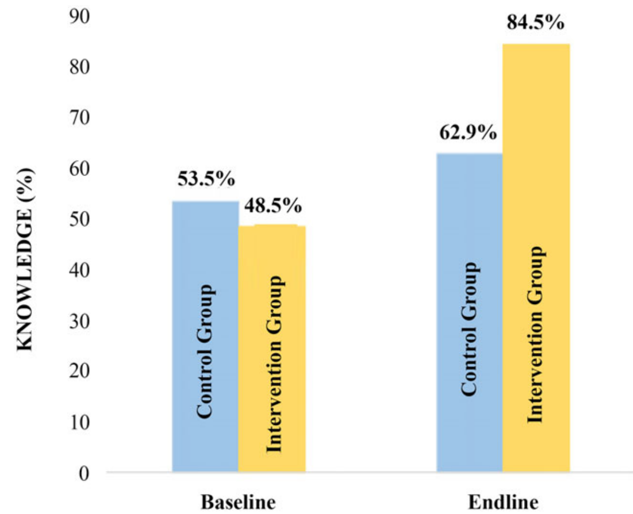

Fig. 2. Maternal knowledge towards IFAS during study period for both the control and intervention groups. IFAS, iron-folic acid supplementation.

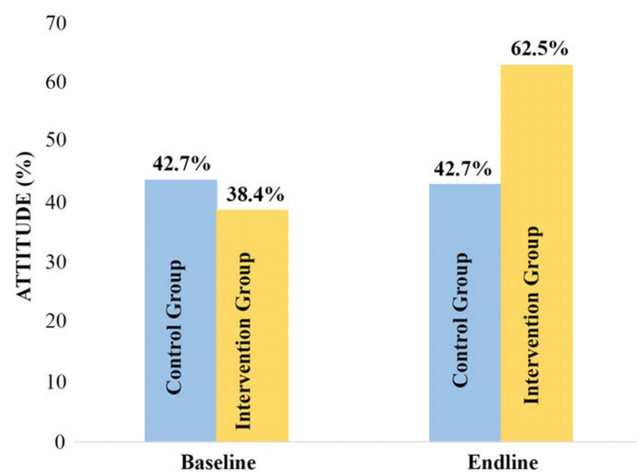

Fig. 3. Maternal attitude towards IFAS during study period for both the control and intervention groups. IFAS, iron-folic acid supplementation.

Results

In this study, 99 pregnant women received nutrition education and counselling as well as IFAS, while 99 pregnant women received usual ANC services. Five pregnant women left the study. Among them, two participants could not be followed up; one shifted residence, and two had abortions (Fig. 1).

There was no significant difference between the intervention and control groups regarding age, parity, educational level, ethnicity, religion, occupation, family size, gravidity, parity, and socio-economic level (Table 1). Both knowledge and attitude scores were lower in the intervention group at baseline compared to the control. However, there were no statistically significant differences between the two groups in terms of knowledge and attitudes toward IFAS (Figs. 2 and 3).

Effect of nutritional education intervention on maternal knowledge of IFAS

Figure 2 shows a comparison between baseline and endline levels of maternal knowledge towards IFAS for both control and intervention groups. The improvement was 35 percentage points in the intervention group (from 48.5% to 84.5%) compared to 7.3 percentage points (from 53.5% to 62.9%) in the control group. The intervention had a net effect of a 27.7 percentage point (35–7.3) improvement in IFAS knowledge level. Therefore, it did yield a statistically significant difference (95% CI: 0.001, 0.015) since the DID between the two groups was <0.001

Factors associated with maternal knowledge on IFAS

GEE results to assess the effect of the intervention and other potential factors on maternal IFAS knowledge are shown in Table 2. There was a highly significant ($P < 0.001$) change in levels of IFAS knowledge between the two time points: the odds of being knowledgeable at endline in the intervention group were 2.6 times that at baseline (OR = 2.67, 95% CI 1.88–3.80), adjusting for other socio-demographic factors. Household economic status significantly influenced maternal knowledge; households with low income ($P = 0.006$) were less likely to be knowledgeable than households with medium income ($P = 0.002$).

Table 2. Factors associated with pregnant women knowledge on iron-folic acid supplementation

Variables	OR (95% CI)	P value
Intervention Vs. control	1.429 (0.905, 2.257)	0.125
End line Vs. Baseline	2.674 (1.882, 3.799)	<0.001[b]
Intervention (group, time)[c]	4.745 (2.410, 9.341)	<0.001[b]
Age		
15–24	1	
25–34	1.029 (0.594, 1.785)	0.918
35–44	1.471 (0.517, 4.188)	0.469
Woman education		
No formal education	1	
Primary education	1.288 (0.722, 2.301)	0.392
Secondary & above	0.705 (0.261,1.907)	0.491
Woman occupation		
Housewife	0.868 (0.353, 2.134)	0.758
Merchant	1.352 (0.328, 5.577)	0.677
Government employee	0.724 (0.156, 3.353)	0.679
Self-employee	0.522 (0.168, 1.617)	0.260
Daily labourer	1	
Family size		
<4	1	
≥4	0.835 (0.456,1.530)	0.560
Household wealth index		
Low	0.444 (0.250, 0.788)	0.006[a]
Medium	2.722 (1.442, 5.137)	0.002[a]
High	1	
Gravidity		
Primi-gravida	1	
Multigravida	1.248 (0.692, 2.249)	0.462
ANC visit		
1st visits	1	
2nd visits	0.905 (0.538, 1.522)	0.707

[a]Statistically significant at P < 0.05.
[b]Statistically significant at P < 0.001.
[c]DID.

Effect of nutritional education intervention on maternal attitudes towards IFAS

Figure 3 shows comparison of maternal attitude towards IFAS between baseline and endline by study group. There was an increase in the proportion of pregnant women who had a favourable attitude towards IFAS during the study period, across the study groups. The increase was higher at 25.8 percentage points (from 37.1% to 62.9%) in the intervention group compared to 0 percentage points (from 42.7% to 42.7%) in the control group. The intervention had a net of 25.8 percentage points (25.8–0.0) of improvement in positive attitude towards IFA supplementation.

Factors associated with favourable maternal attitudes towards IFAS

GEE results to assess the effect of the nutrition education intervention and other potential factors on maternal attitudes towards IFAS are presented in Table 3. There was a significant ($P < 0.001$) change in proportion of those who had a favourable attitude towards IFAS between the two time points: the odds of having a favourable attitude towards IFAS at endline were 1.6 times that of baseline (OR = 1.62: 95% CI 1.08, 2.42), adjusting for other socio-demographic factors. The number of pregnancies significantly influenced maternal attitude; multiparous women were more likely to acquire a favourable attitude.

Table 3. Factors associated with pregnant women attitude on iron-folic acid supplementation

Variables	OR (95% CI)	P value
Intervention Vs. Control	1.349 (0.912, 1.995)	0.134
End line Vs. Baseline	1.619 (1.082, 2.423)	0.019[a]
Interaction (Group, time)[b]	2.207 (1.245, 3.911)	0.007[a]
Maternal knowledge on IFAS		
Good knowledge	1.30 (0.63, 1.84)	0.11
Poor knowledge	1	
Age		
15–24	1	
25–34	1.122 (0.695, 1.811)	0.638
35–44	1.757 (0.544, 5.678)	0.346
Woman education		
No formal education	1	
Primary education	1.136 (0.548,2.356)	0.732
Secondary & above	1.123 (0.724, 1.743)	0.605
Husband education		
No formal education	1	
Primary education	0.831 (0.511, 1.351)	0.455
Secondary & above	0.793 (0.451, 1.396)	0.422
Woman occupation		
Housewife	0.304 (0.070, 1.330)	0.114
Merchant	0.236 (0.047, 1.176)	0.078
Government employee	0.349 (0.057, 2.127)	0.253
Self-employee	0.389 (0.084, 1.805)	0.228
Daily labourer	1	
Family size		
<4	1	
≥4	1.143 (0.662, 1.974)	0.632
Household wealth index		
Low	0.631 (0.371, 1.075)	0.090
Medium	1.311 (0.793, 2.166)	0.291
High	1	
ANC Visit		
1st visits	1	
2nd visits	1.373 (0.898, 2.100)	0.143
Gravidity		
Primi-gravida	1	
Multigravida	1.737 (1.097, 2.751)	0.019[a]

[a]Statistically significant at P < 0.05.
[b]DID.

Discussion

The main aim of the present study was to assess the effect of the community-based nutrition education intervention and counselling based on HBM among pregnant women on knowledge and attitude towards IFAS and to reduce negative impacts on women and neonates in Butajira town, southern Ethiopia. Both knowledge and attitude scores were lower in the intervention group at baseline compared to the control. This may be due to the sensitisation variations that occurred during the initial exposure to the questionnaire and the recall ability differences between the intervention and control groups in answering the baseline questions. The key findings of the present study were, nutrition education and counselling using HBM improved maternal knowledge and positive attitude towards IFAS among pregnant women. At the end of the study, the majority of pregnant women in the intervention group had heard of IFAS, compared with less than half of them who had not heard about IFAS at the baseline of the study. In addition, there was an improvement in the proportion of pregnant women who scored high IFAS knowledge in the intervention group. This shows that

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it is possible to improve the knowledge of pregnant women of IFAS and related consequences during pregnancy through HBM-based nutrition education compared to routine health education through health extension workers.

Several previous studies have shown that a greater proportion of pregnant women were reached and counselled as well as attained more positive behavioural change of IFAS as compared to routine health services alone.[30,34,35] Even though the study settings were different from the present study, our finding is in line with the previous studies.[36–38] The possible explanation might be that nutrition education leads to favourable attitudes and, thus, changes in nutrition behaviour.

Pregnant women who were from low-socio-economic households were less likely to be knowledgeable than upper-socio-economic women. This may be explained by the pregnant women with high income are more likely to have attained high educational level and hold formal employment, or own good income generating activities.[39–41] Formal education creates opportunities for women to have better knowledge and access to information about personal healthcare including IFAS and benefits of supplements, and pregnancy related health specifically.[22,42] The present findings are consistent with the studies conducted in Ethiopia and Kenya where there was a positive association between household socio-economic status and nutritional knowledge of pregnant women.[30,43] This calls for more opportunities to empower women since economically empowered women use increased number of IFA supplementation than the poorest women as evidenced in studies conducted in Indonesia[44] Tanzania[45] and Ethiopia.[16]

Effective community-based nutrition education to affect behaviour change not only should provide knowledge, but also should change beliefs in order to bring about behaviour change. The attitude and perception of pregnant women are based on the information they have, which in turn dictates their practices. We had assessed the attitudes of mothers towards IFAS, and the result revealed a significant change in the attitude score regarding IFA supplementation in the intervention group over the comparison group. There was an overall positive change in the beliefs, opinions, and perceptions of pregnant women towards IFAS during this study. These findings are consistent with nutrition education intervention studies done among women that demonstrated a significant improvement in overall positive health beliefs and practices in relation to IFAS.[25,36,37] The result of the study found that there was no association between increased maternal knowledge and attitude towards IFAS. Several factors may explain this apparent incongruence. These findings can be explained by the fact that by giving adequate information about the benefits of IFAs and the consequences of not taking them during pregnancy, there is a high possibility that pregnant women can develop a positive attitude and perception towards IFAS. The significant improvement in knowledge and positive attitude towards IFAS among the intervention group may be related to the repetition of the key messages of nutrition education during the study period with individual counselling[46] as repetition of messages remained longer in memory according to the information process theory.[47] A previous study found a significant favourable effect of using HBM constructs during prenatal counselling to encourage healthy behaviour.[48] This could be because women who attend nutrition education using the HBM believe that the repercussions of malnutrition are severe, and they also believe that they are perceived to suffer the consequences of malnutrition. In addition, the pregnant women perceived that the benefits of consuming IFA outweighed the hurdles to obtaining it and its side effects. Their perspective can then influence their attitude and actions.

The present study has a number of strengths and limitations that need to be considered when interpreting the findings. First, the study participants were only followed during their pregnancy period, giving a limited time for follow-up. Second, the intervention was conducted without randomisation. However, the effect of non-randomisation was minimised by the use of a control group. Another limitation is that the findings may be prone to recall bias and subjectivity because the study mainly relied on verbal reports from the interviewees. Lastly, the findings may not be extrapolated to other setting in the country with different socio-demographic characteristics since the study was limited to one town.

Notwithstanding the above limitations, the present study has a significant practical implication for improving maternal knowledge and attitude towards IFAS, which will avert the risk of developing anaemia during pregnancy and related birth defects in order to achieve the Sustainable Development Goal (SDG-2). The findings indicated that tailoring current nutrition policies, strategies, and initiatives is justified to integrate the health behaviour model into nutrition education within Ethiopia's current health system. Moreover, this approach has a significant positive impact on improving pregnant women's attitudes towards IFAS, which will in turn increase IFAS intake and decrease maternal and child mortality rates in Ethiopia. Furthermore, beyond the health extension workers (HEWs), there is a need to explore and strengthen other strategies for creating awareness and educating pregnant women on IFAS benefits and consequences that can complement HEWs' activities, as HEWs have wide-ranging responsibilities for community-based health promotion. Their workload is diverse, and they spend time on activities relating to family health, disease prevention and control, hygiene, and sanitation, as well as other community-based activities.[49] Community health leaders (CHLs) provide such an opportunity since they are also able to closely follow-up pregnant women and provide health and nutrition education at the community level, as revealed in this and elsewhere.[30,44] However, CHLs' effectiveness is exceedingly dependent on proper training and continuous supportive supervision. If properly trained and facilitated, CHLs could play a vital role in increasing the knowledge and attitude of pregnant women and increasing maternal health service utilisation.[30,50] Although large-scale community-based studies with a larger sample size are needed to assess the cost-effectiveness of CHL-based interventions, training CHLs in IFAS, routine monitoring, and integrating their operation in a wider framework that promotes maternal health are critical.

Conclusion and implications

The community-based nutrition education intervention approach has significantly improved maternal knowledge and a favourable attitude towards IFAS among pregnant women. The HBM is effective in improving knowledge and attitude among pregnant women. It is necessary to integrate theory-based nutrition education interventions with the economic empowerment of pregnant women. Moreover, existing health facilities in Ethiopia can provide a link between community and health facilities, which is a feasible opportunity for providing information and raising women's awareness of IFAS benefits during pregnancy, as well as distributing IFAS in order to increase IFAS intake. We strongly recommend that HBM-based nutrition interventions be widely implemented because of the well-structured primary healthcare systems in Ethiopia. To realise this approach, health workers need to be properly trained and closely supervised.

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Authors' contributions

AA and MR conceptualised and designed the study and conducted statistical analysis, MR supervised the field data collection, and AA and MR did the interpretation and wrote the manuscript. AA and MR reviewed manuscript drafts. Both authors read and approved the final version of the manuscript.

Competing interest

There are no competing interests for any author.

Data sharing

Data are available upon reasonable request from the corresponding author.

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RESEARCH ARTICLE

Dietary cholesterol increases body levels of oral administered vitamin D_3 in mice

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Abstract

Vitamin D and cholesterol share the same intestinal transporters. Thus, it was hypothesized that dietary cholesterol adversely affects vitamin D uptake. The current studies investigated the influence of cholesterol on the availability of oral vitamin D. First, 42 wild-type mice received a diet with 25 μg/kg labelled vitamin D_3 (vitamin D_3-d_3), supplemented with either 0% (control), 0.2%, 0.4%, 0.6%, 0.8%, 1.0% or 2.0% cholesterol for four weeks to investigate vitamin D uptake. In a second study, 10 wild-type mice received diets containing 0% (control) or 1% cholesterol over four weeks to determine cholesterol-induced changes in bile acids. Finally, we investigated the impact of cholesterol versus bile acids on vitamin D uptake in Caco-2 cells. Surprisingly, dietary cholesterol intake was associated with 40% higher serum levels of vitamin D_3-d_3 and 2.3-fold higher vitamin D_3-d_3 concentrations in the liver compared to controls. The second study showed that cholesterol intake resulted in higher concentrations of faecal bile acids (control: 3.55 ± 1.71 mg/g dry matter; 1% dietary cholesterol: 8.95 ± 3.69 mg/g dry matter; $P < 0.05$) and changes in the bile acid profile with lower contents of muricholic acids ($P < 0.1$) and higher contents of taurodeoxycholic acid ($P < 0.01$) compared to controls. *In-vitro* analyses revealed that taurocholic acid ($P < 0.001$) but not cholesterol increased the cellular uptake of vitamin D by Caco-2 cells. To conclude, dietary cholesterol seems to improve the bioavailability of oral vitamin D by stimulating the release of bile acids and increasing the hydrophobicity of bile.

Key words: Bile acid: Cholesterol: Mass spectrometry: Mice: Vitamin D

Introduction

Vitamin D deficiency is a global public health problem among all age groups.[1] To prevent or treat vitamin D deficiency, many health authorities have established guidelines on vitamin D intake. The National Academy of Medicine recommends a daily oral intake of 15 μg of vitamin D.[2] However, the response of 25-hydroxyvitamin D (25(OH)D), which is used as a vitamin D status marker, to vitamin D supplementation depends not only on endogenous factors such as genetics,[3,4] age[5] and body fat,[6] but also on dietary compounds such as the type of dietary fatty acids,[7] phytosterols[8] or fungal ergosterol.[9] While multiple studies have investigated the role of vitamin D on cholesterol metabolism in humans, the influence of dietary cholesterol on the bioavailability of oral vitamin D is currently unknown. In 2004, Altmann *et al.*[10] identified Niemann-Pick C1-like 1 (NPC1L1) as a transmembrane protein that is crucial for the intestinal absorption of dietary and biliary cholesterol. In 2011, Reboul *et al.* observed that the cellular uptake of vitamin D was significantly reduced when Caco-2 cells were incubated with ezetimibe, a specific inhibitor of NPC1L1.[8] Additionally, inhibition of NPC1L1 in mice resulted in markedly lower concentrations of vitamin D in the liver, adipose tissues, skeletal muscle, kidney and heart.[11] These data indicate that cholesterol and vitamin D compete for the same absorption mechanism in the gut. Thus, it is tempting to speculate that dietary cholesterol could impact vitamin D status by modulating the intestinal uptake of vitamin D.

Data from the National Health and Nutrition Examination Survey (NHANES) reported that the mean dietary cholesterol intake of U.S. adults in the 2013–2014 survey cycle was

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293 mg/day (348 mg/day for men and 242 mg/day for women).[12] However, the dietary cholesterol intake of a person largely depends on the foods and diets that are consumed because foods of animal origin such as eggs and meat are major sources of cholesterol. Since many individuals depend, at least temporarily, on vitamin D supplementation, the question arises whether foods or diets rich in cholesterol may reduce the efficacy of oral vitamin D to improve vitamin D status. Based on the similar chemical structures of cholesterol and vitamin D and the fact that both molecules share the same intestinal transporter, we hypothesized that the consumption of cholesterol could adversely affect vitamin D uptake and in turn vitamin D status.

Methods

The experimental protocols of the mouse studies were approved by the animal welfare committee of the Martin Luther University Halle-Wittenberg (approval numbers: H1-4/T3-19, H1-4/T1-15). The experimental protocols followed the established guidelines for the care and handling of laboratory animals[13] and were in accordance with the German animal welfare regulations. The studies adhered to the ARRIVE Guidelines for reporting animal research.

The impact of dietary cholesterol on vitamin D status in mice

The impact of dietary cholesterol on vitamin D status was first examined in a mouse study. All mice were kept in pairs in Macrolon cages in a room with a constant temperature (22 ± 2°C), light cycle (12-h light, 12-h dark with lamps that did not emit UV light) and relative humidity (50 – 60%). The animals had free access to feed and water.

Forty-two male 6-week-old wild-type mice (C57BL/6N) were purchased from Charles River (Sulzfeld, Germany). Mice were given five days to acclimate to their environment before they were randomly assigned into seven groups of six animals each (initial body weight of 21.6 ± 0.93 g). During the study, mice were fed diets with 25 µg/kg triple-deuterated vitamin D_3 (vitamin D_3-d_3, Sigma-Aldrich, Steinheim, Germany) that contained 0% (control group), 0.2%, 0.4%, 0.6%, 0.8%, 1.0% or 2.0% cholesterol for four weeks. The basal diet contained (per kg) 297 g of starch, 200 g of sucrose, 200 g of casein, 150 g of coconut oil, 50 g of soybean oil, 50 g of a vitamin and mineral mixture, 50 g of cellulose, and 3 g of DL-methionine. Varying amounts of cholesterol were added to the diet in exchange for starch. Vitamins and minerals were added to the diet according to the recommendations of the National Research Council.[14]

After four weeks of treatment, the mice were deprived of feed for four hours, anaesthetised and decapitated. Feed withdrawal four hours before sampling and dissection of mice were carried out during the light phase of the light-dark cycle, starting between 6:00 am and 10:00 am. The blood samples were taken and collected in tubes (Sarstedt, Nümbrecht, Germany) to obtain serum. Additionally, intestinal mucosa, livers, kidneys and retroperitoneal adipose tissues were harvested. All samples were immediately snap-frozen in liquid nitrogen and stored at -80°C until analyses.

The effects of dietary cholesterol on bile acids in mice

To investigate the impact of dietary cholesterol on bile acids, which in turn can influence the digestibility and uptake of fat-soluble nutrients, a second study with mice was conducted. The care and handling of the mice were in line with the protocol described above.

Ten male 4-week-old wild-type mice (C57BL/6N; Charles River) with an initial body weight of 14.3 ± 1.49 g were randomly allotted to two groups (n = 5). The mice were fed diets with 25 µg/kg vitamin D_3 (Sigma-Aldrich) that contained either 0% (control group) or 1.0% cholesterol. The basal diet contained (per kg) 397 g of starch, 200 g of sucrose, 200 g of casein, 100 g of lard, 50 g of a vitamin and mineral mixture, 50 g of cellulose, and 3 g of DL-methionine. Cholesterol was added to the diet in exchange for starch. Vitamins and minerals were added to the diet according to recommendations of the National Research Council.[14]

After four weeks of treatment, the mice were deprived of feed for four hours, anaesthetized and decapitated in accordance to mouse study 1 described above. The intestinal mucosa samples were harvested to analyse the intracellular vitamin D concentration, bile was obtained from the gallbladder, and faeces was collected from the rectum to quantify bile acids. The samples were immediately snap-frozen in liquid nitrogen and stored at -80°C until analyses.

Cell culture study on the effects of cholesterol and bile acids on vitamin D uptake

To elucidate the impact of cholesterol versus bile acids on the cellular uptake of vitamin D_3, a cell culture study using human colorectal adenocarcinoma Caco-2 cells (ACC 169, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was conducted. The Caco-2 cells, which formed monolayers, were cultivated in Minimal Essential Medium, GlutaMAX® (MEM), supplemented with 1% non-essential amino acids (NEAAs), 10% foetal bovine serum (FBS) and 0.5% gentamycin (all from Gibco, Life Technologies GmbH, Darmstadt, Germany) at 37°C in a humidified atmosphere (95% air and 5% CO_2). For intracellular vitamin D_3 analysis, cells were seeded in dishes (diameter: 3.5 cm) at a density of 0.8×10^6 cells per dish. For relative mRNA expression analysis, cells were seeded in 24-well plates at a density of 0.15×10^6 cells per well. The seeded cells were cultured for seven days, to differentiate them into small intestinal epithelial-like cells.[15,16] Eighteen hours prior to incubation, the medium was replaced by FBS-free MEM supplemented with 1% NEAAs. For uptake experiments, cells were incubated in FBS-free MEM with 1 µM vitamin D_3 for 60 min at 37°C. To this end, vitamin D_3 was incorporated in micelles as described by Reboul *et al.*[8,17] All micelles consisted of 0.04 mM L-α-phosphatidylcholine, 0.5 mM oleic acid, 0.3 mM monoolein, 0.16 mM 1-α-lysophosphatidylcholine and 1 µM vitamin D_3 (all from Sigma-Aldrich). Then, the cells were treated in a two-factorial

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design with taurocholic acid (1 mM vs. 5 mM) and cholesterol (0 vs. 100 μM cholesterol) (all from Sigma-Aldrich). Taurocholic acid and cholesterol were added to the micellular components. For preparation of the micelles, appropriate volumes of lipids and vitamin D_3 stock solutions in absolute ethanol were transferred to a glass tube. The solvent was evaporated under nitrogen, and the dried residues were dissolved in MEM containing taurocholic acid. All micelle components were vigorously mixed in a sonication bath at room temperature for 5 min. The viability of the treated cells was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. None of the incubation conditions affected cell viability. After the treatments, the cells were washed twice with ice-cold phosphate-buffered saline (PBS), harvested with a cell scraper and centrifuged. The cell pellets were stored at -20°C until further analysis. Protein concentrations of the cell pellets were determined by the Bradford assay.[18] The experiment was independently repeated three times. Analyses from each experiment were run in duplicate (gene expression) or triplicate (vitamin D_3).

Analysis of vitamin D metabolites in plasma, tissues and cells

The concentrations of vitamin D_3, vitamin D_3-d_3 and triple-deuterated 25-hydroxyvitamin D_3 (25(OH)D_3-d_3) were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as recently described.[11] In brief, sevenfold deuterated vitamin D_3 (Toronto Research Chemicals, Inc., Toronto, Canada) and sixfold deuterated 25(OH)D_3 (Chemaphor Chemical Services, Ottawa, Canada) were added to the samples as internal standards. Subsequently, the samples were saponified with potassium hydroxide, extracted with n-hexane and washed with ultrapure water. Tissue samples were further purified by normal-phase HPLC (1100 Series, Agilent Technologies, Waldbronn, Germany). All types of samples were subjected to derivatization with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD; Sigma-Aldrich) and analysed by LC-MS/MS (1260 Infinity Series, Agilent Technologies; QTRAP 5500, SCIEX, Darmstadt, Germany) with positive electrospray ionization. For quantification of vitamin D_3 and vitamin D_3-d_3, a Hypersil ODS C18 column (120 A, 5 μm, 150 × 2.0 mm^2; VDS Optilab, Berlin, Germany) was used, and for quantification of 25(OH)D_3-d_3, a Poroshell C18 column (120 A, 2.7 μm, 50 × 4.6 mm^2; Agilent Technologies) was used. Quantifier mass transitions of the PTAD adducts were vitamin D_3 560 > 298, vitamin D_3-d_3 563 > 301, sevenfold deuterated vitamin D_3 567 > 298, 25(OH)D_3-d_3 579 > 301, and sixfold deuterated 25(OH)D_3 582 > 298. Calibration curves were constructed with standard solutions for vitamin D_3-d_3 and 25(OH)D_3-d_3 (both from Sigma-Aldrich) by plotting the ratio of the analyte peak area to the internal standard peak area versus the concentration of the analytes.

Analysis of triglycerides in liver

Liver samples were prepared as described elsewhere[19] and the triglyceride concentration of the extracts was quantified using an enzymatic reagent kit according to the manufacturer's manual (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

Analysis of the relative mRNA expression of genes involved in vitamin D uptake and metabolism

Relative mRNA expression was analysed by real-time RT-PCR as described previously.[19] Prior to analysis, total RNA was isolated from tissue samples and Caco-2 cells using peqGOLD TriFast™ (VWR International GmbH, Darmstadt, Germany). The concentration of RNA in the samples was determined at a wavelength of 260 nm with a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific GmbH, Schwerte, Germany). Reverse transcription reactions were performed using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) to yield cDNA, and real-time RT-PCR was performed with GoTaq® Flexi DNA-Polymerase (Promega) on a Rotorgene 6000 cycler (Corbett Research, Mortlake, Australia) according to a protocol described elsewhere.[19] After each PCR run, melting curve analysis and gel electrophoresis verified the amplification and product size. The relative mRNA expression of the target genes was calculated by the method of Pfaffl.[20] Glyceraldehyde-3-phosphate dehydrogenase *(Gapdh/GAPDH)* and ribosomal protein lateral stalk subunit P0 (*Rplp0/RPLP0)* were used as the appropriate reference genes. Primers of the target and reference genes are summarised in Table 1.

Analysis of faecal bile acids

The concentration of bile acids was determined in the faeces of mice obtained from the rectum by MS-Omics (Vedbaek, Netherlands). The freeze-dried samples were extracted with methanol, transferred to centrifuge tube filters and centrifuged for purification. The filtrate was then subjected to a Thermo Scientific Vanquish LC coupled to Thermo Q Exactive HF mass spectrometer for bile acid analysis. An electrospray ionisation interface was used as the ionisation source. The system was operated in negative ionisation mode. Chromatographic separation of the bile acids was carried out on a Waters Acquity HSS T3 1.8 μm 2.1 × 150 mm. The column was thermostatted at 30°C. The mobile phases consisted of (A) ammonium acetate (10 mmol/l) and (B) methanol/acetonitrile (1/1, v/v). Bile acids were eluted by increasing the concentration of B in A from 45 to 100% over 16 min. The flow rate was 0.3 ml/min. Peak areas were extracted using TraceFinder 4.1 (Thermo Fisher Scientific, Waltham, USA). Identification of the compounds was based on the accurate mass and retention time of the authentic standards.

Statistical analysis

Data are expressed as the mean ± standard deviation. Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Data obtained from the first mouse study were tested for normal distribution (Shapiro-Wilk test) and homoscedasticity (Levene's test). Treatment effects were identified by one-way ANOVA for normally distributed data. In the case of significant treatment effects, an appropriate post hoc group comparison was performed (Tukey's test for equal variances or Games-Howell for unequal variances). For parameters that were not normally distributed, the effects of treatment were analysed by the non-parametric Kruskal-Wallis

Table 1. Primers of target genes involved in vitamin D uptake and metabolism and appropriate reference genes

	Obtained from	Accession number	Product size [bp]
Mouse primer			
Npc1l1	Eurofins MWG Synthesis	NM_207242.2	76
Cd36	Eurofins MWG Synthesis	NM_001159558.1	207
Abcg5	Eurofins MWG Synthesis	NM_031884.1	77
Abcg8	Eurofins MWG Synthesis	NM_026180.2	73
Cyp27a1	Sigma-Aldrich	NM_024264.4	126
Cyp2r1	Sigma-Aldrich	NM_177382.4	196
*Gapdh**	Eurofins MWG Synthesis	XM_001473623.1	177
*Rplp0**	Eurofins MWG Synthesis	NM_007475.5	146
Human primer			
NPC1L1	Eurofins MWG Synthesis	NM_001101648.1	164
CD36	Eurofins MWG Synthesis	NM_001001548.2	172
ABCG5	Eurofins MWG Synthesis	NM_022436.3	234
ABCG8	Sigma-Aldrich	NM_001357321.2	199
SCARB1	Sigma-Aldrich	NM_016741.2	149
*GAPDH**	Eurofins MWG Synthesis	NM_002046.3	453
*RPLP0**	Eurofins MWG Synthesis	NM_001002.3	223

*Reference gene. Abcg5/ABCG5, ATP-binding cassette subfamily G member 5; Abcg8/ABCG8, ATP-binding cassette subfamily G member 8; Cd36/CD36, CD36 molecule; Cyp2r1, vitamin D 25-hydroxylase; Cyp27a1, sterol 27-hydroxylase; Gapdh/GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Npc1l1/NPC1L1, Niemann-Pick C1-like 1; Rplp0/RPLP0, ribosomal protein lateral stalk subunit P0; SCARB1, scavenger receptor class B member 1.

Table 2. Body weight, liver weight, relative liver weight, liver triglyceride and feed intake of mice fed different doses of cholesterol

	Dietary cholesterol content							Effect of treatment
	0.0 %	0.2 %	0.4 %	0.6 %	0.8 %	1.0 %	2.0 %	*P* value
Body weight [g]	26.1 ± 1.89	25.7 ± 0.90	24.5 ± 1.54	26.2 ± 2.35	24.3 ± 2.35	25.8 ± 1.42	25.4 ± 1.63	Ns
Liver weight [g]	1.08 ± 0.10	1.04 ± 0.14	0.98 ± 0.20	1.19 ± 0.18	1.00 ± 1.18	1.03 ± 0.15	1.07 ± 0.10	Ns
Liver:body weight ratio [%]	4.14 ± 0.22	4.04 ± 0.53	4.00 ± 0.70	4.53 ± 0.37	4.12 ± 0.37	4.02 ± 0.57	4.22 ± 0.21	Ns
Liver triglyceride [mg/g]	37.9 ± 12.3	30.1 ± 6.16	50.0 ± 15.0	47.3 ± 24.9	52.8 ± 27.6	32.3 ± 6.20	37.6 ± 3.30	Ns
Feed intake [g/d][a]	4.79 ± 0.18	4.95 ± 0.03	4.18 ± 0.18	4.99 ± 0.02	4.79 ± 0.07	5.35 ± 1.63	4.95 ± 0.28	Ns

Ns, not significant.
[a]Feed intake was assessed from two mice per cage. Data are expressed as the means ± standard deviation. Treatment effects were analysed by one-way ANOVA or Kruskal-Wallis test.

test. Individual group comparisons were performed by the Mann-Whitney U test with Bonferroni's correction. A correlation analysis of liver triglyceride concentration and liver vitamin D_3-d_3 concentration was performed by Spearman correlation as a non-parametric measure of correlation. If not otherwise stated, all mice (N = 42) were included in the analysis. For the second mouse study, data were subjected to Student's t-test in cases of normal distribution or the non-parametric Mann-Whitney U test. All mice (N = 10) were included in the analysis. Data from the cell culture experiment were analysed by two-way ANOVA including the factors of taurocholic acid treatment, cholesterol treatment and their interaction (taurocholic acid × cholesterol). $P < 0.05$ was designated as a significant difference and $P < 0.1$ as a trend toward significance.

Results

The impact of dietary cholesterol on vitamin D status

Body weight, liver weight, liver triglycerides and feed intake. The final body weights, liver weights and the liver:body weight ratios as well as the concentrations of triglycerides in the liver of the mice were not differentially affected by the dietary treatments (Table 2). Also, daily feed intake (assessed from two mice per cage) did not differ between the groups (Table 2).

Dietary cholesterol increases vitamin D_3-d_3 in serum and tissues. To assess the effects of dietary cholesterol on vitamin D uptake and vitamin D status, the amounts of deuterated vitamin D metabolites were determined in serum, liver, kidney and adipose tissue. The data showed that the serum and tissue concentrations of vitamin D_3-d_3 were significantly influenced by cholesterol in the diet (Fig. 1). Interestingly, the mice fed cholesterol-containing diets had higher serum and tissue concentrations of vitamin D_3-d_3 than mice fed the cholesterol-free diet, although no clear dose-response relationship between dietary cholesterol and levels of vitamin D_3-d_3 was observed. The increase in vitamin D_3-d_3 in response to dietary cholesterol was most pronounced in the serum and liver (Fig. 1a and b). To test whether the higher vitamin D_3-d_3 levels in the cholesterol-fed mice were associated with higher triglyceride concentrations in the liver, a correlation between both parameters was performed. As depicted in Fig. 2, no significant

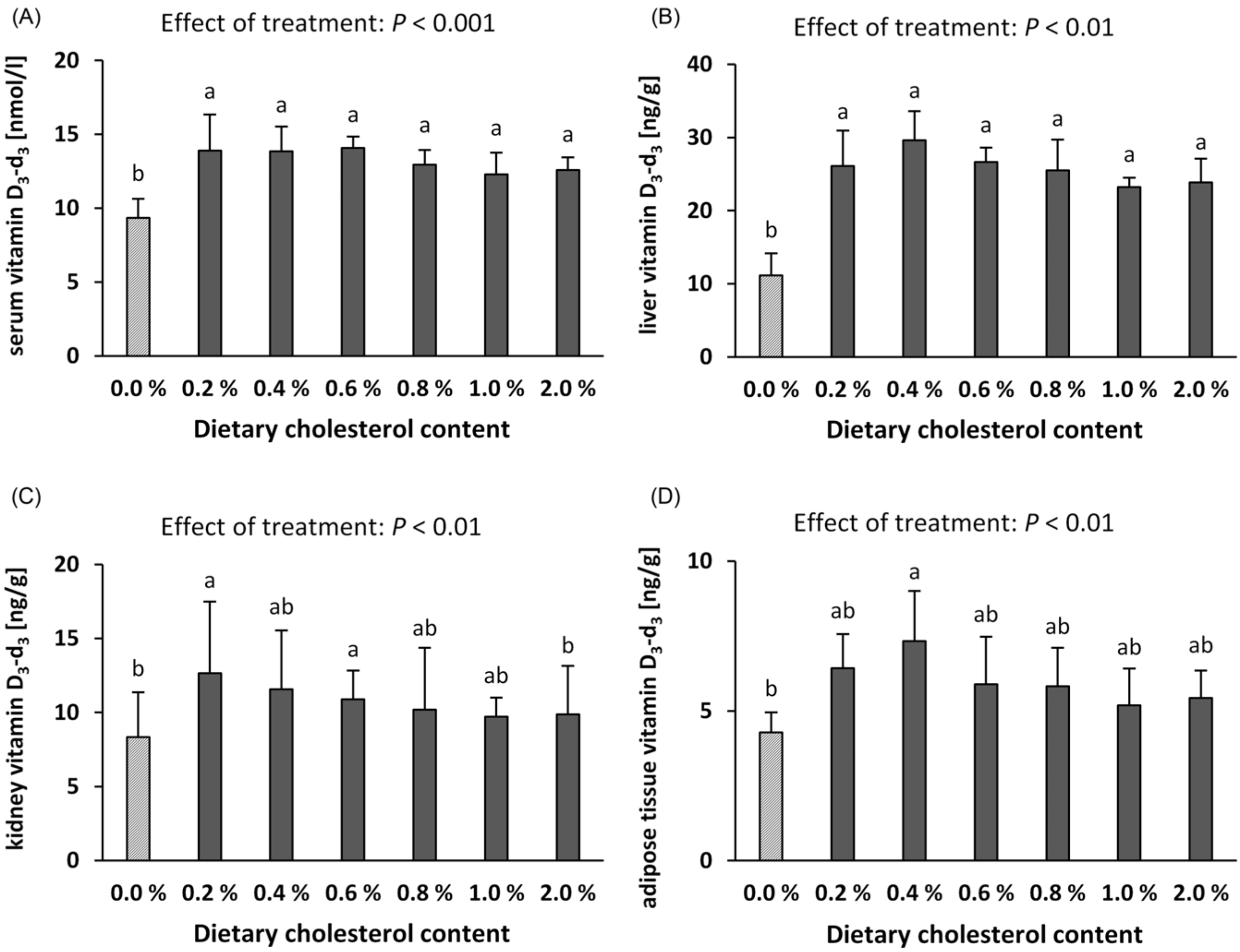

Fig. 1. Concentration of vitamin D_3-d_3 in the serum (a), livers (b), kidneys (c) and adipose tissues (D) of mice fed different doses of cholesterol. Data are expressed as the means ± standard deviation. Treatment effects were identified by one-way ANOVA or the Kruskal-Wallis test. [a,b] Different letters indicate differences between groups (multiple group comparison, $P < 0.05$). Vitamin D_3-d_3, triple-deuterated vitamin D_3.

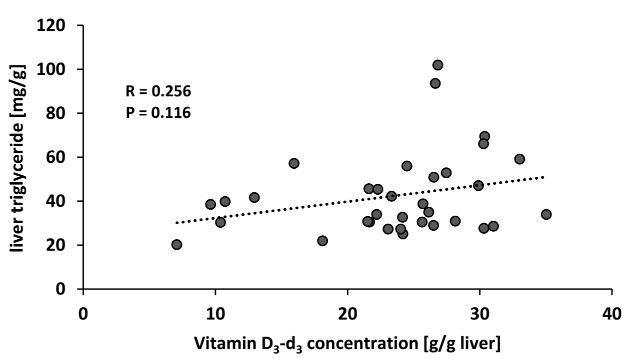

Fig. 2. Correlation between concentrations of liver triglycerides and liver vitamin D_3-d_3 of mice that were fed different doses of cholesterol. No correlation was identified by Spearman correlation, N = 39.

correlation was observed between liver triglycerides and the liver vitamin D_3-d_3 concentration. Due to the high variance, the increase in vitamin D in the kidney and adipose tissue of mice fed the cholesterol diets did not consistently reach statistical significance (Fig. 1c and d). The concentrations of 25(OH)D_3-d_3 in the serum (0% cholesterol group: 59.1 ± 8.89 nmol/l; 0.2%: 57.5 ± 6.94 nmol/l; 0.4%: 54.1 ± 11.7 nmol/l; 0.6%: 68.5 ± 10.6 nmol/l; 0.8%: 57.9 ± 9.56 nmol/l; 1.0%: 64.7 ± 8.56 nmol/l; 2.0%: 61.8 ± 6.80 nmol/l) and kidneys (0% cholesterol group: 2.90 ± 0.23 ng/g; 0.2%: 3.30 ± 0.21 ng/g; 0.4%: 3.39 ± 0.41 ng/g; 0.6%: 3.57 ± 0.49 ng/g; 0.8%: 3.15 ± 0.68 ng/g; 1.0%: 3.52 ± 0.71 ng/g; 2.0%: 3.52 ± 0.55 ng/g) were not affected by dietary cholesterol. The levels of 25(OH) D_3-d_3 in the liver and adipose tissue were lower than the limit of quantification (6 ng/g and 2 ng/g, respectively).

Dietary cholesterol did not modify the mRNA expression of genes involved in vitamin D uptake and metabolism. To determine whether the increased serum and tissue levels of vitamin D were caused by a higher abundance of uptake transporters, we analysed the mRNA expression of intestinal vitamin D transporters. These data did not indicate any consistent effect of dietary cholesterol on the mRNA expression of transporters involved in vitamin D uptake (Table 3). In addition, we analysed the mRNA expression of the most important hydroxylases in the liver but found no differences between the groups (Table 3).

Table 3. Relative mRNA expression of genes involved in vitamin D_3 uptake and metabolism in mice fed different doses of cholesterol

	Dietary cholesterol content							Effect of treatment
	0.0%	0.2%	0.4%	0.6%	0.8%	1.0%	2.0%	(P value)
Intestinal mucosa								
Npc1l1	1.00 ± 0.43	0.89 ± 0.19	0.95 ± 0.29	0.80 ± 0.27	0.85 ± 0.23	0.67 ± 0.09	0.97 ± 0.25	Ns
Cd36	1.00 ± 0.34	1.22 ± 0.26	1.35 ± 0.62	1.30 ± 0.61	1.24 ± 0.31	1.13 ± 0.53	0.80 ± 0.16	Ns
Abcg5	1.00 ± 0.26[ab]	1.16 ± 0.28[ab]	1.46 ± 0.37[ab]	1.60 ± 0.64[a]	1.34 ± 0.26[ab]	0.78 ± 0.28[b]	1.68 ± 0.33[a]	< 0.01
Abcg8	1.00 ± 0.40	1.19 ± 0.51	0.88 ± 0.35	1.43 ± 0.46	0.89 ± 0.25	1.02 ± 0.30	0.74 ± 0.36	Ns
Liver								
Cyp27a1	1.00 ± 0.21	1.05 ± 0.32	0.93 ± 0.26	0.74 ± 0.21	0.96 ± 0.26	0.81 ± 0.16	0.99 ± 0.12	Ns
Cyp2r1	1.00 ± 0.46	1.05 ± 0.39	0.78 ± 0.35	0.87 ± 0.10	0.73 ± 0.34	0.86 ± 0.41	1.47 ± 0.60	Ns

Abcg5, ATP-binding cassette subfamily G member 5; Abcg8, ATP-binding cassette subfamily G member 8; Cd36, CD36 molecule; Cyp2r1, vitamin D 25-hydroxylase; Cyp27a1, sterol 27-hydroxylase; Npc1l1, Niemann-Pick C1-like 1; ns, not significant.
Data are expressed as the means ± standard deviation. Treatment effects were identified by one-way ANOVA or Kruskal-Wallis test. [a,b] Different letters indicate differences between groups (multiple group comparison, $P < 0.05$).

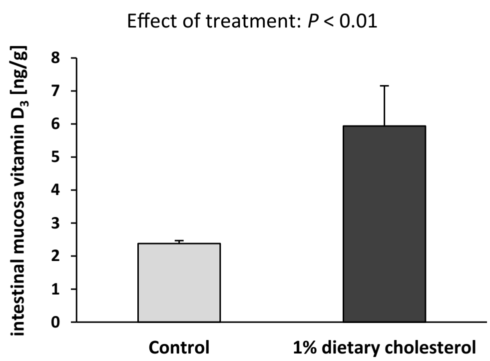

Fig. 3. Concentration of vitamin D_3 in the intestinal mucosa of mice in response to dietary cholesterol supply. Data are expressed as the means ± standard deviation. Treatment effect was identified by Student's t-test.

The impact of cholesterol on intestinal vitamin D and bile acids

To elucidate whether cholesterol feeding is associated with higher intestinal bile acid concentrations and in turn, higher vitamin D concentrations in enterocytes *in vivo*, we conducted a subsequent study. Mice that were fed a vitamin D-adequate diet with 0 or 1% cholesterol over four weeks did not show differences in their final body weights (0% cholesterol: 22.2 ± 0.99 g, 1% cholesterol: 22.4 ± 1.62 g).

Here, we found that the vitamin D_3 concentration in the intestinal mucosa was significantly higher in mice fed 1% cholesterol than in those fed 0% cholesterol ($P < 0.01$, Fig. 3). In addition, the faecal concentrations of total and secondary bile acids were significantly higher in the mice fed 1% cholesterol than in those fed 0% cholesterol. The primary and tertiary bile acids showed a trend towards higher levels in the cholesterol group (Table 4).

The three major types of bile acids found in bile obtained from the gallbladder were muricholic acids, taurocholic acid and taurodeoxycholic acid. Dietary cholesterol intake was associated with a change in the bile acid composition in the bile (Fig. 4). The bile acid profiles of the mice fed 1% cholesterol were characterized by a higher proportion of hydrophobic taurodeoxycholic acid ($P < 0.01$) and a trend towards less hydrophilic muricholic acids ($P < 0.1$, Fig. 4).

Taurocholic acid but not cholesterol increases the uptake of vitamin D in Caco-2 cells

To investigate whether cholesterol can directly improve the cellular uptake of vitamin D or indirectly by the changes in bile acids, we treated Caco-2 cells with micelles differing in cholesterol and taurocholic acid concentrations. Treatment of cells with 100 µM versus 0 µM cholesterol did not change the intracellular vitamin D_3 concentration, suggesting that cholesterol is not capable of modifying vitamin D uptake in Caco-2 cells per se (Fig. 5). In contrast, the intracellular vitamin D_3 concentration was approximately fourfold higher after incubation with 5 mM taurocholic acid than with 1 mM taurocholic acid, indicating a strong effect of taurocholic acid in improving vitamin D_3 uptake into intestinal cells (Fig. 5). Neither cholesterol nor taurocholic acid altered the relative mRNA expression of vitamin D transporters in Caco-2 cells (Table 5).

Discussion

In the current study, we tested the hypothesis that cholesterol could affect the intestinal uptake of oral vitamin D and in turn vitamin D status. Surprisingly, we found significant increases in vitamin D in the serum and livers of mice fed cholesterol, indicating that cholesterol is able to increase the uptake of orally administered vitamin D, although 25(OH)D, which is normally used as a biomarker of vitamin D status, remained unaffected. This finding contradicts the hypothesis that cholesterol and vitamin D compete for the same intestinal transporter and that cholesterol, which was in excess compared to vitamin D, may hinder the absorption of vitamin D. NPC1L1 plays a crucial role in the absorption of cholesterol by enterocytes[(10)] and is important for vitamin D uptake.[(8,11)] However, the current mRNA data were not indicative that the improvement in vitamin D status that we observed in the cholesterol-fed groups was caused by cholesterol-induced changes in the expression of NPC1L1 and other transporters, such as CD36 molecule (CD36) or ATP-binding cassette subfamily G member 5/8 (ABCG5/8), which have been suggested to be involved in vitamin D absorption.[(8,21)] Thus, these data were not indicative of any direct effect of cholesterol on vitamin D transporter

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Table 4. Faecal bile acid concentration [µg/g dry matter] of mice in response to dietary cholesterol supply

	Control	1% dietary cholesterol	*P* value
Total bile acids	3545 ± 1708	8951 ± 3685	< 0.05
Total primary bile acids	1402 ± 1498	4765 ± 3361	< 0.1
Cholic acid	43.2 ± 36.1	369 ± 324	< 0.1
Chenodeoxycholic acid	3.45 ± 2.24	6.62 ± 5.38	Ns
Glycocholic acid	< LOQ	18.7 ± 33.3	< 0.05
Glycochenodeoxycholic acid	0.95 ± 0.08	1.57 ± 0.89	< 0.05
Taurocholic acid	83.2 ± 149	343 ± 414	Ns
Taurochenodeoxycholic acid	3.89 ± 4.32	8.86 ± 8.17	Ns
α-Muricholic acid	151 ± 151	348 ± 369	Ns
Tauro-α-muricholic acid	35.5 ± 51.4	53.4 ± 43.6	Ns
β-Muricholic acid	725 ± 372	1721 ± 561	< 0.05
Tauro-β-muricholic acid	402 ± 741	1701 ± 2340	Ns
Hyocholic acid	8.51 ± 0.46	26.0 ± 26.7	Ns
Total secondary bile acids	2420 ± 129	4144 ± 1581	< 0.05
Lithocholic acid	17.9 ± 9.65	61.3 ± 14.2	< 0.001
Isolithocholic acid	2.37 ± 1.42	3.01 ± 0.94	Ns
Ketolithocholic acid	7.56 ± 4.22	26.2 ± 17.3	< 0.01
Taurolithocholic acid	0.54 ± 0.19	4.33 ± 3.86	< 0.05
Deoxycholic acid	314 ± 95.9	990 ± 82.5	< 0.05
Hyodeoxycholic acid	21.2 ± 8.17	61.7 ± 11.4	< 0.001
Glycodeoxycholic acid	1.08 ± 0.02	1.82 ± 0.98	< 0.05
Taurodeoxycholic acid	2.35 ± 4.60	61.6 ± 67.5	< 0.1
ω-Muricholic acid	1943 ± 183	2711 ± 1436	Ns
Total tertiary bile acids	13.8 ± 13.5	42.0 ± 24.1	< 0.1
Ursodeoxycholic acid	7.63 ± 5.06	22.2 ± 15.5	< 0.1
Glycoursodeoxycholic acid	1.46 ± 0.02	1.67 ± 0.27	Ns
Tauroursodeoxycholic acid	6.09 ± 9.32	18.2 ± 22.3	Ns

Ns, not significant.
Data are expressed as the means ± standard deviation. Differences between the two groups were identified by Student's t-test or Mann-Whitney U test.

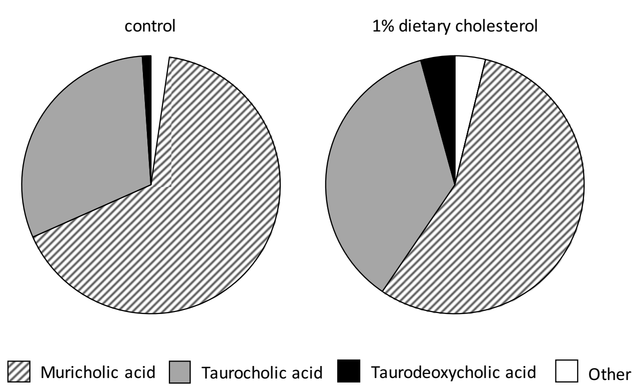

Fig. 4. Major bile acids (weight % of the total) in the bile of mice in response to dietary cholesterol supply. Data are expressed as the means. Taurodeoxycholic acid was significantly different (P < 0.01), and muricholic acids showed a trend towards significance (P < 0.1) in response to dietary cholesterol (Student's t-test).

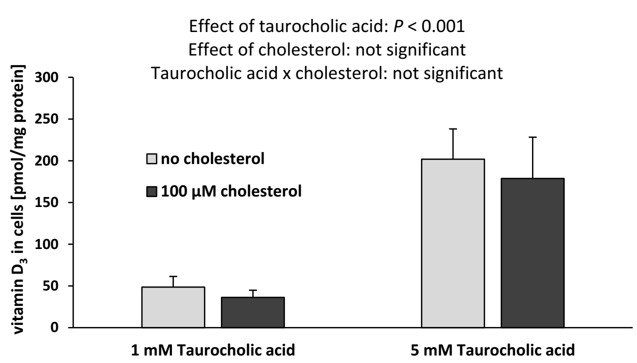

Fig. 5. Concentration of vitamin D_3 in Caco-2 cells in response to micellar cholesterol and taurocholic acid content. Data are expressed as the means ± standard deviation. Treatment effects were identified by two-way ANOVA.

expression. However, it should be noted that the intestinal uptake of cholesterol and vitamin D involves both transporter-mediated uptake and passive diffusion. It is therefore possible that the higher vitamin D_3-d_3 levels in cholesterol-fed mice resulted from higher passive diffusion.

Cholesterol serves as a precursor for bile acid synthesis and stimulates the formation of bile acids in the liver.[22,23] This explains why mice fed a high-cholesterol diet had substantially higher concentrations of bile acids in their faeces than mice fed no cholesterol. Based on these data, we hypothesised that cholesterol could enhance vitamin D uptake indirectly by increasing the formation and release of bile acids into the gut. This assumption was confirmed in the two subsequent studies that we conducted. First, the concentrations of faecal bile acids and vitamin D in enterocytes were significantly higher in mice fed the cholesterol-containing diet than in mice fed the cholesterol-free diet. Second, analysis of the human Caco-2 cells revealed a substantially higher cellular uptake of vitamin D when micelles contained higher concentrations of taurocholic acid but not when they contained cholesterol. This cell culture study clearly indicates a direct effect of bile acids on vitamin D uptake, but the findings need to be confirmed in further *in vivo* studies.

Bile acids serve as micelle-forming surfactants and facilitate the absorption of lipids and hydrophobic nutrients. Micelles are

Table 5. Relative mRNA expression of genes involved in vitamin D uptake in Caco-2 cells

	1 mM TCA		5 mM TCA		*P* value		
	No chol	100 µM chol	No chol	100 µM chol	TCA	chol	TCA×chol
NPC1L1	1.00 ± 0.14	0.98 ± 0.18	0.87 ± 0.05	1.03 ± 0.28	Ns	Ns	Ns
CD36	1.00 ± 0.46	1.29 ± 0.26	1.20 ± 0.32	0.99 ± 0.44	Ns	Ns	Ns
ABCG5	1.00 ± 0.11	1.03 ± 0.08	1.61 ± 0.94	1.08 ± 0.22	Ns	Ns	Ns
ABCG8	1.00 ± 0.38	1.05 ± 0.43	0.99 ± 0.23	0.94 ± 0.49	Ns	Ns	Ns
SCARB1	1.00 ± 0.22	1.44 ± 0.11	1.03 ± 0.08	1.15 ± 0.44	Ns	Ns	Ns

ABCG5, ATP-binding cassette subfamily G member 5; ABCG8, ATP-binding cassette subfamily G member 8; Chol, cholesterol; CD36, CD36 molecule; NPC1L1, Niemann-Pick C1-like 1; ns, not significant; SCARB1, scavenger receptor class B member 1; TCA, taurocholic acid.
Data are expressed as the means ± standard deviation. Treatment effects were identified by two-way ANOVA.

usually self-assembling structures that are formed from lipid digestion products and bile acids,[24] and their shape, size and composition might determine their efficiency in carrying lipophilic compounds. Thus, we assume that cholesterol could have influenced bile acid formation, release and profile and, in turn, the composition of the micelles and the solubility of vitamin D within micelles. A study revealed that mice deficient in 7α-hydroxylase, an enzyme that plays a key role in the synthesis of bile acids, had low serum vitamin D levels, emphasizing an important role of the classic bile acid pathway in intestinal vitamin D uptake.[25] Moreover, the co-supplementation of vitamin D and cholic acid restored the serum vitamin D levels more efficiently than vitamin D supplementation alone in these mice.[25] Bile acids largely differ in their hydrophobicity, which in turn influences sterol absorption.[26] Supplementation with hydrophobic cholic acid was associated with a higher micellar cholesterol concentration and increased absorption of cholesterol in healthy subjects.[27] In contrast, the administration of hydrophilic muricholic acids resulted in low cholesterol absorption in mice in comparison with the most hydrophobic bile acids (cholic acid and deoxycholic acid).[26] In our mouse study, muricholic acids were the most prominent bile acids found in bile, comprising 65% of the total bile acids in the controls. However, it must be mentioned that muricholic acids belong to a group of bile acids primarily found in mice.[28] Since these bile acids are absent in humans, it is to be expected that the effect of cholesterol on vitamin D uptake in humans differs from that of mice. Feeding cholesterol reduced the content of muricholic acids to 55%. Conversely, the percentage of hydrophobic taurodeoxycholic acid increased in response to a cholesterol-containing diet. Thus, we speculate that the improved uptake of oral vitamin D can also be attributed to a more hydrophobic bile acid pool after dietary cholesterol intake.

Notably, our study did not show a clear dose-response relationship between dietary cholesterol intake and vitamin D_3-d_3 concentration in serum and tissue. The maximum levels of vitamin D_3-d_3 in serum and tissue were already reached at the lowest dietary cholesterol concentration. Dietary cholesterol administration usually predisposes mice to develop liver steatosis which is associated with the accumulation of lipid droplets in the liver.[29] Thus, it is tempted to speculate that higher levels of liver lipids may explain the higher levels of vitamin D_3-d_3 in the cholesterol-fed mice. However, data from the current study are not indicative of higher liver triglyceride levels in the cholesterol-fed mice and there was no correlation between triglycerides and vitamin D_3-d_3 in the liver. Instead, we assume that the lowest dose of cholesterol already had the maximum effect on the formation of bile acids or that higher doses of cholesterol had partly displaced vitamin D from the micelles, as has been observed for phytosterols.[30] This would have to be investigated in further studies.

Conclusions

To conclude, the data demonstrate that dietary cholesterol increases body concentrations of vitamin D, shown by the significant rises of vitamin D in the serum and livers of cholesterol-fed mice. However, these findings are not indicative of a direct cholesterol effect on the absorption of vitamin D but indicate that ingested cholesterol might stimulate the formation and release of bile acids, which in turn increases the micellar solubility of vitamin D and its intestinal uptake.

Abbreviations

Abcg5/ABCG5: ATP-binding cassette subfamily G member 5; **Abcg8/ABCG8:** ATP-binding cassette subfamily G member 8; **Chol:** cholesterol; **Cd36/CD36:** CD36 molecule; **Cyp2r1:** vitamin D 25-hydroxylase; **Cyp27a1:** sterol 27-hydroxylase; **FBS:** foetal bovine serum; **Gapdh/GAPDH:** glyceraldehyde-3-phosphate dehydrogenase; **LC-MS/MS:** liquid chromatography-tandem mass spectrometry; **MEM:** minimal essential medium; **NEAAs:** non-essential amino acids; **NHANES:** National Health and Nutrition Examination Survey; **Npc1l1/NPC1L1:** Niemann-Pick C1-like 1; **ns** not significant; **PBS:** phosphate-buffered saline; **PTAD:** 4-phenyl-1,2,4-triazoline-3,5-dione; **Rplp0/RPLP0:** ribosomal protein lateral stalk subunit P0; **SCARB1:** scavenger receptor class B member 1; **TCA:** taurocholic acid; **vitamin D_3-d_3:** triple-deuterated vitamin D_3; **25(OH)D_3-d_3:** triple-deuterated 25-hydroxyvitamin D_3; **25(OH)D:** 25-hydroxyvitamin D

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Competing interests

The authors declare that they have no competing interests.

Authorship

JK, AS, CB and GIS designed the studies; JK, AS and LR performed the studies; JK, LR, MK and AN analysed the samples; JK statistically analysed the data; and JK and GIS wrote the manuscript.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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RESEARCH ARTICLE

Emotional eating and mental health of nurses working in Lebanese hospitals during the double crisis

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Abstract

The 2019 coronavirus (COVID-19) pandemic and strict quarantine increased the likelihood of mental symptoms and abnormal eating behaviours. This study aimed to assess the magnitude of emotional eating (EE) among nurses working in Lebanese hospitals and its association with mental health. A cross-sectional study was conducted among nurses aged between 18 and 50 years working in Lebanese hospitals during the COVID-19 outbreak and the economic crisis. A total of 303 nurses consented to participate. The mean EE score was 28.56 (±8.11). The results of this study revealed that 53.8% of the nurses reported depression, 58.1% suffered from anxiety and 95.1% experienced either moderate or severe stress. The study concluded that females ($\beta = 8.112$, $P = 0.004$), non-smokers ($\beta = -4.732$, $P = 0.01$) and depressed nurses ($\beta = 0.596$, $P = 0.046$) had a higher tendency towards EE. Additionally, it was found that EE was associated with weight gain ($\beta = 6.048$, $P = 0.03$) and increased consumption of fried foods ($\beta = 5.223$, $P = 0.001$). Females experienced more stress ($\beta = 2.244$, $P = 0.003$) and anxiety ($\beta = 1.526$, $P = 0.021$) than their male counterparts. With regard to mental health, depression was associated with weight gain ($\beta = 2.402$, $P = 0.003$) and with lower consumption of healthy foods such as nuts ($\beta = -1.706$, $P = 0.009$) and dishes prepared with sofrito sauce ($\beta = -1.378$, $P = 0.012$). These results can help the health authorities to design preparedness plans to ensure proper mental and physical well-being of nurses during any unforeseen emergencies.

Key words: COVID-19: Eating habits: Emotional eating: Mental health: Nurses

Introduction

The COVID-19 outbreak and the public health policies implemented in reaction to it have had negative physical, psychological and mental health consequences for people from all age groups.[1] In Lebanon, a country already hit by economic crisis and political instability, the effect of the pandemic on mental health has been even more deleterious. Indeed, a study conducted in 2020 revealed that the prevalence of anxiety and depression in Lebanon is about 42% and 42.6% respectively.[2] These numbers are considerably higher than those reported in a sample of Lebanese adults interviewed between September 2002 and 2003, where the prevalence of anxiety disorders was 16.7% and the prevalence of major depression disorders was 9.9%.[3]

Concomitantly with the Covid-19 outbreak, Lebanon was undergoing a severe economic crisis, therefore the country was undergoing a so-called "double crisis". In most countries, even though most restrictions due to the pandemic were lifted by 2022, the stress and the burden on the fragile Lebanese healthcare system wasn't resolved yet due to the ongoing economic depreciation affecting this system. The devaluation of the Lebanese currency has caused major challenges for many hospitals in Lebanon, making it harder for them to buy essential healthcare supplies.[4]

Negative emotions such as worry, irritation or depression can lead to a compulsive need to eat, or so-called emotional eating (EE).[5] EE is characterised by the inability to discern between biological hunger signs and the desire to eat to cope with

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unpleasant feelings.[6] Eating in response to negative feelings, such as those experienced during the COVID-19 pandemic, can therefore lead to weight gain, which can subsequently adversely affect individuals' health. In this regard, a Norwegian cohort study revealed that during the COVID-19 pandemic, psychological distress was associated with weight gain, and this association was partially mediated by emotional eating.[7]

When it comes to the association between mental health and emotional eating, nurses are of particular concern. Working irregular shifts, such as mornings, evenings and, especially, night shifts, can result in increased calorie consumption, obesity and a poor-quality diet.[8] The distribution of energy and nutrient intake has been found to vary based on work shift, with lipid and protein intake for rotating shift workers being associated with higher consumption of beef, eggs, juices, and pasta.[9] This is not consistent with nutritious eating regimens like the Mediterranean diet (MD) which is one of the healthiest dietary patterns, emphasising fruits, vegetables, grains, nuts, and greens intake with low to moderate consumption of dairy products (yogurt, cheese), and poultry, with limited consumption of sweets, bakery goods and red meat.[10] Additionally, the nursing profession is known as being a stressful job, due to the complexity of the assigned tasks.[11] During the pandemic, the mental and emotional health of nurses was seriously compromised. Nurses experienced greater rates of depression, anxiety and insomnia during this pandemic (37%, 35% and 43%, respectively), higher rates compared to those during other pandemics, such as Middle East respiratory syndrome (MERS) and Severe acute respiratory syndrome (SARS).[12] Therefore, nurses are very likely to succumb to stress, as well as to adopt unhealthy dietary and lifestyle habits. Moreover, previous studies have associated their mental health with eating habits. In this regard, a cross-sectional study in Turkey revealed that perceived stress is positively associated with EE among nurses and the authors concluded that special programmes should be developed to enable nurses to make healthy dietary choices.[13] Therefore, exploring the extent of EE and its association with mental status among nurses during pandemics and emergencies in different contexts is important to develop well-tailored and culture-specific preparedness plans that will allow nurses to cope with any unexpected health emergencies in the future without succumbing to EE that has a tremendous effect on their health and well-being. To the best of our knowledge, no previous studies have examined the burden of EE among nurses working in Lebanese hospitals during the double crisis and its association with mental health.

Given the nature of their job and the deleterious effect of the double crisis on their mental health, we hypothesised that nurses working in Lebanese hospitals are more likely to experience high levels of EE during the pandemic and that this eating behaviour is associated with the burden of the crisis on their mental health. Therefore, the main aim of our study is to assess the magnitude of EE among nurses working in Lebanese hospitals and its association with mental health. Factors correlated with each of EE and mental health, including adherence to MD, will also be investigated to gain a better understanding of the situation as a whole. This study will enable the Lebanese Government to design specific measures in order to keep nurses resilient during any unexpected health emergencies.

Methodology

Study design and participants

A cross-sectional study was conducted between March and May 2022 among nurses aged between 18 and 50 years working in Lebanese hospitals during the COVID-19 outbreak. Exclusion criteria included the presence of at least one self-reported non-communicable disease (NCD) (e.g. thyroid disorders, diabetes mellitus, cancer), or being pregnant or lactating. An online questionnaire was developed using Google Forms. A total of 750 nurses working in eleven hospitals (8 private and 3 governmental) were invited to participate via WhatsApp and emails. Only 335 nurses consented to participate in the study (44.6% response rate). Among them, 32 were excluded because they met one of the exclusion criteria or because of incomplete and/or random responses. Our final sample size was 303 participants.

Data collection

Participants completed the questionnaire between March and May 2022. The general aim and information about the ethics of the study were explained at the beginning. The multicomponent questionnaire included four sections that evaluated the degree of emotional eating, sociodemographic characteristics, mental state, health status and eating habits of the participants. The average time for completion was 15 minutes. The questionnaire was pre-piloted with a group of 20 nurses from various departments, to ensure its clarity. Except for four words, which were rephrased, the findings of the questionnaire's pilot suggested that the questions were clear.

Sampling procedure

Sample size was calculated based on the mean emotional eating score of 27.5 in Saudi Arabia, using one mean formula (n = sample size; $z = 1.96$, which corresponds to a 95% confidence level; SD = 16, which corresponds to the standard deviation; $d = 1.8$, corresponding to the precision).[14] A minimum number of 300 nurses was needed to estimate a mean with 95% CI and a precision of 1.8. Through the Order of Nurses in Lebanon official email, an online survey was distributed to nurses working in different Lebanese hospitals from March until May 2022. Additionally, nursing directors in a variety of hospitals were contacted to obtain their approval, and the questionnaire was communicated through WhatsApp and email to working nurses.

Variables

Sociodemographic characteristics. These variables included age, gender (male, female), monthly income (enough, not enough), professional title (nurse, registered nurse, head of department, supervisor, director of nursing), shift pattern (always day, always night, day & night), working department (Covid-19, Non-Covid-19), marital status (married, single, divorced), years of experience (<1 year, 1–5 years, 6–10 years, 11–15 years, >15 years), and educational status (TS2/TB3, registered nurse, Master).

Emotional eating scale (EES). The EES comprises 25 self-reported questions evaluating the desire to eat while experiencing negative emotions, such as anger, anxiety or a low mood state (depression). Participants rated their responses on a five-point Likert scale, ranging from 0 (no desire to eat) to 4 (extreme desire to eat). The final score was obtained by adding all of the item scores together, and these varied from 0 to 100, with higher scores reflecting a higher reliance on food in the management of emotions.[15] The Arabic version of the EES was used in this study; it has demonstrated good test–retest reliability ($r = 0.79$, P < 0.001) and internal consistency of 0.81.[16]

Depressive symptoms (PHQ-9). The validated Arabic version of the Patient Health Questionnaire-9 (PHQ-9) was used in order to evaluate symptoms of depression experienced by the nurses during the COVID-19 pandemic (Cronbach's alpha of 0.857).[17,18] On a four-point Likert scale ranging from 0 (never) to 3 (nearly every day), nine questions assessed the incidence of depression symptoms in the previous week. The final scores ranged from 0 to 27, with values of 10 and higher reflecting the existence of depression.[18,19]

Generalised anxiety disorder (GAD-7). The GAD-7 has a sensitivity value of 0.83 and a specificity value of 0.84 for identifying generalised anxiety disorder.[20] The anxiety level experienced by the nurses during the previous two weeks was assessed using the validated Arabic version of the GAD-7 which possesses an internal consistency reliability of 0.763.[17,18] Symptoms of anxiety were evaluated on a four-point Likert scale, with responses varying from 0 (never) to 3 (almost every day). The total score fluctuated from 0 to 21, with scores above 10 confirming the existence of generalised anxiety disorder.[21]

Perceived stress scale (PSS-10). The Arabic version of the Perceived Stress Scale (PSS) questionnaire was used to quantify the level of stress experienced by the nurses. The scale is composed of ten items, with each question answered on a five-point Likert scale and responses ranging from 0 (almost never) to 4 (very often), with the test-retest reliability having an intra-correlation coefficient of 0.90.[22] Higher values reflected higher levels of perceived stress. The total score was divided into three categories: ≥ 27 = high stress; 14–26 = moderate stress; ≤ 13 = low stress.[23]

Health and eating habits. Weight and height were self-reported by each nurse in order to calculate their body mass index (BMI) using the following formula: weight (kg)/height (m^2). BMI was then categorised based on the World Health Organization (WHO) recommendations into four groups: underweight (< 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese (≥ 30 kg/m^2).[24] Each nurse was also asked to report any weight gain. Furthermore, participants described any change in their physical activity level during the COVID-19 outbreak in the past year.

Regarding eating habits, the Mediterranean Diet Adherence Screener (MEDAS) scale was used in order to evaluate the nurses' adherence to the MD. The MEDAS is a 14-item questionnaire that evaluates food intake frequencies of certain food items, along with food habits related to the MD. The participants were categorised as low adherent (≤ 5), medium adherent (6–8) and high adherent (≥ 9) to the MD pattern.[25] In addition, each nurse was requested to report whether their intake for each of the above-mentioned 14 items was higher, lower or remained the same during the COVID-19 outbreak. Finally, nurses were asked to describe any change in their snacks, fried food, alcohol, or coffee intakes. Smoking status was also investigated (non-smoker, moderate smoker, heavy smoker).

Ethical considerations

The study followed the Declaration of Helsinki guidelines and was approved by Beirut Arab University's Institutional Review Board (date: 20/1/2021, Nb: 2022-H-0136-HS-R-0477). Written approval was obtained from hospital executives. Since the study was web-based, and in order to obtain written consent, the online questionnaire included the sentence: 'I agree to participate in this study'. Participants were also informed that participation was voluntary and that anonymity and confidentiality would be protected.

Statistical analysis

The data was analysed using SPSS software version 22, using mean and standard deviations for continuous variables, and frequencies and percentages for categorical variables. Pearson's correlation was used for linear correlation between continuous variables. A bivariate analysis was conducted to examine factors associated with EE, depression, anxiety and stress, using independent sample t-tests for two means and ANOVA tests for three or more means. Multiple regression analysis was performed for variables significantly correlated with EE, depression, anxiety and stress, and for the correlation between EE and mental health, with a P-value < 0.05 indicating statistical significance.

Results

The baseline characteristics of the study sample are represented in Table 1. The mean age of the participants was 28.56 ± 8.11 years, and the average EE score was 37.9 (± 10.1). More than two-thirds of the nurses (73.9%) reported that their income was insufficient. Unhealthy weight status was evident among the nurses, with 40.9% of them reporting an increase in their weight during the pandemic. Additionally, 44.2% of the nurses were overweight or obese. This fact coincided with a low level of physical activity, with 39.6% reporting a decrease in their level of physical activity. In addition, the nurses reported a poorer-quality diet, with only 11.9% describing good adherence to the MD.

Figure 1 represents the mental state of the participants. A poor mental state was evident among the majority of the nurses, with 53.8% reporting depression, 58.1% suffering from anxiety and 95.1% experiencing either moderate or severe stress.

Table 2 displays the factors associated with emotional eating and the mental state of the participants during the COVID-19 pandemic, through a bivariate analysis. With regard to EE, female nurses manifested a higher EE than males (42.46 ± 7.96, 36.4 ± 6.62, respectively; P = 0.038). Similarly, those with insufficient

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Table 1. General characteristics of the study population (n = 303)

Variables	Frequency	Percentage
Age		
Mean (±SD)	28.56 (8.11)	
Emotional eating (EE)		
Mean (±SD)	37.9 (10.1)	
Gender		
Male	80	26.4
Female	223	73.6
Monthly income		
Enough	79	26.1
Not enough	224	73.9
Professional title		
Nurse	175	57.8
Registered nurse	107	35.3
Head of department	13	4.3
Supervisor	5	1.7
Director of nursing	3	1
Work shift		
Always day	139	45.9
Always night	39	12.9
Day & night	125	41.3
Department		
Covid-19	53	17.5
Non covid-19	250	82.5
Experience in years		
<1	80	26.4
1–5	104	34.3
6–10	50	16.5
11–15	27	8.9
>15	42	13.9
Marital status		
Married	105	34.7
Single	185	61.1
Divorced	13	4.2
Educational status		
TS2/BT3	105	34.7
Registered nurse (LA-LT-BS)	148	48.8
Master	50	16.5
Smoking		
No smoking	192	63.4
Moderate smoking	85	28.1
Heavy smoking	26	8.6
Weight gain		
Yes	124	40.9
No	179	59.1
Body mass index (BMI)		
Underweight	7	2.3
Normal weight	162	53.5
Overweight	84	27.7
Obese	50	16.5
Consumption of 3 regular meals		
Never	69	22.8
Sometimes	181	59.7
Most of the time	20	6.6
Every day	33	10.9
Fried food consumption		
More than usual	78	25.7
Less than usual	155	51.2
No change	70	23.1
Physical activity		
Increase	43	14.2
Decrease	120	39.6
No change	64	21.1
I don't usually practice	76	25.1
Alcohol drinking		
More than usual	2	0.7
Less than usual	11	3.6
No change	14	4.6

Continued

Table 1. Continued

Variables	Frequency	Percentage
Caffeinated beverages intake		
More than usual	132	43.6
Less than usual	38	12.5
No change	96	31.7
Usually No	37	12.2
Med diet adherence		
Low adherence	111	6.6
Moderate adherence	156	51.5
High adherence	36	11.9

TS: Technique Superieur, BT: Baccalaureat Technique, LT: License Technique, BS: Bachelor of Science.
EE: Emotional Eating.

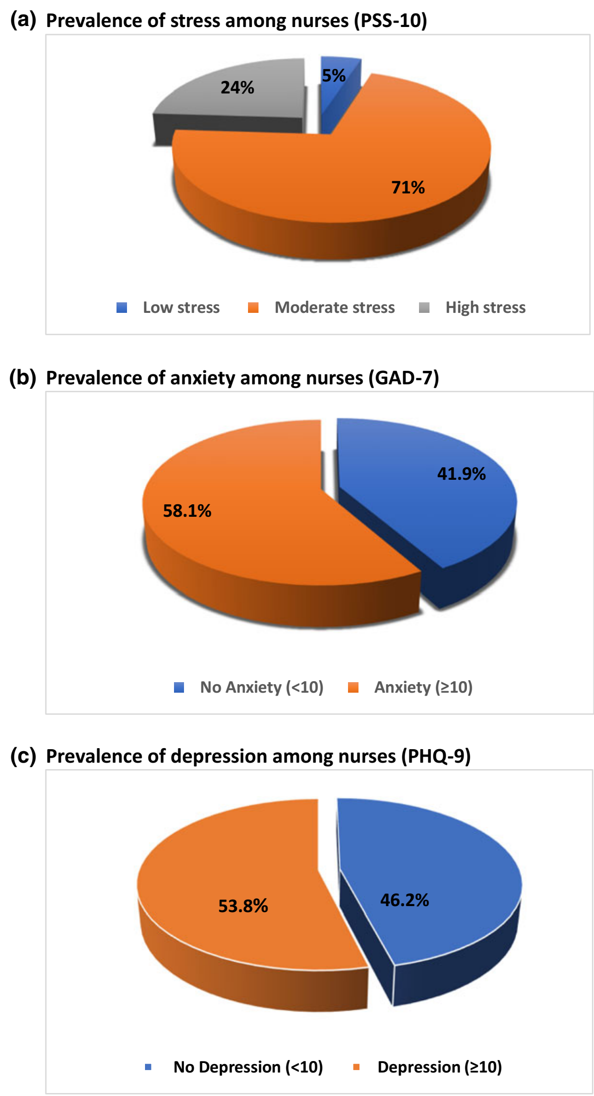

Fig. 1. Mental state of the nurses. (a) Prevalence of stress among nurses (Perceived Stress Scale-10). (b) Prevalence of anxiety among nurses (Generalised anxiety disorder-7). (c) Prevalence of depression among nurses (Patient Health Questionnaire-9).

income reported greater EE (42.97 ± 7.61) than nurses with sufficient income (36.22 ± 5.1) (P = 0.016). An advanced academic degree was associated with higher levels of EE, where

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Table 2. Factors associated with EE and mental state among the participants

Variables	Total EE score (Mean ± SD)	P-value	PSS-10 (Mean ± SD)	P-value	GAD-7 (Mean ± SD)	P-value	PHQ-9 (Mean ± SD)	P-value
Age								
	28.56 (8.11)	0.196	28.5 (8.11)	0.951	11.37 (4.95)	0.224	11.24 (4.30)	0.908
Gender								
Male	36.4 (6.62)	0.038	21.10 (3.59)	0.002	10.09 (3.01)	0.007	10.44 (1.56)	0.207
Female	42.46 (7.96)		23.27 (4.26)		11.83 (3.62)		11.53 (2.81)	
Monthly income								
Enough	36.22 (5.1)	0.016	22.41 (5.81)	0.598	10.81 (2.09)	0.0239	9.89 (2.13)	0.032
Not enough	42.97 (7.61)		22.81 (5.92)		11.57 (3.42)		11.73 (3.59)	
Educational status								
TS2/BT3	32.88 (6.55)	0.006	22.48 (6.11)	0.663	11.22 (3.24)	0.658	10.79 (2.61)	0.422
Registered nurse (LA,LT, BS)	41.24 (8.54)		23.01 (3.31)		11.63 (3.75)		11.76 (3.39)	
Master	39.06 (7.96)		22.28 (5.97)		10.96 (3.05)		10.70 (2.98)	
Professional title								
Nurse	37.3 (5.29)	0.067	22.83 (4.32)	0.236	10.97 (2.58)	0.027	10.90 (2.35)	0.002
Registered nurse	40.91 (6.09)		22.91 (4.87)		12.19 (3.72)		12.46 (3.49)	
Head of department	33.38 (7.19)		20.69 (4.06)		10.15 (2.31)		7.46 (1.43)	
Supervisor	22.60 (7.15)		17.80 (3.67)		7.80 (2.02)		4.20 (1.27)	
Director of nursing	17.33 (7.86)		24.67 (5.09)		16.66 (4.14)		16.67 (4.77)	
Work shift								
Always day	33.66 (5.21)	0.604	22.65 (4.52)	0.608	11.13 (2.26)	0.360	11.80 (1.78)	0.139
Always night	35.43 (4.66)		23.56 (5.89)		12.41 (2.76)		13.17 (2.56)	
Day and night	39.13 (7.84)		22.50 (4.89)		11.33 (2.61)		10.83 (2.13)	
Department								
Non Covid-19	37.06 (7.80)	0.143	22.91 (5.83)	0.178	11.42 (3.52)	0.739	11.27 (2.83)	0.889
Covid-19	42.32 (9.16)		21.71 (6.08)		11.16 (3.31)		11.13 (2.42)	
Experiences in years								
<1year	38.23 (6.18)	0.150	22.25 (5.76)	0.698	11.31 (2.61)	0.815	11.05 (3.52)	0.438
1–5 years	40.96 (8.21)		23.30 (6.73)		11.12 (2.38)		11.37 (3.84)	
6–10 years	38.40 (7.03)		22.86 (6.15)		11.14 (2.58)		11.24 (3.65)	
11–15 years	31.00 (5.90)		22.63 (5.96)		12.22 (3.81)		13.18 (4.51)	
>15 years	34.11 (5.81)		21.98 (5.15)		11.86 (3.02)		10.07 (3.21)	
Marital status								
Married	37.58 (5.45)	0.860	22.08 (4.56)	0.114	11.13 (1.69)	0.069	10.57 (2.47)	0.073
Single	38.38 (6.04)		22.86 (5.47)		11.30 (1.71)		11.37 (2.75)	
Divorced	35.46 (8.32)		25.54 (6.82)		14.46 (3.35)		14.92 (3.44)	
Smoking								
No	39.75 (8.14)	0.032	22.63 (6.11)	0.935	11.21 (2.53)	0.507	11.14 (1.94)	0.771
Moderate	38.55 (9.90)		22.75 (6.67)		11.90 (2.64)		11.65 (2.12)	
Heavy	27.96 (5.98)		23.07 (7.24)		10.92 (2.26)		10.73 (2.67)	
Weight gain								
Yes	46.76 (7.91)	<0.001	22.31 (5.41)	0.157	11.12 (1.82)	0.276	10.57 (2.12)	0.030
No	31.89 (5.40)		23.2 (5.23)		11.75 (2.23)		12.23 (2.56)	
BMI								
Underweight	27.00 (5.14)	0.341	22.57 (4.69)	0.158	14.71 (3.98)	0.251	12.88 (3.11)	0.680
Normal	37.54 (7.23)		23.39 (5.86)		11.12 (2.95)		10.845 (2.25)	
Overweight	37.76 (4.13)		22.11 (5.99)		11.30 (2.65)		11.64 (2.32)	
Obese	41.32 (7.91)		21.50 (5.94)		11.86 (5.84)		11.66 (2.65)	

Continued

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Table 2. Continued

Variables	Total EE score (Mean ± SD)	P-value	PSS-10 (Mean ± SD)	P-value	GAD-7 (Mean ± SD)	P-value	PHQ-9 (Mean ± SD)	P-value
Frequency of eating more								
Yes	43.30 (8.16)	<0.001	22.71 (3.11)	0.985	11.11 (2.31)	0.295	10.91 (2.55)	0.317
No	31.18 (4.94)		22.69 (3.24)		11.73 (2.56)		11.67 (2.31)	
Fried food consumption								
More than usual	49.01 (13.26)	<0.001	22.72 (3.12)	0.318	11.12 (3.18)	0.354	10.91 (2.34)	0.054
Less than usual	34.15 (10.83)		22.69 (3.05)		11.71 (3.25)		11.68 (2.82)	
No change	34.19 (10.05)		22.71 (3.51)		11.11 (3.61)		10.91 (2.56)	
Physical activity								
Increase	41.11 (11.61)	0.003	21.16 (4.12)	0.124	10.12 (3.42)	0.015	10.21 (2.22)	0.157
Decrease	43.95 (12.26)		23.34 (5.45)		22.70 (6.15)		12.00 (3.45)	
No change	34.79 (10.54)		22.01 (5.65)		11.59 (3.95)		10.02 (2.61)	
I don't usually practice	32.34 (9.86)		23.17 (5.25)		10.36 (3.66)		11.68 (3.17)	
Alcohol drinking								
More than usual	59 (8.11)	0.554	26.50 (5.22)	0.455	12.50 (2.54)	0.393	11.50 (1.90)	0.179
Less than usual	38.63 (6.76)		22.90 (4.87)		11.27 (2.86)		14.09 (3.14)	
No change	37.5 (6.88)		24.71 (5.21)		13.57 (3.51)		14.00 (3.24)	
Usually no	37.82 (5.36)		22.57 (5.02)		11.26 (2.62)		10.99 (2.41)	
Caffeinated beverages intake								
More than usual	43.02 (11.37)	0.002	22.43 (4.56)	0.186	11.54 (2.74)	0.501	11.34 (2.95)	0.747
Less than usual	35.00 (9.48)		21.24 (4.23)		10.37 (2.69)		10.16 (2.20)	
No change	33.21 (7.82)		23.21 (5.33)		11.70 (2.56)		11.42 (2.56)	
Usually no	35.43 (8.19)		23.86 (5.47)		10.97 (2.47)		11.59 (2.64)	

TS: Technique Superieur, BT: Baccalaureat Technique, LT: License Technique, BS: Bachelor of Science, EE: Emotional Eating.
PHQ-9: Patient Health Questionnaire-9; GAD-7: Generalised Anxiety Disorder-7; PSS-10: Perceived Stress Scale-10.
$P < 0{\cdot}05$ is considered significant.

registered nurses (41.24 ± 8.54) or master degree holders (39.06 ± 7.96) expressed a greater EE compared to those with a technical degree (32.88 ± 6.55) ($P = 0.006$). In addition, gaining weight during the pandemic (46.76 ±7.91), and eating more fried food in particular (49.01 ±13.26) ($P < 0.001$), tended to be associated with higher levels of EE. Excessive intake of caffeinated beverages (43.02 ± 11.37) ($P = 0.002$) and lower exercise levels (43.95 ± 12.26) ($P = 0.003$) were also linked to higher EE. However, being a non-smoker (39.75 ± 8.14) was correlated with a higher EE score compared to moderate (38.55 ± 9.90) or heavy smoking (27.96 ± 5.98) ($P = 0.032$). With regard to stress, female nurses reported a higher stress level (23.27 ± 4.26) than male (21.10 ± 3.59) ($P = 0.002$). Similarly, anxiety was more prevalent among female nurses than males (11.83 ± 3.62, 10.09 ± 3.01; respectively; $P = 0.007$), those in a higher position than those in a lower position (16.66 ± 4.14, 10.97 ± 2.58, $P = 0.027$; respectively), and those reporting a decrease in their physical activity (PA) compared those who increased their PA (22.70 ± 6.15, 10.12 ± 3.42, respectively; $P = 0.015$). Finally, depression was more prevalent among nurses with insufficient income than those with sufficient income (11.73 ± 3.59, 9.89 ± 2.13, $P = 0.032$; respectively), those in a higher position compared to those in a lower position (16.67 ± 4.77, 10.90 ± 2.5, $P = 0.002$; respectively) and those gaining weight compared to those who didn't gain weight (12.23 ± 2.56, 10.57 ± 2.12, $P = 0.030$; respectively) during the outbreak.

Table 3 represents the association between the EE and mental health of the participants and the MD. It was apparent that nurses with higher EE consumed more olive oil ($P = 0.001$) and red meat ($P = 0.002$), as well as more fats, soft drinks, wine, sweet bakery products, cookies, nuts ($P < 0.001$) and dishes prepared with sofrito sauce ($P = 0.048$), compared to other components of the MD. Regarding mental health, a higher stress level was associated with more intake of fats ($P = 0.002$). In parallel, higher anxiety was correlated with more wine consumption ($P = 0.001$), but with a lower intake of nuts ($P = 0.014$) and dishes prepared with sofrito sauce ($P = 0.046$). Moreover, depression was associated with lower consumption of fruit ($P = 0.017$), nuts ($P < 0.001$) and dishes cooked with sofrito sauce ($P = 0.015$). On the other hand, depressed nurses consumed more red meat ($P = 0.017$), fats ($P = 0.001$), soft drinks ($P = 0.009$) and wine ($P = 0.001$).

Variables significantly associated with EE or mental health in the bivariate analysis were included in a multiple linear regression analysis (Table 4). Female gender ($\beta = 8.112$, $P = 0.004$), smoking ($\beta = -4.732$, $P = 0.010$), weight gain ($\beta = 6.048$, $P = 0.03$), eating more ($\beta = 0.461$, $P = 0.032$) and consuming more fried items ($\beta = 5.223$, $P = 0.001$) were significantly associated with EE in this regression analysis. With regard to stress and anxiety, only the female gender was significantly associated with higher stress ($\beta = 2.244$, $P = 0.003$) and anxiety levels ($\beta = 1.526$, $P = 0.021$). Furthermore, higher levels of depression were correlated with weight gain ($\beta = 2.402$, $P = 0.003$) and with a reduced consumption of nuts ($\beta = -1.706$, $P = 0.009$) and dishes cooked with sofrito sauce ($\beta = -1.378$, $P = 0.012$) (Table 4). Finally, among the studied mental health problems, a positive association was detected only between depression and EE ($\beta = 0.596$, $P = 0.046$) (Table 5).

Discussion

To the best of our knowledge, this is the first study to assess the magnitude of EE among nurses working in Lebanese hospitals during the COVID-19 outbreak and the economic crisis and its association with mental health. Our results showed that during the double crisis witnessed by the Lebanese healthcare system, the mean EE score was 28.56 (±8.11) and the prevalence of mental symptoms was high with 53.8% of the nurses suffering from depression, 58.1% from anxiety, and 95.1% from stress. In addition, the study concluded that depressed nurses, females, non-smokers, those who gained weight, and those who ate more, and in particular those who consumed more fried foods, had a higher tendency towards EE. Regarding mental health, gender difference was noted for the tendency towards stress and anxiety, with females experiencing more stress and anxiety than their male counterparts. Depression was associated with weight gain and with lower consumption of healthy food items (dishes prepared with sofrito sauce and nuts).

Previous studies conducted in various countries found high levels of EE among the healthcare staff during the COVID-19 outbreak. For instance, in Qatar, nurses working during the COVID-19 outbreak had increased odds of experiencing EE, with levels 2.62 times higher than during the pre-pandemic period.[26] In our study the mean EE score in our sample was 37.9 (±10.1) however, due to the use of different tools to assess EE among nurses, comparisons of previous findings to our results were impossible. However, a survey conducted on young Saudi Arabian women during the epidemic revealed a mean EE score of 27.5.[14] These findings spotlight the magnitude of EE among nurses working in Lebanese hospitals during that period.

Our results showed that 43.3% of the nurses were eating more during the double crisis and this increased food consumption was associated with EE. Furthermore, EE was positively associated with weight gain in our sample of nurses. The relationship between EE and weight gain has been extensively highlighted in previous studies. In a two-year cohort study, higher emotional eating among employees predicted more weight gain, independently of other factors such as smoking, alcohol and other dietary habits.[27] Moreover, emotional eaters struggle with losing weight. They are half as likely as non-emotional eaters to accomplish the 10% weight loss goal of standard behavioural weight loss intervention programmes.[28] In addition to excessive food intake, the dietary choices of the emotional eaters could also contribute to weight gain. Emotional eaters usually seek comfort food, and previous research theorised that, during the enforced quarantine, EE arose as a coping mechanism to reduce uncomfortable feelings.[29] In fact, the COVID-19 outbreak impacted people's eating habits in general, with many reporting engaging in harmful eating behaviours, such as reaching for comfort food that is poor in nutrients and high in calories.[30] In China, even among healthcare workers who had a healthy diet prior to COVID-19, a decreased consumption of vegetables ($P = 0.027$) and an increased intake of soft drinks ($P = 0.003$) and convenience food ($P < 0.001$) were reported during the outbreak. Our results showed that among other food choices, higher EE scores were associated with consuming more fried

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Table 3. Factors associated with emotional eating and mental state: the role of the Mediterranean diet

Variables	Total EE score (Mean ± SD)	P-value	PSS-10 (Mean ± SD)	P-value	GAD-7 (Mean ± SD)	P-value	PHQ-9 (Mean ± SD)	P-value
Adherence to MD (total score)								
Low	37.31 (9.86)	0.214	23.46 (6.88)	0.127	11.89 (2.49)	0.144	12.08 (3.05)	0.191
Moderate	37.14 (8.04)		22.50 (5.34)		11.32 (2.12)		10.93 (2.11)	
High	43.66 (11.25)		21.28(4.42)		10.03 (2.05)		10.05 (2.58)	
Olive oil								
Higher	45.95 (9.25)	0.001	23.17 (5.10)	0.460	11.04 (2.19)	0.457	11.51 (3.75)	0.498
Lower	39.62 (7.47)		23.40 (6.88)		12.20 (3.53)		12.17 (2.45)	
As before	35.08 (8.31)		22.39 (5.87)		11.29 (2.04)		10.94 (2.63)	
Vegetables								
Higher	38.98 (6.70)	0.681	21.85 (5.85)	0.141	11.35 (2.74)	0.778	10.90 (2.37)	0.095
Lower	39.52 (7.56)		23.93 (5.31)		11.77 (2.07)		12.91 (2.74)	
As before	37.14 (7.72)		22.59 (6.03)		11.25 (3.01)		10.82 (2.56)	
Fruits								
Higher	40.89 (9.54)	0.224	22.18 (5.48)	0.080	11.41 (2.23)	0.485	11.03 (2.00)	0.017
Lower	39.63 (8.67)		24.10 (6.22)		11.97 (1.97)		13.20 (3.68)	
As Before	36.18 (6.86)		22.33 (5.84)		11.11 (1.00)		10.53 (2.26)	
Red meat								
Higher	50.03 (12.29)	0.002	23.25 (6.28)	0.496	12.46 (2.73)	0.233	12.78 (3.13)	0.017
Lower	36.49 (10.76)		23.03 (5.90)		11.59 (3.17)		12.11 (3.81)	
As before	36.62 (10.60)		22.28 (5.78)		10.93 (2.78)		10.12 (2.09)	
Fats								
Higher	52.87 (13.88)	<0.001	25.84 (5.19)	0.002	12.21 (2.30)	0.278	14.40 (3.98)	0.001
Lower	32.59 (7.86)		22.95 (6.10)		11.75 (2.27)		12.03 (3.90)	
As before	38.48 (10.24)		21.95 (5.68)		10.98 (1.84)		10.14 (2.26)	
Soft drinks or carbonated beverages								
Higher	48.31 (12.16)	<0.001	23.82 (5.84)	0.257	12.27 (4.71)	0.175	13.10 (2.87)	0.009
Lower	32.09 (7.55)		22.60 (6.11)		11.58 (5.28)		11.80 (2.08)	
As before	37.75 (9.45)		22.33 (5.73)		10.89 (4.80)		10.16 (2.97)	
Wine								
Higher	51.20 (14.09)	<0.001	25.30 (7.28)	0.140	15.10 (3.90)	0.001	16.60 (4.13)	0.001
Lower	30.10 (9.69)		23.48 (5.87)		12.66 (3.20)		12.80 (3.68)	
As before	39.77 (12.22)		22.35 (5.79)		10.82 (2.76)		10.53 (3.37)	
Pulses								
Higher	39.01 (11.79)	0.055	23.09 (6.14)	0.596	11.65 (1.10)	0.743	12.26 (3.19)	0.115
Lower	31.92 (9.60)		23.12 (5.90)		11.60 (1.66)		12.03 (3.48)	
As before	39.45 (11.12)		22.41 (5.78)		11.18 (1.00)		10.57 (2.73)	
Fish								
Higher	47.76 (12.42)	0.076	23.82 (6.73)	0.303	12.00 (3.89)	0.159	11.64 (4.35)	0.063
Lower	36.05 (8.43)		23.11 (6.10)		11.89 (3.15)		12.16 (4.83)	
As before	38.65 (9.13)		22.19 (5.55)		10.81(3.11)		10.34 (4.17)	
Sweet bakery and cookies								
Higher	44.04 (11.15)	<0.001	23.78 (6.02)	0.068	12.08 (3.80)	0.051	12.04 (4.04)	0.072
Lower	31.73 (5.73)		22.39 (6.00)		11.74 (3.72)		11.84 (4.60)	
As before	36.91 (8.80)		22.00 (5.60)		10.55 (3.14)		10.20 (3.91)	
Nuts								
Higher	51.50 (12.26)	<0.001	23.40 (6.20)	0.275	11.61 (3.74)	0.014	11.20 (2.86)	<0.001
Lower	32.75 (7.73)		23.12 (6.38)		12.41 (3.45)		13.20 (3.34)	
As before	36.54 (9.27)		22.15 (5.36)		10.56 (3.54)		9.89 (2.96)	

Table 3. Continued

Dishes with sofrito sauce		0.048		0.059		0.046		0.015
Higher	31.08 (5.46)		22.28 (6.02)		11.04 (2.78)		10.49 (2.91)	
Lower	32.52 (8.49)		24.39 (6.24)		12.86 (3.05)		13.52 (2.04)	
As before	38.60 (7.28)		22.35 (5.58)		11.04 (2.96)		10.88 (2.67)	

MD: Mediterranean diet.
PHQ-9: Patient Health Questionnaire-9; GAD-7: Generalised Anxiety Disorder-7; PSS-10: Perceived Stress Scale-10, EE: Emotional Eating.
P < 0·05 is considered significant.

foods in particular; therefore, it could be that fried items are perceived as being comfort food by our population. In fact, serotonin and dopamine, which have been shown to be positive emotion enhancers, are released more readily when fats and foods high in refined carbohydrates are consumed.[31]

Gender emerged as another factor associated with EE, with female nurses reporting more emotional eating than males. This is consistent with previous studies, where female nursing students have experienced significantly higher levels of emotional eating compared to their male counterparts.[32] Similarly, in Turkey, female emergency service workers had higher EE scores than males (P = 0.022).[33] This result can be attributed to gender disparity in food intake, where women generally show less dietary control than men. Women are more inclined than males to report eating compulsively and out of control.[34] For example, the average lifelong prevalence of developing a binge eating disorder is 3.5% for females in comparison to 2.0% in males.[35]

In our study, heavy smokers had a lower EE score compared to non-smokers and those who smoke in moderation (P = 0.018). This could be explained by the fact that when faced with an unusual or unexpected situation, people tend to choose a coping mechanism, which could be either food—favouring empty-calorie items—or smoking, to lessen the burden of their negative feelings. There is compelling evidence that nicotine reduces feelings of tension and rage.[36] Smokers claim that they smoke more frequently when they are upset or angry because they believe that smoking will make them feel better.[37]

Our results shed the light on the extent of mental symptoms among nurses working in Lebanese hospitals during COVID-19 outbreak and the economic crisis. More than half of the nurses were suffering from depression or anxiety (53.8% and 58.1% respectively) and even worse, the big majority were experiencing moderate or severe stress (95.1%). This rate is dramatically higher than the one reported before 2019, where the prevalence of depression was only 36.2% among Lebanese nurses.[38] Mental symptoms were previously reported among nurses during the COVID-19 outbreak, i.e. a study conducted in Iran during the COVID-19 outbreak, found moderate levels of stress, depression and anxiety among a group of nurses working in an educational hospital.[39] However, our findings revealed higher rates of mental symptoms than the ones found in many other countries, in fact a systematic review and meta-analysis revealed an overall prevalence of stress of 43%, while anxiety and depression were 37% and 35%, respectively.[12] Although the toll of COVID-19 cases in Lebanon was significantly lower than the one in China, our rates of mental symptoms were comparable to those found in Wuhan, China, where depression and anxiety symptoms were present in 58% and 54.2% of healthcare workers respectively.[40] This finding is alarming, it pinpoints the fact that the pandemic had a higher-than-expected impact on nurses in Lebanon. At first sight, it may seem that the nurses in Lebanon had an exaggerated response to the emergency situation, however taking into account the economic situation in the country and the realities of the healthcare system at that time, one can understand the magnitude of the mental burden these professionals were experiencing. While the world was facing the COVID-19 pandemic, Lebanon was dealing with a double crisis – the pandemic as well as an economic

Table 4. Factors associated with emotional eating and mental health among nurses: Multiple regression analysis

	Total EE		PSS-10		GAD-7		PHQ-9	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Gender	8.112	0.004	2.244	0.003	1.526	0.021		
Monthly income								
Educational status								
Smoking	–4.732	0.010						
Weight gain	6.048	0.030					2.402	0.003
Eating more during COVID-19	0.461	0.032						
Fried food intake	5.223	0.001						
Physical activity								
Caffeinated beverages intake								
Olive oil intake								
Red meat								
Fats								
Soft drinks								
Wine								
Sweet bakery & cookies								
Nuts							–1.706	0.009
Dishes with sofrito sauce							–1.378	0.012
Adjusted R^2	0.247		0.026		0.032		0.066	
F	6.842		5.035		2.657		3.139	
P-value	<0.001		0.007		0.016		0.001	

PHQ-9: Patient Health Questionnaire-9; GAD-7: Generalised Anxiety Disorder-7; PSS-10: Perceived Stress Scale-10; EE: Emotional Eating *$P < 0.05$ statistically significant.*

Table 5. Association between EE and mental health of the participants

	Total EE	
	Beta	P-value
PSS-10	–0.140	0.584
GAD-7	–0.540	0.187
PHQ-9	0.596	0.046
Adjusted R^2	0.003	
F	0.349	
P-value	0.0258	

PHQ-9: Patient Health Questionnaire-9; GAD-7: Generalised Anxiety Disorder-7; PSS-10: Perceived Stress Scale-10; EE: Emotional Eating.
$P < 0.05$ is statistically significant.

crisis—which has led to the worst rates of unemployment, inflation and poverty that Lebanon has ever experienced.[41,42] Due to the pandemic, Lebanese nurses were forced to take double shifts to replace sick or rotated nurses, in addition to facing medication shortages and high daily death rates.[43] Simultaneously, they were underpaid due to the economic crisis, which led to a remarkable decline in the Lebanese pound that has lost more than 90% of its value, while food prices have increased by more than 50%.[41] In fact, 42.97% of the nurses in our sample reported an insufficient monthly income. Therefore, it is likely that the double crisis significantly affected the emotional well-being of the Lebanese nurses and eventually increased their anxiety, depression and stress levels even beyond that of the general Lebanese population (anxiety and depression of 42% and 42.6%, respectively).[2]

The main aim of our study was to investigate whether EE was associated with mental health. Indeed, our study revealed that EE was associated with depression which is consistent with the existing body of literature that has highlighted the association between depressive symptoms and EE.[44,45] Furthermore, depression was correlated with weight gain in our sample of nurses. According to previous studies, this association could be mediated by emotional eating.[31] Additionally, earlier studies have associated depression with unhealthy dietary choices. For example, a previous study highlighted the correlation between depressive symptoms and lower consumption of healthy items such as fruits and vegetables.[44] In addition, two community surveys have demonstrated that greater severity of depressive symptoms was linked to a decreased probability of adhering to dietary recommendations.[46] These results are consistent with our study outcome, where depressed nurses consumed fewer nuts and dishes prepared with sofrito sauce.

Previous studies have shown that women were more stressed about the pandemic and social isolation than males.[47,48] Likewise, our study detected a gender disparity towards anxiety and stress, with female nurses being more affected than their male counterparts. Our results are consistent with previously reported findings among Iranian nurses and medical staff in China during the COVID-19 outbreak where females were more stressed, anxious and depressed than males.[39] Based on functional magnetic resonance imaging to study brain responses, men and women may be equally susceptible to stress and anxiety,[49,50] although this susceptibility may be revealed through distinct mental conditions. Accordingly, women are more likely than males to have stress-related psychological conditions, such as stress and anxiety disorders, throughout their lifespan,[51] whereas men are more likely to experience behaviours that are externalised (such as substance misuse and aggression.[52]

Strengths and limitations

The current study is the first to our knowledge to assess EE and mental health and their correlating factors in relation to nurses working in Lebanese hospitals during the double crisis.

However, some limitations should be noted. First, the nurses took part voluntarily in this study, and selection bias therefore cannot be overlooked. Second, data was self-reported in order to explore emotional eating and mental states among the participants. Similarly, nurses reported their own weight and height. Finally, due to the cross-sectional design, causal relationships cannot be clearly elucidated, therefore longitudinal studies are warranted toward an in-depth investigation of the relationship between different variables studied.

Conclusion and recommendations

In conclusion, this study revealed that females, non-smokers and depressed nurses had a higher tendency towards EE. Additionally, it was found that EE is associated with weight gain and increased food intake, specifically the consumption of fried foods. With regard to mental health, gender disparity was present, with females experiencing more stress and anxiety than their male counterparts. Furthermore, depression was associated with weight gain and lower consumption of healthy food. Finally, EE appeared only to be correlated with depression. Interventional programmes are thus warranted in order to improve eating habits while reducing psychological discomfort among nurses, especially during crisis. Healthcare systems should offer nutrition education and nutritional counselling if needed and provide healthy snacks and meals at workplace in order to lessen the emotional eating burden experienced during stressful situations. In addition, these programmes could focus on self-management techniques to teach nurses, among other healthcare workers, to self-regulate their eating habits. Self-regulation programmes have been successful in improving physical activity levels and sustaining weight reduction, both of which are associated with mood enhancement.[53] Psychological counselling, stress management sessions and emotional intelligence courses should also be provided to help nurses cope with negative feelings that arise during emergency situations. All these strategies can reduce stress, anxiety and depression levels which will improve the performance of nurses and ultimately lead to improvement in the quality of the healthcare delivered. In fact, previous studies highlighted the importance of emotional intelligence for adequate cognitive performance. In this regard, an Iranian study has highlighted the importance of the ability to control one's emotions for academic achievement.[54] Furthermore, to help nurses face future emergencies without compromising their mental well-being, it is important to empower them with disaster competencies. In fact, nurses may require training in this specific area. For example, a study conducted in Iran revealed that nursing students have poor disaster competencies.[55] Therefore, the curriculum of the nursing programme should be revised to encompass well designed practical courses tackling disaster competencies and emotional intelligence during critical situations. In order to guide the Lebanese authorities to develop well-tailored preparedness plans for future unexpected emergency situations, future studies should focus on gaining an in-depth view of the problem and its nature in the Lebanese context as well as assessing the impact of various intervention programmes on the nurse's well-being.

Abbreviations

EE: Emotional Eating; **MD:** Mediterranean diet; **NCD:** non-communicable diseases; **EES:** Emotional Eating Scale; **TS:** Technique Superieur; **BT:** Baccalaureat Technique; **LT:** License Technique; **BS:** Bachelor of Science; **PHQ-9:** Patient Health Questionnaire-9; **GAD-7:** Generalised anxiety disorder-7; **PSS-10:** Perceived Stress Scale-10; **BMI:** Body Mass Index; **WHO:** World Health Organization.

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Authorship

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Declaration of conflicting interests

All authors declare that there is no potential conflict of interest in this study.

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RESEARCH ARTICLE

Factors influencing the intention of young adults to adopt genotype-based personalised advice on diet and physical activity according to perceived weight status

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Abstract

Genotype-based dietary and physical activity advice can be delivered to young adults before unhealthy lifestyle behaviours or metabolic and physiological conditions have developed. The aim of the present study was to investigate the factors that influence the intention to adopt genotype-based personalised advice on diet and physical activity in young adults who perceive themselves to be a healthy weight versus those who perceive themselves to be overweight or obese. An online survey of 396 young adults (18–25 years) evaluated background factors (participant characteristics (including perception of body weight), psychological factors, belief composites) and constructs of the Theory of Planned Behaviour (TPB) related to the adoption of genotype-based personalised advice. The association between background factors and TPB constructs was assessed using multiple linear regression. The constructs of TPB predicted intention to adopt genotype-based personalised nutrition ($P < 0.001$, adj. $R^2 = 0.54$; attitude: $B = 0.24$, subjective norm: $B = 0.25$, PBC: $B = 0.45$). Background factors including belief composites, health locus of control, gender, physical activity, and food choice motives of 'health', 'price', 'familiarity', 'weight control', and 'convenience' significantly added to models of TPB constructs related to the intention to adopt personalised advice ($P < 0.05$). The influence of background factors varied between TPB constructs and differed based on participants perception of their body weight. The study provides support for the use of the TPB in understanding the intention of young adults to adopt gene-based advice for dietary and physical activity behaviour. In addition to perceived body weight, the background factors identified should help to inform and modify the delivery of advice in behaviour change interventions that seek to use genotype-based personalised advice in young adult populations.

Key words: Intention: Nutrigenomics: Personalised nutrition: Survey: Theory of planned behaviour

Introduction

Modification of lifestyle behaviours, including diet and physical activity, can considerably reduce the prevalence of non-communicable diseases (NCDs), reducing the burden of disease for both the individual and society.[(1)] However, generic public health advice to address dietary and physical activity behaviours is not adhered to.[(2,3)] Compared to this 'one size fits all' approach to dietary and physical activity advice, researchers have hypothesised that personalisation of advice based on an individual's genotype could motivate greater adherence to guidance.[(4)]

Genotype-based personalised advice is usually delivered in combination with other levels of personalisation (phenotypic, clinical, dietary), with the aim to provide more precise and effective advice as well as to encourage behaviour change.[(5)] Studies that have investigated the effect of genotype-based dietary advice on behaviour change have reported contradictory findings, both within and between studies.[(6–9)] Recent systematic reviews and a meta-analysis of studies that have investigated the effect of genotype-based advice to motivate dietary and physical activity behaviour have reported no beneficial effect above that seen with other levels of personalisation.[(10,11)] However, one

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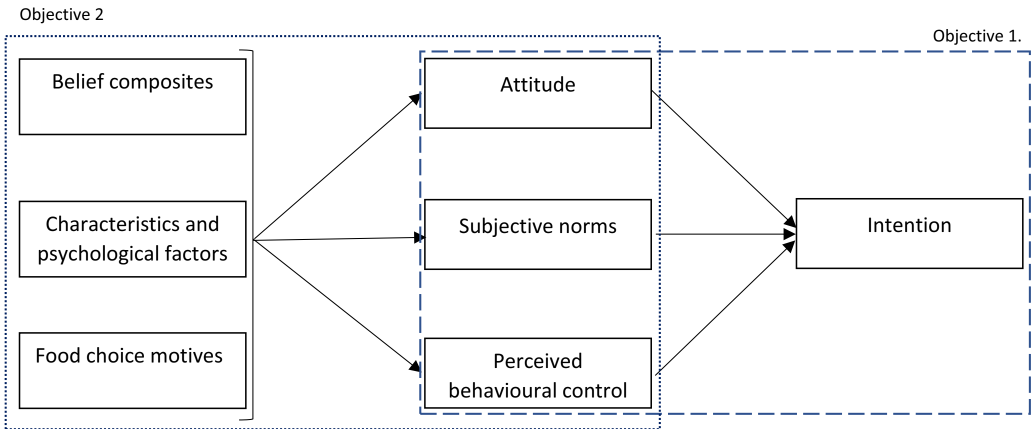

Fig. 1. Specification of theory of planned behaviour model and study objectives. .

benefit of genotype-based personalisation of advice over other levels of personalisation is that it can be delivered earlier in the lifespan, before unhealthy lifestyle behaviours or metabolic and physiological conditions have developed. Therefore, young people stand to benefit the most from genotype-based dietary and physical activity advice.[12,13] Furthermore, young people have been reported to be more likely to adopt personalised nutrition compared to other age groups.[14] However, to effectively implement genotype-based personalised advice to affect behaviour in young adults, an understanding of factors that may encourage or prevent engagement is required.

Interventions designed to change health-related behaviours are more likely to be successful when theoretical links between the intervention and the behaviour have been considered in the design.[12,15–17] One of the most frequently cited behaviour change theories, the Theory of Planned Behaviour (TPB),[15,18] states that 'intention' to perform a behaviour can be predicted from three independent constructs: attitude toward the behaviour, subjective norms, and perceived behavioural control (PBC).[18] 'Attitude toward the behaviour' represents the extent to which an individual has a favourable appraisal of that behaviour, 'subjective norms' is the individual's perceived social pressure to perform or not perform the behaviour and 'PBC' is an individual's perception of how easy or difficult it is to perform the behaviour.[19] Each construct of the TPB is influenced by belief composites: behavioural beliefs, normative beliefs, and control beliefs.[18] Intention and PBC have been demonstrated to account for a significant amount of variation in numerous health-related behaviours including food choice, multiple correlations ranging from 0.20 to 0.78.[18,20] Furthermore, background factors such as demographic characteristics, personality traits, and life values are reported to influence intention to perform a behaviour by affecting TPB constructs.[19] There are several background factors that previous research has identified that may influence intention to engage with personalised advice: optimistic bias, the phenomenon by which an individual underestimates their own risk of developing a disease compared to others[21]; health locus of control (HLC), whether an individual perceives their health to be under their control (internal) or not (external)[22]; food choice motives, nine factors that have been shown to influence food choice[23]; and participant characteristics, such as sex and personal history of NCD.[13,14,24–27]

Although these factors have been associated previously with intention to engage with personalised advice, an understanding of how TPB constructs, belief composites, and background factors relate to the intention to adopt genotype-based personalised nutrition specifically in young adults has not been investigated. A clearer understanding of associations between these background factors and the intention to utilise personalised advice would inform researchers and health practitioners on how best to communicate advice to promote healthy lifestyle behaviours. Therefore, the aim of the present study was to investigate the factors that influence the intention to adopt genotype-based personalised advice for diet and physical activity in young adults; and to determine if factors differ in young adults that perceive themselves to be a healthy weight and those that perceive themselves to be overweight or obese. The overall aim was broken down into two objectives presented in Fig. 1.

Methods

Participants

A total of 414 responses were received for the survey, 18 were screened out due to not meeting the inclusion criteria. Therefore, 396 male and female young adults aged 18–25 years, living in the UK, who were not pregnant, lactating, following a restricted diet, or having a diagnosed eating disorder took part in the survey. Participants were recruited through advertisements shared during lectures at St Mary's University and social media postings (Facebook, Twitter and LinkedIn). Data were collected between March and November 2022 using the Jisc online surveys platform (https://www.onlinesurveys.ac.uk) to ensure data are stored in a secure and GDPR-compliant environment.

Survey development

A pilot survey was conducted in 35 young adults (18–25 years) to assess the usability of the survey and develop the TPB questionnaire.[28] Items used to measure the TPB constructs were assessed for internal consistency[29] and discriminant validity.[30] To measure belief composites, free-response questions were used to elicit behavioural outcomes and experiences (perceived advantages, disadvantages and feelings), normative referents (individuals or groups that would approve or disapprove), and control factors (factors that would make it easy or difficult) in relation to the adoption of genotype-based advice to modify dietary or physical activity behaviour. Content analysis of free-response questions was used to construct items to be used in the final survey[28] (Supplementary Material Tables 1–3).

Final survey

The final survey was divided into three sections. The first section asked participants about characteristics: gender, age, ethnicity, education, perceived health, physical activity behaviour, and their perceived body image. Physical activity was assessed using a single question to determine whether participants were sufficiently active to benefit their health: 'In the past week, on how many days have you done a total of 30 min or more of physical activity, which was enough to raise your breathing rate? This may include sport, exercise, and brisk walking or cycling for recreation or to get to and from places, but should not include housework or physical activity that may be part of your job'.[31] To measure perceived body image, participants were asked to indicate their own body figure by choosing a silhouette of the Stunkard Scale. Based on the selected silhouette participants were classed to perceive themselves as underweight, normal weight, overweight or obese.[32–34]

The second section asked participants about their HLC, motives for food choice, and optimistic bias. For each scale, internal consistency was checked; Cronbach's alpha (α) for all factors indicated adequate internal consistency.[29] To assess HLC, participants were asked to indicate the extent to which they agreed or disagreed with six statements. For example: 'I can be as healthy as I want to be.' Response: Completely disagree, Disagree, Neither disagree/nor agree, Agree, Completely agree.[25,35] The internal HLC was calculated from the average score for the first three items ($\alpha = 0.77$) and external HLC from the second three items ($\alpha = 0.70$).[35] Motives for food choice were measured using the Food Choice Questionnaire.[23] The 36 items represent nine factors and the mean score from 1 to 5 was calculated for each factor (health ($\alpha = 0.86$), mood ($\alpha = 0.88$), convenience ($\alpha = 0.87$), sensory appeal ($\alpha = 0.82$), natural content ($\alpha = 0.88$), price ($\alpha = 0.83$), weight control ($\alpha = 0.86$), familiarity ($\alpha = 0.74$), and ethical concern ($\alpha = 0.79$). Optimistic bias was estimated by asking participants to respond to the following statement 'How do you think your chances of getting cardiovascular disease (CVD) in the future compare with those of the average adult of your age and sex? Response: 7-point Likert scale (much lower than average - much higher than average).[36] Participants were also asked the same question with reference to type 2 diabetes (T2D) and obesity. The mean score of all three items was used to calculate overall optimistic bias ($\alpha = 0.86$), a higher score represented a higher level of optimistic bias.

The final section of the survey asked participants how potential outcomes related to genotype-based personalised advice would increase the likelihood of adopting it.[25] Also, items to determine each construct of the TPB related to the adoption of genotype-based dietary and physical activity advice. The direct measures of TPB constructs (attitude ($\alpha = 0.88$), subjective norms ($\alpha = 0.77$), PBC ($\alpha = 0.81$) and intention ($\alpha = 0.87$) were calculated from the mean score of items for each construct.[19] Belief composites (behavioural, normative, and control beliefs) were calculated as described by Ajzen.[19]

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 26 for Windows (IBM Corp, New York, USA). Measures of centrality and spread are presented as means and SD; categorical data are presented as frequencies and percentages. Comparisons were made between participants who perceived themselves to be normal weight and those who perceived themselves to be overweight or obese. Participants who perceived themselves to be underweight were excluded from analysis (n = 5). Normality of data was assessed using the Shapiro-Wilk test. Baseline continuous measures were not normally distributed ($P \geq 0.05$) and were compared between groups using a Mann-Whitney U test. Categorical variables were compared using a Chi-square Test or when expected counts were less than five, a Fisher's Exact Test. For *post hoc* analyses, a Bonferroni adjustment was made to correct for multiple comparisons. Stepwise linear multiple regression analysis was conducted to identify the relationship between constructs of the TPB and intention to adopt genotype-based personalised nutrition and to determine the relationship between behavioural beliefs, food choice motives, characteristics and psychological factors, with each construct of the TPB. Each multiple regression was conducted with all participants and separately in those who perceived themselves to be normal weight and those who perceived themselves to be overweight or obese. All tests were two-tailed and considered statistically significant when $P < 0.05$.

Results

Participant characteristics

A total of 396 young adults completed the survey; their characteristics are summarised in Table 1. Seventy-six per cent of participants perceived themselves to be normal weight, with 23% overweight or obese, and one per cent underweight. Compared to participants who perceived themselves to be normal weight, participants who perceived themselves to be overweight were more likely to be male (54% v 46%, $P = 0.001$) and reported to be physically active less frequently (3.4 v 4.2 d/week, $P = 0.001$). There was also a significant difference between proportions for how healthy participants considered themselves ($P < 0.001$). Compared to participants who perceived themselves to be overweight or obese, a greater proportion of participants who perceived themselves to be normal weight considered themselves to be very healthy compared to healthy, moderately healthy, or unhealthy. Also, a greater proportion considered themselves to be healthy compared to unhealthy. There was no significant difference between the proportion of participants who perceived themselves to be overweight or

Table 1. Characteristics for all participants (n = 396), for those who perceive themselves to be normal weight (n = 299) and those who perceive themselves to be overweight or obese (n = 92) data presented as n (%) or mean and SD

		Normal weight		Overweight or obese		All participants		
Characteristic		n or mean	% or SD	n or mean	% or SD	n or mean	% or SD	P value
Gender	*Men*	103	34	50	54	153	39	P = 0.001
	Women	196	66	42	46	243	61	
Age	(years)	21	2	21	2	21	2	P = 0.475
Ethnicity	*Asian or Asian British*	29	10	17	19	46	12	P = 0.063
	Black, Black British, Caribbean, or African	27	9	8	9	35	9	
	Mixed or multiple ethnic groups	18	6	8	9	27	7	
	White	214	72	53	58	271	68	
	Other ethnic group	11	4	6	7	17	4	
Country of residence	*England*	293	98	87	95	385	97	P = 0.179
	Wales	1	0	1	1	2	1	
	Scotland	2	1	1	1	3	1	
	Northern Ireland	3	1	3	3	6	2	
Education	*Secondary School (GCSE or equivalent)*	9	3	4	4	14	4	P = 0.317
	Further Education (A Level or equivalent)	187	63	53	58	243	61	
	Bachelor's Degree	86	29	26	28	112	28	
	Master's Degree	16	5	7	8	24	6	
	Prefer not to say	1	0	2	2	3	1	
Health Perception	*Very unhealthy*	3	1	2	2	5	1	P < 0.001
	Unhealthy	5	2	9	10	14	4	
	Moderately unhealthy	48	16	27	29	77	19	
	Healthy	198	66	53	58	253	64	
	Very healthy	45	15	1	1	47	12	
Physical activity	(days/week)	4.2	1.9	3.4	1.9	4.0	2.0	P = 0.001
Perceived body image	*Underweight*	0	0	0	0	5	1	
	Normal weight	299	100	0	0	299	76	
	Overweight	0	0	75	82	75	19	
	Obese	0	0	17	19	17	4	

obese versus those who perceived themselves to be normal weight, based on their age ($P = 0.475$), ethnicity ($P = 0.063$), country of residence ($P = 0.179$), or highest level of education that they had completed ($P = 0.317$).

Psychological factors, motives for food choice, and constructs of the TPB

Mean scores for psychological factors, motives for food choice, and constructs of the TPB were compared between participants who perceived themselves to be normal weight and participants who perceived themselves to be overweight or obese. Participants who perceived themselves to be overweight or obese had a significantly lower internal HLC (3.8 v 4.0, $P = 0.002$), overall optimistic bias (4.2 v 5.2, $P < 0.001$), and optimistic bias for developing CVD (4.3 v 5.0, $P < 0.001$), T2D (4.2 v 5.1, $P < 0.001$) and obesity (4.2 v 5.6, P < 0.001). There were no significant differences between groups for external HLC, food choice motives, or constructs of the TPB ($P \geq 0.05$). Sensory appeal was the highest-rated food choice motive, followed by price and health. Mean scores for attitude, subjective norms and PBC were positive (Table 2).

Objective 1: TPB constructs and intention

Multiple regression analysis revealed that attitude, subjective norm, and PBC explained the intention to adopt genotype-based personalised nutrition for all participants ($P < 0.001$, adj. $R^2 = 0.54$; attitude: $B = 0.24$, subjective norm: $B = 0.25$, PBC: $B = 0.45$), those that perceived themselves to be normal weight ($P < 0.001$, adj. $R^2 = 0.58$; attitude: $B = 0.25$, subjective norm: $B = 0.25$, PBC: $B = 0.46$), and those that perceived themselves to be overweight or obese ($P < 0.001$, adj. $R^2 = 0.40$; attitude: $B = 0.23$, subjective norm: $B = 0.24$, PBC: $B = 0.38$). In all models the largest unstandardised regression coefficient was observed for PBC, followed by subjective norm and attitude which was not a significant predictor in the model for participants who perceive themselves to be overweight or obese (Fig. 2, Supplementary Table 6).

Objective 2

Belief composites and TPB constructs. Belief composites explained attitude, subjective norms and PBC towards genotype-based personalised advice in all participants ($P < 0.001$, adj. $R^2 = 0.49$; $P < 0.001$, adj. $R^2 = 0.20$; $P < 0.001$, adj. $R^2 = 0.08$), participants that perceived themselves to be normal weight ($P < 0.001$, adj. $R^2 = 0.49$; $P < 0.001$, adj. $R^2 = 0.18$; $P < 0.001$, adj. $R^2 = 0.10$), and participants that perceived themselves to be overweight or obese ($P < 0.001$, adj. $R^2 = 0.48$; $P < 0.001$, adj. $R^2 = 0.23$; $F = 4.151$, $P = 0.045$, adj. $R^2 = 0.03$) (Fig. 3, Supplementary Tables 7–9).

Table 2. Psychological factors, motives for food choice and constructs of the Theory of Planned Behaviour for all participants (n = 396), and for those who perceive themselves to be normal weight (n = 299) and those who perceive themselves to be overweight or obese (n = 92); data presented as mean and SD

	Normal weight		Overweight or obese		All participants	
	mean	SD	mean	SD	mean	SD
Internal Health locus of control	4.0	0.6	3.8[a]	0.8	4.0	0.7
External Health locus of control	1.7	0.6	1.8	0.7	1.7	0.6
Optimistic bias	5.2	1.3	4.2[a]	1.3	5.0	1.4
CVD	5.0	1.3	4.3[a]	1.4	4.9	1.4
T2D	5.1	1.5	4.2[a]	1.5	4.9	1.5
Obesity	5.6	1.3	4.2[a]	1.8	5.3	1.6
Food choice motives						
Health	3.5	0.7	3.4	0.8	3.5	0.7
Mood	3.3	0.9	3.4	0.8	3.3	0.9
Convenience	3.2	0.8	3.2	1.0	3.1	0.9
Sensory appeal	3.7	1.0	3.6	0.9	3.7	0.8
Natural content	3.1	0.8	2.9	1.1	3.0	1.0
Price	3.5	0.8	3.6	0.8	3.6	0.9
Weight control	2.8	1.0	3.0	1.1	2.8	1.1
Familiarity	2.5	0.9	2.5	0.9	2.5	0.9
Ethical concern	2.2	0.9	2.1	0.9	2.1	0.9
TPB constructs						
Attitude	5.0	1.1	4.9	1.2	5.0	1.1
Subjective norms	4.8	1.1	4.6	1.3	4.7	1.2
Perceived behavioural control	4.8	1.1	4.7	1.0	4.8	1.1
Intention	4.5	1.3	4.5	1.2	4.5	1.3

CVD, cardiovascular disease; T2D, type 2 diabetes; TPB, theory of planned behaviour.
[a]Significantly different to participants who perceive themselves to have a normal body weight P < 0.05.

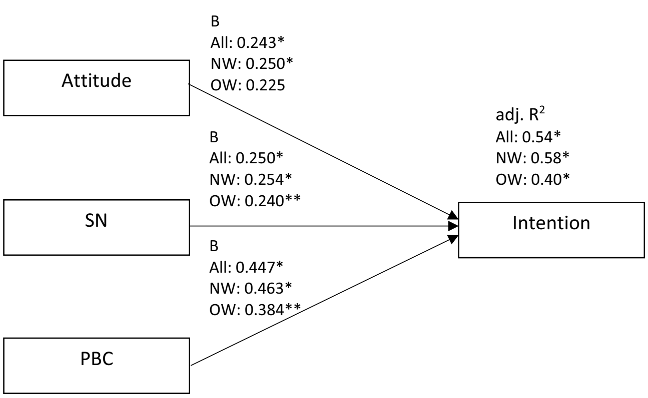

Fig. 2. Objective 1: Summary of unstandardised regression coefficients and adjusted R^2 of constructs of the Theory of Planned Behaviour, for all participants, participants that perceive themselves to be normal weight and participants that perceive themselves to be overweight or obese. B, unstandardised regression coefficient; adj. R^2, adjusted R^2; SN, subjective norms; PBC, perceived behavioural control; All, all participants (n = 391); NW, participants that perceive themselves to be normal weight (n = 299); OW, participants that perceive themselves to be overweight or obese (n = 92). * P < 0.001; ** P < 0.05.

Psychological factors, characteristics and TPB constructs. Psychological factors and characteristics explained attitude, subjective norms, and PBC in all participants ($P < 0.001$, adj. $R^2 = 0.11$; $P < 0.001$, adj. $R^2 = 0.03$; $P < 0.001$, adj. $R^2 = 0.12$), in participants that perceived themselves to be normal weight ($P < 0.001$, adj. $R^2 = 0.13$; $P < 0.001$, adj. $R^2 = 0.07$; $P < 0.001$, adj. $R^2 = 0.13$) and, in participants that perceived themselves to be overweight or obese ($P = 0.001$, adj. $R^2 = 0.10$; $P = 0.042$, adj. $R^2 = 0.03$; $P = 0.028$, adj. $R^2 = 0.04$) (Fig. 4, Supplementary Tables 10–12).

Food choice motives and TPB constructs. Food choice motives predicted attitude, subjective norms and PBC in all participants ($P < 0.001$, adj. $R^2 = 0.10$; $P = 0.001$, adj. $R^2 = 0.03$; $P < 0.001$, adj. $R^2 = 0.11$), in participants that perceived themselves to be normal weight ($P < 0.001$, adj. $R^2 = 0.06$; $P = 0.013$, adj. $R^2 = 0.02$; $P < 0.001$, adj. $R^2 = 0.08$) and participants that perceived themselves to be overweight or obese ($P < 0.001$, adj. $R^2 = 0.20$; $P = 0.032$, adj. $R^2 = 0.04$; $P = 0.001$, adj. $R^2 = 0.15$) (Fig. 5, Supplementary Tables 13–15).

Discussion

The aim of this research was to use the TPB as a model to understand the intentions of young adults to adopt genotype-based personalised advice for dietary or physical activity behaviour. On average, young adults have a positive intention to adopt genotype-based advice for dietary and physical activity behaviour, driven by a favourable attitude, a positive perception of social pressure, and perceived ability to perform the behaviour. These findings were consistent in participants who perceived themselves to be normal weight and overweight or obese. To understand the factors that influence the proximal constructs of intention to adopt genotype-based personalised advice, the relationships between belief composites, characteristics and psychological factors, and food choice motives were determined for each construct.

Attitude towards the behaviour

Behavioural beliefs of 'motivation to eat healthily and exercise' and 'prevent disease' were significant positive predictors of attitude in all models. 'To achieve health and fitness goals' was a

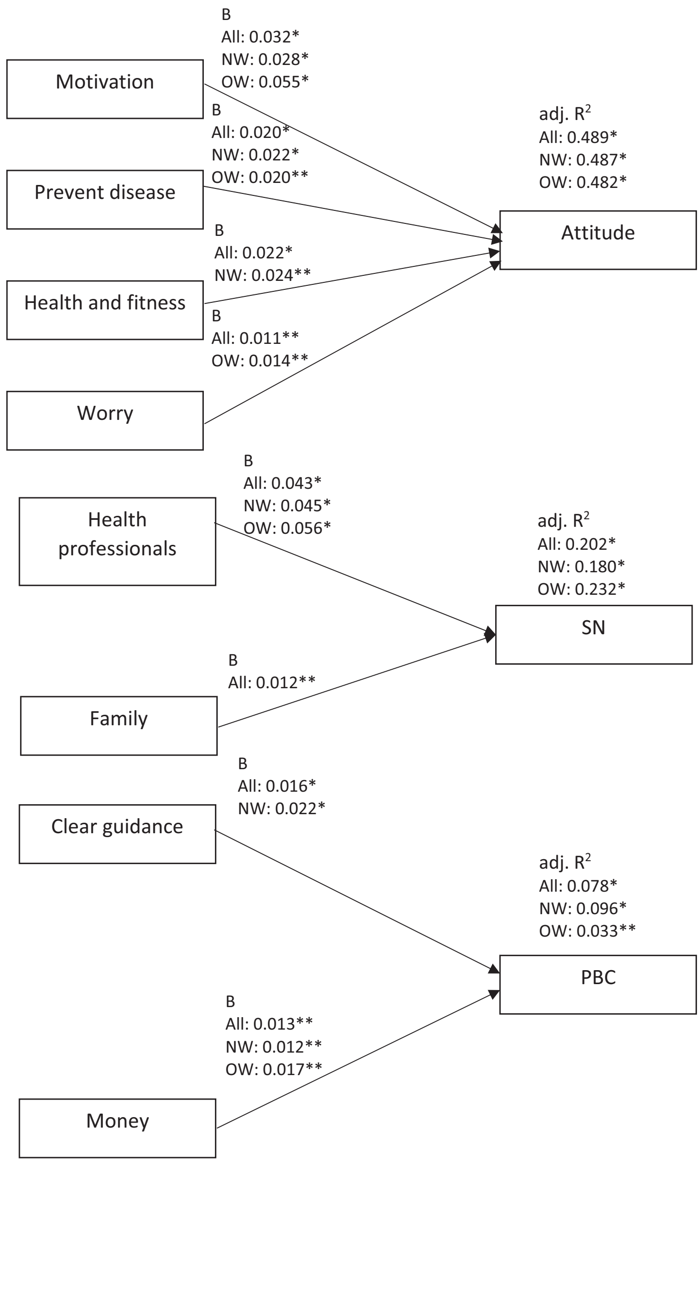

Fig. 3. Objective 2: Summary of unstandardised regression coefficients and adjusted R^2 of constructs of belief composites and Theory of Planned Behaviour constructs, for all participants, participants that perceive themselves to be normal weight and participants that perceive themselves to be overweight or obese. B, unstandardised regression coefficient; adj. R^2, adjusted R^2; SN, subjective norms; PBC, perceived behavioural control; All, all participants (n = 391); NW, participants that perceive themselves to be normal weight (n = 299); OW, participants that perceive themselves to be overweight or obese (n = 92). * P < 0.001; ** P < 0.05.

significant positive predictor of attitude for all participants and participants that perceive themselves to be normal weight; however, 'worry about the risk of developing a disease' was a significant positive predictor for all participants and those that perceived themselves to be overweight or obese. Consequently, when implementing an intervention in young adults who do not perceive themselves to be overweight, highlighting personalised advice as a tool to improve health and fitness may increase uptake, whereas, in a population that deems themselves to be overweight, it may be more effective to highlight the role of personalised advice in disease prevention.

Having an external HLC was a significant negative predictor of attitude towards adoption of genotype-based advice. However, the low mean external HLC score suggested that the majority of participants perceived health to be under their control and scores did not differ significantly between participants based on their body weight perception. Previous research has suggested that internal HLC had a greater capacity to explain variance in diet-related behaviour than external HLC.[37] Internal HLC was significantly positively associated with attitude in the present study ($r = 0.12$) but did not add significantly to the model; furthermore, the negative

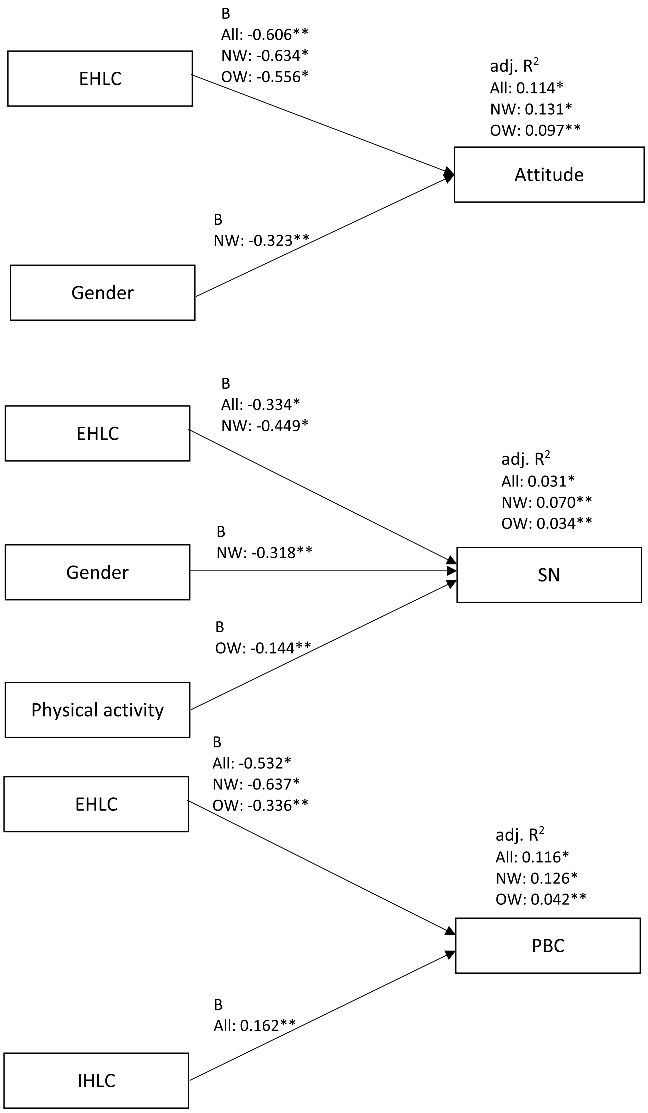

Fig. 4. Objective 2: Summary of unstandardised regression coefficients and adjusted R^2 of psychological factors and characteristics for Theory of Planned Behaviour constructs, for all participants, participants that perceive themselves to be normal weight and participants that perceive themselves to be overweight or obese. B, unstandardised regression coefficient; adj. R^2, adjusted R^2; EHLC, external health locus of control; IHLC, internal health locus of control; SN, subjective norms; PBC, perceived behavioural control; All, all participants (n = 391); NW, participants that perceive themselves to be normal weight (n = 299); OW, participants that perceive themselves to be overweight or obese (n = 92). * P < 0.001; ** P < 0.05.

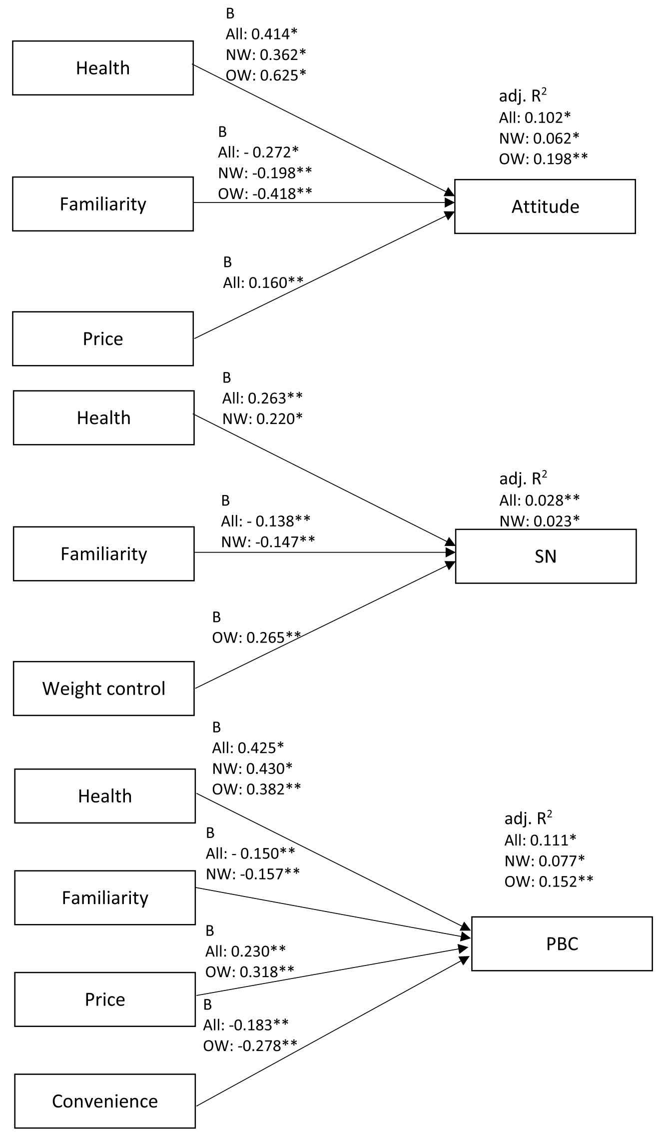

Fig. 5. Objective 2: Summary of unstandardised regression coefficients and adjusted R^2 of food choice motives for Theory of Planned Behaviour constructs, for all participants, participants that perceive themselves to be normal weight and participants that perceive themselves to be overweight or obese. B, unstandardised regression coefficient; adj. R^2, adjusted R^2; SN, subjective norms; PBC, perceived behavioural control; All, all participants (n = 391); NW, participants that perceive themselves to be normal weight (n = 299); OW, participants that perceive themselves to be overweight or obese (n = 92). * P < 0.001; ** P < 0.05.

relationship between external HLC and attitude was stronger ($r = -0.34$). Poínhos *et al.*[24] also reported a stronger association between external, compared to internal, HLC and attitude. Therefore, when investigating personalised nutrition, it appears that external rather than internal HLC has a greater capacity to explain variance in attitude. In the present study, internal HLC was significantly lower in participants who perceived themselves to be overweight or obese compared to those who perceived themselves to be normal weight. Consequently, challenging the perception of young adults that their health is not under their control could improve their attitude towards genotype-based personalised advice. In participants who perceived themselves to be normal weight, men had a significantly less positive attitude towards personalised nutrition than women. Women have been reported to be more conscious of health and demonstrate greater engagement with preventative behaviours.[38] In contrast, men have been reported to have lower adherence to, and belief in, healthy eating recommendations,[39] and are less likely to be willing to have a genetic test.[26,40] In effect, for many aspects of genotype-based personalised nutrition, the advice provided may be more effective if it is personalised by sex.[41,42] Consequently, the findings of the present study are in agreement with the recommendation that interventions to change health behaviours should be developed differently for male and female populations.[38]

Food choice motives explained the greatest percentage of variance in the model which included participants who perceived themselves to be overweight or obese (20%) compared to the model which included participants who perceived themselves to be normal weight (6%). In all models, 'health' had the largest β-coefficient, and this was greatest in the model of participants who perceived themselves to be overweight or obese. Previous research has highlighted a positive association between the food choice motive of 'health' and attitude towards both healthy eating in young adults[43] and attitude towards personalised nutrition in European adults.[44] In the present study, 'health' was the third highest-rated food choice motive after 'sensory appeal' and 'price'. 'Sensory appeal' and 'price' are commonly reported as the highest-rated motives for food choice.[22,43] Consequently, for health motives to be considered in food choice, the food should have sensory appeal and good value. In accordance with previous research, participants who rated 'familiarity' as an important motive for food choice had a less favourable attitude towards genotype-based advice.[44] These participants may perceive that genotype-based advice would require them to consume new or different foods from those they normally eat. Eating context has been investigated in previous research and may overlap with the concept of familiarity.[13,25,45] Eating context may be a barrier to the adoption of personalised nutrition, particularly when eating out of the home or with family members.[13,25,45] Therefore, young adults may have a more favourable attitude towards the use of personalised advice if they are assured that food preferences and eating context will be considered in the advice.[44,45]

Subjective norms

In all models 'health professionals' were a significant positive predictor of subjective norms. In line with other research, communication of information to young adults about the benefits of personalised dietary and physical activity advice may be most effective when delivered by a health professional.[23,46]

Male participants and those who perceived that their health was outside of their own control were less influenced by perceived social pressure to engage with genotype-based personalised dietary or physical activity advice. In participants who perceived themselves to be overweight or obese, a higher level of reported physical activity was associated with lower subjective norms. Since these participants are already engaged in healthy lifestyle behaviours, they may be influenced less by social pressure.

As reported with attitude towards the behaviour, a similar pattern was observed between food choice motives of 'health' (significant positive relationship) and 'familiarity' (significant negative relationship) with subjective norms. However, in participants who perceived themselves to be overweight or obese, 'weight control' was the only significant predictor of subjective norms. Participants who reported 'weight control' as a strong motive in their food choices were more influenced by social pressure to engage with genotype-based personalised advice. Previous research has identified the potential for weight loss as a perceived benefit of personalised nutrition[25] as well as being a significant predictor of attitude, intention[44] and acceptance of personalised nutrition advice.[47]

Perceived behavioural control

Control beliefs explained a significant proportion of the variance in PBC in all models, although the percentage of variance explained was trivial (3–10%). 'Having enough money' was a significant positive predictor in all models and 'having confidence in the effectiveness of guidance' was a positive predictor in the model including all participants and those that perceived themselves to be normal weight. Previous research has reported perceived benefits of personalised advice to have the strongest relationship with attitude, intention,[24,45,48] and acceptance[47] of personalised nutrition. Confidence in the effectiveness of guidance may represent a proportion of what participants would perceive as benefits of personalised advice. Conversely, perceived risk (not measured in the present study) has been reported to have a negative, although less influential, relationship with attitude and intention.[24,48]

Participants who perceived greater control over their own health perceived themselves to have greater control over their health-related behaviour. The consistent finding between external HLC and each construct of the TPB once again highlights the importance of communicating how lifestyle behaviour can be as important as genetics in determining the risk of disease[49] and, in terms of increasing PBC, explaining how individuals can achieve or maintain healthy behaviours.

Food choice motives of 'health', 'price' and 'familiarity' influenced participant's perception of their ability to adopt genotype-based personalised advice to modify their dietary or physical activity behaviour, in a similar manner to attitude and subjective norms. 'Convenience' had a significant negative relationship in the model for all participants and those who perceived themselves to be overweight or obese. Participants who rate 'convenience' as a strong motive for food choice may perceive the adoption of dietary or physical activity advice to be more challenging. 'Convenience' was not identified as a significant factor in the study by Rankin *et al.*[44] and this may be because they only looked at the relationship between food choice motives and attitude and intention to adopt personalised nutrition. The findings of the present study suggest that although there are some consistent patterns between food choice motives and TPB constructs, there are also differences both between constructs and between participants based on their perception of their body weight. An understanding of which factors influence which constructs of the TPB helps to understand the context of how advice should be communicated to young adults. For example, whether it should be phrased to address their appraisal of genotype-based advice (attitude) or their ability to carry out necessary changes in their behaviour (PBC).

Recommendations

There are some recommendations for the delivery of genotype-based personalised advice to motivate healthy dietary and physical activity behaviour in young adults that appear to be generically applicable to this population. To appreciate the need to meet advice, young adults need to accept the strong effect that these lifestyle behaviours can have on their subsequent health and, importantly, that this is under their control. Advice provided should be delivered in the context of improving health. Food preferences should be considered in the delivery of dietary recommendations and advice should preferably be delivered via a health professional. Advice should detail how to meet dietary and physical activity advice; for example, if a reduction in sodium intake is recommended, advice should explain which foods are high in salt and provide alternative food choices to enable the advice to be met. The findings also suggest that to motivate behaviour change, advice should be tailored based on individual characteristics of young adults. Highlighting the role of genotype-based advice to improve health and fitness is more important for young adults who perceive themselves to be normal weight; whereas, in young adults who perceive themselves to be overweight or obese, advice for disease prevention and weight control would likely be more effective for increasing their intention to adopt advice. Of participants who perceive themselves to be normal weight, young men had a less favourable attitude towards the adoption of genotype-based dietary and physical activity advice and were less influenced by social pressure than young women. Therefore, advice that increases their perceived ability to adopt dietary and physical activity advice may be more effective in increasing their intention to adopt advice. Young adults who believe they are already engaged in healthy lifestyle behaviours or perceive themselves to be normal weight are less likely to perceive a need to adopt genotype-based advice.[20] Optimistic bias has been suggested as a potential barrier to the adoption of personalised nutrition advice, particularly in younger populations.[13] Although optimistic bias did not add significantly to any of the models, it was significantly higher in the participants who perceived themselves to be normal weight and was correlated significantly with participants' health perception (r = 0.33), physical activity (r = 0.34), internal HLC (r = 0.35), and external HLC (r = –0.25). Advice provided to this group should highlight how genes can interact with lifestyle behaviours to affect disease risk, in order to challenge their optimistic bias. Adoption of these recommendations would provide more targeted personalised advice to young adults and as a consequence may result in a more effective intervention to change behaviour.

Strengths and limitations

The strengths of this study include a specific focus on a young adult population who stand to benefit most from genotype-based personalised advice. The use of the TPB provided a framework to understand the factors that influence the intention to adopt genotype-based personalised advice. The relationship between background factors and subjective norms and PBC in addition to attitude was included and was novel to this research area. However, the study was not without limitations; in several of the regression models, despite being significant, only a small amount of variance was explained by the factors included. Control beliefs were determined from salient beliefs elicited in the pilot study and explained less than 10% of the variance in PBC; in effect, there may be further control factors that make up PBC in this young adult population. Other potential background factors that may have influenced TPB constructs, and intention to adopt genotype-based advice were not included; the most important of which was a measure of risk and benefit. This has been previously well researched with the relatively consistent finding that benefits have a greater influence than risks on intention to adopt genotype-based advice.[13,24,45,47,48,50] Since the risk/benefit relationship with adoption of personal nutrition is relatively well understood, it was not included as a measure in the present study; however, it may account for a proportion of the unexplained variance in the models.

Conclusions

In conclusion, the current study provides support for the use of the TPB in understanding the intention of young adults to adopt genotype-based advice for dietary and physical activity behaviour. Background factors including belief composites, HLC, gender, physical activity, and food choice motives of 'health', 'price', 'familiarity', 'weight control', and 'convenience' interact with TPB constructs. In addition to perceived body weight, these background factors should be utilised to inform the delivery of advice in behaviour change interventions that seek to use genotype-based personalised advice in young adult populations. Finally, the recommendations for the use of genotype-based dietary and physical activity advice in young adults, based on the findings of the present study, need to be evaluated in a genotype-based personalised nutrition intervention study to change dietary behaviour.

Abbreviations

BMI: body mass index; **HLC:** health locus of control; **NCD:** non-communicable diseases; **PBC:** perceived behavioural control; **T2D:** type 2 diabetes; **TPB:** Theory of Planned Behaviour.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.50

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Conflict of interest

YM is a scientific consultant for MyHealthChecked, a wellness company that uses genetic testing. LP is founder of Optimyse Nutrition, a nutritional advice company that offers genetic testing. KL previously held a paid role as Research Editor for Foodsmatter. She is an Editorial Board Member for the British

Association of Nutritional and Lifestyle Medicine, Nutritional Evidence Database and a Scientific Advisory Board Member for Chuckling Goat, both in an unpaid capacity. She is occasionally paid, or receives hospitality, to deliver talks on her research and infrequently receives sample products related to health and nutrition.

Authorship

AK: formulating the research question, designing the study, carrying out study, analysing the data and writing the article—original draft. YM, LP, MG, and KL—formulating the research question, designing the study and writing the article—review and editing. All authors read and approved the final manuscript.

Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the St Mary's University Research Ethics Sub-Committee (SMEC_2022–23_027). Informed consent was obtained from all participants.

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RESEARCH PAPER

Human immunodeficiency virus exposed child feeding and maternal enriching factors

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Abstract

Globally, each year 1.3 million neonates acquire human immunodeficiency virus during pregnancy, labour, and breastfeeding time. Replacing breastfeeding with recommended safe infant feeding practices significantly reduces the risk of transmission, nearly eliminating it. This study aimed to assess Human immunodeficiency virus exposed child feeding among 314 mothers with infants under 24 months old. Participants were selected using a systematic random sampling technique, and data were collected through a semi-structured questionnaire. Bivariable and multivariable logistic regression analyses employed to identify determinants for safe infant feeding. During interviews, the mean age of women was 32.35 years (standard deviation±4.5), and infants were 10.8(±3.951) months. The overall safe infant feeding was 67.2% (95% CI: 61.7, 72.9), with a mean knowledge score. By the study's end, 9 infants (2.89%) were confirmed to be infected with virus based on dried blood sample test. Maternal promoting factors for safe infant practice included infant age 25–35 years (adjusted odd ratio (aOR) =2.9) completing high school education (adjusted odd ratio = 9.2), having a good knowledge score for infant feeding (adjusted odd ratio = 8.2), and urban residency (adjusted odd ratio = 2.2). On the other hand, being married made it 83% less likely for safe infant feeding practices (adjusted odd ratio = 0.17) compared to those never in a union. Two in three mothers practiced safe infant feeding for their HIV-exposed infants, with a mean knowledge score of 70.3%. Therefore, healthcare providers give accurate information and counselling services to make informed decisions about infant safe feeding.

Key words: Ethiopia: Exposed infant: Infant feeding: Mothers: Virus

Introduction

Mother-to-child HIV transmission is the primary mode of infection for infants during pregnancy, birth, or breastfeeding.[1] Mothers living with human immunodeficiency virus can strive always to breastfeed with negative outcomes for their health and the health of their kids.[2] However, the mixed feeding practices for HIV-infected mothers increase the risk of HIV transmission by 3-4-fold.[2] Reducing this transmission is a critical global public health challenge faced by researchers, healthcare professionals, policymakers, and HIV-infected women worldwide.[3,4]

Globally, around 36.7 million people, primarily in sub-Saharan Africa (71%), are living with HIV and each year, 600,000 infants globally are infected with HIV, averaging 1,600 infections per day.[5,6] Nearly half (42.5%) were infected during pregnancy, labour, and breastfeeding, especially where mixed feeding is predominant in sub-Saharan Africa, strain of economic burden causes postnatal transmission.[2,3,7]

In low- and middle-income countries (LMICs), WHO advises HIV-infected mothers on combined antiretroviral therapy (cART) to breastfeed infants for 12–24 months, supported by the heightened risks of morbidity and mortality in formula-

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fed babies due to infections and malnutrition.[8] Limited access to clean water and the high cost of formula milk in impoverished populations underscore the importance of this recommendation.[9,10] Maternal knowledge of proper newborn safe feeding procedures, including when and how to start, significantly affected the transmission rate of HIV.[6,11] The 2016 Ethiopian Demographic and Health Survey disclosed significant HIV-related insights, with 1.5 million new cases and 680,000 reported deaths, and notably 74% of Ethiopian women are aware of HIV transmission through breast milk.[2,6,11]

Previous systematic reviews[1,12,13] and primary studies[3,4,14–19] have highlighted key factors, including CD4 count, viral load, and ART adherence influencing the prevention of HIV transmission. The Ethiopian government promotes infant health and HIV-free survival through safe infant feeding in Option B+ care for all pregnant women.[19] However, in 2016 an Ethiopia Demographic and Health Survey (EDHS) reported, that children had low rates of dietary diversity (4.3%) and meal frequency (47.7%), with 17% practicing safe infant feeding for all HIV-exposed infants.[4,11,14,17,20,21] In Ethiopia, as of the updated guidelines in 2018, the Prevention of Mother-To-Child Transmission (PMTCT) guidelines recommend breastfeeding as the safest option for HIV-positive mothers, particularly those who have achieved high viral load suppression. However, it is important to note that the gradual introduction of mixed feeding is highly advised for lactating women in such cases.[1,22] However, several economic and peer support challenges caused a significant risk of HIV transmission with a 3.54% to 4.23% rate practiced before 6 months of mixed feeding.[3,6]

Previous research suggests that various maternal factors impact on PMTCT to infants including maternal education (12 instances),[21] employment status (7 instances),[23] maternal knowledge, and attitude (5.9 to 15.2 instances),[23] HIV disclosure status (6.2 times) were identified as significant hindrances to achieving zero transmission.[25] Therefore, this study aimed to estimate levels of HIV-exposed infants' safe feeding practices and maternal enriching factors in Northeast Ethiopia.

Methods

Study area and period

The study was conducted between April 1 and June 20, 2023, in the North Wollo zone, Amhara region, Northeast Ethiopia. The zone is centred on Woldia and is located 521 km from Addis Ababa and 360 km from the regional capital, Bahir Dar. It shares borders with the South Gondar zone in the West, the South Wollo zone in the South, the Afar region in the East, the Tigray region in the Northeast, and the Waghimra zone in the North West sides. The projected population for 2023 was estimated at 1,763,246, with 50.2% females and 13% residing in urban areas. The zone consists of 14 districts, including three town administrative areas. Healthcare facilities in the zone include 6 public hospitals, 69 health centres, 309 health posts, 10 private medium clinics, 42 primary clinics, and 33 pharmacies. Among these, five hospitals and 22 health centres provide ART services.[31]

Study design

A multi-centre, institution-based, descriptive cross-sectional study was conducted among 314 HIV-infected mothers with their dyads less than 24 months.

Source population

All mothers attending antiretroviral therapy (ART) services with infants ≤24 months under Option B+ care in selected health institutions form the sampled population.

Inclusion criteria. From April to June 2023, all HIV-infected women who had infants less than 24 months of age and were receiving treatment at the ART unit were eligible for this study.

Exclusion criteria. Mothers who were severely ill and unable to communicate their HIV status, as well as their children's HIV status, were excluded from the study

Sample size determinations

The sample size was determined using the single population proportion formula using a 95% confidence level and a 5% margin of error, as well as the prevalence of infant feeding practice, which was found to be 75.2%. The formula used to calculate the sample size is as follows: $n = (Z\alpha/2)^2\,[p\,(1-p)]/d^2$. Where: n = required sample size $Z\alpha/2$ = critical value for the normal distribution at a 95% confidence interval, which is equal to 1.96 p = prevalence (75.2%), d = margin of error (5%). Using the given values, the calculation for the sample size is as follows: $n = (1.96)^2\,[0.752(1\text{-}0.752)]/(0.05)^2$, which results in n = 286. However, after accounting for a non-response rate of 10%, the sample size is adjusted by non-response rate by adding10% non-response rate as $286 + (0.10 \times 286) = 314$. Hence, the final sample size was found to be 314 HIV-infected mothers with their dyads were interviewed for final analysis.

Sampling procedure

In the North Wollo zone, there were 27 health facilities providing Option B+ services (PMTCT), comprising 5 public hospitals and 22 health centres. To select the sampled participants, 30% of the facilities were first randomly selected from the total 27 Option B+ services giving centres by using a lottery method. This resulted in a sample of eight health facilities for final sample size selection based on their serving-giving population over the past 3 months. In each health facility, a 3-month file of service was given population divided by our sample size, and we determined K =, then we selected a total of 314, using systematic sampling technique within each facility using the formula (K = N/n, 837/314 = 3) where N represents the total client population (837) and n denotes the required sample size (314). The final sample of 314 participants was selected using a k = 3 interval from each health facility based on their population level.

Dependent variable. This study evaluated WHO-recommended infant feeding practices (Yes/No), emphasising safety for up to 2 years. Unsafe practices include early breastfeeding substitution, introducing complementary foods at 6 months or earlier, or mixed feeding before 6 months. In LMICs, WHO recommends 12–24 months of breastfeeding for HIV-infected mothers cART, and gradual introduction of complementary feeding with stressing strict adherence to ART prophylaxis given for infants.[1,3,16]

Independent variable. Maternal Age, Marital status, Occupation, Educational status, Monthly income, Knowledge of vertical transmission, Disclosure of HIV status, Place of delivery, Antenatal follow-up, Stage of HIV, CD4 Count, Breast problem, Counseling practice, Sex of the child, Age of child

Operational definition

Minimum dietary diversity. The Children's Complementary Food Dietary Diversity Score (DDS) was based on seven food groups: grains/roots/tubers, legumes/nuts, dairy products, flesh foods (meats/fish/poultry), eggs, vitamin A-rich fruits and vegetables (VAFV), and other fruits and vegetables (OFV). The DDS, ranging from 0 to 7, measured dietary diversity by assigning one point to each food group. We considered minimum dietary diversity as consuming food from at least four different groups (DDS $\geq$ 4).[24] **Exclusive breastfeeding;** Exclusive breastfeeding involves giving only maternal breast milk to the infant for the first 6 months, while replacement feeding entails providing a diet with all necessary nutrients for infants not receiving any breast milk. Mixed feeding occurs when infants under 6 months are given liquids or foods alongside breast milk.[1] **Complementary feeding;** HIV-exposed infants require careful feeding, following guidelines for exclusive breastfeeding for the first 6 months, followed by the introduction of complementary foods while breastfeeding up to 12 months. ART is crucial in reducing HIV transmission risk, and decisions on infant feeding should involve healthcare providers, considering specific circumstances and ongoing monitoring for the well-being of these infants during the transition to complementary feeding.[25,26] **Mean Good knowledge;** Good knowledge is defined as respondents who scored equal to or greater than the mean score of maternal safe infant feeding related knowledge question list in WHO guidelines.[26,27]

Data collection procedure and quality control. The questionnaire, initially in English, was translated into Amharic. Six data collectors and three supervisors underwent a 1-day training on study objectives, privacy, and confidentiality. Data collection used a pre-tested semi-structured questionnaire, with a pilot study conducted on a 5% sample size for adjustments. Daily supervision by supervisors and the principal investigator ensured consistency, completeness, clarity, and accuracy in the data collection process.

Data process and analysis. Collected data underwent editing, entry, and coding with EPI info v7.2.5.0 software, then analysed using SPSS v25. Results were presented through frequency tables, figures, and percentages. Maternal knowledge of infant feeding, assessed with nine structured questions, produced a mean score. Bivariate and multivariable logistic regressions were performed with independent variables having P-value$<$0.25 in bivariate analysis. Collinearity effects and data normality were checked, by applying a stepwise backward elimination procedure. Categorical variables with adjusted odds ratios and 95% confidence intervals determined safe infant feeding at P$<$0.05. Model fitness was assessed using the Hosmer–Lemeshow goodness-of-fit test. Maternal knowledge was categorised as poor or good based on the mean score, and the study questions were adapted from a previously published article.[4,6,11,14,17,22,24,28–30] Cronbach's alpha yielded reliability coefficients of 0.76 for maternal knowledge and 0.82 for infant feeding practices, signifying good internal consistency. Tables containing all maternal knowledge and safe infant feeding-related questions were incorporated into the study.[3,5,14,15,17,23,31,32]

Result

Socio-demographic characteristics

The study included 314 HIV-infected women with infants from eight public health institutions, achieving a 100% response rate. The mean age for mothers and children was 32.4 years (SD $\pm$4.4) and 7.8 months (SD$\pm$2.9), respectively. Among live-birth infants, 63.7% were female, and 36.6% were male. The majority of participants (72.3%) lived with their spouses, and 38.2% had no formal education. Urban residents accounted for 60.2%, with 73.6% being housewives. The majority (90.3%) completed their fourth antenatal care (ANC) visit, while 4.6% had a history of home delivery. Additionally, 270 pregnant women had a CD4 count of $\leq$50 cells/mm3.

Maternal and obstetrics characteristics

Nearly half of the 165 respondents (52.5%) had fewer than two children, and the majority of the 304 participant women (96.8%) received counselling on infant feeding options. Among the mothers who gave birth, 283 (90.1%) delivered at health institutions, with 277 (88.2%) having a spontaneous vaginal delivery. During ANC care, 142 women (45.2%) became aware of their HIV status, while 139 (44.3%) knew about their pregnancy before initiating ANC. Additionally, 210 respondent mothers (66.9%) disclosed their HIV status (Table 1).

Maternal knowledge for safe infant feeding practice

Over half of 165 respondents (52.5%) had $<$2 children, and 96.8% of 304 women received feeding counselling. For those giving birth, 90.1% delivered at health institutions, with 88.2% having spontaneous vaginal delivery. During interviews, mothers mentioned HIV transmission: 63.37% (200) during delivery, 7.9% (24) during breastfeeding, and 32.4% (97) during breast pain, oral ulcers of infants, and mother problems (Table 2).

Maternal practice for safe infant feeding

Almost all (99%) of mothers received safe infant feeding demonstrations and counselling during ANC from healthcare providers. During the interview, 203 (64.49%) of them also practiced demonstrated how breastfeeding after the discussion (Table 3).

Table 1. Socio-demographic characteristics of HIV-positive mothers and their infants attending ART service

Variable	Category	Frequency	Percent (%)
Maternal Age	<25	7	2.2
	25-30	98	31.2
	31-35	128	40.8
	> = 36	81	25.8
Residency	Rural	125	39.8
	Urban	189	60.2
Age of the child	<6 month	48	15.3
	>6 month	266	84.7
Sex of the child	Male	115	36.6
	Female	199	63.4
Marital status	Married	227	72.3
	Divorce	53	16.9
	Widowed	34	10.8
Maternal education	Unable to read and write	120	38.2
	Primary school	85	27.1
	High school	61	19.4
	Diploma and above	48	15.3
Occupation status	Housewife	231	73.6
	Government employee	31	9.9
	Private employee	6	1.9
	Daily labourer	1	0.3
	Merchant	24	7.6
	Farmer	21	6.7
Maternal anti-natal care	Yes	283	90.1
	No	31	9.6
Maternal post-natal care	Yes	59	18.8
	No	255	81.2
Counselling for infant feeding	Yes	304	96.8
	No	10	3.2
Place of birth	Health institution	300	95.5
	Home delivery	14	4.6
Mode of Delivery	SVD	277	88.2
	CS	37	11.8
Attending post-natal care	Yes	141	44.9
	No	153	55.1
HIV Disclosed status	Friends	7	3.2
	Husband	307	96.9
Maternal CD4 count (cell/mm3)	≤500 cell/mm3	40	12.7
	>500 cell/mm3	274	83.3
Disease progress	Stage 1	66	21.0
	Stage 2	146	46.5
	Stage 3	102	32.5
	Stage 4	0	0
Ever encountered a breast problem	Yes	101	32.16
	No	213	67.8
If yes Type of breast problem (N = 101)	Burning, tingling-	5	4.9
	Cracked nipples	18	17.8
	Engorgement	59	58.4
	Sore nipples	19	18.8
long term illness	Yes	42	13.4
	No	272	86.6

Mothers' status during an interview

During the study, 67.2% of mothers practiced safe infant feeding, with a mean knowledge score of 70.3%. The majority of mothers (83.3%) had a CD4 count greater than 500 cells/mm3, and 146 (46.5%) were classified as WHO clinical stage II. Among the infants, 81.2% had not experienced any oral ulcers.

Level of safe infant feeding practice

The overall prevalence of safe infant feeding practices was 67.2% (95% CI: 61.7, 72.9), whereas the remaining 32.8% of participants used mixed or unsafe infant feeding options. Among women who used unsafe infant feeding options mainly reported having breast problems with (n = 101), the most common breast problem reported was engorgement (58.4%), followed by sore nipples 19(18.8%), cracked nipples 18(17.8%), and burning or tingling 5(4.9%).

Factors affecting infant feeding practice

During the final multivariable logistic regression of this report, variables with a P-value<0.25 on bivariate analysis were considered candidates for multivariable regression. These included mother and infant age, residence, marital status, education, number of children, ANC visits, place of delivery, timing of breastfeeding, maternal knowledge score of infant feeding practices, HIV disease progression, presence of long-term illness, and infant mouth ulcers.

After controlling certain confounding factors, five variables were significantly associated with safe infant feeding during the PMTCT. These include being maternal age with 25–35 years (adjusted odd ratio (aOR) = 2.9, 95% CI: 1.2, 7.6), completing high school education (aOR = 9.2, 95% CI: 1.3, 6.8), having a good knowledge score for infant feeding (aOR = 8.2, 95% CI: 2.1, 32.7), and urban residency (aOR = 2.2, 95% CI: 1.1, 4.5) are maternal enriching factors as compared with their respective counter groups. On the other hand, infant mothers living with their spouses had an 83% reduced likelihood of safe infant feeding practices compared to those who were never in a union (aOR = 0.17, 95% CI: 0.36, 0.80) but having baby mothers (Table 4).

Discussion

At the end of the study periods, the overall safe infant feeding practice among mothers for their dyads was found to be 67.2%). This finding is consistent with previously reported 63.43% in Gondor Hospital,[14] 63.8% in Samra Hospital,[6] 63.99% in Debre Markos,[11] and 63.8% in Bahir Dar Hospital.[32] This might be related to healthcare providers using similar guidelines for therapeutics, and counselling principles across different study settings similar contextual factors, such as cultural norms, available resources, and healthcare policies, may have influenced safe infant feeding practices across the included healthcare facilities. Conversely, the final report of safe infant feeding practices is higher than previously found at 25.5% in Gondar Hospital,[24] 49.3% in Wolaita Soda Hospital,[2] and 18.2% in Kenya Hospital,[15] but lower than previously reported 86.4% in Gondar hospital.[4] These differences may result from variations in maternal healthcare utilisation across different Ethiopian facilities and the discount could stem from differences in study settings, access to information, and technology, influencing

Table 2. Maternal knowledge of infant feeding practice among HIV-positive women attending ART service

Variables	Categories	Frequency	Percent
1. When can HIV be transmitted from mother to child	During breastfeeding	200	63.7
	During delivery	24	7.9
	During breast pain, oral ulcer of infanta, and mother's problem	97	32.9
2. At which age stage were you informed to start infant complementary?	Before 6 months advisable after birth	94	29.9
	Exactly at 6 months' completion	177	56.4
	After 12 months of only breastfeeding	47	13.7
3. Which is best Prevention means from mother-to-child Transmission (MTCT) after birth	Maternal Good ART adherence with breastfeeding	146	46.5
	Giving ART to the child as prophylaxis	131	41.7
	Taking ART drugs during pregnancy	17	5.5
	Using infant formula feeding after birth	20	9.9
4. Infant Feeding outweighed breastfeeding after birth	Not sure/ don't know	23	7.3
	Yes	291	92.7
5. Do you know infants have acquired HIV through mixed feeding before?	Yes	297	94.6
	No /Not Sure	17	5.4
6. Does breastfeeding outweigh that infant-feeding option?	Yes	289	92.8
	No/Not Sure	25	9.1
7. What are the advantages and disadvantages of using expressed and heat-treated breast milk	The HIV in breast milk is inactivated by heating and yet most of the nutrients are preserved.	159	50.6
	Breast milk is the perfect food for babies even if it has risk	155	49.4
8. How long should exclusive breastfeeding be recommended during counseling by healthcare providers	Up to 6 months	60	19.1
	Beyond 6 months	131	41.7
9. Mothers with HIV should receive evidence-based, patient-centred counselling for decision-making about infant feeding before delivery	No	3	0.99
	Yes	311	98.1
10. Do you know that mixed feeding children can cause HIV	Yes	305	97.1
	No/ I don't know	9	2.9
11. Healthcare providers discuss on risk of mixed infant feeding	Yes	298	94.9
	No/ don't remember	16	5.01
12. Maternal Knowledge score	Good	183	70.3
	Poor	131	39.7

community awareness. Maternal workload and limited time for childcare may contribute to these disparities.

The final report of this study indicated that the mean maternal knowledge score on infant feeding was found to be 70.3%, which is lower compared to previous reports of 86.4% and 65.3% in,[28] 50.1% found in Botswana,[23] 83.6% in Nigeria.[30] This indicates the existence of gaps in mother-to-child transmission and the actual practice in Ethiopia and the healthcare providers sometimes struggle to influence maternal behavioural changes in implementing recommended infant feeding choices which is an urgent need for better counselling for pregnant women on infant feeding options to eliminate transmission.

Regarding maternal enriching factors for safe infant feeding factors identified, accordingly, mothers of HIV-exposed infants within 30–35 years of age were two-fold times more likely to adopt safe infant feeding practices compared with counter groups. The findings of this study are consistent with previous findings in Gondar town,[16] SNNPR regions,[28] Amhara region,[33] Northern Kenya,[19] and southern Nigeria.[34] The possible reasons for the similarity might be due to middle-aged mothers who had exposed infants are more likely to adhere to any recommended medical practice

Consistent with previous study findings in Gondar referral hospital,[16] North America, and Nigeria,[34] married women with HIV-exposed infants were 83% less likely to adopt the safe way of feeding practices compared to divorced women. The possible explanation may be that married women may face unsupportive partners upon disclosing their HIV status, affecting the adoption of safe feeding practices. HIV disclosing can lead to social challenges, including from husbands, friends, and community members when transitioning from exclusive breastfeeding.

The finding of the current study also indicated that getting information or counselling about safe infant feeding from the correct health professionals was significantly associated with adopting the recommended infant feeding practices that counter group. This is consistent with the study done in Addis Ababa,[35] Woldia town,[21] and Oromia regions.[30] This could be because many women find that receiving skilled information on infant feeding options may not be enough for informed decision-making, and it helps them choose appropriate feeding methods, improve adherence, and opt for safer options like exclusive breastfeeding or complete avoidance of breastfeeding.[1]

Consistent with study previous studies done in the Amhara region[33] and Addis Abeba City,[36] mothers with good knowledge about safe infant feeding are more likely to practice it. This might be related to preventing mother-to-child transmission and promoting safe infant feeding, early adoption is encouraged. HIV-positive women need customised counselling to make informed choices about feeding options based on local circumstances, ensuring optimal growth for their babies. Moreover, the findings of this study reveal that individual

Table 3. Maternal practicing related questions for HIV exposed infant feeding

WHO recommendation for safe infant feeding question	Categories	Frequency	Percent
1. Have you practiced Infant Feeding Demonstrations after healthcare providers showed	No/I don't remember	1	0.3
	Yes	313	99.7
2. Infant Age informed for safe infant feeding (n = 313)	During ANC follow-up	94	29.9
	At delivery ward	177	56.4
	After being delivered at PNC	46	13.01
3. Which option do you want to practice with your infant (n = 314)	Mixed method with prophylaxis from infant after birth	3	0.8%
	Exclusive breastfeeding with prophylaxis ceased following complementary feeding.	311	99.01%
3.1 Reasons for EBF with prophylaxis ceased upon complementary feeding (n = 311)	Advised by parents and friends:	24	7.6
	Counselled by health professionals	63	20.1
	Fear of HIV transmission: 129 (40.1%)	129	
	Fear of stigma	48	15.3
	Norm of society	12	3.8
	Lack of information	18	5.7
	Aware of the safety of the baby	17	5.4
	Advised by parents and friends:	27	8.6
4. Breastfeeding the baby at the time of the interview	No	203	64.6
	Yes	111	35.4

Table 4. Factors affecting infant feeding practices among HIV-positive mothers attending ART service

		Infant feeding practice				
Variable	Category	Safe	Unsafe	95% CI for COR	95% CI for aOR	P value
Age of the infant	≤6 month	42(13.5)	6(1.92%)	Reference	Reference	
	>6 month	169(53.8%)	97(30.8%)	1.56(1.14, 4.72)	1.23(0.98, 1.67)	0.057
Infant age	12-24	84 (79.4%)	20(20.6%)	5.80(2.7, 10.3)	0.68(0.3, 1.6)	0.39
	6-18	93(73.1%)	36(27.9%)	3.57(1.9, 6.4)	4.1(1.2, 13.1)	0.026*
	>35	34(42%)	47(58%)	Reference	Reference	
Marital status	Married	161(70.9%)	66(29%)	1.17(0.3, 5.7)	0.17(0.4, 0.8)	0.02*
	Divorce	34(61.2%)	19(38.8%)	2.0(0.8, 4.8)	0.8(0.4, 1.2)	0.09
	Widowed	16(47.1%)	18(52.9%)	Reference	Reference	
Residency	Rural	106(84.8%)	19(15.2%)	Reference	Reference	
	Urban	105(55.5%)	84(44.5%)	4.46(2.5, 7.8)	2.2(1.3, 4.5)	
Source of information	Health institution	208(69.1%)	89(29.9%)	10.9(3.7, 13.8)	5.2(2.3, 16)	0.01*
	Family and friends	3(17.7%)	14(82.3%)	Reference	Reference	
Maternal knowledge	Good mean score	204(69.2%)	91(30.8%)	Reference	Reference	
	Poor mean score	7(36.8%)	12(63.2%)	12.2(3.1, 16)	8.2(2.4, 17.8)	0.003*

* Indicated statistical significant variables after association.

women with permanent urban residency were 2.2 times more likely to adopt safe infant feeding practices compared to rural dwellers. This finding aligns with previous studies conducted in Gondar town[16] and Nigeria.[9] The possible reason is that the majority of urban residents had previous exposure, and those in urban areas tend to be more receptive to the training and guidance provided by medical professionals. They exhibit an eagerness to acquire and apply information, which may contribute to their higher adoption of safe infant feeding practices.

Limitations of the study

This cross-sectional study design limited the ability to establish a cause-and-effect relationship, and there was a possibility of recall bias as mothers were expected to remember the feeding patterns of their children since birth.

Conclusion

This study's findings have important implications for public health interventions targeting infant feeding practices among HIV-positive mothers. The majority (67.2%) followed safe guidelines, but mixed feeding (32.8%) increased HIV transmission risk. Predictors included age, marital status, residency, access to information, and knowledge of recommended options and targeted interventions through healthcare providers give accurate information and counselling services, for mothers to make informed decisions about infant feeding.

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Authorship

BD contributed to conceptualisation, writing the original draft, writing review and editing, investigation, and conceptualisation. ML contributed to writing review and editing, formal analysis, and methodology. FK contributed to formal analysis, methodology, writing review and editing, data curation, software editing, and investigation. All authors have read and agreed to the published version of the manuscript.

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Competing interests

No any conflict of interest for this manuscript

Ethical consideration

The study was conducted in compliance with the Declaration of Helsinki, adhering to relevant guidelines. The Institutional Review Board of Woldia University College of Health Science approved the study after a thorough review of the procedures, objectives, and public health considerations (Ethical Approval Assigned No. 095, dated 17/5/2023). Official letters were obtained from the Zone Health Department, and the significance and objectives of the study were communicated to the selected health facilities. Informed consent was obtained from all participants, ensuring their voluntary participation. Confidentiality was strictly maintained during data collection and throughout all stages of the study.

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RESEARCH ARTICLE

Inequalities in diet quality by socio-demographic characteristics, smoking, and weight status in a large UK-based cohort using a new UK diet quality questionnaire-UKDQQ

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Abstract

The aim of this study was to explore the associations between diet quality, socio-demographic measures, smoking, and weight status in a large, cross-sectional cohort of adults living in Yorkshire and Humber, UK. Data from 43, 023 participants aged over 16 years in the Yorkshire Health Survey, 2nd wave (2013–2015) were collected on diet quality, socio-demographic measures, smoking, and weight status. Diet quality was assessed using a brief, validated tool. Associations between these variables were assessed using multiple regression methods. Split-sample cross-validation was utilised to establish model portability. Observed patterns in the sample showed that the greatest substantive differences in diet quality were between females and males (3.94 points; $P < 0.001$) and non-smokers vs smokers (4.24 points; $P < 0.001$), with higher diet quality scores observed in females and non-smokers. Deprivation, employment status, age, and weight status categories were also associated with diet quality. Greater diet quality scores were observed in those with lower levels of deprivation, those engaged in sedentary occupations, older people, and those in a healthy weight category. Cross-validation procedures revealed that the model exhibited good transferability properties. Inequalities in patterns of diet quality in the cohort were consistent with those indicated by the findings of other observational studies. The findings indicate population subgroups that are at higher risk of dietary-related ill health due to poor quality diet and provide evidence for the design of targeted national policy and interventions to prevent dietary-related ill health in these groups. The findings support further research exploring inequalities in diet quality in the population.

Key words: Dietary assessment: Dietary ill-health: Dietary patterns: Diet quality: Diet quality questionnaire: Disparities: Inequalities: Smoking: Socio-economic: Weight

Introduction

An unhealthy diet is one of the four leading behavioural causes of years of life lost in England alongside smoking, physical inactivity, and alcohol consumption.[(1)] Poor quality diet is associated with obesity, type 2 diabetes, cardiovascular diseases, and some cancers and estimates of the economic cost of the risk of chronic disease on the NHS suggest that poor diet is the behavioural risk factor with the highest impact.[(2)] Prevalence of

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these diseases is unequally distributed in the UK[1,3,4] and socially patterned differences in dietary intake may therefore be a significant contributor to health inequalities.[5] Compared with people living in the least deprived decile of local authority areas, people living in the most deprived areas are more likely to die from preventable cancers, preventable heart disease and are more likely to have type 2 diabetes. Children in Year 6 living in the most deprived communities are around twice as likely to be living with obesity compared with those living in the least deprived.[6]

These inequalities in diet-related ill health are reflected in variations in population-level dietary intake. Amongst UK children and adults, average consumption of total fat, saturated fat, and free sugars exceeds recommended levels while average consumption of fibre is too low.[7] Whilst this is observed across age groups and sexes there is clear evidence that the extent to which recommendations are not met is socially distributed.[8] Higher socio-economic groups tend to eat a greater number of daily grams of fruit and vegetables and fewer daily grams of red and processed meat and non-milk extrinsic sugars.[5] On average adults in the UK with higher incomes consume a greater amount of fruits and vegetables, oily fish, fibre and a lesser amount of sugary drinks and free sugars.[9] Children from families in the lowest 20% of income consume around a third less fruits and vegetables, three quarters less oily fish, and a fifth less fibre per day than children from the most well off 20%.[10] Analysis of empirically derived dietary patterns characterised by higher consumption of fruit, vegetables, and oily fish were more likely to be consumed by higher socio-economic status groups whereas lower socio-economic status groups were more likely to consume dietary patterns characterised by snacks, fast foods and sugary drinks.[11]

Whilst UK studies show clear variation in consumption of individual foods and nutrients across socio-economic groups, exploration and analysis of dietary patterns and composite measures of diet quality can be a better indicator of habitual dietary intake and show stronger associations with health outcomes.[12] This method examines diet as a multidimensional exposure, examining relationships with the whole diet and health rather than individual foods, food groups, or nutrients. However, there is a paucity of studies from the UK that explore dietary quality in the population.

Therefore, the aim of this study was to analyse the relationship between diet quality and its association with socio-economic and demographic characteristics, smoking status, and weight status in a large UK adult cohort. The regional population-based Yorkshire Health Study was used for this purpose. Diet quality was evaluated using a new, brief diet quality assessment tool developed for pragmatic application in large-scale surveys and developed and validated in a representative UK population.[13]

Methods

The Yorkshire health study

The aim of the Yorkshire Health Study was to collect information on the residents from the Yorkshire and Humber region in England to inform local health-related decision making.[14] In the first phase, data were collected from 27,813 individuals aged 16–85 years (15.9% response rate) registered with GP surgeries between 2010 and 2012, in South Yorkshire. The second phase expanded to cover the Yorkshire & Humberside Government Office Region. In this second phase, data were collected from an additional 43,023 individuals between 2013 and 2015 via NHS Trusts, supported by the NIHR Clinical Research Network. A regional media campaign was also used to invite residents to sign up to join the cohort and to complete an online or paper health questionnaire. The questionnaire was used to capture demographic information on sex, age, socio-economic status, employment status, and deprivation level. Data were also collected relating to health related behaviours such as smoking status, height, and weight. BMI was calculated from self-reported valid height and weight data. Data on diet quality were collected in the 2nd phase of YHS data collection using a 13 item Diet Quality Questionnaire (UK-DQQ). This dietary quality assessment tool was designed for pragmatic application and to be brief, low participant burden and easy to analyse and interpret.[13]

Ethical standards

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Leeds East National Health Service (NHS) Research Ethics Committee (ref: 09/H1306/97). An information sheet was sent to participants along with the questionnaire. Consent was implied if a questionnaire was returned to the researchers.

Assessment of diet quality

Thirteen questions relating to the frequency of intake of specific foods were included in the Yorkshire Health Study questionnaire, the UK Diet Quality Questionnaire (UK-DQQ). Eleven food items (oily fish; wholemeal breads; salad and raw vegetables; bacon, ham, sausages, and burgers; sugary drinks; chips; biscuits; cakes and pastries; crisps and savoury snacks; white breads; coated or fried chicken; beer, lager, or cider) were included using a short 'Food Frequency Questionnaire' design. Fruit and vegetable (not raw) intake was assessed using an adapted version of an existing validated two question fruit and vegetable intake screener.[15] The foods included in the questionnaire were generated from empirical dietary patterns analyses undertaken in data from the National Diet and Nutrition Survey 2008–2012.[11] The questionnaire demonstrated that it was predictive of diet quality as measured by a composite measure with a validated Nutrient-based Diet Quality Score that was validated against blood and urine biomarkers of nutritional status and nutrient intake.[15] The design of the UK-DQQ was intended to minimise participant burden, maximise accessibility and acceptability, be easy to analyse and interpret and thus practical for application in population level surveillance by public health professionals as well as academic researchers.

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The scoring for the questions was developed to provide a simple method of analysing responses quantitatively, aiding interpretation and facilitating comparisons of patterns of diet quality across population subgroups. The scoring method reflects the associations that were observed in previous analyses between each food item and diet quality as defined by the Nutrient-based Diet Quality Score.[15] Foods that are positively associated with diet quality such as fruit and vegetables are given greater scores for greater levels of consumption. Foods that are negatively associated with diet quality such as crisps and sugary drinks are given lower scores for greater levels of consumption. For full details of the scoring method please see the Supplementary Material.

Statistical analyses

Diet quality scores were derived from participants who provided 50% or more valid responses to the 13 food-related items included in the derivation of the UK-DQQ. Data relating to participant gender, age, deprivation (measured by the Index of Multiple Deprivation (IMD) decile), weight category (categorised as *Underweight* (BMI $<18\ \text{kg/m}^2$); *Healthy weight* ($18\ \text{kg/m}^2 \leq$ BMI $<25\ \text{kg/m}^2$); *Overweight* ($25\ \text{kg/m}^2 \leq$ BMI $<30\ \text{kg/m}^2$), *Obese* ($30\ \text{kg/m}^2 \leq$ BMI $<40\ \text{kg/m}^2$), and *Severely obese* (BMI $\geq 40\ \text{kg/m}^2$), smoking status (categorised as *current daily smoker*, *current occasional smoker*, *former daily smoker*, *former occasional smoker*, *never smoked*) and employment status (categorised as *unemployed*, *employed in sedentary occupation*, *employed in occasionally physical occupation*, *employed in physical occupation*, *employed in vigorous physical occupation*) was extracted from the Yorkshire Health Study database for analysis and relation to diet quality.

All variables were checked for errors, and cleaning procedures utilised if necessary. The cleaned sample was summarised descriptively. Variables with large amounts of missing data were tested for nature of data missingness. The distribution of all scores was inspected visually, and summary diet quality score statistics were derived for the entire sample and for each category in the categorical factors of interest. The relationship between diet quality scores and each of the demographic factors of interest in turn was investigated initially in a series of unadjusted screening analyses. Factors that appeared to be substantively related to the outcome in the uncontrolled models were carried forward for inclusion in a subsequent main effects multiple regression model. Formulation of the modelling of categorical factors in the multiple model was informed by inspection of plots of diet quality scores by category for linearity of relationship, with specific categories combined as necessary. P-values, parameter estimates, and associated confidence intervals (CIs) were determined for each included variable.

The multiple model was cross-validated using the split-sample method. A randomly selected 80% sample of the data was the training sample. Predicted values from this model were correlated against diet quality scores on this sample and the corresponding 20% validation sample. Similarities in the evaluated correlations were taken as indicative of good model portability.

Results

Descriptive and exploratory analysis

Dietary data were elicited from 43,023 participants with valid diet quality scores obtained from 41,235 respondents (95.8% of the total sample). The mean age of responders was 47 years (range 16–106), 69.0% were female, 54.8% had never smoked, 30.7% were not currently working (i.e. unemployed, retired) and 42.4% of the population self-reported weight and height that placed them in the 'healthy weight' category. Postcode was reported by 73.9% of the cohort, allowing IMD decile to be derived.

Diet quality scores were normally distributed with a mean score of 64.8 (SD 2.72). Theoretical minimum and maximum scores were 20 and 100; with higher scores indicating a better quality dietary pattern. The sample as a whole had generally low variability. A small number of outliers were observed; the range of values obtained from the sample was from 22 to 98; near to the tool minima and maxima. Levels of missing data were generally low on all factors except IMD, due to over a quarter of respondents failing to provide valid postcodes, from which IMD deciles could be calculated. Separate variance *t*-tests conducted on the IMD data revealed no evidence that missing data was not missing at random. Hence complete case analysis was conducted on the data without recourse to data imputation. The descriptive characteristics of the sample are summarised in Table 1.

Diet quality scores in subgroups defined by the categorical variables are summarised in Table 2. Significance levels from one-way analysis of variance (ANOVA) tests on each variable, conducted as screening tests to assess the variables for inclusion in the subsequent multiple model, are also provided.

Hence higher scores were recorded in females; in those from higher IMD deciles; in former smokers and non-smokers; in those with no or limited physical component to their occupation; and in those who were categorised as 'healthy weight' or 'overweight'.

Subgroup diet quality scores are illustrated in Fig. 1.

While all associations tested in univariable screening analyses were significant at the 5% significance level, the substantive magnitude of effects varied. The gender effect was large, with females scoring 3.4 points more than males on the diet quality score scale. A near-monotonic increase in diet quality scores from the 1st decile (most deprived) to the 10th decile (least deprived) was observed, with substantive differences from 62.9 (decile 1) to 67.0 (decile 10). Little substantive difference was revealed in diet quality scores amongst the groups representing current non-smokers (including former occasional or daily smokers and those who have never smoked). Current daily smokers had the lowest dietary scores, while current occasional smokers were at an intermediate level between current daily smokers and non-smokers. The difference in diet quality scores between those of the lowest category (current daily smokers; 59.3) and the highest category (former occasional smokers; 65.9) was substantive. Diet quality scores of current daily smokers were about 2 standard deviations below the overall mean. A near-monotonic decrease in diet quality scores with increasing components of physical activity in employment was

Table 1. Descriptive summary of sample characteristics. Data are shown as %. Age is shown as mean (SD) and range

Variable	Frequency (valid %)
Gender (*n* = 42,477)	
Male	13,148 (31.0)
Female	29,329 (69.0)
IMD decile (*n* = 23,904)	
1 (most deprived)	3128 (13.1%)
2	1849 (7.7%)
3	2150 (9.0%)
4	2080 (8.7%)
5	2241 (9.4%)
6	2417 (10.1%)
7	2703 (11.3%)
8	2502 (10.5%)
9	2366 (9.9%)
10 (most affluent)	2468 (10.3%)
Employment category (*n* = 40,966)	
Unemployed	12,572 (30.7%)
Employed: sedentary occupation	12,509 (30.5%)
Employed: limited physical occupation	7967 (19.4%)
Employed: physical occupation	7279 (17.8%)
Employed: vigorous physical occupation	639 (1.6%)
Age (years) (*n* = 41,630)	47.1 (17.7; 16–106) Mean (SD; range)
Smoking status (*n* = 41,690)	
Daily smoker	4069 (9.8%)
Occasional smoker	1822 (4.4%)
Former daily smoker	7790 (18.7%)
Former occasional smoker	5146 (12.3%)
Never smoked	22,863 (54.8%)
Weight category (*n* = 38,229)	
Underweight (BMI <25 kg/m^2)	527 (1.38%)
Healthy weight (BMI 18–25 kg/m^2)	16,214 (42.4%)
Overweight (BMI 25–30 kg/m^2)	12,313 (32.2%)
Obese (BMI 30–40 kg/m^2)	8009 (21.0%)
Severely obese (BMI >40 kg/m^2)	1166 (3.1%)

observed; however, not all differences between categories were substantive or significant. A monotonic decrease in diet quality scores with increasing weight category was observed for individuals categorised as 'healthy weight', 'overweight', 'obese', and 'severely obese'. Underweight individuals had the lowest diet quality of all weight category groups.

Table 2. Diet Quality Questionnaire scores by gender, deprivation level, smoking status, employment category and weight category. Data are shown as mean score (SD) and P value

Variable	Diet quality score (SD)	P-value[a]
Gender (*n* = 40,778)		<0.001
Male (*n* = 12,495)	62.5 (9.19)	
Female (*n* = 28,283)	65.9 (9.10)	
IMD decile (*n* = 23,098)		<0.001
1 (*n* = 2938)	62.9 (9.80)	
2 (*n* = 1780)	63.8 (9.59)	
3 (*n* = 2085)	64.4 (9.39)	
4 (*n* = 1994)	64.8 (8.98)	
5 (*n* = 2151)	65.2 (8.91)	
6 (*n* = 2359)	65.6 (9.11)	
7 (*n* = 2635)	66.0 (9.00)	
8 (*n* = 2438)	66.0 (8.84)	
9 (*n* = 2305)	66.7 (8.85)	
10 (*n* = 2413)	67.0 (8.39)	
Smoking status (*n* = 40,593)		<0.001
Daily smoker (*n* = 3934)	59.3 (9.75)	
Occasional smoker (*n* = 1783)	62.4 (9.24)	
Former daily smoker (*n* = 7560)	65.2 (8.97)	
Former occasional smoker (*n* = 5022)	65.9 (8.86)	
Never smoked (*n* = 22,294)	65.7 (9.00)	
Employment category (*n* = 39,996)		<0.001
Unemployed (*n* = 11,956)	65.4 (9.37)	
Employed: sedentary occupation (*n* = 12,415)	65.4 (8.74)	
Employed: limited physical occupation (*n* = 7831)	64.8 (9.24)	
Employed: physical occupation (*n* = 7175)	63.8 (9.63)	
Employed: vigorous physical occupation (*n* = 619)	60.7 (10.1)	
Weight category (*n* = 36,888)		<0.001
Underweight (BMI <18 kg/m^2) (*n* = 498)	63.6 (10.6)	
Healthy weight (BMI 18 to 25 kg/m^2) (*n* = 15,961)	65.5 (9.32)	
Overweight (BMI 25 to 30 kg/m^2) (*n* = 11,865)	65.1 (8.92)	
Obese (BMI 30 to 40 kg/m^2) (*n* = 7710)	64.5 (9.13)	
Severely obese (BMI >40 kg/m^2) (*n* = 1124)	64.5 (9.74)	

[a]P-values based on uncontrolled comparisons of diet quality scores across groups defined by levels of controlling variables.

Multiple regression analysis

The screening analyses suggested that gender, age, socio-economic status (as measured by IMD decile), occupation group, smoking status, and weight category may be substantively related to diet quality scores. Inspection of plots derived from the smoking status, occupation status, and weight category suggested that the relationship between diet quality scores and the ordinal levels of these variables could not be assumed to be linear. *Ex-smoker* and *Non-smoker* smoking categories and the *Employed in sedentary occupation* and *Unemployed* occupation categories were hence combined for inclusion in the multiple model (becoming the reference category); in which the effect of levels of categorical variables was modelled using indicator variables. Weight category was modelled using a series of indicator variables compared to the reference category *'healthy weight'*. A main effects multiple regression analysis conducted on the data revealed that all included factors and covariates were statistically significant (at the 5% significance level) except for employment in occasional physical occupation (compared with the reference group of unemployed or sedentary occupation) (Table 3).

Controlling for other factors and covariates, the model revealed that females scored 3.94 more points on the UK-DQQ than males; each increasing IMD decile (i.e. decreasingly deprived) increased scores by 0.243 points; smokers score 4.24 points less than non-smokers; those whose employment involved physical activity scored 0.565 points less than those who were unemployed or in sedentary employment; those whose employment involved vigorous physical activity scored 1.28 points less than those who are unemployed or in sedentary employment; those categorised as 'underweight' score 1.63 points less than those categorised as 'healthy weight'; those categorised as 'overweight' scored 0.706 points less than those of 'healthy weight'; those categorised as 'obese' scored 1.44

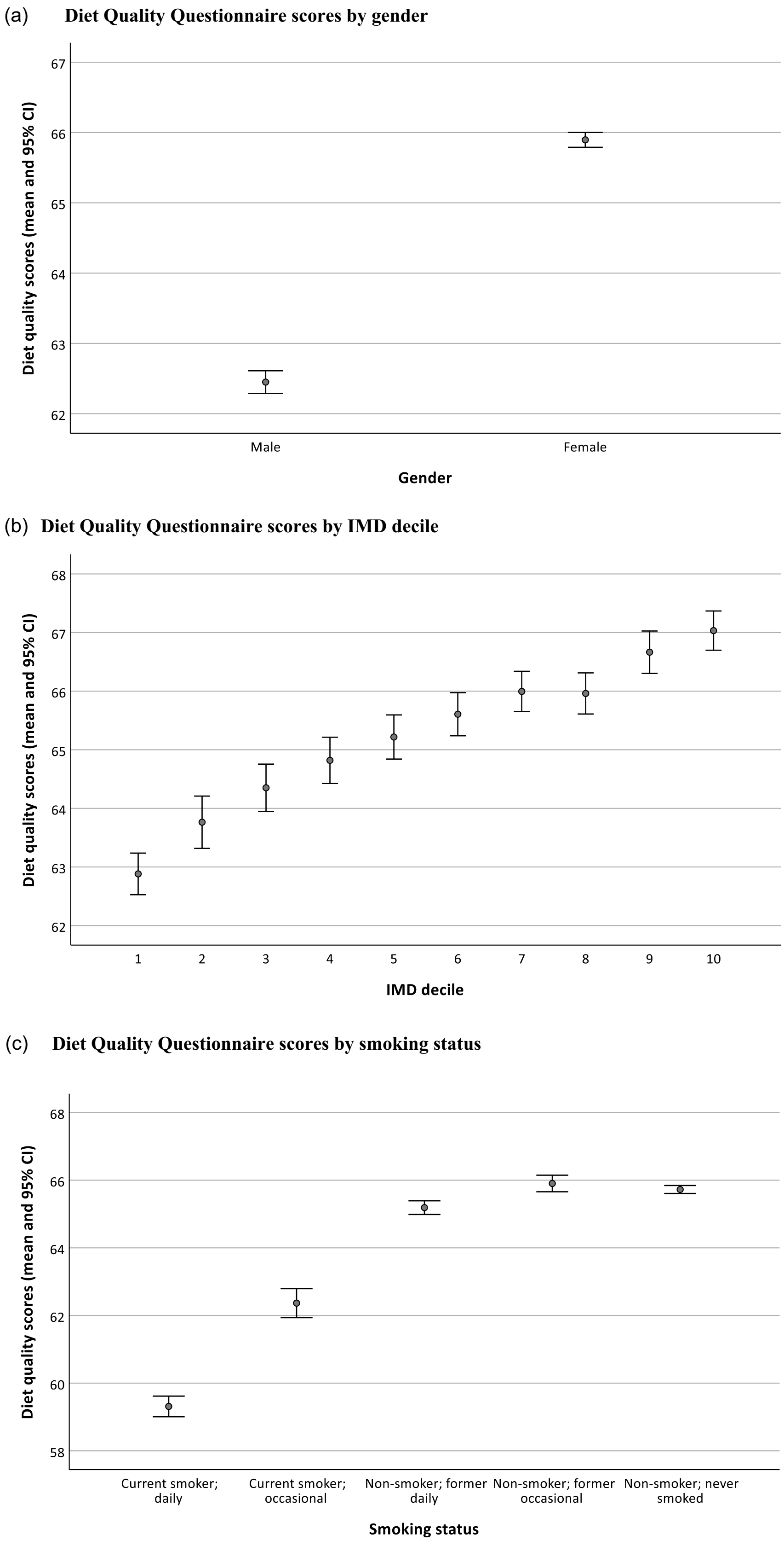

Fig. 1. a–e: Subgroup diet quality scores by gender, IMD decile, smoking status, employment status, and weight category. Data are shown as means and 95% confidence intervals. (a) Diet Quality Questionnaire scores by gender. (b) Diet Quality Questionnaire scores by IMD decile. (c) Diet Quality Questionnaire scores by smoking status. (d) Diet Quality Questionnaire scores by employment status. (e) Diet Quality Questionnaire scores by weight category.

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(d) **Diet Quality Questionnaire scores by employment status**

(e) **Diet Quality Questionnaire scores by weight category**

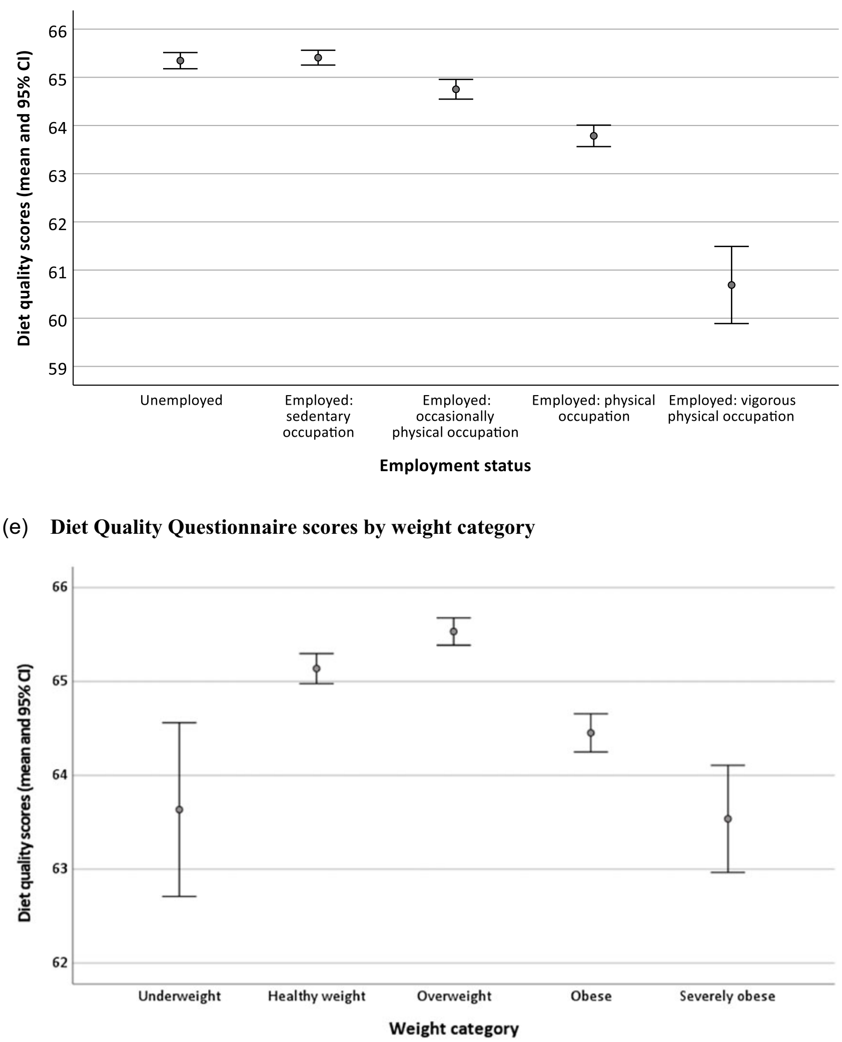

Fig. 1. (Continued).

points less than those of 'healthy weight'; those categorised as 'severely obese' scored 2.59 points less than those of 'healthy weight'; each year of age increased scores by 0.088 points. Differences in scores between subgroups may be interpreted in terms of the theoretical 80-point range of the tool (minimum of 20 to maximum of 100); hence, the largest individual effect noted (that of smoking) represents about a 5 percentage point difference between smokers and non-smokers.

Cross-validation procedures revealed that the model had good portability, with negligible reduction in correlation between predicted values and dietary scores when a model derived from the training sample ($r = 0.159$; $P < 0.001$) was applied to the validation sample ($r = 0.151$; $P < 0.001$).

Discussion

The purpose of this study was to explore the relationship between patterns of diet quality, socio-demographic measures, smoking, and weight status in a large cross-sectional cohort of adults living in Yorkshire & Humber, UK. The main findings were that higher diet quality was associated with being female and the poorest diet quality was associated with being a current smoker. Diet quality scores were higher with increasing socio-economic status and age. Employment in a physically active job, being male, being of obese weight or underweight were each associated with lower diet quality. This provides evidence for diet quality being unequally distributed in the population and indicates population characteristics by which these inequalities are patterned. This provides a focus for targeted resource and intervention to prevent diet-related ill health in these sub groups.

The current study focuses on the relationship between diet quality and population characteristics. Whilst a direct comparison of our results with previous surveys is not possible due to methodological differences, the findings are consistent with observations in the UK where diet quality has been inversely associated with measures of socio-economic status and positively associated with age.[11,16,17]

Table 3. Multiple regression analysis of Diet Quality Scores with gender, age, socio-economic status (as measured by IMD decile), occupation group, smoking status and weight category. Data are shows as parameter estimate, 95% CI and P value

Variable	Parameter estimate	95% CI for estimate	P-value
Gender = female[a]	3.94	(3.69, 4.20)	<0.001
Age (years)	0.088	(0.080, 0.095)	<0.001
IMD decile	0.243	(0.202, 0.284)	<0.001
Employment status = occasional physical occupation[c]	−0.012	(−0.337, 0.313)	0.943
Employment status = physical occupation[c]	−0.565	(−0.899, −0.232)	0.001
Employment status = vigorous physical occupation[c]	−1.28	(−2.29, −0.278)	0.012
Current smoker[b]	−4.24	(−4.60, −3.88)	<0.001
Weight category = underweight[d]	−1.63	(−2.69, −0.583)	0.002
Weight category = overweight[d]	−0.706	(−0.986, −0.426)	<0.001
Weight category = obese[d]	−1.44	(−1.76, −1.12)	<0.001
Weight category = severely obese[d]	−2.59	(−3.27, −1.90)	<0.001

[a]Reference = male.
[b]Reference = former smoker or non-smoker.
[c]Reference = unemployed or sedentary occupation.
[d]Reference = healthy weight.

Our results confirm findings from previous cross-sectional studies of an inverse association between diet quality and smoking status.[18] It is well established that smokers have poorer micronutrient status, an association that is independent of dietary intake and likely explained by increased micronutrient requirements from increased oxidative stress of smoking.[19] The observation that smokers have a poorer diet quality is likely to further exacerbate the risk of chronic disease associated with smoking.

In agreement with our results, studies in the UK have reported better diet quality in women than men.[11,20] We identified a clear linear relationship between socio-economic status (as measured by the Index of Multiple Deprivation) and diet quality (as measured by the UK Diet Quality Questionnaire). Studies in the UK and globally have reported similar findings with better diet quality in more affluent households.[21,22] A lower diet quality, including dietary patterns that were lower in fruit and vegetable consumption and lower in dietary diversity, has been associated with lower socio-economic status, lower income and food insecurity in a number of developed countries including the UK and the US.[23–26] The cost of a healthy diet may account in part for the association between diet quality and socio-economic status,[27–29] and the relationship between cost and diet quality is likely to be bidirectional.

Poorer diet quality was associated with obesity in this study. Finding which reflect those of other UK studies.[22] Diet is known to be a major determinant of morbidity and mortality. Poor diet quality is associated with negative health outcomes across the lifecourse and greater risk of a number of chronic diseases including obesity, a range of cancers, type-2 diabetes, cardiovascular disease, frailty, and mortality risk,[30–35] and more recently, risk and severity of COVID-19 infection.[36]

This study has several strengths. It was undertaken in a large, representative sample.[37] Key findings in this study such as the associations between deprivation and diet quality and smoking and diet quality are consistent with expected observations and provide further evidence on health inequalities in the UK, an important and relevant topic for current public health policy makers. While a small amount of missing data was recorded on most variables in this study, there was no evidence that missing data were not missing at random. The model showed good cross-validation properties and would be expected to be applicable to further datasets with negligible reduction in correlation between predicted values and dietary scores.

The food items included in the UK-DQQ were developed from empirical and theoretical dietary pattern analyses conducted in the National Diet and Nutrition Survey, a nationally representative dataset of nutrient-level dietary data from UK adults.[7,11] Dietary patterns take account of the synergistic relationship between nutrients and foods, and there is evidence that dietary patterns have stronger correlations with health outcomes than analysis of single nutrients or foods.[12,38] The food items included in the UK-DQQ were associated with patterns of diet quality and were predictive of a validated Nutrient-based Diet Quality Score (see Appendix A).[13,15,39] This is the first survey in which this dietary assessment tool has been applied and the findings suggest that it is a suitable tool for UK population-based studies and that it can detect differences in diet quality. The advantage of this dietary assessment tool over others is its brevity, with just 13 questions used to capture diet quality. In addition, its scoring methodology is simple to use and analyse and aids interpretation of findings by non-nutrition experts. This makes it practical for incorporation into population level surveys. The number of questions keeps the time for completion and respondent burden to a minimum and reduces the likelihood of survey fatigue. The tool provides an overall picture of the quality of the diet, rather than detailed nutrient intake. As the tool is independent of an individual's energy intake, energy adjustments are not required, which reduces the chances of measurement error.[40]

The study has some limitations. A substantive proportion of variance in diet quality scores was unexplained by the analysed factors (adjusted $R^2 = 0.111$ for the multiple model); suggesting that diet may also be related to additional factors not included in the current analysis. This is consistent with the fact that diet is a complex, multifaceted behaviour. Our analysis examined factors that determine diet quality separately and assumed the

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impact on diet quality was additive as no significant interactions were found. However, there is good evidence that certain health behaviours such as smoking and dietary intake cluster[41] and whilst some characteristics may be additive it is possible that a combination of characteristics such as socio-economic status, sex, and obesity act synergistically.[42] This could have significant implications for identification of at risk populations, policy development and intervention design.

The cohort in this study represents the population living in the Yorkshire and Humber region of the UK. The cohort was not a random sample and was largely self-selected. Nonetheless, taking into account the proportion of females in the survey, the prevalence of overweight and obesity is in line with what has been reported elsewhere, with the Health Survey for England reporting 63% of adults in the Yorkshire and Humber region are overweight or obese, compared with 56.3% in this survey.[6] It is well recognised that people who participate in such surveys are likely to be better educated and be motivated to participate. Despite this probable self-selection there was good representation across all of the IMD deciles. We anticipate that self-selection would dampen associations; nonetheless, the significant variability in UK-DQQ across the data was apparent.

Further research in this cohort could consider the association between diet quality with use of health services and incidence of disease and long-term health conditions. The YHS is a longitudinal survey and participants have given permission for medical records to be examined. This could facilitate the prospective exploration between the relationship between diet quality and disease. Other factors known to influence diet quality include income, level of education, and household composition, and these factors could be considered in future analysis. It would also be useful to apply the UK-DQQ across different UK regions and in different ethnic groups.

Conclusions

Analysis of diet quality in a large epidemiological dataset has revealed inequalities in patterns of diet quality. Females, non-smokers, those living in areas of lower deprivation yield the highest scores, indicative of a dietary pattern that is better quality. These findings highlight that men, smokers, younger people, and those in lower socio-economic groups are most at risk from diet related ill health. These findings reinforce the evidence for there being multiple layers of inequality in diet quality at population level that contribute to population level health inequalities. These findings are important for informing public health nutrition policy at national and local level and providing evidence for where public health resource and intervention should be directed in order to reduce widening health inequalities. The findings indicate that brief tools can usefully be used in large-scale self-reported surveys in the UK population to assess broad patterns of inequalities in diet quality.

The study findings support further research to understand the association between diet quality in the UK and a wider range of socio-demographic and socio-economic variables such as ethnic group, household composition, and food insecurity status; health-related variables such as physical activity levels and alcohol intake; place-based variables such as access to green space, food access, and access to public transport and other health outcomes such as long-term health conditions and disability, mental health, self-reported health, and self-reported quality of life. It also provides support for further research into the causal determinants of these inequalities in diet quality in different population sub-groups in order to inform intervention development and policy.

Abbreviations

BMI: Body Mass Index; **IMD:** Index of Multiple Deprivation; **UK-DQQ:** UK Diet Quality Questionnaire; **YHS:** Yorkshire Health Study.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.60

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Authorship

KR: data extraction, analysis and interpretation, manuscript preparation and editing; MH, CR, JC study conception and data interpretation, JS statistical analysis and data interpretation. EW data interpretation. All authors contributed to the manuscript preparation and approved the final version.

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Conflict of interest

The authors declare no conflicts of interest.

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RESEARCH ARTICLE

Inoculum microbial mass is negatively related to microbial yield and positively to methane yield *in vitro*

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Abstract

Ruminal microbes catabolise feed carbohydrates mainly into SCFA, methane (CH_4), and carbon dioxide (CO_2), with predictable relationships between fermentation end products and net microbial increase. We used a closed *in vitro* batch culture system, incubating grass and maize silages, and measured total gas production at 8 and 24 h, as well as the truly degraded substrate, the net production of SCFA, CH_4, and microbial biomass at 24 h, and investigated the impact of silage type and inoculum microbial mass on fermentation direction. Net microbial yield was negatively correlated with total gas at 8 h ($P < 0•001$), but not at 24 h ($P = 0•052$), and negatively correlated with CH_4 production ($P < 0•001$). Higher initial inoculum microbial mass was related to a lower net microbial yield ($P < 0•001$) but a higher CH_4 production ($P < 0•001$). A significant difference between grass silage and maize silage was detected within the context of these relationships ($P < 0•050$). The metabolic hydrogen (2H) recovery was 102.8 ± 12.3 % for grass silages and 118.8 ± 13.3% for maize silages. Overall, grass silages favoured more substrate conversion to microbial biomass and less to fermentation end products than maize silage. Lower inoculum microbial mass facilitated more microbial growth and, because of the 2H sink by microbial synthesis, decreased CH_4 production.

Key words: Degraded substrate partitioning: Initial microbial mass: Metabolic hydrogen: Methane: Stoichiometric relationship

Introduction

The ruminal microbiome breaks down plant carbohydrates primarily into SCFA, methane (CH_4), and carbon dioxide (CO_2). During the breakdown of the substrates, electrons are released and bound in different metabolic pathways, transferred by redox cofactors such as NAD^+/NADH and $NADP^+$/NADPH. These cofactors transfer two electrons and hydrogen atoms; therefore, it is suggested that metabolic hydrogen (2H) could be used as a unifying principle to study rumen fermentation as a whole.[1,2] Based on previous research, it is indicated that the production of one mole of acetate and one mole of butyrate produces two moles of 2H each, while the production of one mole of propionate consumes one mole of 2H; further, the production of one mole CH_4 consumes four moles of 2H.[3–5] Nevertheless, other factors could potentially influence the electron flow, such as unsaturated fatty acids and nitrate, dihydrogen gas, or valerate.[5] However, under natural feeding situations, these factors are of minute quantity. Because the produced 2H needs to be consumed within the ruminal fermentation system, a tight relationship among fermentation end products exists.[6,7]

However, another metabolic pathway might have a significant impact on ruminal fermentation balance and electron flow and was relatively rarely mentioned in existing studies — the synthesis of microbial biomass; this process will consume 2H (e.g. to produce fatty acids for microbial cell membranes) and, therefore, reduces CH_4 emission.[1,8] Blümmel *et al.*[9] reported a negative relationship between the production of microbial mass

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and gas production per unit of degraded substrate based on the Hohenheim gas test (HGT). The same study also defined the partitioning factor (PF) as the ratio of truly degraded organic matter (OM) to total gas production; a higher PF indicates more degraded substrate is partitioned to microbial synthesis and less to fermentation end products. The negative relationship between microbial mass and gas production might stem from CH_4 due to competition for 2H. However, quantifying the potential of 2H consumption by microbial synthesis is challenging; different magnitudes were estimated by different authors, which were mainly based on assumptions of microbial composition and stoichiometric calculations.[10–12]

The relationship between SCFA and CH_4, as well as the potential 2H consumption by microbial synthesis, is challenging to verify *in vivo* due to difficulties in measuring accumulated amounts during fermentation. Therefore, we used the closed *in vitro* system HGT modified by Blümmel *et al.*[9] for the incubation of grass and maize silages to measure the net production of SCFA, CH_4, and microbial mass. Across six different experimental runs, we observed variations in the initial inoculum microbial mass; this was likely caused by unintended variation in the time between the last feeding or drinking event and the inoculum sampling or by inadvertent variation in the exact sampling site in the rumen. These variations emerged as influential factors in *in vitro* fermentation.

We assumed that 100% of 2H released during acetate and butyrate production is consumed by propionate and CH_4 production (the impact of isobutyrate, valerate, and other metabolites on redox balance was omitted because of the assumed minute quantity), and therefore, the amount of CH_4 could be calculated through the amount of acetate, propionate, and butyrate as $CH_4 = 0.5 \times \text{Acetate} - 0.25 \times \text{Propionate} + 0.5 \times \text{Butyrate}$ (simply derived based on the previously mentioned relationship; unit: mol[3,7]). The calculated CH_4 production will be compared to the actually measured amounts. A negative relationship between microbial and CH_4 yield was expected, and PF was expected to be a useful indicator of the metabolic state of the *in vitro* microbiome, that is, whether the microbiome was more in a growth or a maintenance stage. By doing these, we aimed to acquire novel insights into ruminal fermentation balance and electron flow and the effects of the initial microbial mass and feed types as influencing factors.

Materials and methods

Hohenheim gas test (HGT)

Fifteen grass silage samples and eight maize silage samples were collected from ten commercial dairy farms in the northwest of Germany, dried to constant weight at 60°C (Memmert ULE 500, Memmert GmbH + Co.KG, Schwabach, Germany), and incubated in HGT syringes for 24 h (each sample in duplicate). The dry matter (DM) was determined by drying subsamples, at 103°C to constant weight (Memmert ULE 500, Memmert GmbH + Co.KG, Schwabach, Germany). Rumen fluid was collected from two rumen-fistulated Holstein-Friesian cows before the morning feeding (cows were fed 9 kg mixed grass hay, 600 g compound feed, and 75 g mineral supplement per animal and day), filtered through two layers of cheesecloth, placed in a prewarmed CO_2-filled container, transferred to the laboratory, and then mixed with the medium described by Menke *et al.*[13] The donor animal care and experimental procedures were conducted according to the German Guidelines and Regulations on Animal Care (Deutsches Tierschutzgesetz) (LAVES-Aktenzeichen 33.9-42502-05-18A269). The dried silage samples (about 200 mg DM) were ground through 1 mm sieve (cutting mill SM300; Retsch Ltd., Haan, Germany) and put into glass syringes and mixed with 30 ml inoculum. Three blank syringes without substrates were also injected with 30 ml of inoculum to calibrate the gas production. The gas volume produced was recorded at 8 and 24 h after initiation. After 24 h, the incubation was terminated by placing the syringes on ice.

Measurements

The nutrient composition of silage samples was analysed according to the standard methods of the Association of German Agricultural Analysis and Research Institutes[14] for DM, OM, crude ash, crude protein (CP; N × 6.25), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) (Table 1). The neutral detergent fibre was analysed after adding amylase, and NDF, ADF, and ADL were corrected for residual ash.

The gas sample after 24 h was collected from one of the duplicate HGT syringes; 15 ml of gas sample were collected and injected into the 5.9 ml vacuum tube (Exetainer® 5.9 ml Evacuated Flat Bottom Vial; Labco Limited, Lampeter, United Kingdom) for gas composition (H_2, O_2, N_2, CH_4, CO_2) measurement by GC (TRACE™ 1300 Series GC, Thermo Scientific, Waltham, MA, USA) equipped with a thermal conductivity detector. The used column was a 30 m × 0.53 mm in size TracePLOT TG-BOND Msieve 5A (Thermo Scientific, Waltham, MA, USA), and argon was used as a carrier gas.

The *in vitro* apparently and truly degraded substrate was measured; the procedure of Blümmel *et al.*[9] was modified, collecting the entire content after incubation from one of the duplicate HGT syringes, gently injecting it into a 50 ml plastic centrifuge tube (Carl Roth GmbH + Co. KG, Karlsruhe, Germany). The tube was centrifuged at 20000 g for 30 min at 4°C, and the supernatant fraction was sampled for SCFA measurement. The remaining supernatant was carefully discarded to minimise any loss of the solid fraction. The former HGT syringe was then rinsed with totally 40 ml NaCl (4 g/l) two to four times, ensuring no particles attached to the glass surface. All the rinse liquid was introduced to the centrifuge tube. The tube was again centrifuged at 20000 g for 30 min at 4°C, and the supernatant fraction was carefully discarded. Subsequently, the tube was dried at 60°C for 2 d, followed by drying at 103°C overnight. Organic matter was determined afterwards via muffle furnace combustion at 550°C for 4 h. The apparently degraded OM was calculated as the difference between the initial OM before incubation and the collected OM after incubation.

The truly degraded OM was calculated as the difference between the initial OM before incubation and the collected and

Table 1. The feed nutrient composition, metabolisable energy, true and apparent degradability (fifteen grass silages and eight maize silages samples)

		OM	CP	EE	NDF_{om}	ADF_{om}	ADL_{om}	ME	AOMD	TOMD
		g/kg DM						MJ/kg DM	%	
Grass silage	Mean	891.7	173.8	42.5	520.1	319.1	33.0	9.9	42.7	80.8
($n = 15$)	SD	27.2	29.70	6.04	48.04	35.67	13.58	0.84	6.97	6.74
Maize silage	Mean	966.7	73.6	28.8	408.2	227.2	25.0	10.8	43.2	75.4
($n = 8$)	SD	3.2	7.17	3.89	23.09	10.55	3.70	0.33	3.65	2.34

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF_{om}, neutral detergent fibre corrected for residual ash; ADF_{om}, acid detergent fibre corrected for residual ash; ADL_{om}, acid detergent lignin corrected for residual ash; ME, metabolisable energy; AOMD, apparent organic matter degradability; TOMD, true organic matter degradability; SD, standard deviation.

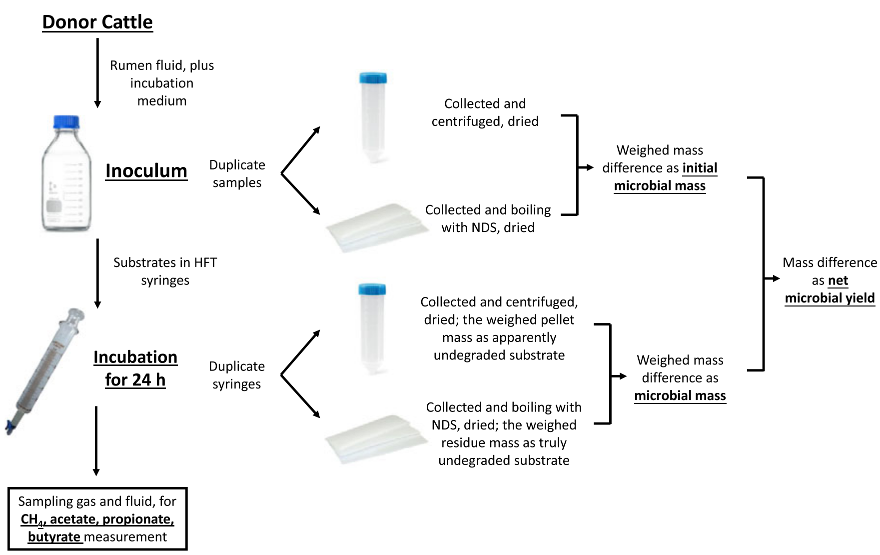

Fig. 1. Determination of microbial mass and sampling of gas and fluid. Microbial mass is expected to be removed through boiling with a neutral detergent solution (NDS).

boiled OM after incubation. Therefore, the entire content from the other duplicate HGT syringe was carefully transferred into a Gerhardt fibre bag (FibreBags ADF, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). The HGT syringe was rinsed with a total of 80 ml NaCl (4 g/l) three times, to ensure no particles attached to the glass surface. All the rinsing liquid was introduced into the Gerhardt fibre bag. The bags were boiled with a neutral detergent solution (NDS) for 1 h to remove microbial mass and then dried at 103°C overnight. Organic matter content was determined via muffle furnace combustion at 550°C for 4 h.

The microbial mass in OM was considered as the difference between the apparently and truly degraded OM (Fig. 1). For each of the six runs, the initial microbial mass before incubation was measured in the same way in the inoculum. Net microbial yield was calculated as the microbial mass at the end of fermentation minus the initial microbial mass (Fig. 1).

For analysis of SCFA, 800 µl of the supernatant was injected into an Eppendorf tube, filled with 150 µl 25% metaphosphoric acid and 50 µl ISTD (4% 2-methylvaleric acid in formic acid). The mixture was again centrifuged at 16600 g for 20 min at 10°C and analysed via GC (GC-17A, SHIMADZU, Kyoto, Japan) equipped with a flame ionisation detector. The Stabilwax w/Integra-Guard column (Restek Corporation, Bellefonte, United States) was 30 m × 0.25 mm in size, and nitrogen gas was used as a carrier gas.

Calculation and Statistics

The PF after 24 h was calculated as suggested by Blümmel *et al.*[9] as:

$$\text{Partitioning factor } (mg/ml) = \textit{truly degraded organic matter } (mg) \div \textit{total gas production}(ml);$$

it serves as a measure of the variation between fermentation end products (gas and SCFA) and microbial mass per unit degraded substrate. A higher PF indicates more degraded substrate was directed towards microbial synthesis.

We did not measure the initial SCFA concentration in the inoculum; therefore, we estimated it by establishing a linear regression between true OM degradability as an independent variable and SCFA concentration as a dependent variable for grass silage samples (in which the fitting of this relationship was higher than in the maize silage samples); the intercept was assumed to be the initial SCFA concentration in the inoculum (Appendix). The reliability of this assumption is grounded in the reported linear relationship between SCFA and digestibility.[15,16] The net SCFA concentration was calculated as the difference between the measured SCFA concentration after incubation and the estimated initial SCFA concentration in the inoculum.

Table 2. The fermentation parameters

		Grass silage ($n = 15$)	Maize silage ($n = 8$)	P value
In 8 h				
Gas volume	ml/200mg DM	13.1 ± 4.14	26.5 ± 3.05	<0.001
In 24 h				
Gas volume	ml/200mg DM	41.0 ± 5.63	50.1 ± 1.99	<0.001
CH_4:CO_2 ratio	ml/ml	0.22 ± 0.03	0.22 ± 0.02	0.471
Microbial yield	mg/200mg DM	35.6 ± 5.81	31.0 ± 4.77	<0.001
Partitioning factor	mg/ml	3.6 ± 0.33	2.9 ± 0.10	<0.001
Acetate production	mmol/200mg DM	0.67 ± 0.11	0.67 ± 0.09	0.867
Propionate production		0.41 ± 0.07	0.44 ± 0.05	0.012
Butyrate production		0.13 ± 0.04	0.16 ± 0.04	<0.001
CH_4 production		0.31 ± 0.06	0.38 ± 0.04	<0.001
2H recovery[a]	%	102.8 ± 12.28	118.8 ± 13.29	<0.001

Note: Values are arithmetic means ± standard deviation and presented as net production during 24 h of fermentation.
[a]2H recovery was calculated as (4 × CH_4 + Propionate)/(2 × Acetate + Butyrate) × 100%.

The theoretical values of CH_4 yield were calculated as[3,7]:

$$Theoretical\ yield_{CH_4}[mmol\ per\ 200\ DM] = yield_{acetate}[mmol\ per\ 200\ DM] \times 0.5 - yield_{propionate}[mmol\ per\ 200\ DM] \times 0.25 + yield_{butyrate}[mmol\ per\ 200\ DM] \times 0.5.$$

The 2H recovery was calculated as[1,2]:

$$2H\ recovery\ rate\ [\%] = (4 \times yield_{CH_4}[mmol\ per\ 200\ DM] + yield_{propionate}[mmol\ per\ 200\ DM]) \div (2 \times yield_{acetate}[mmol\ per\ 200\ DM] + 2 \times yield_{butyrate}[mmol\ per\ 200\ DM]) \times 100\%.$$

The truly degraded OM and the net microbial, SCFA, and gas yields were standardised on the base of 200 mg DM substrate.

The average values between grass and maize silages were compared with a one-way ANOVA. The relationships among variables were analysed using R (version 3.5.2) with a linear mixed model. Net microbial yield and silage type were used as fixed effects, and individual silage sample was used as a random effect with dependent variables of gas production in 8 h, gas production in 24 h, ratio between gas production in 8 and 24 h, CH_4 production in 24 h, CO_2 production in 24 h, and ratio between CH_4 and CO_2 production in 24 h as:

$$Y = \mu + silage\ type + net\ microbial\ yield + (1|individual\ silage\ sample) + e\ .$$

Initial microbial mass and silage type were used as fixed effects, and individual silage sample was used as a random effect with dependent variables of net microbial yield, CH_4 production in 24 h, ratio between CH_4 and CO_2 production in 24 h, gas production in 8 h, and gas production in 24 h as:

$$Y = \mu + silage\ type + initial\ microbial\ mass + (1|individual\ silage\ sample) + e.$$

The μ represents the overall mean, and e represents the residual error. The model's explanatory power was estimated using marginal R^2 (R^2_m) and conditional R^2 (R^2_c), where R^2_m represents the variance explained by the fixed effects alone and R^2_c represents the total variance explained by the model for both fixed and random effects. The number of independent observations was 144 (24 silage samples × 6 replicates); the significance level was set as 0•050.

Results

Maize silages produced more gas than grass silages in 8 h and 24 h, but the difference was greater after 8 than after 24 h ($P < 0•001$; Table 2). However, the proportions of CH_4 and CO_2 after 24 h were similar between the two types of silage ($P = 0•471$). The true OM degradability of maize silages after 24 h was lower than of grass silages ($P < 0•001$; Table 1), and fermentation of maize silages produced more propionate, butyrate and CH_4, and less microbial mass ($P < 0•050$; Table 2). The initial microbial mass ranged from 21.7 to 44.5 mg per 30 ml inoculum in six runs. The estimated initial SCFA concentration in the inoculum ranged from 19.8 to 28.3 μmol/ml for acetate, from 0.13 to 5.20 μmol/ml for propionate, and from 0 to 3.52 μmol/ml for butyrate in six runs.

The 2H recovery calculated through SCFA and CH_4 yields were 102.8% and 118.8% for grass and maize silages, respectively (Table 2). As shown in Fig. 2, the linear regression equation between measured (Y) and calculated (X) CH_4 is $Y = 0.09 + 0.71 \times X$ ($R^2 = 0.53$; 95% CI 0•05, 0•14 for the intercept, and 0•56, 0•86 for the slope) for grass silages and $Y = 0.25 + 0.42 \times X$ ($R^2 = 0.46$; 95% CI 0•21, 0•29 for the intercept and 0•28, 0•56 for the slope) for maize silages.

In all relationships examined via linear mixed models, a significant effect was identified for silage type as a categorical variable ($P<0•050$), which indicates a notable difference between grass and maize silage within the context of these relationships (with the exception when the CH_4:CO_2 ratio acted

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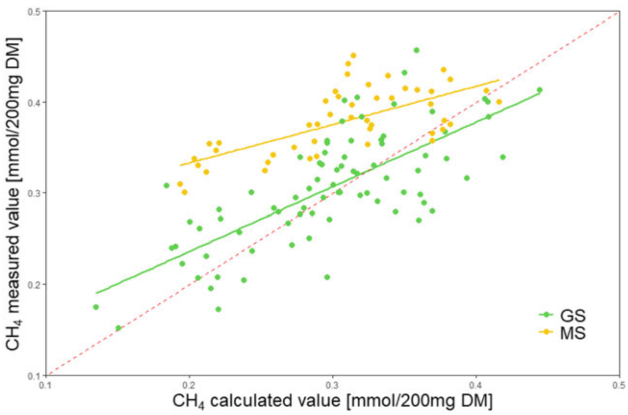

Fig. 2. Comparison of measured and theoretical values of CH_4 production. The dashed line represents when measured and theoretical values are equal. GS, grass silage; MS: maize silage. The linear regression equation between measured (Y) and calculated (X) CH_4 is $Y = 0.09 + 0.71 \times X$ ($R^2 = 0.53$) for grass silage and $Y = 0.25 + 0.42 \times X$ ($R^2 = 0.46$) for maize silage.

as the dependent variable). A strong negative relationship was detected for gas production after 8 h and the net microbial yield ($P < 0{\bullet}001$; $R^2_m = 0.75$, $R^2_c = 0.91$), but not for gas production after 24 h ($P = 0{\bullet}052$; $R^2_m = 0.44$, $R^2_c = 0.97$); a negative relationship was also detected between the net microbial yield and the ratio between gas production after 8 h and 24 h ($P < 0{\bullet}001$; $R^2_m = 0.75$, $R^2_c = 0.88$) (Fig. 3). In other words, when more microbial mass was produced, gas production was particularly low in the initial phase of fermentation. For the gas produced up to 24 h, a negative relationship with net microbial yield was detected for CH_4 ($P < 0{\bullet}001$; $R^2_m = 0.37$, $R^2_c = 0.82$), but not for CO_2 ($P = 0{\bullet}192$; $R^2_m = 0.45$, $R^2_c = 0.91$); correspondingly, the ratio between CH_4 and CO_2 production correlated negatively with net microbial yield ($P < 0{\bullet}001$; $R^2_m = 0.30$, $R^2_c = 0.56$) (Fig. 4).

Initial inoculum microbial mass was negatively related to the net microbial yield ($P < 0{\bullet}001$; $R^2_m = 0.46$, $R^2_c = 0.61$) (Fig. 5). In other words, net microbial yield was greater if the initial microbial mass was low. Consequently, a higher initial microbial mass lead to a higher CH_4 yield ($P < 0{\bullet}001$; $R^2_m = 0.45$, $R^2_c = 0.91$) and a higher $CH_4{:}CO_2$ ratio ($P < 0{\bullet}001$; $R^2_m = 0.46$, $R^2_c = 0.76$) (Fig. 5). The initial inoculum microbial mass correlated positively with gas production after 8 h ($P < 0{\bullet}001$; $R^2_m = 0.79$, $R^2_c = 0.95$) and after 24 h ($P < 0{\bullet}001$; $R^2_m = 0.45$, $R^2_c = 0.97$) (Fig. 6).

Discussion

This study modified the incubation experiment of Blümmel *et al.*[9] measuring degraded substrate, SCFA (acetate, propionate, butyrate), CH_4, and microbial mass. While Blümmel *et al.*[9] only incubated straws, our study also included maize silage; according to our knowledge, the starch content in maize silage is typically fully degraded after 24 h.[16,17] It must be noted that microbial mass measurements were only taken at 24 h, not at 8 h; therefore, the measurements used in this study should not lead to an overestimation of microbial mass for maize silages due to the starch content. As a batch culture system, HGT offers advantages for studying fermentation balance and electron flow,

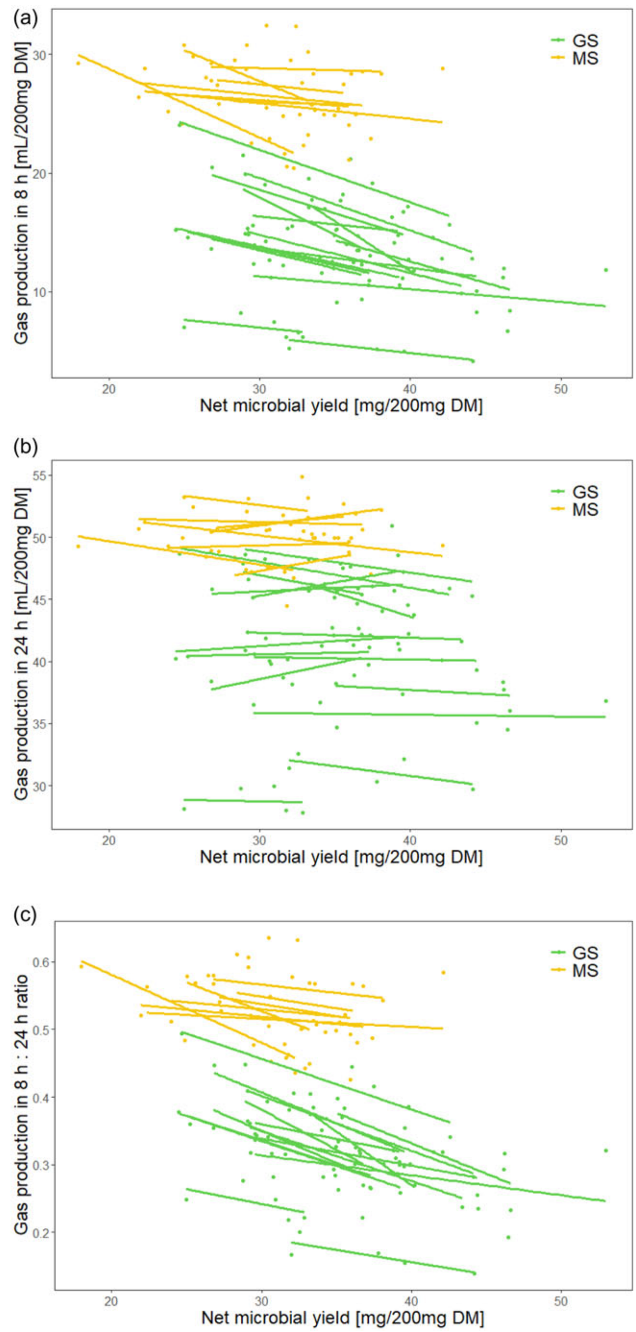

Fig. 3. Comparison between net microbial yield and gas production in 8 h and 24 h and the ratio between gas production in 8 h and 24 h. The linear regression fitted line for each individual silage sample across six runs is given. GS, grass silage; MS, maize silage. The overall R^2 for the linear mixed model with silage type and net microbial yield as fixed effects and silage individual as a random effect was (a) gas production in 8 h: $R^2_m = 0.75$, $R^2_c = 0.91$; (b) gas production in 24 h: $R^2_m = 0.44$, $R^2_c = 0.97$; and (c) ratio between gas production in 8 h and 24 h: $R^2_m = 0.75$, $R^2_c = 0.88$.

facilitating complete and accurate collection of fermentation end products to investigate fermentation direction and electron flow under controlled experimental conditions. In doing so, it is important to base calculations concerning microbial mass on OM instead of DM, to standardise values by the amount of incubated substrate, and to focus on net production instead of static concentration values that do not differentiate between the

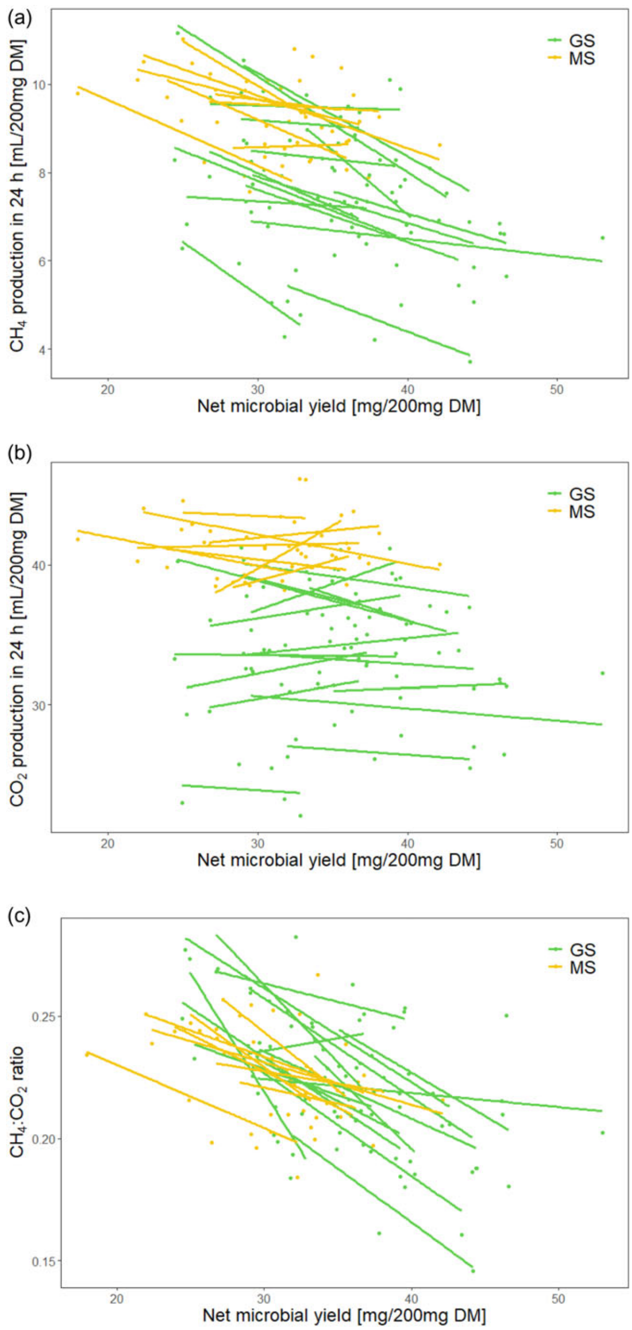

Fig. 4. Comparison between net microbial yield and CH_4 production, CO_2 production, and the ratio between CH_4 and CO_2 production in 24 h. The linear regression fitted line for each individual silage sample across six runs is given. GS, grass silage; MS: maize silage. The overall R^2 for the linear mixed model with silage type and net microbial yield as fixed effects and silage individual as a random effect was (a) CH_4 production in 24 h: $R^2_m = 0.37$, $R^2_c = 0.82$; (b) CO_2 production in 24 h: $R^2_m = 0.45$, $R^2_c = 0.91$; and (c) ratio between CH_4 production and CO_2 production: $R^2_m = 0.30$, $R^2_c = 0.56$.

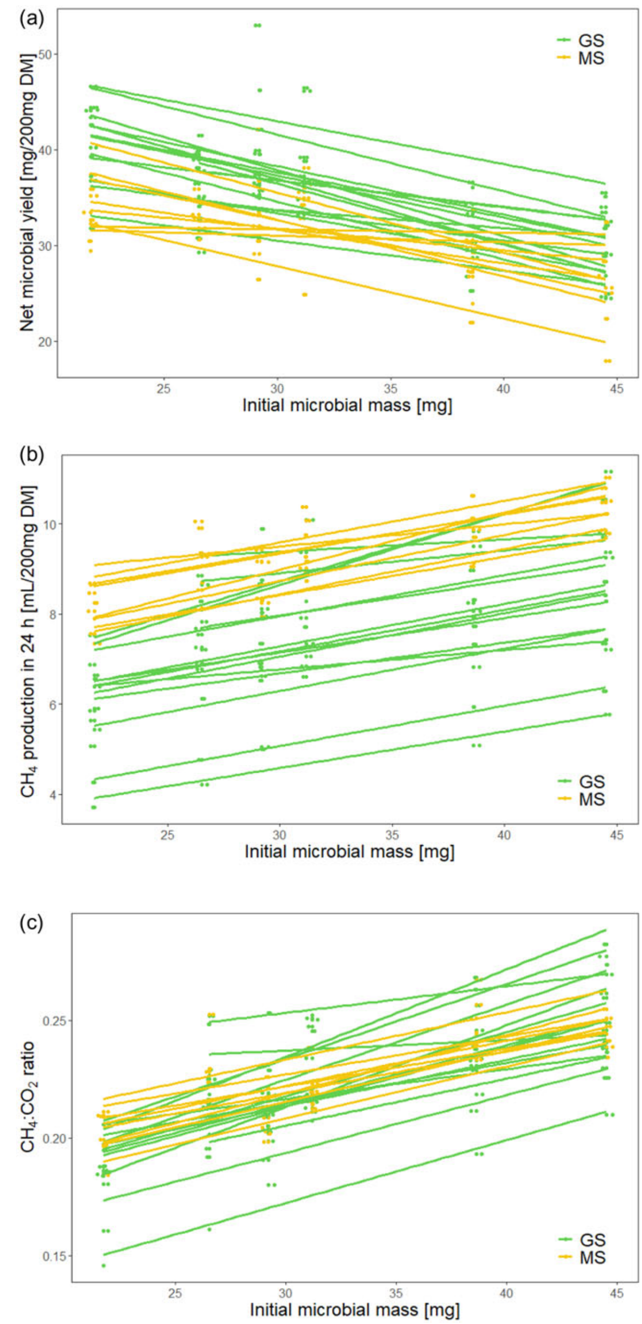

Fig. 5. Comparison between initial microbial mass and net microbial yield, CH_4 production, and the ratio between CH_4 and CO_2 production in 24 h. The linear regression fitted line for each individual silage sample across six runs is given. GS, grass silage; MS: maize silage. The overall R^2 for the linear mixed model with silage type and initial microbial mass as fixed effects and silage individual as a random effect was (a) net microbial yield: $R^2_m = 0.46$, $R^2_c = 0.61$; (b) CH_4 production in 24 h: $R^2_m = 0.45$, $R^2_c = 0.91$; and (c) ratio between CH_4 production and CO_2 production: $R^2_m = 0.46$, $R^2_c = 0.76$.

baseline introduced by the inoculum and the change during fermentation.

The higher CH_4 production by maize silages is suggested to be led by higher total gas production (because the ratio between CH_4 and CO_2 was similar). Thus, for grass silages, less degraded substrate was directed to gas production but more to microbial synthesis, which is also indicated by a higher partitioning factor (Fig. 7). Higher microbial synthesis by grass than maize silage was also reported by several other studies[18–20]; for example, the *in vitro* trial by Boguhn *et al.*[20] incubating grass and maize silage reported similar results, with higher protein and fibre content for grass silage leading to higher OM degradability and microbial yield compared to maize silage, although they reported no difference in gas production. The higher crude protein content in grass silages might promote the proliferation of microbial biomass. Therefore, a higher microbial yield might

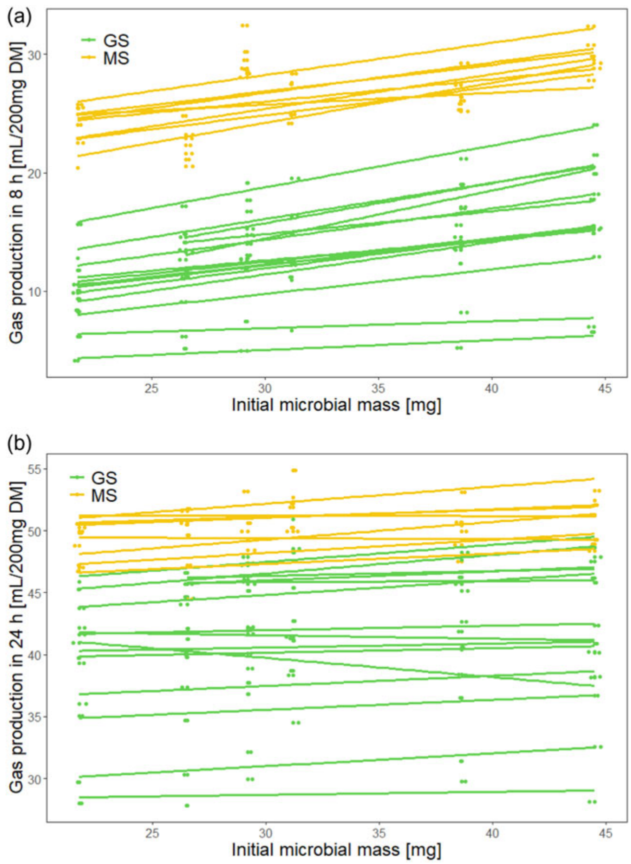

Fig. 6. Comparison between initial microbial mass and gas production in 8 h and 24 h. The linear regression fitted line for each individual silage sample across six runs is given. GS, grass silage; MS, maize silage. The overall R^2 for the linear mixed model with silage type and initial microbial mass as fixed effects and silage individual as a random effect was (a) gas production in 8 h: $R^2_m = 0.79$, $R^2_c = 0.95$ and (b) gas production in 24 h: $R^2_m = 0.45$, $R^2_c = 0.97$.

also be related to a higher OM degradability for grass silage. Another cause might be differences in the polysaccharide structure between cellulose and starch. The transformation from polysaccharide to monosaccharide is mainly accomplished by hydrolysis, which is a non-redox reaction; however, the complex structure of plant cell wall might postpone the principal metabolic pathways and also select the microbial population. Although not measured in our experiment, differences in the microbial population after the fermentation of grass and maize silages are expected,[21,22] which is assumed to be responsible for the different fermentation patterns.

The stoichiometric relationship between SCFA and CH_4 has been suggested by several studies,[1,3,6] though few studies have verified this experimentally. Robinson *et al.*[23] concluded that *in vivo* SCFA concentrations could hardly serve as an indicator to predict CH_4 production, probably because of the difficulty in measuring the net production of fermentation end products, of which the SCFA is absorbed through the rumen wall. By contrast, in a batch culture system, the produced fermentation end products accumulate and can be measured relatively precisely. Theoretically, the 2H recovery should be 100% in a system (such as the rumen or HGT incubation system); this is because all produced reducing power must be utilised (according to the principle of redox conservation). Our results of 2H recovery (only considering SCFA and CH_4) support our assumption that the main 2H recovery is accomplished by the production of SCFA (acetate, propionate, and butyrate) and CH_4. Discrepancies exist with other studies concerning the calculated 2H recovery, and a lower or much lower than a 100% recovery is reported in batch culture[5,24] and continuous culture.[5,25] Our results showed that the initial microbial mass, as well as SCFA concentration, can constitute more than half of the end-stage. For example, Ungerfeld *et al.*[24] minimised the effect of the initial values through dilution of the inoculum from 20% to 0.1% — but this might influence the fermentation pattern values. With respect to our findings, a dilution of the inoculum (i.e. a reduction of microbe mass) might lead to a fermentation process directed more towards microbial growth and less towards gas production. This could represent a promising area of future research.

Microbial synthesis pathways like fatty acids elongation and NH_3 incorporation consume 2H; many *in vitro* and *in vivo* studies indicated that increasing microbial synthesis helps redirecting 2H flow and, therefore, reduces CH_4 production.[8,26–28] The negative relationship between microbial and CH_4 production was also confirmed by our results. Demeyer *et al.*[10] estimated that the synthesis of 1 kg microbial cells consumed 6.1 mol 2H; later work by Baldwin *et al.*[29] and Benchaar *et al.*[11] reported the hydrogen requirement of microbial growth without preformed amino acids is 2.71 mol 2H per kg microbes, while Mills *et al.*[12] reported 0.41 mol 2H per kg microbes. The estimation range of 0.41–6.1 mol is due to varying methodologies utilising stoichiometric relationships based on assumptions, such as Demeyer's assumption regarding the chemical composition of ruminal microbes, which is suggested to be not constant. If we take the estimation of Demeyer *et al.*,[10] then the amount of 2H consumed by microbial synthesis accounted for 19.7% and 13.9% of the 2H consumption by CH_4 production for grass and maize silages, respectively. However, it is not possible to verify this through our experiment, since we obtained a higher than 100% 2H recovery. Thus, other 2H production pathways likely exist beyond acetate and butyrate generation. For example, Baldwin *et al.*[29] and Benchaar *et al.*[11] estimated that microbial growth with preformed amino acids produced 0.42 mol 2H per kg microbes; although this value of 2H production is substantially lower than their estimation of 2H consumption, the extent to which it affects electron flow remains a subject for further discussion.

Under the situation of limiting amounts of available nutrients, the lower initial microbial mass might direct more energy and nutrients towards microbial growth and thus attain a higher growth rate, as indicated by our results. A higher microbial gain indicates more truly degraded substrate was directed to microbial synthesis and less to SCFA, resulting in a higher PF, and because 2H is consumed in the process, there is less CH_4 production (as shown in Fig. 7, for grass silage, higher microbial yield might direct truly degraded OM more from fermentation end products to microbial growth, resulting in less CH_4 production). When assessing the difference between measured gas production and the predicted gas production based on SCFA release during NDF digestion *in vitro*, Doane *et al.*[30] found that this difference was only partially explained by

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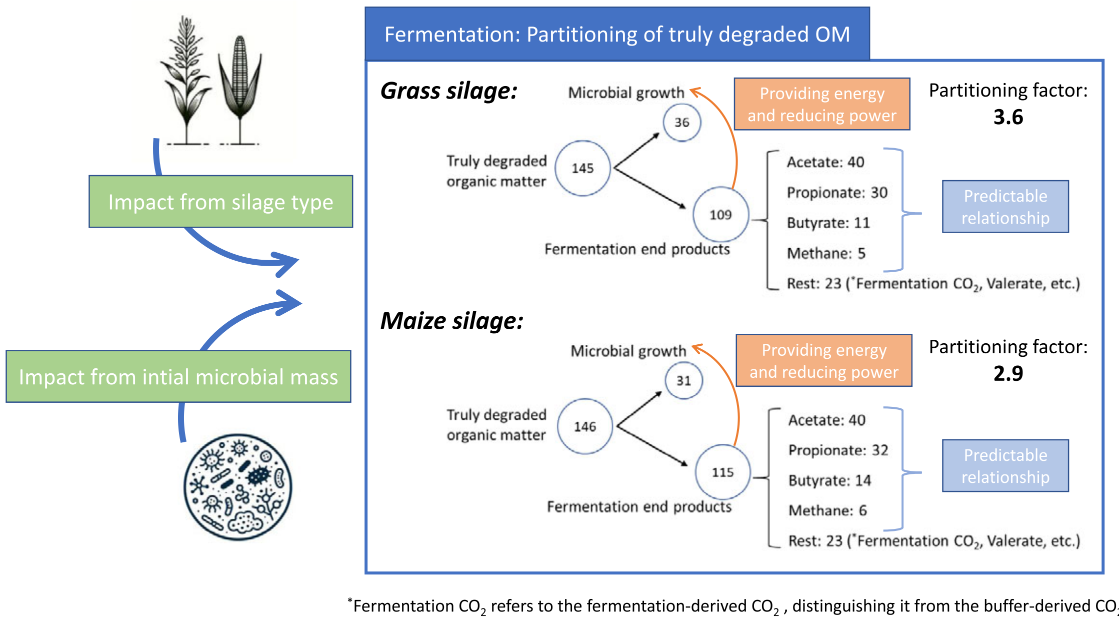

Fig. 7. Schematic of effect of silage type and initial microbial mass on fermentation partitioning. The partitioning of truly degraded OM is presented as net production in mass (mg per 200 mg DM of incubated substrate) during 24 h of fermentation, calculated as the product of the substance's amount and its molar mass. The images of grass, maize, and microbes on the left were generated by ChatGPT version GPT-4.

the amount of propionate released and suggested that microbial synthesis might be responsible for the remaining discrepancy. In their study, this discrepancy was mainly observed in the initial stages of *in vitro* fermentation, similar to our findings that initial microbial mass and gas production are negatively related at the initial 8 h gas reading. Assuming that *in vitro* microbial biomass production will be constrained at some point by the limited habitat available in the *in vitro* system used, as neither microbes nor gases are removed during the assay, this may be particularly relevant *in vivo* where microbes and fermentation gases are removed constantly, and conditions in the reticulorumen might therefore be most represented by assuming conditions of the initial *in vitro* fermentation for extended time periods.

Although there are already studies concerning the fermentation kinetics of total gas production[31–35] and fermentation end products as well as microbiomes,[30,36] there is a need to conduct more kinetic studies with the complete collection of fermentation end products and various microbial types and incorporating transcriptomics to understand the metabolic state of the microbiome at different growth stages. Additionally, it should be further explored how the fermentation direction and electron flow are influenced not only by substrate but also by the inoculum's initial microbial mass or by the constant removal of microbes from open systems.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.37.

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Declaration of interests

The authors declare no conflict of interest.

Authorship

JH and XZ designed the study; XZ performed the study with support from AS and JH; XZ analysed the data, interpreted the findings, and drafted the manuscript, with the supervision of JH; FK, AS, MC, and JH gave critical revisions and important intellectual contents.

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RESEARCH ARTICLE

Intakes of energy, macronutrients, and micronutrients in adult Lithuanian population: a national study of 2019–2020

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Abstract

Dietary surveys are critical in evaluating dietary trends prevailing across the entire population. The aim of this study is to present the results of the latest research into the intakes of energy, macronutrients, and micronutrients amongst the adult population in Lithuania from 2019 through 2020. A cross-sectional study was conducted and dietary data was collected using a 24-h dietary recall method from a total of 2555 Lithuanian adults. Medians, 25th and 75th percentiles were calculated. The diet of Lithuanian adults was found insufficient as the energy intake from fats exceeded the recommended norms, while the energy intake from carbohydrates was below the lower range. The intakes of dietary fibre and most vitamins and minerals were insufficient. Men, compared to women, had a statistically higher total energy intake and energy intake from fats, and a higher intake of dietary fibre. Younger adults, compared to older ones, had statistically lower intakes of energy from fats, including saturated fats, and lower intakes of sodium chloride. Respondents with primary education, compared to those who had attained a higher degree of education, had a statistically lower intake of total energy and dietary fibre. Study showed that intakes of majority of nutrients in the diet of the adult Lithuanian population are not in compliance with the recommended daily intakes. Continuous nationally representative studies into food consumption and nutrient intake of the adult Lithuanian population must be carried out to assess the changes in the population's diet and the effectiveness of policies aimed at promoting healthy diets.

Key words: Dietary survey: Energy intake: Food consumption survey: Nutrient intake

Introduction

Although the life expectancy of the population in Lithuania has increased in recent years, it still remains among the shortest in the European Union (EU) and the national preventable mortality rate is significantly higher than the EU average.[1] Unhealthy nutrition is recognized as one of the preventable risk factors for health. Furthermore, scientific evidence shows that changing one's diet can have significant, both positive and negative, lifelong effects on human health. The consumption of foods high in fat, especially saturated fats and trans fatty acids, sugars, sodium can have a negative effect on health, while the consumption of products high in dietary fibres such as vegetables, fruits, legumes, whole grain products, fish, vitamins is beneficial for health.[2] However, the studies of food and nutrient intake carried out in different European countries, including Lithuania, show that the diet of European residents is still insufficient in nutrients. The intakes of sugars, fats, saturated fats, sodium exceed the recommended norms, however, the intake of dietary fibre is insufficient.[3]

Dietary surveys are critical in evaluating dietary trends prevailing across the entire population. The importance of nationally representative diet and nutrition surveys is highlighted in the WHO's European Food and Nutrition Action Plan, as well as in the Lithuanian Health Strategy for 2014–2025 adopted in 2014.[4,5] These and similar surveys provide essential information about dietary trends of the population, facilitate the identification of inequalities and are important tools in evaluating policies aimed at promoting healthy diets.[2,6]

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Table 1. Recommended daily intakes (RDI) of nutrients for Lithuanian adults

Nutrient	RDI	Vitamins	RDI	Minerals	RDI
Proteins (E%)	10–20	Vitamin A (µg RE/day)	800	Magnesium (mg/day)	325
Fats (E%)	25–35	Vitamin D (µg/day)	10	Phosphorus (mg/day)	700
Saturated fats (E%)	<10	Vitamin E (mg α-TE/day)	11	Potassium (mg/day)	3300
Carbohydrates (E%)	45–60	Vitamin K (µg/day)	75	Calcium (mg/day)	900
Sugars[a] (E%)	<10	Thiamine (vitamin B1) (mg/day)	1.2	Chromium (µg/day)	40
Dietary fibre[b] (g/day)	25–35	Riboflavin (vitamin B2) (mg/day)	1.4	Manganese (mg/day)	3.0
		Vitamin B6 (mg/day)	1.4	Iron (mg/day)	12.5
		Vitamin B12 (µg/day)	3.0	Copper (mg/day)	1.0
		Niacin (PP) (mg NE/day)	17	Zinc (mg/day)	10
		Vitamin C (mg/day)	80	Selenium (µg/day)	55
		Pantothenic acid (vitamin B5) (mg/day)	6.0	Iodine (µg/day)	150
		Biotin (vitamin B7) (µg/day)	50	Sodium chloride (g/day)	5–6

[a]Total sugar intake.
[b]Total daily dietary fibre intake.

The first nationally representative study of food consumption and nutrient intake of the adult Lithuanian population was carried out in 1997, subsequent studies were carried out in 2007, 2013 and 2019–2020. The aim of this article is to present the results of the intakes of energy, macronutrients and micronutrients in the adult Lithuanian population obtained from the latest study carried out in 2019–2020.

Methods

Study population

Based on the latest Lithuanian census data provided by the State Data Agency of Lithuania, a sample of the local adult population was defined to represent the adults of Lithuania based on gender, age, and other socio-demographic characteristics. The current study on food consumption and dietary intake of Lithuanian adults was completed over the period of 2019-2020 and is part of the national study initiated by the Ministry of Health of the Republic of Lithuania in cooperation with Vilnius University and being sustainably carried out into the actual nutrition, dietary and physical activity habits and knowledge of nutrition and physical activity of the Lithuanian adults and elderly.

Dietary intake

The assessment of dietary intake of energy and nutrients was conducted using a single 24-h dietary recall. The data was collected using a face-to-face interview. Interviewers were trained prior to the interviews. Average duration of an interview was 30 minutes. An atlas with photos of commonly consumed foods and their portion sizes was used to record the data on food products and their quantities consumed by each respondent during the previous day. The intakes of energy, macronutrients and micronutrients were estimated using nutritional software which contains a food composition database supplemented with the composition of food products and dishes which are typically consumed in Lithuania.

Nutrient intakes of the adults were compared with the recommended daily intakes (RDI), which have been approved by the order of the Minister of Health of the Republic of Lithuania.[7] These recommended daily intakes are listed in Table 1.

Statistical analysis

The Kolmogorov-Smirnov test was used to examine if variables were normally distributed. Since all the variables were not normally distributed, the Mann-Whitney and Kruskal-Wallis non-parametric tests were used for conducting a comparison between groups. Medians, the 25th and 75th percentiles were calculated for the individual intake of energy, macronutrients and micronutrients categorized by age, gender, living area, and degree of education. All statistical analyses were performed using the SPSS software package for Windows (version 20).

Ethics statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, all individual participants provided verbal informed consent. Verbal consent was witnessed and formally recorded. No approval from the Lithuanian Bioethics Committee was required because only anonymized data were used and the research did not collect health data, according to the Recommendations of the Board of the Lithuanian Bioethics Committee 'Compliance with ethical principles in non-biomedical research involving human health'.

Results

Descriptive statistics

A total of 2555 respondents were surveyed. Women comprised 53.8% of the sample. All the subjects were grouped into the aged 19–34 representing 36.5% of the respondents, aged 35–49 involving 34.1% of the respondents, and aged 50–64 representing 29.4% of the respondents. In all, 78.5% of respondents lived in urban areas while 21.5% in rural areas.

Total energy and macronutrient intake

The median daily energy intake for the entire adult Lithuanian population was determined to be 1691 kcal. In terms of the contribution of macronutrients to dietary energy, fats

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contributed the highest proportion (42.7%) followed by carbohydrates (41.1%) and proteins (14.7%). The actual median intake of protein (14.7 E%) in the diet of Lithuanian adult population was determined to be in compliance with the recommended intake which is 10–20% of the total energy intake (E%). The recommended energy intake from fats for Lithuanian adults should be between 25 and 35 E%. However, our study showed that the fat intake of the adult Lithuanian population (42.7 E%) exceeded the recommended amount. Study also showed that the intake of saturated fats in the diet of Lithuanian adults was above the recommended amount of 10 E%, since the actual median energy intake from saturated fats was determined to be 13.7 E%. It is recommended that for adults carbohydrates should provide 45–60 E%. However, according to our study, the intake of carbohydrates in the adult Lithuanian population was below the lower range as carbohydrates provided 41.1 E%. Our study has demonstrated that the median energy intake from sugars in the diet of Lithuanian adults is 9.8 E% and this is in line with the recommended intake because energy intake from sugars for Lithuanian adults be below 10 E%. The estimated daily intake of total dietary fibre was 15.1 g which is significantly lower than the recommended intake of 25–35 g of dietary fibre per day.

Table 2 shows the total energy intake, the contribution of macronutrients to the total energy intake and the dietary fibre intake according to respondents' gender and age. Men had statistically higher total energy intake than women. The energy intake from fats, including saturated fats, was statistically higher for men than women. The energy intake from carbohydrates, including sugars, was statistically higher for women compared to men. The comparison of the energy contribution of different macronutrients according to respondents' age showed that fat intake, including saturated fats, was statistically lower for the youngest group (aged 19–34) of adults than for older adults. Men, compared to women, and older adults compared to the youngest group of adults, had a statistically higher intake of dietary fibre.

Table 3 shows the results of the total energy and macronutrient intakes according to the respondents' living area and degree of education. While analysing the differences in macronutrient contribution to the total energy intake with respect to the respondents' living area, it was found that the respondents who lived in urban areas had a statistically higher energy intake from sugars. The respondents with primary education, compared to respondents who had attained a higher degree of education, had statistically lower intakes of total energy and dietary fibre.

Vitamin intake

The study showed that the intakes of vitamin A, vitamin E, thiamine, riboflavin, vitamin B6, and vitamin B12 in the diet of Lithuanian adult population were in adherence or almost in adherence to the recommended daily intakes. However, study found inadequate intakes of vitamin K, vitamin D, niacin, vitamin C, pantothenic acid, and biotin.

Table 4 shows the results of the total vitamin intakes and the intakes according to respondents' gender and age. For all

Table 2. Daily energy and macronutrient intakes by gender and age group (median (25th and 75th percentiles))

Macronutrients	Total	Gender			Age group (years)			
		Men	Women	*P-value*	19–34	35–49	50–64	*P-value*
Energy (kcal/day)	1691 (1263–2237)	1956 (1471–2585)	1492 (1148–1948)	<0.0001	1672 (1249–2241)	1700 (1269–2317)	1707 (1267–2199)	0.552
Proteins (E%)	14.7 (12.4–18.2)	14.6 (12.4–17.8)	14.7 (12.4–18.5)	0.391	14.6 (12.1–18.1)	14.7 (12.4–18.3)	14.7 (12.5–18.0)	0.615
Fats (E%)	42.7 (35.3–50.4)	43.5 (36.3–50.9)	41.9 (34.6–50.1)	<0.0001	41.6 (34.4–49.3)	43.2 (36.4–50.7)	43.2 (35.3–51.0)	0.002
Saturated fats (E%)	13.7 (11.3–16.1)	13.9 (11.6–16.3)	13.4 (11.1–16.0)	<0.0001	13.3 (11.0–15.8)	13.8 (11.7–16.2)	13.8 (11.3–16.3)	0.002
Carbohydrates (E%)	41.1 (33.1–49.4)	40.2 (32.6–47.9)	41.7 (33.6–50.5)	<0.0001	41.8 (33.9–50.8)	40.6 (32.9–48.1)	40.6 (32.7–49.5)	0.001
Sugars[a] (E%)	9.8 (6.0–14.2)	8.8 (5.3–12.9)	10.8 (6.9–15.3)	<0.0001	9.7 (6.2–14.4)	9.9 (6.0–14.0)	9.6 (5.8–14.1)	0.938
Dietary fibre[b] (g/day)	15.1 (10.2–21.3)	16.2 (11.1–22.9)	14.4 (10.0–19.6)	<0.0001	14.4 (9.4–20.5)	15.3 (10.5–21.8)	15.6 (11.0–22.1)	0.001

[a]Total sugar intake.
[b]Total dietary fibre intake.

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Table 3. Daily energy and macronutrient intakes by the living area and degree of education (median (25th and–75 percentiles))

Macronutrients	Living area			Degree of education				
	Urban	Rural	*P-value*	Primary	Secondary	College, vocational	Tertiary	*P-value*
Energy (kcal/day)	1689 (1251–2312)	1693 (1254–2317)	0.744	1590 (1245–1908)	1693 (1253–2303)	1767 (1329–2347)	1663 (1258–2181)	0.004
Proteins (E%)	14.6 (12.3–18.2)	14.7 (12.6–18.1)	0.777	14.3 (12.6–17.5)	14.4 (12.1–18.3)	14.7 (12.5–17.9)	14.7 (12.4–18.2)	0.503
Fats (E%)	42.6 (35.3–50.4)	43.3 (35.5–50.8)	0.201	39.8 (34.6–49.8)	43.3 (35.8–50.8)	42.9 (35.6–50.3)	42.3 (35.1–50.4)	0.325
Saturated fats (E%)	13.6 (11.3–16.1)	13.8 (11.4–16.2)	0.231	12.7 (11.1–15.9)	13.8 (11.4–16.2)	13.7 (11.4–16.1)	13.5 (11.2–16.1)	0.325
Carbohydrates (E%)	41.1 (33.4– 49.4)	40.8 (32.3–49.1)	0.225	45.9 (34.0–50.8)	40.6 (33.2–49.4)	40.8 (33.8–48.6)	41.4 (32.5–49.7)	0.683
Sugars[a] (E%)	10.0 (6.2–14.3)	8.9 (5.5–13.8)	0.004	9.2 (6.2–13.6)	8.9 (5.6–13.6)	9.7 (5.9–14.0)	10.3 (6.4–14.7)	0.062
Dietary fibre[b] (g/day)	15.1 (10.3–21.1)	14.8 (9.9–22.1)	0.701	13.0 (9.7–20.7)	14.3 (9.2–20.8)	16.1 (11.0–20.7)	15.0 (10.3–20.7)	0.001

[a]Total sugar intake.
[b]Total dietary fibre intake.

Table 4. Daily vitamin intakes by gender and age group (median (25th and 75th percentiles))

Vitamins	Total	Gender			Age group (years)			
		Men	Women	*P-value*	19–34	35–49	50–64	*P-value*
Vitamin A (µg RE/day)	718.5 (438.0–1278.5)	787.7 (477.4–1400.4)	675.4 (408.1–1152.3)	<0.0001	690.6 (419.2–1258.4)	731.8 (449.2–1289.5)	757.6 (464.8–1297.5)	0.098
Vitamin D (µg/day)	5.2 (3.9–8.3)	5.6 (4.1–9.1)	4.9 (3.8–7.7)	<0.0001	5.2 (4.0–8.3)	5.2 (3.9–8.2)	5.2 (3.9–8.4)	0.853
Vitamin E (mg α-TE/day)	11.1 (6.7–18.6)	12.2 (7.6–19.8)	10.3 (6.3–17.6)	<0.0001	10.5 (6.4–18.0)	11.4 (6.9–18.8)	11.4 (6.9–19.2)	0.106
Vitamin K (µg/day)	49.1 (18.9–108.2)	50.9 (19.3–108.0)	45.8 (18.5–108.3)	0.183	40.7 (15.5–97.0)	51.6 (20.4–117.2)	51.5 (21.6–112.8)	<0.0001
Thiamine (vitamin B1) (mg/day)	1.0 (0.7–1.5)	1.2 (0.8–1.7)	0.9 (0.6–1.3)	<0.0001	1.0 (0.6–1.4)	1.1 (0.7–1.6)	1.1 (0.7–1.6)	<0.0001
Riboflavin (vitamin B2) (mg/day)	1.2 (0.8–1.6)	1.3 (0.9–1.7)	1.1 (0.8–1.4)	<0.0001	1.1 (0.8–1.5)	1.2 (0.9–1.6)	1.2 (0.9–1.6)	0.103
Vitamin B6 (mg/day)	1.5 (1.1–2.2)	1.7 (1.2–2.4)	1.4 (1.0–2.0)	<0.0001	1.6 (1.1–2.2)	1.6 (1.0–2.2)	1.5 (1.1–2.2)	0.987
Vitamin B12 (µg/day)	2.7 (1.1–4.8)	3.0 (1.1–5.3)	2.5 (1.1–4.4)	<0.0001	2.7 (1.2–4.7)	2.8 (1.1–4.9)	2.6 (1.1–4.8)	0.530
Niacin (PP) (mg NE/day)	12.1 (8.0–18.1)	14.2 (9.7–20.6)	10.5 (7.0–15.9)	<0.0001	11.7 (7.8–18.2)	12.4 (8.1–18.4)	12.3 (8.3–17.8)	0.659
Vitamin C (mg/day)	57.5 (33.8–90.4)	60.2 (35.9–93.5)	54.4 (32.6–87.2)	0.010	53.3 (31.7–88.0)	59.8 (35.1–93.1)	58.6 (34.5–90.8)	0.018
Pantothenic acid (vitamin B5) (mg/day)	2.9 (2.0–4.1)	3.3 (2.3–4.7)	2.6 (1.8–3.7)	<0.0001	2.9 (1.9–4.0)	2.9 (2.0–4.2)	2.9 (2.0–4.2)	0.543
Biotin (vitamin B7) (µg/day)	17.8 (11.3–27.9)	20.9 (13.6–31.7)	15.7 (10.1–24.7)	<0.0001	17.5 (11.0–28.0)	18.1 (11.2–27.9)	18.1 (11.5–27.9)	0.715

vitamins, with the exception of vitamin K, men tended to have a statistically higher intake than women. Significant differences were found between the age groups for the intake of vitamin K, thiamine, and vitamin C – the respondents from the youngest age group, compared to older respondents, had a statistically lower intake of these vitamins.

Table 5 shows the results of vitamin intake according to the respondents' living area and degree of education. No significant differences were observed in vitamin intake between urban and rural respondents. The intake of pantothenic acid statistically differed in different groups by degree of education. The respondents who had attained a primary degree of education had a lower intake of pantothenic acid, compared to those, who had attained a higher degree of education.

Mineral intake

According to our study, the intakes of magnesium, manganese, and zinc in the diet of Lithuanian adult population were in line with the recommended daily intakes, while the intakes of phosphorus and copper were higher than the recommended daily intakes. Inadequate intakes in the diet of Lithuanian adults were observed for potassium, calcium, chromium, iron, selenium, and iodine. The median intake of sodium chloride (salt) for Lithuanian adults was determined to be 5.9 g/d which is in line with the recommended daily intake of 5–6 g/d.

Table 6 shows the results of the total mineral intake and the intake according to respondents' gender and age. As for all minerals, with the exception of calcium, men tended to have statistically higher intakes than women. While analysing the differences in mineral intake with respect to respondents' age, it was found that the respondents from the youngest age group, compared to older respondents, had a statistically lower intake of chromium and iodine. Women, compared to men, as well as younger respondents, compared to older, had a statistically lower intake of sodium chloride.

Table 7 shows the results of mineral intake according to the respondents' living area and degree of education. Respondents living in rural areas, compared to those, living in urban areas, had a statistically higher intake of sodium chloride (salt). The intakes of magnesium, phosphorus, and potassium were statistically lower in respondents with a degree of primary education, compared to those, who have attained a higher degree of education. Respondents with a degree in college or vocational education had a statistically higher intake of chromium compared to those with a degree in secondary education. The intake of sodium chloride (salt) was determined to be statistically higher in respondents with college, vocational education in comparison to respondents with secondary or tertiary education.

Discussion

Evidence shows that a healthy diet has a positive influence on health and promotes the prevention of common non-communicable diseases (NCDs). Several NCDs, such as cardiovascular diseases, type 2 diabetes, some cancers, and others, are associated with unhealthy diets.[8,9] Despite the fact

Table 5. Daily vitamins intakes by the living area and degree of education (median (25th and 75th percentiles))

Vitamins	Living area			Degree of education				
	Urban	Rural	*P-value*	Primary	Secondary	College, vocational	Tertiary	*P-value*
Vitamin A (μg RE/day)	713.3 (438.1–1264.0)	740.0 (432.5–1301.4)	0.646	682.0 (406.9–1703.0)	671.9 (398.8–1264.0)	780.6 (482.0–1301.4)	711.8 (430.6–1257.4)	0.054
Vitamin D (μg/day)	5.1 (3.8–8.1)	5.7 (4.4–9.2)	0.311	2.6 (2.0–4.2)	4.9 (3.7–7.7)	5.2 (4.0–8.5)	5.4 (4.0–8.5)	0.271
Vitamin E (mg α-TE/day)	11.1 (6.7–18.4)	11.3 (6.5–19.3)	0.562	8.6 (6.1–17.1)	10.2 (6.5–18.2)	11.8 (7.0–19.2)	11.1 (6.5–18.5)	0.140
Vitamin K (μg/day)	48.9 (19.2–106.2)	49.5 (18.2–112.6)	0.779	39.8 (12.3–91.6)	44.7 (16.3–94.1)	52.6 (21.4–118.9)	47.7 (19.6–105.1)	0.075
Thiamine (vitamin B1) (mg/day)	1.0 (0.7–1.5)	1.1 (0.7–1.6)	0.191	1.0 (0.6–1.4)	1.1 (0.7–1.5)	1.1 (0.7–1.6)	1.0 (0.7–1.4)	0.0005
Riboflavin (vitamin B2) (mg/day)	1.2 (0.8–1.6)	1.2 (0.8–1.7)	0.527	1.0 (0.7–1.4)	1.1 (0.8–1.6)	1.2 (0.9–1.6)	1.1 (0.8–1.5)	0.091
Vitamin B6 (mg/day)	1.5 (1.1–2.2)	1.5 (1.0–2.3)	0.653	1.4 (0.8–2.1)	1.5 (1.0–2.2)	1.6 (1.1–2.3)	1.5 (1.1–2.2)	0.199
Vitamin B12 (μg/day)	2.7 (1.1–4.8)	2.7 (1.1–4.8)	0.496	2.3 (1.5–4.4)	2.5 (0.9–4.7)	2.7 (1.1–4.9)	2.8 (1.2–4.8)	0.863
Niacin (PP) (mg NE/day)	12.1 (8.1–17.9)	12.2 (7.7–19.3)	0.766	10.5 (6.5–16.2)	12.7 (8.2–18.9)	12.4 (8.5–17.8)	11.7 (7.8–18.1)	0.381
Vitamin C (mg/day)	58.0 (34.3–91.3)	54.1 (32.4–87.2)	0.092	42.4 (27.7–69.4)	52.7 (31.5–88.9)	59.8 (36.6–92.9)	58.5 (34.5–89.5)	0.062
Pantothenic acid (vitamin B5) (mg/day)	2.9 (2.0–4.1)	2.8 (1.0–4.3)	0.554	2.0 (1.5–3.3)	2.9 (1.9–4.2)	3.1 (2.2–4.3)	2.9 (1.0–4.0)	0.003
Biotin (vitamin B7) (μg/day)	17.8 (11.5–27.3)	17.9 (10.4–29.9)	0.917	12.4 (9.9–25.0)	18.1 (10.8–29.2)	18.6 (11.5–28.0)	17.5 (11.3–27.4)	0.269

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Table 6. Daily mineral intakes by gender and age (median (25th and 75th percentiles))

		Gender			Age group (years)			
Minerals	Total	Men	Women	*P*-value	19–34	35–49	50–64	*P*-value
Magnesium (mg/day)	276.6 (202.1–381.2)	308.7 (224.8–409.1)	254.0 (188.2–346.8)	<0.0001	272.7 (199.4–374.9)	280.0 (199.2–384.5)	279.8 (206.1–385.4)	0.276
Phosphorus (mg/day)	1044.0 (754.6–1370.1)	1161.7 (849.1–1509.7)	926.8 (698.7–1236.3)	<0.0001	1004.5 (728.4–1337.6)	1057.3 (768.4–1400.3)	1054.8 (775.9–1373.0)	0.084
Potassium (mg/day)	2458.5 (1826.4–3292.8)	2705.2 (2000.5–3645.7)	2263.3 (1737.6–2988.8)	<0.0001	2406.7 (1789.1–3221.3)	2486.5 (1829.8–3350.9)	2473.7 (1884.3–3299.1)	0.087
Calcium (mg/day)	522.8 (347.0–757.2)	561.6 (377.7–850.3)	492.4 (324.3–684.4)	<0.0001	505.0 (332.4–760.2)	531.4 (363.6–747.2)	542.4 (349.7–767.1)	0.208
Chromium (µg/day)	29.4 (16.9–45.5)	33.1 (19.5–50.3)	26.7 (15.5–41.1)	<0.0001	27.1 (15.3–41.9)	31.1 (17.3–48.4)	30.8 (19.5–46.7)	<0.0001
Manganese (mg/day)	2.9 (1.7–4.6)	3.1 (1.9–5.1)	2.7 (1.6–4.2)	<0.0001	2.8 (1.7–4.6)	2.9 (1.8–4.7)	2.9 (1.8–4.5)	0.254
Iron (mg/day)	9.8 (7.0–13.6)	11.2 (8.0–15.6)	8.8 (6.4–11.9)	<0.0001	9.6 (6.6–13.0)	10.0 (7.1–13.8)	9.7 (7.2–13.6)	0.177
Copper (mg/day)	1.6 (1.0–2.2)	1.7 (1.2–2.4)	1.4 (1.0–2.0)	<0.0001	1.5 (1.0–2.1)	1.6 (1.0–2.3)	1.6 (1.1–2.3)	0.162
Zinc (mg/day)	8.4 (5.8–11.5)	9.6 (6.6–13.3)	7.5 (5.2–10.2)	<0.0001	8.1 (5.6–11.4)	8.5 (5.8–11.5)	8.5 (6.0–11.4)	0.419
Selenium (µg/day)	27.8 (26.0–30.9)	34.6 (32.4–37.9)	21.9 (20.3–24.7)	<0.0001	22.0 (20.4–24.8)	32.6 (30.8–35.8)	29.4 (27.6–32.6)	0.332
Iodine (µg/day)	18.8 (2.3–39.9)	21.1 (2.6–45.1)	16.4 (2.1–36.9)	<0.0001	17.0 (2.1–36.1)	18.4 (2.4–43.6)	20.0 (3.8–41.7)	0.019
Sodium chloride (g/day)	5.9 (4.1–8.6)	7.0 (4.8–9.8)	5.3 (3.7–7.3)	<0.0001	5.6 (3.8–8.2)	6.4 (4.3–8.8)	6.1 (4.3–8.8)	<0.0001

Table 7. Daily mineral intakes by the living area and degree of education (median (25th and 75th percentiles))

	Living area			Degree of education				
Minerals	Urban	Rural	*P*-value	Primary	Secondary	College, vocational	Tertiary	*P*-value
Magnesium (mg/day)	278.2 (202.5–381.1)	271.8 (199.1–381.6)	0.661	220.6 (172.0–364.4)	270.7 (188.4–370.1)	283.8 (208.1–391.6)	276.7 (205.9–381.2)	0.003
Phosphorus (mg/day)	1044.0 (760.5–1361.0)	1044.6 (724.3–1405.8)	0.694	900.3 (588.7–1220.3)	1024.0 (722.7–1405.8)	1084.2 (784.7–1412.7)	1012.3 (755.2–1335.6)	0.003
Potassium (mg/day)	2466.6 (1827.3–3318.1)	2423.8 (1825.6–3318.1)	0.821	1921.8 (1521.3–2811.4)	2392.0 (1758.2–3304.5)	2563.9 (1921.9–3411.3)	2439.4 (1820.0–3233.3)	0.002
Calcium (mg/day)	524.7 (347.1–757.2)	513.5 (346.9–757.7)	0.993	536.5 (352.1–725.1)	515.2 (346.1–779.3)	544.8 (357.4–807.4)	515.3 (344.0–730.9)	0.084
Chromium (µg/day)	29.3 (16.6–44.9)	29.5 (18.5–47.5)	0.075	23.4 (15.9–37.4)	27.6 (15.6–42.3)	33.6 (19.4–48.8)	29.2 (16.6–44.0)	<0.0001
Manganese (mg/day)	2.9 (1.8–4.6)	2.7 (1.6–4.7)	0.239	1.6 (1.4–4.0)	2.8 (1.7–4.8)	3.0 (1.8–4.6)	2.8 (1.7–4.6)	0.133
Iron (mg/day)	9.8 (7.1–13.6)	9.6 (6.6–13.4)	0.281	9.2 (5.7–11.1)	9.6 (6.6–13.8)	10.1 (7.2–13.9)	9.7 (7.0–13.3)	0.094
Copper (mg/day)	1.5 (1.1–2.2)	1.6 (1.0–2.3)	0.551	1.5 (0.9–2.3)	1.5 (1.0–2.2)	1.7 (1.1–2.3)	1.5 (1.0–2.2)	0.421
Zinc (mg/day)	8.4 (5.8–11.4)	8.4 (5.5–11.6)	0.893	8.4 (6.2–10.5)	8.4 (5.6–11.5)	8.5 (6.0–11.6)	8.2 (5.6–11.3)	0.218
Selenium (µg/day)	27.0 (25.3–30.0)	26.9 (24.9–30.7)	0.462	27.5 (25.9–30.5)	28.4 (26.6–31.6)	30.4 (28.6–33.9)	25.0 (23.3–27.8)	0.273
Iodine (µg/day)	18.4 (2.4–39.2)	18.9 (2.1–45.1)	0.449	25.1 (1.2–62.3)	16.9 (2.1–38.0)	20.0 (3.3–39.3)	18.0 (2.2–40.3)	0.346
Sodium chloride (g/day)	5.8 (4.0–8.3)	6.6 (4.5–9.7)	<0.0001	6.4 (5.1–8.5)	5.9 (4.0–9.1)	6.5 (4.6–9.3)	5.6 (3.9–8.2)	<0.0001

that a healthy diet is recognized as a very important determinant of disease risk, studies show that the dietary intakes of residents of Lithuania and other countries still does not fully comply with the recommended daily intakes..[10]

The energy intake of the adult Lithuanian population was determined to be 1691 kcal and is similar to the energy intake of adults from other Baltic states, however, the energy intake of Lithuanian adults is lower than the energy intake of adults from Northern and Western Europe regions.[3,11,12] Our study revealed that men had a statistically higher intake of energy and this is comparable to what has previously been reported in other population-based surveys.[11,13]

Proteins are necessary for development and growth as well as the production of essential amino acids. Furthermore, with an aging world population, consuming enough protein in the diet is a crucial dietary component for preventing disorders like sarcopenia.[8,9] Our study showed that in the diet of Lithuanian adults proteins provide the recommended amount of energy (14.7 E%) and these results are in line with the data obtained by studies carried out in the Nordic and Baltic countries where the intake of protein differs from 14 to 19 E%.[14]

Studies show that excessive fat intake is a risk factor for unhealthy weight gain and many NCDs. However, our study showed that the fat intake of the adult Lithuanian population was 42.7 E% and it is one of the highest in Europe since fat intake in Nordic countries typically does not exceed 40 E%.[14] It is important to note that previously carried out studies of nutrient intakes of Lithuanian adults showed similar results for percentage of energy intake from fats, for instance, in the 2014 national study fats contributed 41.7% of energy, and in the 2007 study – 43.2%. One of the possible explanations for high energy intake from fats in the diet of Lithuanian adults could be the reason that Lithuanians tend to frequently consume foods that are high in fat, such as meat, milk, and their products and low fat versions of these products are chosen more rarely.[15,16] It is recommended to limit intake of saturated fats to less than 10% of total energy intake because limiting the intake of saturated fats has been proven to be beneficial for health since it reduces the risk of many cardiovascular diseases.[17] However, our study showed that the intake of saturated fats for Lithuanian adults was 13.7 E% and our findings are similar to those published by studies carried out in other Baltic and Nordic countries where the intake of saturated fats ranges from 13.0 to 15.0 E%.[14]

In the previously carried out national studies of nutrient intakes of Lithuanian adults the energy intake from carbohydrates has always been established to be below the lower range of recommended intake (45 E%). It was determined to be 44.4 E% in the 2014 study and 41.1 E% in 2007 study.[15,16] Our study showed that the energy intake from carbohydrates in the diet of Lithuanian adult population was 41.1% and, as in previous studies, it remained below the lower range of the recommended intake. Also, our study showed that women had a higher energy intake from carbohydrates compared to men, which is in line with the findings of other studies carried out in European countries.[14,18]

Many adverse health effects including unhealthy weight gain, dental caries or diabetes are associated with excessive intake of sugars.[8] Our study has demonstrated that the median energy intake from sugars in the diet of Lithuanian adults was 9.8 E% and it is one of the lowest among other European countries. However, there are differences among countries when comparing the amounts of energy intake from sugars. For example, in Spain, the energy intake from sugars for adults is 16.7 E%, while in the Netherlands it is 19.5 E%.[12,19] Gender had a significant effect on the energy intake from sugars: women had a statistically higher energy intake from sugars which exceeded the recommended daily intake. Higher energy intake from sugars for women, compared to men, was also observed in other population-based studies.[20]

Scientific evidence shows that dietary fibre is crucial for a healthy and balanced diet. Dietary fibre is proven to be important for preventing a number of NCDs, including diabetes, cancer, cardiovascular diseases, and others.[21] Since the first national food consumption and dietary intake study of Lithuanian adult population carried out in 1997, the intake of dietary fibre in the diet of Lithuanian adults has been shown to be insufficient. In 1997 study it was 16.2 g/d, in 2014 study – 15.7 g/d, and our study showed that the intake of dietary fibre remained insufficient – 15.1 g/d.[15,16] The low intake of dietary fibre and the fact that men had a higher intake of dietary fibre compared to women, which was observed in our study, is in accordance with studies carried out in other European countries.[3]

The study of the adult Lithuanian population diet showed that the intakes of vitamin A, vitamin E, thiamine, riboflavin, vitamin B6, and vitamin B12 were in adherence or almost in adherence to the recommended intakes. The intakes of these vitamins range across Europe, for example, the intake of vitamin A differs from 692 μg RE/d in the Czech Republic to 1200 μg RE/d in France.[10] The intake of vitamin B6 in Lithuanian adults is similar to the intake of vitamin B6 in other European populations which is between 1.5 mg/d to 2.0 mg/d.[14,22]

Insufficient intakes in our study were found for vitamin K, vitamin D, niacin, vitamin C, pantothenic acid, and biotin. Vitamin deficiencies can cause many adverse health effects so a healthy diet with adequate intakes of vitamins ensures the prevention of these adverse health effects. Our study determined that the intake of vitamin D in Lithuanian adults was only 5.2 μg/d which is only half of the recommended intake of 10 μg/d. Several health benefits are associated with an adequate intake of vitamin D, including the health of bones and the proper functions of muscles, nerves, and the immune system.[23] Vitamin D intakes vary across Europe from 4.8 μg/d in Denmark to 11.5 μg/d in Finland.[14]

Inadequate intakes in the diet of Lithuanian adults were observed for potassium, calcium, chromium, iron, selenium, and iodine. When comparing the intakes of these minerals among the population of Lithuanian adults and other European adult populations, it was observed that Lithuanian adults had lower intakes of these minerals than adults from other European populations.[10,23,24] Selenium is an essential mineral for the normal function of the cardiovascular, endocrine, and immune systems, therefore, adverse health conditions, including type 2 diabetes, thyroid autoimmune disease and others, are associated with selenium deficiency.[25] It is recommended that

Lithuanian adults should consume 50–60 μg of selenium per day (depending on gender and age), however, our study showed that the median intake of selenium is only half of the recommended norm, as it is 27.8 μg/d. Selenium intake in Lithuanian adults is one of the lowest in Europe since in other European countries it differs from 46 μg/d in Sweden to 78 μg/d in Finland.[14] Iodine intakes in the adult Lithuanian population were determined to be 18.8 μg/d which is below the recommended amount. In order to protect public health against the ailments caused by the Lithuanian geographical location, such as iodine deficiency, table salt should contain 20–40 mg/kg of iodine when used in food production to compensate for iodine deficiency.[26]

Our study showed that sodium chloride (salt) consumption of Lithuanian adults was 5.9 g/d and it complied with the recommended daily intake. Salt intake differs across Europe from 5.4 g/d in Estonia to 8.2 g/d in Austria.[3,14] Significant differences were observed between salt intakes in women and men – women had a significantly lower sodium chloride intake than men which is in line with other population-based surveys.[3,22]

The main advantage of this study is that it uses data from a nationally representative survey and data was collected by well-trained interviewers. However, this study has several limitations. First, a single 24-h recall was used in this study and it is known to have a high within-person variability. However, it is frequently used to estimate food and nutrient intakes in a large group of participants, since multi-day recalls place more of a burden on respondents and can eventually increase drop-out rates. Second, since this study was a cross-sectional look at the diet of Lithuanian adults, results should be interpreted with caution, also there is no accounting for seasonality in food consumption. Lastly, the current paper does not describe foods consumed from which nutrient intakes were calculated, however, the authors are planning to report this in the future publications.

Conclusions

In conclusion, study showed that intakes of majority of nutrients in the diet of the adult Lithuania population are not in compliance with the recommended daily intakes. Continuous nationally representative studies into food consumption and nutrient intake of the adult Lithuanian population must be carried out to assess the changes in the population's diet and the effectiveness of policies aimed at promoting healthy diets.

Abbreviations

E%: percentage of the total energy intake; **EU:** European Union; **NCD:** non-communicable diseases; **RDI:** recommended daily intake.

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Conflict of interest

None.

Authorship

Conceptualization, G.B., R.B., A.B. and R.S.; methodology, R.B. and R.S.; formal analysis, G.B. and R.B.; investigation, R.B. and A.B.; data curation, G.B. and A.B.; writing – original draft preparation, G.B.; writing – review and editing, R.S.; visualization, G.B.; supervision, R.B and R.S. All authors have read and agreed to the published version of the manuscript.

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RESEARCH ARTICLE

Menu provision in a young offenders institution, comparison with dietary guidelines, and previous menu allocation: a cross-sectional nutritional analysis

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Abstract

Objective: This study aimed to assess and comparatively analyse two menus from a Young Offenders Institution (YOI). One menu from 2019, and one from 2022, with the objective of identifying any improvements in meeting dietary guidelines. *Design:* Cross-sectional and comparative analysis. *Setting:* United Kingdom, a YOI in Northern England. *Participants:* YOI Menus. *Results:* Analysis of 30 dietary components identified that 25 exceeded the dietary guidelines ($P < 0.05$) for the 2022 menu, with five failing to meet the guidelines ($P < 0.05$). When compared to the 2019 menu, the 2022 menu showed improvements in saturated fat, sodium, and vitamin D. Despite the improvement, vitamin D levels remained below dietary guidelines ($P < 0.01$). Salt and energy content were reduced in the 2022 menu ($P < 0.05$); however, they were still above the dietary guidelines ($P < 0.01$). Free sugars were significantly above dietary guidelines for both menus, with no significant change between the 2019 and 2022 menu ($P = 0.12$). *Conclusion:* The 2022 menu has demonstrated progress in alignment with meeting dietary guidelines, particularly in reducing calories, fat, saturated fat, salt, sodium, and chloride, as well as increasing vitamin D. Despite improvements, calories, free sugars, salt, saturated fat, sodium, and chloride are still exceeding dietary guidelines, posing as potential health risks.

Key words: **Incarceration: Nutritional analysis: Nutrition: Prisoners: Public health: Sugar: Vitamin D: Vitamin D deficiency: Vulnerable population: Young offenders institution**

Introduction

A healthy diet is widely known for its importance to overall human health and development. Nutrient deficiencies are linked to a higher risk of poor physical and mental health,[1,2] reduced cognitive function, lower educational outcomes, and diminished productivity.[3,4] Specific population groups face a higher risk of poor nutrition, including populations in poverty, experiencing food insecurity, and those lacking the ability to control their own diet.[5–7] Prisoners are an example of such a vulnerable population.

Prisoners' loss of liberty results in a reduction of autonomy over aspects of their lives, with one key aspect being their diet. Although prisoners are presented with menu choices, for example, five options at lunch and dinner, they remain restricted to a diet institutionally provided for them.[8]

This food provision is constrained by the restricted budget which the catering manager must work with, estimated to be approximately only £2 per prisoner per day, though this can be less in some cases.[8] For comparison, National Health Service (NHS) hospitals, on average, spent £4.56 per patient meal in 2018–19, though as with prisons, this budget can vary.[9] While on the surface, hospitals also appear to suffer from a low budget, it is worth noting that hospital patients have a much shorter stay, with 5.2 months in 2020–21, compared with the average custodial sentence of 21.4 months in 2022.[10,11]

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In 2022, it was estimated in England and Wales that just over 13,000 children aged between 10 and 17 received cautions or sentences from the courts.[12] The majority of those sentenced will serve this within a Young Offenders Institution (YOI), designed for individuals aged 15–21. As of March 2022, the average monthly population in these institutions was 454.[12,13]

Prisoner health is compromised, with them suffering a disproportionately higher burden of physical and mental health conditions compared to the general population. Up to 90% across the prison estates are estimated to have at least one undiagnosed mental health condition.[14] For those in youth justice, practitioners have reported that just over 70% of young offenders present with mental health concerns and that they suffer from a higher burden than adolescents in the general population.[15,16] In many cases, this is attributable to traumatic life experiences, such as witnessing family violence, abuse, substance misuse issues, and neglect.[16]

A high cost is associated with youth justice, estimated at £300 million across the youth estates in 2019, encompassing YOIs, secure children's homes, and training settings.[17] For YOIs, the expenditure was estimated at around £76,000 per young offender per year.[18] This represents a substantial cost to taxpayers, though it is a cost which could be reduced through improved diet quality and living conditions.[14]

Nutrition is crucial for one's overall health, and institutions housing vulnerable young offenders must ensure that they meet nutritional standards. Therefore, it is critical to identify the nutritional profile of the food offered to prisoners, ensuring they have access to adequate nutrition. This is not only beneficial for their health but also for their future in society. His Majesty's Prison and Probation Service (HMPPS) is currently developing new menus for male and female adult prisons, as well as Young Offenders Institutions (YOIs). The aim of these new menus is to ensure that dietary options enable prisoners to meet the Government Dietary Recommendations (GDRs).[19]

The objective of this study was to assess the nutritional content of a current and previous menu, developed by HMPPS for a male YOI in Northern England. Specific aims include comparing both menus' nutritional content to (i) the UK GDRs, an update on the formerly used dietary reference values (DRVs),[19] (ii) Tolerable Upper Intake Level (ULs), which represent the maximum daily intake of a nutrient which is unlikely to pose a negative risk to one's health, as defined by the European Food Safety Authority (EFSA),[20] (iii) and a comparison in nutritional content between the 2019 and 2022 menus.

Materials and methods

Two menus were provided by HMPPS, both of which were used in a YOI in Northern England, one from 2019 and one from 2022. While YOIs across England are provided with food from the same supplier, and most follow a similar menu format, we cannot state that this menu analysed will be fully representative of other YOIs in England. This is due to the freedom catering managers have in what main meals they prepare, in addition to meeting the demands of prisoners with special dietary needs, i.e. halal, vegetarian etc, which can vary across the prison estates. However, typically what is provided for breakfast, snack, and dessert options will remain similar across prisons, with only lunch and dinner options open to some variation.

Each menu consisted of 28 days over a four-week cycle. The nutrient profile of the menus was compared to the GDRs, with additional comparisons to the UL by EFSA due to an absence of guidelines provided by the UK Government. Regarding the UL, there is only data for n = 10 dietary components, in the cases where there is no UL provided, this is usually due to limited data to derive a UL for the nutrient in question.[20] This analysis used the UL values provided for the age groups of 15–17 and 18+, as the YOI houses prisoners aged between 15 and 18 years.

Statistical analyses

Recipes were provided by the catering supplier and entered into the nutrition software Nutritics (v5.80, Dublin, Ireland) to calculate the nutritional content of each recipe for an individual portion.[21] Recipe data were organised in Microsoft Excel and exported to IBM SPSS Statistics (v29.0, New York, NY, USA) for Macintosh.[22] The final analysis was a comparison of the nutrient profiles between the new (2022) and old (2019) menu to identify changes in nutritional content between the two. Data were assessed for normality using the Shapiro–Wilk test. When comparing macro and micronutrient menu data to the GDRs, and ULs, the One-Sample *T*-Test was used for normally distributed data, and the Wilcoxon One-Sample Signed-Rank test for non-normal data. When comparing the old (2019) and new (2022) menu an Independent Samples *t*-Test was conducted for normally distributed data, and The Mann–Whitney *U* test for non-normally distributed data. Data were considered significant at P-value ($P < 0.05$).

Results

Comparison of new (2022) menu provision to GDRs

Out of the n = 30 dietary components tested, all results were found to differ from their GDR target ($P < 0.05$), with n = 24 exceeding their respective GDR and n = 6 failing to meet their target. See Table 1 for a full list of dietary components, and results.

Comparison of new (2022) menu to upper intake limit

UL values are provided for only n = 10 dietary components. N = 9 were found to be below their UL value ($P < 0.01$), while only magnesium was found to be exceeding its UL figure ($P < 0.01$) (Table 2).

Comparison of old (2019) menu provision to GDRs

Out of the n = 30 dietary components tested, n = 29 were found to be significantly different, with n = 25 found to be exceeding their respective GDR target, and n = 4 significantly below ($P < 0.05$) (Table 3).

Comparison of old (2019) menu to upper intake limit

Of the 10 UL values provided, n = 9 dietary components were found to be below their UL value ($P < 0.01$) (Table 4). While

Table 1. Comparative analysis of macro and micronutrient composition of the 2022 28-day menu provision and UK Government dietary recommendations for young males aged 15–18 years old

Dietary component	Mean	SD	GDR target*	Mean difference	Sig.
Energy (kcal)	2696	97	2500	196	<0.01[a]
Macronutrients					
Carbohydrates (g)	388	19	333	55	<0.01[a]
Free sugars (g)	63‡	8	33†	30	<0.01[b]
Fat (g)	88	7	97†	–9	<0.01[a]
Monounsaturated fat (g)	27	3	36	–9	<0.01[a]
Polyunsaturated fat (g)	12	2	18	–6	<0.01[a]
Saturated fat (g)	33	3	31†	2	<0.01[a]
Fibre (g)	39	4	30	9	<0.01[a]
Protein (g)	84.8	5.4	55.2	29.6	<0.01[a]
Salt (g)	8‡	1	6†	2	<0.01[b]
Vitamins					
Folates (µg)	390	25	200	190	<0.01[a]
Niacin (mg)	39.1	1.9	16.5	22.6	<0.01[a]
Riboflavin (mg)	2.2	0.2	1.3	0.9	<0.01[a]
Thiamine (mg)	3	0.4	1	2	<0.01[a]
Vitamin A (µg)	981‡	218	700	281	<0.01[b]
Vitamin B12 (µg)	4.9	0.5	1.5	3.4	<0.01[a]
Vitamin B6 (mg)	2.8	0.1	1.5	1.3	<0.01[a]
Vitamin C (mg)	129	21	40	89	<0.01[a]
Vitamin D (µg)	8	0.8	10	–2	<0.01[a]
Minerals					
Calcium (mg)	1118	103	1000	118	<0.01[a]
Chloride (mg)	4960‡	79	2500	2460	<0.01[b]
Copper (mg)	2.2	0.2	1	1.2	<0.01[a]
Iodine (µg)	131	17	140	–9	<0.01[a]
Iron (mg)	21	2	11	10	<0.01[a]
Magnesium (mg)	371	25	300	71	<0.01[a]
Phosphorus (mg)	1494	84	775	719	<0.01[a]
Potassium (mg)	4260	338	3500	760	<0.01[a]
Selenium (µg)	47	6	70	–23	<0.01[a]
Sodium (mg)	2986‡	452	2400	586	<0.01[b]
Zinc (mg)	15.3	0.6	9.5	5.8	<0.01[a]

SD, standard deviation; GDR, Government Dietary Recommendations; kcal, calories; g, grams; µg, micrograms; mg, milligrams.
[a]One-Sample *t* Test.
[b]One-Sample Wilcoxon Signed Rank Test.
*GDR target is based on the values for males aged between 15 and 18.
†Indicates that the GDR is the maximum allowance for this dietary component.
‡Median.

Table 2. Comparison of macro and micronutrient content in the 2022 28-day menu with EFSA tolerable upper intake Levels for young males aged 15–18 years old

	Mean	UL (ages 15–17)	Mean Difference	UL (age 18)	Mean Difference	Within UL	Sig.
Minerals							
Calcium (mg/day)	1118	N.D.	N.D.	2500	N.D.	Yes	<0.01[a,c]
Copper (mg/day)	2.2	4	–1.8	5	–2.8	Yes	<0.01[a,c]
Iodine (µg/day)	131	500	–369	600	–469	Yes	<0.01[a,c]
Folates (µg/day)	390	800	–410	1000	–610	Yes	<0.01[a,c]
Magnesium (mg/day)	371	250[d]	121	250[d]	121	No	<0.01[a,c]
Selenium (µg/day)	47	250	–203	300	–253	Yes	<0.01[a,c]
Zinc (mg/day)	15	22	–7	25	–10	Yes	<0.01[a,c]
Vitamins							
Vitamin A (µg/day)	981‡	2600	–1619	3000	–2019	Yes	<0.01[b,c]
Vitamin B6 (mg/day)	3	20	–17	25	–22	Yes	<0.01[a,c]
Vitamin D (µg/day)	8	100	–92	100	–92	Yes	<0.01[a,c]

UL, tolerable upper intake level; mg, milligrams; N.D., no data; µg, micrograms; EFSA, European Food Safety Authority.
[a]One-Sample *t* Test.
[b]One-Sample Wilcoxon Signed Rank Test.
[c]Result is significant for both UL (15–17), and UL (18).
[d]Does not include magnesium naturally present in food or beverages.
‡Median.

Table 3. Comparative analysis of macro and micronutrient composition of the 2019 28-day menu provision and UK Government dietary recommendations for young males aged 15–18 years old

Dietary component	Mean	SD	GDR target*	Mean difference	Sig.
Energy (kcal)	2903‡	240	2500	403	<0.01[b]
Macronutrients					
Carbohydrates (g)	406‡	37	333	73	<0.01[b]
Free sugars (g)	61‡	14	33†	28	<0.01[b]
Fat (g)	102	11	97†	5	<0.01[a]
Monounsaturated fat (g)	33	4	36	–3	<0.01[a]
Polyunsaturated fat (g)	12‡	2	18	–6	<0.01[b]
Saturated fat (g)	38	6	31†	7	<0.01[a]
Fibre (g)	34	3	30	4	<0.01[a]
Protein (g)	83.1‡	6.4	55.2	27.9	<0.01[b]
Salt (g)	10	2	6†	4	<0.01[a]
Vitamins					
Folates (µg)	321	28	200	121	<0.01[a]
Niacin (mg)	39.8‡	2.9	16.5	23.3	<0.01[b]
Riboflavin (mg)	2.1	0.3	1.3	0.8	<0.01[a]
Thiamine (mg)	2.4	0.3	1	1.4	<0.01[a]
Vitamin A (µg)	937‡	355	700	237	<0.01[b]
Vitamin B12 (µg)	3.7‡	0.9	1.5	2.2	<0.01[b]
Vitamin B6 (mg)	2.3	0.2	1.5	0.8	<0.01[a]
Vitamin C (mg)	103‡	18	40	63	<0.01[b]
Vitamin D (µg)	2‡	1	10	–8	<0.01[b]
Minerals					
Calcium (mg)	1252‡	202	1000	252	<0.01[b]
Chloride (mg)	6146	1080	2500	3646	<0.01[a]
Copper (mg)	1.5	0.2	1	0.5	<0.01[a]
Iodine (µg)	147‡	33	140	7	<0.05[b]
Iron (mg)	15	2	11	4	<0.01[a]
Magnesium (mg)	327	30	300	27	<0.01[a]
Phosphorus (mg)	1467‡	161	775	692	<0.01[b]
Potassium (mg)	3934	270	3500	434	<0.01[a]
Selenium (µg)	46	6	70	–24	<0.01[a]
Sodium (mg)	3849	597	2400	1449	<0.01[a]
Zinc (mg)	9.3	0.8	9.5	–0.2	0.22[a]

SD, standard deviation; GDR, Government Dietary Recommendations; kcal, calories; g, grams; µg, micrograms; mg, milligrams.
[a]One-Sample *t* Test.
[b]One-Sample Wilcoxon Signed Rank Test.
*GDR target is based on the values for males aged between 15 and 18.
†Indicates the GDR is the maximum allowance for this dietary component.
‡Median.

only magnesium was found to be exceeding its UL figure ($P < 0.01$).

Comparison of new (2022) and old (2019) menu

Regarding the results of macro and micronutrients, there were $n = 22$ significant differences between the two menus ($P < 0.05$). Of these, $n = 13$ was higher in the new menu, while $n = 9$ was lower (Table 5; Fig. 1). Of those which were higher in the new menu, these included copper, fibre, folates, iron, magnesium, potassium, riboflavin, saturated fat, zinc, vitamin B12, B6, C, and D ($P < 0.05$). The $n = 9$ which was lower in the new menu included calcium, chloride, energy, fat, iodine, monounsaturated fat, salt, sodium, and thiamine ($P < 0.05$).

Discussion

This study aimed to profile the menu offered to young male offenders in an English YOI and to identify changes between the new and old menus. Overall, the results indicated a positive improvement in the content of the 2022 menu, although there are still areas for improvement to note.

2022 menu and GDRs

In the 2022 menu, numerous instances were identified where dietary components exceeded the GDRs. These components included calcium, carbohydrates, chloride, copper, energy, fibre, folates, free sugars, iron, magnesium, niacin, phosphorus, potassium, protein, riboflavin, salt, saturated fat, sodium, thiamine, and vitamins A, B12, B6, C, and zinc. Most of these exceedances do not raise concerns, as further demonstrated by a comparison to the ULs, where for calcium, copper, folates, magnesium, vitamins A, B6, and zinc, these were below their respective ULs. Only magnesium exceeds both limits, however, the UL for magnesium applies to magnesium compounds found in nutritional supplements or added to food, not the naturally occurring magnesium in foods.[23] Therefore, in this analysis, which only considered nutrients naturally present in foods, magnesium exceeding its UL limit is not of concern. It is worth noting that for 20 out of the 30 dietary components

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Table 4. Comparison of macro and micronutrient content in the 2019 28-day menu with EFSA tolerable upper intake Levels for young males aged 15–18 years old

	Mean	UL (ages 15–17)	Mean difference	UL (age 18)	Mean difference	Within UL	Sig.
Minerals							
Calcium (mg/day)	1202‡	N.D.	N.D.	2500	–1298	Yes	<0.01[b,c]
Copper (mg/day)	1.5	4	–2.5	5	–3.5	Yes	<0.01[a,c]
Iodine (μg/day)	147‡	500	–353	600	–453	Yes	<0.01[b,c]
Folates (μg/day)	321	800	–479	1000	–679	Yes	<0.01[a,c]
Magnesium (mg/day)	327	250[d]	77	250[d]	77	No	<0.01[a,c]
Selenium (μg/day)	46	250	–204	300	–254	Yes	<0.01[a,c]
Zinc (mg/day)	9	22	–13	25	–16	Yes	<0.01[a,c]
Vitamins							
Vitamin A (μg/day)	937‡	2600	–1663	3000	–2063	Yes	<0.01[b,c]
Vitamin B6 (mg/day)	2	20	–18	25	–23	Yes	<0.01[a,c]
Vitamin D (μg/day)	2‡	100	–98	100	–98	Yes	<0.01[b,c]

UL, tolerable upper intake level; mg, milligrams; N.D., no data; μg, micrograms; EFSA, European Food Safety Authority.
[a]One-Sample *t* Test.
[b]One-Sample Wilcoxon Signed Rank Test.
[c]Result is significant for both UL (15–17), and UL (18).
[d]Does not include magnesium naturally present in food or beverages.
‡Median.

Table 5. Comparative analysis of the dietary components from the 2019 and 2022 menus for male young offenders aged 15–18 years old

	2019 Menu		2022 Menu				
Dietary component	Mean	SD	Mean	SD	Mean diff.	95% CI	Sig.
Energy (kcal)	2903*	240	2705*	97	–198	–239, –104	<0.05[d]
Macronutrients							
Carbohydrates (g)	405*	37	390*	19	–15	–24, 0.8	0.06[d]
Free sugars (g)	61*	14	63*	8	2	–2, 10	0.12[d]
Fat (g)	102	11	88	7	–14	–19, –9	<0.01[a,b]
Monounsaturated fat (g)	33	4	27	3	–6	–8, –4	<0.01[a,b]
Polyunsaturated fat (g)	12.1*	1.6	11.9*	1.8	–0.2	–1, 0.9	0.96[d]
Saturated fat (g)	38	5.7	33.4	3.3	4.6	–7, –2	<0.01[a,c]
Fibre (g)	34	3	39	4	5	2, 6	<0.01[a,b]
Protein (g)	83.1*	6.4	83.9*	5.4	0.8	–0.5, 5.6	0.12[d]
Salt (g)	9.8*	1.5	7.5*	1.1	–2.3	–3, –1	<0.01[d]
Vitamins							
Folates (μg)	321	28	390	25	69	55, 83	<0.01[a,b]
Niacin (mg)	39.8*	3	39.2*	1.9	–0.6	–1.7, 0.8	0.51[d]
Riboflavin (mg)	2.1	0.3	2.2	0.2	0.1	0.02, 0.3	<0.05[a,c]
Thiamine (mg)	2.4	0.3	2.8	0.4	–0.4	0.2, 0.6	<0.01[a,b]
Vitamin A (μg)	936.5*	355.2	980.8*	216.7	44.3	–124, 101	0.90[d]
Vitamin B12 (μg)	3.7*	0.9	4.9*	0.5	1.2	0.7, 1.7	<0.01[d]
Vitamin B6 (mg)	2.3	0.2	2.8	0.2	0.5	0.5, 0.7	<0.01[a,b]
Vitamin C (mg)	103*	18	129*	21	26	13, 36	<0.01[d]
Vitamin D (μg)	2*	1	8*	1	6	6, 7	<0.01[d]
Minerals							
Calcium (mg)	1252*	202	1134*	103	–118	–186, –34	<0.05[d]
Chloride (mg)	6203*	1080	4960*	793	–1243	–1604, –443	<0.01[d]
Copper (mg)	1.5	0.2	2.2	0.2	0.7	0.6, 0.8	<0.01[a,b]
Iodine (μg)	147*	33	132*	17	–15	–41, –10	<0.01[d]
Iron (mg)	18	2	21	2	3	2, 4	<0.01[a,b]
Magnesium (mg)	327	30	371	25	45	30, 60	<0.01[a,b]
Phosphorus (mg)	1467.4*	161	1489.5*	83.5	22.1	–35, 99	0.39[d]
Potassium (mg)	3934.3	270.4	4259.5	338.4	325	161, 489	<0.01[a,b]
Selenium (μg)	46.1	6.1	47.1	6.4	–1	–2, 4	0.56[a,c]
Sodium (mg)	3937.1*	597.4	2986.2*	52.1	–950.9	–1107, –544	<0.01[d]
Zinc (mg)	9.3*	0.8	15.3*	0.6	6	5.6, 6.4	<0.01[a,b]

SD, standard deviation; CI, confidence intervals; kcal, calories; g, grams; μg, micrograms; mg, milligrams.
[a]Independent Samples *T* Test.
[b]Equal variances assumed.
[c]Equal variances not assumed.
[d]Independent-Samples Mann–Whitney *U* Test.
*Median value.

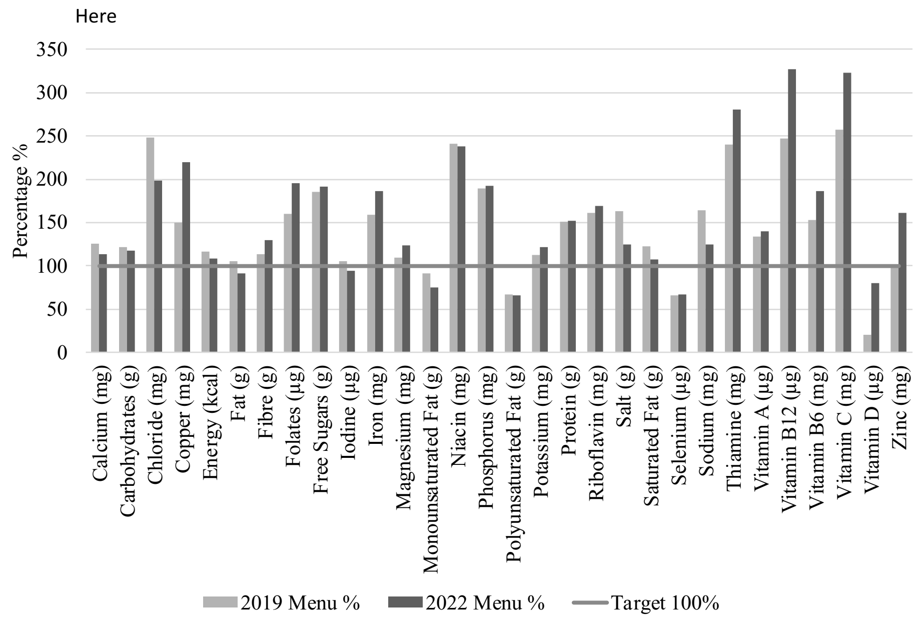

Fig. 1. 2022 and 2019 menu dietary components as a percentage, identifying those which have met their GDR target.

analysed, no ULs are established by the EFSA. The absence of UL values can be attributed to insufficient evidence, or the available evidence suggests that consuming these components at higher quantities than their respective GDR targets does not pose a major health risk.[20,24]

Carbohydrates, chloride, energy, fibre, free sugars, niacin, phosphorous, potassium, protein, riboflavin, salt, saturated fat, sodium, thiamine, vitamin B12, and C significantly exceeded their GDRs without an associated UL. Among these, saturated fat, free sugars, sodium, and salt standout as concerns as the GDRs state these should not be consumed above their targets. While the average quantities for saturated fat and salt exceed their respective GDR values by only 2 g, sodium and free sugars present a more significant concern, with free sugars nearly doubling the recommended 33g at 66g. These findings highlight a potential concern due to the associated health risks of a diet high in fat, sugar, and salt. High salt and sodium intake in adults can lead to hypertension and coronary heart disease, with evidence further suggesting similar risks for adolescents.[25–29]

With salt exceeding the GDR, it is then unsurprising that sodium is also exceeding its respective GDR. The health risks associated with high salt are similar to that of high sodium in the diet, including increasing risk of hypertension, chronic kidney disease, cardiovascular disease and potentially osteoporosis.[30] Though these health risks are primarily evidenced in adults, a high salt diet from a young age can contribute to these potential health risks over time. The issues of high sodium in prisoners have been previously identified and reported as an area of concern. For example, Chrisostomou *et al.* (2019) investigated UK prisoner food choice, where choices led to sodium intake being significantly above the recommended intake (mean = 3056 mg, SD = 345, 95% CI = 2899–3214, $P < 0.01$).[31] A more recent study by Johnson *et al.* (2022) looked at the nutrition content of menus in Canadian penitentiaries. Here sodium was also highlighted as exceeding the Canadian dietary reference intakes (DRIs) (1500 mg; mean = 3404.2), and this figure would also be exceeding UK recommendations.[32]

However, we did find that the menu was offering less salt and sodium than the intake of males (19–64y) based on the latest results from the National Diet and Nutrition Survey (NDNS). The estimated salt intake from the NDNS was reported as 9.2 g, and the sodium intake of 3256 mg.[33] Though the results of the NDNS were for male adults, and this menu analysis is for adolescents, it was worth noting the general population intake to put the menu results into the context of the wider population. While no UL is provided by the EFSA for sodium, they do highlight evidence indicating sodium's potential role in hypertension, putting one at risk of renal and cardiovascular issues.[34] Johnson *et al.* (2022) noted that achieving a diet with sodium below the recommendations can be found unpalatable for many, with added salt found to improve the sensory properties of most foods for human consumption at a low cost.[32,35] This becomes a difficult trade-off between providing a diet for prisoners which is palatable while minimising potential health risks from dietary components like sodium. However, the overall recommendation would be to reduce sodium or use low-sodium food alternatives.

Along with sodium, the high levels of free sugar (mean = 63 g, GDR = 33 g) almost double the maximum GDR allowance, pose potential health risks for young offenders due to increasing risk of weight gain, leading to obesity.[36,37] In adult life, this can pose further risks for coronary heart disease and type-2 diabetes.[38] Food items included in menus leading to this risk include sugar packets, and dessert items such as sponge cake, doughnuts, and biscuits. However, the problems with free sugars may be far worse, as we are only considering menu items. Prisoners also have the option to purchase their own foods from a prison shop (known in prison as the 'canteen') where, for example, Morley *et al.* (2019) demonstrated that prison shop food contained 12 times the amount of foods containing high levels of sugar, fat and salt based off the NHS Eatwell Guide reccomendations.[39] Although shop purchases were not assessed in the current study, it can be assumed based on general prison food choice studies, that this will contribute negatively to total free sugar consumption. Future research should endeavour to assess all possible dietary provisions in prison settings, including dietary intake from the menu provided, and any shop purchases made. This will allow for the identification of food options available to prisoners which are contributing the most to the intake of free sugars, salt, sodium, and saturated fat, and in turn revaluation of these food items inclusion in prisons.

While the menu content identifies that energy intake was exceeding the GDR, it was only exceeded by 196 kcal. Any future changes to the menu would need to be mindful of this, as altering food options may reduce the average calories on offer. For example, the breakfast pack provided contained items such as cereal, sugar packets, tea, coffee, whitener, and hot chocolate. This was found to be a major cause for the high level of free sugars. When adjusting the breakfast pack and removing most of the high-sugar items, the menu almost met the GDR for free sugar with 34 g. However, this led to the new issue of the menu being below the GDR for energy intake with 2395 kcal. This highlights both the concern that many calories were coming from high-sugar items, as well as that any further changes to the menu would need to be mindful of a knock-on effect on the content of other dietary components.

Though much of this discussion has revolved around what is exceeding the GDRs, consideration must also be given to those below. In particular, we found monounsaturated fat, polyunsaturated fat, iodine, selenium, and vitamin D all below targets. There is a lower reference nutrient intake for selenium, which indicates that for males 18 + 40 μg per day will suffice, and the mean selenium in the menu was 47 μg.[40] Therefore, selenium is not a huge concern in this menu, though improvements could be made. A broad recommendation would be to increase the menu in food items such as tofu and various fishes such as salmon, mackerel tuna, and herring, which would increase the levels of selenium, iodine, vitamin D, polyunsaturated and monounsaturated fat. Replacing red meat on the menu with options such as tofu and fish could decrease the total saturated fat content of the menu. However, given the budget in prisons, these options may not be financially feasible, but we would recommend further investigation of what foods are available by the suppliers to the prisons, to keep dietary choices in closer alignment with the GDRs.

2019 to 2022 menu comparison

Looking at the 2019 and 2022 menu comparison, although the 2022 menu analysis identifies areas for further improvement, overall, there were positive changes highlighting the efforts made to improve the nutrition on offer.

For example, fat content exceeded the GDR in 2019, and this has now been corrected in the 2022 menu with fat significantly below the GDR. The 2019 menu contained more use of beef in recipes, with eight beef-containing recipes, compared with the 2022 menu which reduced the total number down to just three. This would have had a major impact on the total fat content of each menu, and possibly a cause for the reduction of total fat, saturated fat, and monounsaturated fat. This also allows the new menu to be more aligned with government recommendations regarding reducing the consumption of red meat.[41] By providing fewer options containing beef, the menu has improved upon this.

Many of the recipes containing beef were also high in carbohydrates, with recipes such as beef baguette, and lasagne, which would have contained high carbohydrate items like bread and pasta. Changing this is likely one of the reasons why there was a reduction in carbohydrates seen in the new menu, and although this change did not reach significance it still represents a movement towards overall improvement.

Although energy, salt, sodium, and saturated fat all exceeded their GDR in the 2022 menu, these four have significantly improved from the 2019 menu, likely due to the changes mentioned previously. Energy is now 196 kcal over the GDR, whereas previously it was 313 kcal. This improvement seen for energy was in part due to the greater variation in menu items in 2022. The 2019 menu had many food items frequently repeated across the 4-weeks. Examples include pitta bread, sandwiches, baguettes, and other carbohydrate-heavy options. While the 2022 menu has these same items, they are not repeated as frequently and do not often appear in the dinner menu options. New dinner options include recipes with higher quantities of vegetables, such as homemade pies containing vegetables and protein (e.g. fish, chicken). This reduction potentially could lead to a decrease in the risks associated with a diet high in calories, such as reducing the risk of obesity.[42] Given that the prisoner populations tend to have a greater restriction for exercise opportunities, it is important to be mindful of a diet exceeding the recommendation for calorie intake. However, in this new menu the average content of calories was only slightly higher, so whether this does pose a great risk would depend on further research identifying exactly what prisoners are consuming.

Considering salt and sodium, the reduction between the two menus is significant, however, the levels of both are still quite high given the known risks associated with a diet high in salt and sodium.[26] The prison population overall is dealing with the burden of being an aging population, and evidence indicates the increase in health risks associated with high salt and sodium diet for older people.[43] While for young offenders the concern of aging and high salt and sodium diet may not be apparent at first, it is important to consider that there is a roughly 32.5% reoffending rate amongst young offenders as of 2021.[44] The majority of those who reoffend were between the ages of 15–18, and it's likely with continued reoffences these young offenders will move into the adult population. Regardless of whether a young offender reoffends, or stays within the general population, as earlier identified the general population does consume a high salt diet, X g above the recommendations. It would therefore be important for young offenders to consume a diet within the dietary requirements in addition to instilling healthier behaviours prior to release, to reduce the risk of high blood pressure, coronary heart disease, and osteoporosis in the long-term.[45–47]

Additional risks to osteoporosis include a diet low in vitamin D.[48] Vitamin D is difficult to obtain through diet, and in keeping with this vitamin D was significantly below the GDR in the 2019 and 2022 menus. These results reflect previous research, for example, Mommaerts *et al.*[49] (mean = 4.84), a menu analysis by Stanikowski *et al.*[50] (mean = 5.10), and a dietary analysis by Gesch *et al.*[51] (mean = 3.50). While vitamin D was below the GDR in both menus, the 2022 menu was significantly higher than in 2019. This demonstrates the efforts made by stakeholders responsible for catering to improve the new menu. The key effort was the inclusion of a vitamin cordial juice drink, with two offered per day for prisoners, containing an additional 5ug of vitamin D. This vitamin juice drink was also a contributing factor to why zinc is now meeting the GDR in the 2022 menu. Given the status of vitamin D being difficult to obtain through diet, the use of supplements offers an alternative to achieving dietary requirements. This would be particularly beneficial in a prison environment due to prisoners' limited access to outside spaces, which further restricts their ability to obtain vitamin D through sunlight.[52]

As with the improvement in vitamin D, zinc was also found to meet its GDR in the new menu, in part due to the vitamin juice drink. This now reduces the potential impacts on the immune system caused by zinc deficiency.[53] Overall, the idea of supplementation in prisons is not new, and there have been many studies which have used supplements which aimed to identify improvements in aspects of mental health and behaviours.[51,54,55] However, a key issue is many of these studies did not include a baseline dietary analysis, therefore whether improvements were due to participants now meeting GDRs through supplements is unclear. One study which did include blood measures at baseline, did identify that the group taking omega-3 supplements increased their blood levels of omega-3 by the end of the intervention period.[56] Currently, within the author's research group, there is further work in progress investigating the impact of vitamin D supplements in prisons, however, this work is not yet complete.

While this work has focused on assessing the menus of a YOI, the importance of food does extend beyond providing nutrition. Food is linked to social interactions and can be affected by social, cultural, and religious identities, playing a pivotal role in celebrations and bonding.[47,57] During an offender's sentence, meals provided can be a focal point of the day and provide a break from the routine and often a chance to be social with each other. With food options provided having the potential to be of varied quality, and quantity, with limited options, this can not only lead to poor physical health but have a role in mental wellbeing. Surveys of YOI offenders found that they often commented on the menu content and food quality in a negative manner. This included a lack of fresh fruit and vegetables, too many high-salt foods, processed foods which were too fatty, too many carbohydrates, and a lack of protein.[47] These YOI offender comments mirroring many outcomes of this analysis.

While overall this work is able to recognise the efforts of the justice service in improving diets and a commitment towards meeting the GDRs. We do identify a few areas which need continued work to ensure that offenders are achieving optimal nutrition and health.

Limitations and next steps

These results are specific to a single YOI, and therefore not necessarily generalisable to male or female adult prisons, or female YOIs. While similar budgets for providing food to prisoners exist across the UK, a budget of around £2 per prisoner per day, the menus are subject to the individual prisoners catering manager to devise.[8] Secondly, this study consisted of a menu content analysis, therefore, these results reveal what nutrient content, on average, is available to prison residents, but cannot offer information on prisoner consumption. The collection of food diary data can be difficult in a prison setting due to issues of low literacy, as well as general limitations of food diaries such as misreporting portion sizes.[58] Therefore, performing an overall menu analysis can give an indication as to the nutrition content available to prisoners.

Additionally, prisoners can purchase food items through the onsite canteen (prison shop), and these items were not factored into this menu analysis. An important next step would be to conduct a food diary analysis, within this prison, which would identify what prisoners are consuming from the 2022 menu, as well as considering any food items purchased via the canteen. Finally, a further menu analysis could be performed for male and female adult prisons.

Conclusion

Though areas of concern were identified, the new menu has, however, made numerous attempts to offer prisoners a diet more in line with recommendations. Key areas of improvement include the provision of the vitamin juice drink which supports prisoners in meeting their recommendations for vitamin D and zinc. Additionally, the added variety in prison meals has increased fruit and vegetables and reduced total and saturated fat. Moving forward, the results of this study have led to a new breakfast pilot, where sugar will be removed from the breakfast packs, aiming to reduce total sugar content of the menu. Additionally, the results of this study have led to further efforts to improve recipes to provide prisoners with nutritious food that meets dietary guidelines.

Abbreviations

DRIs: Dietary reference intakes; **DRVs:** Dietary reference values; **EFSA:** European Food Safety Authority; **GDR:** Government dietary recommendations; **HMPPS:** His Majesty's Prison and Probation Service; **NDNS:** National Diet and Nutrition Survey; **UL:** Tolerable upper intake level; **YOI:** Young Offenders Institution.

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Conflict of interest

The authors have no conflicts of interest to declare.

Authorship

This study was conceptualised by J.T, S.C, C.A-M.G, and M.P, which involved the research question and study design. M.P collected and analysed the data and wrote the initial manuscript. J.T, S.C, C.A-M.G, and B.L provided further comments, and editing of the final draft prior to final manuscript submission.

Ethical standards disclosure

This work did not involve the use of human participants.

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RESEARCH ARTICLE

Nutritional status and its associated factors among infants and young children aged 6–23 months in Addis Ababa, Central Ethiopia, 2021: a cross-sectional study

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Abstract

The aim of this study is to assess nutritional status and associated factors among infants and young children aged 6–23 months in Yeka sub-city, Ethiopia, 2021. An institution-based cross-sectional study was conducted in selected health centres found in the Yeka sub-city from May 2021 to July 2021. In total, 396 systematically selected infants and young children aged 6–23 months attended the selected health centres were included in the study. Data were collected by using a structured questionnaire and anthropometric measurements. A multinomial logistic regression model was used.

The overall magnitude of undernutrition and overnutrition among infants and young children were 24.7% and 5.5%, respectively. Dietary diversity score (DDS) ((adjusted odd ratio (AOR) = 5.65; 95% CI = 2.301, 10.87; P value = 0.003), minimum meal frequency (MMF) (AOR = 5.435; 95% CI = 2.097, 11.09; P value = 0.0052), and diarrhoea (AOR = 2.52; 95% CI = 1.007, 6.310; P value = 0.002) were statistically significantly associated factors for nutritional status among infants and young children. Malnutrition (undernutrition and overnutrition) is a public health problem among infants and young children in Yeka sub-city, Ethiopia. DDS, MMF, and diarrhoeal disease were associated with higher odds of undernutrition.

Key words: **Cross-sectional study: Ethiopia: Nutritional status: Yeka sub-city: Young children**

Background

Child malnutrition is a major public health and development concern in most poor communities leading to high morbidity and mortality throughout the world.[1] Inadequate nutrition during the first 2 years of life leads to childhood morbidity including inadequate brain development and mortality. Infants are at increased risk of malnutrition by 6 months when breast milk alone is no longer sufficient to meet their nutritional requirements.[2] Globally, 149.2 million children under 5 years of age are stunted, 45.4 million are wasted, and 38.9 million are overweight.[3] The prevalence of undernutrition in the rural parts of Ethiopia was 47.6% stunted, 29.2% underweight, and 13.4% wasted.[4] The prevalence of undernutrition in the urban parts of Ethiopia was 33.8% stunted, 12.6% underweight, and 8.3% wasted.[5]

The nutritional status of children from the lower socio-economic class was poor as compared to their counterparts in the upper socio-economic class.[1] Many infants in sub-Saharan Africa start getting cereal-based supplemental feeds well before the age of 6 months, and in rare cases, they do not get them until the second year.[2]

Infant and young child feeding (IYCF) practices are multidimensional and change rapidly in short intervals in the first year of life, socio-demographic, and socio-economic factors and feeding practices affect the nutritional status of children aged 6 months and older. The Infant and Child Feeding Index is a composite index that measures complete feeding practices for infants and young children.[6] Inappropriate IYCF practices in the first 2 years of life are among the major causes of childhood malnutrition in developing countries, including

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Ethiopia.[7] Early introduction of solid foods and transitional feeding affect the early origin of weight gain and obesity risk.[8]

Poor feeding patterns, low socio-economic position, rural residency, insufficient health service coverage, and diseases are some of the factors that have an impact on nutritional status. A significant indicator of newborns' and early children's health status is their nutritional status.[9] Children's obesity is one of the twenty-first century's most critical public health problems in the world.[10]

In Ethiopia, malnutrition is one of the most serious health and welfare problems among infants and young children commonly high prevalence of chronic malnutrition and undernutrition.[11] Undernutrition of infants and young children is affected by inappropriate IYCF practices.[12] The nutritional status of infants and young children is affected by several factors including the socio-economic status of the family.[13] The nutritional status of infants and young children is affected bilaterally, which results in overnutrition and undernutrition. Undernutrition has short-term and long-term impacts. The short-term impact of undernutrition is immunosuppression and increase risk of infection, which is caused by, inadequate food intake and repeated infection (diarrhoeal diseases and acute upper respiratory tract infection (AURTI)) and also, the long-term impact of undernutrition is growth and development retardation, decreased school performance, and decreased productivity at a later age.[14] Infants and young children who initiate early mixed feedings with breast milk are more likely to become obese at a young age.[8]

Even though there are a number of studies conducted in different parts of the world, there is limited information on the magnitude of nutritional status and associated factors among infants and young children aged 6–23 months in Ethiopia. The objective of this study was to assess nutritional status and associated factors among infants and young children aged 6–23 months in order to address the aforementioned gap in the area.

Methods and materials

Study design and setting

An institutional-based cross-sectional study was conducted. This study was conducted in four selected health centres (Hiddassie Health Center, Yeka Abado Health Center, woreda 12 Health Center, and Woreda 13 Health Center) in the Yeka sub-city from May 2021 to July 2021. Yeka sub-city is one of the sub-cities located North East of Addis Ababa, Ethiopia.

Sample size determination and sampling procedure

The sample size was calculated by using the formula for estimation of single population proportion, considering 43% prevalence,[15] with a 95% CI and allowable error of 5%, and the final sample size was 396.

Study participants were selected using a systematic sampling technique among infants and young children, who were attending four selected health centres during the study period. Proportional allocation was used to maintain proportionality among the four selected health centres. A systematic sampling technique with a sampling interval of two was used to select infants and young children aged 6–23 months from each institution.

Data collection procedure and measurement/instruments

Data were collected using an interviewer-administered structured questionnaire (supplementary file) and anthropometric measurements. Data were collected by trained clinical nurses. The interview was conducted in a separate room.

The questionnaire was adapted from previous literature and it was modified to the context of this study. The training was given to both supervisors and data collectors. The pre-test was conducted on 5% of the sample size at Hiddassie Health Center before the actual data collection period. A necessary correction was made based on the results of the pre-test data. The questionnaire was translated into the local language (Amharic) and back to English by fluent speakers of the two languages. Strict supervision was done by supervisors, and the overall quality of the data collection was also monitored by the principal investigator. The collected data were checked for completeness and consistency before starting, processing, and analysing data.

Dietary diversity score (DDS) and minimum meal frequency (MMF) were assessed using the 24-h dietary recall method. The MMF was fulfilled if the food was received two to three times per day at 6–9 months of age, three to four times per day at 9–11 months of age, and three to four times at 12–24 months of age, with additional nutritious snacks offered one to two times per day between meals in the last 24 h. The dietary diversity score was fulfilled if infants and young children consumed five or more food groups in the last 24hrs from the nine food groups.[16]

Weight and length were taken for each study participant. Length was measured using a length board and recorded to the nearest 0.1 cm. The bodyweight was measured using a weight scale when the participant wore light clothing, was barefoot, and recorded the weight to the nearest 0.1 kg. Measuring instruments were checked and calibrated before the procedure to make measurements more reliable. Finally, weight-for-length, weight-for-age, and length-for-age were also measured, and it was classified according to WHO classification to determine the nutritional status of the respondents.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board of Addis Ababa Public Health Research and Emergency Management Directorate with reference number PHREM 1342/2021. Written informed consent was obtained from all participants.

Operational definition

Undernutrition: When the body does not have adequate amount of one or more nutrients reflected in biochemical tests like haemoglobin level for anaemia, in anthropometric indicators such as stunting (low height-for-age) or wasting (low weight-for-height) and/or weight-for-age (underweight).[17,18]

Wasting: weight-for-length < -2 Z score of the median WHO child growth standards.[17,18]

Stunting: length-for-age < -2 Z score of the median WHO child growth standards.[17,18]

Underweight: weight-for-age < -2 Z score of the median WHO child growth standards.[17,18]

Overweight: weight-for-height > 2 standard deviations above the median.[17,18]

Obese: weight-for-height > 3 standard deviations above the median.[17,18]

Complementary feeding: refers to a process of introducing the infant to additional sources of nutrition other than breast milk, usually at the age of 6 months.[19]

Dietary diversity score: the standard guideline (FAO) for the individual determinant DDS should be focused on the amount and type of food consumed at the individual level.[17,18]

Low family size: family size means the number of persons counted as members of an individual's household; when a family have four or fewer persons, we classify it as low family size.[20]

Improved source of water: improved drinking water sources are those which by nature of their design and construction have the potential to deliver safe water. This includes piped water, boreholes or tube wells, protected dug wells, protected springs, rainwater, and packaged or delivered water.[21]

Improved latrines: are those designed to hygienically separate excreta from human contact. These include wet sanitation technologies (flush and pour flush toilets connecting to sewers, septic tanks, or pit latrines) and dry sanitation technologies (ventilated improved pit latrines, pit latrines with slabs, or composting toilets).[22]

Good solid waste management: good solid waste management practices are considered a success in properly segregating solid waste and/or disposing of it in an authorized location.[23]

Diarrhea: having three or more loose/watery stools in a 24-h or more loose/watery stool than are normal for the individual as entirely reported by the mother/caretaker of the child.[24]

Occupation: is defined as the main work undertaken by the participant/husband. If a participant/husband has more than one job, we report their main job.[25]

Ethics approval and consent to participate

Ethical approval was obtained from the Addis Ababa Public Health Research and Emergency Management Directorate with a reference number PHREM 1342/2021. Permission was obtained from concerned stakeholders. Written informed consent was obtained from parents (mothers/caregivers) of children who attended a selected health centre, in the Yeka sub-city during data collection. The study participants were assured of confidentiality by excluding their names during the period of data collection. The rights were given to study participants to refuse, stop, or withdraw from the interview at any time. Confidentiality was maintained throughout the study.

Statistical analysis

The data were coded, entered using Epi-Data Version 3.1, and exported to SPSS version 25 for analysis, and anthropometric measurements were measured for WHO Z score classification using WHO Anthro plus software (version) v3.2.2. The descriptive summary was presented using frequencies, proportions, figures, and tables. A multinomial logistic regression model was used to analyse the association. Both the bi-variable and multinomial logistic regression analyses were performed to assess the association between dependent and independent variables. All covariates with a P value <0.25 during bi-variable analysis were considered for further multinomial logistic regression analysis to control possible confounders and identify true predictors of nutritional status. Finally, those variables that showed a P value < 0.05, with 95% CI and adjusted odds ratio were considered to declare the variables were significantly associated factors of the dependent variables.

Results

Socio-demographic characteristics of study participants

A total of 396 infants and young children who had attended the selected health centres were included for the study with a response rate of 96%. A total of 335 (88.2%) mothers belonged to the age group of 21–34 years, and 327 (86.1%) mothers delivered their child at the age of 21–34 years. A majority of 196 (51.6%) mothers had 1 child. Among them, mothers/caregivers who had 3 or fewer children, and 4 and above were 353 (92.9%) and 27 (7.1%), respectively. The age interval of delivery below 2 years was 8 (2.1%) and three and above was 176 (46.3%). A majority of 371 (97.6%) mothers were married and 321 (84.5%) had a low family size.

About half, 191 (50.3%) of the mothers were educated at the primary educational level, 53 (13.9%) of mothers were unable to read and write, and 136 (35.8%) of mothers were educated at the secondary and higher educational level. A majority of 318 (83.7%) mothers were unemployed and 293 (77.1%) of mothers were housewives by their occupation. About three-fourth, 297 (78.2%) of mothers had <1000-birr monthly income. A majority of 191 (50.3%) of the husbands were educated at the secondary educational level. A majority of 233 (61.3%) of the husbands were unemployed and 123 (32.4%) of the husbands were in daily labour. A majority of 203 (53.4%) of the husbands have 1000–5000-birr monthly income (Table 1).

Infant and young child characteristics

Of the total 194 (51.1%) infants and young children, 48.9% were male. Approximately 47.9% of children belong to 12–17 months. The mean age of the children was 14.7 months (±SD 4.4) (Table 1).

Breastfeeding practices

A majority of 347 (91.3%) mothers currently practiced breastfeeding. Among mothers who currently practiced breastfeeding, 81.1% practiced exclusive breastfeeding to their infants. A majority of 347 (91.3%) mothers breastfed after 6 months, 19 (5%) infants and young children were fed <6 months, and 14 (3.9%) never breastfed. A total of 132 (34.7%) infants and young children were fed with bottle-feeding. Among the total bottle-feeding, eighty-two (21.6%) infants and young children started bottle-feeding after 6 months and fifty

Table 1. Socio-demographic characteristics of respondents and infants and children characteristics in Yeka sub-city, Ethiopia, 2021 (*n* = 380)

Variables	Category	Frequency	Percentage
Age of mother	15–20 years	8	2.1
	21–34 years	335	88.2
	>34 years	37	9.7
Maternal age at	15–20 years	33	8.7
the time of	21–34 years	327	86.1
delivery	>34 years	20	5.3
Marital status	Married	371	97.6
	Divorced	9	2.4
Number of	Three and less than	353	92.9
children	three	27	7.1
	Four and more		
Birth spacing	No age interval	196	51.6
	Two and more	176	46.3
	Less than 2 years	8	2.1
Number of family	Four and less	325	84.5
size	Five and more	59	15.5
Educational level	Unable to read and	55	14.5
of mother	write	189	49.7
	Primary	136	35.8
	Secondary and		
	higher educational		
	level		
Mother/caregiver	Housewife	288	75.8
occupation	Government and	66	17.4
	private employed	26	6.8
	worker		
	Others		
Mother's monthly	Had no income by	281	73.9
income (birr)	own	14	3.7
	<1000	49	12.9
	1000–5000	36	9.5
	>5000		
Husband's	Unable to read and	39	10.3
educational level	write	151	39.1
	Primary	190	50
	Secondary and		
	higher educational		
	level		
Husband's	Government and	147	38
occupation	private employed	123	32.4
	workers	110	29.6
	Daily labour		
	Others		
Husband's	<1000	26	6.8
monthly income	1000–5000	203	53.4
(birr)	>5000	151	39.7
Infants and	**Age**		
children	6–11 months	82	22.6
characteristics	12–17 months	182	47.9
	18–23 months	112	29.5
	Sex		
	Male	194	51.1
	Female	186	48.9

(13.2%) of them started bottle-feeding before 6 months. On the majority of them, 308 (81.1%) started at the appropriate time of complementary feeding than those who did not start, 72 (18.9%) (Supplementary Table 1).

Dietary diversity score and minimum meal frequency

A majority of 362 (95.3%) infants and young children were fed starchy food. Fish and organ meat was poorly consumed by infants and young children. Dark green leaf and vitamin A-rich fruits and vegetables were consumed by 213 (56.1%) and 299 (78.7%), respectively. A majority of 266 (70%) infants and young children received adequate dietary diversity (DD). A majority of 335 (88.2%) infants and young children meet adequate MMF (Supplementary Table 2).

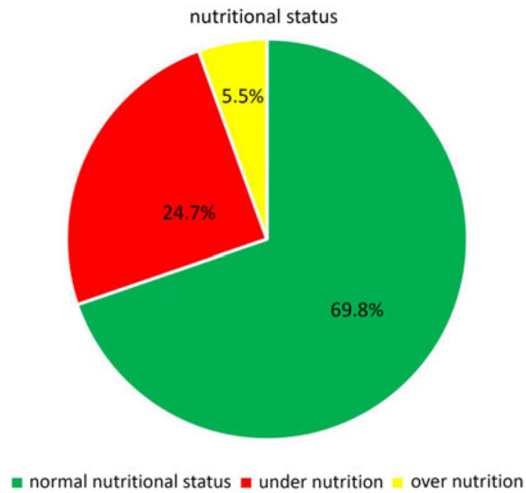

Fig. 1. Nutritional distribution among infants and young children at the age of 6–23 months in Yeka sub-city, Ethiopia, 2021.

Maternity and child health care services

A majority of 278 (73.4%) mothers used contraceptives and 101 (26.6%) mothers did not use contraceptives. All 380 (100%) mothers had ante natal care (ANC) follow-up during pregnancy and all of them delivered at health institutions. A majority of 362 (95.7%) mothers had post natal care (PNC) follow-up and 18 (2.3%) of them did not have PNC follow-up. All 380 (100%) infants and young children were vaccinated, and among them, 231 (60.8%) were fully vaccinated and 149 (39.2%) were vaccinated appropriately depending on their age. Among the total, fifty (13.2%) of infants and young children developed AURTI and forty-seven (12.4%) of them developed diarrhoeal disease (Supplementary Table 3).

Hygiene and sanitation

On the majority, 353 (92.9%) of the mothers/caregivers have an improved source of water and 337 (88.7%) used improved latrines. A majority of 359 (94.5%) mothers/caregivers used a good solid waste management system disease (Supplementary Table 4).

Nutritional status among infants and young children at the age of 6–23 months of study participants

In general, ninety-four (24.7%) and twenty-one (5.5%) of infants and young children were undernourished and over-nourished, respectively (Fig. 1).

Anthropometric measurement result

The median and interquartile range of length, weight, and mid-upper-arm circumference (MUAC) of the study participants were 75.5 ± 8 cm, 9.5 ± 1.65 kg, and 13.6 ± 1.1 cm,

Table 2. Factors associated with undernutrition among infants and young children using multinomial logistic regression in Yeka sub-city, Ethiopia, 2021 ($n = 380$)

Variables	Category	Frequency	COR	95% CI COR	AOR	95% CI AOR	P value
Undernutrition							
DDS	Inadequate	114 (30%)	6.5	2.1, 6.6	5.65	2.3, 10.87	0.003
	Adequate	266 (70%)	1		1		
MMF	Inadequate	45 (11.8%)	11.92	2.4, 12.1	5.44	2.1, 11.09	0.0052
	Adequate	335 (88.2%)	1		1		
Diarrhoea	Yes	47 (12.4%)	5.53	1.55, 7.02	2.52	1.0, 6.3	0.002
	No	333 (87.6%)	1		1		
Overnutrition							
DDS	Inadequate	114 (30%)	0.19	0.03, 1.45	0.14	0.02, 1.16	0.069
	Adequate	266 (70%)	1		1		
MMF	Inadequate	45 (11.8%)	2.43	0.50, 6.76	4.08	0.77, 9.52	0.097
	Adequate	335 (88.2%)	1		1		
Diarrhea	Yes	47 (12.4%)	1.44	0.31, 6.69	1.48	0.31, 7.04	0.62
	No	333 (87.6%)	1		1		

DDS, dietary diversity score; MMF, minimum meal frequency; COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

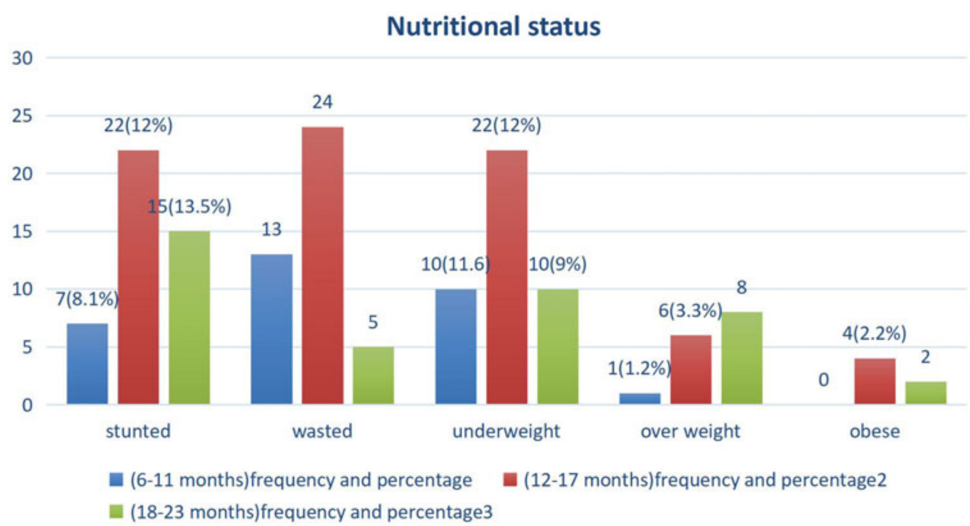

Fig. 2. Nutritional status distribution among infants and young children at the age of 6–23 months in Yeka sub-city, Ethiopia, 2021.

respectively. Mean Z score values of infants and young children, WAZ, LAZ, and WLZ scores were −0.75, −1.25, and −0.26, respectively. The mean LAZ, WLZ, and WAZ scores were negative in all age groups (Fig. 2, Supplementary Table 5, and Supplementary Figs. 1–3).

Factors associated with nutritional status among infants and young children

In binary logistic regression, maternal age at the time of delivery, occupation of the husband, starchy staples food, dark green vegetables, vitamin A-rich food, other fruits and vegetables, legumes, nuts, and seeds, milk and milk products, DDS, MMF, postnatal care visit, AURTI, diarrhoea, water source, latrine, and solid waste management were some of the determinant factors (with P value < 0.25) for undernutrition. Those variables having a P value of <0.25 in the binary analysis were taken to multinomial logistic regression analysis. In binary logistic regression, the age of the respondent, mother's employment, husband's employment, husband's occupation, and DDS were some of the determinant factors (with P value < 0.25) for overnutrition. In multinomial logistic regression analysis, those variables with P value < 0.05 were considered significant predictors for undernutrition and overnutrition.

Multinomial logistic regression analysis results revealed that DDS (AOR = 5.7; 95% CI = 2.30, 10.87; P value = 0.003) was significantly associated with the nutritional status of infants and young children in this study. Infants and young children who did not obtain adequate DDS were more likely to develop undernutrition 5.7 times more than those who obtained adequate DDS (AOR = 5.7; 95% CI = 2.30, 10.87; P value = 0.003). As the DDS decreases by 1 unit, the odds of undernutrition risk increase by 5.7 times (Table 2).

This study shows that MMF (AOR = 5.44; 95% CI = 2.10, 11.09; P value = 0.0052) was significantly associated with undernutrition. Infants and young children who did not meet adequate MMF are more likely to develop undernutrition 5.4 times than those who meet adequate MMF. Decreasing by one unit of MMF, there was increasing 5.4 times being odd of undernutritional status (Table 2).

The result of multinomial logistic regression analysis showed that diarrhoea within 2 weeks (AOR = 2.52; 95% CI = 1.01, 6.31; P value = 0.002) was significantly associated with the nutritional status of infants and young children. Children who developed diarrhoea within 2 weeks are more likely to develop undernutrition 2.5 times than those who did not develop diarrhoea. As the episode of diarrhoea increased by one unit being odd of undernourished increased by 2.5 times (Table 2).

Discussion

In this study, the magnitude of undernutrition and overnutrition among infants and young children were 24.7% and 5.5%, respectively. DDS, MMF, and diarrhoea were significantly associated with nutritional status among infants and young children.

This study indicated that the magnitude of undernutrition and overnutrition were 24.7% and 5.5%, respectively. The finding was similar to the report of EDHS 2019, which was reported to be 21% and 4.5%, respectively.[7] Among undernourished infants and young children 11.58%, 11.05%, and 11.05% were stunted, wasted, and underweight, and among overnourished, 3.95% and 1.2% were overweight and obese, respectively.

The magnitude of undernutrition among infants and young children in India was 65.2%, 43.3%, and 11.9% stunted, underweight, and wasted, respectively. When it was compared to this study, the magnitude of stunted and underweight was higher, due to poor DD and MMF practices, but in line with wasting prevalence.[6] The magnitude of undernutrition among infants and young children in this study was consistent with a study conducted in rural China, which was 24.37%.[26] The magnitude of underweight and stunted was lower in this study when compared to the study done in Harar, which were 21% and 19.3%, respectively.[11] This might be due to the differences in these two studies where different determinant factors, such as breastfeeding practices, health status, and vaccination status of infants and children were associated factors that affect the nutritional status of infants and young children in Harar.

The prevalence of undernutrition in this study was lower than the prevalence of undernutrition in Tanzania.[26,27] The cause of this difference was due to poor consumption of animal sources of food in Tanzania. The magnitude of stunted was high in Myanmar, which was 20%, due to poor DD and MMF.[28] The magnitude of overnutrition in this study was 3.9% overweight and 1.6% obese, respectively, overweight was lower when it was compared to Kuwait, which was 6.5%, due to early initiation of complementary feeding that made the difference, but the prevalence of obese was similar to this study, which was 1.6%.[29] The magnitude of stunted and underweight in this study was lower when it was compared to Ghana which were 20.5% and 21.1%, respectively.[30] The factors that made the difference were poor DD and MMF which were 34.8% and 58.2%, respectively, in Ghana, but the magnitude of wasted was consistent, which was 11.5%.

The magnitude of undernutrition was low in this study when compared to research that was done in rural Ethiopia, which was 48.5%.[31] The main cause of the difference was pre-lacteal feeding practices, high family and children size, and the high proportion of diarrhoea were significantly associated with the nutritional status of infants and young children.

The prevalence of overweight (WLZ > +2) and obesity (WLZ > +3) were 3.9% and 1.6% in this research, respectively, which were lower compared to the research done in South Africa, which were 11% and 5%, respectively.[32] This might be due to anthropometric measurement difference which was used by MUAC for classification.

In this study, the magnitude of stunted was lower, which was 18.5% in Gamo Gofa, due to breastfeeding practice being highly related. Wasting was lower in Gamo Gofa, which was 3.9% when it was compared to this study. Underweight was consistent with this study which was 9.1%.[33] The main difference was due to different determinant factors. Breastfeeding practices determined the magnitude of stunting in Gamo Gofa. In this study, the magnitude of stunted and underweight was lower when they were compared to the study which was done in Southern Ethiopia, which were 43.8% and 15.8%, respectively.[34] The factor that made the difference was poor DDS compared to this study.

The magnitude of underweight, wasting, and stunting was lower than research which was done in Indonesia, which were 26%, 23%, and 28%, respectively.[35] The factors that made the difference were poor DD and MMF. The magnitude of undernutrition in this study was lower when compared to the research done in Nigeria.[36] The factors that made the difference were poor DDS and MMF compared to this study. The magnitude of undernutrition in this study was lower compared to that in Bule Hora, due to diarrhoea in the past 2 weeks and pre-lacteal feeding.[4]

DDS and MMF of this study were 70% and 88.2%, respectively, which were higher than the magnitude of minimum DD and MMF practices, which were 28.5% and 68.4% in Bale, Ethiopia, respectively.[37] Similarly, DD and meal frequency were 17% and 72.2%, respectively, in Holeta town and 7% and 47%, respectively, in Bahir Dar.[38,39] The DDS of this study was 70% which was nearly five times higher than the study that was done in Dejen, Ethiopia 13.6%.[40] DD and meal frequency were 23.7% and 32.7%, respectively, in Pawie District, Benishangul Gumuz.[41] They were lower than this study, due to the strong significance of the residence and PNC check-up. DD and meal frequency were 18% and 56%, respectively, in Ethiopia.[42] They were lower than this study, mainly affected by poor timely initiated complementary feeding.

Minimum DD and meal frequency were 45% and 33%, respectively, in North Shoa, Ethiopia, which were lower than this study, due to postnatal care visits, child feeding practices, and getting media exposure.[43] DD was 29.9% in North West Ethiopia,[44] which was lower than this study; it was significantly associated with maternity services and child vaccination. The DDS of this study was 70% which was higher compared to 59.9% in Addis Ababa, Ethiopia.[45] Increasing DD may be an approach to reduce the burden of stunting and chronic malnutrition among young children.[46] DD and MMF were very low in Afar when compared to this study due to different predictors, maternal education, maternal occupation, sex of the child, and history of postnatal care visit, which were significantly associated factors.[7]

In this study, diarrhoea was significantly associated with the nutritional status of infants and young children. This finding is supported by studies conducted in Filtu town, Somali Region, Ethiopia,[2] in Tigray,[47] and rural parts of Ethiopia.[4] Diarrhoea contributes to malnutrition through the reduction in food intake, decrease in absorption of nutrients, and increase in catabolism of nutrient reserves.

Limitations of the study

A single 24-h recall dietary data might not reflect the usual intake of participants and recall bias was one of the limitations of this study. Moreover, the shortcomings of the cross-sectional study design may not enable the determination of causal relationships.

Conclusion

High numbers of infants and young children suffered from malnutrition with low DDS, MMF, and diarrhoea. Large numbers of mothers/caregivers were housewives and did not get income on their own. As a result, infants and young children feeding practices were poorly practiced which was significantly associated with infants and young children nutritional status in the study area. The nutritional status of infants and young children should be assessed at a community level, so researchers should take the responsibility to determine the determinant factors by qualitative and quantitative study design.

Acronym and Abbreviations

AA: Addis Ababa; **DD:** Dietary Diversity; **ETB:** Ethiopian Birr; **FANTA:** Food and Nutrition Technical Assistance; **FAO:** Food and Agriculture Organization; **ICFI:** Infant and Child Feeding Index; **IDDS:** Individual Dietary Diversity Score; **IMNCI:** Integrated Management of Newborn and Childhood Illness; **EPI:** Ethiopia Program Immunization; **MAM:** Moderate Acute Malnutrition; **MUAC:** Mid-Upper-Arm Circumference; **NACS:** Nutrition Assessment; Counselling; and Support; **NNP:** National Nutritional Program; **OPD:** Outpatient Department; **OTP:** Outpatient therapeutic feeding program; **SAM:** Severe Acute Malnutrition; **WFP:** World Food Program; **WHO:** World Health Organization.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.20

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Conflict of interests

The authors declare that they have no conflict of interests.

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Author Contributions

Conceptualization: **SH**. Formal analysis: **SH, EK and DJ.** Development or design of methodology: **SH, DJ, CY and EK.** Entering data into software: **SH.** Supervision: **DJ, CY and EK**. Validation **SH, DJ, CY and EK**. Writing original draft: **SH**. Review and editing report: **SH, DJ, CY and EK**. Manuscript preparation: **EK** and **SH**. Edited the overall improvement of the manuscript: **CY and DJ.** All authors read and approved the final submitted paper

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RESEARCH ARTICLE

Prenatal vitamin C and fish oil supplement use are associated with human milk microbiota composition in the Canadian CHILD Cohort Study

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Abstract

Maternal diet may modulate human milk microbiota, but the effects of nutritional supplements are unknown. We examined the associations of prenatal diet and supplement use with milk microbiota composition. Mothers reported prenatal diet intake and supplement use using self-administered food frequency and standardised questionnaires, respectively. The milk microbiota was profiled using 16S rRNA gene sequencing. Associations of prenatal diet quality, dietary patterns, and supplement use with milk microbiota diversity and taxonomic structure were examined using Wilcoxon signed-rank tests and multivariable models adjusting for relevant confounders. A subset of 645 mothers participating in the CHILD Cohort Study (originally known as the Canadian Healthy Infant Longitudinal Development Study) provided one milk sample between 2 and 6 months postpartum and used prenatal multivitamin supplements ≥4 times a week. After adjusting for confounders, vitamin C supplement use was positively associated with milk bacterial Shannon diversity ($\beta = 0.18$, 95% CI = 0.05, 0.31) and *Veillonella* and *Granulicatella* relative abundance ($\beta = 0.54$; 95% CI = 0.05, 1.03 and $\beta = 0.44$; 95% CI = 0.04, 0.84, respectively), and negatively associated with *Finegoldia* relative abundance ($\beta = -0.31$; 95% CI = –0.63, –0.01). Fish oil supplement use was positively associated with *Streptococcus* relative abundance ($\beta = 0.26$; 95% CI = 0.03, 0.50). Prenatal diet quality and dietary patterns were not associated with milk microbiota composition. Prenatal vitamin C and fish oil supplement use were associated with differences in the milk microbiota composition. Future studies are needed to confirm our findings and elucidate mechanisms linking maternal supplement use to milk microbiota and child health.

Key words: Breastmilk: CHILD Cohort Study: Diet: Microbiome: Pregnancy: Supplements

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Introduction

Human milk confers numerous benefits to the infant's health including guiding the maturation of the immune system and modulating the gut microbiome development.[1,2] It does so through its diverse constituents, including nutrients and bioactive components such as human milk oligosaccharides, lactoferrin, growth factors, and cytokines.[3] In addition, the milk microbiome potentially impacts the infant microbiome development and guides the maturing immune system, adding to the list of pathways through which human milk can influence infant health.[4,5]

Numerous factors can shape the human milk microbiota composition, including maternal body mass index (BMI), delivery mode, lactation stage, mode and exclusivity of breast milk feeding, and peripartum antibiotic use.[4,6,7] Although it is well established that maternal diet affects the human milk composition,[8,9] especially the fatty acid profile,[10] few studies investigated the effect of maternal diet on the milk microbial communities,[11–14] and none have examined the effects of supplement use. Existing studies of maternal diet have been conducted among a small sample or among women with high rates of gestational glucose intolerance, while others did not adjust for potential confounding factors that influence the milk microbiota composition or utilised human milk samples prior to milk maturation.[11–14]

Pregnant and breastfeeding women are recommended to use multivitamin supplements since the nutrient needs of these women are generally greater than those of non-pregnant women, and the recommended intakes of some nutrients, such as folate and iron, are difficult to meet from food alone.[15] Despite such recommendations and the widespread use of supplements during pregnancy,[16] the effect of supplement use on the human milk microbiota composition is unknown.

To help fill this gap in the literature, we conducted a study among a diverse sample of 645 mothers participating in the CHILD Cohort Study in Canada who provided one milk sample between 2 and 6 months postpartum. The aim of this study is to examine the associations between prenatal diet quality, dietary patterns, and supplement use and the human milk microbiota composition. Findings on how prenatal maternal diet and supplement use are potentially associated with the human milk microbiome can help better inform nutrition guidelines of maternal intake during pregnancy.

Subjects and methods

Study design and subject selection

The CHILD Cohort Study (originally known as the Canadian Healthy Infant Longitudinal Development Study) is a longitudinal, prospective, population-based birth cohort study conducted across four regions in Canada: Vancouver, Edmonton, Manitoba, and Toronto.[17] The main aim of the study is to examine the developmental origins of paediatric asthma and allergy. This study was approved by the Human Research Ethics Boards at McMaster University, the Hospital for Sick Children (Toronto) and the Universities of Manitoba, Alberta, British Columbia, and Purdue University.

Women ($n = 3608$) in their second or third trimester of pregnancy were enrolled in the study between 2008 and 2012 (Fig. 1). Eligible subjects ($n = 3455$, 95.8%) had singleton pregnancies and delivered a healthy infant >35 weeks of gestation. A total of 2598 (75.2%) mothers provided human milk samples, 1194 (46.0%) of whom had their milk samples analysed for microbiota composition. Among those, 482 (40.4%) mothers were randomly selected from a representative subset of mother-infant dyads across the cohort,[18] and the remaining 712 (59.6%) were from an additional subset enriched for maternal and infant health conditions (atopy, asthma, obesity).[4] After microbiome pre-processing, the human milk samples of 887 (74.3%) mothers were retained in the analysis. Because lactation stage influences the human milk microbiota composition,[19] we limited our analysis to the 811 (91.4%) mothers with milk samples collected between 2 and 6 months postpartum. Among the 811 mothers, 645 (79.5%) used multivitamin supplements ≥4 times a week throughout pregnancy. The 645 mothers constituted our primary sample of analysis because the chronic use of multivitamin supplements creates a relatively steady state in the body for each of the micronutrients in the multivitamin supplement and provided a more homogenous background to assess the role of additional individual micronutrient supplements on the microbiota.[20]

Maternal and infant characteristics

Demographic characteristics of the mother including age, ethnicity, parity, and antibiotic use around the time of milk sample collection, and those of the infant including sex, delivery mode, gestational age at birth, and age at milk sample collection were documented from hospital records or from standardised questionnaires. Maternal pre-pregnancy BMI in kg/m^2 was calculated using self-reported pre-pregnancy weight from standardised questionnaires and measured height abstracted from medical records.

Mothers completed standardised questionnaires about infant feeding practices at 3, 6, and 12 months postpartum. At the time of milk sample collection, breastfeeding status was classified as exclusive (human milk only) or partial (human milk supplemented with infant formula), and the mode of breast milk feeding was classified as all direct from the human (no feeding of pumped milk) or some pumped human milk (at least one serving of pumped human milk in the two weeks prior to milk sample collection).

Prenatal diet quality, dietary patterns, and supplement use

Prenatal diet was assessed using a validated semi-quantitative food frequency questionnaire (FFQ) adapted from the Fred Hutchinson Cancer Center tool.[21] The FFQ was self-administered and completed between 24 and 28 weeks of pregnancy. Details on the diet analysis were described elsewhere.[22] Briefly, responses to the FFQ were linked to the United States Department of Agriculture nutrient composition database and modified for a Canadian setting[23] to estimate total energy intake. Diet quality was assessed using the Healthy Eating Index 2010 (HEI-2010) score[24] and dietary

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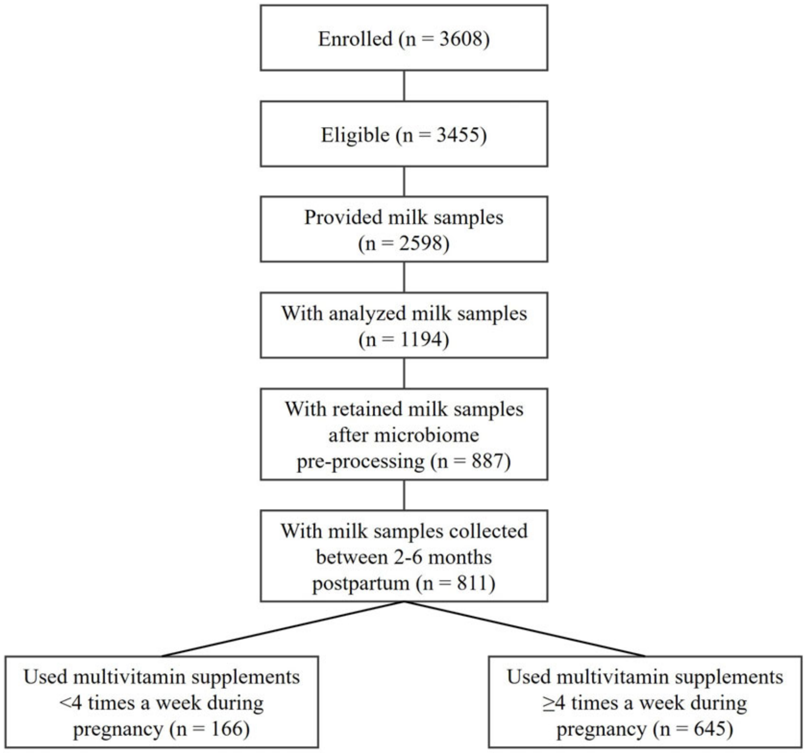

Fig. 1. Flowchart for selecting mother-infant dyads in the CHILD Cohort Study included in the current analysis.

patterns were identified using principal component analysis (PCA) using the "psych" package (version 1.5.6) within R (version 3.1.2). Three dietary patterns emerged among the CHILD Cohort Study subjects: plant-based (characterised by dairy, legumes, vegetables, whole grains, and an aversion to meats), Western (characterised by fats, meats, processed foods, and starchy vegetables) and balanced (characterised by diverse sources of animal proteins [especially fish], vegetables, fruits, nuts and seeds).[25] The PCA scores for each of the three patterns represented how close the mothers' dietary intakes were to the dietary patterns. Positive PCA scores indicated adherence to the specific dietary pattern while negative scores indicated avoidance. Certain mothers had positive scores for more than one dietary pattern and were thus considered adherent to more than one pattern. Dietary pattern scores were adjusted to the mean energy intake of the cohort (2500 kcal/d) using the residual method.[26] Women with implausible energy intakes (<500 or >6500 kcal/d) were excluded from the analysis of diet quality and dietary patterns.[22]

Mothers completed self-administered standardised questionnaires during pregnancy and after delivery about the frequency of use and dosage of supplements throughout pregnancy. Frequency of individual and multivitamin supplement use was reported based on the following options: never, 1–3 times a month, 1–3 times a week, 4–6 times a week, and every day. Dosage of prenatal supplements per day was reported for several but not all supplements and responses varied by questions for the individual supplements. Due to the unavailability of comparable responses on the frequency and dosage of supplement use, we categorised supplement use as never (reported never using the supplement during pregnancy) vs. ever (reported using the supplement at least once a month during pregnancy).

Milk sample collection and microbiota profiling

Details on milk sample collection and analysis are described elsewhere.[18] Briefly, mothers provided one milk sample which was a mix of fore- and hind milk from several feeds during a 24-hour period in a sterile milk container provided by the CHILD study. The milk samples were collected using hand expression or a pump and stored in the refrigerator at home for up to 24 hours. The study staff then transported and processed the milk samples and stored them at –80°C until analysis.

Milk microbiota was analysed at the University of Manitoba using 16S rRNA gene sequencing of the V4 hypervariable region on a MiSeq platform (Illumina, San Diego, CA, USA).[18] Negative controls composed of sterile DNA-free water were used in sequencing library preparation, while positive controls consisted of DNA extracted from 8 species with known theoretical relative abundances (Zymo Research, USA, Cat# D6005).

Microbiota data pre-processing was previously described.[27] Briefly, overlapping paired-end reads were processed with the DADA2 pipeline[28] using the open-source software QIIME 2 v.2019.10 (https://qiime2.org).[29] Unique amplicon sequence variants (ASVs) were assigned taxonomy and aligned to the 2019 release of SILVA v.138 SSURef NR99 at 99% sequence similarity.[30] A three-step framework including (1) verification of sequencing accuracy, (2) contaminant removal and correction of batch variability, and (3) assessment of microbiome analysis repeatability was used for comprehensive quality control.[27]

Samples were rarefied to 8,000 sequencing reads per sample. ASVs with mean relative abundance ≤0.01% were discarded. ASVs were then agglomerated at the genus level and genera with mean relative abundance ≤0.1% and those present in ≤10% of the samples were discarded. ASVs and genera abundances were centred log-ratio transformed using the CoDaSeq package[31] after zero imputation using a Bayesian-multiplicative replacement method.[32] This dataset was used for downstream analysis unless otherwise stated.

Statistical analysis

Frequency (%) and mean ± standard deviation (SD) were computed to describe the characteristics of the mother-infant dyads in this study. Differences between included vs. excluded mother-infant dyads and associations between maternal and infant characteristics and prenatal diet quality, dietary patterns, and supplement use were examined using χ^2 test. Diet quality was categorised as below vs. above the 50th percentile (74.82) for HEI-2010 scores. Dietary patterns were categorised as adherence (positive PCA scores) vs. avoidance (negative PCA scores).[25]

Associations of prenatal diet quality, dietary patterns, and supplement use with human milk α diversity (Shannon index) and genera relative abundance were examined using Wilcoxon signed-rank tests and multivariable linear regression permutation models using lmPerm package v.2.1.0.[33] Variables tested for their potential confounding effect included: parity, pre-pregnancy BMI, infant sex, delivery mode, and gestation age at birth as well as maternal antibiotic use, infant age, breastfeeding exclusivity, and mode of breast milk feeding at the time of milk sample collection. Based on the literature[4,18,34] and the results of the univariable analysis, parity, pre-pregnancy BMI, and breastfeeding exclusivity and mode of breast milk feeding at the time of milk sample collection were adjusted for in the final multivariable models. The models also adjusted for the batches of milk samples, which were analysed separately.

The association with milk microbiota β diversity was examined using permutational analysis of variance (PERMANOVA) of a Bray-Curtis dissimilarity matrix derived using the ASV dataset via the *vegan* package (version 2.5.7).[35] The Benjamini-Hochberg's false discovery rate (FDR) was computed to correct the *P*-values for multiple comparisons of the genera relative abundance. A two-sided *P*-value <0.05 and a confidence interval (CI) not including 0 were considered statistically significant. Data analysis was conducted in R (version 4.0.3)[36] using the *Phyloseq* package (version 1.36.0).[37]

Results

Maternal and infant characteristics, prenatal diet quality, dietary patterns, and supplement use

The 645 mothers included in this study had a mean ± SD age at delivery of 33.1 ± 4.2 years (range, 21.0–46.3 years); 80.0% were Caucasian, 58.0% were primiparous, 12.4% were obese, and 11.5% used antibiotics at the time of milk sample collection (Table 1). Fifty-four per cent of the infants were boys, 72.9% were born vaginally, 51.9% were exclusively breastfed, and 62.5% received some pumped milk at the time of milk sample collection. The mean ± SD age of the infants at the time of milk sample collection was 3.7 ± 0.8 months. As for maternal diet, 51.5% consumed a high-quality diet, 40.2% were adherent to a plant-based dietary pattern, 38.9% were adherent to a Western dietary pattern, and 43.7% were adherent to a balanced dietary pattern. In addition to using multivitamin supplements ≥4 times a week, mothers used the following supplements at least once a

Table 1. Characteristics of mother-infant dyads from the CHILD Cohort Study included in the current analysis

	Mother-infant dyads N = 645
Maternal characteristics	
Recruitment study centre, *n* (%)	
Edmonton	134 (20.8)
Toronto	174 (27.0)
Vancouver	171 (26.5)
Manitoba	166 (25.7)
Age at time of delivery (years)	
Mean ± SD	33.1 ± 4.2
Range	21.0 – 46.3
Ethnicity, *n* (%)	
Caucasian	516 (80.0)
Asian	98 (15.2)
Other	31 (4.8)
Primiparity, *n* (%)	374 (58.0)
Pre-pregnancy BMI, *n* (%)	
Normal weight (<25 kg/m^2)	403 (62.5)
Overweight (25–29.99 kg/m^2)	141 (21.9)
Obese (≥30 kg/m^2)	80 (12.4)
Missing[a]	21 (3.2)
Antibiotic use at time of milk sample collection, *n* (%)	74 (11.5)
Infant characteristics	
Boys, *n* (%)	348 (54.0)
Vaginal delivery, *n* (%)	470 (72.9)
Term birth (≥39 weeks), *n* (%)	570 (88.4)
Exclusive breastfeeding at time of milk sample collection, *n* (%)	335 (51.9)
Some pumped breast milk feeding at time of milk sample collection, *n* (%)	403 (62.5)
Age at time of milk sample collection (months), mean ± SD	3.7 ± 0.8
Maternal prenatal diet	
High-quality diet[b], *n* (%)	332 (51.5)
Adherence to plant-based pattern[c], *n* (%)	259 (40.2)
Adherence to Western pattern[c], *n* (%)	251 (38.9)
Adherence to balanced pattern[c], *n* (%)	282 (43.7)
Maternal prenatal supplement ever use[d]	
Vitamin D, *n* (%)	150 (23.3)
Vitamin C, *n* (%)	32 (5.0)
Fish oil, *n* (%)	133 (20.6)
Calcium, *n* (%)	125 (19.4)
Folate, *n* (%)	108 (16.7)
Iron, *n* (%)	78 (12.1)

BMI, Body mass index; SD, standard deviation.
[a]Missing was analysed as a separate category in the multivariable models.
[b]High-quality diet: Healthy eating index-2010 scores ≥50th percentile of 74.8.
[c]Adherence to a dietary pattern: Positive principal component analysis scores indicating that the mother's dietary intake was similar to the components of the dietary pattern, which were as follows: plant-based (dairy, legumes, vegetables, whole grains, and an aversion to meats), Western (fats, meats, processed foods, and starchy vegetables) and balanced (diverse sources of animal proteins (especially fish), vegetables, fruits, nuts and seeds); categories are not mutually exclusive
[d]Supplement ever use: Use of the supplement at least once a month during pregnancy.

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month: vitamin D (23.3%), vitamin C (5.0%), fish oil (20.6%), calcium (19.4%), folate (16.7%), and iron (12.1%).

Mothers who consumed a high-quality diet and those who used prenatal vitamin D or fish oil supplements were less likely to have pre-pregnancy overweight or obesity (P-value < 0.001 for diet quality and P-value = 0.01 for vitamin D and fish oil supplement use). Furthermore, mothers who consumed a high-quality diet and those who used prenatal fish oil supplements were more likely to exclusively breastfeed at the time of milk sample collection (P-value = 0.02 for diet quality and P-value < 0.001 for fish oil supplement use). No other associations were observed between maternal or infant characteristics and prenatal diet quality, dietary patterns, and supplement use.

Few differences were noted in the characteristics of the mother-infant dyads included in our study compared to those who were excluded ($n = 2963$) (Supplementary Table 1). A larger proportion of the mothers included resided in Toronto (27.0% vs. 21.2%) and Vancouver (26.5% vs. 19.1%, P-value = 0.001), were Caucasian (80.0% vs. 70.0%, P-value = 0.005), and consumed a high-quality diet (51.5% vs. 34.8%, P-value <0.001). Further, more mothers included in the analysis used vitamin D supplements (23.3% vs. 13.9%, P-value = 0.001), fish oil supplements (20.6% vs. 10.4%, P-value <0.001), calcium supplements (19.4% vs. 11.6%, P-value = 0.003), and prenatal folate supplements (16.7% vs. 9.6%, P-value = 0.01) compared to those who were excluded.

Human milk microbiota diversity and prenatal diet quality, dietary patterns, and supplement use

Mean ± SD of milk bacterial Shannon diversity was 1.74 ± 0.67. The milk bacterial Shannon diversity of mothers who used prenatal vitamin C supplements was higher (2.05 ± 0.61) than that of mothers who never used them (1.71 ± 0.65, P-value = 0.01) (Fig. 2a). The milk bacterial Shannon diversity of mothers who used prenatal fish oil supplements was lower (mean ± SD of milk bacterial Shannon diversity: 1.61 ± 0.64) than that of mothers who never used them (1.78 ± 0.68, P-value = 0.01). No significant differences in mean milk Shannon diversity were noted by diet quality or dietary patterns or use of other prenatal supplements.

After adjusting for relevant confounding factors, vitamin C supplement use was positively associated with milk bacterial Shannon diversity ($\beta = 0.18$; 95% CI = 0.05, 0.31) (Fig. 2b).

Significant, albeit small, differences were observed in milk microbiota β diversity for vitamin C supplement use ($R^2 = 0.33\%$, P-value = 0.04) (Fig. 3).

We conducted a sensitivity analysis among terms infants and mothers who did not use antibiotics. The results were materially unchanged: vitamin C supplement use was positively associated with milk bacterial Shannon diversity among term infants ($\beta = 0.17$; 95% CI = 0.04, 0.30) and women without antibiotic use ($\beta = 0.19$; 95% CI = 0.05, 0.32). In addition, vitamin C supplement use was associated with differences in milk microbiota β diversity among term infants ($R^2 = 0.344\%$, P-value = 0.05) and women without antibiotic use ($R^2 = 0.34\%$, P-value = 0.05).

Taxonomic structure of human milk microbiota and prenatal diet quality, dietary patterns, and supplement use

Firmicutes were the predominant phylum in human milk in our study (mean relative abundance ± SD: 60.65% ± 35.17), followed by Proteobacteria (31.52% ± 37.39), Actinobacteria (5.99% ± 7.33), Bacteroidetes (1.57% ± 4.51) and Fusobaceria (0.13% ± 0.63). At the genus level, *Streptococcus* (mean relative abundance ± SD: 40.07% ± 31.39) and *Staphylococcus* (13.48% ± 21.59) were most abundant, followed by *Actinobacter* (10.11% ± 20.05) and *Pseudomonas* (7.45% ± 19.40). *Streptococcus, Staphylococcus,* and *Acinetobacter* were present in ≥90% of the 645 milk samples.

The relative abundance of *Streptococcus* was higher in the milk of mothers who used fish oil supplements compared to those who did not (mean relative abundance ± SD: 44.33% ± 32.24% vs. 38.59% ± 30.91%, $P_{FDR} = 0.03$). Similarly, the relative abundance of *Bifidobacterium* was higher in the milk of mothers who used fish oil supplements compared to those who did not (mean relative abundance ± SD: 0.52% ± 2.12% vs. 0.26% ± 1.33%, $P_{FDR} = 0.03$).

After adjusting for relevant confounding factors, vitamin C supplement use was positively associated with *Veillonella* and *Granulicatella* relative abundance ($\beta = 0.54$; 95% CI = 0.05, 1.03 and $\beta = 0.44$; 95% CI = 0.04, 0.84, respectively), and negatively associated with *Finegoldia* relative abundance ($\beta = -0.31$; 95% CI = –0.63, –0.01) (Fig. 4). Fish oil supplement use was positively associated with *Streptococcus* relative abundance ($\beta = 0.26$; 95% CI = 0.03, 0.50).

Discussion

In one of the largest studies of human milk microbiota, we report for the first time that prenatal vitamin C and fish oil supplement use were associated with milk microbiota composition, even after adjusting for confounding factors. Specifically, vitamin C supplement use was positively associated with milk bacterial Shannon diversity and with *Veillonella* and *Granulicatella* relative abundance and negatively associated with *Finegoldia* relative abundance. Fish oil supplement use on the other hand was positively associated with *Streptococcus* relative abundance. We limited our analysis to mothers who used multivitamin supplements ≥4 times a week since the chronic use of multivitamin supplements creates a relatively steady state in the body for each of the micronutrients in the multivitamin supplement.[20]

Despite the recommendations and widespread use of nutritional supplements during pregnancy,[16] no previous studies have examined the associations between supplement use and the human milk microbiota composition. The few studies on maternal diet and the milk microbiome, although they do not account for prenatal supplement use, provide some insight into the associations between vitamin C and fish oil and the milk microbiota composition.[13,14] The vitamin C composition of the prenatal diet of 94 healthy women in Brazil was associated with differences in milk microbiota β diversity,[13] similar to our study. Further, vitamin C intake in the diet during pregnancy was positively correlated with *Staphylococcus* presence,[13] while in our

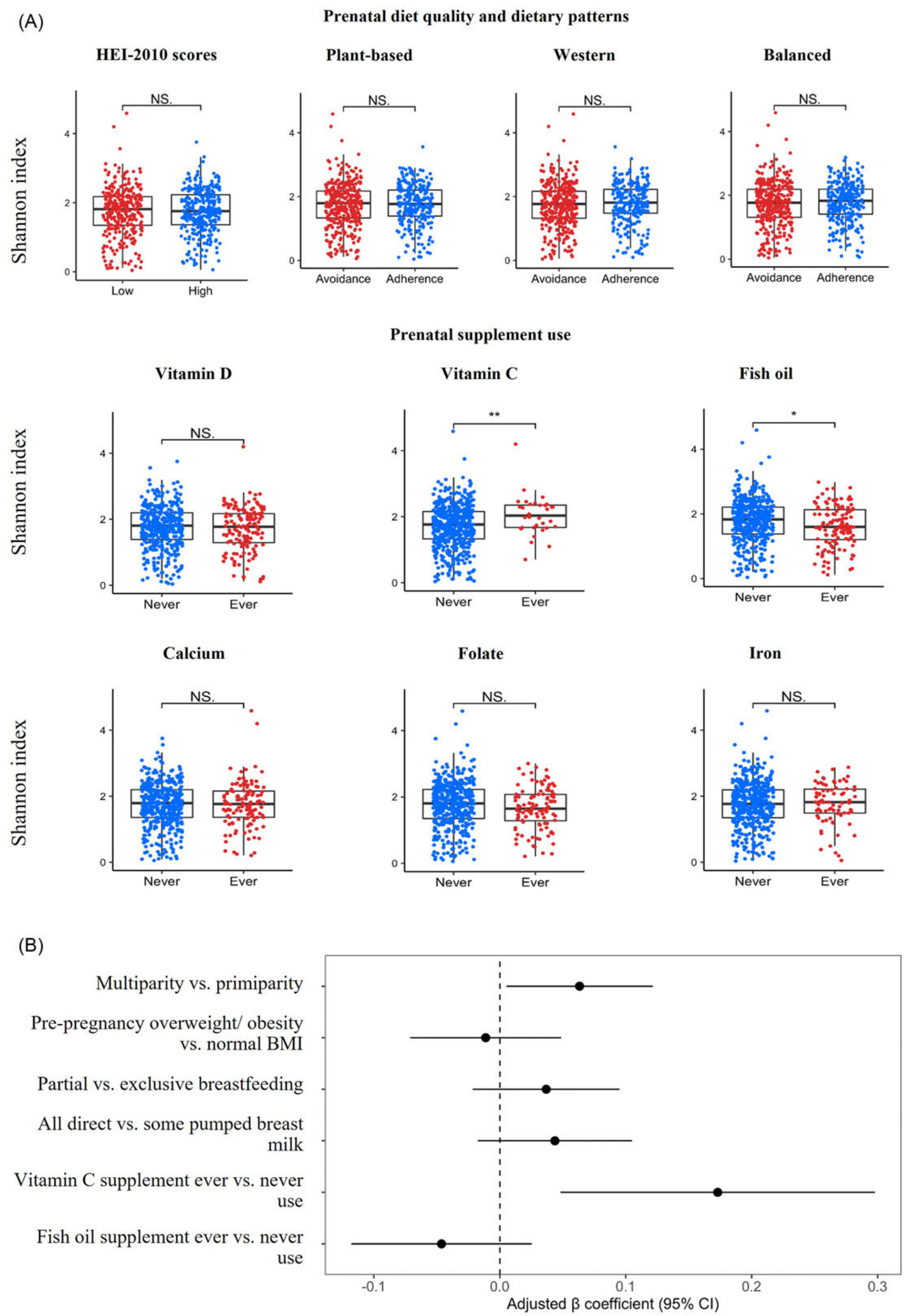

Fig. 2. Human milk bacterial Shannon diversity by prenatal diet quality, dietary pattern, and supplement use among mothers in the CHILD Cohort Study. (a) Unadjusted associations examined using Wilcoxon-sign rank test. (b) Adjusted associations examined using multivariable linear regression permutation models adjusted for parity, pre-pregnancy BMI, breastfeeding exclusivity, mode of breast milk feeding at the time of milk sample collection, and fish oil and vitamin C supplement use; models were additionally adjusted for batch of analysis. Diet quality was examined using HEI-2010 scores categorised as low ($<50^{th}$ percentile) vs. high ($\geq 50^{th}$ percentile). Dietary patterns derived using PCA were examined as adherence (positive PCA scores) vs. avoidance (negative PCA scores). PCA scores reflected how closely the mother's dietary intake was similar to the components of the dietary pattern, which were as follows: plant-based (dairy, legumes, vegetables, whole grains, and an aversion to meats), Western (fats, meats, processed foods, and starchy vegetables) and balanced (diverse sources of animal proteins (especially fish), vegetables, fruits, nuts and seeds). Supplement ever use was defined as use at least once a month during pregnancy. BMI, body mass index; CI, confidence interval; HEI, healthy eating index; PCA, principal component analysis. NS, not significant; *: P-value < 0.05; **: P-value < 0.01.

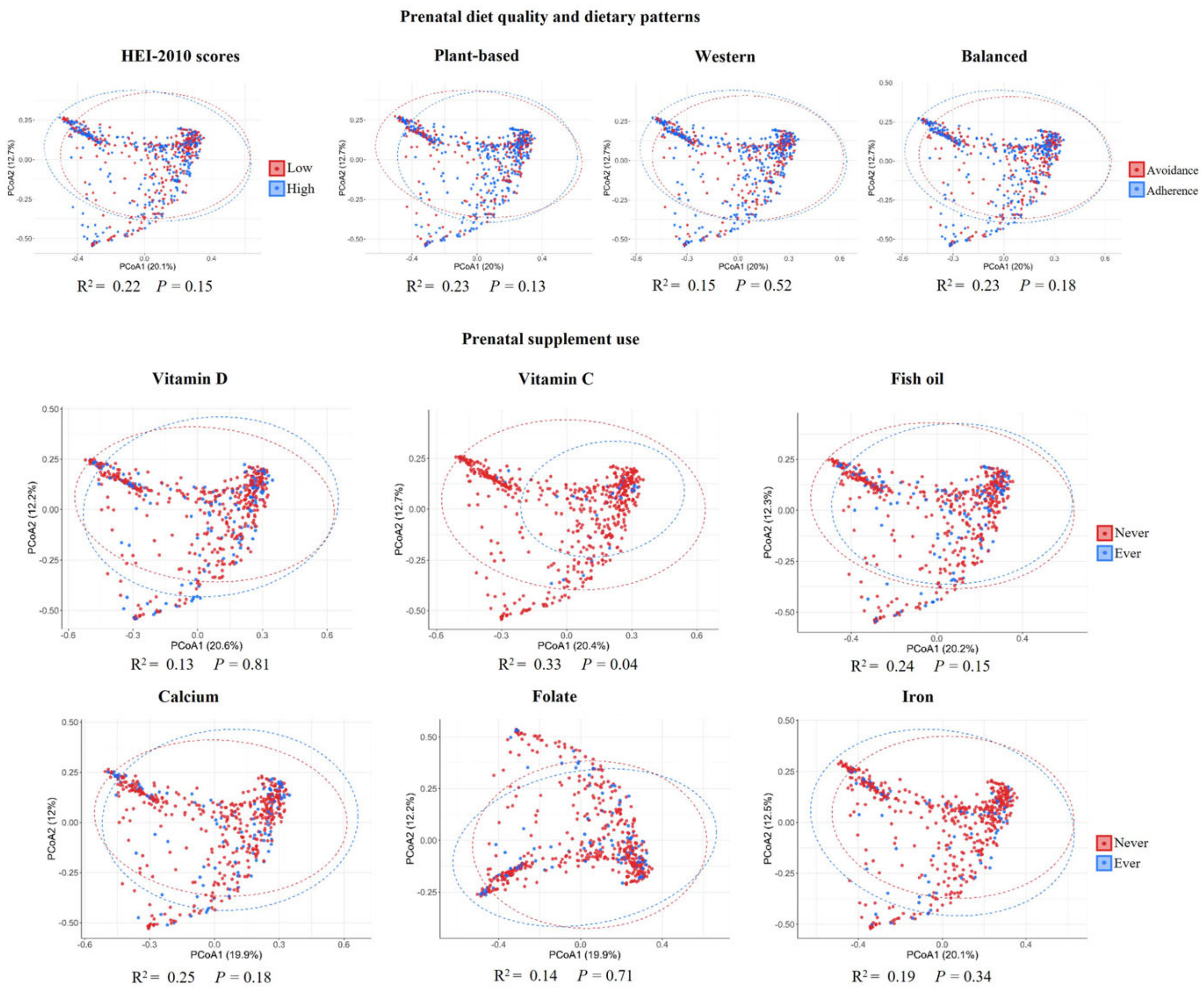

Fig. 3. Human milk microbiota *β* diversity by prenatal diet quality, dietary patterns, and supplement use among mothers in the CHILD Cohort Study. *β* diversity assessed on Bray–Curtis dissimilarity matrix using permutational analysis of variance (PERMANOVA). Diet quality was examined using HEI-2010 scores categorised as low (<50th percentile) vs. high (≥50th percentile). Dietary patterns derived using PCA were examined as adherence (positive PCA scores) vs. avoidance (negative PCA scores). PCA scores reflected how closely the mother's dietary intake was similar to the components of the dietary pattern, which were as follows: plant-based (dairy, legumes, vegetables, whole grains, and an aversion to meats), Western (fats, meats, processed foods, and starchy vegetables) and balanced (diverse sources of animal proteins (especially fish), vegetables, fruits, nuts and seeds). Supplement ever use was defined as use at least once a month during pregnancy. PCA, principal component analysis.

study, vitamin C supplement use was positively associated with *Veillonella and Granulicatella* and negatively associated with *Finegoldia*. It is worth noting that the sample size of mothers using vitamin C supplements in our study is small (5%); yet the consistent association of vitamin C in prenatal supplements and diet with the human milk microbiota composition warrants further investigation. In the MAternal MIcrobes (MAMI) study among 120 healthy mothers in the Spanish Mediterranean area, prenatal dietary omega-3 polyunsaturated fatty acids, including eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid are major fatty acids in fish oil, were positively associated with *Streptococcus* and *Gemella* abundance, while total polyunsaturated fatty acids were negatively associated with *Bifidobacterium* abundance.[14] We found positive associations between fish oil supplement use and *Staphylococcus* and *Bifidobacterium* abundance. *Bifidobacterium* is the hallmark of breastfed infant gut microbiota, suggesting a possible link between human milk and the neonatal gut;[38] however, the association with *Bifidobacterium* abundance was not significant after adjusting for relevant confounders. More studies are needed to reproduce our findings, to examine the combined effects of these nutrients from diet and supplements during pregnancy and lactation on human milk nutrient and microbiota composition, and to elucidate the functional significance of the observed associations in relation to maternal and infant health.

We did not find significant associations between prenatal diet quality and dietary patterns (plant-based, Western, and balanced) and the human milk microbiota composition. Few studies examined the association of maternal diet with the milk microbiota composition.[11–14] In the study among Brazilian women, stronger associations were noted between the milk microbiota composition and the nutrient composition of the diet during pregnancy compared to that during lactation, supporting our analysis of maternal diet and supplement use

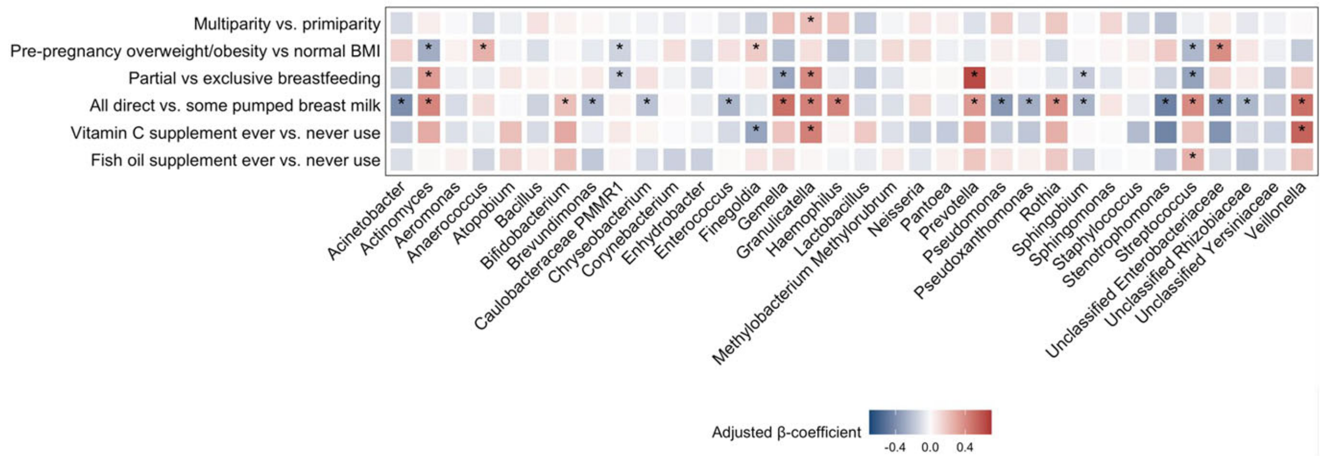

Fig. 4. Genus relative abundance in the human milk of mothers in the CHILD Cohort Study by prenatal supplement use. Associations were examined using multivariable linear regression permutation models adjusted for parity, pre-pregnancy BMI, breastfeeding exclusivity, mode of breast milk feeding at the time of milk sample collection, and fish oil and vitamin C supplement use; models were additionally adjusted for batch of analysis. Supplement ever use was defined as use at least once a month during pregnancy. Genera relative abundance was centred log-ratio transformed. BMI, body mass index. *: Confidence interval not including 0.

during pregnancy.[13] The MAMI study examined prenatal diet in terms of diet clusters (Cluster I characterised by plant protein, fibre, and carbohydrates and Cluster II characterised by animal protein and lipids) rather than individual nutrients and found that the diet clusters were associated with the milk microbiota composition more than other factors including breastfeeding exclusivity and delivery mode.[14] Cluster I was associated with higher bacterial Shannon diversity and higher relative abundance of *Staphylococcus, Lactobacillus,* and *Bifidobacterium* and lower abundance of *Bacteroides* and *Escherichia/Shigella* compared to Cluster II.[14] The nutrient composition of Cluster I seems to be most similar to the plant-based dietary pattern derived among the CHILD Cohort Study subjects; however, we did not detect any associations between this pattern and milk microbiota composition. Future studies of the associations between prenatal dietary patterns and the human milk microbiota composition are needed.[39]

Study strengths and limitations

Our study has several strengths. The CHILD Cohort Study is conducted among a diverse sample of mother-infant dyads and is among the largest studies with available human milk microbiota data to date. The longitudinal prospective cohort study design provided the opportunity to examine prenatal maternal diet and supplement use prior to milk sample collection. Some limitations are worth noting. This is an observational study among a Canadian cohort of women whose place of residence might have influenced both maternal diet and supplement use and the milk microbiota, thus the findings may not be generalisable to other settings. However, the characteristics of the women included in our analytic sample were similar to those in the overall CHILD cohort, and the majority of the women consumed a healthy diet and used multivitamin supplements prenatally, which enhances comparability of our findings to other similar cohorts. We did not account for the dose, frequency, and form of the prenatal supplements nor did we account for postnatal supplement use. Nevertheless, previous studies that examined prenatal and postnatal diet noted stronger associations between prenatal diet and the milk microbiota composition.[13] Our dietary patterns were also not mutually exclusive—i.e. one participant received scores for all three patterns; and only the patterns for which a person "positively" adhered to were ascribed to them. This enhanced interpretation and accommodated the fact that dietary patterns are not "all or nothing." Future studies are ongoing to explore alternative approaches to dietary pattern analyses in the CHILD cohort. Finally, we did not examine how dietary sources of vitamin C and fish oil were associated with the human milk microbiota composition. Future studies are needed to better understand the combined effect of maternal nutrient intake from diet and supplements on the human milk microbiota composition. Despite the current limitations, our exploratory study begins to fill a gap in the understanding of the associations between maternal dietary patterns and supplement use and the human milk microbiota.

In conclusion, fish oil and vitamin C supplement use were associated with the human milk microbiota composition among mothers in the CHILD Cohort Study who used multivitamin supplements ≥4 times a week during pregnancy. Such findings can inform future hypotheses and intervention trials to elucidate mechanisms linking maternal diet and supplement use to the human milk microbiota. A better understanding of these mechanisms and their effects on child health will help inform recommendations on maternal intake during pregnancy. Further research is warranted to test whether our findings are reproducible in other populations and to examine the effects of frequency and dose of supplement use on the associations with the human milk microbiota and child health. Future research is also needed to examine the associations with other human milk components and the infant gut microbiota and overall health, as well as to examine the associations beyond the community profile to explore function and metabolites produced by the microbes.

Abbreviations

ASVs, amplicon sequence variants; **CI**, confidence interval; **FDR**, false discovery rate; **FFQ**, food frequency questionnaire; **HEI-2010**, Healthy Eating Index 2010; **PCA**, principal component analysis; **PERMANOVA**, permutational analysis of variance; **SD**, standard deviation.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.58

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Authorship

MBA, TH, PM, ST, PS, and EK designed research. KF and SM conducted research. RFC analysed data and wrote the paper. MRF, MBA, RJdS, KF, and SM provided guidance with data analysis and write-up. All authors read and approved the final manuscript.

Data share statement

A list of variables available in the CHILD Cohort Study is available at https://childstudy.ca/for-researchers/study-data/. Researchers interested in collaborating on a project and accessing CHILD Cohort Study data should contact the Study's National Coordinating Centre (NCC) to discuss their needs before initiating a formal request. To contact the NCC, please email child@mcmaster.ca. More information about data access for the CHILD Cohort Study can be found at https://childstudy.ca/forresearchers/data-access/.

Conflict of interest

The authors report no conflicts of interest.

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RESEARCH ARTICLE

Promoting healthful and diverse eating behaviours through an extracurricular culinary skills intervention in Philadelphia

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Abstract

In the current study we evaluated an afterschool nutrition education programme, called Vetri Cooking Lab (VCL), for promoting healthy and diverse eating habits among at-risk children in the Greater Philadelphia area. To understand potential programme impacts, we conducted a longitudinal analysis of survey data collected before and after participation in VCL. Main study included cooking confidence, cooking knowledge, changes in dietary consumption behaviours, and changes in vegetable preferences. Participants included students in grades 3–11 enrolled in VCL during the 2018–19 school year at VCL sites (n = 60) throughout Philadelphia, PA, and Camden, NJ. Eligible participants completed surveys both before and after participating in the programme. We found that students' confidence and knowledge increased ($P < 0.001$) after the cooking intervention. Knowledge and confidence were positively associated ($r = 0.55$; $P < 0.001$). Confidence was correlated with consumption behaviour changes ($r = 0.18$; $P = 0.022$). Confidence was positively associated with consumption changes in both our adjusted (OR = 1.81; $P < 0.001$) and unadjusted models (aOR = 1.88; $P = 0.013$). Compared to Black students, White students were more likely to report consumption changes (aOR = 5.83; $P = 0.013$). Hispanic/Latino participants and participants who spoke Spanish had nearly three times higher odds of consumption behaviour changes (Hispanic/Latino OR = 2.55; $P = 0.007$; Spanish OR = 3.04; $P = 0.005$). Student age and gender were not associated with behaviour changes. Our research demonstrates that programmes integrating practical cooking skills education along with nutrition, food, and cooking education can improve confidence and knowledge about healthy food choices amongst children driving an overall improvement in children's eating habits.

Key words: **Child and adolescent nutrition: Cooking skills: Eating habits: Extracurricular education**

Introduction

Nutritious diets play a major role in the growth and development of children and prevention of future chronic diseases, such as obesity, which affects 19% of those aged 2–19 and 40% of adults in the US, and costs the US healthcare system $147 billion dollars annually.[1] Despite nutritional efforts such as the Federal Child Nutritional programmes (e.g. National School Lunch Program, School Breakfast Program), the US still faces a childhood obesity crisis.[2] Childhood obesity elevates risk of chronic health and worsening outcomes for affected children, including cardiovascular disease, asthma, and psychological disorders, with a linkage to the persistence of obesity into adulthood.[3,4] Obesity rates among youth have been increasing steadily over the past decade, generating an increased burden of

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disease and negative health outcomes for affected populations.[3] Furthermore, these complications disproportionately increase risk in racially and economically marginalised children.[4–8]

Nutrition also plays a major role in the management of chronic disease with those eating a healthy diet living longer and having a lower risk of chronic health conditions.[1,5] According to the US Departments of Health and Human Services and Agriculture, a healthy diet includes a variety of plant- and animal-based proteins, fruits, vegetables, and whole grains and contains a minimum of added sugars, sodium, trans and saturated fats, and cholesterol.[9] Since 1999, there has been an upward trend in consumption of ultra-processed foods among US youth.[10] Contrarily, studies have shown that when foods with high nutrient density are consumed, they can help break the intergenerational cycle of malnutrition and obesity.[11] Nutrition education and cooking skills in the younger population can provide long-term benefits later in life.[12] Thus, one approach to combating childhood obesity has focused on providing nutrition education to children, particularly amongst at-risk populations. The approach is founded on the idea that increasing knowledge of food and eating habits can subsequently increase dietary consumption of healthier foods and decrease consumption of their unhealthy counterparts.[8,13,14]

Educational interventions including general food knowledge, gardening lessons, and interactive culinary skills sessions have proven to increase participant knowledge and awareness of healthy food choices; however, anthropometric changes have been variable.[4,6,13,15,16] Yet if initiated in the early stages of life, there is evidence that nutrition education coupled with cooking skills can be used to prevent and reduce diet-related disease and overall obesity.[17] Inconsistency in study findings suggests multifactorial influences on eating patterns in the paediatric population. Home food availability, parental diet, familial eating habits, and length and sustainability of intervention have been identified as factors that play an important role in the diet quality of children.[7,15] Research shows that healthy eating leads to healthier weights and better long-term health outcomes.[3] Nonetheless, the benefits of integrating food and cooking skills (e.g. food safety, preparation) into nutrition education programmes have not been widely explored in urban paediatric populations, serving as the novel focus of this study. For example, while programmes such as SNAP-Ed and EFNEP have integrated skills-based aspects like food and cooking safety, there is limited research on their specific impact on paediatric populations, particularly in terms of longitudinal outcomes. We sought to fill that gap by exploring the benefits of integrating such skills-based components into nutrition education programmes.

In Philadelphia, approximately 41% of youth aged 6–17 are overweight or obese and more than a third (37%) live in poverty.[1,18] Vetri Community Partnership (VCP) is a nonprofit organisation in Philadelphia that strives to educate and empower students and families to lead healthier lives.[19] Using the kitchen as the classroom, Vetri Cooking Lab (VCL) — on of several VCP community engagement initiatives — teaches students from under-resourced communities how to cook while reinforcing STEAM (science, technology, engineering, art, and math) core concepts.[20] Culinary and education experts combine hands-on cooking experiences with nutrition education to foster the next generation of educated food consumers. Each lesson includes the creation of one to three recipes that complement a nutrition lesson, such as 'Eating a Rainbow', the 'Importance of Breakfast', and 'Food Comes from the Earth'.

Previous research shows that nutrition education and cooking skills interventions can lead to improved food knowledge and confidence, which are positively correlated with healthier dietary behaviours.[13,14] Our study builds on these findings by examining the correlation between increased cooking confidence and knowledge with changes in dietary consumption behaviours among children. Specifically, in the current study, we aimed to determine if children enrolled in a culinary skills programme promoting healthy food choices (i.e. VCL) experienced subsequent changes in: (1) knowledge and confidence; (2) vegetable preferences; and (3) dietary consumption behaviours.

Methods

Study design, setting, and participants

To assess the effectiveness of the VCL programme, we conducted a longitudinal observational study comparing students' food knowledge and confidence, vegetable preferences, and dietary consumption behaviours student before and after VCL participation.

Eligible participants were enrollees in VCL during the 2018–19 academic year. Each VCL consisted of 8–10 sessions held weekly during both in-school and out-of-school time. Each class was conducted once a week, and the same cohort of students attended either in-school or out-of-school sessions, but not both, allowing for flexibility in implementation while maintaining consistent delivery of the programme's curriculum. There were a total of 60 VCLs at 30 sites throughout Philadelphia and Camden, with semesters running in the fall and spring of each school year. At each site, one class of up to 15 students from grades three and up were selected by the school liaison or after-school programme liaison with any methods of their choosing — hand-selecting, nominating, or utilising an application process.

Ethical review

Analysis of de-identified data was performed according to International Review Board (IRB) protocol approved by the Thomas Jefferson University IRB. Consent was obtained from parents/guardians of participating students through a signed consent form approved by partner schools.

Educational cooking lab programme

The programme's curriculum offered participants a sequence of lessons aimed to allow them to taste new foods in a positive setting and develop meal planning, food preparation, and cooking safety skills.[4] VCL provided students with hands-on cooking lessons that complemented nutrition topics and highlighted the STEAM concepts learned in school.

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Participants received extracurricular programming with a goal of increased understanding of healthy eating and food preparation. Teachers and school liaisons were encouraged to observe classes and often received recipe samples and recipe books. As an opt-in programme, students were enrolled by school staff or partners and able to withdraw at any time. Appendix A presents an overview of 2018–2019 VCL curriculum.

Measurement

A 46-item survey questionnaire was developed to be administered at baseline and post-intervention (see Appendix B). Additional demographic items were also collected (e.g. age, race/ethnicity, gender). Survey items were adapted from previously validated research instruments including the Healthy Eating Index (HEI), Block Kids Questionnaire, and Home Environment Survey.[21–26] The survey included 10 items to capture students' culinary knowledge (e.g. 'I can identify foods that are made of whole grains'.); 10 items to capture students' culinary confidence (e.g. 'I feel confident measuring ingredients correctly'); six items for consumption behaviours (e.g. 'How often do you eat fruits?'); and 20 items for vegetable consumption preferences (e.g. 'How do you feel about cabbage?'). To support comparison among the 20 vegetables included in the consumptive preferences measure, half were presented during VCL sessions and half were not presented (presented: cabbage, avocado, sweet potato, radish, cauliflower, kale, chickpeas, Swiss chard, mushroom, and turnips; not presented: eggplant, peas, Brussels sprouts, bell pepper, asparagus, cucumber, green beans, celery, butternut squash, carrots).

Knowledge and confidence items were responded to on a 3-point scale: '0 = not at all', '1 = sometimes', or '2 = often'. Frequency of food consumption behaviour items were presented with the options '0 = never', '1 = 1–2 times a week', '2 = 3–5 times a week', and '3 = daily or almost daily'. Consumption preferences item response included '0 = I don't like it', '1 = I like it', and '2 = I don't know what it is'. Confidence and knowledge items scores were totalled, creating a scale ranging 0 (low confidence/knowledge) to 20 (high confidence/knowledge). The first scale measured student confidence in meal planning and preparation. The second measured knowledge of nutrition and safety. Consumption behaviour questions were totalled for maximum score of 18. We assessed scale reliability of the included measures using Cronbach's alpha,[27] concluding that each exhibited high reliability scores (confidence = 0.77; knowledge = 0.73; consumption behaviours = 0.75).

Data collection

Programme instructors and volunteers administered baseline and post-intervention surveys in the first and last classes, respectively. If students were absent in the first class, the baseline assessment was administered during the first class attended by the student. If any student was absent on the last day, a post-intervention survey was not administered or collected, and their baseline survey was not included in our matched analysis. To address varied reading and comprehension skill levels, instructions for administering the surveys were provided for all VCL instructors and volunteers. In particular, instructors and volunteers were available to read survey questions orally. Assistance was provided for students who had questions about answering the surveys.

Table 1. Sample characteristics (n = 171). Frequency and proportion are presented for characteristics of the study participants including gender, grade, race, ethnicity, and language

Characteristic	Category	#	%
Gender	Female	104	60.8
	Male	63	36.8
Grade	1	1	0.6
	3	10	5.9
	4	46	26.9
	5	53	31.0
	6	31	18.1
	7	11	6.4
	8	8	4.7
	9	5	2.9
	11	1	0.6
Race	White	21	12.3
	Black	88	51.5
	Asian	1	0.6
	Other	42	
Ethnicity	Not Hispanic or Latino	73	42.7
	Hispanic or Latino	67	39.2
	Missing	31	18.1
Language	English	99	57.9
	Spanish	40	23.4

Statistical analysis

Following data collection, survey responses were compared at baseline and post-intervention to identify changes in cooking confidence, nutritional knowledge, consumption preferences (i.e. vegetable likes), and consumption behaviours. Students who skipped more than a quarter of questions in either survey were omitted from analysis. Median imputation was used to account for missing responses to individual items (percent missing: mean = 3.8; maximum = 7.6).

We used paired *t*-tests (alpha = 0.05) to identify pre-post changes in knowledge, confidence, consumption preferences, and consumption behaviours. Pearson's correlation coefficients (*r*) and Cohen's d, a standardised measure of effect size,[28] were also generated among each of the aggregate measures. For consumption preference questions, we calculated percentages of vegetables liked, not liked, and hadn't tried, stratified by whether or not vegetables were presented during VCL classes.

Using multivariable logistic regression, we modelled how consumption behaviours were associated whether changes in knowledge and confidence while adjusting for participants' age, race, ethnicity, gender, and language, as well as VCL site. An indicator variable was created if students had positive changes in consumption behaviours (0 = no change, 1 = change). To account for correlation between change in knowledge and confidence, we included an interaction variable in the final regression model to explore their potentially synergistic effect.[29,30] Student grade was not included because of collinearity with age.

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Table 2. Pre-post score and scale descriptive statistics (total n = 171). Summary of pre- and post-intervention scores for confidence, knowledge, and consumption behaviours among participants. Table columns includes mean scores, standard deviations, Cohen's d effect sizes, and P-values for paired *t*-tests comparing baseline and post-intervention results. Alpha level is set at 0.05 for significance. Alpha = 0.05

		Mean (SD)				
Score/Scale (Range)	N	Pre	Post	Δ	Cohen's d	P
Confidence (0–20)	159	14.00 (4.00)	15.52 (3.46)	1.64 (0.30)	0.45	< 0.001
Knowledge (0–20)	163	11.65 (3.75)	13.29 (3.97)	1.52 (0.27)	0.43	< 0.001
Consumption Behavior (0–18)	159	9.20 (3.65)	9.02 (3.62)	–0.05 (1.02)	0.05	0.5386
Liked Vegetable (%)	165	36.05 (19.87)	43.01 (19.53)	6.96 (6.39)	0.32	< 0.001
Disliked Vegetable (%)	165	30.44 (6.65)	32.34 (6.59)	1.90 (3.98)	0.32	0.0458
Haven't Tried Vegetable (%)	165	33.51 (20.40)	24.65 (18.66)	–8.86 (5.63)	0.48	< 0.001

Results

Sample characteristics

A total of 904 students enrolled in VCL in the 2018–19 school year. Of enrollees, 171 completed pre- and post-surveys and were eligible for our analysis (response rate = 18.9%; see Table 1). Among the 171 respondents, more than half identified as Black (51.5%) and female (60.1%). Nearly four in ten also identified as Hispanic or Latino (39.1%). Student ages ranged from 8 to 16 (mean = 10.6; SD = 1.5; median = 10) and grade levels from 4 to 11. More than half were in grades four or five, 26.9% and 31.0%, respectively.

Confidence, knowledge, and consumption behaviours

On average, students scored higher on post-survey confidence ($P < 0.001$; $d = 0.45$) and knowledge ($P < 0.001$; $d = 0.43$) scales (see Table 2). Half of students reported at least some changes in individual consumption behaviours (n = 85, 49.7%). As shown in Table 3, knowledge and confidence were positively correlated ($r = 0.55$; $P < 0.001$). Increased confidence was also positively correlated with consumption behaviour change ($r = 0.18$; $P = 0.022$). Cohen's d scores and *t*-tests revealed small to medium effects on students' food confidence and knowledge that were significant at the alpha = 0.05 level (see Table 2).

When accounting for differences in student demographics and VCL site, we identified several predictors of changes in consumption behaviours (see Table 4). Increased knowledge was independently associated with behaviour changes (OR = 1.46; P = 0.026). Confidence was positively associated with consumption changes in both our adjusted (OR = 1.81; P < 0.001) and unadjusted models (aOR = 1.88; P = 0.013). Students who gained confidence in their ability to add vegetables to every meal had nearly three times higher odds of reporting consumption behaviour changes, an association that increased in strength in the adjusted model (aOR = 2.65; P = 0.030). Similarly, students who gained confidence in understanding the positive benefits of fruits and vegetables also had nearly three times higher odds (aOR = 2.88; P = 0.045).

Compared to Black students, White students were more likely to report consumption changes (aOR = 5.83; P = 0.013). In unadjusted modelling, Hispanic/Latino participants and participants who spoke Spanish had nearly three times higher odds of consumption behaviour changes (Hispanic/Latino OR = 2.55; P = 0.007; Spanish OR = 3.04; P = 0.005). Student age and gender were not associated with behaviour changes.

Table 3. Pearson's correlation coefficient (*r*) for survey components. Correlation coefficients are presented for selected components of the survey, including confidence, knowledge, consumption behaviours, and vegetable likes. Significance levels are indicated: ***P < 0.001, *P < 0.05. Alpha = 0.05

	Vegetable Likes	Confidence	Knowledge
Confidence	–0.10	–	–
Knowledge	–0.13	0.55***	–
Consumption Behaviors	–0.15	0.18*	0.04

Vegetable preference changes

Table 5 presents changes in vegetable preferences from pre- to post-class as well as between vegetables presented in class versus those not presented. Among vegetables presented during class, bell peppers, chickpeas, and mushrooms exhibited the greatest changes in percentage liked, 88%, 67%, and 58%, respectively. On average, vegetables presented during class had higher percentage increase in students who liked them (P < 0.001) and decreases in students who hadn't tried them (P < 0.001).

Discussion

Students who participated in VCL showed noticeable improvements in cooking confidence and knowledge, indicating the programme's potential benefits and impact on their dietary habits. Most students also reported individual changes in their consumption behaviours and preferences for certain vegetables. There was a strong positive correlation between students' knowledge and confidence levels, suggesting that as students gained more knowledge, their confidence also increased. Our analysis identified several predictors of student behaviour changes highlighting potential demographic disparities. Taken together, our findings help to close a gap in understanding the impact of nutritional education in paediatric populations, pointing to multiple opportunities for improving VCL programming and future research and evaluation.

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Table 4. Logistic regression models. Results of logistic regression models analysing the association between changes in consumption behaviours and various predictors, including knowledge change, confidence change, age, race, gender, ethnicity, and language. An interaction term between knowledge and confidence is also included to explore their combined effect. Unadjusted and adjusted odds ratios (i.e. OR, aOR), 95% confidence intervals, and P-values presented at right (Alpha = 0.05).

	OR	95% CI		P	aOR	95% CI		P
Knowledge Change	1.46	1.05	2.04	0.026	1.04	0.61	1.79	0.880
Vegetables Likes	0.98	0.72	1.33	0.884	0.92	0.62	1.36	0.676
Knowledge x Confidence	1.01	0.86	1.19	0.908	0.75	0.59	0.96	0.025
Confidence Change*	1.81	1.26	2.61	0.001	1.88	1.14	3.08	0.013
I feel confident making a meal for my family	1.34	0.62	2.90	0.459	1.98	0.72	5.41	0.184
I think about adding a vegetable to every meal	2.13	1.08	4.21	0.03	2.65	1.10	6.41	0.030
I feel confident measuring ingredients correctly	0.97	0.43	2.16	0.938	0.74	0.23	2.38	0.617
I feel confident reading and following a recipe	0.94	0.41	2.15	0.886	0.51	0.15	1.71	0.278
I feel confident when I use a knife	2.31	0.79	6.77	0.126	1.00	0.21	4.76	0.998
I feel confident when I use a hand-held can opener	1.51	0.77	2.95	0.23	2.16	0.64	7.27	0.216
I feel confident when I use a vegetable peeler	1.08	0.45	2.55	0.869	0.87	0.26	2.94	0.819
I follow safety rules when I carry a knife	2.28	1.02	5.10	0.046	1.71	0.55	5.29	0.351
I feel confident that I could find nutritious foods in my corner store	2.18	1.15	4.14	0.018	2.04	0.96	4.33	0.062
I think eating fruits and vegetables makes me feel good	3.42	1.50	7.81	0.004	2.88	1.02	8.12	0.045
Age (years)	0.99	0.97	1.02	0.62	1.02	0.92	1.14	0.654
Race (ref = Black/AA)								
White	3.61	1.28	10.19	0.015	5.83	1.44	23.61	0.013
Other	1.51	0.73	3.15	0.268	0.86	0.28	2.62	0.785
Gender (ref = Female)								
Male	0.76	0.41	1.42	0.391	0.72	0.32	1.63	0.432
Ethnicity (ref = Not Hispanic/Latino)								
Hispanic/Latino	2.55	1.29	5.04	0.007	1.11	0.33	3.76	0.871
Language (ref = English)								
Spanish	3.04	1.39	6.66	0.005	2.87	0.82	10.07	0.099
Other	1.82	0.54	6.14	0.332	3.03	0.58	15.91	0.190

*Excluded from model with individual items.

Table 5. Vegetable preferences pre- versus post-programme. Comparison of participants' preferences for various vegetables before and after the intervention. The table includes percentages of vegetables liked, disliked, and not tried, stratified by whether the vegetables were presented during the VCL sessions. Paired *t*-tests were used to evaluate pre-post differences, with significance levels indicated: ***P < 0.001, *P < 0.05. Alpha = 0.05

	% Liked			% Did Not Like			% Did Not Try		
Vegetable	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ
Presented in Class									
Chickpeas	19.9	37.4	88.3	38.6	39.8	3.0	41.5	22.8	–45.1
Bell Pepper	35.1	58.5	66.7	33.9	31.0	–8.6	31.0	10.5	–66.0
Mushroom	22.2	35.1	57.9	43.3	46.2	6.8	34.5	18.7	–45.8
Eggplant	18.1	23.4	29.0	30.4	36.3	19.2	51.5	40.4	–21.6
Cabbage	45.6	55.6	21.8	28.7	26.9	–6.1	25.7	17.5	–31.8
Cucumber	56.7	64.9	14.4	28.7	28.7	0.0	14.6	6.4	–56.0
Celery	59.1	62.6	5.9	29.2	29.2	0.0	11.7	8.2	–30.0
Sweet Potato	62.0	64.9	4.7	28.1	26.9	–4.2	9.9	8.2	–17.6
Cauliflower	42.1	43.9	4.2	32.8	38.6	17.9	25.2	17.5	–30.3
Carrots	73.7	74.9	1.6	21.6	22.8	5.4	4.7	2.3	–50.0
Not Presented in Class									
Swiss Chard	7.6	15.2	100.0	16.4	21.1	28.6	76.0	63.7	–16.2
Kale	25.2	34.5	37.2	24.0	29.2	21.9	50.9	36.3	–28.7
Avocado	40.4	53.2	31.9	29.2	28.7	–2.0	30.4	18.1	–40.4
Butternut Squash	10.5	13.5	27.7	28.7	32.8	14.3	60.8	53.8	–11.5
Peas	48.0	59.1	23.2	43.3	36.3	–16.2	8.8	4.7	–46.6
Turnips	13.5	15.8	17.4	25.7	26.9	4.5	60.8	57.3	–5.8
Brussels Sprouts	30.4	35.7	17.3	35.7	39.8	11.5	33.9	24.6	–27.6
Asparagus	32.2	36.3	12.7	33.9	32.8	–3.4	33.9	31.0	–8.6
Radish	14.6	15.2	4.0	31.6	42.1	33.3	53.8	42.7	–20.7
Green Beans	64.3	60.8	–5.5	25.2	31.0	23.2	10.5	8.2	–22.2
Mean — Presented in Class	*43.5*	*52.1*	*29.5****	*31.5*	*32.6*	*3.3**	*25.0*	*15.3*	*–39.4****
Mean — Not Presented in Class	28.7	33.9	26.6***	29.4	32.0	11.6*	42.0	34.0	–22.8***
Mean — Total	36.1	43.0	28.0***	30.4	32.3	7.5*	33.5	24.6	–31.1***

Exposure to healthy foods and culinary skills education can have a positive impact on confidence in meal planning and preparation and knowledge of nutrition and safety.[31] Our study highlights the importance of integrating cooking lessons and kitchen skills into educational programmes as a means to improve food preferences, cooking attitudes, self-efficacy, nutrition knowledge, and long-term healthy eating habits in paediatric populations. It emphasises the practical and tangible benefits that can be derived from hands-on cooking experiences and underscores the potential of such interventions in promoting positive dietary behaviours in childhood.

Constraints to behavioural dietary changes are usually multifactorial and are influenced by socioeconomic status, access to food resources, cultural effects, home food availability, and overall food preferences.[7,32,33] For example, children are limited to the types of food their parents provide them which can be impacted by parental nutrition education, cooking skills, familial eating habits, and time and availability to prepare meals. And a child's larger nutrition environment — for example, school cafeteria, grocery stores, restaurants — determines food choices and quality of foods consumed and this entails areas of food deserts, food swamps, and overall food insecurity.[32] Given VCL was conducted in the surrounding regions of Philadelphia with an unfavourable nutrition environment,[34–36] the lack of an effect among students who didn't report behaviour changes could be explained by how much control children actually have over their dietary intake outside of school.

Showing vegetables during lessons was important for exposing students to previously untried items, and could also serve as an opportunity to change negative perceptions and preferences. Prior research shows that incorporating cooking lessons and the teaching of kitchen skills as a component improves participants' preference for vegetables, positively impacts food and cooking attitudes, and increases self-efficacy and enthusiasm towards assisting with food preparation at home.[5,6,17] Additionally, studies amongst college students have found that incorporating hands-on components such as cooking safety to these sessions is effective in increasing nutrition knowledge, cooking confidence, and ability even further and may promote long-term healthy eating habits.[18]

Cooking skills interventions like VCL that improve cooking confidence and increased fruit and vegetable consumption can promote healthy dietary behaviours, an effect particularly beneficial for economically marginalised populations.[31] Although we stratified our results by age, we found no differences across age groups in terms of improvements in cooking confidence, knowledge, or dietary behaviours, contrasting with findings from other studies. For example, Lavelle and colleagues[17] noted that children under 12 years old and teens 13–18 years old had greater confidence in their cooking and food skills, cooking practices, cooking attitudes, diet quality, and health. The authors underscored the 'importance of learning cooking skills at an early age for skill retention, confidence, cooking practices, cooking attitude and diet quality'.[17] Based on our evaluation of VCL participants, the benefits of cooking skills interventions may be experienced consistently across a wide age range, supporting the inclusion of such programmes at various developmental stages.

Our findings suggest several potential follow-up studies to further understand and improve food confidence and consumption behaviours. Firstly, a long-term follow-up study to track the sustained effects of VCL on students' food confidence, knowledge, and consumption behaviours over time. Secondly, an intervention modification study that tailors VCL for specific subgroups that showed lower engagement or less demonstrable behaviour changes, aiming to optimise the intervention's effectiveness and address disparities. Lastly, we propose a qualitative study to explore students' experiences, perceptions, and barriers, providing valuable insights into the factors influencing confidence, knowledge, and behaviour changes.

The current study is not without limitations. First, as our dataset included only self-reported response from VCL participants, we could not confirm behaviour changes otherwise. Furthermore, our findings may not be generalisable beyond students who participated in VCL in Philadelphia, although they may be transferrable to similar populations in other diverse urban settings, and it is possible that the findings are not generalisable to students who participated in VCL but didn't complete evaluation surveys. Second, our sample was comprised of VCL participants who completed both baseline and end line surveys, less than 20% of the total participants in the programme. In the absence of information about those who participated in VCL but didn't complete the surveys, it is possible that there were differences between our sample and the larger pool of VCL students both demographically and as it relates to changes in knowledge, confidence, and consumption behaviours. Third, although HEI has been rigorously evaluated for validity and reliability as a scoring metric that utilises dietary intake data collected from other instruments (e.g. 24-hour recalls, food records or food frequency questionnaires) to determine overall diet quality as well as quality of various dietary components, it does not collect dietary intake data in and of itself as we adapted it for in the current study. Finally, we lacked a control group with which to draw comparisons about the impact of VCL. The lack of a control group limits our ability to establish causality and draws attention to the need for more robust study designs in future research endeavours.

Conclusion

Although family members are usually the primary source of culinary skills for the younger generation, there is great variability with regard to access and knowledge of culinary skills within families. Extracurricular cooking lessons and kitchen skills teaching can supplement and enhance participants' self-efficacy and enthusiasm towards assisting with food preparation at home. When individuals gain confidence in their ability to cook and prepare meals, they are more likely to engage in these activities independently, leading to a greater sense of empowerment and autonomy in making healthy food choices. As VCL is done as a peer-educational model, it could ultimately be part of larger normative restructuring efforts of food and food preparation culture.

Here we demonstrated that programmes integrating practical cooking skills education along with nutrition can improve

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confidence and knowledge about healthy food choices amongst children. With this improved confidence and knowledge of healthy foods, we hope to improve attitudes towards healthier foods and in the long term inspire healthier food behaviours. We encourage larger studies to be done in the paediatric setting to see how we can improve and expose children to healthier foods to create long term healthier lifestyles. This has the long-term goal of preventing adolescent and adult obesity.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.31

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Conflict of interest

None.

Authorship

MB, AN, and SA conceived of and conducted the intervention. AM and MB collected and prepared data for analysis. MK, PC, and SA conducted data analysis. AM, MB, AN, and SA developed the first version of the manuscript draft, and MK, PC, and SA completed the final version of the manuscript draft. All authors reviewed, commented on, and approved of the final draft of the manuscript.

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RESEARCH ARTICLE

Relationship between coronary artery disease with dyslipidaemia and trace mineral intake: a cross-sectional analysis of the Shika study

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Abstract

Although the relationship between dyslipidaemia (DL) and coronary artery disease (CAD) or between trace minerals intake and CAD is well known separately, the exact nature of this relationship remains unknown. We hypothesize that the relationship between trace mineral intake and CAD may differ depending on whether or not the individual has DL. The present study analysed the relationships among trace mineral intake, DL, and CAD in middle-aged and older adults living in Shika town, Ishikawa prefecture, Japan. This study included 895 residents following the exclusion of those with genetic risk carriers for familial hypercholesterolemia. Trace mineral intake was evaluated using the brief-type self-administered diet history questionnaire. Interactions were observed between DL and CAD with zinc ($p = 0.004$), copper ($p = 0.010$), and manganese intake ($p < 0.001$) in a two-way analysis of covariance adjusted for covariates such as sex, age, body mass index, and current smokers and drinkers. Multiple logistic regression analysis showed that zinc (odds ratio (OR): 0.752; 95% confidence interval (CI): 0.606, 0.934; $p = 0.010$), copper (OR: 0.175; 95% CI: 0.042, 0.726; $p = 0.016$), and manganese (OR: 0.494; 95% CI: 0.291, 0.839; $p = 0.009$) were significant independent variables for CAD in the dyslipidaemic group. The present results suggest that DL with a low trace mineral intake is associated with CAD. Further longitudinal studies are required to confirm this relationship.

Key words: **Angina pectoris: Lipoproteins: Logistic models: Myocardial infarction: Triglycerides: Zinc**

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Introduction

Coronary artery disease (CAD) is estimated to affect 1655 per 100000 individuals worldwide (126 million people) and accounts for 9 million deaths annually. It is regarded as one of the leading causes of death and disability globally.[1] Risk factors for CAD include diabetes, hypertension, smoking, dyslipidaemia (DL), obesity, and social stress.[2] DL is a well-established risk factor for CAD and is estimated to account for more than 50% of CAD cases worldwide.[3] The diagnosis of DL is based on high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), and triglycerides (TG).[4]

One of the nutrients related to serum lipids is trace minerals, such as zinc,[5,6] copper,[7,8] and manganese.[9,10] Zinc plays an essential role in many biochemical pathways as a cofactor for more than 300 enzymes, such as the expression of several genes, and in immune function regulation in many types of cells.[11,12] A cohort study by Chen *et al.*[13] showed that an adequate nutritional zinc intake was associated with lower CAD mortality. Moreover, a systematic review by Ranasinghe *et al.*[6] revealed that zinc supplementation significantly reduced total cholesterol, LDLC, and triglycerides. Regarding the relationship between copper and lipids, a cross-sectional study by Song *et al.*[14] demonstrated that high serum copper levels were associated with elevated serum concentrations of total and HDLC. Additionally, a review by Blades *et al.*[15] found that an increased copper intake ameliorated the detrimental effects of a high-fat diet. Regarding manganese, a cohort study by Meishuo *et al.*[9] showed a relationship between its high intake and a lower risk of CAD. Even though the relationship between these trace minerals and serum lipids or CAD has been examined separately, the exact nature of this relationship remains unknown.

Although the relationship between DL and CAD has been extensively examined, the effects of trace mineral intake on this relationship remain unclear. We hypothesize that the relationship between trace mineral intake and CAD may differ depending on whether or not the individual has DL. Therefore, the present study analysed the relationships among trace mineral intake, DL, and CAD in middle-aged and older adults living in Shika town, Ishikawa prefecture, Japan.

Materials and methods

Study population

We used data from the Shika study.[16–18] Participants were recruited between October 2011 and January 2017. The target inhabitants were residents living in 4 model districts (Tsuchida, Higashimasuho, Togi, and Horimatsu districts) in Shika town, Ishikawa prefecture, Japan. The population of Shika town was 18786 as of November 2022, with an average age of 55.8 years.[19] The main industries in Shika town are manufacturing and services, with an ageing population of 39.9% at the time of the survey, making it a typical Japanese community in a superaged society. Written informed consent was obtained from all 4546 participants. One thousand and one hundred and seventy-four people who underwent a medical examination agreed to participate in this study. Of these, 7 had no blood tests, 12 had at least one of risk alleles for two single nucleotide polymorphisms (SNPs) which have been frequently observed among Japanese familial hypercholesterolemia (FH) patients as described below, 239 had not responded to the comorbidity and demographics questionnaire, 11 had not responded to the brief-type self-administered diet history questionnaire (BDHQ),[20,21] and 10 had a daily intake outside the range of 600–4000 kcal on the BDHQ and, thus, were excluded. Figure 1 shows inclusion criteria. In total, 895 participants (422 men and 473 women with mean (standard deviation, SD) ages of 62.32 (11.19) and 62.91 (11.41) years, respectively) were included in the analysis. The present study was conducted following the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Kanazawa University (No. 1491; Dec. 28, 2021). Informed consent was obtained from all participants involved in the study.

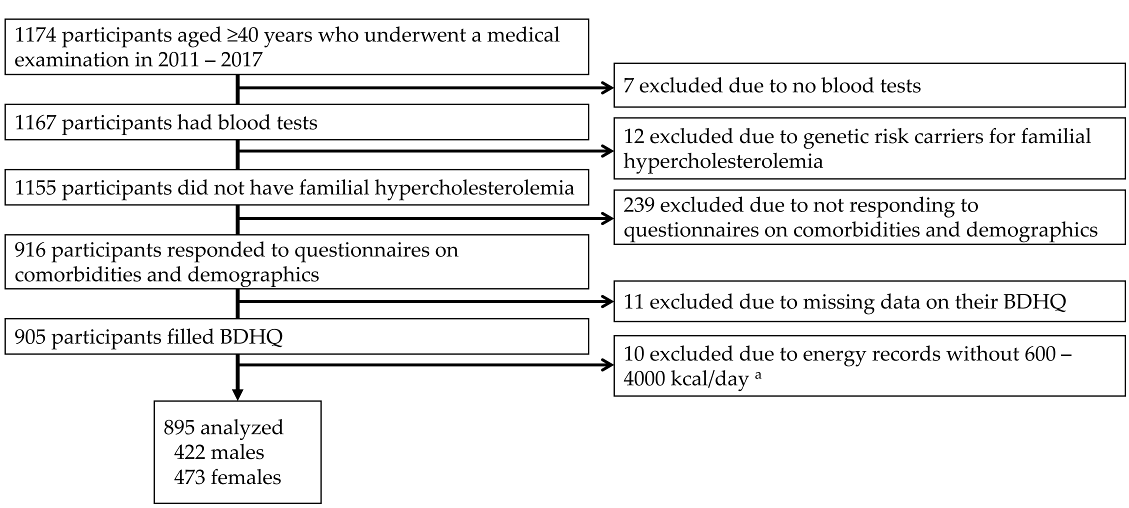

Fig. 1. Participant recruitment chart. [a] This range is due to the following reasons: less than 600 kcal/day is equal to half the energy intake demanded by the lowest physical activities; more than 4000 kcal/day is equivalent to 1.5-fold the energy intake needed for the medium physical activities. Abbreviations: BDHQ, Brief-type Self-Administered Diet History Questionnaire.

Evaluation of trace mineral intake

Zinc, copper, and manganese intakes were investigated using the BDHQ, which uses an analytical algorithm to estimate the intake of adult Japanese nutrients from the daily food consumed habitually, excluding supplements. The BDHQ collects data on the previous month and seasonal food intake from a structured four-page dietary intake questionnaire. The filled-out questionnaire is sent to the DHQ Support Center (Tokyo, Japan), where a computer system algorithm estimates the food consumed by the participant as the intake of each nutrient. The validity of the BDHQ has been demonstrated in previous studies.[22,23]

Evaluation of DL

Blood samples were collected during annual medical check-ups in the Shika study, mostly between December and January. DL was defined as hypertriglyceridaemia (≥150 mg/dL), hyper LDLC (≥140 mg/dL), or low HDLC (<40 mg/dL),[4] and/or receiving DL medication ($n = 123$). Genetic data, which were obtained in the previous study,[24] were utilized to identify the risk carriers for familial hypercholesterolemia (FH). Briefly, genomic DNA was isolated from peripheral blood samples using QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions or consigning the company specialized in clinical laboratory testing (SRL, Inc., Tokyo, Japan). Genome-wide SNP typing was performed using the Japonica Array v2[25] (TOSHIBA Co., Ltd. Tokyo, Japan). We defined individuals carrying at least one of the risk alleles for rs564427867 or rs879255211, which have been frequently observed among Japanese FH patients,[26] as genetic risk carriers and excluded them from the statistical analysis. For these two SNPs, deviations from the Hardy–Weinberg equilibrium were not observed ($p = 1.00$).

Evaluation of CAD and demographic data

A self-administered questionnaire was used to obtain data on comorbidities and lifestyle behaviors. CAD was defined as having a comorbidity of angina or myocardial infarction. Hypertension and diabetes questionnaire items were used as comorbidities associated with DL. Demographic data included age, sex (1: man, 0: woman), body mass index (BMI), smoking status (1: current smoker, 0: non- or ex-smoker), and drinking status (1: current drinker, 0: non-drinker or occasional drinker).

Statistical analysis

Participants were classified into the normolipidaemic and dyslipidaemic groups or the non-CAD and CAD groups. For statistical analyses, IBM SPSS Statistics version 25 for Windows (IBM, Armonk, NY, USA) was utilized. The Student's t-test was applied to examine the relationships between continuous variables, while the chi-squared test was adopted to investigate those between categorical variables. A two-way analysis of covariance (ANCOVA) was performed to analyse the main effects and interactions between the two lipidaemic groups and two CAD groups with trace mineral intake. Adjustments were performed for the following confounding factors: age, sex, BMI, current drinkers, and current smokers. To confirm the trace minerals that showed an interaction between the two lipidaemic groups and two CAD groups, a multiple logistic regression analysis stratified by the lipidaemic status with CAD as the dependent variable was performed. Variables were chosen using the forced entry method. The significance level was set to 5%.

Sample size

We used the free software, G-power, to calculate the sample size. In the F-test for ANCOVA, effect size, alpha error probability, power, number of covariates, and number of groups were set 0.25, 0.05, 0.95, 4, and 5, respectively. The total sample size and actual power were 400 and 0.950. For the Z-tests for logistic regression, tails, odds ratio, mull hypothesis, alpha error probability, power, X distribution, X parm π were set to two, 2.5, 0.20, 0.05, 0.95, binomial, and 0.5, respectively. The total sample size and actual power were found to be 315 and 0.950, respectively. Therefore, the sample size of this study was confirmed to be sufficient.

Results

Participant characteristics

Table 1 shows participant characteristics. Among 895 participants, the mean age of 62.32 years (standard deviation: SD = 11.19) in 422 men was not significantly different from that of 62.91 years (SD = 11.41) in 473 women. The percentages of men who were current drinkers ($p < 0.001$), current smokers ($p < 0.001$), with hypertension ($p = 0.012$), and with diabetes ($p = 0.004$) were significantly higher than those of women. Triglycerides ($p < 0.001$), total energy ($p < 0.001$), zinc ($p < 0.001$), copper ($p = 0.037$), and manganese ($p = 0.001$) were significantly higher in men that in women. The percentage of participants with CAD did not significantly differ by sex.

Comparisons between normolipidaemic and dyslipidaemic groups

Table 2 shows the characteristics of the two lipidaemic groups. The mean age of 420 patients in the dyslipidaemic group (63.56 years) was significantly higher than that of 475 patients in the normolipidaemic group (61.80 years, $p = 0.027$). In the lipid assessment, triglyceride ($p < 0.001$) and LDLC ($p < 0.001$) levels were significantly higher in the dyslipidaemic group than in the normolipidaemic group. The percentage of participants with CAD and the intake of trace minerals did not significantly differ between the two lipidaemic groups.

Comparison between non-CAD and CAD groups

Table 3 shows the characteristics of the two CAD groups. The mean age of 38 patients in the CAD group (72.61 years) was significantly higher than that of 857 patients in the non-CAD group (62.19 years, $p = 0.031$). The percentage of participants with hypertension was significantly higher in the CAD group than in the non-CAD group ($p = 0.047$). HDLC levels were significantly lower in the CAD group than in the non-CAD group ($p = 0.009$). Copper ($p = 0.041$) and manganese

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Table 1. Participant Characteristics

	Men ($n = 422$)		Women ($n = 473$)		
	Mean/n	SD/%	Mean/n	SD/%	p-value[a]
Age, years	62.32	11.19	62.91	11.41	0.819
BMI, kg/m^2	23.93	3.11	22.72	3.26	0.376
Current drinker, n (%)	305	72.27	117	24.74	<0.001
Current smoker, n (%)	349	82.70	70	14.80	<0.001
Hypertension, n (%)	145	34.4	126	26.6	0.012
Diabetes, n (%)	50	11.8	30	6.3	0.004
TG, mg/dL	131.50	87.50	106.50	56.07	<0.001
HDLC, mg/dL	60.82	16.03	68.50	17.01	0.053
LDLC, mg/dL	119.75	32.96	128.36	29.65	0.213
DL, n (%)	192	45.50	228	48.20	0.418
CAD, n (%)	20	4.74	18	3.81	0.489
Total energy, kcal	2084.70	604.45	1666.15	518.03	<.001
Zinc, mg/day	8.91	3.01	7.74	2.65	<0.001
Copper, mg/day	1.27	0.42	1.12	0.40	0.037
Manganese, mg/day	3.25	1.22	2.79	1.03	0.001

[a]The variables of means and SD are the Student's *t*-test, and those of n and percent are chi-square tests. Abbreviations: BMI, body mass index; CAD, coronary artery disease; DL, dyslipidaemia; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; SD, standard deviation; TG, triglycerides.

Table 2. Comparisons between normolipidemic and dyslipidaemic groups

	NL ($n = 475$)		DL ($n = 420$)		
	Mean/*n*	SD/%	Mean/n	SD/%	p-value[a]
Age, years	61.80	11.65	63.56	10.84	0.027
Sex (Men), n (%)	230	48.42	192	45.70	0.418
BMI, kg/m^2	22.68	3.08	23.97	3.29	0.196
Current drinker, n (%)	229	48.21	193	45.95	0.499
Current smoker, n (%)	236	49.68	183	43.57	0.063
Hypertension, n (%)	155	32.6	116	27.6	0.103
Diabetes, n (%)	48	10.1	32	7.6	0.193
TG, mg/dL	85.45	29.58	155.43	89.28	<0.001
HDLC, mg/dL	69.38	16.62	59.79	15.93	0.187
LDLC, mg/dL	109.31	18.70	141.25	34.40	<0.001
CAD, n (%)	18	3.80	20	4.80	0.471
Total energy, kcal	1864.51	605.94	1862.40	589.32	0.583
Zinc, mg/day	8.29	2.88	8.29	2.90	0.727
Copper, mg/day	1.19	0.42	1.19	0.41	0.561
Manganese, mg/day	3.00	1.20	3.02	1.08	0.053

[a]The variables of means and SD are the Student's *t*-test, and those of n and percent are chi-square tests. Abbreviations: BMI, body mass index; CAD, coronary artery disease; DL, dyslipidaemia; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; NL, normolipidaemia; SD, standard deviation; TG, triglycerides.

($p < 0.001$) intakes were significantly higher in the CAD group than in the non-CAD group. The percentage of participants with DL did not significantly differ between the two CAD groups.

Interaction between DL and CAD with trace mineral intake

Table 4 shows the results of an analysis of the main effects and interactions of DL and CAD with trace mineral intake using a two-way ANCOVA. Covariates were adjusted for age, sex, BMI, current drinkers, and current smokers. The trace minerals that showed a main effect in the two lipidaemic groups were zinc ($p = 0.008$), copper ($p = 0.022$), and manganese ($p = 0.002$). No trace minerals showed a main effect in the two CAD groups. The trace minerals that showed an interaction between the lipidaemic and CAD groups were zinc ($p = 0.004$), copper ($p = 0.010$), and manganese ($p < 0.001$). In multiple comparisons of the dyslipidaemic group using the Bonferroni method, the intake of zinc ($p = 0.010$) and copper ($p = 0.023$) was significantly lower in the CAD group than in the non-CAD group. In contrast, no significant differences were observed in these two trace mineral intakes between the two CAD groups in the normolipidaemic group. Therefore, these results indicate that zinc and copper intake was significantly lower in the CAD group than in the non-CAD group in the dyslipidaemic group, but did not significantly differ in the normolipidaemic group.

Logistic regression analysis of CAD with trace mineral intake stratified by DL

Table 5 shows the results of the multiple logistic regression stratified by normolipidaemic and dyslipidaemic groups, with

Table 3. Comparisons between non-CAD and CAD groups

	Non-CAD ($n = 857$)		CAD ($n = 38$)		
	Mean/*n*	SD/%	Mean/n	SD/%	*p*-value[a]
Age, years	62.19	11.22	72.61	8.35	0.031
Sex (male), n (%)	402	46.90	20	52.63	0.489
BMI, kg/m^2	23.27	3.26	23.59	2.99	0.465
Current drinker, n (%)	409	47.72	13	34.20	0.102
Current smoker, n (%)	403	47.02	16	42.10	0.548
Hypertension, n (%)	254	29.6	17	44.7	0.047
Diabetes, n (%)	74	8.6	6	15.8	0.130
TG, mg/dL	117.68	73.29	132.00	80.64	0.999
HDLC, mg/dL	65.25	17.07	56.63	12.31	0.009
LDLC, mg/dL	0.30	0.46	0.24	0.43	0.380
DL, n (%)	400	46.67	20	52.63	0.471
Total energy, kcal	1866.59	593.58	1793.91	692.81	0.268
Zinc, mg/day	8.29	2.86	8.28	3.40	0.137
Copper, mg/day	1.19	0.41	1.23	0.51	0.041
Manganese, mg/day	3.00	1.13	3.20	1.51	<0.001

[a]The variables of means and SD are the Student's *t*-test, and those of n and percent are chi-square tests. Abbreviations: BMI, body mass index; CAD, coronary artery disease; DL, dyslipidaemia; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; SD, standard deviation; TG, triglycerides.

Table 4. Interactions between DL and CAD with trace mineral intake

	NL ($n = 475$)				DL ($n = 420$)				*p*-value[a]		
	Non-CAD ($n = 457$)		CAD ($n = 18$)		Non-CAD ($n = 400$)		CAD ($n = 20$)		Main effect		Interaction
	EMM	95%CI (lower, upper)	EMM	95%CI (lower, upper)	EMM	95%CI (lower, upper)	EMM	95%CI (lower, upper)	*P*1	*P*2	*P*3
Zinc, mg/day	8.26	(8.00, 8.52)	9.29	(7.97, 10.60)	8.36	(8.08, 8.64)	6.69	(5.45, 7.94)	0.008	0.502	0.004
Copper, mg/day	1.18	(1.15, 1.22)	1.32	(1.13, 1.51)	1.20	(1.16, 1.24)	0.99	(0.81, 1.17)	0.022	0.572	0.010
Manganese, mg/day	2.97	(2.87, 3.07)	3.61	(3.09, 4.12)	3.04	(2.94, 3.15)	2.42	(1.94, 2.91)	0.002	0.972	<0.001

[a]Two-way ANCOVA. Covariates were adjusted for age, sex, BMI, current smokers, and current drinkers. *P*1: Lipidaemic groups, *P*2: CAD groups, *P*3: lipidaemic × CAD groups. Abbreviations: ANCOVA, analysis of covariance, BMI, body mass index; CAD, coronary artery disease; CI, confidence interval, DL, dyslipidaemia; EMM, estimated marginal means; NL, normolipidaemia.

Table 5. Multiple Logistic Regression Analysis

		Model 1					Model 2				
					95%CI					95%CI	
		B	*p*-value	OR	Lower	Upper	B	*p*-value	OR	Lower	Upper
NL (n = 475)	Zinc	0.116	0.147	1.123	0.960	1.314	0.112	0.159	1.119	0.957	1.309
	Copper	0.834	0.142	2.302	0.756	7.014	0.831	0.144	2.296	0.753	7.005
	Manganese	0.377	0.046	1.458	1.007	2.110	0.380	0.043	1.462	1.012	2.111
DL (n = 420)	Zinc	–0.285	0.010	0.752	0.606	0.934	–0.289	0.010	0.749	0.600	0.934
	Copper	–1.744	0.016	0.175	0.042	0.726	–1.779	0.015	0.169	0.040	0.713
	Manganese	–0.704	0.009	0.494	0.291	0.839	–0.717	0.009	0.488	0.285	0.835

The dependent variable is CAD. Model 1: Covariates were adjusted for age, sex, BMI, current smokers, and current drinkers. Model 2: Covariates in model 1 plus hypertension and diabetes. Abbreviations: B, partial regression coefficient; CI, confidence interval; DL, dyslipidaemia; NL, normolipidaemia; OR, odds ratio.

the dependent variable of CAD. In model 1, covariates were adjusted for age, sex, BMI, current smokers, and current drinkers, with each trace mineral imputed individually as independent variables. Significant independent variables for CAD were zinc (OR: 0.752; 95% CI: 0.606, 0.934; $p = 0.010$), copper (OR: 0.175; 95% CI: 0.042, 0.726; $p = 0.016$), and manganese (OR: 0.494; 95% CI: 0.291, 0.839; $p = 0.009$) in the dyslipidaemic group. The Bonferroni correction divided by the number of individually input trace minerals showed that zinc and manganese were significant independent variables. There was no trace mineral for CAD in the normolipidaemic group.

Model 2 was the same analysis as model 1, with the addition of hypertension and diabetes as covariates. Significant independent variables in the dyslipidaemic group were zinc (OR: 0.749; 95% CI: 0.600, 0.934; $p = 0.010$), copper (OR: 0.169; 95% CI: 0.040, 0.713; $p = 0.015$), and manganese (OR: 0.488; 95% CI: 0.285, 0.835; $p = 0.009$). In the Bonferroni correction for the number of items, zinc and manganese were significant independent variables. In the normolipidaemic group, trace minerals other than manganese were not independent variables for CAD. Therefore, low zinc and manganese intake inversely correlated with CAD in the dyslipidaemic group, but not in the normolipidaemic group.

Discussion

The present results demonstrated that zinc intake was significantly lower in the CAD group than in the non-CAD group in the dyslipidaemic group, but not in the normolipidaemic group in multiple comparisons by the Bonferroni method after a two-way ANCOVA or in the multiple logistic regression analysis.

A review by Mihăilă[22] that examined the relationship between DL and CAD revealed that HDLC, the TC/HDLC ratio, and TG/HDLC ratio were strongly related in a population with cardiovascular risk factors. Andreadou *et al.*[23] showed that patients with hypercholesterolemia were predisposed to myocardial dysfunction and myocardial infarction through increased oxidative stress, mitochondrial dysfunction, and apoptosis induced by inflammation. The present study revealed that HDLC was significantly lower in the CAD group than in the non-CAD group. Previous reviews[27,28] on the relationship between HDLC and CAD reported that low HDLC increased the risk of CAD. In contrast, the present results showed no increase in TG or LDLC in the CAD group. Although the underlying reason is unclear, dietary habits represented by the Japanese diet may be involved because we targeted middle-aged and older adults in a rural Japanese community.

The present results on zinc, copper, and manganese showed that its lower intake in the dyslipidaemic group was associated with CAD after adjustments for age, sex, BMI, current smokers, and current drinkers. A case–control study by Ghayour-Mobarhan *et al.*[29] showed that dyslipidaemic patients with CAD had a lower zinc intake and zinc/copper ratio than patients without CAD or healthy university/hospital personnel as controls. It has been reported that lower zinc intake is associated with CAD[29] and CAD mortality.[13] Furthermore, a systematic review by Ranasinghe *et al.*[11] revealed that zinc supplementation significantly reduced TC, LDLC, and TG. Therefore, improvements in HDLC and LDLC reduce the risk of CAD, indicating that the high zinc intake in the dyslipidaemic group decreased the risk of developing CAD. A reason why the low zinc intake was associated with CAD in the dyslipidaemic group only may be attributed to an insufficient trace mineral intake, which did not improve blood lipids and, thus, failed to reduce the risk of developing CAD. Alternatively, copper[14,15] and manganese[9] have been associated with a decrease in serum lipids. In our multiple comparisons using the Bonferroni method, zinc and copper correlated with CAD in the dyslipidaemic group only. Additionally, in multiple logistic regression analyses using two different models, zinc and manganese were significant independent variables in the dyslipidaemic group after the Bonferroni correction. Therefore, a novel result in the present study is that a low zinc intake with DL was strongly associated with CAD in the simultaneous evaluation of several trace minerals. As a possible mechanism, the combination of low zinc intake increasing total cholesterol, LDLC, and triglycerides,[6] as well as low copper intake decreasing HDLC[14] and low manganese intake increasing CAD risk,[9] suggests that low intake of these trace minerals is comprehensively associated with CAD only in the DL group.

Typical comorbidities related to CAD include hypertension[30,31] and diabetes.[32,33] A systematic review and meta-analysis by Nicoll *et al.*[33] demonstrated the importance of hypertension and diabetes as predictors of the presence and extent of coronary artery calcification. Even in our multiple logistic regression analysis with hypertension and diabetes as covariates in addition to lifestyle, zinc was a significant independent variable that negatively correlated with CAD in the dyslipidaemic group. Additionally, zinc has been shown to improve insulin sensitivity.[34,35] Therefore, another novel result of the present study is that a high zinc intake was identified as a beneficial factor for CAD, even in the presence of diabetes and hypertension comorbidities in addition to DL.

Among DL, FH is a common hereditary autosomal dominant disorder characterized by high plasma cholesterol levels, with an estimated frequency of 1 in 200.[36] The most common causes of FH are pathogenic variants of the LDL receptor (*LDLR*) gene, as well as deleterious mutations in the apolipoprotein B (*APOB*) gene decreasing the binding of LDL to the *LDLR* and gain-of-function mutations in the gene for proprotein convertase subtilisin/kexin 9 (*PCSK9*) resulting in the increased destruction of *LDLR*.[37] The presence of *APOB* or *PCSK9* mutations is included in the diagnostic criteria of the Simon Broome Criteria for the Diagnosis of FH. A genetic analysis by Koyama *et al.*[38] revealed that rs564427867 in *PCSK9* and rs879255211 in *LDLR* were associated with elevated serum levels of TC in the Japanese population. Since hereditary hypercholesterolemia may not be associated with trace mineral intake or serum lipid levels, we excluded residents with rs564427867 or rs879255211 risk alleles from the statistical analyses.

The limitations of the present study ought to be addressed. First, since this was a cross-sectional study, the causal relationship between trace minerals, serum lipids, and CAD cannot be elucidated. Secondly, comorbidities other than DL were self-reported by a questionnaire and not diagnosed by data. Thirdly, trace mineral intake by the BDHQ may lack objectivity and does not include supplement intake, Fourthly, due to the small number of CAD participants, further analysis with a larger sample size is needed. Fifthly, we did not examine the trace mineral intake from living environments. Sixthly, we did not evaluate the trace minerals in serum. Finally, since the results are from one rural town, it may not be representative of Japan.

Conclusions

Results of the present study showed that in the dyslipidaemic group, trace mineral intake was significantly lower in the CAD group than in the non-CAD group, whereas in the normolipidaemic group, this association was not observed between the two CAD groups. Further longitudinal studies are needed to confirm this relationship.

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Conflict of interest

None.

Author contributions

Conceptualization, HN; formal analysis, TK (Takayuki Kannon), TS, AN: data curation; KK (Kei Kimura); funding acquisition, HN; investigation, KK (Kei Kimura), FS, HT (Hiromasa Tsujibuchi), SM, KS, YS, TTTN, KK (Koji Katano), AA, TK (Tomoko Kasahara), MN, CT, KH, TH, AS, HT (Hirohito Tsuboi); methodology, HN; project administration, HT (Hiromasa Tsujibuchi), HN; resources, HN; supervision: AH, TK (Tadashi Konoshita), YK, AT, TK (Takayuki Kobayashi); visualization, FS; Writing — original draft, KK (Kei Kimura), FS; writing — review and editing, HT, AH, SM, TK (Takayuki Kannon), KS, YS, TTTN,KK (Koji Katano), AA, TK (Tomoko Kasahara), MN, CT, KH, TH, AS, TS, AN, TK (Tadashi Konoshita), YK, HT (Hirohito Tsuboi), AT, TK (Takayuki Kobayashi), HN. All authors have read and agreed to the published version of the manuscript.

Data availability

Data in the present study are available upon request from the corresponding author. Data are not publicly available due to privacy and ethical policies.

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RESEARCH ARTICLE

Spatial variation and risk factors of the dual burden of childhood stunting and underweight in India: a copula geoadditive modelling approach

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Abstract

India has one of the highest burdens of childhood undernutrition in the world. The two principal dimensions of childhood undernutrition, namely stunting and underweight can be significantly associated in a particular population, a fact that is rarely explored in the extant literature. In this study, we apply a copula geoadditive modelling framework on nationally representative data of 104,021 children obtained from the National Family Health Survey 5 to assess the spatial distribution and critical drivers of the dual burden of childhood stunting and underweight in India while accounting for this correlation. Prevalence of stunting, underweight and their co-occurrence among under 5 children were 35.37%, 28.63% and 19.45% respectively with significant positive association between the two (Pearsonian Chi square = 19346, P-value = 0). Some of the factors which were significantly associated with stunting and underweight were child gender (Adjusted Odds Ratio (AOR) = 1.13 (1.12) for stunting (underweight)), birthweight (AOR = 1.46 (1.64) for stunting (underweight)), type of delivery (AOR = 1.12 (1.19) for stunting (underweight)), prenatal checkup (AOR = 0.94 (0.96) for stunting (underweight)) and maternal short-stature (AOR = 2.19 (1.85) for stunting (underweight)). There was significant spatial heterogeneity in the dual burden of stunting and underweight with highest prevalence being observed in eastern and western states while northern and southern states having relatively lower prevalence. Overall, the results are indicative of the inadequacy of a "one-size-fits-all" strategy and underscore the necessity of an interventional framework that addresses the nutritional deficiency of the most susceptible regions and population subgroups of the country.

Key words: **Copula geoadditive model: India: NFHS 5: Stunting: Underweight**

Background

Childhood undernutrition is one of the most pressing public health crises facing the world today. It is estimated that nearly 50% of deaths in children under 5 are due to some form of undernutrition, while majority of these deaths occur in low and middle-income countries of South Asia and sub-Saharan Africa.[1,2] Undernutrition is broadly categorised into stunting, wasting, underweight and vitamin and mineral deficiency. Of these, stunting, wasting and underweight are collectively known as child growth failure (CGF) and corresponds to low height-for-age, low weight-for-height and low weight-for-age, respectively. As per 2020 estimates, 149.2 million children under 5 are stunted, 45 million are wasted and 462 million are underweight. These correspond to 22%, 6.7% and 68.18% of all children under 5 globally.[3] Due to its far-reaching negative consequences on childhood mortality, morbidity as well as on various aspects of health and wellbeing that continue into adulthood,[4–11] childhood undernutrition is now considered a global health priority with various global targets being set by international bodies, all aimed towards its reduction and eventual eradication. For instance, the first of the six global nutrition targets set by the World Health Assembly Resolution 65.6 in 2012 specifies a 40% reduction in stuntedness among under-5 children by 2025.[12–14] The aforementioned target is also part of the second Sustainable Development Goals (SDGs) set by the United Nations alongside ending hunger and all forms of malnutrition by 2030.

India has some of the highest rates of childhood undernutrition worldwide. As per current estimates, 31.7% of under 5

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children are stunted, 18.7% are wasted while underweight incidence ranges from 39% to as high as 74% across the country. Despite the success of various government-run large-scale interventions, India is still way behind in achieving the aforementioned global nutritional targets.

The antecedents of childhood undernutrition are multifarious and there exists considerable literature on the same in the context of India.[15–18] In all these studies, the different verticals of undernutrition are treated as independent and modelled separately. However, existing research has shown that these dimensions can be associated in a particular population and the degree of association can vary spatially.[19–21] To our knowledge, rigorous analysis of the co-occurrence of various dimensions of childhood undernutrition is a relatively under-researched area, especially in the context of India. The current study endeavours to address this gap by modelling the dual burden of childhood stunting and underweight across the different states and union territories of India using nationally representative data from the National Family Health Survey-5 (NFHS-5) carried out during 2019–2021. Since rigorous modelling of multiple dimensions of childhood undernutrition is not possible in the conventional modelling framework, we have used a joint modelling approach in this study since it incorporates the implicit association between the various dimensions while modelling their co-occurrence. In doing so, it enables us to understand the spatial heterogeneity in their co-occurrence as well as in their association parameter.

Specifically this study provides four novel contributions to the literature. First, we identify the various drivers of childhood stunting and underweight while accounting for the implicit association between the two measures. Second, we use a flexible modelling approach to incorporate non-linear association between the determinants and the undernutrition indices. Third, we account for spatial variations of the association between stunting and underweight across the states of India. Finally, we quantify the joint likelihood of all possible combinations of stunting and underweight across all the states and union territories and produce spatial maps of the same. We implement these through a comprehensive modelling framework based on the copula geoadditive modelling approach.[22,23] To our knowledge, this is one of the first studies that implements a joint modelling framework to analyse the co-occurrence of multiple dimensions of childhood undernutrition based on current nationally representative data from India. We hope that insights from this study will inform policymakers about regional variation in joint prevalence of childhood stunting and underweight and will aid in the formulation of effective nutritional interventions targeted towards the most susceptible regions and population subgroups of the country.

Methods

Data source

This study uses nationally representative data from the latest round of NFHS-5 carried out from June 2019 to April 2021. Like its previous counterparts, NFHS-5 provide estimates of key indicators of health and family welfare such as fertility, infant and child mortality, family planning practices and maternal and child health. The estimates are based on nationally representative data from 636,699 households, 724,115 women and 101,839 men selected using a two-stage stratified-cluster sampling scheme. The current study uses the children's recode file of NFHS-5, which contains data on various nutrition and health indicators of children who were less than 5 years of age during the time of the interview. Data on maternal health and household characteristics for the sampled children were also available. Of the total of 232,920 children included in the survey, data on 104,021 children and their mothers were used for the analysis after necessary data cleaning. Informed consent was taken from all the participants either in the form of written or verbal means.

Outcomes and covariates of interest

The outcomes of interest were childhood stunting and underweight. Anthropometric measures of children's height-for-age and weight-for-age *Z*-scores (HAZ and WHZ) were used to construct indicators of these indices. Specifically, children whose standardised HAZ and WAZ scores were below –2 were labelled as stunted and underweight respectively.[24,25] This corresponds to having height-for-age and weight-for-age values less than –2 standard deviations from the World Health Organization child growth standards median.[26] Children with scores more than 6 or less than –6 for either of these measures were treated as outliers and dropped. For children aged 2 years or below, height was measured using Seca 417 infantometer while for children aged 24–59 months, a Seca 213 stadiometer was used. Weight was measured using Seca 874 digital scale. Thus the dependent variables were binary with categories "stunted" and "not stunted" and "underweight" and "not underweight" respectively. Having said that, the main purpose of this study is to explore the spatial variation and determinants of the dual burden of malnutrition among Indian children characterised by the co-occurrence of stunting and underweight.

The antecedents for stunting and underweight for each child included age of the child (in months), age of mother at first birth (in years), duration of breastfeeding (in months), source of drinking water (1 if improved, 0 otherwise), quality of sanitation (1 if improved, 0 otherwise), prenatal care from doctor (1 if yes, 0 otherwise), whether child had low birth- weight (less than 2500 gms of weight at birth) (1 if yes, 0 if no), number of children in the household aged 5 years or below, child gender (1 for male, 2 for female), gender of household head (1 for male, 2 for female), mode of delivery (1 for caesarean, 0 otherwise), short stature of mother (height less than 145 cm) (1 if yes, 0 if no), place of residence (0 if urban, 1 if rural), whether mother is underweight (body mass index less than 20) (1 if yes, 0 if no), maternal educational attainment (0 if no education, 1 if primary education, 2 if secondary education and 3 if higher secondary education with 0 being the baseline), household wealth quintile (1 if poorest, 2 if poorer, 3 if middle, 4 if richer and 5 if richest with 1 being the baseline), caste (1 if scheduled caste, 2 if scheduled tribe, 3 if other backward classes and 4 if none of those with 1 being the baseline), perceived size at birth (1 if very large, 2 if larger than average, 3 if average, 4 if smaller than average and 5 if very small with 1 being the baseline), maternal anaemia level (1 if haemoglobin(Hb) 8 g/dl, 2 if 8 Hb 11, 3 if 11 Hb 12 and 4 if Hb 12 with 1 being the baseline) and childhood anaemia (1 if haemoglobin(Hb) 7 g/dl, 2 if 7 $\leq$ Hb <10, 3 if 10$\leq$ Hb < 11 and 4 if Hb $\geq$ 11 with 1 being the baseline).

Copula geoadditive models

Our empirical strategy hinges on a framework that enables the joint modelling of two binary responses, namely childhood stunting and underweight while accommodating the implicit association between the two. This is achieved using a copula geoadditive modelling framework that incorporate spatial heterogeneity in the stunting-underweight association as well as flexible non-linear functions of covariates.[22,27] In this setup, the dependence structure between the two responses is modelled using a special class of functions that enable flexible specification of the marginal models of each of the responses separately from that of the joint distribution governing their dependence structure. These functions are known as copulas.[28] Although a relatively new concept, copulas have been extensively used for modelling association between diverse class of responses across multiple fields. Applications range from modelling mixed binary-continuous data,[29] continuous and discrete longitudinal data,[30] censored data[31] and count data.[32] Copula models have been used in finance and insurance,[33,34] forestry and environment[35] and marketing[36] and public health.[37,38] Excellent reviews of copula models are provided by Trivedi and Zimmer (2007) and Genest (2007).

Let Y_{is} and Y_{iu} be the stunting and underweight status of the i^{th} child such that $Y_{is} = 1$ (0) if the child is stunted (not stunted) and $Y_{iu} = 1$ (0) if the child is underweight (not underweight). The copula framework enables the specification of the joint probability of the i^{th} child being stunted as well as underweight as

$$P(Y_{is}=1, Y_{iu}=1|\boldsymbol{x}_{is}, \boldsymbol{x}_{iu}) = C(P(Y_{is}=1|\boldsymbol{x}_{is}), P(Y_{iu}=1|\boldsymbol{x}_{iu}); \theta)$$

where $\boldsymbol{x}_{is}$ and $\boldsymbol{x}_{iu}$ are the attribute vectors corresponding to stunting and underweight respectively. Here $C\colon [0,1]^2 \rightarrow [0,1]$ is a two-place copula function while θ is the copula parameter that quantifies the association between stunting and underweight prevalence.[23] The marginal probabilities of being stunted or underweight i.e $P(Y_{is} = 1 \mid \boldsymbol{x}_{is})$ or $P(Y_{iu} = 1 \mid \boldsymbol{x}_{iu})$ is parameterised using the following latent variable representation

$$P(Y_{is} = 1|\boldsymbol{x}_{is}) = P(Y^*_{is} > 0|\boldsymbol{x}_{is}) = 1 - F_s(-\eta_{is})$$

where Y^*_{is} is a continuous latent variable expressible as $Y^*_{is} = \eta_{is} + \varepsilon_{is}$, η_{is} being the linear predictor consisting of linear, non-linear as well as structured and unstructured spatial effects while ε_{is} is a white noise error. $F_s(-\eta_{is})$ is the cumulative distribution function of the error and determines the structure of the marginal model linking the stunting indicator Y_{is}, to the corresponding linear predictor. The flexibility of the copula approach lies in the fact that $F(.)$ can correspond to a broad class of univariate distributions (Gaussian, logistic, Gumbel for instance) depending on the assumed distributional form for the error term. For instance, a standard normal distributional assumption for ε_{is} would lead to a probit specification for the corresponding marginal model. A similar setup can be replicated for the underweight indicator, Y_{iu}.

The flexibility of the copula geoadditive framework lies in its ability to incorporate a diverse class of effects in the marginal model specifications. In our setup, we accommodate the following four types of effects in the marginal models for stunting and underweight:

(i) regular fixed effects of the categorical variables and of those continuous variables which are linearly related to the response; (ii) flexible non-linear effects for variables which have a curvilinear association with the response; (iii) within-region (unstructured) spatial effects to account for the presumed similarity in stunting and wasting prevalence among children residing in the same region and lastly; (iv) between-region (structured) spatial effects to account for the assumed dependence in stunting and wasting prevalence among children residing in adjacent regions. The non-linear effects are estimated by thin-plate regression splines while the structured spatial effects are estimated by Markov random field smoother which is based on the neighbourhood structure of the regions.[23,29] For the purpose of modelling, we used the Frank copula to model the dependence between stunting and underweight and the logit specification for the marginal models of stunting and underweight. We chose the Frank copula for its flexibility in accommodating the full range of correlation values for the two responses.[39] The Frank copula has the following expression

$$C(F_s(Y_{is}), F_u(Y_{iu})\theta) = \frac{1}{\theta}\ln\left[1 + \frac{\left(e^{-\theta\times Fs} - 1\right)\left(e^{-\theta\times Fu} - 1\right)}{e^{-\theta} - 1}\right]$$

In addition to the marginal models of stunting and underweight, we modelled the copula parameter, θ with respect to the state boundaries in order to capture any spatial variation in the association between stunting and underweight across the states and union territories of India. This may enable the identification of particular states where stunting and underweight prevalences are strongly or weakly associated, which, in turn, can provide valuable insights to policymakers on the need for region-specific interventions. For ease of interpretation, the copula parameter was transformed to Kendall's tau correlation coefficient, τ which is a measure of the degree of concordance between two variables.[23] Finally, we create joint probability maps depicting the between-state variation in the co-occurrence of stunting and underweight.

Analysis was carried out using the R package *GJRM* (Generalized Joint Regression Modelling)[23,40] while mapping was carried out in QGIS 3.22 using shapefiles freely obtainable from the Spatial Data Repository maintained by the DHS programme (https://spatialdata.dhsprogram.com/boundaries). All estimates have been weighted using the sampling weights provided in the NFHS-5 data file. The standard errors of all the estimates have been suitably adjusted to account for the multistage cluster sampling carried out in the survey.

Results

We present our results in four stages. First, we discuss the prevalence and distribution of stunting and underweight across various socio-economic and demographic subgroups as identified by the child, maternal and household-specific attributes. We next present the linear as well as non-linear effects of the various covariates on the likelihood of stunting and underweight as derived from the marginal models. Next, we discuss the unstructured and structured spatial effects of stunting, underweight and their association across India. Finally, we elaborate on the insights obtained from joint probability maps of the dual burdens of stunting and underweight.

Stunting and underweight prevalence

Overall, 35.37% of the sampled children were stunted, 28.63% were underweight and 19.45% were both stunted and underweight. The prevalence of all the three conditions was higher among males (36.8% stunted, 29.9% underweight, 20.5% both) than females (33.7%, 27.2%, 18.2%). Children belonging to the scheduled caste category had the highest prevalence of all the three conditions (39.1%, 32.1%, 22.2%) followed by those in the scheduled tribe (38.6%, 30.3%, 21.1%), other backward classes (35.3%, 29.3%, 19.9%) and unreserved categories respectively (27.5%, 21.3%, 13.5%). Caesarean children had lower incidence of all the three conditions (29.2%, 22.3%, 14.5%) compared to those born through normal means (37.2%, 30.5%, 20.9%). Children whose perceived size at birth was "very small" had the highest incidence of all the three conditions (46.4%, 42.3%, 30.7%). Similarly, severely anaemic children had the highest incidence of the three conditions (46.1%, 36.5%, 27.8%) while the incidence gradually reduced with improvements in anaemia status. Finally, children having low birthweight had higher incidence of the three ailments (44.1%, 39.4%, 28.4%) compared to those having normal birthweight (33.7%, 26.6%, 17.7%). As far as maternal attributes are concerned, prevalence of each of the three conditions was substantially higher among children whose mothers had short stature (54.4%, 44.2%, 34.6%) or were underweight (42.2%, 37.8%, 26.4%) compared to those whose mothers had normal physique. Children whose mothers did not receive prenatal treatment from a certified physician had higher incidence of the conditions (39.2%, 32.5%, 22.6%) compared to those whose mothers received such care (33.1%, 26.4%, 17.6%). Children whose mothers had at least a higher secondary education had the lowest incidence of all the three conditions (23.7%, 17.4%, 10.3%) while the incidence steadily increased with lower maternal educational attainment. Highest incidence of the three conditions was observed among children whose mothers had severe anaemia (43.0%, 35.7%, 25.5%) followed by those whose mothers were moderately anaemic (38.0%, 31.1%, 21.6%), mildly anaemic (34.7%, 28.9%, 19.5%) and non-anaemic respectively (33.5%, 26.3%, 17.6%). As far as locational attributes were concerned, incidence of all the three ailments was lower among children hailing from urban regions (29.6%, 23.5%, 15.1%) compared to those from rural areas (37.0%, 30.1%, 20.7%). Children belonging to the richest households had the lowest incidence of all the three ailments (23.2%, 16.7%, 10.0%) while those belonging to the poorest households had the highest incidence (46.2%, 39.8%, 28.6%). Finally, lower incidence of the ailments were observed in households with improved sanitation facility (32.8%, 25.8%, 17.1%) and those with availability of clean drinking water (35.3%, 28.6%, 19.4%) compared to households lacking such facilities (Table 1).

In order to assess the strength of association between stunting and being underweight, we performed a Pearsonian chi-square test which yielded a P-value of 0 indicating strong positive association between the two. This validates the necessity of joint modelling of the ailments using the copula modelling framework.

Linear covariate effects

Adjusted for all other variables, boys had a significantly higher odds of being stunted as well as underweight compared to girls (AOR = 1.13, 95% CI 1.10–1.16 for stunting and AOR = 1.12, 95% CI = 1.09–1.16 for underweight). Similarly, children born through caesarean section had a significantly lower odds of being stunted and underweight compared to those born through normal delivery (AOR = 0.89, 95% CI = 0.86–0.92 for stunting and AOR = 0.84, 95% CI = 0.81–0.87 for underweight). Children who had low birth weight had a significantly higher odds of both the conditions (AOR = 1.46, 95% CI = 1.41–1.52 for stunting and AOR = 1.64, 95% CI = 1.58–1.71 for underweight). Interestingly, children hailing from rural regions had significantly lower odds of both stunting and underweight (AOR = 0.94, 95% CI = 0.91–0.97 for stunting and AOR = 0.91, 95% CI = 0.87–0.94 for underweight). As far as maternal attributes are concerned, children whose mothers received prenatal checkup from a doctor were significantly less likely to be either stunted or underweight than those whose mothers did not receive such treatment (AOR = 0.94, 95% CI = 0.92–0.98 for stunting and AOR = 0.96, 95% CI = 0.93–0.99 for underweight). Likewise, children whose mothers were short-statured had significantly higher odds of being stunted or underweight (AOR = 2.19, 95% CI = 2.1–2.28 for stunting and AOR = 1.85, 95% CI = 1.78–1.93 for underweight) and similar effects were observed for underweight mothers (AOR = 1.30, 95% CI = 1.26–1.34 for stunting and AOR = 1.57, 95% CI = 1.53–1.62 for underweight) as well. Higher maternal educational attainment, higher household wealth quintile and availability of clean drinking water and improved sanitation facilities on-premise correspond to significantly lower odds of stunting and underweight while higher severity of maternal and childhood anaemic status correspond to significantly higher odds of both the conditions. Newborns with perceived birthsize "smaller than average" and "very small" have a significantly higher odds of being stunted as well as underweight compared to the complement groups. As far as caste is concerned, children belonging to other backward classes and non-reserved (general) caste have significantly lesser odds of being stunted as well as underweight compared to those belonging to scheduled castes. However, children belonging to the scheduled tribes have a significantly higher odds of being underweight compared to those from the scheduled castes. Finally, the odds of stunting and underweight prevalence are significantly higher in

Table 1. Prevalence of stunting, underweight, and their co-occurrence among under 5 children

	Stunting		Underweight		Stunting & underweight	
	Proportion/Mean	Std. Dev	Proportion/Mean	Std. Dev	Proportion/Mean	Std. Dev
Overall	0.354	0.478	0.286	0.452	0.194	0.396
Child characteristics						
Gender						
Male	0.368	0.482	0.299	0.458	0.205	0.404
Female	0.337	0.473	0.272	0.445	0.182	0.386
Caste						
Scheduled caste	0.391	0.488	0.321	0.467	0.222	0.415
Scheduled tribe	0.386	0.487	0.303	0.460	0.211	0.408
Other backward classes	0.353	0.478	0.293	0.455	0.199	0.399
Non-reserved/General	0.275	0.447	0.213	0.410	0.135	0.342
Mode of delivery						
Caesarean	0.292	0.454	0.223	0.416	0.145	0.352
Normal	0.372	0.483	0.305	0.460	0.209	0.406
Perceived size at birth						
Very large	0.352	0.477	0.282	0.450	0.191	0.393
Larger than average	0.337	0.473	0.264	0.441	0.179	0.383
Average	0.348	0.476	0.279	0.448	0.188	0.391
Smaller than average	0.410	0.492	0.358	0.479	0.254	0.436
Very small	0.464	0.499	0.423	0.495	0.307	0.461
Anaemia						
Severe	0.461	0.499	0.365	0.482	0.278	0.448
Moderate	0.400	0.490	0.318	0.465	0.224	0.417
Mild	0.343	0.475	0.285	0.452	0.188	0.391
Normal	0.299	0.458	0.243	0.429	0.157	0.364
Low birthweight						
Yes	0.441	0.496	0.394	0.489	0.284	0.451
No	0.337	0.473	0.266	0.442	0.177	0.382
Age	29.95	14.57	30.58	14.95	31.19	14.52
Breastfeeding duration	20.09	12.41	20.38	12.69	20.87	12.73
Maternal characteristics						
Age at first birth	21.17	3.59	21.11	3.58	20.96	3.51
Prenatal care by doctor						
Yes	0.331	0.471	0.264	0.441	0.176	0.381
No	0.392	0.488	0.325	0.468	0.226	0.418
Shortstature						
Yes	0.544	0.498	0.442	0.497	0.346	0.476
No	0.330	0.470	0.267	0.442	0.176	0.380
Underweight						
Yes	0.422	0.494	0.378	0.485	0.264	0.441
No	0.319	0.466	0.239	0.427	0.159	0.366
Education						
No	0.453	0.498	0.390	0.488	0.282	0.450
Primary	0.425	0.494	0.346	0.476	0.246	0.431
Secondary	0.338	0.473	0.270	0.444	0.179	0.384
Higher secondary	0.237	0.425	0.174	0.379	0.103	0.305
Maternal characteristics						
Anaemia						
Severe	0.430	0.495	0.357	0.479	0.255	0.436
Moderate	0.380	0.485	0.311	0.463	0.216	0.411
Mild	0.347	0.476	0.289	0.453	0.195	0.396
Normal	0.335	0.472	0.263	0.440	0.176	0.381
Household characteristics						
Region						
Urban	0.296	0.457	0.235	0.424	0.151	0.358
Rural	0.370	0.483	0.301	0.459	0.207	0.405
Household head						
Male	0.351	0.477	0.287	0.452	0.194	0.396
Female	0.366	0.482	0.281	0.449	0.196	0.397
Household characteristics						
Wealth						
Poorest	0.462	0.498	0.398	0.489	0.286	0.452
Poorer	0.399	0.490	0.323	0.468	0.227	0.419
Middle	0.343	0.475	0.270	0.444	0.180	0.385
Richer	0.284	0.451	0.226	0.418	0.139	0.346
Richest	0.232	0.422	0.167	0.373	0.100	0.300

Continued

Table 1. Continued

	Stunting		Underweight		Stunting & underweight	
	Proportion/Mean	Std. Dev	Proportion/Mean	Std. Dev	Proportion/Mean	Std. Dev
Toilet facility						
Improved	0.328	0.469	0.258	0.438	0.171	0.377
Unimproved	0.443	0.497	0.383	0.486	0.274	0.446
Drinking water						
Available	0.353	0.478	0.286	0.452	0.194	0.396
Not available	0.369	0.482	0.295	0.456	0.200	0.400
Under 5 child	1.56	0.76	1.56	0.76	1.58	0.76

households with a higher number of under-5 children. This is indicative of the benefits of family planning measures in reducing the prevalence of childhood undernutrition (Table 2).

Non-linear covariate effects

Table 3 and Fig. 1 depict the significance of the non-linear covariate effects corresponding to child age, maternal age at first birth and duration of breast feeding. It is apparent that these covariates have significant non-linear association with the incidence of stunting while child age and duration of breastfeeding have significant non-linear association with underweight.

Structured and unstructured spatial effects

Table 4 depicts the structured and unstructured spatial effects of stunting and underweight. The unstructured spatial effects are significant at 5% level implying considerable within-state variation in the prevalence of stunting and underweight. The structured spatial effect of underweight is also significant at 5% level implying considerable between-state variation in its prevalence. Figure 2 depicts the structured spatial effect of underweight across India. Specifically, childhood underweight seems to have the lowest prevalence in the north-eastern states followed by states in northern India while the prevalence is highest in central and western India. The copula parameter was found to be significant at 1% level implying significant spatial heterogeneity in the stunting-underweight association across states and union territories. The Kendall's tau coefficient was 0.4 with a 95% confidence interval of (0.384, 0.418) implying moderately strong stunting-underweight association across India.

Joint probability maps

In order to better understand the spatial variation in the dual burden of stunting and underweight, we produced maps depicting the joint probabilities of a child being (i) neither stunted nor underweight, (ii) stunted but not underweight, (iii) underweight but not stunted and (iv) stunted as well as underweight in Fig. 3. The highest probability of co-occurrence of stunting and underweight was observed in the relatively impoverished eastern state of Bihar closely followed by the adjacent state of Jharkhand as well as in the considerably wealthier western states of Gujarat and Maharashtra. This apparent "anomaly" is indicative of the fact that economic development may not always go hand-in-hand with improvements in maternal and child health. The lowest co-occurrence of stunting and underweight is observed in the agriculturally rich northern state of Punjab, the thinly populated mountainous state of Uttarakhand, union territories of Andaman and Nicobar and Lakshadweep islands as well as in the progressive southern state of Kerala and north-eastern states of Manipur and Mizoram. Along similar lines, children belonging to Bihar, Jharkhand and Gujarat have the lowest probabilities of being neither stunted nor underweight while those hailing from southern states of Kerala, Tamilnadu, northern states of Punjab, Uttarakhand and Jammu and Kashmir and eastern states of Mizoram, Manipur, Arunachal Pradesh and Sikkim have the highest propensity of being free of both these ailments.

A few insights can be derived from these maps. Firstly, there is noticeable spatial heterogeneity in the co-occurrence of stunting and underweight across the states of India. Secondly, there is moderately strong pan-India association between childhood stunting and being underweight. Lastly, the drivers of stunting and underweight may not necessarily overlap as indicated by the moderately strong probability of the occurrence of one ailment in the absence of another in some states. This points to the necessity of incorporating regional attributes for effective policy formulation and impact maximisation.

Discussion

This study relates to the modelling of the dual burden of childhood stunting and underweight and its antecedents in India. This is achieved using a copula geoadditive modelling framework[23,29] which accounts for the implicit association between the two dimensions of undernutrition as well as their spatial heterogeneity across the country. To the best of our knowledge, this is one of the first and the largest studies carried out on nationally representative data from India that employs a joint modelling framework to analyse the dual burden and geographical variation of two major dimensions of childhood undernutrition.

Marginal prevalence of stunting and underweight were 35.37% and 28.63% while their dual prevalence was 19.45%. Stunting and underweight had significant positive correlation at the pan-India level which called for the necessity of their joint modelling. Childhood underweight had significant between-state spatial variation while the variation was lesser for childhood stunting. This partly corroborated the findings from previous studies which have demonstrated significant spatial variation in both childhood stunting and underweight across

Table 2. Estimated odds ratios and 95% confidence intervals of stunting and underweight

	Stunting OR (95% CI)	Underweight OR (95% CI)
Child characteristics		
Gender (Reference category: male)		
Female	0.89*** (0.86–0.91)	0.89*** (0.86–0.91)
Mode of delivery (Reference category: normal delivery)		
Caesarean	0.89*** (0.86–0.92)	0.84*** (0.81–0.87)
Birthweight (Reference category: high)		
Low	1.46*** (1.41–1.52)	1.64*** (1.58–1.71)
Caste (Reference category: scheduled caste)		
Scheduled tribe	0.97 (0.92–1.02)	1.06** (1.00–1.11)
Other backward classes	0.95*** (0.92–0.99)	0.96** (0.92–0.99)
General	0.82*** (0.78–0.85)	0.82*** (0.78–0.86)
Perceived size at birth (Reference category: very large)		
Larger than average	0.95 (0.89–1.02)	0.99 (0.93–1.06)
Average	1.04 (0.98–1.09)	1.11*** (1.05–1.17)
Smaller than average	1.18*** (1.11–1.27)	1.27*** (1.18–1.37)
Very small	1.21*** (1.09–1.34)	1.54*** (1.39–1.69)
Anaemia (Reference category: severe)		
Moderate	0.78*** (0.71–0.85)	0.74*** (0.67–0.81)
Mild	0.63*** (0.58–0.69)	0.67*** (0.61–0.74)
Normal	0.54*** (0.49–0.59)	0.58*** (0.53–0.64)
Maternal characteristics		
Education (Reference category: no education)		
Primary	0.94*** (0.89–0.98)	0.91*** (0.87–0.96)
Secondary	0.81*** (0.78–0.84)	0.81*** (0.78–0.84)
Higher secondary	0.66*** (0.62–0.70)	0.67*** (0.63–0.71)
Shortstature (Reference category: no)		
Yes	2.19*** (2.10–2.28)	1.85*** (1.78–1.93)
Prenatal care by doctor (Reference category: no)		
Yes	0.94*** (0.92–0.97)	0.96*** (0.93–0.99)
Underweight (Reference category: no)		
Yes	1.30*** (1.26–1.34)	1.57*** (1.53–1.62)
Anaemia (Reference category: severe)		
Moderate	0.90** (0.82–0.98)	0.89** (0.81–0.98)
Mild	0.85*** (0.78–0.94)	0.88** (0.80–0.97)
Normal	0.87*** (0.79–0.95)	0.87*** (0.79–0.96)
Household characteristics		
Residence (Reference category: urban)		
Rural	0.94*** (0.91–0.97)	0.91*** (0.87–0.94)
Household head (Reference category: male)		
Female	1.04* (1–1.08)	0.93*** (0.89–0.97)
Wealth (Reference category: poorest)		
Poorer	0.89*** (0.85–0.93)	0.86*** (0.82–0.90)
Middle	0.78*** (0.74–0.82)	0.72*** (0.68–0.75)
Richer	0.64*** (0.60–0.67)	0.62*** (0.59–0.66)
Richest	0.56*** (0.53–0.60)	0.50*** (0.47–0.54)
Toilet facility (Reference category: unimproved)		
Improved	0.92*** (0.89–0.96)	0.95*** (0.92–0.99)
Drinking water (Reference category: not available)		
Available	1.02*** (1.10–1.18)	1.08** (1.00–1.16)

*Significant at the 10% level.
**Significant at the 5% level.
***Significant at the 1% level.

Table 3. Statistical significance of non-linear effects of child age, maternal age at first birth and breastfeeding duration on childhood stunting and underweight in India

	Stunting		Underweight	
	Chi-square	P -value	Chi-square	P -value
Age of child (months)	739.49	< 0.001	422.55	< 0.001
Age at first birth	54.62	< 0.001	5.46	0.16
Duration of breastfeeding (months)	90.44	< 0.001	156.14	< 0.001

Table 4. Statistical significance of structured and unstructured spatial effects of stunting and underweight among under 5 children in India

	Stunting		Underweight	
	Chi-square	P -value	Chi-square	P -value
Unstructured spatial effect	418.20	< 0.001	447.13	< 0.001
Structured spatial effect	2.31	0.545	19.88	< 0.01

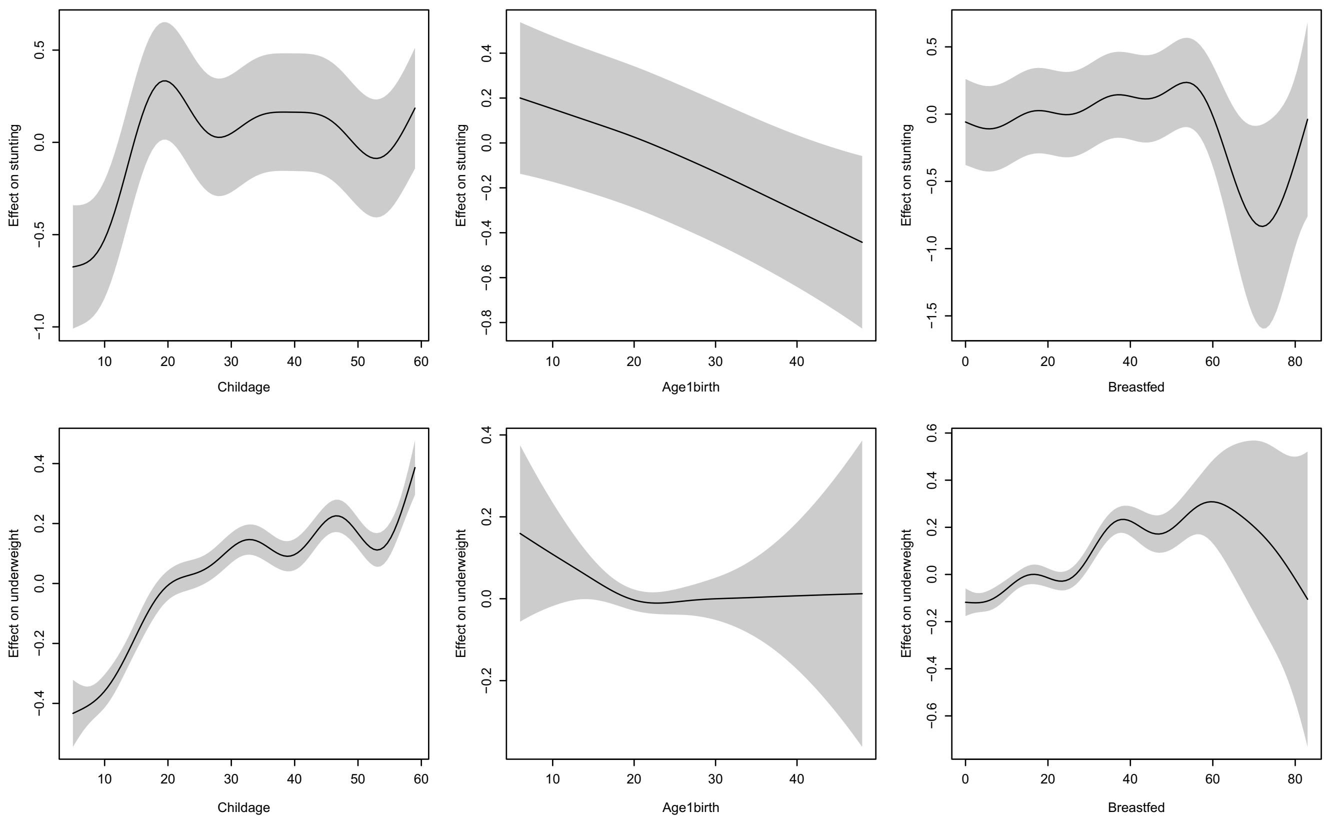

Fig. 1. Estimated non-linear effects of child age, maternal age at first birth and duration of breastfeeding on the likelihood of stunting (top row) and underweight (bottom row). Shaded regions correspond to 95% confidence bands.

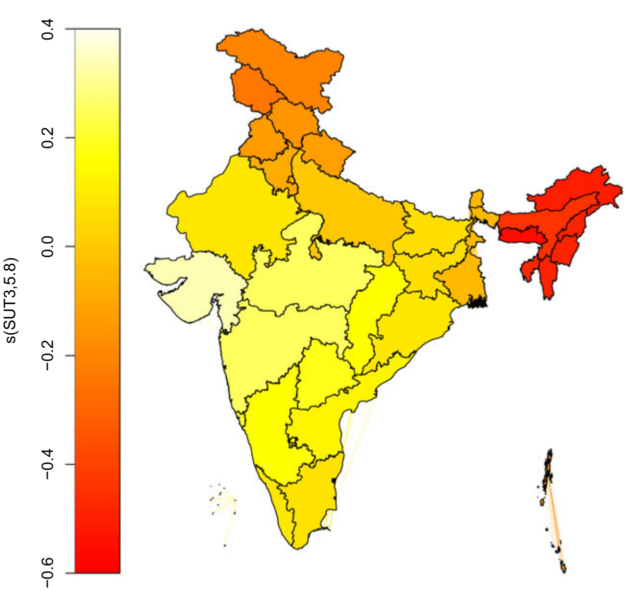

Fig. 2. Structured spatial effect for childhood underweight.

various sub-national boundaries of India.[16,18,41–43] Interestingly, childhood underweight has higher prevalence in the relatively wealthier western states of India while it is less prevalent in the north-eastern and northern parts of this country. Spatial maps of the joint probabilities of stunting and underweight depicted higher co-occurrence of stunting and underweight both in the poorer eastern states of Bihar and Jharkhand as well as in the relatively wealthier western states of Gujarat and Maharashtra. Lower prevalence was observed in relatively prosperous, less populated and developed states of the northern, north-eastern and southern parts of the country. This is an interesting finding since it is indicative of the fact that economic growth may not always go hand-in-hand with improvements in maternal and child health. The joint prevalence of underweight but not stunting was uniformly low across India which validated their close association. The co-occurrence of stunting but not of underweight is lowest in the Andaman and Nicobar Islands and highest in Arunachal Pradesh, Meghalaya and Lakshadweep islands. This is indicative of the presence of other drivers of stunting apart from

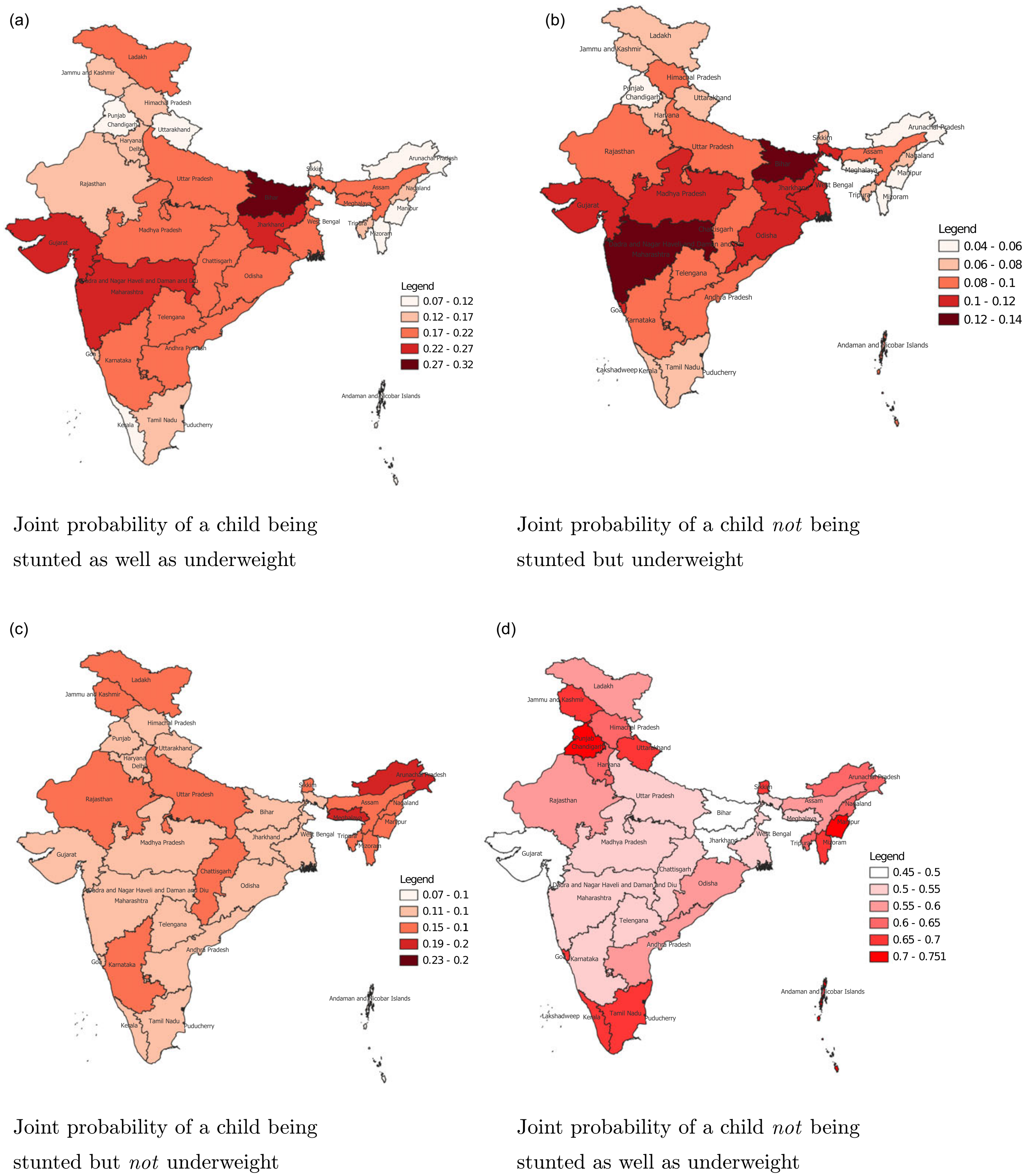

Fig. 3. Joint probability maps depicting the co-occurrence of stunting and underweight across states and union territories of India.

underweight in these regions. To our knowledge, these insights are unavailable in any previous studies on childhood undernutrition in India.

Various drivers at the child, maternal and household levels had significant association with the prevalence of stunting and underweight. As far as the child-specific drivers were concerned, girls had a significantly lesser odds of being stunted as well as underweight compared to boys. This is well aligned with previous studies from developing countries.[44–46] Children having low birthweight had significantly higher odds of stunting as well as underweight compared to those having normal birthweight. This is broadly consistent with that observed in previous studies.[46,47] Children who were perceived to be very small or smaller than average during birth had a significantly higher odds of stunting and underweight compared to those whose perceived birth size was normal. This is well established in the undernutrition literature as well.[44,45] Children born through caesarean section had significantly lesser odds of being either stunted or underweight compared to those born through normal delivery. Similarly, severity of childhood anaemia had a direct association with undernutrition with higher severity corresponding to higher odds of both forms of undernutrition. Finally, children belonging to unreserved categories had a significantly lesser odds of both stunting and underweight compared to those belonging to scheduled castes. To our knowledge, these observations are fairly new specifically in the context of joint analysis of stunting and underweight of current nationally representative data from India.

Children whose mothers were underweight or short-statured have significantly higher odds of being both stunted or underweight compared to children of healthy mothers. Maternal educational attainment had a significant negative influence on the incidence of childhood undernutrition with higher levels of attainment leading to significantly lower odds of both forms of undernutrition markers. These results align with those from previous studies on India and other developing countries.[16,44,45,47–49] Children belonging to non-anaemic mothers had a significantly lower odds of being either stunted or underweight compared to those belonging to severely anaemic mothers. This extends the findings of previous studies which observed similar association patterns between maternal anaemia and childhood stunting.[17] Finally, mothers who have received prenatal care from a registered physician have a significantly lower odds of having a stunted or underweight baby compared to those who did not have access to such care. This improves upon the findings from previous studies which have shown a positive impact of receiving prenatal care in reducing the likelihood of childhood stunting.[47]

Children from rural areas had a significantly lower odds of being either stunted or underweight compared to those from urban areas. This is in divergence with findings from previous studies which reported lesser odds of stunting for children hailing from urban localities.[17,45,47] However, this is consistent with a previous study on childhood undernutrition in Bangladesh which depicted higher prevalence of stunting among children in urban localities than their counterparts in rural regions.[44] One reason for this can be the significantly lower pollution and healthier environmental conditions prevailing in rural parts of India as opposed to the urban metros many of which suffer from some of the highest levels of pollution in the world. Another important factor can be the improved implementation and better penetration of various government schemes aimed at meeting the nutritional requirements of susceptible population subgroups primarily located in the rural areas. Households having a female head have a significantly lower odds of having underweight children although no such association exists for stunting. Finally, households belonging to higher wealth quintiles and those having better access to clean drinking water and improved sanitation facilities have significantly lesser odds of having a stunted or underweight child. These are broadly consistent with findings from previous studies carried out in developing countries.[16,17,44,46,48,49]

As far as continuous predictors were concerned, child age, maternal age at first birth and breastfeeding duration had significant non-linear association with the likelihood of stunting as well underweight. Specifically, children who are breastfed beyond 5 years seem to have lower odds of stunting as well as underweight while higher maternal age at first birth has a positive impact in reducing the onset of stunting. To our knowledge, these are relatively novel findings in the context of India and add to those obtained from previous studies on childhood undernutrition in low and middle-income countries.[47,48,50]

The key strength of our study is that it is one of the first and largest studies in India that jointly models the co-occurrence of childhood stunting and underweight while accounting for their spatial heterogeneity across sub-national boundaries. Previous studies that jointly modelled multiple undernutrition indicators for India[37,38] were based on much smaller sample sizes and also accounted for a smaller number of predictors compared to the current study. Having said that, this study is not free from limitations which may provide pointers for future research. First and foremost, the study uses cross-sectional data from a single round of NFHS thus limiting the scope for causal inference. Second, environmental predictors such as cluster height, vegetation index and land surface temperature have not been accounted for in the modelling framework, which, if done, can further our understanding of the impact of environmental drivers on the co-occurrence of childhood stunting and undernutrition. Third, socio-economic and demographic variables can be incorporated into the model for the copula parameter which would better help us in understanding how the stunting-underweight association varies across socio-economic strata.

Conclusion

In conclusion, the current study provides interesting data-driven insights about regional variation in the dual occurrence of childhood stunting and underweight based on the latest nationally representative data from India. The resulting spatial maps can help in the identification of states with high propensity of both stunting and underweight as well as high propensity of one and low propensity of the other ailment. This study also identifies various risk factors at the child, maternal and household levels which have significant association with stunting and underweight as well as the exact nature of the association pattern.

Overall, the results of this study are indicative of the fact that a "one-size-fits-all" strategy may be sub-optimal for a vast and diverse country like India and calls for a more nuanced, region-specific intervention framework to effectively tackle the scourge of childhood undernutrition. The considerable within and between-state heterogeneity in malnutrition also points to the importance of designing state or even region-specific nutritional intervention plan that accounts for the specific needs and characteristics of that state or region. One such example is the Rajmata Jijau Mother-Child Health and Nutrition Mission launched in the western state of Maharashtra in 2005 which has been credited with successfully reducing stunting rates in children aged under 2 years, from 44% in 2005 to 22.8% in

2012. One can also examine similar success stories from Brazil, Peru and Bolivia.[14] These case studies stand testament to the importance of effective policy formulation, careful implementation and diligent monitoring and supervision as weapons in the fight towards ending all forms of hunger and malnutrition specifically in high-burden countries like India.

Abbreviations

NFHS: National Family Health Survey; **CGF:** child growth failure; **SDG:** sustainable development goals; **AOR:** adjusted odds ratio; **GJRM:** generalised joint regression modelling; **DHS:** Demographic Health Survey; **HAZ:** height-for-age; **WHZ:** weight-for-height; **WAZ:** weight-for-age; **IIPS:** Indian Institute of Population Sciences.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authorship

D.B. conceptualised the study and performed data curation, statistical analysis and writing and revision of the original draft. D.B. interpreted the results and compiled all tables and figures. D.B. supervised the study, performed review and editing of the manuscript and approved the final version. D.B. has directly accessed and verified the underlying data reported in the manuscript and was responsible for the decision to submit the manuscript for publication.

Ethics declaration

The National Family Health Survey 5 was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures and survey protocols involving the study participants were reviewed and approved by the IIPS Institutional Review Board. Written/verbal informed consent (or thumbprint from illiterate participants) was obtained from all participants involved. Verbal consent was witnessed and formally recorded. The confidentiality and anonymity of the names, addresses and locations of the study participants were kept by the data source.

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RESEARCH ARTICLE

The development of sodium reduction targets for New Zealand fast foods and a comparison with the current sodium contents of products

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Abstract

Sodium intake attributed to fast food is increasing globally. This research aims to develop maximum sodium reduction targets for New Zealand (NZ) fast foods and compare them with the current sodium content of products. Sodium content and serving size data were sourced from an existing database of major NZ fast-food chains. Target development followed a step-by-step process, informed by international targets and serving sizes, and previous methods for packaged supermarket foods. Sodium reduction targets were set per 100 g and serving, using a 40% reduction in the mean sodium content or the value met by 35–45% of products. Thirty-four per cent (1797/5246) of products in the database had sodium data available for target development. Sodium reduction targets were developed for 17 fast-food categories. Per 100 g targets ranged from 158 mg for 'Other salads' to 665 mg for 'Mayonnaise and dressings'. Per serving targets ranged from 118 mg for 'Sauce' to 1270 mg for 'Burgers with cured meat'. The largest difference between the current mean sodium content and corresponding target was for 'Other salads' and 'Grilled Chicken' (both –40% per 100g) and 'Fries and potato products' (–45% per serving), and the smallest, 'Pizza with cured meat toppings' (–3% per 100 g) and 'Pies, tarts, sausage rolls and quiches' (–4% per serving). The results indicate the display of nutrition information should be mandated and there is considerable room for sodium reduction in NZ fast foods. The methods described provide a model for other countries to develop country-specific, fast-food sodium reduction targets.

Key words: **Benchmarking: Fast-foods: New Zealand: Salt: Sodium: Target**

Introduction

Excess sodium consumption leads to hypertension, which in turn is associated with cardiovascular disease, the leading cause of preventable mortality in New Zealand (NZ) and globally.[1,2] New Zealand adults 15 years and over consume 3,035 mg of sodium per day,[3] far more than the World Health Organization (WHO) upper limit of 2,000 mg per day; this is despite NZ committing to a 30% relative reduction in mean population sodium intake by 2025, as part of the WHO Global Action Plan for reducing non-communicable diseases.[4] A recent WHO report shows that NZ, like many other countries, is not on track to meet its commitment to the WHO to reduce population sodium.[5]

Like other high-income countries with Western diets, NZ adults consume around 75% of sodium from processed foods, defined as foods that have been altered from their natural state by industrial processes and including ultra-processed foods, formulations of ingredients not commonly found in a home kitchen and often with added salt, sugar and chemical additives.[6] The higher sodium content of processed and

[†]A related abstract was presented at the Annual Scientific Meeting of the Nutrition Society of New Zealand and published in Multidisciplinary Digital Publishing Institute (MDPI). It included the development of the sodium reduction targets (presented here), and semi-structured interviews regarding the implementation of the targets (not presented here).
[a]Research completed as part of the University of Auckland MHSc in Dietetics.
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ultra-processed foods found in supermarkets and the out-of-home sector (defined as outlets where food or beverages are prepared for immediate consumption) means that reformulation considered a 'Best Buy' by the WHO, is an important strategy for reducing sodium intake.[7,8] Furthermore, reducing the sodium content of processed foods if done gradually has little impact on consumer acceptability and does not require consumer agency, meaning it is more likely to deliver pro-equity benefits.[5,9,10]

Equitable interventions are particularly important for NZ which is a diverse country with considerable health inequities.[11] However, NZ does not have a national sodium reduction strategy, and the reformulation of processed foods is currently driven by two voluntary programmes, both of which focus only on supermarket products, that is, the Heart Foundation food reformulation programme including sodium reduction targets for 40 categories of supermarket foods,[12] and the Health Star Rating, a Trans-Tasman voluntary front-of-pack label scheme of which one component is sodium.[13]

However, in 2020 the average NZ household spent 27% of its weekly food budget on restaurant meals and takeaways, an increase of 5% since 2000,[14] and with the rise of online food delivery applications making fast food even more accessible,[15] this percentage is likely to continue to rise. In addition, the sodium content of NZ fast foods has been consistently high, with a 2018 study reporting that while the sodium per 100 g remained consistent between 2012 and 2016, an increase in serving size means consumers are receiving more sodium per serving than before.[16] Combination meals (combo meals), which are meal deals that bundle individual products together, are particularly problematic, with half of these types of meals in 2020 found to contain close to the WHO 2,000 mg/d upper daily sodium intake limit.[17]

Two countries with world-leading sodium reduction programmes are the United Kingdom (UK) and the United States of America (USA); their success is based on multi-stakeholder collaboration with industry partnership, Government leadership, and consumer advocacy.[18,19] Both countries have implemented sodium reduction targets for out-of-home foods, encompassing both independent fast-food outlets and chains.[18,20] Despite challenges such as resistance from retailers, consumer preferences, and lack of mandate, these targets have driven a decrease in the sodium content in out-of-home products in the UK.[21] As such, well-implemented and monitored sodium reduction targets for fast foods are an essential component of country-specific sodium reduction strategies.

In NZ the 2020 Food Environments Policy Index, backed up by considerable local evidence,[16,22,23] noted a significant implementation gap for published sodium reduction targets for out-of-home foods.[24] However, there is no published data on how to develop such targets, and while many fast-food companies are international, significant inter-country discrepancies exist in the sodium content of fast food, meaning country-specific targets are needed.[25] Therefore, this study aimed to describe the development of maximum sodium reduction targets for NZ fast foods and compare them with the current sodium content of products.

Methods

This study was conducted in 2020 and was an analysis of cross-sectional individual-product data from the NZ Nutritrack fast-food database.

Development of the sodium reduction targets

The Nutritrack database. The Nutritrack database contains annually updated information on the nutrient content of packaged foods and beverages sold at major NZ supermarkets and fast-food chains.[26] A major fast-food chain is defined as a chain with 20 or more outlets nationwide with outlets where people buy and pay for food at the counter i.e. table service is not provided; independent outlets such as fish and chips shops are excluded. Based on Euromonitor data Nutritrack fast-food data are estimated to comprise approximately 24% of the NZ fast-food market share based on sales.[27]

Fast-food data for the Nutritrack database are collected in a standardised format on Microsoft Excel at the same time each year (February to May). Fieldworkers copy data for all products sold directly from company websites and make one store visit to collect any additional information available e.g. on a menu board. The following data are collected from each chain and product: product name, package size, serving size, and all nutritional information available (per 100 g or mL and per serve or serving, the latter considered identical). However, complete data are not available for all products because NZ labelling laws do not mandate the provision of nutritional information for fast foods unless they are making a health claim.[28] Nutritional information is provided by manufacturers; no laboratory analysis is completed and it does not include discretionary salt added by consumers. All products collected are manually categorised into 16 food groups and 39 major categories consistent with the global food monitoring group.

The following fields from the original 2019 Nutritrack fast-food database, which included 28 fast-food chains and 5246 products (Supplementary Table 1), were used for estimating the sodium reduction targets: sodium per 100 g and per serve (mg), energy per 100 g and per serve (kJ), serving size (g or ml), and package size (g or ml specifically for multi-serve products such as buckets of fried chicken).

Data preparation and development of food groups and categories for the sodium reduction targets. Figure 1 presents the process used to prepare the fast-food data, develop and refine the food categories, and complete the initial summary sodium data for estimating the sodium reduction targets. First, when possible, missing values were calculated using related existing data. For example, if sodium per serving was not available, it was calculated from serving size and sodium per 100 g data. However, no missing values were imputed. Second, to develop sensible food groupings containing similar types of products for sodium reduction, the food groups and categories in Nutritrack were compared with those used for the UK out-of-home sodium reduction targets; this was because considerable work and consultation with stakeholders had gone into enhancing the practicality of the UK Targets and the types of fast foods and dietary patterns in the two countries are

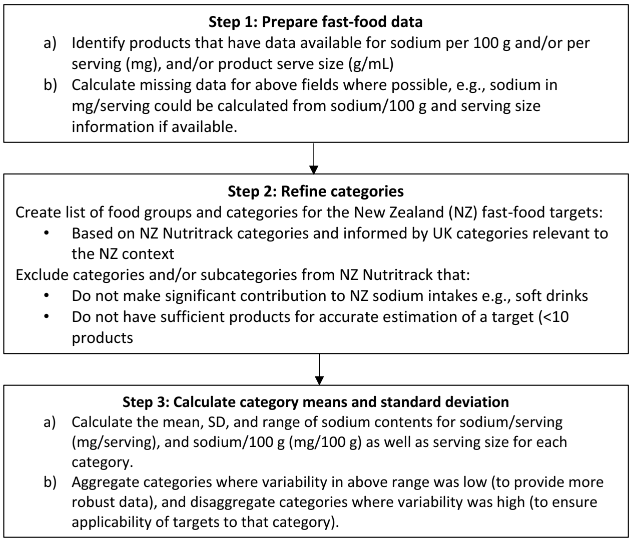

Fig. 1. Preparation of fast-food data and development of food categories for the targets.

similar.[29] For example, products in the general 'Burgers, pizzas, and sandwiches' category in Nutritrack were recategorized into the UK categories because the latter were split by type of meat, and cured meat products have a higher baseline sodium content before reformulation. Food groups and categories that do not make a large contribution to population sodium intakes in NZ (<5%) such as desserts were excluded.[30] Sodium reduction targets were only calculated for categories with ≥10 products; this pragmatic approach maximised the validity of the estimated target while ensuring products that were likely to be frequently consumed, were still included e.g. 'Burgers with cured meat', 'Grilled chicken', and 'Sauce'. The mean and standard deviation sodium content per 100 g and per serving were then calculated for each category. The original Nutritrack food groups and categories are displayed in Supplementary Table 2, and the final food groups and categories are displayed in Table 1.

Development of the sodium reduction targets. Figure 2 shows the process used to develop feasible fast-food sodium reduction targets for the NZ context. Because sodium reduction targets per 100 g are required to drive decreases in the sodium concentration of products, and sodium reduction targets per serving are necessary to ensure any reduction in sodium concentration is not 'undone' by increases in serving size,[16] sodium reduction targets were estimated both per 100 g and per serving; both types of targets are intended to be the maximum level for an individual product and the maximum mean for that fast-food category.

Development of sodium reduction targets per 100 g. First, draft sodium reduction targets per 100 g were calculated by reducing the mean sodium per 100 g for each category by 40%. Methods were based on the intention to estimate sodium reduction targets that were feasible from a food technology and consumer perspective, yet aspirational from a health perspective, with the 40% cut-off informed by the methods used by the Australia Healthy Food Partnership (HFP)[31] and previous NZ research by Eyles *et al.*[32] The NZ research involved the development of a salt reduction model to estimate the required reduction in sodium consumed from all sources for the population to meet the WHO 30% relative reduction target; the model indicated that alongside salt reduction targets for packaged foods, a 40% decrease in the sodium content of foods consumed away from the home would be required to reduce total population salt intake in NZ by 35% (which was also assumed in the initial model developed by England).[33] Therefore, draft sodium reduction targets per 100 g were calculated for each category by reducing the mean sodium per 100 g by 40%.

The Australian HFP, when creating their packaged foods targets, determined that a target was technically feasible and appropriate if approximately 33.3% ± 10% of products already met the target. However, as fast foods have generally struggled more to achieve sodium reductions when compared to packaged foods both nationally and internationally including in the UK,[34] a more lenient approach was adopted here i.e. the cut-offs were adjusted to 35–45%, that is, a sodium reduction target was determined to be feasible yet aspirational if 35–45% of products already met the sodium reduction target. If > 45% of products in the category met the draft sodium reduction target, then the sodium reduction target was feasible but not aspirational enough, and if <35% of products in the category met the draft sodium reduction target, then the sodium reduction target was considered infeasible.

Therefore, if not enough products (<35%) or too many products (>45%) met draft target, then the sodium reduction target was revised to a level met by 40% of products; this was achieved by arranging products from lowest to highest sodium per 100 g and selecting the sodium value at the 40% mark in the distribution. This value was then set as the final sodium reduction target.

Development of sodium reduction targets per serving. The same process as for the development of the sodium reduction targets per 100 g was used for the development of the sodium reduction targets per serving (Fig. 1). However, no standardised serving sizes are available meaning that fast food in NZ use serving sizes recommended by manufacturers. Therefore, to estimate sodium reduction targets by serving size we used the mean serving size in the Nutritrack data and international standard serving sizes (if available) from the USA's Food and Drug Administration (FDA)[35] and the Australian HFP programme[36]; this was to ensure serving sizes were not artificially inflated by those recommended by manufacturers, the smallest size was chosen for the latter. A sodium reduction target was set by adjusting the estimated sodium reduction target for sodium per 100 g to the international standard serving size on a proportional basis (a) if the international standard serving size was available and was smaller than the Nutritrack means, and (b) if a reduction in serving size was (subjectively) considered health-promoting for that category, for example, it did not reduce potential intake of vegetables in a salad or wrap.

Statistical analysis. Descriptives were used to describe the current mean (standard deviation (SD)) sodium content per

Table 1. Descriptions of the food groups and categories for the final NZ fast-food sodium targets

Food group	Category	Description
Pasta, rice, and risotto dishes	All	Includes Any meal dish containing pasta, rice and risotto, meat on rice dishes e.g. katsu chicken on rice Excludes Curry on rice dishes, 'bites' with pasta, e.g. mac and cheese bites and risotto bites, salads with pasta, rice and noodles
Pies, tarts, sausage rolls and quiches	All	Includes Baked goods with pastry Vegetarian pastry rolls e.g. feta rolls Excludes Savoury muffins and cakes and toppas (savoury filling wrapped in pasta and breadcrumbs)
Asian	Sushi and rice paper rolls	Includes All sushi and rice paper rolls
Burgers	Burgers with cured meat	Includes Single or multiple beef/pork/lamb) patty burgers and chicken burgers with cured meat additions such as bacon or chorizo (e.g. bacon and cheese)
	Single patty burgers	Includes Single meat (beef/pork/lamb) patty burgers and chicken burgers Excludes Burgers with cheese or cured meat (e.g. bacon) additions
	All other burgers	Includes Single patties with cheese, multiple patties with or without cheese and vegetarian/bean or fish alternatives
Chicken	Crumbed Chicken	Includes All breaded chicken portions and pieces
	Grilled chicken	Includes Chicken that is not crumbed e.g. grilled, roast and buffalo chicken
Pizzas	Cured meat Toppings	Includes All pizza toppings with cured meat
	All other toppings	Includes All pizza toppings without cured meat e.g. chicken, beef, fish and margherita
Salads	Salads with meat	Includes All salads containing meat
	Other salads (excluding garden salads)	Includes Salads with a grain-based, bean and/or lentil addition
Sandwiches	Cured meat sandwiches	Includes Sandwiches that do contain cured meat
	All other sandwiches	Includes Sandwiches that do not contain cured meat e.g. tuna, cheese, and vegetables
Condiments	Mayonnaise and dressings	Includes Mayonnaise, aioli, ranch, cheesy, and dressings
	Sauce	Includes Tomato, sweet and sour, BBQ, and other non-creamy dipping sauces
Sides	Fries and potato products	Includes Fries, hash browns, and baked potatoes

food category per 100 g and serving. Serving sizes were calculated as the mean or standard serving size relevant to that category based on the serving size data available. Forty-percent reductions calculated during the development of the targets were calculated as the current mean sodium content per 100 g or per serving multiplied by 0.6. The percentage of products meeting the target for each category was calculated by taking the maximum sodium target value in mg per 100 g away from the current sodium content for each product in mg per 100 g; products with a zero or negative result were considered to 'meet the target', and those with a value greater than zero were considered to 'not meet the target'.

Results

Of the 5246 products in Nutritrack, 3449 (66%) did not have sodium content information available, either per 100 g or per serving. For 10/28 chains in Nutritrack, there was no sodium information available (Supplementary Table 1). Food groups with the largest number of products also had the largest number of products with missing sodium information, that is, 'Pizzas' ($n = 956$ total, $n = 533$ missing) and 'Sandwiches and wraps' ($n = 744$ total, $n = 621$ missing). There were sufficient data i.e. ten or more products within each category to estimate sodium reduction targets for 17 categories within 10 food groups.

Current mean sodium content per 100 g

Mean (SD) sodium per 100 g ranged from 220 mg (54 mg) for 'Sushi and rice-paper rolls' to 729 mg (192 mg) for 'Mayonnaise and dressings'. There was a wide range in sodium content for all 17 categories, with 'Grilled chicken' having the largest range of 1146 mg/100 g, 'Sushi and rice paper rolls' the smallest (198 mg/100 g).

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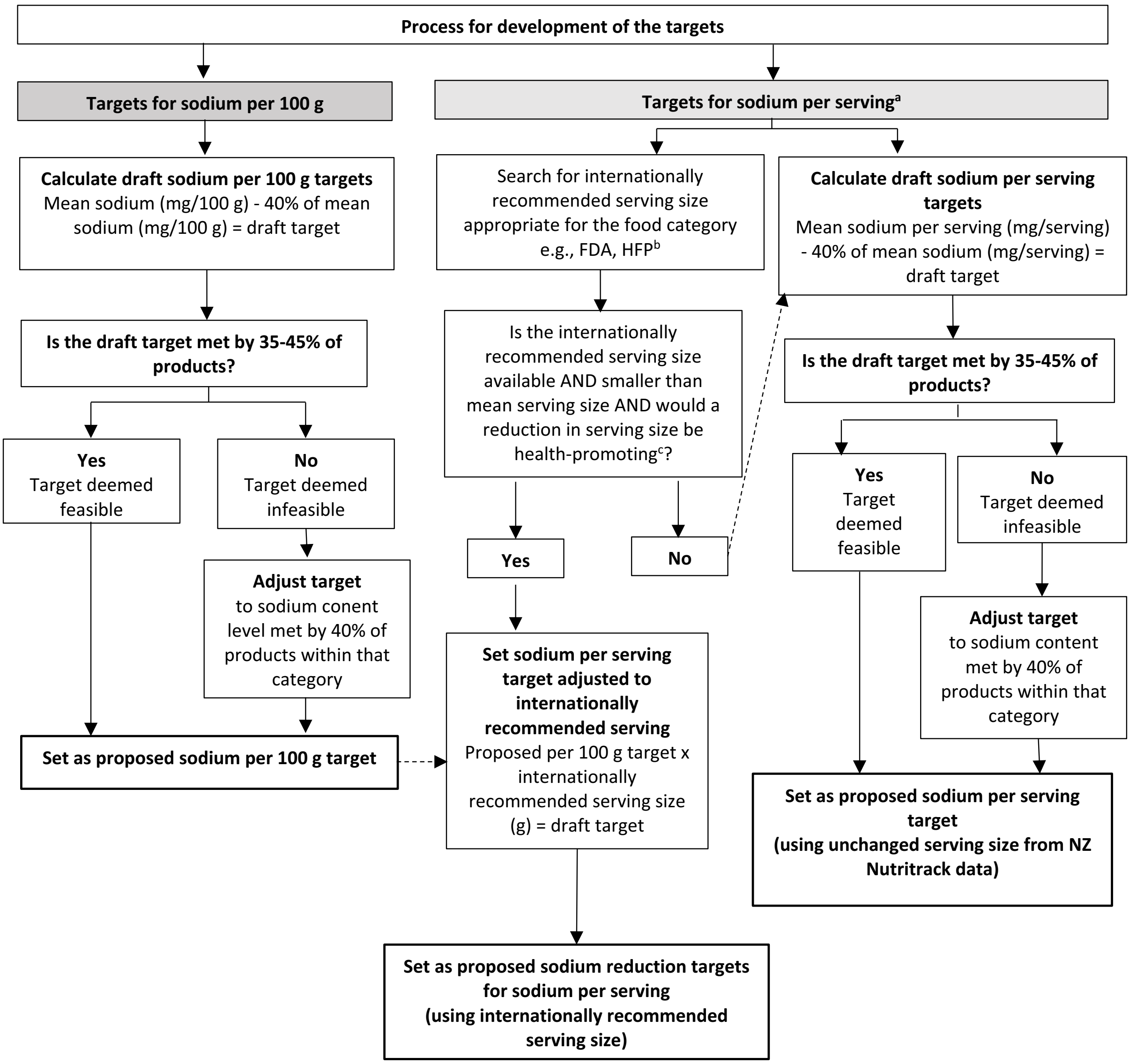

Fig. 2. Process for development of fast-food sodium reduction targets.

Current mean sodium content per serving

The mean (SD) sodium per serving ranged from 148 mg (86 mg) for 'Sauce' to 1416 mg (522 mg) for 'Burgers with cured meat' (Supplementary Tables 3 and 4).

Sodium reduction targets per 100 g

The final estimated sodium reduction targets per 100 g ranged from 158 mg/100 g for 'Other salads' to 665 mg/100 g' for 'Mayonnaise and dressings'. The mean reduction in sodium required to meet the sodium reduction targets (per 100 g) across all categories was 67 mg/100 g (15%). 'Grilled chicken' and 'Other salads' were the only two categories where a 40% reduction of the mean was considered feasible and set as the final estimated target (Table 1). The estimated sodium reduction targets per 100 g for the remaining 15 categories were adjusted to the level met by 40% of products (Table 1), resulting in the majority of the remaining 15 categories needing to reduce sodium by between 9% and 26% to achieve the sodium reduction target. However, the 'Pizzas with cured meat toppings' category would only require a 14 mg/100 g (3%) reduction, and 'Pizzas with all other toppings' a 25 mg/100 g (6%) reduction.

Sodium reduction targets per serving

A 40% reduction in the mean sodium per serving was set as the final estimated sodium reduction target for six categories (Table 1). The final estimated sodium reduction targets for the remaining 11 categories were adjusted to the level met by 40% of products. For the majority of these remaining 11 categories, the sodium content would need to be reduced by between 10% and 26% to achieve the sodium reduction target, except for 'All pies, tarts, sausage rolls and quiches' which would only need to reduce sodium by 30 mg/serving (4% reduction) (Table 1). The sodium reduction targets for sodium per serving (which used the serving size in the Nutritrack database) ranged from 118 mg/serving for 'Sauce' to 1270 mg/serving for 'Burgers with cured meat'.

Overall, setting the sodium reduction targets for sodium per serving using international standard serving size was only possible for two categories i.e. 'Mayonnaise and dressings' (target: 200 mg/serving with a serving size of 30 g) and 'Fries and potato products' (360 mg/serving with a serving size of 150g) (Table 2) (Supplementary Table 5).

Difference between the current mean sodium content and the sodium reduction targets

The food categories with the biggest difference between the mean sodium content per 100 g and the target per 100 g were 'Other salads' and 'Grilled chicken' (both –40%), and per serve was 'Fries and potato products' (using sodium reduction target using internationally recommend serving size) (–45%), and 'Mayonnaise and dressings' (–43%). The food category with the smallest difference between the mean sodium content per 100g and the target per 100g was 'Pizza with cured meat toppings' (–3%) and per serve was 'Pies, tarts, sausage rolls and quiches' (–4%) (Table 2).

Discussion

We have outlined a step-by-step process for the development of feasible, yet aspirational country-specific sodium reduction targets for sodium per 100 g, and sodium per serving. Sodium reduction targets were developed for 17 categories of NZ fast food and compared with the current sodium content of products sold by major national chains.

The current sodium content of NZ fast foods was more variable per serving than per 100 g and more products were able to meet a 40% reduction in sodium per serving versus per 100 g. This can likely be attributed to the wide range of serving sizes within each category and the use of predominantly manufacturer, rather than standard, serving sizes which would have affected the percentage of products meeting the target.

The categories with the highest current sodium content per 100 g were 'Mayonnaise and dressings' (729 mg/100 g), 'Sauces' (710/100) mg, and 'Crumbed chicken' (540 mg/100 g), similar to those of an earlier analysis of the 2016 Nutritrack fast-food database, which found that found the food groups with the highest mean sodium content per 100g were 'Sandwiches', 'Dressings', 'Pizza', 'Chicken' and 'Burgers'.[16] Chicken and dressings were also reported by Prentice *et al.*[23] in their analysis of 2008/2009 National Nutrition Survey data as being the most consumed NZ fast foods with the highest sodium content per 100 g. Furthermore, chicken was found to be in the top three fast-food categories with the highest sodium per 100 g in a study across six countries using data from 2010; the remaining two were savoury breakfast items and pizza and burgers (note that sauces and dressings were not part of this analysis).[25]

A limitation of the data used for the development of the sodium reduction targets is that it excluded independent retail outlets. Nevertheless, focusing only on fast-food chains is most feasible in terms of setting targets and monitoring sodium, as the foods are comparable across chains, recipes from chains are standardised, and there is (at least) some nutritional information available for the sector. However, it is important to consider how to address independent outlets particularly for commonly consumed products such as battered fish, a popular product for independent outlets, and one of the four categories that contribute most to sodium intake from fast foods according to the NZ analysis by Prentice *et al.*[23] Reducing the salt added to battered fish and chips, through either avoiding pre-salting or using reduced-hole saltshakers has also been recommended by a previous sodium reduction intervention.[37]

Other limitations of our analysis include the low availability of sodium and serving size data and the lack of retailer sales data, the latter of which would provide a better understanding of the potential impact of sodium reduction targets on the diets of New Zealanders and greater confidence that the sodium reduction target values reflect the wide range of products available to NZ consumers. Sales data are particularly important to monitor dietary intakes in NZ because there is a lack of up-to-date nationally representative data on population dietary and sodium intake, including sodium consumption from fast food[23]; the most recent adult National Nutrition Survey was in 2008/09[38] and children's Nutrition Survey was in 2002,[39] although an analysis of savoury foods consumed by fast-food consumers in the 2008/09 adult nutrition survey found hamburgers followed by filled rolls and pizza contributed the most to sodium intake (194, 183, and 129 mg/d, respectively).[23]

Although not unusual globally[34,40,41] the low availability of nutritional information for fast foods is a major barrier to developing sodium reduction targets and monitoring their impact. Other barriers to monitoring include changing menus, seasonal products, and variances in preparation if done manually by food handlers.[16] As such, mandating the provision of nutritional information for fast foods would greatly benefit monitoring efforts, and enable customers to identify healthier options as recommended by the WHO.[5] Further, nutritional information provided by the industry, if based on recipes, may include errors, and thus laboratory analysis of the sodium contents of commonly consumed fast-food items may be considered.

Our analysis was also limited by an absence of national standard serving sizes for fast foods, which meant that our sodium reduction targets were based primarily on serving sizes set by manufacturers. To ensure sodium reduction targets per serve represent realistic serving sizes related to dietary energy and nutrient requirements, the development of NZ-specific serving sizes should be investigated, which if applied would also help to manage the high amount of total energy, added sugar, and saturated fat in NZ fast foods.[23] However, it is important to consider that reductions in serving sizes are not to the extent that they encourage consumers to purchase multiple packs instead of just one.[42]

Nonetheless, the key strengths of this research were the structured step-by-step process and clear criteria based on previous studies used to estimate feasible yet aspirational sodium reduction targets. The categories were also informed by the UK's out-of-home sector which considered the variability in sodium contents of individual products within each category. Further, analysis was completed using up-to-date sodium content data for NZ fast foods, although if retailers were able to

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Table 2. The current sodium contents of New Zealand fast foods and the final estimated sodium reduction targets per 100 g and per serving

			Current sodium content and serving size (Mean(SD))				Estimated target		
Food group	Category	*n*[a]	Na/serving (mg)	Serving size (g)	Serving size (kJ)	Na/100 g (mg)	Na/100 g target (mg)	Na/serving target: serving size from national data (mg)	Na/serving target (mg) (standard serving size (g) from guidelines)[b]
Pasta, rice, and risotto dishes	All	21	1127 (402)	381 (97)	2456 (842)	298 (90)	256[c]	895[c]	
Pies, tarts, sausage rolls, and quiches	All	45	719 (255)	168 (61)	1835 (463)	389 (148)	343[c]	689[c]	
Asian	Sushi and rice paper rolls	25	677 (269)	302 (56)	1897 (833)	220 (54)	200[c]	607[c]	
Burgers	All other burgers	40	962 (365)	252 (92)	2651 (1060)	393 (109)	358[c]	577[d]	
	Burgers with cured meat	29	1416 (522)	289 (70)	3186 (1254)	499 (138)	447[c]	1270[c]	
	Single patty burgers	30	938 (409)	196 (74)	1727 (673)	508 (177)	432[c]	830[c]	
Chicken	Crumbed Chicken	36	568 (394)	107 (82)	970 (598)	540 (146)	440[c]	341[d]	
	Grilled chicken	16	691 (480)	214 (153)	1209 (1069)	445 (279)	267[d]	415[d]	
Pizzas	All other toppings	228	426 (162)	107 (44)	950 (457)	414 (104)	389[c]	350[c]	
	Cured meat Toppings	193	527 (198)	107 (39)	991 (364)	508 (131)	494[c]	430[c]	
Salads	Other salads (excluding garden salads)	10	745 (630)	244 (109)	1521 (947)	264 (179)	158[d]	447[d]	
	Salads with meat	18	649 (330)	258 (88)	1288 (524)	256 (115)	190[c]	483[c]	
Sandwiches	All other sandwiches	98	916 (441)	252 (103)	1977 (699)	376 (157)	315[c]	725[c]	
	Cured meat sandwiches	34	1157 (459)	214 (93)	1937 (746)	554 (151)	497[c]	998[c]	
Condiments	Mayonnaise and dressings	15	236 (291)	37 (56)	373 (519)	729 (192)	665[c]	134[c]	200 (30g)
	Sauce	22	148 (86)	20 (8)	141 (71)	710 (285)	561[c]	118[c]	
Sides	Fries and potato products	31	655 (592)	232 (123)	1690 (915)	288 (158)	240[c]	393[d]	360 (150g)

[a]Total number of products with Na/serving information available.
[b]Used 40% reduction of mean Na/100 g multiplied by international standard serving size where available (either Australian Healthy Food Partnership,[36] USA: Food and Drug Administration[35]).
[c]Targets calculated as the value met by 40% of products.
[d]Targets calculated as a 40% reduction of the mean.

provide more comprehensive sodium data and sales information, then the sodium reduction targets developed in this research could and should be revised using the step-by-step methods we describe, and alongside an implementation plan with the industry.

Concerning implementation, evidence suggests that it is possible to reduce the sodium content of processed food products by 40−50% without consumers noticing if done in a stepwise manner.[43] Therefore, the sodium reduction targets developed here may be more likely to be adopted if divided into less ambitious reductions over set time frames. For example, a 25–30% decrease in the current sodium contents over the first four to five years, followed by a further 10–15% reduction over the next four to five years, as recommended by other authors including those of the New York National Sodium Reduction Initiative.[44,45] Most importantly, whether the programme begins as a voluntary initiative or is mandated, progress toward the targets will need to be closely and independently monitored, with the potential to hold the fast-food industry to account if progress is slow.[8] This process is supported by sodium reduction initiatives in both the UK and Australia, where the first set of targets was set to be achieved within four years of implementation, with a mid-point review every two years, aided by independent monitoring.[31,46] Further, implementation would ideally be government-led with resources committed to taking a long-term approach.

In addition to the adequately monitored, structured, Government-led approach, both sodium reduction targets per 100 g and serving must be implemented, and targets for commonly consumed fast foods from independent outlets such as fish and chips, should be added. Per 100 g targets are required to drive decreases in the sodium concentration of products within each category and sodium reduction targets per serving are required to ensure the reduction in sodium concentration is not ‘undone’ by increases in serving size, as has been previously observed for NZ fast-food products.[16] The control of serving size is particularly important for fast-food products and in particular combo meals because these offerings are generally intended to be consumed in one sitting; this was illustrated by a 2020 analysis of NZ combo meals created using previous fast-food data from Nutritrack, which found that most combos (88.6%) offered by chains provided more than the maximum recommended daily intake of sodium (2,000 mg/d) in one meal.[17]

In summary, the burden of disease, in particular hypertension, is associated with excess sodium consumption, a major contributor to which is the increasing consumption of sodium-dense fast food. Therefore, there is a need for country-specific fast-food sodium reduction targets to be developed and implemented. The methods outlined here provide a step-by-step process for the development of feasible, yet impactful country-specific sodium reduction targets per 100 g and serving and include recommendations for research, monitoring, and implementation. While it should not prevent immediate action, the mandatory provision of nutritional information by fast-food manufacturers is critical to ensure the relevance of sodium reduction in fast foods and to help consumers make informed choices. Finally, reformulation schemes need to be a part of a wider package of interventions that improve population nutrition and the food system, enabling a shift away from processed foods towards more whole and fresh foods alongside food preparation in the home.

Abbreviations

NZ: New Zealand; **WHO:** World Health Organization; **Combo meal:** Combination meal; **UK:** United Kingdom; **USA:** United States of America; **HFP:** Healthy Food Partnership; **FDA:** Food and Drug Administration; **SD:** Standard deviation

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.35

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Conflict of interest

All authors declare no conflict of interest.

Authorship

Conceptualisation, H.E.; methodology, H.E., S.M., S.G. (Shona Gomes), and S.G. (Sarah Gerritsen); formal analysis, S.G. (Shona Gomes); investigation, S.G. (Shona Gomes); writing—original draft preparation, S.G.; writing—review and editing, H.E., S.M., and S.G. (Sarah Gerritsen); supervision, H.E., S.M., and S.G. (Sarah Gerritsen). All the authors have read and agreed to the published version of the manuscript.

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REVIEW

The neutropenic diet and its impacts on clinical, nutritional, and lifestyle outcomes for people with cancer: a scoping review

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Abstract

The neutropenic diet (ND) is often recommended to people with cancer to reduce infection risk despite recommendations of clinical guidelines advising against its use. While recent literature suggests the ND does not reduce infection risk, other outcomes related to health, nutrition, and lifestyle are unknown. The aim of this review is to systematically scope the literature on the ND in people with cancer for all outcomes related to clinical health, nutrition, and lifestyle. Scientific databases were systematically searched. Eligible studies were in English, people with any cancer type, consuming an ND, any age group, date, or setting. Eligible study types were randomised control trials, observational studies, systematic reviews, and meta-analyses. Twenty-one studies met the inclusion criteria. Outcomes of interest found were infection rates, fever, mortality, antibiotic use, gastrointestinal side effects, comorbidities, biochemistry, hospitalisation, nutritional status, quality of life (QoL), well-being, and financial costs. Most research has focused on infection and mortality rates with few assessing hospitalisation rates, nutritional status, financial costs, and QoL. Most included studies found no significant differences between ND and comparator diet for mortality, antibiotics use, comorbidities, and QoL; however, several studies reported the ND significantly increased the risk of infection. Gaps in the literature included effect of ND on QoL in an adult population, microbiome, lifestyle changes, and financial burden. Further research is needed regarding how the ND affects the microbiome and QoL of its consumers, but in the interim, it is important for hospitals providing an ND to their patients to liberalise the ND wherever possible.

Key words: Cancer: Infections: Neutropenic diet: Quality of life

Introduction

Haematological cancers originate in blood forming tissue, such as bone marrow or immune cells, and utilise chemotherapy as first line treatment followed by haematopoietic stem cell transplant (HSCT). HSCTs involve infusing stem cells from the bone marrow of the individual, taken prior to treatment (autologous) or from a matched donor (allogeneic). HSCT also involves a high dose of chemotherapy to destroy cancerous blood cells and suppress the immune system to allow the body to accept the stem cell transplant.[1,2] This treatment may cause other healthy cells to be destroyed including neutrophils, which are integral in preventing infections.[3] Due to the high dose of chemotherapy received, these individuals generally become neutropenic characterised by neutrophil counts below 1.5×10^9 neutrophils per litre of blood, and reduction below 0.5×10^9 neutrophils/l classified as severe neutropenia.[4] The decline in neutrophil cell counts leads to an increased risk of infection, prolonged bleeding time due to low platelet count, increased pain, and reduced nutritional intake due to mucositis and tiredness.[1]

Due to the increased infection risk, individuals receiving cancer treatment in hospital in the 1960s would often be placed

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in sterile environments including laminar flow rooms and receive gut decontamination with antibiotics in addition to a 'sterile' diet.[5] However, an early literature review in 1984 determined that protective environments did not reduce infection rates, but had many negative effects including poor psychological impact, increased costs, and increased staff labour associated with their use.[6] Although complete protective environments are no longer employed, many healthcare institutions continue to provide patients with a neutropenic diet (ND),[7] which have changed from very restrictive 'sterile' diets to one that limits 'high-risk' foods: raw fruits and vegetables, raw/undercooked meat, fish and eggs and unpasteurised dairy. Restriction of these foods is widely considered the basis of the ND; however, no set guidelines exist.[7]

In recent years the validity of the ND has been questioned. Numerous systematic reviews and meta-analyses[8–14] have shown that the ND does not significantly reduce the risk of infection or mortality within this population. Additionally, The European Society for Clinical Nutrition and Metabolism (ESPEN), a major society in nutrition, do not recommend an ND.[15,16] Despite this, clinicians[17,18] and hospitals that perform HSCTs[19–22] continue to provide patients with NDs in Switzerland,[19] China,[20] Italy,[21] Germany,[22] Austria,[22] UK,[17] and the US.[18] All studies reported between 50 and 80% usage of the ND.[17–22] Interestingly, the studies with >80% usage are the most recent studies within this area, published between 2018 and 2021.[20–22]

Although there are numerous studies that have assessed the ND and its impacts on infection and mortality rates, other outcomes aside from infection rates that are of importance in relation to diet during cancer have not been considered. To our knowledge, many of these additional areas have not been systematically reviewed and need to be considered as part of the overall impact of NDs in the health and lifestyle of people with cancer. Therefore, the aim of this research is to systematically scope the current evidence-base to identify studies on the ND and any outcomes for people with cancer that relate to their medical, nutritional, social, psychological, or physical health as well as costs associated with treatment. Additionally, gaps in the evidence and opportunities for future research will be identified.

Methods

Protocol

The methods for this scoping review were prospectively designed and registered with Open Science Framework on 19 July 2022 and can be accessed at https://osf.io/gan2p.

Selection criteria

Included studies were required to be (i) in English, (ii) human studies assessing people with cancer of any type, (iii) consuming a ND as defined by the paper authors, (iv) any age group, date of study, or setting. There were minimal restrictions in the included studies in attempt to capture as many studies as possible. The eligible study types included randomised control trials (RCTs), retrospective, prospective, cohort, observational, comparative, systematic review, and meta-analyses.

Studies were excluded if (i) in a language other than English, (ii) non-human, (iii) assessed the wrong diet or (iv) outcomes were not related to the clinical health or lifestyle outcomes of the scoping review. Excluded study types included letters, conference proceedings, books, book chapters, and guidelines.

Search strategy

The following databases were searched CINAHL Complete (EBSCO Publishing, Inc), The Cochrane Library (John Wiley & Sons, Ltd), Embase (Ovid), Emcare (Ovid), MEDLINE (Ovid), Scopus (Elsevier Science Publishers), and Web of Science (Clarivate Analytics).

The following search terms with Boolean operators were used in all databases with no other filters applied: "neutropenic diet*" or "low bacteria* diet*" or "low-bacteria* diet" or "low microbial diet*" or "low-microbial diet*" or "germ free diet*" or "germ-free diet*" or "sterile diet*". Databases were searched from inception to 19th July 2022 with additional papers added from search alerts of the above searches in all databases between 20 July 2022 and 23 August 2023.

Screening sources and data extraction

Screening was undertaken in Covidence (Veritas Health Innovation Ltd). Title and abstract screening were completed independently by two reviewers (TG + one of MH, PK, LT, EM) with any conflicts being resolved by discussion (TG + EM). Full text screening was completed independently by two authors (TG + one of MH, PK, LT, EM) with any conflicts being resolved by discussion (TG + EM) or by a third author if needed. Reasons for exclusion were given for each study at the full-text screening stage.

Data from included articles was extracted into Excel (Microsoft Corporation, Washington, US) using standardised tools formulated by one reviewer (TG) and checked by one reviewer (EM). Extraction was completed by one reviewer (TG) and checked by a second reviewer for accuracy (one of MH, PK, LT, or EM). The outcomes of interest that were extracted were infection rate, mortality rate, fever, antibiotic use, side effects (including diarrhoea, nausea, vomiting), comorbidities (including neutropenic enterocolitis, graft vs host disease, mucositis), hospitalisation, quality of life, diet acceptability, nutritional status, and costs. Data was extracted and used as it was presented in the corresponding paper, and the results are presented in a narrative summary.

Critical appraisal

Critical appraisal was completed for all included studies using the most suitable JBI checklist for study type (https://jbi.global/critical-appraisal-tools). Appraisal was completed by TG and checked by EM. JBI critical appraisal checklist for descriptive/case series was used for all retrospective studies included in the review. This checklist is no longer listed on the JBI website but is however considered a key tool for critical appraisal of descriptive studies.[23]

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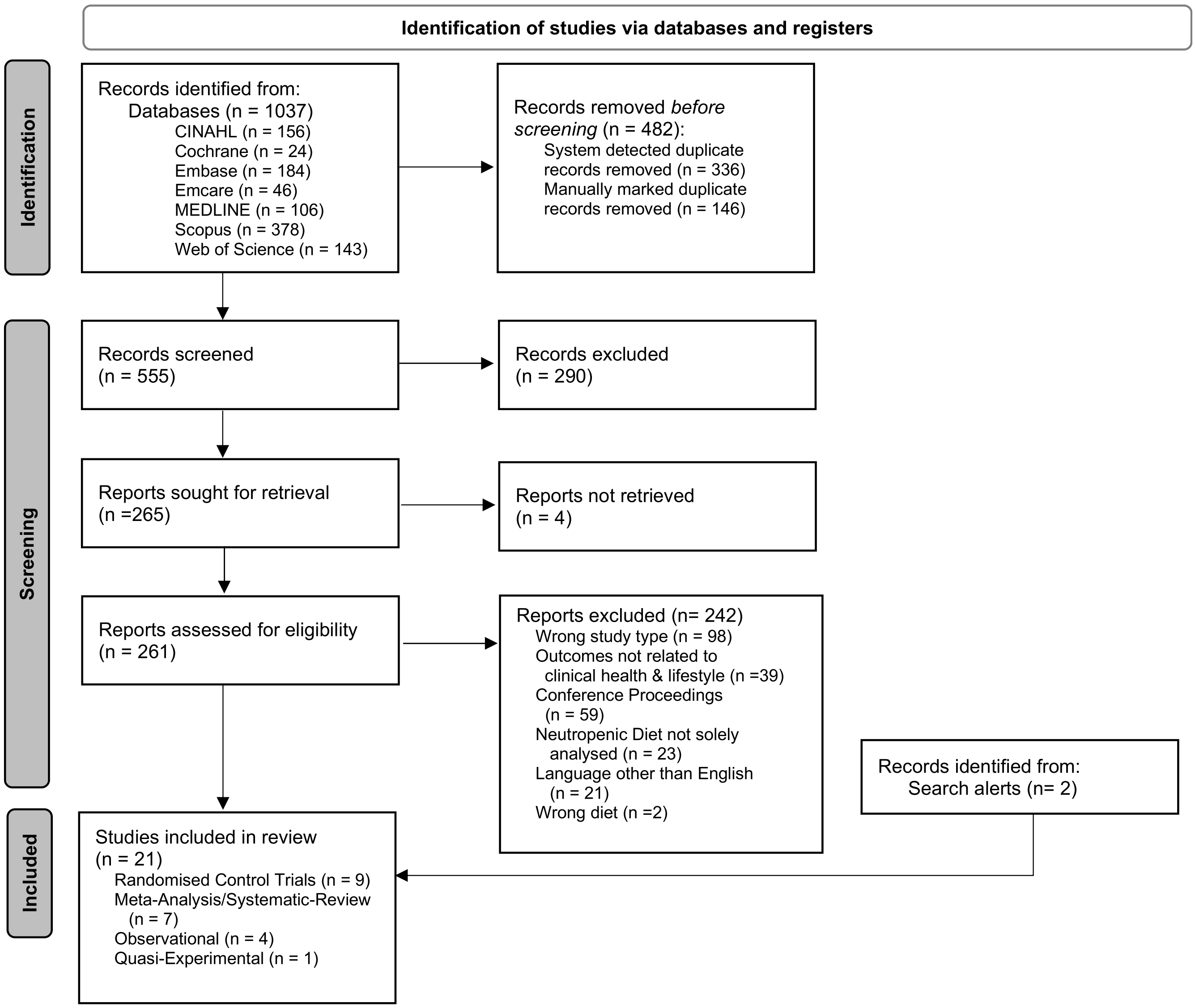

Fig. 1. PRISMA Flow Diagram.

Results

The initial search in July 2022 retrieved 1037 citations and after the removal of 481 duplicates, a total of 550 articles were available for title and abstract screening. Following initial screening, 264 articles were eligible for full-text screening. Following full-text screening 19 articles were deemed eligible for inclusion. Two articles were added from email alerts from the databases for a total of 21 included articles. Figure 1 shows the PRISMA Flow Diagram.

Nine studies were conducted in the United States,[8,9,24–30] three in India,[11,31,32] two in The Netherlands[10,33] and Iran[34,35] and one in Australia,[36] China,[12] Germany,[37] The Philippines[13] and Italy.[14] Four studies[24,30,32,35] included only paediatric participants and nine studies included only adult participants.[14,25–28,33,34,36,37] Eight studies,[8–13,29,31] including the seven systematic reviews/meta-analysis, included all age groups. Eleven studies were conducted in an inpatient hospital setting, and one in an outpatient setting.[25] Two studies included both inpatients and outpatients[24,29] and six of seven systematic reviews/meta-analyses[8–13] included one or more of the outpatient studies whereas one[14] only included studies with an inpatient population. All studies included participants with haematological cancers and four also included participants with oncological cancers[24,25,29,32] however, none analysed results by cancer type. A summary of the characteristics of the included studies are shown in Table 1.

There are no guidelines for the use of the ND and the diet can vary between hospitals in the foods restricted. This is reflected in the differences between the NDs in the included studies. All included studies restricted most raw fruits and vegetables – some allowed fruits and vegetables with thick skin or that could be hand-peeled.[24,28,29,35] Other restricted foods included raw grains,[27,30,37] raw nuts/nut butters,[29,37] raw seeds,[27] raw miso,[27] yoghurt,[29,36] dairy from bulk machines (i.e. soft serve ice cream, frozen yoghurt), raw honey,[30] dried/raw herbs and spices,[25,27,33,36] yeast,[27,30] cold desserts[36] tap/un-boiled water,[33–35,37] takeaway/fast food,[24,29,30] buffet/street food,[25,30] and sharing food.[30]

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Table 1. Characteristics of Included Studies

Study (country)	Design (setting)	Participants		Diet specifications		Critical appraisal
		Number	Age	Neutropenic	Control	
DeMille 2006(25) (United States)	Prospective (Outpatient)	ND: 16 CD: 7	18–70	American Dietetic Association food safety guidelines in addition to restricted foods: – Fresh and dried fruit – Raw vegetables – Unpasteurised juice – Raw/undercooked meat, fish and eggs – Dried and raw herbs – Buffet and street food	Those non-compliant with neutropenic diet.	6/9
Trifilio 2012(27) (United States)	Retrospective (Inpatient)	ND: 363 CD: 363	ND: 18–78 CD: 18–76	Diet used during neutropenic period only with restricted foods: – Fresh fruit – Raw vegetables – Black pepper – Raw/undercooked meats and cheeses – Cold smoked fish – Raw/unpasteurised dairy – Raw miso, grains and seeds – Brewer's yeast	Academy of Nutrition and Dietetics approved hospital diet with restricted foods: – Raw tomatoes – Raw/undercooked meats and cheeses – Cold smoked fish – Raw/unpasteurised dairy – Raw miso, grains and seeds – Brewer's yeast	6/9
Taggart 2019(30) (United States)	Prospective (Inpatient)	ND: 49 CD: 53	ND: 11.7 CD: 9.2	Restricted Foods: – Fresh fruit – Raw vegetables – Unpasteurised dairy – Raw/undercooked eggs, meat and fish – Raw blue cheese – Dairy from bulk machines – Deli meats – Raw grains, yeast and honey – Buffet meals – Fast food (if not freshly prepared) – Sharing food	Modified Bone Marrow Transplant Diet Restricted Foods: – Unpasteurised dairy – Raw blue cheese – Dairy from bulk machines – Undercooked meat and fish	8/9
Heng 2020(36) (Australia)	Retrospective (Inpatient)	ND: 79 CD: 75	ND: 50–63 CD: 53–69	Hospital food safety guidelines in addition to restricted foods: – Fresh fruits – Raw vegetables – Undercooked meat and eggs – Cold meats – Yoghurt – Cold desserts – Fresh herbs – Black pepper (added after cooking)	Hospital food safety guidelines consisting of restricted foods: – Undercooked meat and eggs	6/9
Jakob 2021(37) (Germany)	Retrospective (Inpatient)	ND: 1043 CD: 1043	ND: 40–63 CD: 37–61	Restricted Foods: – Fresh fruits – Raw vegetables – Raw grains and nuts – Raw dairy and eggs – Tap water	Hospital food safety guidelines consisting of restricted foods: – Unpasteurised dairy – Raw/undercooked eggs, poultry, fish, and meat	7/9
Moody 2006(24) (United States)	RCT (Inpatient + Outpatient)	ND: 9 CD: 10	ND: 4.4 CD: 4.1	FDA restrictions in addition to restricted foods: – Fresh fruit (excluding fruits able to be hand peeled) – Raw vegetables – Aged cheeses – Cold cuts	FDA Food Safety Guidelines consisting of restricted foods: – Unpasteurised milk, cheese, juice – Raw/undercooked eggs, poultry, fish, and meat	11/13

Continued

Table 1. Continued

Study (country)	Design (setting)	Participants: Number	Participants: Age	Diet specifications: Neutropenic	Diet specifications: Control	Critical appraisal
				– Fast foods – Takeaway		
van Tiel 2007(33) (Netherlands)	RCT (Inpatient)	ND: 10 CD: 10	ND: 40–69 CD: 30–68	Restricted foods: – Raw vegetables – Soft cheeses – Raw meats – Most fresh fruits – Tap water – Raw spices Use of single serve containers for food	Normal hospital diet. Did not specify dietary restrictions.	9/13
Gardner 2008(26) (United States)	RCT (Inpatient)	ND: 75 CD: 78 CDN: 53	ND: 17–88 CD: 47–84 CDN: 49–81	Restricted foods: – Fresh fruit – Raw vegetables	Fresh fruits and vegetables not restricted. Did not specify dietary restrictions.	8/13
Lassiter 2015(28) (United States)	RCT (Inpatient)	ND: 25 CD: 21	ND: 45 CD: 45	FDA Food safety guidelines. Allowed to consume cooked foods and thick-skinned fruits	FDA Food safety guidelines with no further food restrictions	10/13
Moody 2017(29) (United States)	RCT (Inpatient + Outpatient)	ND: 77 CD: 73	ND: 12 CD: 11	FDA restrictions in addition to restricted foods: – Raw vegetables – Fresh fruit (excluding fruits able to be hand peeled) – Aged cheeses – Cold cuts – Raw nuts and nut butters – Yoghurt – Unpasteurised dairy/fruit juice – Undercooked food – Fast foods and Takeaway	FDA Food Safety Guidelines consisting of restricted foods: – Unpasteurised milk, cheese, juice – Raw/undercooked eggs, poultry, fish, and meat	11/13
Jalali 2018(34) (Iran)	RCT (Inpatient)	ND: 25 CD: 25	ND: 40 CD: 41	Restricted Foods: – Fresh fruit (excluding fruits able to be hand peeled) – Water not boiled – Unpasteurised dairy – Raw/undercooked meat and eggs	Mediterranean Neutropenic Diet – Consumed 30mL olive oil daily – Same restrictions as ND group	9/13
Hosseini 2020(35) (Iran)	RCT (Inpatient)	ND: 25 CD: 25	ND: 40.80 CD: 38.36	Restricted Foods: – Raw fruits (excluding fruits able to be hand peeled) – Raw vegetables – Raw/undercooked eggs and meat – Unpasteurised dairy – Un-boiled water	Neutropenic Diet + Vitamin C (500 mg tablet daily) Restricted Foods: – Raw fruits (excluding fruits able to be hand peeled) – Raw vegetables – Raw/undercooked eggs and meat – Unpasteurised dairy – Un-boiled water	7/13
Gupta 2022(32) (India)	RCT (Inpatient)	ND: 21 CD: 21	ND: 3–13 CD: 3–12	Hospital food safety guidelines in addition to restricted foods: – All raw foods	Hospital food safety guidelines	11/13
Radhakrishnan 2022(31) (India)	RCT	ND: 102 CD: 98	ND: 1–60 CD: 1–59	American Cancer Society Food safety guidelines in addition to restricted foods: – Fresh fruit – Unpasteurised fruit juice – Raw vegetables	American Cancer Society food safety guidelines	10/13

Study (Country)	Design	Included Studies	Inclusion Criteria	Exclusion Criteria	Critical Appraisal
van Dalen 2012(10) (Netherlands)	Systematic Review	3	– Randomised Control Trials – Comparing neutropenic diet to control – Cancer patients receiving chemotherapy causing neutropenia – Adult and paediatric population	– Children under 1 year of age	9/11

Continued

Table 1. Continued

Study (Country)	Design	Included Studies	Inclusion Criteria	Exclusion Criteria	Critical Appraisal
Ball 2019(9) (United States)	Meta-Analysis	5	– Randomised Control Trial – Compares liberal diet to a neutropenic diet – Assessed rates of infection	– Did not report outcomes relating to neutropenic diet compared to unrestricted diet	5/11
Sonbol 2019(8) (United States)	Systematic Review/Meta-Analysis	6	– Neutropenic diet compared to regular diet in neutropenic patients with cancer Adult and paediatric populations – Comparative, retrospective, prospective studies	– Single-arm trials	10/11
Ramamoorthy 2020(11) (United States/ India)	Systematic Review	11	– Infection rates: bacteraemia, pneumonia, fungaemia – Infection related mortality and all-cause mortality	NA	4/11
Ma 2022(12) (China)	Systematic Review/Meta-Analysis	6	– Diagnosed with leukaemia and neutropenic – Randomised Control Trial only – Neutropenic diet intervention – Standardised diet control	– Duplicates – Letters and abstracts	8/11
Ng 2022(13) (Philippines)	Meta-Analysis	3	– Paediatric population – Undergoing chemotherapy – Randomised Control Trial only – No limits on treatment phase – No language limits	– Adult population – All other study types	7/11
Matteucci 2023(14) (Italy)	Systematic Review	12	– Adult population – Haematological malignancy – Meta-analyses and observational studies – Published from May 2015 to January 2023	– Paediatric population – All other study types – Published prior to May 2015	11/11

ND, neutropenic diet; CD, comparator diet; RCT, randomised control trial; CDN, comparator diet not randomly assigned, ages presented as mean ages or age range.

The major comparator diet in the included studies was the food safety diet, used by nine studies.[24,27–32,36,37] This diet restricts unpasteurised eggs, dairy, raw/undercooked meat and fish and has regulations on food handling, washing, preparation, and storage.[38] One study termed the comparator diet ‘Modified Bone Marrow Transplant Diet’ however restrictions were similar to the food safety diet.[30] Two studies[27,33] used a standard hospital diet which followed the food safety diet with some additional restrictions: raw tomatoes, cold smoked fish, raw miso, raw grains/seeds, and brewer’s yeast. One study did not impose any restrictions for the comparator diet,[26] and another included participants in the comparator group if they were non-compliant with the ND.[25]

The incidence of infections was a major outcome in all but two of the 21 included studies. However, the type of infections, grouping of infection types, and the method of reporting varied amongst studies. As shown in Table 2, we report these results as they have been reported in the included studies.

Total infection, reported in 13 studies[9,11,12,14,24–27,29,31,32,36,37] included infection of any body-site with any bacteria, fungi, or virus. One RCT[32] reported a significantly higher rate of infection in the ND group with 12 infections (n = 21) compared to nine in the comparator group (n = 21) (P = 0.049).[32] A retrospective study[27] found a significant difference between the diets for total diagnostically confirmed infections – 135 infections in the ND group compared to 106 in the comparator group (P = 0.03). Infections present when neutropenia had resolved was also significantly higher in the ND group compared to the comparator group (P = 0.01). However, infection during neutropenia was not significantly different (P = 0.22).[27] Ten of the 13 studies which reported on total infection did not find any significant difference between diet groups. Overall, from the included studies the ND either results in no significant difference in infection rates compared to the comparator diet (n = 12 studies) or increases the infection rate (n = 2 studies).

A further three studies reported combined rates of total infection and/or fever.[8,10,26] A systematic review/meta-analysis[8] found higher rates of infection in the ND group for the total population (RR 1.17, 95% CI (1.04–1.32)). When haematopoietic stem cell transplant (HSCT) recipients following the ND were compared to those following the comparator diet, significantly higher rates of infection in the ND group were demonstrated (RR 1.25, 95% CI (1.02–1.54)). However, no significant difference was seen between diet groups for participants who were not recipients of HSCT.[8]

Major infection was assessed in three studies;[8,26,31] however, as there is no clinical definition of what constitutes a major infection, there were differences in the definition between studies. All three studies included pneumonia, bacteraemia, or fungaemia as major infections,[8,26,31] whilst one study also

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Table 2. Effect of the Neutropenic Diet on Rates of Infection

Study	Total infection	Total infection + fever	Fever	Major infection	Minor infection	Gut colonisation	Pneumonia	Bacteraemia	Fungaemia	Bacteraemia + fungaemia	Viral infection
DeMille 2006[25]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Trifilio 2012[27]	–[a] ↑[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Taggart 2019[30]	NA	NA	NA	NA	NA	NA	NA	–	NA	NA	–
Heng 2020[36]	–	NA	–[f] ↑[g]	NA	NA	NA	NA	–	NA	NA	NA
Jakob 2021[37]	–	NA	–	NA	NA	NA	NA	–	NA	NA	NA
Moody 2006[24]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
van Tiel 2007[33]	NA	NA	–	NA	NA	–	NA	–	–	NA	NA
Gardner 2008[26]	–	–	–	–	–	NA	–	NA	NA	↓	NA
Lassiter 2015[28]	NA	NA	NA	NA	NA	NA	NA	–	NA	NA	NA
Moody 2017[29]	–	NA	–	NA	NA	NA	NA	NA	NA	NA	NA
Gupta 2022[32]	↑	NA	–	NA	NA	NA	–	NA	NA	NA	NA
Radhakrishnan 2022[31]	–	NA	–	–	–	↓[j] –[k]	–	–	NA	NA	NA
van Dalen 2012[10]	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ball 2019[9]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sonbol 2019[8]	NA	↑[d] –[e]	NA	↑[h] –[i]	NA	NA	NA	NA	NA	–	NA
Ramamoorthy 2020[11]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ma 2022[12]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ng 2022[13]	NA	NA	–	NA	NA	NA	NA	–	NA	NA	NA
Matteucci 2023[14]	–[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA, Not Assessed.
[a]During neutropenia.
[b]Overall and when neutropenia had resolved.
[c]Significant differences not reported however of the six studies 4/6 found no significant difference 1/6 did not include a comparator and 1/6 ND increased infection risk for HSCT transplant recipients only.
[d]HSCT recipients and overall.
[e]Non-HSCT recipients.
[f]Cases of febrile neutropenia.
[g]Number of febrile days.
[h]HSCT recipients.
[i]Non-HSCT recipients and overall.
[j]At baseline.
[k]Day 15 of study.
– No significant difference between Neutropenic Diet and comparator, ↑Neutropenic Diet increases risk, ↓Neutropenic Diet decreases risk.

included urinary tract infection, meningitis, cellulitis, or diarrhoea.[31] A systematic review/meta-analysis reported a significantly higher rate of major infection in the ND group when only assessing participants who were HSCT recipients (RR 1.25, 95% CI (1.02–1.54)). No significant difference was seen in participants who were not transplant recipients or when all participants were assessed together.[8]

Minor infection was assessed by two studies;[26,31] however, as there is no clinical definition of what constitutes a minor infection, both studies included all other infections not defined as a major infection. Both studies found no significant difference between diet groups.[26,31]

Gut colonisation by pathogenic bacteria or yeasts was assessed in two RCTs by faecal analysis.[31,33] Radhakrishnan, Lagudu[31] reported significantly higher rates of colonisation in the comparator group (n = 40/96) compared to the ND group (n = 29/102) at baseline (P = 0.05). Bacteria isolated from stool included multidrug resistant (MDR) *Escherichia coli*, MDR *E. faecalis*, MDR *Klebsiella pneumoniae*, MDR *E. faecium*, Vancomycin resistant *E. faecium*. A positive stool culture at baseline is considered more likely to be reflective of the diet consumed while participants are in the community. At the end of the study (day 15), there were no differences, indicating that the diet did not significantly affect gut colonisation.[31] No significant differences were reported in the study by van Tiel, Harbers.[33]

Pneumonia is the inflammation of the lungs caused by bacteria, fungi, or viruses[39] and while there is no evidence linking it to diet, it was assessed in three studies.[26,31,32] Two studies found no significant difference between diet groups[26,31] and the third did not analyse statistical significance, however they reported two pneumonia cases in the ND group (n = 21) and one in the comparator group (n = 21).[32]

Bacteraemia, presence of bacteria in the bloodstream was assessed in seven studies, none of which found a significant difference between diet groups.[13,28,30,31,33,36,37] *Fungaemia, the presence of fungi in the bloodstream was assessed by o*ne RCT which found no significant difference between diet groups.[33] Combined bacteraemia or fungaemia was assessed in two studies,[8,26] a quasi-experimental study found significantly higher rates in the comparator group (n = 17) than the ND (n = 7) (P = 0.03);[26] however, a systematic review/meta-analysis found no significant differences between diet groups.[8]

Norovirus, which causes gastrointestinal infection[40] was assessed in an observational study which reported no significant differences between diet groups.[30]

Neutropenic fever or febrile neutropenia is characterised as a high temperature (>38.3°C or >38°C on two occasions).[41] Eight studies assessed fever with five reporting on neutropenic fever,[13,24,31,32,36] four reported on fever of unknown origin (>38.3°C on multiple occasions for three weeks, with diagnosis unclear after one week[42]),[26,31,36,37] one assessed persistent fever[37] and one assessed high or low temperature,[33] with none of the eight studies reporting significant differences between diet groups.

Antibiotic use was assessed in two studies with different metrics; length of antibiotic use (the consecutive number of days which an antibiotic is used)[32,33] and antibiotic duration (the total amount of time in which antibiotics are used).[32] Neither study found a significant difference between diet groups for use of antibiotics (see Table 3).

Gastrointestinal side effects such as diarrhoea, nausea, and vomiting during cancer treatment may be caused by chemotherapy,[43] use of antibiotics which causes healthy bacteria of the gut to be destroyed and/or, use of other medications such as opioids.[43] These symptoms are also commonly attributed to *Clostridium difficile* infection.[44] Diarrhoea was assessed in five studies[14,24,27,36,37] and only one study found a significantly higher rate in the ND group (P < 0.001). The remaining studies saw no significant differences. Two studies reported on *Clostridium difficile* – with both reporting no significant difference between groups – however, no link was made between the incidence of diarrhoea and *Clostridium difficile* infection.[27,36] Nausea was assessed in two studies, a retrospective study reported a significantly higher rate of nausea in the ND group[37] and a systematic review included only findings from the retrospective study.[14] Vomiting was assessed in one study which reported two of nine participants in the ND group and two of ten in the comparator diet group had instances of vomiting, however, significance was not reported[24] (see Table 3).

Neutropenic enterocolitis is the inflammation of the gastrointestinal tract occurring in a neutropenic individual. Two studies[32,36] assessed neutropenic enterocolitis, one study reported higher rates in the ND group (P = 0.044) and the other reported no significant difference between groups.[36]

Graft vs Host Disease (GvHD) can occur post-stem cell transplant when donor T-cells attack healthy cells of the recipient. Two observational studies assessed GvHD;[27,30] however, neither saw a significant difference between diet groups.

Mucositis, defined as, inflammation of the mouth and/or gut, was assessed by one RCT[29] which reported four cases in the ND group and two in the comparator group, however this was not significant.

Mortality was assessed in ten studies;[8,11,12,14,26,27,31,32,36,37] however, no studies reported any significant differences between groups (see Table 3).

Serum albumin, whilst no longer used as a sole indicator of nutritional status, recent literature suggests decreased levels may be associated with gut dysbiosis.[45] Two RCTs[34,35] assessed serum albumin levels and both found significantly lower levels in ND groups compared to the comparator diets post-intervention (see Table 4). This may indicate a decreased dietary intake or gut dysbiosis. Neither study reported on fluid status, inflammation, microbiome, or other GI related side effects associated with decreased serum albumin levels.

Four studies assessed hospitalisation by length of stay[27,32,36] or admissions.[25,32,36] A retrospective study[27] reported that those in the ND group (n = 363) who underwent HSCT spent, on average, one day longer in hospital than those following the comparator diet (n = 363), but level of significance was not reported.[27] No significant difference was seen in the remaining two studies.[32,36] None of the three studies which assessed hospital admissions found a significant difference between diet groups[25,32,36] (see Table 4).

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Table 3. Effect of the Neutropenic Diet on Clinical Factors

	Mortality		Antibiotic use		Gastrointestinal			Comorbidities		
Study	Actual	Probability	Length	Duration	Diarrhoea	Nausea	Vomiting	NE	GvHD	Mucositis
Trifilio 2012[27]	–	NA	NA	NA	–	NA	NA	NA	–	NA
Taggart 2019[30]	NA	NA	NA	NA	NA	NA	NA	NA	–	NA
Heng 2020[36]	–	NA	NA	NA	–	NA	NA	–	NA	NA
Jakob 2021[37]	NA	–	NA	NA	↑	↑	NA	NA	NA	NA
Moody 2006[24]	NA	NA	NA	NA	–	NA	–	NA	NA	NA
van Tiel 2007[33]	NA	NA	–	NA	NA	NA	NA	NA	NA	NA
Gardner 2008[26]	NA	–	NA	NA	NA	NA	NA	NA	NA	NA
Moody 2017[29]	NA	NA	NA	NA	NA	NA	NA	NA	NA	–
Gupta 2022[32]	–	NA	–	–	NA	NA	NA	NA	NA	NA
Radhakrishnan 2022[31]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sonbol 2019[8]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ramamoorthy 2020[11]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ma 2022[12]	–	NA	NA	NA	NA	NA	NA	NA	NA	NA
Matteucci 2023[14]	–[a]	NA	NA	NA	↑[b]	↑[b]	NA	NA	NA	NA

NA, not assessed; NE, neutropenic enterocolitis.
[a]Significant difference not reported 3/3 studies included had no significant differences between groups.
[b]Only included results from Jakob, Classen.[37]
– No significant difference between Neutropenic Diet and comparator, ↑Neutropenic Diet increases risk.

Table 4. Effect of the Neutropenic Diet on Biochemical Factors, Hospitalisation, Nutritional Status, and Well-being

	Biochemical	Hospitalisation		Nutritional status		Wellbeing		
Study	Serum albumin	Length of Stay	Admissions	Weight change	PG-SGA	Quality of life	Diet acceptability	Diet adherence
DeMille 2006[25]	NA	NA	–	NA	NA	NA	NA	NA
Trifilio 2012[27]	NA	–	NA	NA	NA	NA	NA	NA
Taggart 2019[30]	NA	NA	NA	NA	NA	NA	–	NA
Heng 2020[36]	NA	–	–	NA	NA	NA	NA	NA
Jakob 2021[37]	NA	NA	NA	↑[c]	NA	NA	NA	NA
				–[d]	NA	NA	NA	NA
Moody 2006[24]	NA	NA	NA	NA	NA	↑[e]	–	SNR
						–[f]	NA	NA
Lassiter 2015[28]	NA	NA	NA	NA	NA	–	NA	NA
Moody 2017[29]	NA	NA	NA	NA	NA	–	NA	NA
Jalali 2018[34]	↑[a]	NA	NA	–	↑	NA	NA	NA
	–[b]							
Hosseini 2020[35]	↑[a]	NA	NA	–	↑	NA	NA	NA
	–[b]							
Gupta 2022[32]	NA	–	–	NA	NA	NA	NA	–
van Dalen 2012[10]	NA	NA	NA	NA	NA	NA	–	NA
Matteucci 2023[14]	NA	NA	NA	↑[c,g]	NA	NA	NA	NA
				–[d,g]				

NA, Not Assessed; SNR, Significance Not Reported.
[a]Neutropenic Diet significantly decreases serum albumin when compared to diet with additional Vitamin C or olive oil and when compared pre and post intervention.
[b]Neutropenic Diet is not significantly different from diet with additional Vitamin C or olive oil pre intervention and diet with additional Vitamin C olive oil is not significantly different pre and post intervention.
[c]Neutropenic diet significantly increases risk of weight loss of between 1 and 3 kg.
[d]No significant difference between diets for weight loss of greater than 3 kg.
[e]Neutropenic diet significantly lower QoL score for PEDS core module.
[f]No significant difference between diet groups for PEDS cancer module.
[g]only included results from Jakob, Classen.[37]
↑Neutropenic Diet increases risk, – No significant difference between Neutropenic Diet and comparator.

Patient Generated Subjective Global Assessment (PG-SGA) is a common, validated tool used in cancer patients to evaluate nutritional status.[46] The PG-SGA categorises individuals into three groups: 'appropriate nutrition', 'prone to malnutrition' or 'severe malnutrition'. Two RCTs[34,35] reported a significantly higher proportion of the ND group being placed into the 'prone to malnutrition' or 'severe malnutrition' categories.

Weight change was assessed in four studies.[14,34,35,37] A retrospective study reported a significantly greater proportion of those in the ND group losing between 1 and 3 kg ($P = 0.05$), however further sub-analysis showed no significance for weight loss greater than 3 kg.[37] This study only reported absolute weight loss and did not report percentage weight loss, hence making it difficult to determine if this weight loss is clinically significant. Weight loss was an outcome in the systematic review by Matteucci, De Pasquale[14] and the only included study for this outcome was Jakob, Classen.[37] As no further analysis was conducted in the systematic review, results were the same.[14]

One study published in 2007[33] assessed the total financial costs as a secondary outcome of the ND contrasted with the comparator diet at different stages of care – including hospital costs, other healthcare costs, and inability to work. The areas in which costs are associated were identified, however the reason for cost differences were not determined. Whilst they reported higher costs for the comparator diet during hospitalisation, in contrast during follow-up and in total, the ND had higher costs, however significance of these results was not reported.[33]

Quality of Life (QoL) is defined by the World Health Organisation as 'an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns'.[47] Two RCTs from the same research group[24,29] assessed QoL in paediatric cancer patients (Table 4) using the Paediatric Quality of Life Inventory. These two studies were similar in design and in the outcomes assessed, however the 2017 study[29] had more participants (ND = 77, CD = 73) compared to the 2006 study (24) (ND = 9, CD = 10). In the 2006 study, they found the Core QoL of the ND group was significantly lower (indicating lower overall QoL) than the comparator group ($P < 0.05$), however, this was not significant for cancer specific QoL.[24] In the 2017 study, they did not find a significant difference between diet groups.[29]

Diet acceptability was assessed by three different metrics across three studies[10,24,30]; ease of following assigned diet, food not tasting how participants remembered, and inability to consume desired foods. All three RCTs found no significant difference between ND and comparator diet groups for diet acceptability.[10,24,30]

Diet adherence was measured by two different metrics in two RCTs; number of meals for which participants (n = 19) were following their assigned diet[24] and number of weeks participants (n = 42) were following the assigned diet.[32] Both studies found significantly better adherence in the comparator groups. Participants were reported to have adhered to the ND (n = 9) 94.10% of the time and to the comparator diet (n = 10) 99.99% of the time.[24] Participants followed the ND for 93 of the 98 weeks (n = 21) and the comparator 94 of the 98 weeks (n = 21), however, this was not significantly different.[32]

Discussion

The aim of the present scoping review was to systematically search the literature and identify articles which assessed the use of the ND for those undergoing cancer treatment on any outcomes relating to medical, nutritional, social, psychological, physical health, and all associated costs. We identified 21 relevant articles which covered outcomes including participants' clinical health with most assessing infection and mortality rates. Ten of sixteen outcomes included in the present study have not previously been included in systematic reviews.

Clinical outcomes identified in this scoping review were related to risk of infection, mortality, and fever in 19 of the 21 studies. The majority of studies observed no significant difference between groups, which aligns with the rationale for recommendations of the clinical guidelines for nutrition in cancer patients from The European Society for Clinical Nutrition and Metabolism (ESPEN) published in 2016[15] and updated in 2021.[16] Based on the evidence base at the time, ESPEN recommended that "There are insufficient consistent clinical data to recommend a low bacterial diet for patients more than 30 days after allogeneic transplant".[15,16] As such the ESPEN guideline for hospital nutrition.[48] was "Neutropenic diets (also called 'germ-free', 'no microbial' or 'sterilised' diets) shall not be used (e.g. in neutropenic patients with cancer including haematopoietic stem cell transplant patients)",[48] it received an A grade recommendation as it was supported by a strong evidence base, including a meta-analysis[8] and a Cochrane review.[10] The recommendation received strong consensus from ESPEN members, and it was recommended that this population follow food safety guidelines.[48]

The ND generally includes well-cooked meat and excludes raw fruit and juices, however, within a population of Haematopoietic Stem Cell Transplant (HSCT) recipients the main food aversions were shown to be meat, specifically beef and chicken, due to the association with dysphagia.[49] Preferred foods included fruit, fruit juices, and soup due to the association with improved gastrointestinal symptoms i.e. nausea.[49] The ND has been shown to have reduced Vitamin C[50,51] and fibre[50] due to restrictions on raw fruits and vegetables, which increases the risk of nutrient deficiencies in this population.[50,51] These nutrient deficiencies coupled with a decreased overall energy intake could lead to malnutrition. Malnutrition associated with cancer is common, with rates of 30–40% in Australia and was an outcome in two included studies.[34,35] The combination of loss of taste and gastrointestinal symptoms, such as nausea could lead to decreased intake of food which may result in malnutrition.[49]

Weight loss during cancer treatment may be caused by a decreased intake of food due to loss of taste and other common side effects from treatment such as nausea and vomiting;[49] however, this scoping review determined that weight loss was seen in people with cancer following an ND.[34,37] Low weight in people with cancer is concerning as it has been shown to decrease overall survivorship in allogeneic HSCT recipients[52] and may be a sign of cancer cachexia, characterised by lower skeletal muscle mass. Moreover, cachexia requires medication, nutrition therapy, exercise, and psychosocial interventions and if untreated can reduce positive chemotherapy outcomes, increase side effects, and decrease survivorship.[53]

The impact on the microbiome and associated health problems have been identified in this scoping review as one of the major gaps in ND research. The understanding of the importance of the microbiome is developing, and it can affect cancer development, prevention, and treatment efficacy, conversely, treatment can also affect the microbiome.[54] Problematically, chemotherapy can cause gut dysbiosis – loss of diversity or changes to the gut microbiota, which affects the immune system and increases infection risk.[55] Antibiotics, commonly prescribed during cancer treatment, alter the balance of bacteria in the gut and negatively impact immunotherapy.[55] One study found that multi-drug resistant bacteria were detected in the faeces of ND patients in greater quantities than those following the comparator diet at baseline.[31] Interestingly, the participants included only those receiving induction chemotherapy, therefore, they would not have begun consuming their study diet, and it was not specified whether they had any prior use of antibiotics. Participants' diets prior to the study were not assessed. No studies have been conducted to demonstrate the impact of the ND on the gut microbiome, however the ND has been shown to have reduced fibre when compared to a standard hospital diet.[50] As raw fruits and vegetables are limited in the ND, this may limit the number of sources of fibre and probiotics. This may have an impact on the microbiome but as no research has been conducted this remains unknown.[50]

Evaluation of differences in financial costs between the ND and the comparator diet was only assessed in one study. Overall, the ND had higher costs (hospital costs, other healthcare costs and, inability to work) of EU€1,760 more compared to a standard hospital diet for the duration of treatment (EU€41,769 vs EU€40,009 in 2007). These costs were determined from hospital records, questionnaires and estimated from expert opinion.[33] Additionally, haematological cancers have been identified as some of the most expensive cancers to treat.[56] Further research is needed to determine financial costs associated with use of the ND in a broader context including the financial impact on people following the ND.

Understanding the impact of dietary quality on the quality of life (QoL) among individuals with cancer is paramount. QoL when consuming an ND has only been assessed in populations of children and young people with cancer, which found following an ND was associated with a decreased QoL compared to the comparator diets.[24,29] More research is needed as QoL is shown to be improved by eating with other people[57] and this social connection remains critical – potentially more so – in people with cancer.[58] Currently there is limited data on how the ND affects the way people eat with others. A qualitative study of older people with cancer[57] found they experienced taste alterations and decreased appetite due to treatment and decreased social interactions around food but had increased family connection irrespective of food.[57] As QoL is lower in people with cancer[59,60] it is important that future studies consider the impact of ND on QoL in all populations.

A key finding was of the small number of studies that looked at diet acceptability and diet adherence there was no significant difference between the ND and comparator for acceptability[10,24,30] however, adherence was significantly greater for the comparator diets.[24,32] As these studies were conducted in 2006, 2012, and 2019, more recent studies may be needed to confirm these findings with contemporary menu designs.

Another major gap identified was how the ND impacts (i.e. procurement, cooking and safe handing, and storage) the normal routine of those with cancer, as well as their friends and family. None of the included studies reported on this area. While studies have considered what impact cancer treatment has had on families and lifestyle,[61,62] none have assessed the impact of the ND.

A major strength of the present research is that, to our knowledge, this is the first scoping review of the ND for those with cancer, to scope the literature for all health-related outcomes using a systematic search process. The present scoping review is not without limitations. Samples sizes of the included studies – mainly of RCTs – were relatively small. Few studies assessed each included outcome making it challenging to draw conclusions from this. Due to variability of the ND, each study had a different definition in addition to the comparator diet used, however, most were a form of the food safety diet. Additionally, each study had implemented prophylactic measures in their study population in addition to the ND to reduce infection making it difficult to draw conclusions across the data. Included studies did not have consistent units or measures across outcomes particularly for the outcome of weight loss. It was difficult to determine whether there was a true difference in costs between the ND and the comparator due to ambiguity in the included study.

Conclusion

Despite the need for further research into several areas related to cancer and the administration of the ND, the current evidence suggests that the ND does not serve its original purpose: to reduce the risk of infection in this population. Additionally, the ND may lead to malnutrition due to it lacking variety and providing an unpleasant experience at mealtimes, may be costing us more in the long-term, and has been shown to decrease paediatric patient quality of life. This is of particular importance as the ND is used for patients with cancer by more than 50% of hospitals in Europe and China.[17–22] Further research is needed regarding how the ND affects the microbiome and quality of life of its consumers as well as associated costs, but in the interim, it is important for hospitals and other institutions providing an ND to their patients to liberalise the diet wherever possible.

Abbreviations

ND: Neutropenic Diet; **QoL:** Quality of Life; **RCT:** Randomised Control Trial; **HSCT:** Haematopoietic Stem Cell Transplant; **ESPEN:** European Society for Clinical Nutrition and Metabolism; **MDR:** Multidrug Resistant; **GvHD:** Graft vs Host Disease.

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Author contributions

TG: Conceptualisation, methodology, article screening, formal analysis, investigation, data curation, writing: original draft, writing: review and editing, visualisation, and project administration. MH: Screening, writing: review and editing. PK: article screening, writing: review and editing. LT: article screening, writing: review and editing. EM: Conceptualisation, methodology, validation, article screening, and writing: review and editing.

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Conflict of interest

None.

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RESEARCH ARTICLE

Using co-design to identify intervention components to address unhealthy dietary and activity behaviours in New Zealand South Asians

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Abstract

There is an urgent need to develop sustainable and impactful interventions to mitigate the high risk of diet-related non-communicable diseases (diet-NCDs) in South Asians living in high-income countries. The current study using a co-design methodology aimed to identify community-led intervention components (solutions) to address barriers and enablers of disease-promoting dietary and physical activity behaviours in New Zealand South Asians. Data were collected from South Asian immigrants aged 25–59 years via three focus group discussions ($n = 21$) and 10 telephone or face-to-face interviews between 2018 and 2019. The thematic analysis resulted in identifying 22 barrier and enabler codes and 12 solution codes which were summarised under five themes. The key solutions (intervention components) to mitigate the identified target behaviours were providing recipes for using local vegetables in South Asian cuisine, information on the nutritional quality of frozen vegetables and canned lentils, simple home gardening techniques, the saturated fat content of dairy foods, interpreting nutrition labels, optimal portion sizes of foods, and framing low-fat messages positively. Similarly, group-based activities with peer support such as walking, cultural dancing and community sports like cricket, football, and tennis were the identified solutions to increase physical activity levels. The identified solutions for health promoting dietary habits and physical activity levels could be part of any targeted multicomponent health promoting programme to reduce the risk of diet-NCDs in South Asian immigrants.

Key words: **Co-design methods: Dietary practices: Intervention components: Physical activity: Solutions: South Asians**

Introduction

The South Asian diaspora in high-income countries is at high risk for diet-related non-communicable diseases (diet-NCDs).[1–7] The Global Burden of Disease study has highlighted a strong association between suboptimal diets, predominantly low in fibre from whole grain, fruits and vegetables and high in saturated fats, and diet-NCDs.[8] Adopting a 'western diet' that is high in saturated fats is a common phenomenon observed among South Asian immigrants[9] and New Zealand South Asians are no exception.[1,10] Among New Zealand South Asians, fruit and vegetable consumption decreased, whilst consumption of meat, poultry, processed meat, potato chips, cakes, pastries, festival foods, takeaways, and alcohol increased as the duration of residence in New Zealand increased.[10] Not surprisingly, body mass index (BMI) also increased as the duration of residence increased.[10] Similar findings have been reported in the Metabolic Syndrome and Atherosclerosis in South Asians Living in America Study (MASALA),[4] which indicate that in contrast to 'fruits, vegetables, nuts and legumes' dietary pattern, 'animal protein', and 'fried snacks, sweets and high-fat dairy' dietary patterns were associated with greater insulin resistance and lower HDL cholesterol.[4] A strong positive association also exists between regular physical activity and adherence to healthy eating habits;[11] nevertheless, levels of physical activity in the South Asian diaspora are inadequate, for example, the New Zealand health survey data findings indicate that only 46% of South Asians aged 15 + years were physically active.[2]

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A review of dietary changes and their impact on the trajectory of health among South Asian immigrants in Western countries has highlighted the first five years post-migration as a window of opportunity to promote healthier dietary habits to reduce the risk of diet-NCDs in the South Asian diaspora.[12] Hence, designing and implementing culturally appropriate health-promoting interventions to promote healthier dietary habits of South Asian immigrants could prolong their healthy migrant status and reduce the diet-related disease burden. The InnvaDiab-DEPLAN study conducted in Norway on Pakistani immigrants showed that a health-promoting intervention was effective in changing intentions to make their dietary habits healthier and resulted in modest changes in dietary habits[13] with a longer-term maintenance of these changes.[14]

Given the uptake of universal health-promoting interventions is much higher among European people in comparison to people of South Asian origin,[15] probably due to recruitment challenges[16] and cultural inappropriateness of the developed intervention,[17] developing culturally appropriate interventions is prudent to address health disparities prevalent in the South Asian diaspora. Moreover, there is substantial evidence that interventions developed using a shared decision-making approach are more beneficial than culturally adapted ones.[18] Co-design is a participatory action research method that has been used to develop culturally appropriate interventions targeting minority populations.[19,20] Studies have shown that using co-design throughout the intervention development process will facilitate tracing significant intervention components to the generated solutions from the co-design research process[21] and being participant centered, co-design research is more likely to avoid resource wastage due to ineffective interventions and reduce the gap between research and practice. In this paper, we report the outcomes of a study that used a co-design approach to identify ethnic specific intervention components to reduce the risk of diet-NCDs in New Zealand South Asians. First, we report the barriers and enablers of disease-promoting dietary and activity behaviours followed by the solutions (intervention components) generated by the participants to address disease-promoting dietary and physical activity behaviours.

Methods

This study used a community-centric approach, inherent to co-design methodology[22] described previously.[23] Briefly, this involved extensive engagement with the South Asian community in 2017 to obtain community buy-in, a key building block towards addressing health disparities.[24] Three community meetings were held in a culturally appropriate manner and included the attendance of community leaders and cultural food. For this study, stakeholders were consulted at two time points to ensure cultural safety, cultural appropriateness, and cultural integrity were upheld throughout the research process. Adhering to the principles of co-design, which is underpinned by the theoretical framework of participant action research[25] and founded on the principle that the community of interest are the experts,[26] the researchers went beyond just stakeholder consultation and facilitated active participation of the target population in generating solutions (intervention components) for the identified barriers. Using an iterative process first, three focus group discussions (FGDs) followed by ten semi-structured interviews were conducted. All participants provided written informed consent before participation. The FGDs and interviews were audio recorded and conducted by ethnically matched personnel to ensure cultural appropriateness. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants signed an approved consent form prior to participation. Ethics approval was obtained from the University of Otago Human Ethics Committee (18/205; dated 14/12/2018).

Study participants and recruitment

The participants for this study were first generation South Asians, i.e. people from India, Sri Lanka, Nepal, Pakistan, Bangladesh, and Afghanistan, aged 25–59 years both ages inclusive, who understood and could communicate in English and were living in Dunedin or Auckland, New Zealand. Participants meeting the study's inclusion criteria were recruited via advertising through established links of the stakeholders and researchers to various South Asian cultural and religious groups in New Zealand. Data for this study were collected between 2018 and 2019.

Study design, data collection and analysis

This study used qualitative research methods on a convenient sample of New Zealand South Asians to achieve the study objectives. Participants recruited for the FGDs, and the semi-structured interviews completed a short demographic questionnaire before participation. Three FGDs (~90 min) with 5–9 participants of different duration of residence (DoR) ($\leq$ 5 years (n = 9); $>5<10$ years (n = 5) and $\geq$ ten years (n = 7); total n = 21) in each were conducted to capture the experiences of immigrants of different DoR on changing dietary habits and physical activity patterns post-migration, barriers to adopting healthy dietary habits and physical activity and generating solutions to address unhealthy dietary patterns and physical activity levels. FGDs were commenced by general discussions on post-migration changes in food habits, concerns regarding changing dietary habits and long-term health, sources of health information, and prioritisation of health as migrants in a western country. Following this, findings of a previous study (data collected in 2017)[10] were used as a starting point for more focused discussions. For example, in the previous study, consumption of vegetables had decreased post-migration, with only 15% of males and 36% of females meeting the three-plus-a-day recommendation for vegetable consumption.[10] This was discussed in the three FGDs, and participants were asked about the barriers they faced in consuming adequate vegetables and the solutions they perceived from their own experiences and knowledge that would be effective to increase vegetable consumption. Following the FGDs, ten semi-structured interviews (45–60 min) were conducted face-to-face and via telephone with the target population aiming to triangulate the FGDs findings. The topic list for the interviews was generated from the findings of FGD data.

SMY and SMP transcribed all the recordings verbatim, and the transcribed data were thematically analysed. The transcripts and field notes were continually re-read to become familiar with the data and identify relevant codes to the research questions. Transcripts were initially manually coded by one researcher (SMY), and the codes were used to generate themes and subthemes following an inductive thematic analysis approach. The analysis begins with familiarisation, generating initial codes, searching for themes, reviewing the themes, defining and naming themes, and producing the report.[27] Microsoft Excel® was used to organise the transcripts so that the initial coding process could be completed. All the transcripts were also imported and analysed into NVivo 12 Plus by SSA, a qualitative analytic software program for data storage, coding and retrieval, and text analysis.[28] Coded transcripts were subsequently compared, and areas of disagreement were resolved through regular debriefing meetings held between SMY, SSA, and SMP to verify the codes and reach a consensus. This iterative process permitted reviewers to closely examine significant issues raised in the focus groups and interviews to identify specific themes, subthemes, and subtopics. The coding guide was revised to include additional themes due to these consensus meetings. Once coded, all transcripts were merged, and frequently used codes were sorted into categories. Finally, one researcher coded and analysed all transcripts (SMY). Through a final round of selective coding, the study team reviewed significant quotes within each code category, aggregated across all transcripts, to facilitate the identification of significant themes. All study authors then met to agree on the significance of themes, subthemes, and subtopics and to select and agree upon quotations to illustrate themes. Participant quotations throughout the manuscript are italicised, some were edited for length and clarity and indicate whether the participant attended the FGD or in-depth interview along with the associated unique identifier number.

Results

The majority of South Asians who participated in the FGDs and the interviews were early immigrants (≤5 years in New Zealand), women (>60%), from India (>60%), identified as "Hindu" (>55%), had a tertiary level qualification (>90%), were employed (>80%), were from a middle to high-income household (NZ$ 20,000+; 33–60%), were aged below 35 years (61% in FGD and 80% in Interview), with a mean age of 38.3 (SDM10.9) years in the FGD and 33.6 (SDM 3.6) years in the interview (Table 1). The thematic analysis resulted in 22 barrier and enabler codes and 12 solution codes summarised under five themes, which are discussed below. Detailed illustrative quotes of the barriers and enablers of unhealthy behaviours (Suppl Table 1), and the solutions to mitigate these behaviours (Suppl Table 2) are provided as supplementary data.

Theme 1: inadequate consumption of fruits, vegetables, and lentils

The critical barriers to adequate consumption of fruit and vegetables primarily were the high cost of fruit and vegetables, lack of availability of fresh traditional vegetables, and difficulty in developing a taste for new varieties of fruit and vegetables in New Zealand.

In 9: New Zealand does have vegetables, but the price of the vegetables are (is) humongous. . . . ., like obviously we all go through a budget in a week and that is the hardest time that when we know that half cauliflower is like three or four dollars or an eggplant is like five dollars.

Participants felt accepting what was locally available and trying new fruits and vegetables was the solution to increasing fruit and vegetable consumption. This was also true for increasing lentil consumption. Lack of knowledge about cooking locally available vegetables was also identified as a barrier to adequate vegetable consumption. Substituting local vegetables in South Asian cuisine was identified as a solution with participants sharing their experiences, for example, substituting Kale for traditional greens in cooking Pulau (Pilaf).

FGDP7: we don't have (the) kind of greens here (which we had in India), tried to cook the available greens in similar way, tasted really good. Kale tasted really good, I made pulau and aroma is quite good.

Another suggested solution for increasing vegetable consumption was to use frozen or canned traditional vegetables available from local ethnic stores. However, many participants were skeptical of the nutritional quality of both frozen and

Table 1. Demographic characters of the focus group discussions (n = 21)* and in-depth interview participants (n = 10)

Demographic characteristics	Focus group discussion	Interview
Mean age (SD)	38.3 (10.9) years	33.6 (3.6) years
Sex (n (%))		
Men	8 (44)	4 (40)
Women	10 (66)	6 (60)
Country of origin (n (%))		
India	12 (67)	6 (60)
Pakistan	1 (5.5)	1 (10)
Sri Lanka	2 (11)	1 (10)
Nepal	2 (11)	1 (10)
Bangladesh	1 (5.5)	1 (10)
Religion (n (%))		
Hindu	10 (55)	7 (70)
Muslim	3 (17)	1 (10)
Christian	2 (11)	2 (20)
Buddhist/other	3 (17)	–
Education (n (%))		
Completed Tertiary	18 (100)	9 (90)
Some Tertiary	–	1 (10)
Employment (n (%))		
Employed	10 (56)	8 (80)
Not Employed	8 (44)	2 (20)
Household income per year (n (%))		
Less than $NZ20,000	3 (17)	2 (20)
$NZ20,000–50,000	6 (33)	6 (60)
More than $NZ50,000	9 (50)	2 (20)
Duration of residence in NZ (n (%))		
Early migrant (≤ 5 years)	13 (72)	9 (90)
Established migrant (>5 years)	5 (28)	1 (10)

*Demographic data was missing for n=3.

canned vegetables. The lack of variety of traditional vegetables also had a follow-on effect on less rice consumption, which was replaced by more pasta and noodle consumption, with little or no vegetables.

In 3: lack of variety is the main reason; you don't want to eat the same thing (vegetables) all week . . . if you had to get seven varieties of vegetable shares for seven days, then I would probably have eaten more rice. And if you eat rice, then we can do vegetables as well . . . . In pasta, there are no vegetables. Usually, pasta is some meat, some onions and some garlic.

Participants also suggested growing some vegetables would be a solution to overcome the high cost and lack of availability of some traditional vegetables, especially herbs and greens. However, many participants raised the issue of lack of knowledge and space for gardening as barriers to having a vegetable garden. The suggested solution to overcome this barrier was providing information on how to grow their own using containers, especially re-using common household items such as rice bags.

Theme 2: increased consumption of poultry and meat

Most participants reported that their meat intake had increased post-migration due to a change in lifestyle in New Zealand. Enablers of increased consumption of poultry and meat were affordability, taste, ease of cooking, and longer frozen life. Participants attributed the increase in meat consumption to a decrease in consumption of vegetables and lentils.

In7: I think it's changed a lot. I would say it's worse because my diet pattern back home was more of vegetarian food. Non-vegetarian was very rare. And even if it was non-vegetarian, we used to have a lot of variety of vegetables every day. But here, it's very much limited. Even considering my diet, I've started eating more non-vegetarian, and the consumption of vegetables has dramatically reduced.

Theme 3: increased consumption of dairy fats, oils and fast foods

Generally, oil consumption increased post-migration, and most participants indicated that they did not keep track of how much oil they consumed daily, weekly, or monthly. The choice of oil varieties had changed for participants after shifting to New Zealand, primarily due to cost factors or based on the perception that certain oils were healthier.

In4: Yeah, so I don't have to be too conscious that I'm using extra, you know, more oil or anything so I can use it (olive oil). Otherwise, I would feel guilty that oil is not good so I try and use extra virgin olive oil.

Consumption of coconut oil had increased significantly among those from Southern India and Sri Lanka, particularly due to recent surge in social media promotion. Participants perceived that coconut oil was healthy, although some were uncertain whether it was good for their health.

In10: I have started using coconut oil recently because it's good for you. And I've got even a big bucket of coconut oil, so I use it . . . but I still don't know if . . . I don't know if it's good.

Most participants maintained their consumption of traditional dairy foods such as ghee and yoghurt and, in addition, consumed more ice-creams and cheese.

FGD P15: I am from Punjab (India or Pakistan). So I love milk products and cut them (reduced consumption). When I started (living in New Zealand), I ate cheese a lot. I used to buy an entire block, and then I'll finish it in two weeks.

Strong belief in the health benefits of ghee was an enabler for the continued consumption of ghee. Enablers of increased ice-cream consumption, cheese and butter were the numerous varieties of ice creams, easy access and affordability, and the overall high quality of dairy products in New Zealand. Yoghurt consumption had also increased among most participants, nevertheless participants were unsure of low-fat options of yoghurt.

In 6: I generally buy a natural yoghurt, so I don't know whether it has a high or low fat.

The consumption of Indian fast-food decreased among participants after moving to New Zealand; in contrast, their consumption of other fast foods such as fish and chips, KFC, Dominoes and Chinese increased. Dietary acculturation played an essential role in the increased consumption of western fast foods such as fried chicken and pizzas, primarily due to their affordability.

In9: When you come to a new country, you think about the cost maximization and minimization. And that's the reason we focus on KFC and McDonald's because of the price, the reasonable price.

Solutions suggested by participants to reduce consumption of high-fat dairy foods and oils were educating people on the optimal portion size of dairy foods and oils, framing low-fat messages positively, providing more information on the saturated fat content of foods and general information on recommended levels of fat intake.

In3: If you say cheese is bad for me, that will give a negative effect on me. But if you say like, a one slice of cheese or a gram or whatever you need (recommended), you can say like, okay, if you eat that, that's good for health. But if you increase that, (that might not be good for your health). If you say that then that's good for me. The word (framing the message positively) used for the thing (dairy food) that we are consuming, and we feel like that is good.

Theme 4: increased consumption of cakes, biscuits, chips, and snacks

Most participants reported that they had increased their consumption of cakes and biscuits in New Zealand more as a substitute for traditional sweets. Dietary acculturation increased consumption of cakes, biscuits, chips, and snacks. Increased accessibility and affordability also enabled increased consumption of cakes, chocolate biscuits, butter cookies, and chips. Some participants reported regular consumption of salty fried foods such as crisps/potato chips, with some describing these as comfort foods.

In 9: Yeah, because I couldn't get used to this country's food habits and didn't know what to do. So, those (chips) were my comfort foods to make me feel happy and good, but then I realised that that was increasing my weight.

Many participants were unaware of the health impact of consuming deep-fried and highly salted snacks regularly and did not read the label for nutritional information. Those participants who read the nutrition labels did so to know if the product contained animal fats (vegetarians), was Halal (Muslims), or contained preservatives.

FGD P9: So, when I look at nutrition labels, it was more of what I would be concerned about the preservatives .... everything that's happening to us, our health and environmental issues, is because of all these preservatives which are going in our body.

Solutions identified by participants to address excessive consumption of high-fat, high-sugar, and salty snacks were to increase awareness and knowledge on reading and interpreting nutritional labels on packed foods and optimal portion sizes.

FGDP4: yeah that kind of information (nutrition labels) is really important. Especially because people from India for example, based on my experience, we get everything from family and our mother would take care of us. So we do not know about nutrients and what a serving should look like.

Theme 5: lack of adequate physical activity

Many participants felt that a lack of physical activity among South Asian immigrants was an important issue to be addressed. Although participants were aware of the importance of staying active, they felt that they were not being active enough. Lack of knowledge of the benefits of physical activity was not a barrier to being physically active in New Zealand. However, the change in the living conditions in New Zealand was a barrier.

In7: Yes, I know about that (activity and health), but where will I fit it? I was, I would say, more physically active because I used to walk to the bus stop and even travel by bus. We used to do physical activity (back home). There was more walking and climbing and stuff like that. But here, lazy lifestyle for me.

Key barriers faced were frequent cold and wet weather, time constraints due to prioritising work and family responsibilities, lack of peer support, cultural barriers, and the high cost of organised sports.

In6: My major concern here, only one thing I find it is the cold weather, which is I'm still getting adjusted to the weather. So, as it's very cold, I try my best not to step out walking.

Participants indicated that having peer support would encourage them to be regularly active via walking, trekking, cultural activities such as dancing, and knowledge about community sports and walking tracks. Preferred activities included walking, cultural dancing, and community sports like cricket, football and tennis.

In 4: I want company. I think that's my problem. I think (walking) anywhere is fine as long as I have some company ....Put together a walking group or something like that. you know, I mean having our community would be somewhat better because we can just enjoy more fun talks (as we walk).

Discussion

In this paper, we discuss in detail the barriers and enablers of unhealthy dietary and physical activity behaviours in New Zealand South Asians who have a high risk for diet-NCDs such as diabetes. One risk factor for diet-NCDs consistently reported in national and international studies is poor fruit and vegetable intake among South Asians living in Western countries.[1,3,8,10] Hence, understanding the modifiable factors to improve fruit and vegetable intake is critical to reduce the burden of diet-NCDs in this population. The finding that the high cost of fruit and vegetables in New Zealand was a deterrent to adequate consumption was similar to an Australian study on the health behaviours of South Asian immigrants[29] and is likely to impact similarly for other population groups in New Zealand and elsewhere. However, key modifiable barriers identified for adequate fruit and vegetable consumption specifically among New Zealand South Asians in the current study were the poor availability of fresh traditional vegetables, lack of knowledge about cooking locally available vegetables in South Asian cuisine, and skepticism on the nutritional quality of frozen and canned vegetables and lentils. Among other factors, such as affordability, increased meat and poultry consumption was reported as a replacement for the lack of traditional vegetables. In a study of Pakistani migrant women in Oslo, Norway, increased affordability of meat and poultry resulted in daily meat consumption in contrast to eating meat once or twice a week while they lived in Pakistan.[30,31]

In the current study, South Asians were more concerned about the quality of oil rather than the quantity of oil consumed, and overall oil consumption and dairy fat consumption increased post-migration. The finding that fat consumption increased post-migration is consistent with other studies on South Asians in Europe[30,31] and North America.[32] The current study indicates that the increase in total fat consumption was primarily due to the consumption of ice cream and cheese in addition to traditional fat sources. In the Oslo study, Pakistani South Asians increased their oil intake, whereas Sri Lankan South Asians increased their butter and oil consumption.[30,31] A previous New Zealand study also reported increased fast food (takeaway) consumption among South Asian males.[1] The current study indicates that the types of fast food consumed by South Asians, which were predominantly high-fat options such as Kentucky Fried Chicken, Pizzas, and deep-fried fish and chips, are significant contributors to the increased fat consumption observed among South Asian immigrants in Western countries. Similarly, the increased consumption of

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cakes, biscuits, potato chips, and other fried snacks observed in the current study was primarily to replace traditional sweets and snacks. In the MASALA study, those with strong cultural beliefs were more likely to consume a diet high in fried snacks, sweets, and high-fat dairy.[32] The findings of the current study, for example, "ghee" was considered healthy, the excessive use of coconut oil and that participants were not concerned about the quantity of oil or the fat content in yoghurt, all resonate with the findings of the MASALA study, which reported a strong association between cultural beliefs and high-fat consumption.

The co-design methodology adopted in the current study enabled the generation of solutions as intervention components to address unhealthy dietary and physical activity behaviours. The participants identified several solutions to address detrimental dietary behaviours. One of the solutions generated for improving vegetable consumption was providing tips and techniques for home gardening. Home gardening has been shown to be perceived as an effective measure to increase consumption of fresh home grown produce, reduce fast-food consumption, increase motivation to be healthy, increase physical activity, mental health, and the ability to better manage stress by an ethnic minority population of low-socioeconomic status in the US.[33] Similar associations have been shown between fruit and vegetable consumption and being involved in community gardening.[34] Community gardeners significantly increased their intake of total vegetables by 0.63 servings ($P = 0.047$) and garden vegetables by 0.67 servings ($P = 0.02$) in a randomised controlled trial in the US.[34] Recipes to substitute locally available vegetables in South Asian cuisine were another solution generated for increasing vegetable consumption. Demonstration of recipes via culinary instruction maybe one method of achieving this. Community-based nutrition education that included culinary instruction has shown promise in changing nutrition behaviours and improving cardiometabolic biomarkers in a sample of low socio-economic ethnic minority participants.[35] Educating people on the optimal portion size of dairy foods and oils, framing low-fat messages positively, providing more information on the saturated fat content of foods, and general information on recommended levels of fat intake were solutions suggested for reducing consumption of high-fat dairy foods and oils. Solutions identified by participants to address excessive consumption of high-fat, high sugar, and salty foods were to increase awareness and knowledge on optimal portion sizes and read and interpret nutritional labels on packaged foods. Studies have demonstrated that, if empowered with the correct knowledge regarding diet, South Asians can bypass cultural obligations and fulfil healthier choices, highlighting the need for greater education in the community.[36–40] Several reviews have indicated that culturally adapted or tailored interventions have the potential to improve nutrition-related outcomes including reduced portion sizes and saturated fat.[41,42] These analyses suggests that co-creation practices hold potential for tailoring nutrition interventions in collaboration with Indigenous and ethnic minority populations.

Increasing physical activity was considered necessary for South Asians, albeit a lack of knowledge of the health benefits of physical activity was not a barrier among the South Asian participants of the current study. A review of healthy lifestyle changes in minority ethnic populations in the UK found that knowledge about the positive impact of physical activity on health was poor among South Asian communities in the studies reviewed.[43,44] Previous studies among South Asian participants showed that whilst they were generally aware of the health benefits associated with physical activity,[40,45–47] there was limited understanding of the actual levels of physical activity required to gain health benefits.[40,45,47] Studies on South Asians have indicated that the barriers to increased physical activity include prioritising work over physical activity to provide for the family[29,43,44,48,49] the need to serve and eat traditional foods, and the different perceptions of healthy body weight.[44] In the current study, in addition to prioritising work over allocating time for physical activity, other barriers included the changed living conditions post-migration, cold weather, and the cost of organised sports. These findings are similar to those reported by a U.S. study, where significant barriers to adequate physical activity among South Asians included changes in post-migration living conditions, the cold and wet weather, and the financial cost of being engaged in organised sporting events.[50] Other studies have also identified cultural barriers[29,43,50] and cultural beliefs[29] on physical activity to be significant barriers to adequate physical activity among the South Asian diaspora.

A key solution suggested by participants for increasing physical activity was peer support. Peer-led interventions to increase physical activity levels have been shown to be as effective as those led by professional physical activity providers across several population groups.[51] Participants felt that having peer support or doing physical activity with other South Asians would encourage them to be regularly active. Kalavar *et al.* found that lack of peer support was a key barrier to increasing physical activity among South Asians in the U.S.[48] The finding from the current study resonates well with other studies investigating enablers of physical activity in South Asians. Nisar *et al.* found peer modelling was a motivating enabler for walking, cycling, and group sports activities.[29] Other studies have found that group physical activity sessions had health and social benefits for South Asians.[42] A meta-analysis of the effectiveness of interventions to promote physical activity in the general population has found group delivered interventions to be more effective in changing behaviour compared to individually delivered interventions.[52] More specifically, walking in groups was found to be efficacious at increasing physical activity across different populations in different countries.[53] Preferred activities identified in the current study for increasing physical activity were walking, cultural dancing, and community sports like cricket, football, and tennis, which are all group based. A feasibility study among South Asian women in the Netherlands has shown some promising results in increasing physical activity levels by implementing cultural dancing (Bollywood) as a form of physical activity.[54] A study among South Asians in Scotland found that physical activity that provided a platform for socialising and enjoyment was a key motivator for engaging in physical activity.[46]

The current study is unique in identifying participant generated ethnic-specific intervention components to address established risk factors of diet-NCDs. The generated solutions have the potential to develop a multicomponent intervention to reduce diet-NCDs in South Asians. Multicomponent interventions have been shown to have a higher level of effectiveness in achieving the targeted behaviours.[55] The key strengths of the current study were the strong community buy-in, which resulted in a high level of participation by the South Asian community as stakeholders and participants. SP, SA, and SMY are all South Asian, are multilingual, and have extensive connections with the South Asian community in New Zealand of various faiths and socio-economic backgrounds. Hence, common limitations such as cultural sensitivity and language barriers did not hinder recruitment, data collection, or analysis. Although the FGDs and interviews were all conducted in English, most participants expressed themselves in their mother tongue frequently during the FGDs and interviews. An inherent limitation of the current study is the generalisability of the findings to the broader South Asian communities in New Zealand and elsewhere. The participants of the current study were highly educated; hence, the findings of this study may not be the same for South Asian immigrants with low education levels. This was evident in the data regarding knowledge about the benefits of physical activity on health, which was high among the participants of this study. Nevertheless, this knowledge did not necessarily translate into behaviour, and the barriers to adequate physical activity were similar to those found in other studies.[26,40,41,45,46] Moreover, the depth of enquiry that was undertaken generated information broadly transferable to the South Asian diaspora worldwide to enable a targeted health promotion initiative for prolonging the healthy migrant status of South Asians and reducing the burden of diet-related chronic diseases in this population. Currently, the evidence for lifestyle interventions developed using non co-design methods to reduce the risk of diet-NCDs in South Asians is at the best, moderate.[56] The question whether interventions developed using co-design are more effective in reducing the risk of diet-NCDs in the South Asian diaspora is yet to be answered. The next phase of this project is to develop a co-designed, multicomponent, health promoting intervention incorporating the intervention components from this study and test its effectiveness in promoting healthier diet and physical activity levels in New Zealand South Asians.

Conclusions

The current study identified key modifiable barriers for low consumption of vegetables and fruits, high consumption of fats, unhealthy takeaway foods, and inadequate physical activity among South Asian immigrants in New Zealand. The identified solutions to mitigate these barriers were providing information on simple home gardening techniques, the nutritional quality of frozen vegetables and canned lentils, recipes to substitute local vegetables in South Asian cuisine, educating people on the optimal portion size of dairy foods and oils, framing low-fat messages positively, providing more information on the saturated fat content of foods, knowledge on optimal portion sizes and reading, and interpreting nutritional labels on packaged foods. Similarly, group-based activities with peer support such as walking, cultural dancing and community sports like cricket, football, and tennis were the identified solutions to increase physical activity levels in South Asians. The identified solutions for health promoting dietary habits and physical activity levels contribute to creating a multicomponent intervention that is achievable and can be part of any targeted health promoting programmes to reduce the risk of diet-NCDs in South Asian immigrants.

Abbreviations

diet-NCDs: diet-related non-communicable diseases; **FGDs:** focus group discussions; **DoR:** duration of residence; BMI: body mass index; **MASALA:** Metabolic Syndrome and Atherosclerosis in South Asians Living in America Study.

Supplementary material

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Conflict of Interest

Dr Parackal, Dr Akthar, Mr Boyina, and Prof Brown declare no conflicts of interest.

Ethical Standards Disclosure

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants signed an approved consent form prior to participation. Ethics approval was obtained from the University of Otago Human Ethics Committee (18/205; dated 14/12/2018).

Author contributions

S.M.P. developed the concept, obtained funding, developed the data collection tools, collected data, contributed to the analyses and interpretation of data, led the writing of the manuscript; S.S.A contributed to the coding and analysis of the data and writing of the methods and discussion; S.Y.B. contributed to the thematic analysis of the data and editing of the manuscript; R.B contributed to developing the concept, obtaining funding, data interpretation, writing the discussion, and overall editing of the manuscript. All authors approved the final manuscript.

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RESEARCH ARTICLE

Workers' healthy eating practices during the COVID-19 pandemic and their relationship with physical activity and quality of life

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Abstract

The lifestyle of the population has undergone significant changes due to the COVID-19 pandemic, which could have influenced alterations in dietary habits and overall well-being among workers. This study aimed to evaluate healthy eating practices and their relationship with the workers' quality of life and physical activity during the COVID-19 pandemic. This was a cross-sectional investigation involving workers in the city of Curitiba, southern Brazil. The study was conducted through the application of an online questionnaire. The data were evaluated using non-parametric tests and fitting a logistic regression model. A total of 123 workers participated in the study, most of them male (53.2%), aged between 31 and 40 years (42.2%), with a predominance of workers with postgraduate degrees (62.6%, $n = 77$), and the majority of workers (68.2%, $n = 84$) were performing their professional activities remotely for at least one day during the week, and 73.2% ($n = 90$). It was observed that 52.8% had excellent healthy eating practices, and the older their age and the greater the practice of physical activity (time and frequency), the better the workers' healthy eating practices. When assessing quality of life, the lowest average score for healthy eating practices was in the domain of social relationships. A direct relationship of older age, social relationships, and the practice of physical activity with the best individuals' healthy eating practices was detected. Considering that remote work continues to be adopted post-pandemic, evaluating the dietary practices, physical activity, and quality of life of workers is necessary to understand this new labour phenomenon.

Key words: **Coronavirus infections: Eating behaviour: Food guides: Health promotion: Nutritional status: Worker's health**

Introduction

In March 2020, the World Health Organization (WHO) declared a global pandemic due to the new coronavirus (Sars-Cov-2) that causes the COVID-19.[1] By the end of January 2022, more than 356,955,803 cases of COVID-19 and 5,610,291 deaths had been confirmed worldwide.[2] In Brazil, the number of confirmed COVID-19 cases exceeds 24 million, with more than 623 thousand deaths during the same period.[2]

During the pandemic, measures such as movement restrictions and lockdowns negatively impacted eating habits and physical activity, leading to negative psychological impacts.[3] Public health measures such as quarantines and social distancing were implemented to contain the virus spread, drastically altering our lifestyle and affecting our physical and mental health.[3,4]

It is noteworthy that the main risk factors of COVID-19 are associated with the presence of comorbidities such as Chronic Non-Communicable Diseases (NCDs), which can increase the probability of death, especially in vulnerable groups of all ages.[5]

Thus, other important health factors must be addressed, such as diet and physical activity,[6] which may have changed in connection with the new population's life framework. Thus, the world has experienced two health conditions, coronavirus infections and NCDS increase, and COVID-19 is no longer just a pandemic but is part of a syndemic, that is, a synergy of epidemics.[7]

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This change in the lifestyle of the population is partly, due to the need for measures to contain the spread of COVID-19, such as social isolation. This has proven to be effective[8] and has led many people to perform their work remotely.[9] This mobility restriction has had direct effects on psychological factors, such as an increase in cases of anxiety and depression and a reduction in the practice of physical activities.[10–12] In addition, eating habits were also influenced both by economic factors, due to the reduction in the population's income, as well as by the consumption of foods with higher energy density.[13,14]

In the period before the pandemic, the consumption of fresh and minimally processed foods represented approximately 70% of the total caloric intake by the Brazilian population, and ultra-processed foods contributed to one-fifth of the calories consumed.[15] Such studies enhance the need for attention to dietary practices, as the consumption of ultra-processed foods tends to have a greater energy density, greater amounts of saturated and *trans* fat, sodium, sugar, and less fibres, which suggests an increased risk of developing obesity and other chronic diseases related to food intake.[16]

Changes in eating habits were also observed during the COVID-19 pandemic. Staying at home has also led to the development of unbalanced eating habits The COVID-19 pandemic has resulted in a sedentary life for people under lockdowns and excessive food consumption due to spending much time at home.[17] Due to confinement, some individuals increased the number of meals eaten throughout the day and consumed more snacks between meals.[18] Additionally, an increase in the consumption of ultra-processed products was also observed among people with lower education.[19] On the other hand, other studies did not show changes in the eating patterns of individuals during the pandemic.[10,13]

In addition, some individuals improved their diet during this period,[13,20] with a greater consumption of vegetables, fruits, beans, and other legumes.[19] One of the factors that may be associated with this diet improvement is the fear of health problems and the need to stay healthier to face COVID-19.[13] It is also worth noting that due to the pandemic and social isolation measures,[1] food consumption at home increased. This fact, as well as having meals with the family, may have resulted in a positive association to improve the nutritional quality of these meals.[16]

Social isolation measures also cause conditions that favour physical inactivity and sedentary behaviour.[18] Yet, the maintenance or improvement of physical activity levels became essential during the pandemic, as it maintains the physiological functions that help to reduce the physical and mental consequences of COVID-19,[11,21] besides contributing to the reduction of anxiety symptoms, improving sleep and the immune system stimulation.[12] For example, Taheri *et al.*[3] found that increased physical activity during lockdowns was linked to reduced rates of depression, likely because of the beneficial effects of vigorous exercise. The research underscored a link between emotional eating and mood disorders like depression, anxiety, and stress, particularly among elite athletes with decreased physical activity. Emotional eating, prompted by negative emotions such as stress and loneliness, can impact dietary decisions, and vice versa.

Although some studies have assessed the diet[10,12,13,18,19] and some isolated factors such as physical activity[11,12,20] and the individuals' psychological factors[10,21] during the pandemic, little is known about the association between eating habits and health-promoting actions adopted by people during this period, especially by those who work remotely. Therefore, this study aimed to evaluate the workers' healthy eating practices and verify their relationship with the quality of life and physical activity of these individuals during the COVID-19 pandemic.

Materials and methods

Type of study, sample design and ethics committee

Exploratory, quantitative, descriptive, and cross-sectional study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Research Ethics Committee of the Federal University of Paraná (CAAE Nº. 98205318.2.0000.0102). Written informed consent was obtained from all subjects.

The study was conducted online with adult workers (≥ 18 years old) who were allowed to have meals at the Food and Nutrition Units (FNU) of two companies accredited to the Worker's Food Program (WFP), located in the city of Curitiba, Brazil, which were selected for convenience.

Data collection

Data collection was carried out during November and December 2020, when the number of COVID-19 cases in Brazil approached 7 million, and deaths reached 200,000, with a daily average of 35,000 new cases.[22]

The survey was carried out through an online questionnaire available on the Google Forms® platform that investigated the workers' description, healthy eating practices,[23,24] physical activity practice,[25] and quality of life.[26] Information regarding the investigation was distributed by e-mail or through telephone contact with the workers. The invited workers were informed about the objectives of the study, voluntary and unpaid participation, preservation of identity, and harmless refusal and/or withdrawal. After reading and agreeing to participate in the survey, the workers answered the questions.

Workers' description. In this section, workers answered questions about age, gender, education, income, eating frequency at the company's restaurant (FNU), pre-existing medical conditions, consumption of alcoholic beverages and days worked remotely (home office). Information on weight and height were self-reported and the measurements were used to calculate the Body Mass Index (BMI).[27] These variables were structured in the online questionnaire by researchers.

Healthy eating practices. The assessment of healthy eating practices was carried out through a questionnaire that is based on the recommendations of the Dietary Guidelines for the Brazilian Population (DGBP)[23] and considers the current healthy eating practices of individuals.[24] This questionnaire validated consists of 24 questions divided into four domains:

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planning (9 questions), domestic organisation (3 questions), eating modes (5 questions), and food choices (7 questions). The answers to the questions were distributed on a psychometric scale of four options, namely: strongly disagree, disagree, agree, and strongly agree. These answers compose a score from 0 to 72 points and classify the respondents according to their healthy eating practices; the individuals who attained 41–72 points were classified as 'Excellent'; 'Keep going' for those attaining between 31 and 40 points and 'Attention' for those who scored 0–30 points.[16,24] The questionnaire assesses current food consumption.

Physical activity practice. The level of physical activity practice was measured through the International Physical Activity Questionnaires (IPAQ) short version validated, consisting of eight questions that assess the frequency, intensity, and duration of physical activities reported in the last seven days mat.[25] These activities were divided, according to their intensity, into three categories: walking, moderate physical activity, and vigorous physical activity. After answering the questionnaire, the participants were classified, according to their physical activity practice, as sedentary, irregularly active, active, and very active.[26] Additionally, the frequency and time spent in the practice of physical activity were also considered alone to estimate their association with healthy eating practices.

Quality of life. To assess the quality of life, the WHO Quality of Life (WHOQOL-bref) was used, which has 26 questions, consisting of four domains: physical (7 questions), psychological (6 questions), social relations (3 questions), and environment (8 questions).[28] This is a questionnaire validated. In addition, two more questions were evaluated separately, which consisted of the individual's general perception of quality of life and health.[28] For the assessment, the last two weeks before filling out the questionnaire were taken as a reference. The participants answered the questions according to a psychometric scale of five responses: 'very good', 'good', 'not bad nor good', 'bad', and 'very bad'. When answering the questions, a score was generated according to the domains for each individual.[29]

Data analysis

Data analysis was performed using the Statistica software version 7. To describe the variables observed, descriptive and frequency measures were calculated. Then, to compare the categories of the characterisation variables in relation to the index of healthy eating practices, the Kruskal-Wallis Test was applied, complemented by the Minimum Significant Difference (MSD) test. When the characterisation variable was dichotomous, the Mann-Whitney test was applied. To assess the association between the healthy eating practices index and other quantitative indices, the Spearman's coefficient was calculated. The same analysis was performed with the classification of healthy eating practices by applying the Chi-square test, when the other variable was categorical, or the Kruskal Wallis test when the variable was quantitative. The lack of normality of the data was confirmed through the Shapiro-Wilk test, which justifies the use of non-parametric tests. To adjust a model that explains the classification of healthy eating practices, logistic regression was used and the Odds Ratio (OR) was identified for the significant variables considering workers' description, the physical activity practice and the quality of life. Regarding the model's codes 0 and 1, and 1 represented the excellent level and 0 represented the attention level. The significance limit was considered to be $P < 0.05$.

Results

A total of 123 workers participated in the study; most of them were men (53.7%, $n = 66$). There was a predominance of workers with undergraduate and graduate degrees (93.5%, $n = 115$) and 43.1% ($n = 53$) their monthly income was between 2 and 5 minimum wages. In addition, more than half of the workers (52.9%, $n = 65$) were carrying out their professional activities remotely for five or more days during the week, and less than 27.0% ($n = 33$) reported having had meals at the company's cafeteria during the previous month (Table 1).

Most participants (71.5%, $n = 88$) stated they did not have preexisting ailments. The main pre-existing diseases reported, were dyslipidemia (8.1%, $n = 10$), hypertension (7.3%, $n = 9$), and diabetes (3.2%, $n = 4$) (Table 1). Regarding the consumption of alcoholic beverages, almost 61.0% ($n = 75$) of the workers stated that they had drinks at least one day a week (Table 1).

Considering the nutritional status, 65.0% ($n = 80$) of the workers were overweight (overweight or obese) (Table 1). The Kruskal-Wallis test showed a significant difference between the classifications of nutritional status with the mean score referring to the workers' healthy eating ($P = 0.03$). However, the MSD test did not confirm this difference. There was also a statistical difference between the age group ($P = 0.01$) in connection with the workers' healthy eating practices score. Individuals with and over 41 years of age had an average score of healthy eating practices significantly higher than the group least 41 years of age (Table 1).

Regarding healthy eating practices, in general, the mean score obtained by the total number of participants was 41.0 ± 9.9 (Table 1), and 52.8% ($n = 65$) of the workers had healthy eating practices classified as 'Excellent', which represents healthy eating in different aspects according to the DGBP. Then, 33.3% ($n = 41$) had their practices classified as 'Keep going', which are those that need to make adjustments in their diet but are moving towards healthy eating. The others had their healthy eating practices classified as 'Attention' (13.7%, $n = 17$) and received the lowest score, indicating the need to foster greater changes to make their diet healthier and more adequate to the DGBP recommendations (Table 1).

In the distribution of the responses to the healthy eating practices questionnaire with the separation by domains (Fig. 1), it was found that in the domain referring to 'Food choices' (Fig. 1a), most participants used to eat candies, chocolates, and others sweets (55.3% agreed), but they were not in the habit of using sugar to sweeten their drinks (54.5% strongly disagreed). In addition, soft drink consumption and the switch of meals for snacks were less common among the participants.

Regarding the 'Eating modes' domain (Fig. 1b), the question regarding the practice of having meals sitting at the table was the one that showed the highest adherence, with 83.0% of the

Table 1. Comparison between the characterisation of workers with the score and categories of healthy eating practices ($n = 123$)

	Total		Healthy eating practices								
					Classification						
			Score	*P* value	Attention		Keep going		Excellent		*P* value
	%	*n*	Mean ± SD		%	*n*	%	*n*	%	*n*	
General	100	123	41.0 ± 9.9		13.7	17	33.3	41	52.8	65	
Gender				0.58							0.52
Female	46.3	57	40.2 ± 11.6		15.8	9	36.8	21	47.4	27	
Male	53.7	66	41.6 ± 8.4		12.1	8	30.3	20	57.6	38	
Income				0.15							0.64
1–2 minimum wages	12.2	15	36.7 ± 9.1		20.0	3	40.0	6	40.0	6	
2–5 minimum wages	43.1	53	39.5 ± 10.7		17.0	9	37.7	20	45.3	24	
5–10 minimum wages	28.5	35	42.8 ± 9.2		14.3	5	28.6	10	57.1	20	
Above 10 minimum wages	8.1	10	43.9 ± 7.5		0.0	0	30.0	3	70.0	7	
Did not answer	8.1	10	46.6 ± 10.4		0.0	0	20.0	2	80.0	8	
Education				0.20							0.17
Graduation	30.9	38	39.2 ± 10.9		23.7	9	26.3	10	50.0	19	
Postgraduation	62.6	77	42.3 ± 9.7		9.1	7	35.1	27	55.8	43	
Technical/High School	6.5	8	37.8 ± 6.1		12.5	1	50.0	4	37.5	3	
Group Age				0.01*							0.01*
20–30 years	29.3	36	38.9 ± 9.6[b]		13.9	5	47.2	17	38.9	14	
31–40 years	42.2	52	40.3 ± 10.8[b]		21.2	11	32.7	17	46.2	24	
41–50 years	12.2	15	41.1 ± 9.0[ab]		6.7	1	26.7	4	66.7	10	
Above 50 years	16.3	20	46.7 ± 7.0[a]		0.0	0	15.0	3	85.0	17	
Nutritional status											
Low weight	1.6	2	55.6 ± 0.7[a]		0.0	0	0.0	0	100.0	2	
Eutrophic	33.3	41	42.2 ± 10.1[a]	0.03*	14.6	6	24.4	10	60.9	25	0.10
Overweight	45.5	56	41.6 ± 9.1[a]		8.9	5	39.3	22	51.8	29	
Obese	19.5	24	36.4 ± 10.5[a]		25.0	6	37.5	9	37.5	9	
Alcohol consumption				0,05							0,24
No consumption	39.0	48	38.6 ±10.0		18.7	9	37.5	18	43.7	21	
1–2 d a week	51.2	63	42.5±10.0		11.1	7	28.5	18	60.3	38	
3–4 d a week	8.1	10	39.3±10.5		0.0	0	40.0	4	60.0	6	
5–6 d a week	1.6	2	30.0 ±12.3		50.0	1	50.0	1	0.0	0	
Meals at the workplace											0.65
None	73.2	90	40.5 ± 9.9		14.4	13	36.6	33	48.8	44	
1–2 d a week	13.0	16	40.7 ± 10.0		18.7	3	25.0	4	56.3	9	
3–4 d a week	4.1	5	40.2 ± 10.6	0.19	20.0	1	20.0	1	60.0	3	
5–6 d a week	7.3	9	43.1 ± 9.6		0.0	0	33.3	3	66.6	6	
Every day	2.4	3	53.3 ± 10.9		0.0	0	0.0	0	100.0	3	
Home office/ Remote work											0.13
None	31.7	39	41.9 ± 10.2		10.2	4	30.7	12	58.9	23	
1–4 d	15.5	19	38.4 ± 10.1	0.44	31.5	6	21.0	4	47.4	9	
5–7 d	52.9	65	41.3 ± 10.3		10.8	7	38.4	25	50.7	33	
Pre-existing diseases											
Reported some illness	28.5	35	40.8 ± 9.9	0.98	20.0	7	25.7	9	54.2	19	0.33
Did not report any illness	71.5	88	41.1 ± 10.1		11.3	10	36.7	32	52.2	46	
Diseases reported											
Diabetes	3.2	4	42.0 ± 11.6	0.87	0.0	0	50	2	50.0	2	0.63
Hypertension	7.3	9	35.5 ± 10.3	0.08	33.3	3	22.2	2	44.4	4	0.20
Dyslipidemia	8.1	10	43.7 ± 10.5	0.47	20.0	2	20.0	2	60.0	6	0.61
Other (anaemia. gastritis. hypothyroidism. etc)	12.2	17	41.8 ± 9.9	0.48	29.4	5	17.6	3	52.9	9	0.99
Physical activity											
Sedentary	13.0	16	33.8 ± 10.4[b]		25.0	4	50.0	8	25.0	4	0.00*
Irregularly active	30.8	38	38.1 ± 10.0[b]	0.00*	23.7	9	36.8	14	39.5	15	
Active	29.2	36	43.0 ± 10.0[a]		11.1	4	27.8	10	61.1	22	
Very active	26.8	33	45.6 ± 10.0[a]		0	0	27.3	9	72.7	24	

Note: Food practice classification- Attention: 0–30 points. Keep going: 31–40 points. Excellent: above 41 points.
$P < 0.05$* with statistical significance.
Differences not evidenced by MSD test; For the association of the variables regarding the score, Spearman's coefficient was applied and the Chi-square test for classification; a, b: Different letters indicate the statistical difference.

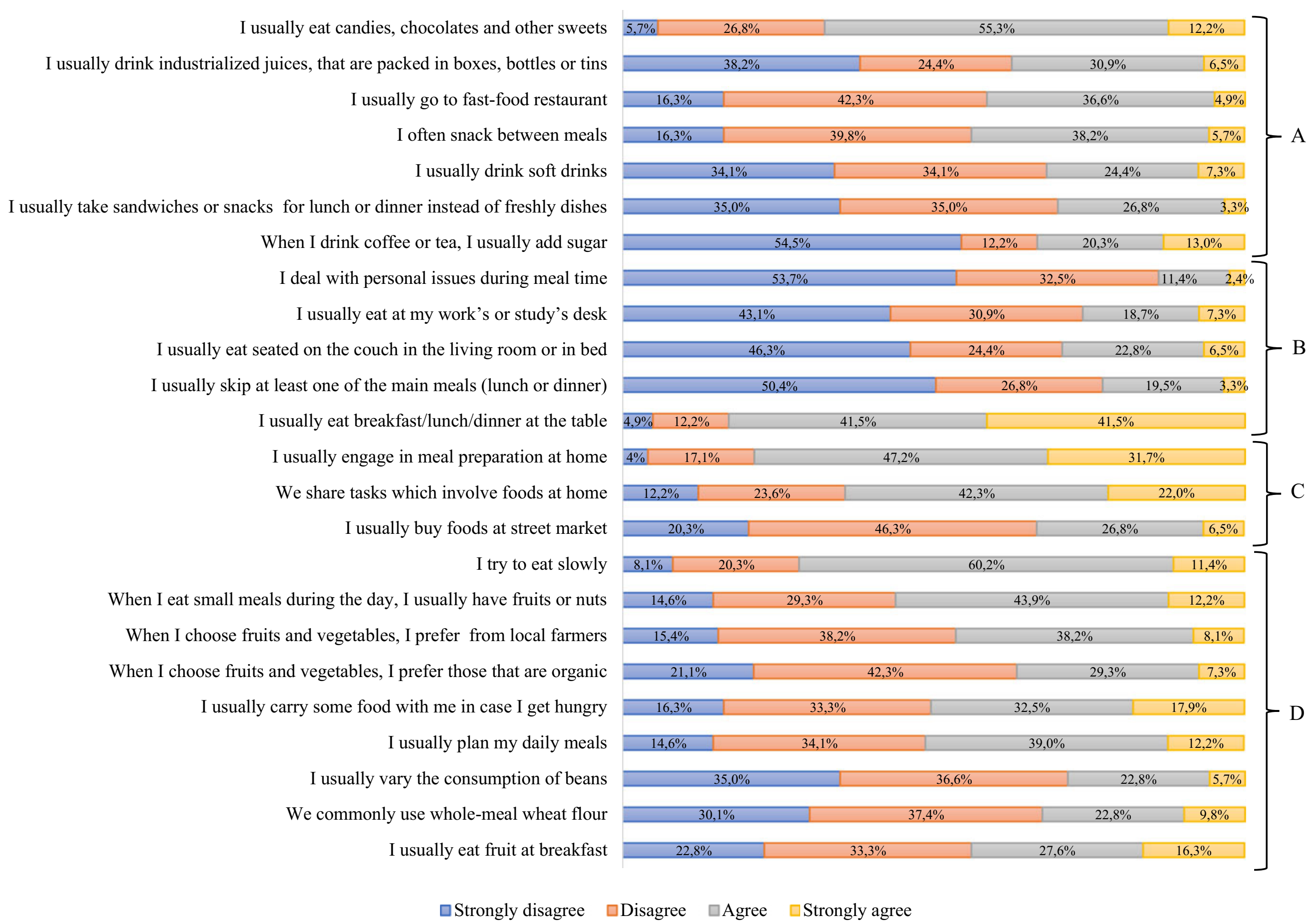

Fig. 1. Distribution by percentage of workers' responses to the questionnaire on healthy eating practices by domains (n=123).

participants reporting this practice. Furthermore, the practice of skipping main meals and taking advantage of mealtimes to deal with other things was little practiced among workers, with the responses 'disagree' (26.8%) and 'strongly disagree' (50.4%) standing out.

As for the domain 'Domestic organization' (Fig. 1c), the item with the highest agreement referred to the preparation of food at home, for which the answers 'agree' (47.2%) and 'strongly agree' (31.7%) were more prominent. The sharing of tasks involving the preparation of food at home was also frequent among the participants (42.3% agreed). In contrast, the purchase of food in street markets was the item with lower adherence, where most responses were 'disagree' (46.3%) and 'strongly disagree' (20.3%) for this practice.

Regarding the domain 'Planning' domain (Fig. 1d), it was found that the habit of varying the pulses in the diet and the consumption of whole wheat flour occurs seldom, as demonstrated by the greater number of 'disagree' (36.6%) and 'strongly disagree' (30.1%) answers. The purchase of organic products also showed low adherence, with 42.3% disagreeing and 21.1% strongly disagreeing with the statement about this practice. On the other hand, as positive points of this domain, most workers used to take meals calmly (60.2% agreed; 11.4% strongly agreed) and consumed fruits and nuts as snacks (43.9% agreed and 12.2% strongly agreed).

When the level of physical activity practiced by the workers was evaluated, most were irregularly active (30.8%, $n = 38$) and active (29.2%, $n = 36$) (Table 1). According to Spearman's association coefficient, a significant relationship was identified ($P < 0.00$), demonstrating that the more active the individual, the better their healthy eating practices (Table 1).

The median time spent on physical activities was 190 minutes a week, with a median frequency of 6 times a week. A strong association was found between the classification of healthy eating practices with the frequency of physical activity (Fig. 2a) and with the time spent with the practice of physical activity (Fig. 2b).

When assessing the quality of life of workers, the domain with the highest mean was physical (15.7 ± 2.3), followed by psychological (14.3 ± 2.3) and the one with the lowest mean was social relationships (13.9 ± 2.9) (Table 2). The self-assessment of quality of life had a mean of 14.0 ± 3.6.

When the assessment of the quality of life was related to healthy eating practices, there was a significant association between the categories of healthy eating practices and the physical ($P = 0.03$), psychological ($P = 0.00$), and environment ($P = 0,00$). Self-assessment of quality of life is also associated ($P = 0.00$) with healthy eating practices (Table 2).

To identify the strength of the association between the study variables and the classification of workers regarding healthy eating practices, the logistic regression model was applied. The

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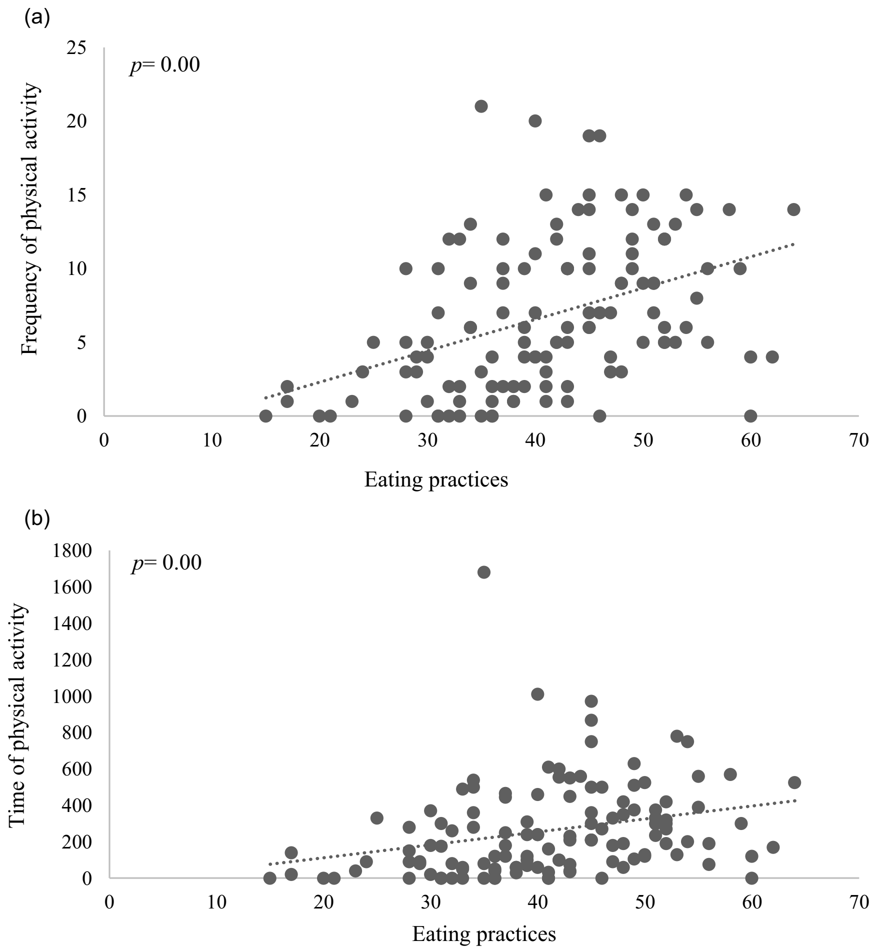

Fig. 2. Association between healthy eating practices and physical activity. Note: Statistical significance was accepted as p<0.05 for the association between healthy eating practices and time and frequency of Physical Activity.

Table 2. Comparison between the categories of healthy eating practices in relation to the scores of the quality of life domains

Quality of Life Domains	Attention (*n* = 17) Mean ± SD	Keep going (*n* = 41) Mean ± SD	Excellent (*n* = 65) Mean ± SD	P value	General (*n* = 123) Mean ± SD
Physical	14.70 ± 2.7	15.30 ± 2.4	16.3 ± 1.9	0.03*	15.70 ± 2.3
Psychological	13.00 ± 2.1	13.70 ± 2.5	15.0 ± 2.1	0.00*	14.30 ± 2.3
Social relationships	13.60 ± 3.8	13.30 ± 2.9	14.5 ± 2.5	0.20	13.90 ± 2.9
Environment	13.50 ±1.7	14.26 ± 1.8	15.3 ± 1.8	0.00*	14.70 ± 1.9
QOL self-assessment	11.41 ± 4.2	13.56 ± 3.5	14.9 ± 3.1	0.00*	14.00 ± 3.6

Note: SD = Standard deviation. QOL = Quality of Life. *Statistical significance was accepted as P < 0.05 for the association between QOL domains and the classification of healthy eating practices.

significant variables for the logistic regression model were age, physical activity frequency, and home office work (Table 3). However, no evidence was found when verifying the differences between the home office categories.

When the logistic regression model was applied, two variables were significant in influencing the classification of healthy eating practices at the excellent level (Table 3). The first variable was age, where those aged above the sample mean (44.8 ± 11.2 years) are 2.11 times more likely to achieve an excellent classification in healthy eating practices, compared to those with a lower age (Table 3). The second variable that showed more significance was the social relationships domain of quality of life; in this case, respondents with the highest score in the domain (13.9 ± 2.9) (Table 2) are 1,88 times more likely to achieve an excellent rating in healthy eating practices, in relation to those with lower scores in this domain (Table 3).

Discussion

The study evaluated the workers' healthy eating practices and their relationship with the quality of life and physical activity during the COVID-19 pandemic. Workers with and over 41

Table 3. Logistic regression for the classification of healthy eating practices and study variables

Variables	Classification of healthy eating practices		'Excellent' Rating		
	Chi square	P value	Estimate	P value	OR
Intercept	0.0	–	–0.043	0.965	
Age	11.6	0.003*	0.749	0.009*	2.11
Nutritional status	2.5	0.28	0.547	0.072	
Physical activity frequency	18.3	0.00*	0.349	0.177	
Physical domain	2.4	0.28	–0.037	0.900	
Psychological domain	2.5	0.28	–0.237	0.496	
Social relations domain	2.4	0.29	0.633	0.046*	1,88
Environment domain	2.3	0.30	0.515	0.085	
Self-assessment domain	2.5	0.27	0.260	0.343	
Gender	1.8	0.40	–0.032	0.928	
Education	4.7	0.31	0.043	0.861	
Wage	12.9	0.11	–0.028	0.917	
Health Problems	2.1	0.33	–0.281	0.430	
Diabetes	3.6	0.16	0.700	0.344	
Hypertension	0.3	0.83	0.064	0.906	
Dyslipidemia	0.0	0.98	0.211	0.693	
Home office	9.9	0.04*	–0.020	0.945	
Meal at work	1.5	0.46	–0.136	0.646	

*Statistical significance was accepted as $P < 0.05$.

years of age had better average scores for healthy eating practices. The research demonstrated that people of older age and with better social relationships are more likely to achieve an excellent rating in healthy eating practices. Furthermore, the practice of physical activity frequency is related to the workers' best healthy eating practices.

Healthy eating practices

The present study evaluated the adoption of workers' healthy eating practices during the COVID-19 pandemic. The average healthy eating score was 41.0 ± 9.9, with 52.8% classified as having 'Excellent' practices. In specific domains, participants commonly ate sweets but avoided sugary drinks. They often sat down for meals but rarely skipped them or multitasked while eating. Domestic food preparation was preferred over street market purchases. Planning-wise, there was low variation in the consumption of organic products, but there were consumption regular of fruits and nuts as snacks.

It was observed that most participants had their meals at home and were involved in the preparation of meals, which is largely due to the fact that they are performing their work activities remotely (home office). When analysing the workers' healthy eating practices, using the DGBP recommendations as a parameter, in the domain referring to 'Planning', the consumption of fruits or nuts was prominent, a fact that was also identified in another study with Brazilians during the pandemic.[10] This result enhances the idea that some healthy eating practices may have improved during that period. On the other hand, there are also studies indicating that staying at home has led to the development of unbalanced eating habits, resulting in a sedentary lifestyle for people under lockdowns and excessive food consumption due to spending much time at home.[3,17]

In addition, in the 'Planning' domain, having meals 'calmly' also showed high adherence. This statement can be complemented by the habit of having meals sitting at the table, which was highlighted in the 'Eating modes' domain. This way of eating during the evaluated period showed that individuals are attentive to the meal period, avoiding performing other activities that interfere with this occasion. In addition, eating behaviours are also influenced by factors such as the place of consumption, which can stimulate stressful situations, leading to the consumption of more energy-dense foods.[30] However, when eating at home, there may be a greater sense of well-being, favouring better healthy eating practices, a fact that occurred more frequently during the pandemic.[10]

With regard to preparing meals at home and sharing activities involving food preparation, these were also reported more frequently by workers in the domain of 'Domestic organization'. This set of healthy eating practices may have benefited from the longer stay of workers in their homes during periods of social isolation, as observed by Coulthard *et al.*,[31] where those who were more involved in the preparation of meals were also able to intake healthier and more adequate food. Low adherence to the purchase of food in street markets was also highlighted in this domain, which may be related to the discontinuation of street market activities, since during the pandemic period some services were discontinued in public places, and were even avoided by the population to prevent the coronavirus contagion.

The domain referring to 'Food choices' showed another important factor: the consumption of candies, sweets, and other treats that were greatly appreciated among workers. However, the addition of sugar to beverages and the consumption of soft drinks had low adherence. Data from the Household Budget Surveys (HBS) 2017–2018 already identified that the consumption of soft drinks had decreased among Brazilians.[15] As for the consumption of foods with higher energy density, such as treats and sweet products, this has also been reported in other studies.[17,31] This may be associated with the use of comfort food, related to conditions of anxiety, stress, and depression during the pandemic period.[32] In addition, the prolonged time of social isolation and long remote work routines have also favoured the consumption of these processed foods.[33]

The logistic regression model applied in the study demonstrated that age and social relationships are more likely to be related to the workers' best healthy eating practices. Adherence to national dietary guidelines (DGBP) by older individuals has already been observed in Brazil[16] and France.[34] This may be related to the fact that younger people consume more processed and ultra-processed foods, as these are part of their daily lives since childhood.[35,36] In this younger group, it is already observed that one out of four people between 18 and 34 years of age consumes five or more types of ultra-processed foods, especially margarines and industrialised breads.[37]

In addition, young people tend to be less concerned about food quality[14,38] and have greater difficulty with cooking skills.[39] Although this study was carried out during the time of the pandemic, changes in eating habits should be carefully observed, as individual habits tend to be maintained.[10,13] These results enhance the importance of developing good healthy eating practices and practices throughout life, from childhood and adolescence.[10] Thus, the study serves as a warning to pay greater attention to this group in public policies that aim to reduce the consumption of ultra-processed foods.[37] As a result of an unhealthy diet, individuals may become overweight, which is a risk factor for NCDs, responsible for 71% of deaths occurring globally, among adults aged 30–69 years.[22]

Physical activity and healthy eating practices

Just like age, the frequency of physical activity practice was a variable associated with healthy eating practices, where it was observed that the better the eating practices, the greater the practice of physical activity among workers. In the United States, during the pandemic, the practice of physical activity was kept similar to what was current in the previous period; however, a part of the participants (30%) reported having reduced their activity in this period.[12] In a study carried out in the period before the pandemic, with more than 50 thousand adults in Brazil, 44.1% of the population did not reach a sufficient level of physical activity which was less than 150 min per week.[40] The results of the present study show that almost half of the participants needed to improve their physical activity practice during the pandemic.

In order to improve health indicators and encourage the practice of physical activity, the WHO updated its recommendation regarding the time and frequency of physical activity, in 2020, during the pandemic. It was recommended an ideal practice of 150–300 min per week of physical activities, considering those of light or moderate intensity; for intense workouts, the recommendation is 75–150 min per week.[4] This guidance aimed to encourage the practice of physical activity among the population, especially the workers, as a strategy to improve the quality of life and prevent NCDs.[4] There are many reports of the negative psychological impacts related to the COVID-19 pandemic, where restrictions such as home confinement negatively affected levels of physical activity.[3,41]

Quality of life and healthy eating practices

Quality of life assessments showed physical well-being as the highest and social relationships as the lowest. However, as for quality of life, the domain of social relationships was more associated with the workers' healthy eating practices in the logistic regression model; this conclusion stands out since this was also the domain that exhibited the lowest score. These results demonstrate the potential effects of social isolation, such as the reduction of activities in groups and the imposition of restrictive measures at the state and municipal levels, due to the rules that limited access and permanence in public and private spaces, such as schools, clubs, workplaces, parks, following the WHO recommendations.[1] The emotional support and social network support are important to adherence to a better diet.[42] The DGBP highlights the importance of commensality, as human beings are social beings and having meals with other people favours more suitable environments for food consumption.[23]

Also with regard to quality of life, the environment domain was also associated with healthy eating practices, signalling that the environment in which the individual lives can affect their health and individual well-being.[43] Furthermore, the relationships with the environment, when altered, can increase the feeling of insecurity, a fact that occurred during the pandemic due to the fear of contamination by COVID-19.[32] Those individuals who have negative feelings such as insecurity are less willing to improve their diet, while those who have positive feelings are more likely to improve their diet.[10]

As observed in the results presented, to promote workers' health, an approach with different focuses is important in order to improve the workers' living conditions, avoiding emphasising only weight loss.[44] An adequate work environment can improve the quality of life and help reduce the workers' NCDs; public authorities' support is important for these actions.[45] When applied to remote work, specific measures that consider the reality of the worker at home are required.

Some limitations of this research include that due to the high prevalence of COVID-19 disease at the time of the study in the country, it was not possible to use a larger sample and a follow-up test. For this reason, it is suggested that a larger and more diverse sample be used in future research. In addition, the instruments used could be subject to errors as they rely on participants' memory. However, despite its limitations, the study is innovative, as it is the first to assess healthy eating practices recommended by the DGBP, focusing on workers during the COVID-19 pandemic period. It is further pioneer in verifying the relationship between the healthy eating practices guided by the DGBP with the practice of physical activity and quality of life. Considering that remote work continues to be adopted post-pandemic, future research evaluating the dietary practices, physical activity, and quality of life of workers is necessary to understand this new labour phenomenon.

Conclusion

Most workers, especially those aged 41 or over and who practiced physical activity, followed the healthy eating practices recommended by the DGBP during the pandemic. The adoption of appropriate healthy eating practices was also strongly associated with the psychological, physical, environmental, and social relationships domains. However, when considering all the study variables, the logistic regression model showed a higher probability of a relationship for the age indicator.

The study also enhances the importance of evaluating the workers' diet by considering factors such as healthy eating practices, physical activity, and quality of life, in order to guide decision-making and the development of public policies in the framework of promoting health and food and nutritional safety for this population. The study results suggest several implications for public health policies and interventions, such as implementing wellness programmes by companies that encourage healthy behaviours, providing resources for nutritious eating, and promoting physical activity both at work and remotely. Public health campaigns can also educate the population, especially those with pre-existing conditions, about the importance of healthy eating habits, regular physical activity, and lifestyle choices. Additionally, strategies can be developed to integrate health promotion into the workplace culture, including flexible work arrangements that support physical activity and healthy eating habits. Lastly, addressing factors that contribute to overall quality of life, such as social relationships and psychological well-being, can indirectly influence healthy behaviours like eating habits and levels of physical activity.

Highlights

Most workers had excellent healthy eating practices.

Age was strongly related to the adoption of healthy eating practices.

The older their age, the better the workers' healthy eating practices.

The greater the practice of physical activity, better the workers' healthy eating practices.

Adequate healthy eating practices were associated with some quality of life domains.

Abbreviations

WHO: World Health Organization; **NCDs:** Chronic Non-Communicable Diseases; **FNU:** Food and Nutrition Units; **WFP:** Worker's Food Program; **BMI:** Body Mass Index; **DGBP:** Dietary Guidelines for the Brazilian Population; **IPAQ:** International Physical Activity Questionnaires; **WHOQOL-bref:** World Health Organization Quality of Life; **MSD:** Minimum Significant Difference; **OR:** Odds Ratio; **HBS:** Household Budget Surveys.

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Competing interests

The authors declare no conflict of interest.

Authorship

A. do N. O.: Methodology, Formal analysis, Validation, Investigation, Writing—Original Draft. L. S.-F.: Methodology, Validation, Supervision, Writing—Review & Editing. C. O. M.: Conceptualisation, Methodology, Formal analysis, Validation, Funding acquisition, Supervision, Project administration, Writing - original draft, Writing—Review & Editing. All authors reviewed and commented on subsequent drafts of the manuscript.

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