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REVIEW

A systematic review of the implementation of healthy food retail interventions in settings with multiple food retail outlets (complex food retail settings)

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Abstract

Complex food retail settings, where multiple food retail outlets operate in close proximity are common. Despite their ubiquity, there remains a significant knowledge gap regarding healthy food retail interventions implemented within these settings. Furthermore, understanding the factors affecting the implementation of interventions in these settings remains limited. This systematic review aimed to (1) identify and describe complex food retail settings where interventions were implemented to promote the healthiness of foods purchased, (2) synthesise the evidence on the effectiveness of the interventions implemented, and (3) identify enablers and barriers to the implementation of the interventions in these settings. Four databases, namely, MEDLINE Complete, Global Health, Embase, and Business Source Complete, were searched until December 2022. The Effective Public Health Practice Project quality assessment tool was used. Six studies reported on the implementation of interventions promoting healthy food purchases across multiple food retail outlets. Three studies each described two complex food retail settings: university and hospital. Interventions including promotion and promotion plus price improved the healthiness of foods purchased. There was limited description of institutional food policies, conceptual frameworks, formative research, or evaluation outcomes to inform the implementation of interventions in these settings. No study analysed enablers and barriers to the implementation of interventions. No study identified their settings as complex food retail settings. There is limited evidence describing complex food retail settings, their impact on intervention effectiveness, and associated enablers or barriers. Investigating factors influencing the effectiveness of interventions implemented within complex food retail settings is critical to support their implementation at scale.

Key words: **Complex food retail settings: Food purchase: Food retail: Health promotion: Implementation science**

Background

Unhealthy diet is a leading risk factor for non-communicable diseases that causes substantial societal and economic burden.[1] Food environments are known to drive dietary choices and associated population health outcomes.[2] There is a comprehensive body of evidence that has tested the effectiveness of marketing mix strategies[3] implemented in single food retail outlets such as a supermarket or cafe on diet-related outcomes[4] including consumer purchasing behaviour, dietary intake, and health. For example, front-of-package labels and point-of-sale signage are among the most commonly and successfully implemented strategies in single food retail outlets worldwide to inform healthy food choices.[5] There is also evidence illustrating a range of factors at different levels of the socio-ecological model that influence the implementation, sustainability, and scalability of these strategies in single food retail outlets,[6] both from consumer and retailer perspectives[7–9] — for example, retailers' knowledge and skills, retailer-consumer relationship, consumer demand and food preferences, and store infrastructure including product availability, space, and resources among others.

The effectiveness of similar food retail interventions to promote the purchase of healthy foods, as well as factors that

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influence the uptake and success of such interventions in more complex food retail settings, is unclear. Food retail environments where multiple food retail outlets are operating in close proximity including in shopping malls, on high streets, in airport food lounges, or within institutions such as a hospital or an educational institution are more complex than a single food retail outlet.[10,11] This is because such settings typically include a governing body that not only leases out the space to food retail outlets but may also guide and monitor the activities of the food retail outlet in a desired direction. Further, in settings with multiple food retail outlets, there is likely to be a competitive nature between the food outlets (inner settings) which can be affected by external structures (outer settings) and processes and can impact the retailer's capacity to embrace business risk and become an early adopter of healthy food options.[12] These interactions between the inner and outer settings create complexity, which is likely to vary in different settings. For example, a mall compared with a hospital will typically have different institutional practices and policies, organisational commitment to healthy food retail, culture, and customer demands.[13] There is some evidence suggesting that implementing multi-component population-based interventions such as education, policy, or advocacy at multiple levels of the socio-ecological model (individual, societal, and environmental) may improve health outcomes.[14] However, the evidence is inconclusive on the effectiveness of this type of intervention when implemented in settings with multiple food retail outlets.

As the implementation success of any intervention largely depends on the context in which it is implemented, and because most food retail outlets do not exist in isolation, it is imperative to understand the factors that influence the implementation and effectiveness of interventions in complex food retail settings. This systematic review aimed to (1) identify and describe complex food retail settings where interventions were implemented to promote the healthiness of foods purchased, (2) synthesise the evidence on the effectiveness of the interventions implemented in complex food retail settings, and (3) identify enablers and barriers associated with the implementation of the interventions in complex food retail settings. Evidence from this review will offer an understanding of how and what healthy food retail interventions are implemented in more complex settings. This will then help improve the design, uptake, and compliance of the healthy food retail interventions by multiple food retail outlets situated in a particular setting, for a positive impact on population diet and health.

Methods

A review protocol was developed *a priori* and registered in PROSPERO (The International Prospective Register of Systematic Reviews; registration number CRD42021258235).[15] For reporting of this systematic review of reviews, the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline[16] was followed.

Search strategy

A complex food retail setting is defined as a commercial or non-commercial establishment whose primary purpose may or may not be to sell food and drinks (e.g. hospitals/health services, sports, and recreational centres; shopping strip), and that includes multiple food retail outlets operating within the setting. A range of keywords and MeSH terms (Appendix A) were chosen to capture multiple food retail outlets across diverse settings, globally to capture complex food retail settings. Public health experts in the United States (US), United Kingdom (UK), and Australia were consulted to identify terminologies used to refer to complex food retail settings in different countries. For example, 'shopping strips' in Australia are often referred to as 'high streets' in the UK and 'street intersections' in the US. The search was conducted in four databases: Medline Complete, Global Health, Embase and Business Source Complete. Database search terms were categorised under the following hedge terms: settings with multiple food retail outlets, healthy food retail interventions, and healthy eating measures. Using the same search terms, an online search using Google Advanced was also conducted to identify grey literature published from inception up to December 2022 to expand the scope of the search. The search was limited to the first 100 uniform resource locators (URLs) depending on relevancy. A citation search of included papers was performed ('forward search'), and the reference lists of all relevant articles were searched to capture any potentially relevant paper missed by electronic search ('backwards search'). All articles (English language only) identified were subjected to selection criteria as described in the below section.

Study selection

A modified PICOS (population, intervention/exposure, comparison, outcome, and study context) criterion was developed to inform the study selection (Table 1). Articles were eligible for inclusion if they examined interventions (including but not limited to the '4Ps' of the marketing mix strategies, i.e. price, promotion, product, and placement)[3] implemented across multiple food retail outlets within a setting to promote the healthiness of foods purchased. Information on enablers and barriers to implementation and effectiveness of food retail interventions were either extracted wherever reported or inferred from the discussion section of the selected papers.

After duplicate articles were removed, titles, abstracts, and full texts were examined against the inclusion and exclusion criteria by two authors independently. Any discrepancies were resolved via consensus between the two screening authors.

Data extraction

Data was extracted from all included articles by two authors independently, and their results were compared. Data were collated into a predetermined matrix table that included publication details (author, title, year, study design, and aim), type of setting, type and number of food retail outlets, type of

Table 1. PICOS criteria

Criteria	Inclusion	Exclusion
Population	Studies that included and identified multiple food retail outlets within a setting *(defined as an establishment primarily engaged in retailing a general line of food, e.g. cafeteria, grocery store located in a hospital, university, etc.).*	Single food retail outlets operating or not operating within a setting, for example, supermarkets, dining halls or food pantries, online food retail outlets, and online experiments. No exclusions were based on race, culture, ethnicity, or geographical location of the food retail or retailers.
Intervention/ exposure	In-store food retail interventions (including but not limited to 4Ps, i.e. price, promotion, product, and placement) to promote healthy food purchased within food environment settings with food retail outlets.	Studies that do not include a relevant food retail intervention or online experiments.
Comparator	No restrictions	No restrictions
Outcome	Primary outcome: Studies reporting on the effectiveness of food retail interventions on healthy food purchased measured as food purchased, food consumed, and dietary intake. Secondary outcome: Studies reporting on implementation outcomes (e.g. adoption and engagement)	Studies that only reported on changes in knowledge and attitude; intention to purchase or consume healthy food.
Type of studies	All studies published in the English language from inception to December 2022	Commentaries, conference abstracts, editorials, laboratory-based or modelling studies

food sold, mention of any governing policy, and type and description of intervention including behaviour change theories underpinning the intervention, information on formative research conducted to inform the intervention, and key findings on the effectiveness of healthy food retail interventions on the healthiness of foods purchased (measured as food purchased, food consumed, or dietary intake), evaluation outcomes, and reported enablers and barriers to implementation healthy food retail interventions.

Quality appraisal

To ensure credibility, relevance, and value, each included article was critically appraised independently by two authors using the Effective Public Health Practice Project (EPHPP) Quality Assessment Tool.[17] The rating was based on the quality assessment across all six domains of the EPHPP tool — selection bias, study design, confounding, blinding, data collection, and withdrawal/dropouts.

Data analysis

A narrative synthesis describing the characteristics of complex food retail settings that implemented interventions to promote the healthiness of foods purchased, the effectiveness of interventions implemented, and the associated enablers and barriers to implementation across different types of settings with multiple food retail outlets was conducted.

Results

A total of 11,546 published peer-reviewed articles were identified from database searches. Following the removal of duplicates (n=4,167), title and abstract screening was conducted for 7,379 articles. Of these, 154 articles underwent full-text review. Following full-text screening, 148 articles were excluded based on reasons listed in the PRISMA flowchart (Fig. 1: PRISMA flowchart). A total of six distinct articles that implemented food retail interventions in two different settings to promote the healthiness of foods purchased were considered eligible for inclusion in the review. No grey literature met the eligibility criteria and hence was not included.

Study design and study participants

Two of the six included studies were quasi-experimental studies[18,19] (with no randomisation and no control group), of which one was conducted in two canteens in a university in Belgium[18] and the other at two cafeterias in a hospital in the US.[19] One of the six was a quasi-experimental study (with no randomisation) with a control group conducted at two cafeterias in a hospital in Canada,[20] one a cluster randomised controlled trial at 30 shops (food outlets) in a hospital in the UK,[21] one a non-experimental programme evaluation at three food outlets in a university in the US,[22] and one a cross-sectional observational study conducted at five food outlets in a university in New Zealand (NZ).[23]

Within studies conducted in universities, participants were largely students and staff, aged 17–35 years, and the sample size ranged from 111 to 244 participants. Participants in studies conducted in hospitals included staff and visitors, aged 18 years and above (only adults participated in the studies), and the sample size ranged from 1013 to 2800 participants. Table 2 provides a detailed description of the characteristics of the six included studies.

Characteristics of complex food retail settings

No study referred to the settings in which the studies were conducted as being a multiple food retail outlet setting or similar. Broadly, the studies described the characteristics of the university and hospital settings regarding the size, location, population demographics, characteristics of food retail outlets, and pre-existing food policy/health guidelines. Two studies[22,23] reported that the universities were in urban areas and considered themselves as a large public higher education setting hosting thousands of students and staff with middle-to-high socioeconomic levels. Regarding the characteristics of multiple food retail outlets where the interventions were tested, the number of food outlets in the university and hospital settings varied from

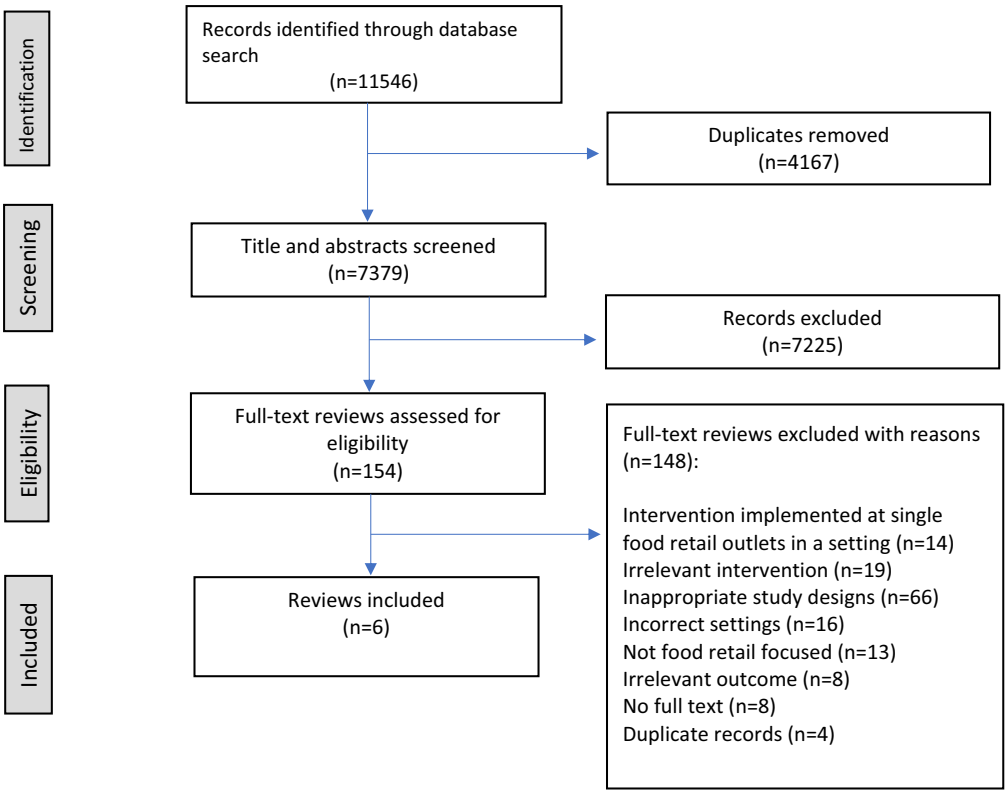

Fig. 1. PRISMA flowchart.

two to thirty. The food outlets varied in terms of size, catering for <100 to >1000 consumers, types of food sold (from pre-packaged foods, hot meals, salads, snacks, desserts, hot sides, and fruits), vendor properties (trading hours generally ranged from morning to evening on weekdays, except for food retail outlets at one hospital[19] that remained open on weekends and served food up to midnight), and customers (staff, students, and visitors). In two studies,[19,20] the hospitals where the interventions were implemented were large hospitals located in urban or metropolitan city areas. All studies conducted in hospitals (n=3) and one in a university mentioned an existing policy in place (including healthcare retail standards,[21] hospital check criteria,[20] 'healthy food' punch card policy,[19] and preparing standardised meals[18]) to influence the type of food sold at food retail outlets within their settings. Only two studies were conducted in hospitals in the UK, and the US reported that their intervention strategies were developed through some form of formative research including survey data,[19] qualitative interviews,[19,21] and a pilot study.[21] No study explicitly reported on whether their intervention was underpinned by a health behaviour change framework. However, the authors of one study conducted in a hospital in the UK[21] stated that their intervention design included different behaviour change techniques[24] to cognitively simplify healthier snack choices by facilitating cross-product comparison.

Effectiveness of healthy food retail interventions to promote the purchase of healthy foods, implemented in complex food retail settings

All studies tested the effectiveness of marketing mix strategies,[3] either as a standalone strategy or in combination with other strategies on healthy food purchased. The healthiness of the food purchased was collected using objective sales data from the participating food retail outlets. Three studies measured the energy (calorie or kilojoule) content of purchases,[18,20,21] one measured the average and proportional change in sales,[19] and two studies measured food purchased through a customer intercept survey.[22,23]

Two of the four[18,20–22] studies that tested the impact of a promotion strategy (defined as food and beverage marketing practices that promote products that adhere to healthy dietary guidelines) reported conflicting results. Promotion interventions delivered at food outlets in universities in the US[22] (as health star ratings) and in Belgium[18] (as an infographic and slogan front-of-package labelling) reported no change in the proportion of healthy meals purchased. In contrast, promotion interventions delivered at food outlets in hospitals in Canada[20] and the UK[21] reported healthier food purchases. In Canada,[20] digital menu boards with prominent displays of nutrition information coupled with an educational campaign (posters and pamphlets) to encourage customers to make healthy decisions at the point of choice or point of purchase were associated with a substantial reduction in foods purchased with high energy (-21%; P<0.001), high sodium (-23%; P<0.001), high saturated fat (-33%; P<0.001) and total fat (-37%; P<0.001) content. In the UK, an intervention trial in thirty hospitals (fifteen intervention and fifteen control) tested a warning label sign displaying all of the available single-serve snacks (>1 million) in order from lowest calorie on the left to highest calorie on the right resulted in significant reductions in snacks purchased with high energy content (total energy and sugar).[21] The authors stated that the intervention was designed to cognitively

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Table 2. Characteristics of the six included studies grouped by settings

Publication details	Type and number (n) of food retail outlets	Type of food sold at the food retail outlets	Existing governing policy (yes/no)	Type of intervention	Key findings		Risk of bias
					Effectiveness of food retail interventions on diet	Enablers and barriers to implementation[a]	
University settings Hoefkens et al. 2011[18] Belgium Quasi-experimental studies (with no randomisation and no control group)	Canteens (n=2)	Four protein sources (e.g. meat), one or two warm sauces, two cooked vegetables, one salad, and five carbohydrate components (e.g. French fries); dressings, fruit, other desserts, and drinks	Yes (Food preparation methods and menus were standardised in all canteens of the universities)	At the point of purchase, stars based on energy content, saturated fat, sodium, and vegetable portions are posted on large poster boards at the entrance of the canteens and next to example dishes at the buffet counter. Besides the number of stars, noncomplying nutrients or food groups were posted in a red font and followed by an exclamation mark or verbal descriptor. PROMOTION	The proportion of meals chosen in the different star-rating categories remained constant after posting the nutrition information (P=0.820). Posting nutrition information did not affect meal choices and nutrient intakes.	*Enabler:* Commitment to promote health; organisational policy	Weak quality
Magdaleno et al. 2021[22] United States Non-experimental programme evaluation (process and impact evaluation)	Markets (n=3)	A variety of food and beverage options including many 'grab and go' items in cold cases.	No	Front-of-pack labelling: The sticker is bright red with a heart graphic and a slogan that states 'Live Well [the university's mascot]'. The items that were selected to display the sticker contained one or more of the following nutritional benefits based on FDA guidelines: 100% whole grain, lean protein, fruits, vegetables, high fibre, low sodium, low saturated fat, and/or low sugar. PROMOTION	Participants did not purchase an item with the sticker.	*Enabler:* Collaborations and leadership at the organisation level	Weak quality
Roy et al. 2021[23] New Zealand A cross-sectional observational study	Food outlets (n=5)	Meals (food and beverages)	No	Price-reduced meals: The 'Budgie Meal' is a price-reduced meal option available at most food outlets, offering staff and students the opportunity to purchase a substantial meal from an array of cuisines across campus. The meals are $6·50 or under and consist of protein, vegetables, and carbohydrates. PROMOTION, PRICE	The 'Budgie Meal' had higher sales volumes at each outlet than other items.	*Enabler:* Commitment to promote health *Barrier:* No organisational support and governance; Retailer's profit-oriented approach and unwillingness to host interventions	Weak quality

Continued

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Table 2. Continued

Publication details	Type and number (n) of food retail outlets	Type of food sold at the food retail outlets	Existing governing policy (yes/no)	Type of intervention	Key findings: Effectiveness of food retail interventions on diet	Key findings: Enablers and barriers to implementation[a]	Risk of bias
Hospital settings Vanderlee et al. 2014[20] Canada Quasi-experimental studies (with no randomisation but a control group)	Cafeteria (n=2)	Overall nutrition intake	Yes (Hospital check criteria)	• Five digital menu boards with prominent displays of nutrition information at the point of sale next to the price of items on the menu board, featuring information on energy (calories), sodium, saturated fat, and total fat • A health logo (an apple with a check mark) for items that met the developed nutritional standards; • A Healthier Menu Plus Sante at the entrance to the cafeteria highlighting healthier menu items available on the menus; • An educational campaign (posters and pamphlets) promoting the programme • Reformulated recipes for some food items and removed the deep fryer from the cafeteria PROMOTION	Nutrition information on menus and improved nutrition profile of food offerings were positively associated with substantial reductions in energy, sodium, and fat consumption.	*Enabler:* Commitment to promote health; organisational policy *Barrier:* Retailer's profit-oriented approach	Weak quality
Patsch et al. 2016[19] United States Quasi-experimental studies (with no randomisation and no control group)	Cafeteria (n=2)	Burgers and salads	Yes ('Healthy food' punch card policy—where employees earned a free healthy meal after 10 healthy purchases)	Marketing and price incentives/ disincentives for healthy and unhealthy items, with a 35% price differential. Point-of-purchase marketing included the following: (1) Better Bites (BB) logo on all food items meeting nutritional criteria and (2) signage highlighting taste, cost, and health benefits. Intervention strategy was developed by combining evidence-based practices with hospital-specific formative research, including key informant interviews, the Nutrition Environment Measures Study in Restaurants, hospital employee surveys, and nutrition services staff surveys. PROMOTION, PRICE	Average weekly turkey burger sales increased 13-fold (10.85–145.59); healthy salads were popular at baseline and intervention. Cafeteria gross sales and burger profit ($P < .001$) increased at both cafeterias.	*Enabler:* Commitment and moral obligation to promote health; leadership at the organisation level; organisational policy	Weak quality

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Table 2. Continued

Allan et al. 2020[21] United Kingdom Cluster randomised controlled trial	Shops (n=30)	Snack food	Yes (Healthcare Retail Standards)	Theory-based point of purchase prompts (a form of cognitive nudge) — sign displaying all the available single-serve snacks in order from lowest calorie on the left to highest calorie on the right. Intervention design was underpinned by different components of behaviour change technique[24] to cognitively simplify healthier snack choices by facilitating cross-product comparison. Intervention strategy was developed by formative research including consultation with a multidisciplinary team of psychologists, public health scientists, and nutritionists (formative research) and a successful pilot study. PROMOTION	Snacks purchased from intervention sites were on average significantly lower in calorie and sugar.	*Enabler:* Commitment and moral obligation to promote health; collaborations and leadership at the organisation level; organisational policy *Barrier:* Retailer's profit-oriented approach; instore competition between health versus profit-oriented interventions	Weak quality

[a]Inferred from the discussion section of the included studies.

simplify healthier snack choices by facilitating cross-product comparison.

Two studies that tested a combination of promotion and price manipulation strategies at two cafeterias in a hospital in the US[19] and five food outlets in a university in NZ[23] resulted in significant reductions in unhealthy food purchases. In the two hospital cafeterias, the promotion component included a 'Better Bites' logo on food items including burgers (traditional hamburger vs 'Better Bites' turkey burger), salads (traditional salad vs 'Better Bites' healthy salad)), and signage highlighting taste, cost, and health benefits. The prices of healthy items were decreased to incentivize purchases, and prices of unhealthy items were increased to disincentivize purchases at the point of purchase. A 35% intervention price differential was applied. The intervention resulted in a 13-fold increase in the average weekly Better Bites turkey burger sales and increased popularity of the Better Bites salads, leading to increased gross sales and profit at both cafeterias.[19] A study evaluating five food outlets at a university in NZ found that the promotion and price-reduced meal choice initiative 'Budgie Meal' resulted in higher purchases of these meals.[23]

Associated enablers and barriers to implementation of food retail interventions in complex food retail settings

None of the six included articles reported on the enablers and barriers to the implementation of the various food retail interventions. One study conducted in a university in the US[22] included process evaluation outcome assessment on the completeness of programme delivery and the degree to which participants were engaged with the intervention via audits and surveys with customers. Some authors speculated on enablers and barriers in the discussion section of their article to explain the outcomes of the study.

Quality appraisal

All six studies included two or more weak quality ratings across the tool criteria and therefore were evaluated as weak in the global rating. Briefly, five studies were rated as moderate or weak quality due to high selection bias[18,19,21–23] and weak study design.[18–20,22,23] Three studies did not adjust for, or did not report on, confounders and were rated as weak. Three other studies[19–21] were rated as strong for treating confounders. Blinding in all studies was assessed as weak, except for one study that was rated as moderate.[21] Data collection was primarily through either sales data (n=4) or self-reported data (n=2). The validity and reliability of the data collection tool were reported in only one study.[22] Only three studies reported achieving more than 60% of participants completing the study.[18,20,22] For details on the assessment across each of the tool criteria, please refer to Appendix B.

Discussion

This is the first systematic review to summarise the evidence on food retail interventions to promote the purchase of healthy foods implemented in complex food retail settings, that is, where multiple food retail outlets operate together in close proximity in a particular setting. We identified six studies where a relevant food retail intervention had been implemented in a complex food retail setting; however, none of the studies identified the surrounding food retail outlets in their setting as relevant to the implementation or effectiveness of the interventions. The intervention outcomes along with associated enablers and barriers were neither analysed nor discussed in the context of the interrelationship between the setting and the multiple food retail outlets.

The six included studies were conducted in university and hospital settings where two marketing mix strategies, promotion (as a standalone strategy) and price (in combination with promotion), were implemented in multiple food retail outlets and their effect on food purchased was assessed. University and hospital settings were defined here as complex food retail settings as they included multiple food outlets such as cafés, restaurants, and vending machines, offering diverse food options to their consumers. The studies described these settings regarding their size, location, population demographics, and characteristics of food retail outlets more broadly. Furthermore, there was limited description of formative research and process evaluation, to inform intervention design and development. While previous literature suggests that these characteristics are likely to influence the implementation of interventions in single food retail outlets,[12,25] there is no comparable literature to confirm whether these are also characteristics relevant to complex food retail settings. This is critical information for policymakers and researchers to better plan the implementation of the intervention specific to such settings to achieve maximum success.[26]

Studies[20,21] that implemented promotion interventions in the form of warning labels and displayed nutrition information including a health logo on a digital menu board at food outlets in hospitals reported a positive effect on healthy food purchasing behaviour. Studies[19,23] that implemented a combination of promotion and price (in the form of a display sign highlighting taste, cost, and health benefits) at food outlets in both university and hospital settings reported a positive effect on healthy food purchasing behaviour and overall sales of healthy foods. These findings are consistent with the findings of previous studies[4,27–30] that have reported positive effects of combined promotion and price strategies on the healthiness of foods purchased implemented in small (i.e. convenience stores) and large (i.e. supermarkets or recreation and sports facilities) settings but with single food retail outlets. While some of the studies in our review reported the presence of an existing health-enabling food policy,[18–21] none mentioned their impact on the implementation of the interventions. Furthermore, the studies did not report on whether and how the intervention design and implementation factors were adapted according to the characteristics of complex food retail settings. This is important as the settings may have their own culture, structures, policies, and practices, serving a certain interest,[12,31] and this may impact the level of uptake of the interventions or intervention success in multiple food retail outlets. For example, there was no discussion of the impact of a potential competitive disadvantage if an outlet becomes an early adopter in their broader setting.

While no study in our review analysed enablers and barriers to the implementation of food retail interventions in the context of

complex food retail settings, some authors made inferences in their discussion sections on potential enablers and barriers that influenced intervention outcomes. Factors such as collaboration and leadership[19,21,22] at the setting level and existing health-enabling food policy[18–21] were assumed as enablers that led to the positive impact of interventions on the outcomes. Barriers to implementation were speculated to be the lack of organisational support to implement healthy food policy[23] at the setting level and the retailer's concern for profitability.[19–21,23] Several reviews have previously summarised similar factors (from retailer and consumer perspectives) influencing the implementation of food retail interventions at a single food retail outlet.[6,12] However, factors influencing the implementation of food retail interventions specific to complex food retail settings remain an area open to exploration. To advance knowledge in this context, using a combination of the Consolidated Framework for Implementation Research (CFIR) tool[32] and socio-ecological model[6] can be useful to not only identify the characteristics of outer setting (i.e. the external influence of the organisation on implementation of the intervention) and inner setting (i.e. characteristics of the food retail outlets implementing interventions) domains but also explain the interrelationship between the factors within the two domains. Moreover, CFIR comprehensively encompasses both barriers and enablers to the implementation of interventions, contributing to an in-depth understanding of the processes involved. For example, in Singapore, strong leadership in the Health Promotion Board's Healthier Dining Programme initiative in 2019[33,34] (outer setting) led the food retailers within some hawker centres (i.e. a complex food retail setting) to cook with healthier amounts of oil and salt and shifting the culture of the hawker centres (inner settings) towards more health-promoting. Given the limited description of formative research to inform the implementation of the intervention in the included studies, future studies could better report on steps and processes undertaken with key stakeholders to unpack the complex interplay between the settings and food outlet level to help inform the implementation strategies for effective and sustained implementation of interventions in complex food retail settings.

The overall lack of evidence on complex food retail settings that implemented interventions to promote the purchase of healthy foods illustrates the need for better investigation of the characteristics of complex food retail settings (beyond universities and hospitals) for context and factors (at the setting level and the retailer level) influencing the procurement, preparation, and provision of food at multiple food retail outlets for three reasons. First, to enable identifying and undertaking a shared/collaborative approach (between key actors — organisational contractors and retailers) to developing a comprehensive plan for programme and policies tailored (to the context) to promote healthy food choices culture within their settings.[31,35] Second, understanding the context and factors (at the setting level and the retailer level) influencing the implementation of interventions in complex food retail settings is important to identify appropriate ways to mitigate context-specific challenges and harness opportunities to facilitate sustained implementation of healthy food retail interventions.[11,36] Last, better characterising complex food retail settings can help authorities in power such as local governments to develop standardised healthy food policies that can be implemented consistently across the multiple food retail outlets operating within a setting to make a population-level impact.[31] For example, healthy food policies can be linked to lease agreements, contracts, and tenders applicable to the food outlets within a setting.[37] Existing resources such as the Victorian 'Healthy Choices: healthy eating policy and catering guide for organisations'[38] can be useful resources to adapt and implement as a healthy food policy in a complex food retail setting. Another likely benefit of implementing a setting-level food policy is that consumers are less likely to indulge in compensatory unhealthy food purchases[39] in the vicinity of complex food retail settings. However, this warrants future investigation.

Strengths and limitations

A strength of this review is the identification and inclusion of a broad range of search terms to capture complex food retail settings. The main limitation is that only six studies of weak quality were identified and included that lacked randomisation, lacked assessor and participant blinding raising concerns regarding internal validity, and used a convenience sample of students and hospital visitors which may not be representative of the general population. However, within a real-life university or hospital setting, quasi-experimental studies are deemed more appropriate considering problems of contamination. Second, the review only found universities and hospitals as two examples of complex food retail settings, the findings of which may not be generalisable to other complex food retail settings such as shopping strips. Last, we excluded non-English language papers and studies where the authors did not report the settings in which the interventions were implemented; this may have led to the exclusion of studies with insights relevant to complex food retail settings.

Research implications

First and foremost, good quality studies investigating the complex food retail setting, and giving consideration to the nature of that environment, are needed. Exploring complex food retail settings regarding their governance structure, existing nutrition policies, and the interaction between factors at the settings and the retailer level is needed to understand the implementation of healthy food retail interventions in complex food retail settings. This will also enable the development of context-specific support for complex food retail settings. Food retail interventions need to be tested for their fit and evaluated (using process and outcome evaluation measures) to better understand the barriers and enablers to implement within the context of complex food retail settings. Identifying ways to mitigate barriers to successful implementation of interventions will then be the logical next step. Multi-stakeholder collaboration between public health researchers, policymakers, and the various actors within complex food retail settings, including consumers, is needed to collectively inform public health interventions.[40] Future research can investigate existing food environment monitoring and benchmarking initiatives[36] in the

context of complex food retail settings. This may also identify potential levers of success to create healthy complex food retail settings. Finally, with an increasing number of food retail outlets that co-exist both in conventional brick-and-mortar-type settings and within emerging online food retail settings,[41] there is an opportunity to adapt the learnings from complex food retail settings to influence the emerging online forms of food environment towards health promotion in their infancy.

Conclusion

This systematic review demonstrates a significant gap in the existing evidence regarding the clear conceptualisation and description of healthy food retail interventions implemented in settings where multiple food retail outlets are co-located (i.e. complex food retail settings). Universities and hospitals are two examples of such settings identified in this review where healthy food retail interventions have been implemented. Evidence suggests that healthy food retail interventions including promotion and price strategies can lead to healthier purchasing behaviour among consumers in university and hospital settings where multiple food retail outlets are co-located. However, exploring complex food retail settings regarding their governance structure, existing nutrition policies and other characteristics are fundamental to inform the development and successful implementation of healthy food retail interventions at the settings level.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.52.

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Competing interests

All the authors confirm that they have no competing interests to declare.

Authorship

AG was responsible for leading the review including research inception, study design, data analysis, manuscript writing, and revisions. AP contributed to the research inception and study design and provided detailed feedback on the manuscript. GS contributed to the research inception and search terms and provided detailed feedback on the manuscript. JG contributed to the search terms. All authors provided feedback and suggestions on multiple drafts of the manuscript. Ethics of human subject participation: Not applicable.

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REVIEW

Adolescent nutrition in Nigeria: a systematic review

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Abstract

In this systematic review, we scrutinise adolescent nutrition in Nigeria, focusing on dietary patterns, intake, and nutritional status. Through a systematic examination of observational studies across three major databases, we analysed data from 67,844 adolescents. Our exploration revealed 102 studies, predominantly cross-sectional, addressing various nutritional dimensions. However, only 13% of these studies demonstrated low risk of bias, with none offering national representation and most concentrated in specific, school-based regions. The findings underscore a complex nutritional landscape with widespread malnutrition and highlight the critical need for high-quality, comprehensive data. The dominance of cross-sectional designs and regional biases in existing research calls for cautious interpretation and suggests a pressing need for more robust, nationally representative studies to guide future nutritional interventions and policy-making in Nigeria.

Key words: **Adolescents: Anthropometry: Nigeria: Nutrition: Systematic review**

Introduction

Promoting health and well-being of adolescents in low- and middle-income countries (LMICs) is a global health priority, given that they account for more than 90% of the world's 1.2 billion adolescent population.[1] This age group, spanning from 10 to 19 years, represents a unique life period marked by physical, cognitive, psychosocial and emotional changes. The health needs of adolescents, particularly those living in LMIC, are distinct from other age groups and overlooking this can have lasting impacts on growth and development of future generations. Thus, adolescence offers a window of opportunity for interventions that can greatly shape health outcomes later in life and foster a sustainable and equitable future for societies.

Recognising the importance of this life phase, the United Nations Sustainable Development Goals (SDGs) explicitly mention adolescence in 12 health-related SDG indicators.[2] Research has further reinforced the role of nutrition on adolescent growth and development, the influence of the food environment on their food choices and which interventions might lead to healthier nutrition and growth.[3] These efforts have renewed the interest in investing in nutrition - a major modifiable risk factor with significant implications for public health.[4] Indeed, large prospective human studies have shown that nutritional status in adolescence is a strong predictor of adult cardiovascular health.[5]

However, in LMIC settings usually, adolescents have limited control over their food choices. In Nigeria, the limited investment in nutritional research, particularly regarding adolescent nutrition, poses a great challenge in monitoring the state and trajectories of nutrition indicators across regions

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and over time.[6] Nevertheless, such nutrition surveillance data can be a valuable guide for formulating policies and interventions tailored to this demographic group. According to the latest Global Nutrition Report 2022, Nigeria has only made slight progress towards diet-related non-communicable disease (NCD) targets.[7] Given the effects of the post-pandemic era and the ongoing influence of food prices on household finances, along with the challenges pertaining to food affordability, accessibility of nutritious diets and livelihoods, a careful assessment of the state of adolescent nutrition in Nigeria is warranted.[8]

Therefore, high-quality evidence on Nigerian adolescent nutrition can inform tailored solutions and support better nutrition and health. Findings and insights can also be used as an invaluable tool in shaping the development and establishment of evidence-based guidelines and nutrition surveillance systems for monitoring dietary risks among Nigerian adolescents. Taking this into account, we conducted a systematic review aiming to comprehensively summarise and examine the state of adolescent nutrition in Nigeria, including dietary intake profile, habits and nutritional status.

Methods

This work was carried out in accordance with established guides for conducting evidence syntheses for medical and health research,[9,10] as well as PRISMA guidelines for reporting findings from systematic reviews and meta-analyses.[11] We systematically searched three electronic databases namely: PubMed, Web of Science and Google Scholar, from inception to March 6, 2023. The protocol of this work is registered in the international prospective register of systematic reviews (PROSPERO) with identification code **CRD42023399668**.

Selection of studies

Studies were considered for inclusion if they met the following criteria: (i) carried out in Nigeria, (ii) included adolescents between the ages of 10–19 years and (iii) reported dietary intake, patterns, adherence to a healthy diet, and/or nutritional status (i.e. anthropometry and other related measures). Only observational studies were considered for inclusion. There have been recent proposals[12] to extend the definition of adolescence from 10–19 years to 10–24 years, noting delays in the transition age to adult roles (e.g. marriage and parenthood) in many societies as the main motivation. However, in this work, we use the definition of 10–19 years. Studies were not included if: (i) they were not conducted in Nigeria and (ii) did not report any nutrition-related indicator (e.g. nutritional status, dietary intake or adherence to healthy diet standards). In addition, we did not consider case studies/reports, letters to the editor, conference proceedings, posters, abstracts, reviews or preprints.

Data extraction

Three reviewers independently evaluated the titles and abstracts according to the inclusion and exclusion criteria. For each eligible study, three reviewers assessed the full-text. In cases of disagreement, a decision was made by consensus or, when necessary, a fourth reviewer was consulted. Information was extracted from studies in triplicate and was categorised according to the following variables: study design, study area (region, state), target population, age, sex, sample size, setting, dietary assessment tool used, journal details, intake of macro- and micro-nutrients, etc.

Quality assessment of included studies

We evaluated the methodological rigour of included studies using the Joanna Briggs Institute (JBI) corresponding tool for assessing methodological quality of studies and provided answers to the relevant questions, based on study design.[13] Detailed assessment can be found in supplementary Table S4. The methodological quality was rated on a scale of maximum 8 points. Based on this evaluation, studies were classified as '*low risk of bias*' (>= 7 points), '*some concerns*' for bias (>=4 points), and '*high risk of bias*' (< 4 points). We included all studies in the synthesis, irrespective of their JBI evaluation classification.

Data synthesis

We synthesised the extracted data from each study and described the information on dietary assessment tool and/or nutritional status (e.g. anthropometry classification) for the target group (10–19 years) with no discrimination to setting (e.g. in school or out of school), location or sex. We extracted primary and composite anthropometric parameters, and dietary intake in comparison to internationally established reference values and the primary aims of each included study were reported. Nutrient, energy and other metrics were converted to same units (e.g. energy in kcal, protein intake in grams, etc.). We also report the food and food group consumption and dietary habits among Nigerian adolescents. In order to show the nutritional status trends over time a summary of results from studies reporting anthropometric indicators ordered by year of publication was produced using R Studio 2022.07.2 Build 576 (Fig. 2). Data used to produce this figure can be found in supplementary Table S3.

Results

Based on the systematic search (Fig. 1), we retrieved 238 articles from Web of Science and 542 from PubMed search engines. In Google Scholar, the first 200 results were considered. Supplementary Table S1 outlines the search strategy and strings used for each database. Based on the bibliographic searches, 980 records were retrieved in total. Following deduplication and merging 837 studies were left. 302 studies were excluded after title and abstract screening, leaving 535 records for full-text screening. Of those, 446 studies were excluded for various reasons. Eventually, 89 studies were included for qualitative synthesis. In addition, 34 studies were found after screening the reference lists of the 89 studies included in the qualitative synthesis. Of these 34 records, only 13 studies were eligible to be added to the previous 89 studies, bringing the number of studies contributing to the systematic review to 102 records.

Table 1 shows the characteristics of included studies. A total of 102 cross-sectional studies have been examined and

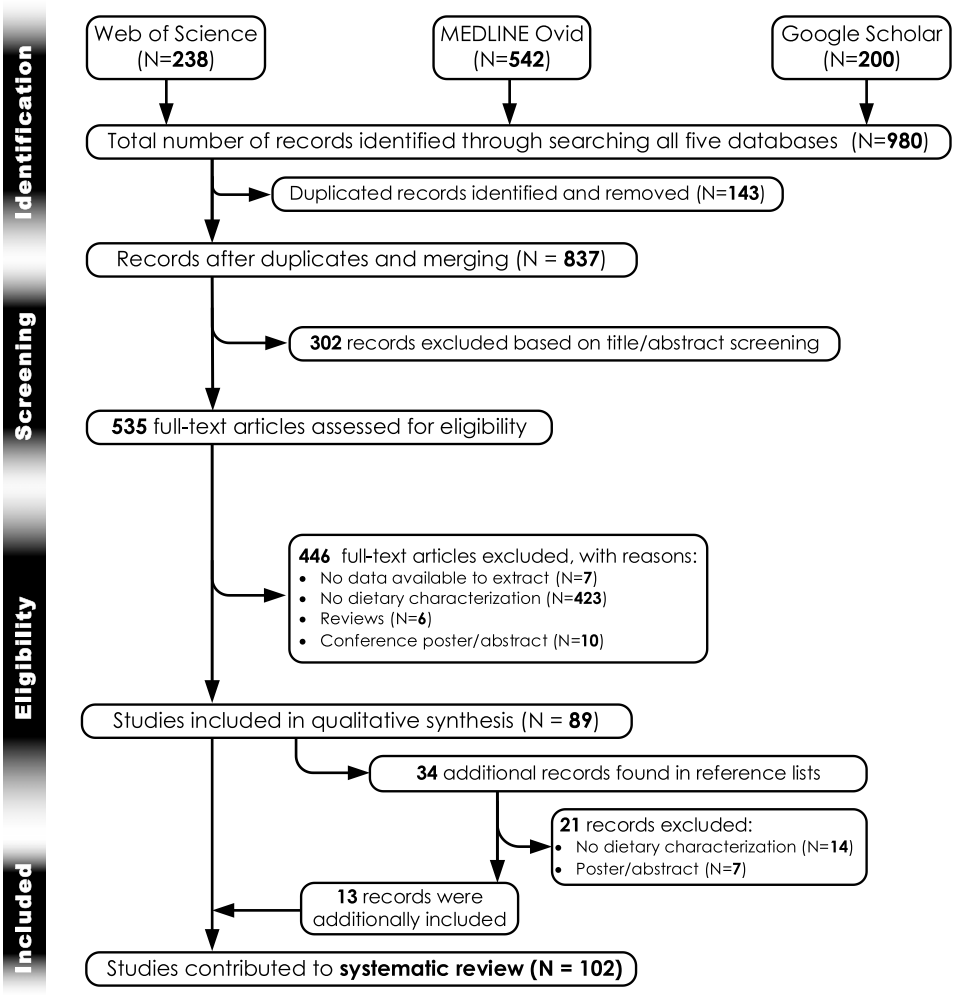

Fig. 1. PRISMA flowchart.[14]

reported. Sample sizes ranged from as small as 22 to as large as 7,625. Approximately half of the study sites, representing (n = 51), were located in the south-western region of the country. Conversely, the north western region was the least represented with merely 3.9% of the studies conducted there (4 out of 102 studies). A visual map with study locations and population density of all included studies is available in supplementary Fig. S1. The majority of studies (86%) included both male and female participants. Educational institutions were predominantly utilised as the research setting in over 70% (77 out of 102) of the studies. Substantial heterogeneity was observed with regards to the methodological approach to dietary assessment and anthropometric classification tools adopted in these studies. Questionnaires were the most widely employed data collection instrument, featured in nearly 81 out of 102 studies. Based on the JBI tool evaluation, 13 studies were classified as 'low risk of bias', representing 12.7% of the total 102 studies. Conversely, 46 studies, accounting for 45.1%, were assessed as having 'some concerns' for bias, while 43 studies, or 42.2%, were rated as at 'high risk of bias'. The most commonly used dietary assessment tool was a questionnaire. Other tools included the 24-hour dietary recall, food frequency questionnaire, and diet diversity score. The administration method was predominantly self-administered or researcher-administered. Validation: There was a mixed validation status for the instruments used. While many of the questionnaires were not validated, a minority of studies used validated tools. Most of studies (84.3% or 86/102) were published in journals that were not indexed and consequently had no impact factor at the time of publication.

Table 2 provides a summary of anthropometric findings of nutritional epidemiology studies conducted in Nigeria among adolescents. Out of the total, sixty-six studies documented either primary or composite anthropometric measures or a combination thereof. Composite anthropometric measures encompassed various indicators such as underweight, overweight, stunting, obesity, thinness and wasting. Of note, 77.2% of the studies reported different manifestations of undernutrition, such as stunting, wasting and/or underweight. Furthermore, 68.2% of the studies indicated the prevalence of overweight and obesity, which constitute forms of over nutrition. Additionally, the height-for-age z-score (HAZ) and weight-for-age z-score (WAZ) were reported in about 39% of the studies (25 out of 66). The overall range for body mass Index (BMI, in kg/m^2) across all studies that reported it, ranged from 15.7 to 24.6. Height (in metres) varied within a range of 1.2–1.7 and weight (in kilograms) ranged from 19 to 70. With regard to the prevalence of simple nutritional status phenotypes (expressed as percentages), the overall range was as follows:

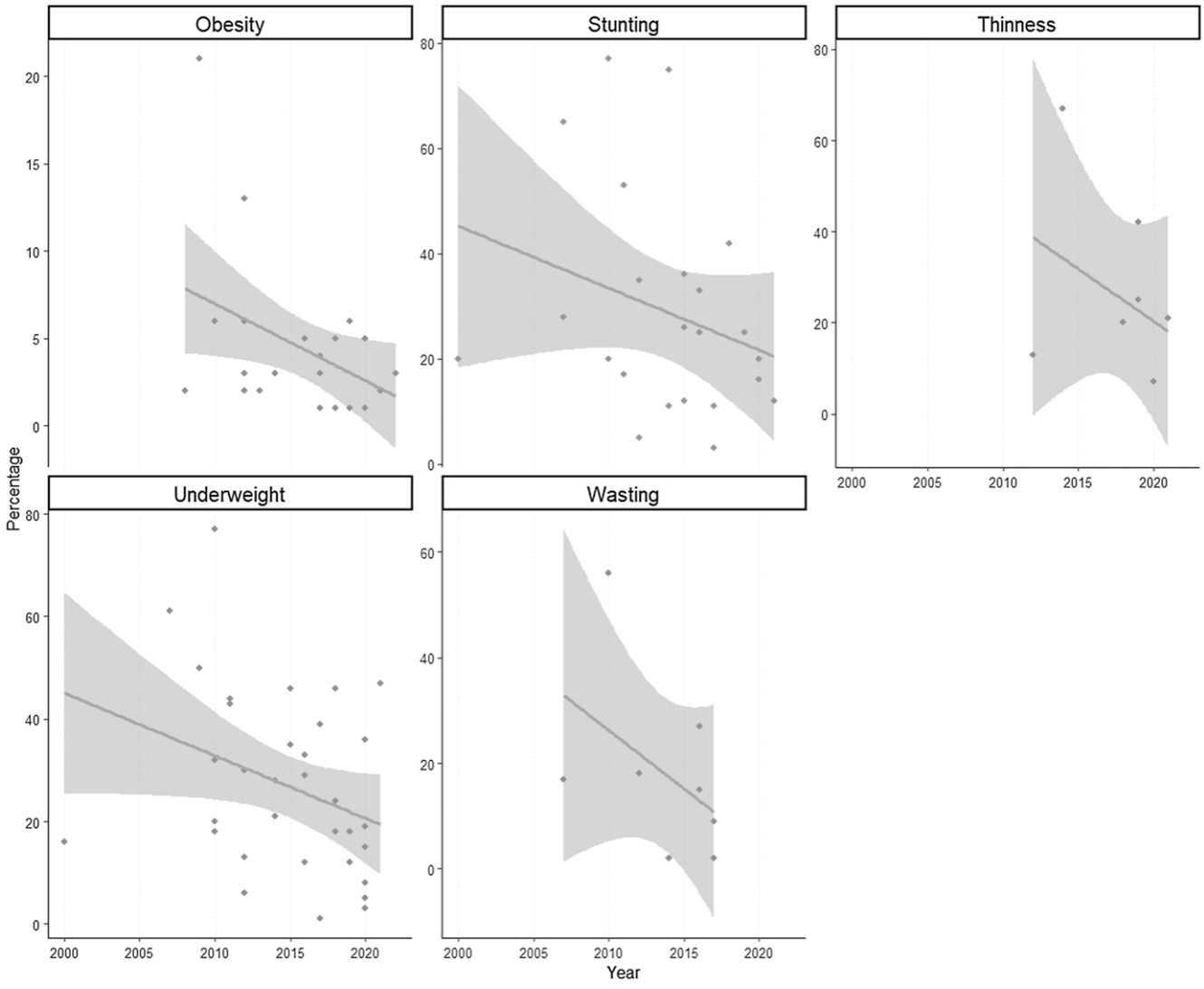

Fig. 2. Summary of the individual studies reporting anthropometric indicators over time.

underweight from 2.5% to 78.3%; overweight from 1.4% to 25.2%; stunting from 2.5% to 77.1%; wasting from 1.7% to 56%; and obesity from 0.2% to 21%.

Table 3 presents findings on food and food group consumption for Nigerian adolescents, from 29 nutritional epidemiology studies that reported consumption data for at least one food and/or food group.[25–27,31,38–40,56,60,70,71,74,76,77,80,94–96,101,102,104,105,110,113,117–120] Starchy staples, encompassing cereals and starchy roots/tubers, emerged as a commonly consumed dietary component, were reported by eleven studies and consumption levels ranged from 28.2% to 96.7%.[25,26,38,70,74,76,77,96,101,113,117] However, only two studies reported consumption levels below 50%.[25,76] Legumes, nuts, and seeds were reported in six studies.[25,38,60,70,105,117] Similarly, consumption of meat, poultry, fish, and seafood ranged from 19.1% to 62.1%, reported in nine studies.[25,38,60,70,74,76,96,101,117] Vegetable consumption was documented across eleven studies with a range from 10.1% to 83.0%, with seven studies indicating proportions below 50%.[25,38–40,70,71,96,113,117–119] Fruit intake ranged from 9.7% to 77.4% and was reported across seventeen studies.[25,26,31,38,40,60,70,71,74,77,94,96,101,105,117–119] Conversely, sweets' consumption was described in six studies[26,71,74,94–96] showing variation across the studies (range: 35.3–62.4%). Snacking habits were recorded and reported in ten studies (range: 33–96.8%).[56,60,70,76,77,80,93,96,104,117] Additionally, consumption of sugar-sweetened beverages was indicated in 13 studies.[27,31,40,71,76,94–96,102,110,117,119,120] Consumption of such beverages exhibited a wide spectrum, ranging from 8.5% to 99.4%. Only two studies reported egg consumption (range: 3.3–14.3%).[26,38]

Table 4 summarises dietary intake among adolescents in Nigeria. Results show that the amount of protein (range: 27–93.5 g), energy (range: 903–5754.7 kcal) and carbohydrate (range: 82–937.60 g) consumed by majority of adolescents were inadequate[38,46,68–70] also one study reported an inadequate consumption of fat (range: 6.0–157.1 g) by this age group.[70] In contrast, some studies reported an excessive intake of carbohydrate, energy and protein beyond recommended nutrient intake.[70,82,109,112] Only two studies[30,116] reported on

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Table 1. Study characteristics of the studies included in the systematic review

Ref.	Author, year	Location	Target population	Age, mean (SD)	Sex	Setting	Sample size	Dietary assessment instrument (validation/ administration)	Intake quantified?	Risk of bias	Journal indexed in MJL
[15]	Abdulkarim *et al.*, 2014	Abuja	Adolescents only	14.4 ± 1.9	♀♂	Primary school	1700	Questionnaire (Q), Not validated (NV), self-administered (SA)	No	Some concerns	No
[16]	Abidoye and Akande, 2000	Lagos State.	Adolescent/Children	NR	♀♂	Primary school	120	Q (NV/SA)	No	High Risk	No
[17]	Adebimpe, 2019	Osogbo	Adolescents only	14.6 ± 1.7	♀♂	Community	480	24 HDR (NV/SA)	Yes	High Risk	Yes
[18]	Adeomi *et al.*, 2019	Ile-Ife, Osun state.	Adolescents only	14.4 ± 2.0	♀♂	Community	313	Q (NV/SA)	No	Low Risk	No
[19]	Adeomi *et al.*, 2022a	Southwest, Nigeria	Adolescents only	♀14.4 ± 1.9 & ♂14.8 ± 2.1	♀♂	Community	400	Q (Validated (V)/SA)	Yes	Low Risk	No
[20]	Adeomi *et al.*, 2022b	Osun and Gombe states	Adolescent/Children	NR	♀♂	Rural and urban	1200	Food frequency questionnaire (FFQ) (V/ SA)	Yes	Low Risk	Yes
[21]	Adeomi *et al.*, 2022c	Osun and Gombe states.	Adolescent/Children	NR	♀♂	Rural and urban	1200	Q(NV/Researcher-administered (RA))	Yes	Some concerns	Yes
[22]	Adesina *et al.*, 2012	Port- Harcourt	Adolescents only	14.5 ± 2.3	♀♂	Secondary school	960	Q(NV/SA)	No	Some concerns	Yes
[23]	Adinma *et al.*, 2020	Nnewi, Anambra state.	Adolescents only	14.5 ± 3.0	♂	Secondary school	311	FFQ (V/RA)	Yes	High Risk	No
[24]	Adu *et al.*, 2009	Lagos State	Adolescent/Adults	NR	♀♂	University	100	Q (NV/SA)	Yes	Some concerns	No
[25]	Afolabi *et al.*, 2013	Abeokuta	Adolescents only	NR	♀♂	University	140	24-hour dietary recall (24 HDR) (NV/SA)	Yes	High Risk	No
[26]	Agofure *et al.*, 2021	Delta State	Adolescents only	NR	♂	Secondary school	201	Q(NV/SA)	No	Some concerns	No
[27]	Agoreyo *et al.*, 2002	Benin City, Edo state	Adolescents only	NR	♂	University	500	Q(NV/SA)	Yes	High Risk	No
[28]	Ajuzie *et al.*, 2018	Ogun State.	Adolescent/Children	NR	♀♂	Primary school	1200	Q(NV/RA)	Yes	High Risk	No
[29]	Akinbodewa *et al.*, 2020	Ondo state	Adolescents only	13.0 ± 2.0	♀♂	Primary school	160	Q(NV/SA)	No	Low Risk	No
[30]	Akinlade *et al.*, 2014	Oyo state	Adolescents only		♀♂	Secondary school	821	Q(V/SA)	Yes	Some concerns	No
[31]	Akinola *et al.*, 2022	Lagos state	Adolescents only	14.3 ± 2.1	♀♂	Secondary school	1120	FFQ (NV/SA)	Yes	Low Risk	No
[32]	Akinyemi *et al.*, 2009	Lagos state.	Adolescents only	NR	♂	Secondary school	40	Q (NV/SA)	Yes	High Risk	No
[33]	Ansa *et al.*, 2008	Calabar Cross river state.	Adolescents only	NR	♀♂	Secondary school	1000	Q (NV/SA)	No	High Risk	No
[34]	Anyika *et al.*, 2009	Abia State	Adolescents only	NR	♂	Secondary school and university	160	Q (NV/SA)	Yes	High Risk	No
[35]	Atawodi *et al.*, 2015	Kaduna state.	Adolescent/Children	NR	♀♂	Secondary school	141	Q (NV/SA)	No	High Risk	No
[36]	Ayogu *et al.*, 2016	Nsukka, Enugu state.	Adolescents only	NR	♀♂	Secondary school	400	Q(NV/SA)	Yes	Low Risk	No
[37]	Ayogu *et al.*, 2018	Enugu state	Adolescent/Children	NR	♀♂	Primary and secondary school	450	Q (V/RA)	Yes	Low Risk	No
[38]	Ayogu *et al.*, 2019	Ede-Oballa, Enugu state	Adolescent/Children	NR	♀♂	Primary and secondary school	450	Diet Diversity Score (DDS) (NV/SA)	Yes	Some concerns	Yes
[39]	Ayogu *et al.*, 2021	South-east Nigeria.	Adolescents only	NR	♀♂	Rural	401	Q (NV/SA)	No	Low Risk	Yes
[40]	Bamidele *et al.*, 2016	Lagos State	Adolescent/Children	NR	♀♂	Primary school	529	Q (NV/RA)	Yes	Some concerns	No
[41]	Charles *et al.*, 2020	Borno State	Adolescents only	NR	♂	Secondary school	612	Q (NV/RA)	No	Some concerns	No

Continued

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Table 1. Continued

Ref.	Author, year	Location	Target population	Age, mean (SD)	Sex	Setting	Sample size	Dietary assessment instrument (validation/ administration)	Intake quantified?	Risk of bias	Journal indexed in MJL
(42)	Cole *et al.*,1997	Ibadan	Adolescents only	NR	♀♂	Secondary school	22	Q (NV/SA)	Yes	High Risk	No
(43)	Darling *et al.*, 2020	Sub-Saharan Africa including Nigeria (Ibadan)	Adolescents only	NR	♀♂	Secondary school	7625	Q (NV/RA)	Yes	Low Risk	No
(44)	Ekekezie *et al.*, 2012	Lagos State	Adolescent/Children	NR	♀♂	Primary school	529	Q (NV/RA)	No	Some concerns	No
(45)	Elizabeth *et al.*, 2009	Makurdi, Benue State	Adolescent/Children	NR	♀♂	Secondary school	600	Q (NV/SA)	No	Some concerns	No
(46)	Ene-Obong *et al.*, 2003	Enugu State	Adolescents only	NR	♀♂	Community	135	Q (NV/SA)	Yes	Some concerns	Yes
(47)	Ene-Obong *et al.*, 2012	Lagos, Rivers, Enugu and Abia state.	Adolescent/Children	NR	♀♂	Community	1,599	Q (V/RA)	No	High Risk	Yes
(48)	Eneobong, 1993	Nsukka, Enugu	Adolescents only	NR	♀♂	Community	50	Q (NV/SA)	Yes	High Risk	Yes
(49)	Erinoso *et al.*, 1992	Olodo, Oyo state	Adolescent/Children	NR	♀♂	Community	400	Q (NV/SA)	Yes	High Risk	Yes
(50)	Esimai *et al.*, 2015	Port Harcourt	Adolescents only	♂:12.5 ± 0.1; ♀:12.6 ± 0.1	♀♂	Secondary school	182	Q (NV/SA)	No	Low Risk	No
(51)	Essien *et al.*, 2014	Sokoto	Adolescent/Adults	♂:18.0 ± 1.9; ♀:15.7 ± 1.2	♀♂	Secondary school	240	Q (V/SA)	No	High Risk	No
(52)	Eze *et al.*, 2017	Enugu State	Adolescent/Adults	NR	♀♂	Secondary school	2616	Q (NV/SA)	No	Some concerns	Yes
(53)	Fadipe *et al.*, 2017	Lagos, state.	Adolescent/Adults	21.4	♀♂	University	1,054	Q (NV/SA)	Yes	Some concerns	Yes
(54)	Fagbamigbe *et al.*, 2019	Oyo state	Adolescent/Children	NR	♀♂	Secondary school	390	Q (NV/RA)	No	Some concerns	Yes
(55)	Folashade *et al.*, 2016	Ogun State	Adolescents only	NR	♀♂	Secondary school	572	Q (NV/SA)	No	Low Risk	No
(56)	Funke and Ajayi, 2007	Osun State	Adolescents only	NR	♀♂	Secondary school	450	Q (NV/SA)	Yes	Some concerns	No
(57)	Goon *et al.*, 2011	Makurdi, Benue State	Adolescents only	NR	♀♂	Secondary school	2015	Q (NV/SA)	No	Some concerns	Yes
(58)	Henry-Unaeze *et al.*, 2011	Nnewi, Abia state	Adolescents only	NR	♀♂	Secondary school	200	Q (NV/SA)	No	High Risk	No
(59)	Ikorok *et al.*, 2012	Akwa Ibom	Adolescents only	NR	♀♂	Secondary school	450	Q (V/RA)	No	Some concerns	No
(60)	Ikujenlola and Adekoya, 2020	Osun state	Adolescents only	NR	♂	University	200	FFQ (V/SA)	Yes	High Risk	Yes
(61)	Iyalomhe *et al.*, 2018	Ekpoma, Edo state	Adolescents only	NR	♀♂	Secondary school	400	Q (V/SA)	Yes	Some concerns	No
(62)	Kayode *et al.*, 2020	Ede, Osun State	Adolescent/Adults	19.8 ± 1.3	♀♂	University	268	FFQ (NV/SA)	Yes	Some concerns	No
(63)	Kelvin and Sanusi, 2016	EKITI state	Adolescents only	14.4 ± 1.9	♀♂	Secondary school	789	24 HDR (NV/RA)	Yes	Some concerns	No
(64)	Kola-Raji *et al.*, 2017	Ibadan, Oyo state.	Adolescents only	♂:12.9 ± 2.05; ♀:14.0 ± 1.49	♀♂	Secondary school	490	Q (NV/RA)	No	High Risk	No
(65)	Lateef *et al.*, 2016	Kwara state	Adolescents only		♀♂	Secondary school	515	FFQ (NV/SA)	Yes	High Risk	No
(66)	Nnanyelugo *et al.*, 1982	Anambra	Adolescent/Children	NR	♀♂	Community	2036	Q (NV/SA)	Yes	Some concerns	No
(67)	Nwokoro *et al.*, 2006	Benin city, Edo state.	Adolescent/Adults	NR	♀♂	Secondary school	2012	Q (NV/SA)	No	High Risk	No
(68)	Ogechi *et al.*, 2007	Umuahia, Abia State	Adolescents only	NR	♀♂	Secondary school	190	Q (NV/SA)	Yes	High Risk	No

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Table 1. Continued

[69]	Ogechi *et al*., 2012	Umuahia, Abia State	Adolescents only	NR	♀♂	Secondary school	416	Q (NV/SA)	Yes	High Risk	No
[70]	Ogunkunle and Oludele, 2013	Ila Orangun, Osun state	Adolescents only	NR	♀♂	Secondary school	302	FFQ (NV/RA)	Yes	High Risk	No
[71]	Ogunsile,2012	Ekiti state	Adolescents only	NR	♀♂	Secondary school	128	Q (NV/SA)	Yes	Low Risk	No
[72]	Oguntona and Kanye, 1995	Ogun State	Adolescents only	NR	♀♂	Secondary school	187	Q (NV/SA)	Yes	High Risk	No
[73]	Okeke *et al*., 1989	Anambra	Adolescent/Children	NR	♀♂	Community	387	Q (NV/SA)	Yes	High Risk	Yes
[74]	Okoro *et al*., 2016	Ibadan, Oyo state	Adolescent/Children	13.6 ± 3.6	♀♂	Secondary school	464	FFQ (V/SA)	Yes	Some concerns	Yes
[75]	Okpokowuruk *et al.,* 2017	Uyo, Akwa Ibom	Adolescent/Children	NR	♀♂	(I+II)	195	Q (NV/SA)	No	Some concerns	No
[76]	Olatona *et al*., 2018	Lagos state.	Adolescent/Adults	20.3 ± 3.5;	♀♂	University	506	FFQ (V/RA)	Yes	High Risk	Yes
[77]	Olatona *et al*., 2020	Lagos state	Adolescents only	3.6 ± 2.3	♀♂	Community	682	Q (NV/RA)	Yes	High Risk	No
[78]	Olatona *et al*., 2022	Lagos State.	Adolescents only	13.8 ± 1.7	♀♂	Secondary school	397	Q (NV/SA)	Yes	High Risk	Yes
[79]	Olorunfemi *et al*., 2019	Kaduna, State	Adolescents only	14.3 ± 2.8	♀♂	University	50	Q (V/RA)	No	Some concerns	No
[80]	Olumakaiye *et al*., 2010	Osun State	Adolescents only	NR	♀♂	Secondary school	401	Q (V/SA)	Yes	High Risk	Yes
[81]	Olumakaiye, 2013	Osun State	Adolescent/Children	NR	♀♂	Secondary school	600	24 HDR (V/SA)	Yes	High Risk	No
[82]	Olumuyiwa *et al*., 2012	Ile-Ife, Osun State	Adolescent/Children	NR	♀♂	Primary school	160	Q (NV/SA)	Yes	High Risk	No
[83]	Oluyinka *et al*., 2020	Iwo Osun State	Adolescents only	NR	♀♂	University	216	Q (NV/SA)	Yes	Some concerns	No
[84]	Omigbodun *et al*., 2010	Ibadan, Oyo state	Adolescents only	NR	♀♂	Secondary school	1799	Q (NV/SA)	Yes	Some concerns	Yes
[85]	Omobuwa *et al*., 2014	Ibadan, Oyo state	Adolescents only	15.67 ± 1.25	♀♂	Secondary school	93	Q (NV/SA)	Yes	Some concerns	No
[86]	Omuemu and Oko-Oboh, 2015	Benin city, Edo state	Adolescents only	15.4 ± 3.6	♀♂	Secondary school	797	Q (NV/RA)	Yes	High Risk	No
[87]	Omuemu *et al*., 2010	Benin-city	Adolescents only	15.3 ± 1.9	♀♂	Community	300	Q (NV/RA)	No	High Risk	Yes
[88]	Onabanjo and Balogun, 2014	Ogun State	Adolescents only	16.0 ± 2.3	♀♂	Secondary school	127	Q (V/RA)	Yes	Low Risk	No
[89]	Onimawo *et al*., 2010	Abia state	Adolescent/Children	NR	♀♂	Primary school	249	FFQ (NV/SA)	Yes	Some concerns	No
[90]	Oninla *et al*., 2007	Ile-Ife,Osun State	Adolescent/Children	10.2 ± 2.7	♀♂	Rural	749	Q (NV/SA)	No	Some concerns	Yes
[91]	Onofiok *et al*., 1996	Emene, Nsukka	Adults/Adolescents/ Children	NR	♀♂	Community	1030	Q (NV/SA)	Yes	High Risk	No
[92]	Onuoha and Eme, 2013	Aba, Abia state	Adolescents only	4.6 ± 1.8	♀♂	Secondary school	600	Q (NV/SA)	Yes	High Risk	No
[93]	Onyechi and Okolo, 2009	Enugu, State	Adolescents only	NR	♀♂	University	620	Q (V/SA)	Yes	High Risk	No
[94]	Onyiriuka *et al*., 2013	Benin City, Edo state	Adolescents only	14.8 ± 1.9	♂	Community	2,097	Q (NV/SA)	Yes	Some concerns	No
[95]	Onyiriuka *et al*., 2013	Edo State	Adolescents only	14.5 ± 2.0	♂	Secondary school	2,304	Q (NV/SA)	Yes	Some concerns	No
[96]	Onyiriuka *et al*., 2013	Edo State	Adolescents only	NR	♂	Secondary school	2 097	Q (NV/SA)	Yes	Some concerns	No
[97]	Opara and IEE, 2010	UYO, Akwa Ibom state	Adolescent/Children	NR	♀♂	Community	500	Q (NV/SA)	No	Some concerns	No
[98]	Oranusi *et al.,* 2007	Zaria, Kaduna state	Adolescent/Adults	NR	♀♂	Community	44	Q (NV/SA)	Yes	High Risk	No

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Table 1. Continued

Ref.	Author, year	Location	Target population	Age, mean (SD)	Sex	Setting	Sample size	Dietary assessment instrument (validation/ administration)	Intake quantified?	Risk of bias	Journal indexed in MJL
(99)	Orisa and Wordu, 2021	Portharcourt, Rivers state	Adolescents only	NR	♂	Secondary school	236	Q (V/RA)	Yes	High Risk	No
(100)	Otekunrin and Otekunrin,2022	Ogun and Oyo States	Adolescents only	13.6 ± 2.7	♀♂	Community	160	Q (NV/SA)	Yes	Some concerns	No
(101)	Otemuyiwa and Adewusi, 2012	Osun and Ondo State	Adolescent/Adults	NR	♀♂	University	402	FFQ (NV/SA)	Yes	High Risk	Yes
(102)	Otuneye *et al.*, 2017	FCT – Abuja	Adolescents only	14.4 ± 1.9	♀♂	Secondary school	1550	Q (NV/SA)	Yes	Some concerns	No
(103)	Samuel *et al.,* 2015	Ibadan, Oyo state	Adolescents only	NR	♀♂	Community	190	FFQ (NV/RA)	Yes	High Risk	No
(104)	Samuel *et al.,* 2021	Ibadan, Oyo state	Adolescents only	16.9 ± 1.9	♀♂	Community	300	FFQ (NV/SA)	Yes	Some concerns	No
(105)	Sanusi *et al.*, 2021	Ibadan, Oyo state	Adolescents only	NR	♀♂	Community	433	24 HDR (NV/RA)	Yes	High Risk	Yes
(106)	Senbanjo *et al.*, 2011	Abeokuta, Ogun state	Adolescent/Children	12.2 ± 3.4	♀♂	Secondary school	570	Q (NV/SA)	No	Some concerns	Yes
(107)	Senbanjo *et al.*, 2014	Abeokuta, Ogun state	Adolescent/Children	NR	♀♂	Secondary school	570	Q (NV/SA)	No	Some concerns	Yes
(108)	Shapu *et al.*, 2020	Borno State	Adolescents only	NR	♂	Secondary school	612	Q (NV/SA)	No	Some concerns	Yes
(109)	Shokunbi and Ukangwa, 2021	Ogun State	Adolescents only	NR	♀♂	Secondary school	488	Q (NV/SA)	Yes	Some concerns	Yes
(110)	Sholeye *et al.*, 2018	Sagamu, Ogun state	Adolescents only	15.6 ± 1.2	♀♂	Secondary school	620	Q (NV/SA)	No	High Risk	No
(111)	Silva *et al.*, 2017	Lagos State	Adolescents only	12.4	♀♂	Secondary school	220	Q (NV/RA)	Yes	Some concerns	No
(112)	Tassy *et al.*, 2021	Ibadan, Osun state	Adolescent/Children		♀♂	Community	955	24 HDR (NV/RA)	Yes	Some concerns	Yes
(113)	Uba *et al.*, 2020	Bauchi State	Adolescents only	15.9 ± 0.9	♂	Secondary school	250	Q (V/SA)	No	Some concerns	No
(114)	Umeokonkwo *et al.*, 2020	Ebonyi State	Adolescent/Children	NR	♀♂	Primary school	780	Q (NV/RA)	No	Some concerns	Yes
(115)	Wariri *et al.,* 2020	Gombe State and Uyo, Akwa Ibom state	Adolescents only	NR	♀♂	Community	2100	Q (V/RA)	No	Some concerns	Yes
(116)	Yunusa *et al.*, 2014	Nigeria	Adolescents only	NR	♀♂	Community	270	Q (NV/SA)	Yes	High Risk	No

Note: ♀, Male; ♂, Female; NR, Not Reported.

Table 2. Anthropometric findings from Nigerian nutritional epidemiology studies

Ref.	Author, year	Primary anthropometric measures	Composite anthropometric measures (%)
(26)	Agofure *et al.*, 2021	NR	Underweight: 46.8 Normal weight: 31.8 Overweight: 21.4
(104)	Samuel *et al.*, 2021	NR	Stunting (males): 20.5 Stunting (females): 6.8 Stunting: 12.1 Overweight/obese: 9.5
(74)	Okoro *et al.*, 2016	Average BMI (kg/m^2): 17.8	Underweight occurrence was high
(60)	Ikujenlola and Adekoya, 2020	BMI(kg/m^2) : Private University 22.2 ± 3.5, Public University 21.9 ± 4.2	Normal weight: 67.5 Underweight: 15 Overweight: 12 Obesity: 4.5
(18)	Adeomi *et al.*, 2019	NR	Overweight and obesity: 10.2 Underweight: 12.1 Overweight: 8.9 Obesity: 1.3 Overweight/Obesity (females): 12.5 Overweight and Obesity (males): 7
(102)	Otuneye *et al.*, 2017	NR	Overweight: 12.6 Stunting: 11.2 Obesity: 2.8 Wasting: 1.7
(21)	Adeomi *et al.*, 2022c	NR	Thinness: 10.3 Overweight/Obesity: 11.4 Rural; Thinness: 12.8 Urban; Thinness: 7.7 Rural; Obesity/Overweight: 7 Urban; Obesity/Overweight: 15.8
(20)	Adeomi *et al.*, 2022b	NR	Thinness: 10.3 Overweight/Obesity: 11.4
(89)	Onimawo *et al.*, 2010	Triceps (m) (mean ± standard deviation): 0.0043 ± 1.8; Height (m): 1.3 ± 9.7; Weight (Kg): 28.4 ± 5.8; Subscapular (m):0.0043 ± 1.4; Suprailiac (m): 0.0035 ± 1.4; waist circumference (m): 0.0045 ± 1.8	Stunting: 77.1 Wasting: 56 Underweight: 77.1
(35)	Atawodi *et al.*, 2015	NR	Underweight: 35 Stunting: 26.4
(36)	Ayogu *et al.*, 2016	12-14yrs.: Weight (Kg): 48.2; Height(m): 1.5 15–18 yrs.: Weight (Kg): 56.3; Height (m): 1.6	Stunting: 33.3; Thinness: 31
(32)	Akinyemi *et al.*, 2009	Weight (Kg): 58.9 Height(m): 1.7 BMI(Kg/m^2): 21.4	NR
(45)	Elizabeth *et al.*, 2009	BMI(Kg/m^2): 16.3 ± 2.4 for males and for females, 16.7 ± 2.7	L.G.E.A Wurukum: Underweight: 73.3 C.A.C Wadata: Underweight: 78.3 B.S.Urban: 34.16 Underweight: 34.2 Urban.A.M: Underweight: 38.3 Nativity School: underweight: 28.3
(17)	Adebimpe, 2019	NR	3.8 Obese/Overweight
(79)	Olorunfemi *et al.*, 2019	Weight (Kg): 35.9 ± 10.4	Overweight: 6 Obesity: 6 Thinness: 42 Severe thinness: 38
(39)	Ayogu *et al.*, 2021	NR	Male: Overweight: 3.7 Female: Overweight: 2.8
(47)	Ene-Obong *et al.*, 2012	NR	Overweight: 11.4 Obesity: 2.8 Thinness: 13
(53)	Fadipe *et al.*, 2017	Weight (Kg):63.3 (± 11.14); Height (m) :1.7 (± 0.9); BMI (Kg/m^2): 22.2 (± 3.6)	NR
(54)	Fagbamigbe *et al.*, 2019	NR	**Males:** Underweight: 19, Stunting: 27, Thinness: 27 **Females:** Underweight: 17, Stunting: 23, Thinness: 23
(101)	Otemuyiwa and Adewusi, 2012	Mean BMI (kg/m^2): Males (AAU): 23.6; Males (OAU): 22.3	Overweight: 29 Obesity: 6 Underweight: 13
(76)	Olatona *et al.*, 2018	NR	Overweight: 15 Obesity: 5

Continued

Table 2. Continued

Ref.	Author, year	Primary anthropometric measures	Composite anthropometric measures (%)
(93)	Onyechi and Okolo, 2009	NR	Obesity: 21
(24)	Adu *et al*., 2009	BMI(Kg/m^2): 24.6 ± 3.3; Mid arm circumference (m): 0.3 ± 3; Weight (Kg): 64 ± 9.1; Height(m): 1.6 ± 0.23	NR
(106)	Senbanjo *et al*., 2011		Stunting: 17.4 Severely Stunted: 22.2
(75)	Okpokowuruk *et al*., 2017	Mean weight (Kg): 37.4 Mean height(m): 1.4 Waist circumference(m): 0.6	NR
(112)	Tassy *et al*., 2021	NR	Obesity: 1.6 Overweight: 3.0 Thinness: 21.0 Stunted: 18.2
(88)	Onabanjo and Balogun, 2014	Weight (kg): 43.3 ± 7.8 (male 41.9 ± 8.5, female 44.6 ± 9.3); Waist (m): 0.6 ± 13.7 (male 0.6 ± 12.6, female 0.7 ± 13.9)	Underweight: 21.3; Overweight: 14.2; Waist/hip(m): 0.82 ± 0.3 (male 0.8 ± 0.3, female 0.9 ± 0.5)
(114)	Umeokonkwo *et al*., 2020	NR	Underweight: 8 Thinness: 7.2 Stunting: 9.9 Overweight: 1.4 Obesity 0.7
(40)	Bamidele *et al*., 2016	NR	Rural: Overweight 15.1; Underweight: 49.6; Wasting: 24.2 Urban : Overweight: 13.2; Underweight: 15; Wasting: 13.6
(44)	Ekekezie *et al.,* 2012	NR	**Rural area:** Underweight: 49.6; Stunting: 50.8; Wasting: 24.2 **Urban area :** Underweight: 15.1; Stunting: 16.6; Wasting: 13.6; Overweight: 5.1; Obesity: 13.2
(16)	Abidoye and Akande, 2000	NR	**Upland (urban):** Stunting: 15.8; Wasting: 3.3; Underweight: 14.2 **Riverine (rural):** Stunting: 30; Wasting: 1.7; Underweight: 18.3
(80)	Olumakaiye *et al*., 2010	NR	Underweight: 20.1 (rural 22.1 and urban 18.7)
(107)	Senbanjo *et al*., 2014	NR	Stunting: 75 Thinness: 66.7
(83)	Oluyinka *et al*., 2020	NR	Underweight: 2.5 Obesity:5 Overweight: 25.2
(55)	Folashade *et al*., 2016	NR	Wasting: 26.7 Stunting: 24.8
(28)	Ajuzie *et al*., 2018	Mean weight (Kg): 19 – 70 Mean height(m): 1.2 – 1.6	Underweight: 45.8
(19)	Adeomi *et al*., 2022a	NR	Overweight/ Obesity: 12.8
(23)	Adinma *et al*., 2020	Weight (Kg): 51.8 ± 10.4 Height(m): 1.6 ± 0.1 BMI (kg/m^2): 20.1 ± 3.5	Underweight: 7.7 Overweight: 9.0 Obesity: 1.3
(30)	Akinlade *et al*., 2014	Height (m): Male 1.6 ± 8.5, female 1.6 ± 6.7 BMI (kg/m^2): Male 19.8 ± 2.8, female 20.5 ± 3.1 Triceps (m): (Male 0.006 ± 3.6, female 0.01 ± 4.5) Biceps (m): male 0.01 ± 4.0, female 0.01 ± 4.1 Waist (m): male 0.7 ± 6.4, female 0.7 ± 7.1 Hip (m): 0.9 ± 8.0, female 0.9 ± 7.9	BMI-for-Age percentile: 82.5: 5th-85th percentiles (Normal); 2.2: 85th-95th percentiles (Overweight); 2.6: 95th percentile (Obesity)
(33)	Ansa *et al*., 2008	NR	Obesity: 1.7 Overweight: 6.8
(57)	Goon *et al*., 2011	NR	Underweight (WAZ < –2): 43.4 Stunting (HAZ < –2): 52.7
(22)	Adesina *et al*., 2012	NR	underweight 6.4, overweight 6.3, obesity 1.8 and stunting 5.4
(61)	Iyalomhe *et al*., 2018	NR	Underweight: 24 Overweight: 3 Obesity: 1
(85)	Omobuwa *et al*., 2014	NR	Overweight and obesity: 7.6
(68)	Ogechi *et al*., 2007	Weight (Kg): 56.5 ± 8.6(boys), 53.5 ± 6.9 (girls) Height (m): 1.7 ± 6.3 (boys) and 1.6 ± 5.3 (girls) BMI (Kg/m^2): 20.0 ± 2.6 boys and 20.50 ± 2.38	Overweight: 4 males and 2 females; Stunting: 67.3 males and 57.8 females
(63)	Kelvin and Sanusi, 2016	Waist(m): 0.8 ± 0.1 Hip circumference(m)0.7 ± 0.1M Waist-hip ratio(m) 0.9 ± 0.1 BMI-for- age 45.1 percentile	Underweight: 11.7 Overweight: 8.7 Obesity: 4.9

Continued

Table 2. Continued

Ref.	Author, year	Primary anthropometric measures	Composite anthropometric measures (%)
(64)	Kola-Raji *et al*., 2017	NR	Stunting: 2.5 Underweight: 39.3 Overweight: 8 Obesity: 0.8
(97)	Opara and IEE, 2010	NR	Private school: Underweight: 27.3; Stunting 17.1; Obesity 11.1 Public school: underweight: 39.4; Stunting: 25.3; Obesity: 0.2
(52)	Eze *et al*., 2017	Height (m): 1.5 ± 10.2 Weight (Kg): 29.7 ± 7.7 BMI (Kg/m^2) 15.7 ± 2.4	Wasting: 9.3 Overweight: 6.3 Obesity: 4.4 Underweight: 0.9 Stunting: 0.4
(43)	Darling *et al*., 2020	NR	Underweight:18.6 Overweight:4.8 Stunting:15.9
(84)	Omigbodun *et al*., 2010	Males: Height (m) mean (SD): 1.6 (0.13); Weight (Kg): 43.7 (10.61); BMI (Kg/m^2): 17.31 (2.29) Females: Height (m): 1.6 (0.1); Weight (Kg): 43.3 (9); BMI (Kg/m^2): 17.9 (2.7)	**Males:** Underweight: 213 (23.1); Height: 213 (23.1); Overweight: 10 (1.1) **Females:** Underweight: 127 (14.5); Height: 69 (7.9); (overweight): 32 (3.7)
(115)	Wariri *et al*., 2020	NR	**Overall**: Thinness:6.8; Overweight/Obesity: 12.4; Stunted: 6.4 **Gombe:** Overweight/Obesity: 16.1; Thinness: 12 **Uyo:** Thinness: 5.3; Overweight/Obesity.: 11.3
(65)	Lateef *et al*., 2016	BMI (Kg/m^2):19.7 ± 2.6	Overweight: 4.7 Obesity: 0.2 Underweight: 29.1 Overweight: male 0.6 and female 6.7 Obesity: (male 0, female 0.3)
(69)	Ogechi *et al*., 2012	BMI (Kg/m^2):19.9 ± 2.6 (boys); Girls: 23.0 ± 3.9	NR
(77)	Olatona *et al*., 2020	NR	Undernutrition: 5.4 Overweight: 10.7 Obesity: 5.3
(78)	Olatona *et al*., 2022	NR	**Males:** Overweight: 7.1; Obesity: 3.3 **Females:** Overweight: 7.1; Obesity: 2.8
(92)	Onuoha and Eme, 2013	NR	Overweight: 3.0 and 6.7, in males and females, respectively Obesity: 1 and 2.5, in males and females, respectively
(103)	Samuel *et al*., 2015	NR	Stunting: 12.1 Overweight/Obesity: 9.5
(113)	Uba *et al*., 2020	NR	Underweight: 36. Overweight: 9.6
(15)	Abdulkarim *et al*., 2014	Mean BMI 20.31 ± 3.07kg/m^2, Weight 51.07 ± 10.80 kg, Height 1.6 ± 9.33 m.	Wasting 1.7, Stunting 11.3, Overweight 13.2, Obesity 2.6
(37)	Ayogu *et al*., 2018	NR	Underweight (18.2), Stunting (41.6), Thinness (20.0)
(50)	Esimai *et al*., 2015	mean height 1.55 m mean weight 45.2 kg mean BMI 18.5 kg/m2	Underweight 46.2, Overweight 6.6 Stunting 36.3
(51)	Essien *et al*., 2014	NR	Underweight 27.9 Overweight 7.5
(58)	Henry-Unaeze *et al*., 2011	mean BMI 19.2 ± 3.06kg/m2	Underweight 44.0 Overweight 5.0
(62)	Kayode *et al*., 2020	NR	Underweight 2.2, Overweight 31 Obese 9.3
(90)	Oninla *et al*., 2007	Mean Weight 25.5 ± 6.5 kg. Mean Height of 1.3 ± 12.9m	Underweight 61.2 Wasting 16.8 Stunting 27.6

Note: Values are given in percentage unless otherwise indicated.

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Table 3. Food and food group-based findings

		Food(s) and food group(s) (proportion of respondents)[a]								Snacking and drinks	
Author, year	**Ref.**	**Starchy staples (cereals, starchy roots/ tubers)**	**Legumes, nuts, and seeds**	**Meat, poultry, fish, and sea-food**	**Vegetables**	**Fruits**	**Eggs**	**Dairy and milk products**	**Sweets**	**Snacking**	**Drinks**
Agofure *et al*., 2021	(26)	47.8 (very often)	NR	NR	NR	38.8 (sometimes)	41.3 sometimes	33.3 (sometimes)	35.3 (sometimes)	NR	NR
Samuel *et al*., 2021	(104)	NR	NR	NR	NR	NR	NR	NR	NR	96.8	NR
Ayogu *et al*., 2019	(38)	96.7	60	46.7	32.1	35.6	13.3	47.8	NR	NR	NR
Okoro *et al.,* 2016	(74)	62.3	NR	62.1	NR	67.6	NR	62	62.3[s]	NR	NR
Ikujenlola and Adekoya, 2020	(60)	NR	9.5 (≥ 3 times/week), 90.5 (≤ 2 times/week)	49.5 (red meat), 22.4 (white meat), 28.1 (fish), 51.8 (≥ 3 times/week), 48.2 (≤ 2 times/ week)	NR	29.1 ≥ 3 times/week; 70.9 ≤ 2 times/week	NR	44.7 (≥ 3 times/week), 55.3 (≤ 2 times/week)	NR	74.9	NR
Orisa andWordu, 2021	(117)	54.1 (1-2 times/ week)	33.9 (1-2 times/week)	51.3 (fish and meat daily)	47.9 (1-2 times/ week)	33.05 (daily)	NR	36.9 (1-2 times/week)	NR	49.6 (daily)	38.1 (1–2 times/ week)
Otuneye *et al*., 2017	(102)	NR	NR	NR	NR	NR	NR	NR	NR	NR	35.2
Sanusi *et al*., 2021	(105)	NR	54.6	NR	NR	60 (≤ 1 daily)	NR	25.8	NR	NR	NR
Onyiriuka *et al*., 2013	(94)	NR	NR	NR	NR	11.3 (1–3 times/week)	NR	NR	53.4 (1–3 times/week)	NR	79.4 (1–3 times/ week)
Ogunkunle & Oludele, 2013	(70)	76.5	52.2	48.80	70.8	69.8	NR	NR	NR	87.2	NR
Ayogu and Nwodo, 2021	(39)	NR	NR	NR	29.7 (males), 45.1 (females)	NR	NR	NR	NR	NR	NR
Afolabi *et al*., 2013	(25)	49.3	18.6	84.4	73.7	73.7	NR	73.8	NR	NR	NR
Otemuyiwa and Adewusi, 2012	(101)	58 (males), 62 (females)	NR	35 (males),42 (females)	NR	20 (males),40 (females)	NR	10 (males), 25 (females)	NR	NR	NR
Olatona *et al*., 2018	(76)	28.2	NR	32 meat, 10 fish	NR	NR	NR	14	NR	44.0	29, Alcohol: 6
Onyechi &Okolo, 2009	(118)	NR	NR	NR	26.7	28.20	NR	NR	NR	33.6	NR
Bamidele *et al*., 2016	(40)	NR	NR	NR	83 (rural), 63.1 (urban)	76.9 (rural), 77.4 (urban)	NR	NR	NR	NR	25.0 (rural), 61.1 (urban)
Olumakaiye *et al*., 2010	(80)	NR	NR	NR	NR	NR	NR	NR	NR	33.00	NR
Oluyinka *et al*., 2020	(119)	NR	NR	NR	10.1	16.10	NR	NR	NR	NR	NR
Agoreyo *et al*., 2002	(27)	NR	NR	NR	NR	NR	NR	22.6	NR	NR	99.4 (beverages except milk), 11.6 (milk beverages), 50.6 (diet soda)
Ansa *et al*.,2008	(120)	NR	NR	NR	NR	NR	NR	NR	NR	NR	97.20

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Table 3. Continued

Akinola *et al.*, 2022	(31)	NR	NR	NR	NR	28	NR	NR	NR	NR	11 (sugar-sweetened beverages daily), 20 (most days of the week)
Funke *et al.*, 2007	(56)	NR	NR	NR	NR	NR	NR	NR	NR	51.40	NR
Ogunsile, 2012	(71)	NR	NR	NR	10.2	16.4	NR	7.0	50 (sweets), 38.3 (chewing gum)	NR	45.3
Olatona *et al.*, 2020	(77)	73.5	NR	NR	NR	9.7	NR	NR	NR	69.6	46.8
Onyiriuka *et al.*, 2013	(96)	NR	NR	NR	NR	11.3	NR	NR	53.4 (sugar), 62.4 (ice cream)	76.4	9.40
Onyiriuka etal., 2013	(95)	89.6	NR	19.1	15.2	NR	NR	8.1	41.2	NR	58.7
Sholeye *et al.*, 2018	(110)	NR	NR	NR	NR	NR	NR	NR	NR	NR	8.5 (carbonated drinks), 44.2 (energy drinks)
Uba *et al.*,2020	(113)	78.4	NR	NR	55.2	NR	NR	88.8	NR	NR	NR

[a]Values are provided in percentage (%), unless otherwise indicated.

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Table 4. Dietary intake by adolescents in Nigeria

Author, year (ref.)	Location	Subject	Method of assessment	Intake reported	Intake value		Nutritional targets		Key findings
Ayogu, 2019 [38]	Ede- Oballa, Southeast	450 in-school children and adolescents	DDS	Energy (Kcal)	Male	Female	Males	Females	Energy, protein and carbohydrate intakes did not meet the recommended nutrients intake for neither sex. However, the girls' mean carbohydrate intake was higher than boys.
					Data for 10–12 years school adolescents				
					1775.6 ± 508.8	1740.1 ± 452.6	260	2350	
				Protein (g)	45.1 ± 14.7	48.5 ± 24.0	47	58	
				Fat (g)	54.1 ± 25.2	60.1 32.6	NA	NA	
				Carbohydrate (g)	274.4 ± 96.1	284.1 ± 164.1	1430	1293	
					Data for 10–12 years school adolescents				
				Energy (Kcal)	1917.8 ± 559.3	1771.1 ± 681.8	2900	2490	
				Protein (g)	40.2 ± 26.6	35.9 ± 23.2	45	49	
				Fat (g)	51.9 ± 35.9	75.9 ± 44.6	NA	NA	
				Carbohydrate (g)	154 ± 94.5	321.3 ± 304.9	1595	1370	
Sanusi et al, 2021 [105]	Ibadan	433 school-aged adolescents	24- hour dietary recall	Prudent Pattern (n = 474)					Foods consumed by the target population does not provide an adequate supply of nutrients
				Energy (kcal)	1592.8 ± 578		NR	NR	
				Protein (g)	55.2 ± 22.7		NR	NR	
				Carbohydrates (g)	259 ± 96.7		NR	NR	
				Traditional South-Western Nigerian Pattern (n = 481)					
				Energy (kcal)	1414.7 ± 494.5		NR	NR	
				Protein (g)	43.2 ± 18.4		NR	NR	
				Carbohydrates (g)	235.8 ± 88.3		NR	NR	
Ogunkunle and Oludele, 2013 [70]	Ila Orangun, southwest	302 public school adolescents	FFQ	Energy (kcal)	Below reference (BR): 24.4; Above reference (AR): 200		NR	NR	Insufficient calcium and iron intake were observed.
				Carbohydrates (g)	BR 43; AR 187		NR	NR	
				Lipids (g)	BR 9; AR 153		NR	NR	
				Proteins (g)	BR 127;AR 73		NR	NR	
				Calcium (mg)	BR 281; AR 21		NR	NR	
				Iron (mg)	BR 145; AR 157		NR	NR	
Onimawo IA *et al*., 2010 [121]	Abia State	249 rural school children	24-hour dietary recall and FFQ	Iron (mg)	6.8		10		According to the findings of this study, children's iron intake was approximately 30% lower than RDA.
				Protein (g)	32		34		
				Fat (g)	32		NR		
				Carbohydrate (g)	250		NR		
				Energy (kcal)	1220		1800–2000		
				Zinc (mg)	4.5		10		
Anyika *et al*., 2009 [34]	Abia State	160 Secondary school and university students	Questionnaire	Energy (kcal)	3838.3 for secondary school students (SS); 5754.7 for university students (US)		1957.4–2158.2		The conclusion of this study indicate that energy intake was higher than the recommended nutrient intake in all the schools studied.
				Carbohydrate(g)	SS: 757.1; US: 937.60		NR	NR	
				Protein (g)	SS: 93.5; US: 135.4		NR	NR	
				Fat (g)	SS: 50; US: 157.1		NR	NR	
				Iron (mg)	SS: 31.6; US: 37.1		NR	NR	
				Vit. A (µg RE)	SS: 6296.1; US: 9135.2		NR	NR	
				Vit C. (mg)	SS: 94.1; US: 179.7		NR	NR	

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Table 4. Continued

Akinyemi& Ibraheem, 2009 [32]	Lagos	40 secondary-school adolescents	Questionnaire	Energy (kcal)	1546.8		2310–2350	2600–3070	This study shows inadequate nutrient intake.
				Protein (g)	56		29–30	30–38	
				Vitamin C (mg)	30.3		20–30	20–30	
Ene-Obong *et al.*, 2003 [46]	Nsukka, Enugu state	135 in-school adolescents	3-day weighted food intake	Vitamin A (ug RE/day)					Vitamin C intake was below recommendations, while vitamin A exceeded recommendations
				13–15 years	820 ± 61	766 ± 26	137	131	
				16–18 years	765 ± 20	776 ± 25	127	126	
				19–20 years	797 ± 19	NR	133	NR	
				Vitamin C (mg/day)					
				13–15 years	24.3 ± 1.2	19.5 ± 5.1	81	65	
				16–19 years	23.7 ± 0.7	15.3 ± 2.8	77	51	
				19–20 years	27.3 ± 8.0	NR	91	NR	
Otemuyiwa and Adewusi, 2012 [101]	Osun and Ondo State	402 university students	FFQ	Obafemi Awolowo University					70% of participants met RDA for iron, whereas just 15% met the calcium RDA. Iron, calcium and phosphorus intake did not differ significantly between sexes or institutions.
				Protein (g)	84 ± 16	83 ± 25	1.1	NR	
				Iron (mg)	15 ± 4b	13 ± 5	10	15	
				Calcium (mg)	311 ± 110	319 ± 203	800		
				Phosphorus (mg)	771 ± 219	795 ± 323	1000		
				Adekunle Ajasin University					
				Protein (g)	83 ± 14	88 ± 22	1.1		
				Iron (mg)	15 ± 4	14 ± 4	10	15	
				Calcium (mg)	343 ± 18	369 ± 250	800		
				Phosphorus (mg)	724 ± 292	849 ± 333	1000		
Shokunbi& Ukangwa, 2021 [109]	Ilishan- Remo, Ogun State	488 secondary school adolescents	Questionnaire	Sodium (mg)	2404 ± 902	2225 ± 971	1200–1500		Sodium intake exceeded RDA, while potassium fell below it.
				Potassium (mg)	1384 ± 972	1298 ± 588	2300–3000		
Tassy *et al.*, 2021 [112]	Ibadan, Oyo state	955 secondary-school adolescents	Multiple-pass 24h dietary recall	Energy (kcal)	1590 ± 295		NR	NR	According to findings of study, there were greater micro-nutrients deficiencies among adolescents.
				Carbohydrates (g)	271.1 ± 50		NR	NR	
				Protein (g)	50 ± 15		NR	NR	
				Calcium (mg)	301 ± 68	1100	NR	NR	
				Iron (mg)	11 ± 2	16.4	NR	NR	
Oguntona and Kanye, 1995 [72]	Ogun state	187 secondary-school adolescents	Questionnaires and 24-hour recall	Energy (kcal)	2593.2 ± 239		NR	NR	This study showed that protein intake was relatively high for both sexes, with 10% of total protein intake coming from animal sources.
				Protein (g)	62.6 ± 12.3		NR	NR	
				Animal protein (g)	4.1 ± 1.4		NR	NR	
				Oils and fats (g)	29.8 ± 0.6		NR	NR	
				Carbohydrates (g)	82 ± 12.4		NR	NR	
				Cholesterol (g)	9.1 ± 0.2		NR	NR	
				Fibre (g)	33.1 ± 0.5		NR	NR	
Akinlade *et al.*, 2014 [30]	Oyo state	821 Secondary School adolescents	Questionnaire	Calorie (kcal)	1726.8 ± 327.5		1040 – 2197		Carbohydrate intake was above RNI for both sexes, while fat, iron and zinc intakes were within RNI. Protein, vitamin A, C and B12, folate, and calcium were below recommendations.
				Protein (g)	39.3 ± 10.7		11 – 57.8		
				Carbohydrate(g)	170.6 ± 56.5		89.8 – 294		
				Fibre (g)	13.1 ± 10.7		0 – 68.9		
				Fat (g)	29.9 ± 14.1		1.2 – 54.2		
				Vitamin A (RE)	478.4 ± 293.7		19.6 – 978.9		
				Vitamin C (mg)	7.2 ± 5.7		0– 89.9		
				Folate (mcg)	136.6 ± 118.1		0– 642.1		
				Vitamin B12 (mcg)	1.4 ± 1.1		0 – 6.4		
				Calcium (mg)	305.3 ± 216.2		27– 885.7		
				Zinc (mg)	6.9 ± 3		0.01 – 15.9		
				Iron(mg)	11.3 ± 4.7		3.2 – 21.1		
				Energy (kcal)	2683.1 ± 113.9		NR	NR	

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Table 4. Continued

Author, year (ref.)	Location	Subject	Method of assessment	Intake reported	Intake value		Nutritional targets		Key findings
Ogechi *et al.*, 2007 [68]	Umuahia, Abia state	190 secondary schools Adolescents	7 d weighed food inventory	Protein (g) Fat (g) Carbohydrate(g)	54.4 ± 7.9 43.7 ± 3.8 518.9 ± 27.7		NR NR NR	NR NR NR	Males consumed significantly more carbohydrates, fats and energy.
Ogechi, 2012 [69]	Umuahia, Abia state	416 secondary School adolescents	7 d weighed food inventory	Protein (g) Fat (g) CHO (g) Energy (kcal)	53.9 ± 7.2 43.7 ± 1.9 517.3 ± 30.1 2673.7 ± 12.8	50.9 ± 9.3 47.4 ± 9.9 426.6 ± 27.5 2343.5 ± 10.9	NR NR NR NR	NR NR NR NR	Boys had higher energy intake but still below recommended levels for both sexes
Oranusi *et al.*, 2007 [98]	Zaria, Kaduna state	44 members (5 families)	6 d food inventory	Energy (kcal)	1194 ± 114.7	1062.6 ± 98.7	NR	NR	Energy intake was below RDA.
Erinoso *et al.*, 1992 [49]	Olodo, Oyo State	400	Questionnaire	Energy (kcal) Protein (g) Iron (mg) Calcium (g) Riboflavin (mg) Vitamin C (mg)	903 ± 134 27 ± 7 13 ± 7 128 ± 71 1 ± 0.4 12 ± 5		36 ± 6 89 ± 24 10 6 ± 75 22 ± 17 72 ± 28 55 ± 20		Results indicate a significant deviation from the established reference values and poor nutritional status.
Onabanjo and Balogun, 2014 [88]	Ogun State	127	Questionnaire	Energy (kcal) Carbohydrates Fats (g) Zinc (mg) Phosphorus (mg) Niacin (mg) Vitamin C (mg)	2028.9 ± 228.7 276.8 ± 15.6 47.7 ± 3.8 10.3 ± 1.8 771.3 ± 106.3 3.7 ± 0.6 80.8 ± 16.4	1738.9 ± 216.7 251.3 ± 10.6 40.3 ± 2.6 8.6 ± 1.2 616.2 ± 101.6 2.5 ± 0.5 72.6 ± 20.1	NR NR NR NR NR NR NR	NR NR NR NR NR NR NR	The majority (59.8%) of adolescents had iron intake below the recommended nutrient intake.
Yunusa *et al.*, 2014 [116]	Nigeria	270	Questionnaire	Energy (kcal) Water Protein (g) Fat (g) Carbohydrate (g) Dietary fibre (g) PUFA (g) Vitamin A (mg) Total folic acid (mg)	2014.4 ± 194.6 36.3 ± 11.8 48.3 ± 9.6 24.5 ± 7.3 393.7 ± 26.1 23.5 ± 8.2 10.8 ± 2.8 132.4 ± 23.1 70.3 ± 21.5	1441.1 ± 169.3 501.7 ± 72.5 36 ± 9.6 6 ± 2.9 305.4 ± 11.2 18.9 ± 2.4 1.8 ± 0.6 158.9 ± 29.6 50.7 ± 6.8	2036.3 2800 60.1 69.1 290.7 30 10.0 900 400	2036.3 2450 60.1 69.1 290.7 30 10 1100 400	Overall energy intake was higher among males, compared to females.

Note: RNI, Recommended Nutrient Intake; NR, Not reported; RDA, Recommended Dietary Allowance; PUFA, Polyunsaturated fatty acids; FFQ, Food Frequency Questionnaire; DDS, Dietary Diversity Score.

fibre intake (range: 13.1–33.1 g) among Nigerian adolescents. One of the studies showed an intake of fibre less than 20 g (when defined as no starch polysaccharide) from foods—or less than 25 g from foods (when defined as total dietary fibre)—which are also the accepted values recommended for the prevention of NCDs.[122] With regards to vitamin intake, the most commonly reported vitamins in the studies included in this systematic review are vitamin A (range: 132.4–9135.2 mg), vitamin C (range: 7.2–80.8 mg), vitamin B_{12} (range: 0–1.4 mg) and folate (range: 0–136.6 mg). Virtually all studies reporting them found insufficient consumption of these vitamins among adolescents.[30,32,46] Information gathered also shows an inadequate intake of minerals and trace elements, particularly calcium, iron, zinc and potassium. There was a report of excessive sodium intake (2225–2404 mg compared to the recommended value of 1200–1500 mg).[109]

Table 5 presents a summary of dietary habits among Nigerian adolescents. The studies included in the table report findings related to key dietary characteristics such as the number of meals, observing breakfast, eating lunch, eating dinner, and skipping meals. Specifically, the table includes findings from twenty-one studies.[17,25,26,31,51,60,61,70,71,76,80,86,94,95,102,104,105,113,117,118,123] Among these studies, nine[26,60,61,71,76,80,86,105,117] reported the number of meals based on a three-square meal basis. Thirteen[26,31,60,70,71,102,105,118] studies provided information on the proportion of participants who observe breakfast, while seven[70,86,94,102,105,113,118] studies reported the proportion of participants who observed lunch meal. Only one[102] study reported the responses of participants regarding the order of importance of meals, fasting to lose weight, using diet pills to lose weight, infrequent intake of fruits/vegetables, consuming alcohol and smoking cigarettes. Furthermore, eight[17,25,51,60,76,94,104,117] studies examined fast-food consumption, and four[26,61,95,102] studies investigated factors that motivate dietary intake.

Table 5 provides information on studies reporting dietary habits of Nigerian adolescents. With regards to meal patterns the proportion of participants reporting consuming three-square meals varied across studies, ranging from 33% to 85%. A majority of participants generally observed breakfast, with proportions ranging from 16.4% to 95%. Lunch and dinner were also commonly consumed meals, with proportions ranging from 6.9% to 95.5%. Skipping meals was prevalent among adolescents, with proportions ranging from 10% to 86%. Breakfast skipping was particularly common, reported by 48% to 86% of participants in different studies. Fast food consumption was widespread among adolescents, with proportions ranging from 16.5% to 87.8%. Some studies highlighted a higher prevalence of fast-food consumption among females. Fruit consumption varied across studies, with proportions ranging from 48.3% to not been reported. The frequency of fruit consumption was generally moderate, with a significant proportion reporting eating fruit sometimes.

Fig. 2 shows a summary of results from studies reporting anthropometric indicators and these studies have been ordered by year, to provide a trend over time. For all 5 indicators (obesity, stunting, thinness, underweight and wasting) the trends appear to decrease over time.

Discussion

To the best of our knowledge, this systematic review represents the most comprehensive observational analysis on dietary profile and nutritional status of adolescents in Nigeria. We reviewed and synthesised results from a total of 102 nutritional epidemiological studies among Nigerian adolescents (10–19 years) with a total population of 67,844 participants. However, only 13 % of the included studies were classified being at low risk of bias in terms of methodological quality and with the majority published in unindexed journals (89.2%). No prospective studies were found on the topic. Since included studies were cross-sectional, their reliance on self-reported, unvalidated tools for dietary assessment or anthropometric classification was common. The majority (i.e. 73.5%) of the surveys took place within education settings (e.g. school or university) and some studies focused only on males (12/102), but no study focusing only on females was found. The rest had both males and females. From the results, it is evident that a significant amount of nutrition research has been conducted involving Nigerian adolescents, but there is large variability with regards to assessment and quality of methods, with a substantial proportion of studies using unvalidated tools and instruments and methodologies with high risk of bias.

Although there were no studies focusing exclusively on male participants, some of the nutritional issues reported among the included studies were related mostly to undernutrition, particularly among adolescent girls. Such findings are in line with a UNICEF report, that reported a significant increase in undernourishment among adolescent girls in Nigeria, with the number rising from 5.6 million in 2018 to 7.3 million in 2021.[124] According to the same data, there are evident malnutrition challenges among school-aged adolescents, with a prevalence of thinness and overweight, 10% and 8% respectively. Further, among girls aged 18 and above, 10% are underweight, while 33% are overweight. The prevalence of anaemia among women aged 15–49 years is 55%. Additionally, 55% of households in Nigeria consume salt with iodine, which is an important indicator for addressing iodine deficiency disorders.

In addition, results from the National Nutrition and Health Survey 2018 show that the prevalence of acute malnutrition was more than four times higher for adolescents (15–19 years) than adult women (20–49 years), 19 per cent compared to 4 per cent, respectively.[125] This report underscored the urgency of developing effective interventions to improve the nutrition of adolescent girls, as they play a crucial role in birth outcomes and subsequent nutrition throughout the lifecycle. Improving nutrition in adolescent girls is critical to improving the nutrition status of the entire population.

Nigeria is one of the 12 countries hit hardest by the global food and nutrition crisis, which has been exacerbated by factors such as COVID-19, conflict, and drought. The dietary diversity of adolescent girls' and women's diets is too low, particularly in rural areas and poor households.[126] A recent situation report from the UN Office for the Coordination of Humanitarian Affairs indicates a nutrition crisis is occurring in 6 regions.[127] According to the same report the number of adolescents with

Table 5. Findings on dietary habits of the available studies

Ref.	Author, year	Primary aim of the study	Findings
(26)	Agofure *et al.*, 2021	This study investigated the dietary patterns and nutritional status of the female adolescents in Amai Secondary Commercial School, Delta State, Nigeria.	- Proportion of meals (three-square meals): 34.4% of participants reported having meals sometimes. - Proportion of participants observing breakfast: 38.3% observed breakfast very often. - Proportion of participants eating fruit after meals: 48.3% ate fruit sometimes. - Proportion of participants skipping meals: 41.8% skipped meals sometimes. - Factors motivating dietary intake: Nutritional values (35.3%), taste (26.9%). - Order of importance: Breakfast (24.40%), Lunch (21.9%), Dinner (14.4%).
(104)	Samuel *et al.*, 2021	Study explored adolescents' dietary patterns (DP) and nutritional status focusing on out-of-school adolescents	- Proportion of participants eating home-cooked meals: 90.5%. - Proportion of participants eating fast food: 87.8%.
(105)	Sanusi *et al.*, 2021	This study examined the contribution of food to nutrient intake, meal, and dietary patterns among children aged 9–13 years in Ibadan, Nigeria	- Proportion of meals (three-square meals): 85%. - Proportion of participants observing breakfast: 95%. - Proportion of participants eating lunch: 85%. - Proportion of participants eating mid-morning meals: 48%. - Proportion of participants eating dinner: 85%.
(60)	Ikujenlola and Adekoya, 2020	This study examined the dietary habits, nutritional status, and socio-demographic characteristics of female undergraduates in selected public and private universities in Osun State, Southwestern Nigeria	- Proportion of meals (three-square meals): 33% of participants reported having meals. - Proportion of participants observing breakfast: 52.4% observed breakfast. - Proportion of participants skipping meals: 86%. - Proportion of participants eating food made outside home: 10.5%. - Proportion of participants eating fast food: 16.5%. - Proportion of participants eating home-cooked meals: 73%. - Proportion of participants eating from school cafeteria: 54.8%.
(117)	Orisa and Wordu, 2021	This study was designed to assess the diet, physical activity, and food consumption patterns of adolescent girls in Port Harcourt, Rivers State	- Proportion of meals (three-square meals): 61.43% of participants reported having meals. - Proportion of participants skipping meals: 55.71% skipped breakfast sometimes. - Proportion of participants eating fast food: 55.71% ate fast food occasionally.
(102)	Otuneye *et al.*, 2017	To determine the dietary eating patterns and nutritional status among adolescents in secondary schools within Abuja Municipal area council	- Proportion of participants eating breakfast every day: 4.6%. - Proportion of participants eating lunch: 15%. - Proportion of participants eating dinner: 16.6%. - Proportion of participants fasting to lose weight: 16.3%. - Proportion of participants inducing vomiting/taking laxatives to lose weight: 5%. - Proportion of participants using diet pills to lose weight: 9%. - Proportion of participants eating less food or fat to lose weight: 28%. - Factors motivating dietary intake: Taste good (35.2%), balanced diet (34.2%), satisfying (15.6%), don't know (15%), good nutritional knowledge (34.8%).
(70)	Ogunkunle and Oludele, 2013	This study was designed to assess the food intake and describe the meal pattern of adolescents attending public secondary schools in Ila Orangun, southwest Nigeria	- Proportion of participants observing breakfast: 62.2%. - Proportion of participants eating lunch: 6.9%. - Proportion of participants eating dinner: 95.1%.
(118)	Onyechi and Okolo, 2009	The objective of this study is to determine the prevalence of obesity among undergraduates living in halls of residence in University of Nigeria Nsukka campus and to obtain information on their feeding pattern, physical activity, and health status	- Proportion of participants observing breakfast: 64.9%. - Proportion of participants eating food made outside home: 40.6%. - Proportion of participants eating home-cooked meals: 46.6%. - Proportion of participants eating lunch: 62.6%. - Proportion of participants eating dinner: 64.1%.
(80)	Olumakaiye *et al.*, 2010	Association between nutritional status of adolescents and food consumption pattern	- Proportion of meals (three-square meals): 66.1%.
(31)	Akinola *et al.*, 2022	To describe dietary habits, physical activity, and sleep patterns among secondary-school adolescents	- Proportion of participants observing breakfast: 68.9% (3–5 d/week). - Proportion of participants skipping meals: 10%.
(61)	Iyalomhe *et al.*, 2018	This study aimed to optimise the health needs of Nigerian adolescents by determining the dietary habits and the nutritional status of adolescents using anthropometry	- Proportion of meals (three-square meals): 69.2%. - Proportion of participants skipping meals: 39.2%. - Factors influencing dietary intake: Parental influence (87%), taste of food (71%), mass media reports (61%), and culture (55%).

Continued

Table 5. Continued

Ref.	Author, year	Primary aim of the study	Findings
(71)	Ogunsile, 2012	The main objective of this study was to determine the effects of dietary patterns and body mass index on the academic performance of in-school adolescents in Ekiti State	- Proportion of meals (three-square meals): 14.1%. - Proportion of participants observing breakfast: 16.4%.
(123)	Olatona *et al.*, 2022	The objective of this study was to determine the relationship between breakfast skipping and the prevalence of obesity among secondary-school adolescents in Lagos State	- Proportion of participants observing breakfast: 57.4%. - Proportion of participants aged 16-19 years having breakfast: 52.2%. - Proportion of participants aged 13–15 years having breakfast: 43%. - Proportion of participants aged <13 having breakfast: 34.7%.
(86)	Omuemu and Oko-Oboh, 2015	This study aimed to determine the pattern of meal consumption among in-school adolescents in Benin City	- Proportion of meals (three-square meals): 71.6%. - Proportion of participants observing breakfast: 85.9%. - Proportion of participants eating lunch: 90.2%. - Proportion of participants eating dinner: 95.5%.
(95)	Onyiriuka *et al.*, 2013	To describe the pattern of snack consumption among adolescent Nigerian urban secondary schoolgirls	- Proportion of participants eating snacks between meals: 74.8%. - Factors motivating dietary intake: Taste (88.2%).
(94)	Onyiriuka *et al.*, 2013	To describe the eating habits of adolescent urban secondary-school girls in Benin City, Nigeria	- Proportion of participants observing breakfast: 53.7%. - Proportion of participants skipping meals: 48%. - Proportion of participants having breakfast: 46 (breakfast), 22 (dinner). - Proportion of participants eating fast food: 60 (with soft drinks) and 76 (without soft drinks). - Proportion of participants eating lunch: 69.6%. - Proportion of participants eating dinner: 78.5%.
(17)	Adebimpe, 2019	The objective of this study was to determine the prevalence and knowledge of risk factors of childhood obesity among school-going children in Osogbo, south-western Nigeria	- Proportion of participants eating fast food: 53.1%. - Proportion of participants taking snacks between meals: 86.7%. - Proportion of participants often taking sweets: 75.6%. - Proportion of participants eating ice cream: 74.8%. - Proportion of participants often taking soft drinks: 87.9%. - Proportion of participants having good nutritional knowledge: 91.9%. - Proportion of participants having low self-esteem: 61.7%. - Proportion of participants taking alcohol: 2.1%. - Proportion of participants smoking cigarettes: 0.8%.
(25)	Afolabi *et al.*, 2013	The aim of the study was to assess the pattern of consumption and the contribution of fast foods to nutrient intake of undergraduates of the Federal University of Agriculture Abeokuta (FUNAAB)	- Proportion of participants eating fast food: 60% (males), 40% (females). - Proportion of participants eating fast food: 53.6% (males), 35.0% (females).
(51)	Essien *et al.*, 2014	The aim of this study was to determine the nutrition knowledge and nutritional status of children attending a secondary school in Sokoto metropolis	- Proportion of participants eating fast food: 68.8%. - Proportion of participants taking snacks between meals: 82.1%. - Proportion of meals (three-square meals)[51]: 3.3% (once), 13.3% (twice), 56.3% (thrice), 27.1% (more than thrice).
(76)	Olatona *et al.*, 2018	This study aimed to assess the dietary pattern and metabolic risk factors of non-communicable diseases among university undergraduate students in Lagos State	- Proportion of meals (three-square meals): 7% (once), 57.1% (twice), 31% (thrice), 5% (more than thrice). - Proportion of participants taking snacks between meals: 44%. - Proportion of participants eating fast food: 22.7% (daily), 18.9% (4–6 times/week), 58.4% (≤3 times/week). - Proportion of participants often taking soft drinks: 29.0%. - Proportion of participants having good nutritional knowledge: 22.9%. - Proportion of participants observing breakfast: 22.5%.
(113)	Uba *et al.*, 2020	This study aimed to investigate the nutritional status of adolescent girls in a selected secondary school in Nigeria	- Proportion of participants observing breakfast: 91.6%. - Proportion of participants eating lunch: 84.8%.

severe acute malnutrition requiring inpatient care between January and April 2023 increased by 61% compared to the same period in 2022. Thinness trends in our study are similar to data from UNICEF, showing a temporal downward trend.(128) According to the 2023 edition of the Joint Child Malnutrition Estimates by UNICEF/WHO/World Bank Group that in 2012, Nigeria had a stunting prevalence rate of 37.7%, indicating a high level of stunting. However, by 2022, this rate decreased to 34.2%.(129) In the same report, Nigeria had an overweight prevalence rate of 2.5% in 2012, which remained relatively stable at 2.2% in 2022. Wasting for 2020 was 6.5 % and considered a "medium" prevalence threshold, i.e. 5–10%. It is important to note that the data provided is limited to specific years (2012, 2020, and 2022), does not include most recent estimates of all indicators and reflect mostly the children's population, not adolescents.

In terms of global targets for nutrition for 2025 outlined in the United Nations Sustainable Development Goals Agenda 2030,(130) Nigeria appears to have made slight progress. This limited progress is also reflected in the updated, Global

Nutrition Report 2021[131] that shows the country being "*on course*" to fulfil one of the global nutrition objectives for which there is adequate data to assess progress. These goals and global programmes have focused mostly on nutrition in childhood and adults, but data on adolescents appear to have been overlooked. National and global policy has also overlooked adolescent and youth nutrition and the UN Decade of Action on Nutrition (2016–25) has no adolescent or youth-specific nutrition indicators. With the current global challenges, malnutrition in all its forms may worsen. Kidnapping, communal conflict, inflation, urbanisation and banditry may have impeded Nigeria's capacity to make progress.[132] A review in 2020[133] reported that Nigeria's stunting rate is 37%, making it the world's second-most-stunted affected nation.

A previous review,[134] in line with ours, pointed out challenges on insufficiency and scarcity of the data on nutritional status. This is consistent with many low and middle-income countries where malnutrition has been a major concern and, in our synthesis, the included studies reported a range of malnutrition issues, starting from underweight and stunting to overweight and obesity. This is a strong signal reflecting the presence of a double burden of malnutrition. The argument is further supported by a recent analysis on temporal trends in overweight and obesity in Nigerian adolescents and young adults,[135] that reported the co-existence of under- and over-nutrition challenges.

Furthermore, the included studies in our review that reported intake across different regions in Nigeria, consistently showed inadequate intake of certain nutrients. In particular, energy, protein, iron and calcium intake were the most reported inadequacies and, in some cases, certain nutrient intakes exceeded established recommendations. Taken together, these findings suggest that current or future efforts targeting adolescent nutrition in Nigeria should consider a region- and context-specific approach to address the identified dietary gaps.

Our findings also illustrate an adolescent population with consumption patterns varying widely, but with an overall picture that indicates frequent and widespread consumption of starchy staples, sugar-sweetened beverages and snacks and a highly variable intake across studies of other essential food groups like vegetables, fruits and dairy products. This is in line with a review on dietary intake of schoolchildren and adolescents in developing countries,[136] where it was reported that in Nigeria this population group had inadequate consumption of vegetables, micro-nutrients, fruits and animal protein. In the same analysis, a significant increase in the consumption of snacks and energy-dense nutrient-poor foods and drinks was reported. Another recent analysis also showed that adolescents, in Ogun state (south-western Nigeria), primarily consumed starchy foods, with limited dietary diversity.[100]

In 2021[6] a position paper from the Nutrition Society of Nigeria called for urgent action"... *to bridge the identified policy and data gaps, enhance coordination and increase delivery platforms to reach adolescents with a minimum package of nutrition interventions giving special consideration for nutritional needs of pregnant adolescent mothers.*" Although adolescents have increasing nutritional requirements and constitute about 21% (more than 41 million) of the Nigerian population, surveillance of their well-being and nutrition remains largely underestimated, inconsistently measured and not prioritised for nutrition interventions. Considering all complexities, efforts to address this situation must be culturally relevant, region-specific and address the identified challenges. Considering earlier findings that highlight the influence of the food environment on adolescents' food choices, it's evident that fast-food establishments offering processed foods rich in fat, salt and sugar are gaining popularity in Nigeria.[137] This trend is especially pronounced among adolescents. Future studies should dissect adolescents' autonomy and agency within the food environment in Nigeria. Irrespective of context, adolescents have a lot to say about why they eat what they eat, and insights into factors that might motivate them to change. Efforts to improve food environments and ultimately adolescent food choice should harness widely shared adolescent values and input beyond nutrition or health.

Limitations

Although our report is the most comprehensive review of adolescent nutrition in Nigeria, concerns about the scarcity of studies and poor methodological rigour undermine establishment of strong inferences. The available observational studies on adolescent nutrition reveal several methodological limitations, including issues with study design, confounder control, statistical analysis, and sampling, highlighting a crucial need for enhanced investment in robust and rigorous nutrition research to better understand and support adolescent health. In addition, further research is currently ongoing and we did not include intervention studies, but based on current findings and in order to address the identified research gaps, we are conducting an epidemiologic study, for which we have obtained approval from the Yobe State Ministry of Health and Human Service (YB/MOH/HREC/04/22/008). More precise evidence to understand the key nutritional challenges and context of food choices of Nigerian adolescents is needed to increase the potential for impactful and tailored actions.

Conclusions

Our review on the state of nutrition of Nigerian adolescents showcases both the inherent strengths and limitations of nutrition research in the country, emphasizing the urgent need for targeted, evidence-based interventions to address the double burden of malnutrition. This is further nuanced due to cultural and regional differences and other socio-cultural determinants. Overall, findings underscore the need for more rigorous research and establishment of nutrition surveillance for malnutrition in all its forms among Nigerian adolescents.

Abbreviations

BMI: Body mass index; **DDS:** Dietary diversity score; **FFQ:** Food frequency questionnaire; **HAZ:** Height-for-age z-score; **JBI:** Joanna Briggs institute; **LMICs:** Low- and middle-income countries; **NCD:** Noncommunicable diseases; **NR:** Not reported; **PRISMA:** Preferred reporting items for systematic reviews and meta-analyses; **PROSPERO:** International prospective register of systematic reviews; **PUFA:** Polyunsaturated fatty acids; **RDA:** Recommended dietary allowance;

RNI: Recommended nutrient intake; **SDGs:** Sustainable Development Goals; **UN:** United Nations; **UNICEF:** United Nations Children's Fund; **WAZ:** Weight-for-age z-score; **WHO:** World Health Organization

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.34

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Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose.

Authorship

EL, TSG and MK were responsible for research conceptualisation, implementation and methodology; TSG, MK and FAO were responsible for data collection and analysis, and contributed to drafting the article and revising it; TM provided technical input and revised the work critically for important intellectual content. All authors contributed to and approved the final version of this manuscript.

Ethical approval

Not applicable.

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RESEARCH ARTICLE

Barriers and enablers to engagement with a type 2 diabetes remission project in the North East of England: qualitative perspectives of patients

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Abstract

This qualitative research sought to identify factors influencing patient choice of, and patient-related internal and external enablers and barriers to engagement with, type 2 diabetes (T2D) remission strategies offered by the Remission in diabetes (REMI.D) project. Patients had a choice of three diets: Total Diet Replacement (TDR)-Formula Food Products, TDR-Food, and Healthy lifestyle approach; and three activity pathways: Everyday life, General Practitioner referral, and Social hub. Semi-structured interviews were recorded and transcribed. Thematic analysis used the Framework Method and NVivo 12 to assist with generation and organisation of codes, inductive and deductive (Theoretical Domains Framework). The REMI.D project was a place-based approach (place in this case being defined as two local authorities with significant rates of deprivation) situated in the North East of England. Twenty patients out of a possible 65 patients took part. Areas of interest included: patient choice, patient intention, patient adherence, patient non-adherence, and patient stigma. Addition of a more moderate dietary strategy (not dissimilar to the diet in the Healthier You NHS Diabetes Prevention Programme) to the existing NHS England T2D Path to Remission programme may enable more patients to achieve remission or delayed progression with deprescribing of diabetes medications. Embedding a tailored physical activity path within or as a bolt-on to the NHS programme requires consideration. Limited resources should be targeted towards patients who identify with more barriers or fewer opportunities for health behaviour modification. Further research on use of virtual programmes in deprived areas is warranted.

Key words: **Patient interviews: Qualitative research: Remission strategy: Type 2 diabetes**

Introduction

The impact of type 2 diabetes (T2D) and its complications places a significant burden on adults living with the condition. Recent evidence offers the possibility of long-term T2D remission through weight reduction.[1,2] The Diabetes UK[1] position statement on T2D remission recommends an individualised approach, recognising that people with T2D have achieved remission using various dietary interventions, including the Mediterranean ('healthy') diet, calorie-controlled (low-fat) diets, low carbohydrate diets as well as the total diet replacement (TDR).[3] Nationally, one quarter of adults with T2D live in the most deprived areas of England.[4] This Sport England-funded primary care-based project questioned whether T2D remission is possible for adults in two of the most deprived local authorities (Middlesbrough, Redcar and Cleveland) in England.[5,6] Adults with T2D may struggle to complete, or decline to take part in, the multiple dietary and lifestyle strategies for the management of T2D. Known patient-related internal and external barriers and enablers to T2D self-management are explored below.

An improvement in physical and psychological wellbeing, coupled with a sense of achievement as a result of weight loss

[1]Diabetes UK is a leading charity for people living with diabetes in the UK.

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and improved glycaemia, motivated lasting health behaviour change.[7,8] Clearly defined intentions and goals, for example, to lose weight, were dominant themes in a recent lifestyle intervention to prevent T2D.[9] Self-distraction, for example, patchwork and quilting, helped some patients overcome poor eating habits.[8] A fear of diabetes-related complications motivated others to manage lifestyle changes.[10] A multinational study, including the views of patients living with type 1 diabetes and T2D, identified a frustration with the diagnosis of diabetes and associated unrealistic expectations which hampered self-management.[11] Similarly, Peng, *et al.*[12] noted blindly optimistic attitudes, and Cheng, *et al.*[13] negative T2D appraisal as dietary barriers. Physical barriers to activity included fatigue, muscle and joint pain, and other co-morbidities. T2D and depression were seen as interrelated.[14] The initial motivation for dietary behaviour change waning as the reality of perceived long-term restraint brought negative emotions. While patients with low health literacy reportedly 'misunderstood diabetes management, showed passive attitudes towards seeking information, and had difficulty obtaining detailed information'.[15] Studies have observed that a reoccurring driver for positive lifestyle behaviour change was social support from family, friends or co-workers.[8,16] Establishing good habits was linked with positive diabetes outcomes. Rehackova, *et al.*[8] cited removing food from the environment, planning, and avoidance of tempting situations as helpful decisions. Preliminary evidence from a narrative review suggests technological advances, in particular the use of real-time blood glucose monitoring devices, may be an adjunct to lifestyle behaviour adherence in patients with T2D[17]; rapid weight loss and positive changes to clinical diabetes markers providing ongoing motivation.[7,16] An ineffective therapeutic relationship between the care provider and the patient, including reports of weight-related stigma, hindered strategies for the management of T2D. Patients described 'inconsistent provision of information and resources to assist them in tackling their weight problems'.[18] Patients described feeling confused by conflicting advice received from health professionals, friends, family, internet and diabetes organisations.[19] Additional reports of feeling 'rushed and not heard' in consultations resulted in poor ongoing engagement with general practice. Other patient barriers were time constraints,[20] incomplete diabetes-related knowledge and skills,[21] social aspects (social functions interfering with dietary regimens, family members diverting patient from dietary goals)[22] and socio-economic factors.[23]

Objectives of this T2D remission project, Remission in diabetes (REMI.D) included (a) to identify factors influencing patient choice of T2D remission strategy, and (b) to explore the internal and external barriers and enablers to adherence to a 12-week dietary and physical activity intervention for patients living in an area of significant deprivation. A process evaluation for REMI.D is published elsewhere.[24]

Methods

This local delivery pilot, REMI.D, was developed following a successful bid by a diabetes development group in the North East of England in 2020. REMI.D sought to tackle health inequalities by working with multiple organisations to demonstrate a way of scaling up an effective T2D remission strategy which included both dietary and physical activity components.

REMI.D deviated from the UK Diabetes Remission Clinical Trial (DiRECT)[2,25] which used TDR-Formula Food Products, by including three dietary remission strategies (1) TDR-Formula Food Products, (2) TDR-Food, and (3) healthy lifestyle approach, and three activity pathways (1) Everyday life (walking, climbing stairs, getting off bus early) (2) General Practitioner (GP) referral (gym, swimming, chair-based exercises), and (3) Social hub (activity indirectly through social activities, day trips). The TDR-Formula Food Products (800-calorie diet based on commercial meal replacements) and TDR-Food (800-calorie prescriptive food-based diet) utilised the existing DiRECT trial remission resources.[26] The healthy lifestyle approach supported participants to set tailored goals based on other commonly used diets for T2D management: Mediterranean diet, portion controlled (lower carbohydrate, higher protein, lower calorie), and lower glycaemic index foods. All three dietary strategies sought weight loss-induced reversal of T2D; Fig. 1 summarises the T2D remission strategies offered.

The REMI.D project eligibility criteria followed the 2019 NHS Low Calorie Diet Pilot Programme model.[27] The REMI.D project intended for GPs and practice nurses within general practices, situated in one of four electoral areas, to identify suitable patients with T2D for inclusion in REMI.D. The dietary interventions were to be delivered by practice nurses at the general practices with initial support from the REMI.D project-funded dietitian based in secondary care. Due to loss of primary care stakeholder engagement (GP and practice nurse) during and following the COVID-19 (coronavirus) pandemic 2020–2022,[28] the REMI.D dietitian took sole responsibility for recruitment and delivery of the dietary interventions, the latter being relocated out of general practice.

The researcher (RCB) conducted semi-structured interviews during the 12-week intervention. Sample size for the qualitative research was 10 per remission strategy. Promotional posters, including information about a prize draw (£250 shopping voucher) for participation, advertised this qualitative research. The posters, written participant information sheets and consent forms were available to the REMI.D dietitian. The REMI.D dietitian actively discussed participation in this qualitative research with eligible patients. The names and contact telephone numbers of interested patients were emailed to the researcher (RCB). The Capability Opportunity Motivation - Behaviour (COM-B) model and Theoretical Domains Framework (TDF)[29] were used to develop the interview schedule (Supporting Information). Interviews were audio- or video-recorded using two devices.

The Framework Method (Ritchie & Lewis, 2003; p220) used in this qualitative research, accommodated both inductive and deductive (TDF domains) coding. Transcription files (cases) were imported and the TDF domains (codes) were added to NVivo 12 (qualitative data analysis software). The patient's voice was coded to the TDF domains. The NVivo codebook was used to add additional descriptions to ensure consistency in coding decisions (Table 1). The researcher (AH) independently coded 10% of the transcripts and looked for inconsistencies in the coding decisions across all of the transcripts. Coding

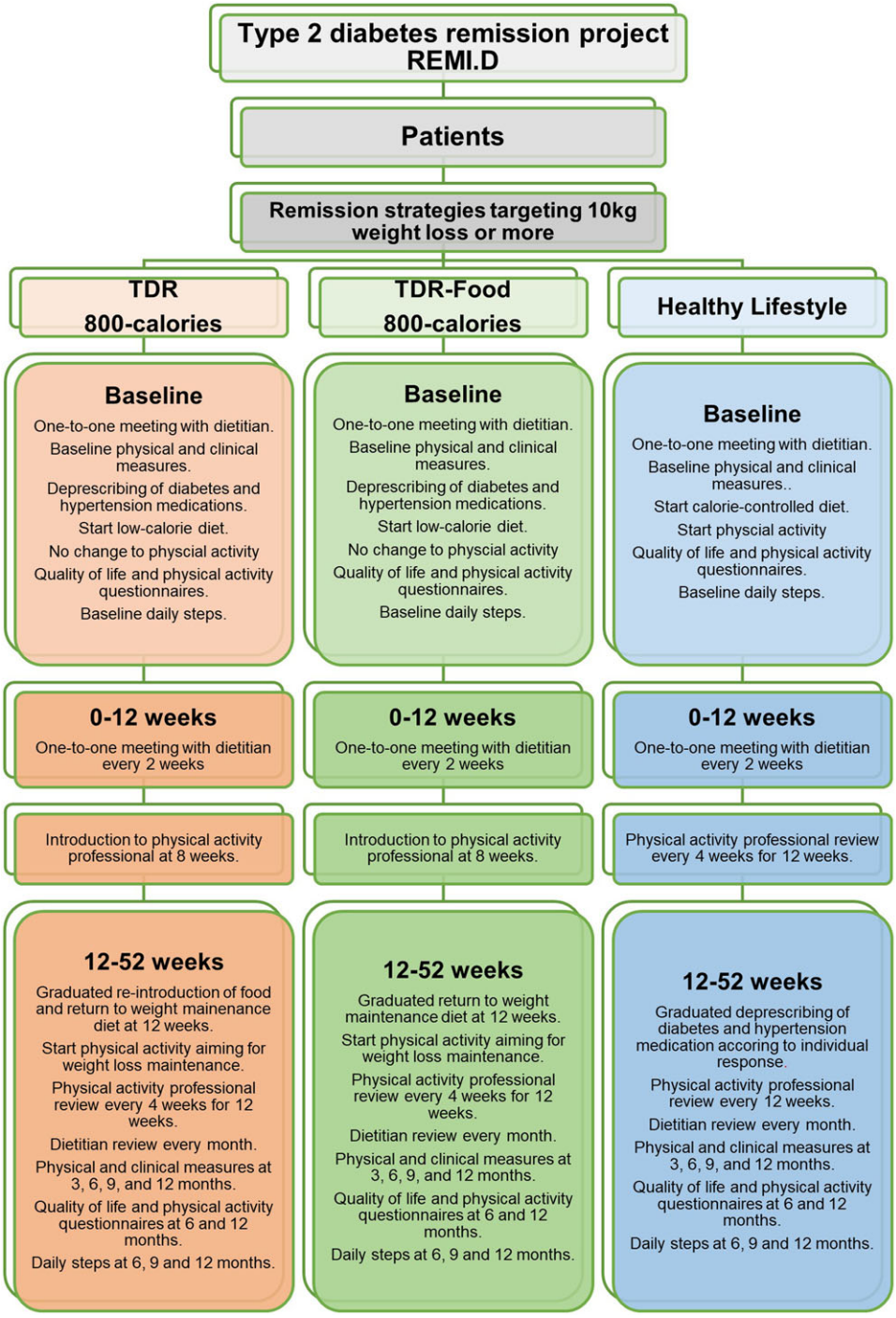

Fig. 1. Type 2 diabetes remission strategies, including the three interview groups, for this qualitative research.

inconsistencies not readily resolved by joint review (RCB, AH, and HJM) were referred to team member (AAL). Case classifications (attributes) were added including gender, age, index of multiple deprivation (IMD), weight lost at 12 weeks, HbA1c at 12 weeks, and diet option chosen. Codes were explored by running coding queries within NVivo 12. For example, one query sought to explore patient views coded to the TDF 'behaviour regulation' domain in patients who chose the diet option 'TDR-Formula Food Products' and achieved an 'HbA1c less than 48' at the end of the 12-week intervention. The creation of an analytical framework steered the application of coding query reports and refinement of data.[30] The patient voice is reported in italics and each different voice noted, by P (for patient) and the participant number, in square brackets, for example [P1]. TDF domains are highlighted in italics.

Results

Twenty patients participated in this qualitative research, 11 patients from the healthy lifestyle approach and nine patients from the TDR-Formula Food Products group. None of the participants chose the TDR-Food strategy following a consultation with REMI.D dietitian. Seventeen patients chose to be interviewed by telephone. One patient opted to be interviewed online using

Table 1. NVivo codebook: The Capability Opportunity Motivation - Behaviour model (3 components of behaviour change) with the Theoretical Domains Framework (14 domains or determinants of behaviour)

Capability component	Description of domains
Behavioural regulation	Self-monitoring, Action planning
Knowledge	Understanding of condition (T2D) and dietary/ activity treatments (including REMI.D and remission strategy)
Memory attention and decision processes	Ability to retain information and make choices Memories which influence choice of whether to do one diet over another
Skills	Competence in following task with practice
Motivation component	**Description of domains**
Belief about capabilities	Self-confidence, Self-efficacy, Self-esteem Why participant feels more confident
Belief about consequences	Expectations in relation to outcomes (diabetes-related complications, weight change)
Emotion	Personal anxiety, stress, depression, stigma, ill-health
Goals	Target setting
Intentions	Conscious decision to perform a behaviour/ act in a certain way Reason for doing REMI.D
Optimism	Confidence in achievement of goals, including unrealistic
Reinforcement	Rewards/ incentives or punishment Feedback from REMI.D team on progress made (bloods, weight, medication changes)
Social-Professional role and identity	Professional confidence, social identity, perception of self
Opportunity component	**Description of domains**
Environmental context and resources	Which help/ hinder change processes Responses relating to positives/ negatives of REMI.D (diet and activity), or other interventions they have participated in
Social influences	Group norms, peer pressure, social support (positives/ negatives) Family, friends (not healthcare professionals)

Table 2. Descriptive statistics for the qualitative sample and the full cohort who participated in the type 2 diabetes (T2D) remission project

	Qualitative sample		Full cohort	
Diet options	Healthy lifestyle	TDR-formula food products	Healthy lifestyle	TDR-formula food products
Number of participants	11	9	39	26
Current age[a] (years)	54 ± 7	50 ± 11	52 ± 11	52 ± 10
Duration of diabetes[a] (years)	1 ± 2	0 ± 1	2 ± 2	1 ± 2
Gender: male/ female (%)	73/27	11/89	54/46	19/81
Ethnicity: white/ other (%)	91/9	100/0	90/10	92/8
Index of Multiple Deprivation decile[a]	4 ± 3	3 ± 3	5 ± 3	3 ± 2
Smokers (%)	18	11	10	4
Current weight[a] (kg)	110.7 ± 18.6	105.7 ± 9.9	101.8 ± 19.4	108.9 ± 22.0
Current body mass index[a] (kg/m^2)	36.8 ± 6.1	38.6 ± 3.2	35.2 ± 5.9	38.7 ± 6.6
Current HbA1c[a] (mmol/mol)	57 ± 18	52 ± 9	57 ± 15	59 ± 16
12-week REMI.D intervention				
Weeks completed[a] *(out of 12)*	12 ± 1	8 ± 4	9 ± 4	6 ± 4
Weight lost[a] (kg)	3.4 ± 2.9	10.5 ± 4.5	3.5 ± 3.2	8.9 ± 5.3
HbA1c achieved[a] (mmol/mol)	50 ± 11	49 ± 18	52 ± 10	50 ± 13
Follow-up post intervention to 1-year				
Weeks completed[a] *(last recorded)*	31 ± 10	20 ± 14	18 ± 12	15 ± 12
Weight lost[a] (kg)	3.8 ± 5.1	8.4 ± 5.7	2.9 ± 3.6	6.2 ± 5.9
HbA1c achieved[a] (mmol/mol)	49 ± 10	50 ± 16	54 ± 12	52 ± 13
Number of participants in T2D remission[b]	2	4	4	5

[a]mean ± SD.
[b]T2D remission was defined as HbA1c <48 mmol/mol for at least 3 months without diabetes medication.[3]

Microsoft Teams, one patient in-person at their home and one patient in a meeting room at the university campus. Interviews lasted 30–60 min. This sample was recruited from the REMI.D project full cohort (n = 65). These patients were referred to the REMI.D project by practice nurses (n = 7; five general practices), Diabetes Specialist Nurses (n = 3) or opted-in following an REMI.D invitational letter to patients on the Diabetes Education and Self-Management for Ongoing and Newly Diagnosed (DIAMOND)[31] waiting list (n = 10).

Table 2 uses descriptive statistics to compare the qualitative patient sample with the full cohort. Mean age and standard

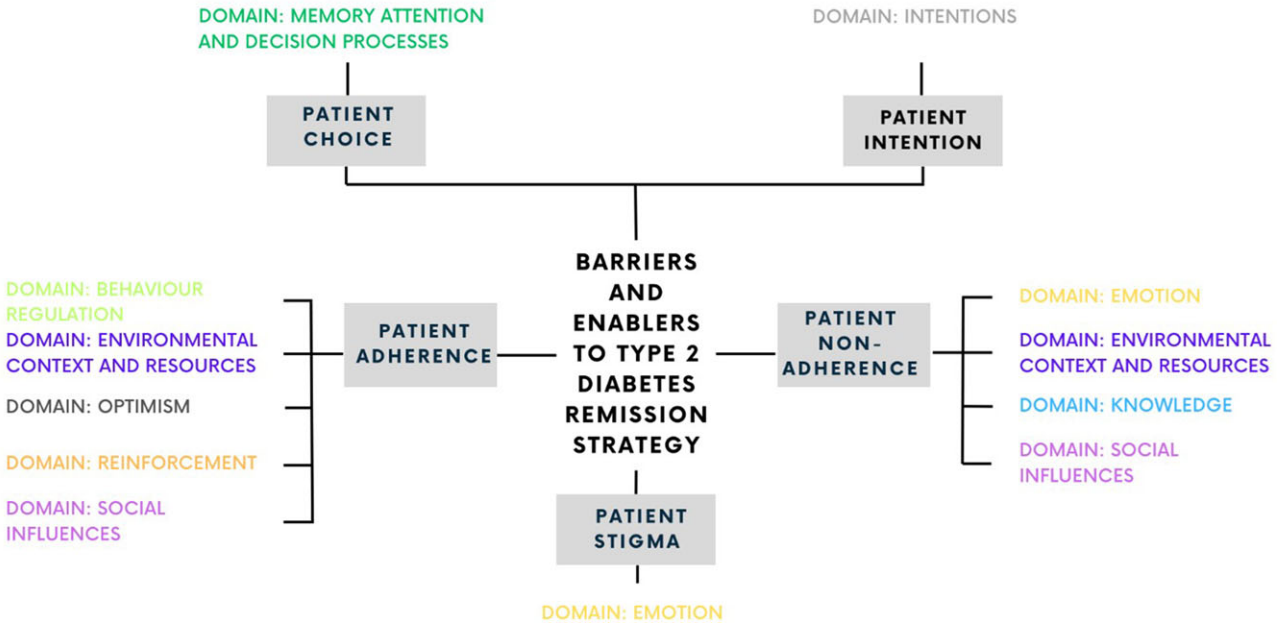

Fig. 2. Visual representation of five areas of interest with associated theoretical domain framework domains.

deviation (SD) for the qualitative sample and full cohort were 52 ± 9 and 52 ± 11 years, respectively. Forty-five and forty per cent of participants in the qualitative sample and full cohort identified as male respectively. Sixty per cent of the full cohort selected the healthy lifestyle approach. Of those participants choosing the TDR-Formula Food Products, 89% of the qualitative sample and 81% of the full cohort identified as female.

Figure 2 illustrates the behavioural barriers and enablers to participation in, and completion of, the initial 12-week REMI.D T2D remission intervention, as identified by the patients who took part in this qualitative research. Five areas of interest emerged: patient choice; patient intention; patient adherence; patient non-adherence; and patient stigma; the results of which are summarised in Table 3 and reported in detail below.

Patient choice

The TDF domain *Memory attention and decision making processes* was used to record factors influencing patient choice, in this case of one diet option over another: TDR-Formula Food Products or healthy lifestyle approach.

The **TDR-Formula Food Products** strategy appealed to patients due to the anticipated large rapid weight losses achievable over the 12-week intervention.

> *'I want to see results and thought that this would be the best way . . . ' [P18].*
> *'I want to lose weight and hopefully reverse diabetes as quickly as possible . . . '[P9].*

One patient described the process as.

> *'a bit like a renovation with a house, strip it down to the foundations and build it up again so you learn the right way' [P8].*

Similarly another patient shared their hope

> *'With this being . . . like cutting everything out. You know, I should be able to do it more sensibly . . . like reintroduce' [P4].*

The experiences of others influenced patient choice too

> *'I have a friend who actually came back from diabetes and that was through protein shakes' [P8].*

In summary, recollection of past success in others and a desire for rapid weight loss/ a fresh start, enabled participation in the TDR-Formula Food Product intervention.

The more popular **healthy lifestyle approach** was chosen largely in response to a negative perception of the TDR-Formula Food Products strategy.

> *'I want to change for life. I don't want to be on a diet' [P16].*
> *'I didn't think I could commit and stick to a tight calorie controlled diet' [P14].*
> *'I'm still living with my mum . . . so it's difficult to say, right you aren't having that because I can't have it' [P10].*

One patient was successfully losing weight so saw no reason to change to the TDR-Formula Food Products.

> *'Because I've been losing weight, we just kept the healthy eating and looked at what I'm eating . . . ' [P2].*

Another patient explained that the healthy lifestyle approach made sense to them.

> *'It's a matter of me having to choose to say . . . can I do some exercises . . . can I reduce my food portions . . . can I increase the healthier options . . . which I found doable' [P7].*

In summary, memories of previous failed diet attempts or recent successes with more moderate dietary strategies were barriers to participation in the TDR-Formula Food Product intervention.

Table 3. Summary of patient barriers and enablers, under the five areas of interest, for the qualitative sample who participated in the type 2 diabetes (T2D) remission project

Areas of interest	Barriers	Enablers
Choice (barriers and enablers to choosing Total Diet Replacement (TDR)-Formula Food Product intervention)	• I want a change for life, not a diet • Food-based diet working • Couldn't stick to TDR • Healthy lifestyle approach more realistic, more cost-effective, and offers a long-term solution	• I want to see results quickly • I want to get it done and dusted • Peer recommended TDR • Professional advised TDR • Previous failed food-based diets
Intention (enablers other than T2D remission and weight reduction)		• I want to get fitter • I want to have a long and healthy retirement • I want to be able to play with my children • I want to care for my family • I want to look better
Non-adherence (barriers to engagement with REMI.D intervention) and **Adherence** (enablers to engagement with REMI.D intervention)	• Online information contradicts itself • I feel ashamed of my diabetes • I eat when I'm stressed • Weight has a negative effect on my mental health • I lost interest in my food diary and feel guilty • I am part of a drinking culture • I end up having a takeaway as I don't plan • Stuff happened and I fell off the wagon • I eat more processed food as live alone • I have issues with my knees so stopped exercising • I don't get breaks at work • I want the professional to tell me what to eat • I work long shifts so can't go to gym • Dizzy spells stop me exercising • I drive for work and stop at cafes • I crave something different at weekends	• Supportive family and friends • Online forums good • REMI.D professionals encourage • I can see the benefits • Walking makes me happy • Results give me drive and motivation • I've got loads more energy • I've dropped two clothes sizes • Family and friends are noticing • I now bring my blood glucose meter to work • I write a meal plan • I walk kids to nursery, walk during my break, park further away • I eat regularly • I've halved my portions
Stigma (barriers to engagement with REMI.D intervention)	• People stare and think I'm lazy • GP called me fat • People assume I can't be exercising • Influencers with their belly out to here • At least I am not as fat as her • Overweight practice nurse judged me	

Patient intention

The TDF domain *intentions* was used to collate patients' reasons for doing the REMI.D project. The primary outcomes for the REMI.D project were greater than 10kg weight reduction and a HbA1c of less than 48mmol/mol without diabetes medication, following the 12-week intervention.

These goals were voiced by many of the patients.

> *'Wanting to lose weight . . . I'm not getting any younger and want to be healthier with my food' [P10].*
> *'To try and get into remission and not have medication that was one of my goals' [P2].*
> *'The main aim is to come off medication and not have diabetes' [P4].*

Other intentions reported by patients included physical appearance.

> *'If I can manage to put on all my t-shirts without looking too big in the tummy that will help' [P7].*

Knowledge of the complications associated with a diagnosis of T2D was voiced as a reason for taking action.

> *'I've had a lot of relatives, friends who I know have been struggling with diabetes, and some of them nearly lost their lives' [P7].*
> *'Think I'm just really worried at getting them diseases associated with diabetes such as your kidneys, your feet, your heart and your eyes . . . they really concern me' [P2].*
> *'I think my motivation was fear . . . I've got my wife and two young daughters and it's not a good position to be in . . . all the health negatives with diabetes . . . I was quite anxious' [P14].*

For many patients, the perceived benefits to physical and mental wellbeing were the strongest drivers for health behaviour change.

> *'To not feel sluggish and lethargic all the time and unwell' [P18].*
> *'Doing things with the grandkids, being able to join in with them' [P4].*
> *'I really want to try and get it sorted so I can have that quality of life with them (2 young children)' [P20].*
> *'I've been reading around mental health and mental health problems but I feel if I do some exercises, I'm doing a lot towards (improving) that [P7].*

In summary, reasons enabling participation in the REMI.D included clinical parameters, physical measures and quality of life indices.

Patient adherence

This area of interest sought to identify behaviours which enabled patients following either diet option to achieve one or

both of the primary outcomes of weight reduction and improved glycaemic control. The TDF domain *Behaviour regulation* saw patients offer up solutions resulting in health behaviour changes.

> *'I have bitten the bullet, I now bring it (blood glucose meter) into work' [P10].*
> *'I'm walking the kids to nursery, rather than driving them' [P20].*
> *'Around week six, I found a guy on YouTube and he does walking videos . . . so I've been doing that maybe once a week' [P18].*
> *'I think you need to plan what you want. Look up recipes, make your list and then go and just get what you need' [P18].*
> *'I've been and asked them if I can take my own sugar-free bottles (to the pub)'[P4].*
> *'I rang ahead and asked if they would be able to do me a bowl of vegetables and they did' [P18].*

Strong *reinforcement* came through positive changes to weight and HbA1c, alongside a reported improvement in quality of life.

> *'I've seen improved results from blood glucose testing . . . currently my seven day average is 6.4 and I think the 90 day average is about 6.7. A few months ago I was getting averages in the mid to high 7s' [P14].*
> *'I've seen a higher blood sugar, I'm looking and I'm thinking, oh, maybe I should have had only one wrap instead of two wraps' [P10].*
> *'I'm taking fasting glucose levels, levels after meals and so on. But yes, by drawing graphs and charts and being able to look at the trend, I can actually say look this is getting better' [P1].*
> *'I've dropped two clothes sizes. Trousers are down to a 36 (inch waist)' [P9].*
> *'I'm feeling a lot more positive about things . . . just getting out, music and just walking . . . I didn't realise how happy you can actually be just doing that' [P10].*

Opportunities were key to success for many patients. In this category, *social influences* played a positive role for patients who met one or both of the primary outcomes.

> *'I want to go up the hills but I won't go on my own. So the hubby is going to come with me' [P16].*
> *'We're trying to help each other (partner), not to reach for the phone and load up Just Eat (app) if we're having a bad day' [P20].*
> *'The girls (at work) are right . . . what are we doing? . . . What shake are you having? . . . You're doing really well' [P18].*
> *'My daughter will research things on the internet because I'm not very good with computers' [P4].*
> *'Very, very supportive group of lads (veterans) round me and they know' [P9].*

Optimism was expressed by patients.

> *'It's working (healthy lifestyle approach), I feel more educated than I have been' [P2].*
> *'I'm very confident (in REMI.D) because of the benefits I can see physically' [P7].*

For patients who saw an improvement in physical and clinical markers, *environmental context and resources* were favourable. One-to-one contact with the REMI.D dietitian and physical activity professional was highly rated compared to previous experiences.

> *'I feel like I'm listened to (by REMI.D dietitian) . . . and not dismissed' [P4].*
> *'He (physical activity professional) gave me loads of tips. He did a plan for me. I'm more or less 90% doing what he suggested' [P16].*
> *'It was nice to have that one-to-one time. Never had that throughout my life if I'm honest, around weight' [P2].*

Work and living arrangements offered space and opportunity for health behaviour change.

> *'I work part-time and I've got time to do extra things as well as exercise . . . I've got an elliptical thing, an exercise bike and a treadmill in the garage which is ideal on days when it's pouring with rain' [P8].*
> *'I live on my own, diet doesn't impact others' [P9].*
> *'I think I'm at an advantage because I'm a working person/ family so we can afford the healthier options as it were' [P7].*
> *'My partner works in Tesco's . . . Slimfast (TDR) was on offer and he got a discount (employee)' [P4].*
> *'We (me and my husband) make sure we don't have a lot of things in the house that we can't eat' [P2].*

In summary, reasons enabling adherence to the REMI.D intervention included a pro-active approach, the support of others, and improvements in health-related outcomes.

Patient non-adherence

This area of interest sought to identify behaviours which prevented patients following either diet option achieving one or both of the primary outcomes of weight reduction and improved glycaemic control.

Opportunity (TDF domains *environmental context and resources* and *social influences*) was the key barrier to health behaviour change for these patients. Work and living arrangements offered barriers to achievement of goals.

> *'You don't get designated breaktimes (at work) anymore now . . . ' [P11].*
> *'I sit at a desk all day long whereas before I'd be out walking (support worker)' [P2].*
> *'I'm out in the van and I'm doing three or four jobs in a day, I know where all the little cafés are' [P19].*
> *'Convenience is a big factor when living alone. The idea of making a Sunday lunch for one is kind of madness, so of course you default to more processed food' [P1].*
> *'Circumstances happened at home . . . I fell off the wagon, went back to eating all the rubbish foods and sweets and it (weight) went back up again' [P19].*
> *'She (daughter) goes in (to work) for about eight o'clock . . . would need to take me to the park (safe place)' [P8].*

Social interactions presented challenges to health behaviour change.

> *'The culture of my friends is around drinking. If the culture was around hiking and healthy eating I would be happy doing that instead' [P1].*
> *'I look after my grandkids two days a week . . . have treats for them. We (patient and partner) buy them and eat them before the kids come' [P16].*

Incomplete *knowledge,* misinformation and poor understanding of role of healthcare professionals likely impacted patient non-adherence.

> *'He (patient) just didn't understand what he could eat and what he couldn't eat . . . I (patient's partner) thought that somebody would need to come in and obviously speak to him' [P13].*
> *'I would find it handy if say somebody (dietitian) had prepared like say meals . . . give us suggestions and maybe for the first couple of weeks just stick to that type of diet' [P11].*
> *'It kind of depends on whether I know why it (blood glucose level) was high or not . . . there are still some days where I can't make any sense of it' [P20].*
> *'I was told about the DESMOND (type 2 patient education), which doesn't do anything for me because I'm not on medication' [P3].*
> *'I went on T2D course. It just bored me silly . . . I used to put it on and walk away . . . I felt that my opinion wasn't worth anything' [P4].*
> *'It's (type 2 education course) all online now. I started to do it and then I just got a bit bored of it so I didn't complete it' [P16].*
> *'I wasn't getting any form of help (from my GP)' [P7].*

The other TDF domain of note in this section was *emotion*. Patients expressed various emotions relating to the diagnosis and management of T2D.

> *'Only two people know about my diabetes . . . this notion of shame to me is a significant thing. I guess with diabetes it's almost a lack of control . . . you're eating excessively and you've given yourself this illness' [P1].*
> *'I look at myself and there's a sort of self-loathing there . . . I don't want to be that massive whale-like person' [P10].*
> *'I started to do it (keep a food diary) then I just lost interest in it . . . I feel guilty' [P11].*

Poor physical and mental health hindered adherence to plans.

> *'With the dizzy spells, they've (physical activity professional) put exercise on hold . . . they are going to find me some sitting exercises to do' [P13].*
> *'He (physical activity professional) phoned me . . . I said, oh yes, I am swimming but this last couple of weeks I haven't with cold and then with my leg it's been difficult' [P10].*
> *'I've just re-joined the gym again, but I've got issues with my knees' [P11].*
> *'I've suffered with weight gain . . . depression and anxiety issues . . . and COVID-19 was a bit of a killer. After what happened last time (regained weight lost), I'm not at all confident' [P14].*

In summary, reasons cited for non-adherence to the REMI.D intervention included an obesogenic environment, the impact of others, ambivalence, poor health literacy, low self-esteem and physical ailment.

Patient stigma

The rich data set coming from the *emotion* TDF domain merited its own area of interest. Weight stigma is a form of discrimination based on a person's body weight, and presented a barrier to engagement with the REMI.D project.

Patients heard or perceived weight-related stigma targeted at them.

> *'I just think everyone wants to have a dig at my weight . . . you are fat'. [P22].*
> *'I'd been speaking to the doctor, he was very blunt with me that because I'm overweight, I can't be exercising . . . that must be the reason. And I'm like, dude, I'm with the personal trainer twice a week . . . you can't just say to me because I have that number on a piece of paper that that's the reason' [P6].*
> *'That (diagnosis of T2D) was horrific . . . I cried. It was a telephone appointment and he (GP) called me fat' [P23].*
> *'I think one of the reasons that I don't go out is I just feel too fat. Nobody says anything but you can see the look' [P6].*

Patients were conscious of noticing the body weight of others and drawing self-comparisons.

> *'Every time I go for my bloods (healthcare assistant talks about my weight) . . . so, I think she's overweight . . . she needs to be on them (beta-blockers) . . . so there is a bit of prejudgement' [P2].*
> *'I always think . . . at least I'm not as fat as her' [P16].*
> *'I know all these influencers and models say, but I'm very healthy. How can you when your belly is out here? It's not right' [P3].*
> *'I was watching something on Facebook (social media) the other day, and some woman was drinking one of these energy drinks, Prime, and she was a big built girl, and I thought, what the hell are you doing' [P11].*

In summary, further reasons for non-adherence to the REMI.D intervention included the concepts of feeling judged and being judgemental.

Discussion

This qualitative research represents the views of patients with a recent diagnosis of T2D. Adherence to either of the two dietary strategies enabled a weight loss-induced improvement in glycaemia, with 1 in 7 of the full cohort achieving short-term reversal of T2D.

Weight loss, recorded in clinical trials, appears to be the best predictor of T2D remission,[32] with the use of TDR-Formula Food Products interventions offering favourable outcomes for people with T2D.[25,33,34] In 2022, Diabetes UK and the National Institute for Health Research (NIHR)[2] announced funding for the NewDAWN project which seeks to expand the range of weight loss programmes with the potential to offer T2D remission.[35] Parallels may be drawn between the NewDAWN project and this REMI.D project. Both include a TDR-Formula Food Products intervention, a TDR-Food, and more conventional diabetes dietary management strategies. At the time of writing, no publications from NewDAWN were available.

Decision making processes related to food choice is complex, influenced by personal behaviour, the food environment and social interactions. There was no uptake for the TDR-Food offer by REMI.D participants despite a previous successful feasibility study, DIAMOND, by Morris, *et al.*.[36] This raises the question of whether a care provider should influence the choice of one diet over another. In this case, it could be argued that the strongest evidence for successful dietary T2D remission is the

[2]NIHR is a major funder of high quality global health research that directly addresses the diverse health needs of people in low and middle income countries.

TDR-Formula Food Products intervention. The NHS England T2D Path to Remission Programme, based on the TDR-Formula Food Products intervention, launched in 2020.[27] While clinical outcomes influenced the *intentions* of the REMI.D patients, mental and physical wellbeing were strong drivers for participation in REMI.D. An area of growing interest within public health nutrition is 'food choice architecture'; how a food choice is framed through multiple means (food environment, patient and provider interactions, analysis of habitual menu cycles) and its influence on subsequent food selection.[37]

Findings reported within the area of interest 'patient adherence' add to the existing evidence base for facilitators; feelings of *optimism* with measurable improvements in physical and psychological wellbeing. The use of technology, including flash glucose monitoring,[38] provided strong *reinforcement* as an adjunct to dietary adherence in REMI.D participants. These findings add to the work of Taylor, *et al.*.[17]

Positive *social influences* of family, friends and co-workers and *resources* (REMI.D team) proving key to successful primary outcomes in REMI.D. Adding to the findings of Rehackova, *et al.*,[8] *behavioural regulation* featured strongly in REMI.D participants who succeeded in achieving a HbA1c below 48mmol/mol and/ or a weight loss of 10kg or more. A novel approach within the REMI.D project was the establishment of good physical activity habits (walking children to school, accessing YouTube walking videos, planning ahead so daughter can take to park) in addition to helpful food and diabetes-related decisions (having TDR-Formula Food Products in cupboard at work, meal planning and making a shopping list, ringing venue to check if they can accommodate diet, seeking permission to bring own drinks to pub, taking blood glucose meter to work). The Diabetes Intervention Accentuating Diet and Enhancing Metabolism (DIADEM)-1 trial[39] which included physical activity support after a TDR intervention reported a remission rate of 61% compared with 46% in the DiRECT trial.[40] Further research on the benefits of combined tailored diet and physical activity strategies during and following interventions, building on the REMI.D project is required.

Findings reported within the area of interest 'patient non-adherence' and 'patient stigma' add to the existing evidence base for barriers. In particular, rich data related to suboptimal *environmental context and resources* and unhelpful *social influences.* Physical activity was supported where active travel (walking) to school was feasible for REMI.D participants accompanying children. For other REMI.D participants assistance to travel, beyond local neighbourhoods, to safe recreational areas was deemed necessary to facilitate walking.

Work arrangements for REMI.D participants (sedentary job roles, work patterns and perceived limit to breaks, cultural pressure) add weight to the general consensus that the physical environment has an important influence on an individuals' weight status. Likewise, reports of cheap, convenient, ultra-processed foods satisfying REMI.D participants' multiple reasons for eating (hunger, habit, social, psychological, sensory) draws attention to the obesogenic environment described by the Foresight report more than 15 years ago[41] but still very much in evidence today.[42] These drivers of excess calorie intakes and low levels of physical activity arising out of this qualitative research, provide ideas for local place-based initiatives to support population-level weight change.

REMI.D participants' *emotions,* associated with the diagnosis and management of T2D offered valuable insights. REMI.D participants were not immune to prevailing social norms of adequate body weight and shape; feelings of shame, self-loathing, low self-esteem, guilt, anger, judgement and defensiveness fuelled unhelpful attitudes and behaviours. Sutin, *et al.*[43] found weight discrimination to be associated with a 60% increased mortality risk, even when body mass index was controlled for. Patient reports of weight discrimination in healthcare are well documented.[44,45] Talumaa, *et al.*[46] offers stigma reduction strategies for healthcare and calls for a move away from a solely weight-centric approach to a health-focused weight-inclusive one. REMI.D, like other T2D management strategies, has a primary outcome of weight reduction; any rollout into primary care warrants compassionate and knowledgeable care providers.

The REMI.D participants were recruited during or immediately following the COVID-19 pandemic; *knowledge* was incomplete even accounting for a recent diagnosis of T2D, and access to online learning evaluated negatively. A reminder that digital innovations in diabetes do not suit everyone. The REMI.D data emphasises the value of an effective patient: care provider relationship and synchronous tailored advice.

Limitations

A patient preference for conducting the qualitative interview by telephone (85% of sample) was unexpected. A recent Swedish study by Azad, *et al.*[47] identified advantages and challenges, experienced by participants and researchers, to using mobile phones for conducting interviews. Advantages included convenience, greater anonymity and emotional distance. Challenges are presented as 'loss of human encounter, intense listening, worries about technology, and sounds or disturbances in the environment'. The quality of some of the audio recordings of the telephone interviews was impacted where the patient's voice did not carry well over speaker phone.

Recruitment to this qualitative research was challenging leading to ethical amendments to improve recruitment through a financial incentive and advertising. The full cohort has a small sample size which limited the interpretation of the quantitative statistics. As researcher (RCB) conducted the qualitative interviews during the first 12 weeks, the patient's voice in relation to weight loss maintenance was limited.

Conclusion

A scalable choice of dietary and physical activity interventions may enable more patients to achieve T2D remission or delayed progression with deprescribing of diabetes medications. Humanising patient: care provider encounters with the dietitian and physical activity professional resulted in a more empathic environment in which patients felt heard and understood. For the narrative around obesity in healthcare settings to change, erroneous assumptions and beliefs about obesity need to be identified and challenged. Limited resources should be targeted

towards patients who identify with more barriers or fewer opportunities for health behaviour modification. At risk patients include those who are deprived and those with poor mental or physical health. Rewards, incentives and patient-centred feedback are strong motivators for diet and physical activity change.

Abbreviations

COM-B: Capability Opportunity Motivation – Behaviour; **COVID-19:** Coronavirus; **DIADEM:** Diabetes Intervention Accentuating Diet and Enhancing Metabolism; **DIAMOND:** Diabetes Education and Self-Management for Ongoing and Newly Diagnosed; **DiRECT:** Diabetes Remission Clinical Trial; **GP:** General practitioner; **IMD:** Index of multiple deprivation; **NIHR:** National Institute for Health Research; **REMI.D:** Remission in diabetes; **TDF:** Theoretical domains framework; **TDR:** Total diet replacement; **T2D:** Type 2 diabetes.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.30

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RESEARCH ARTICLE

Breastfeeding and biomarkers of folate and cobalamin status in Norwegian infants: a cross-sectional study

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Abstract

Folate and vitamin B_{12} (cobalamin) are essential for growth and development. This cross-sectional study aims to describe folate and vitamin B_{12} status according to infant age and breastfeeding practices in Norwegian infants. Infants aged 0–12 months ($n = 125$) were recruited through public health clinics. We registered breastfeeding status and measured serum concentrations of folate, cobalamin, total homocysteine (tHcy), and methylmalonic acid (MMA). The associations between infant age, breastfeeding, and biomarker concentrations were estimated in regression models. The mean (SD) age was 24 (16) weeks, and 42% were exclusively breastfed, 38% were partially breastfed, and 21% were weaned. Overall, median (IQR) folate, cobalamin, tHcy, and MMA concentrations were 47 (35–66) nmol/L, 250 (178–368) pmol/L, 6.99 (5.69–9.27) μmol/L, and 0.35 (0.24–0.83) μmol/L, respectively. None of the infants were folate deficient, 15% were vitamin B_{12} deficient (< 148 pmol/L), and 23% had low vitamin B_{12} status (148–221 pmol/L). Elevated tHcy (> 6.5 μmol/L) and MMA (> 0.26 μmol/L) were found in 62% and 69% of the infants, respectively. Compared to weaned, exclusively or partially breastfed infants were younger and had 46% higher tHcy concentrations ($P < 0.001$), in addition to 47% and 39% lower cobalamin concentrations ($P < 0.001$), respectively. However, the observed biomarker concentrations appeared to be independent of infant age. In conclusion, low vitamin B_{12} status was prevalent and appeared to be more common in the younger exclusively breastfed compared to older weaned infants. The implications of low vitamin B_{12} status in infancy are unknown and require further investigation.

Key words: **Breastfeeding: Folate: Homocysteine: Methylmalonic acid: Vitamin B_{12}**

Introduction

Folate and vitamin B_{12} (cobalamin) play pivotal roles in DNA synthesis, cell division, formation of red blood cells, and myelination of the nervous system.[1] Due to rapid growth and development, these vitamins are especially important during infancy.[2] Infant dietary sources of folate and vitamin B_{12} include breast milk, formula milk, and complementary foods. Even though exclusive breastfeeding is important and recommended during the first 4–6 months of life,[3] breast milk is not a nutritionally complete food due to low concentrations of vitamin D and K.[4] Breast milk is considered a good source of folate;[4] however, concerns have been raised about low vitamin B_{12} concentrations in breast milk, especially in the context of exclusively and prolonged breastfeeding.[5,6]

The vitamin B_{12} concentration of breast milk depends on maternal status and potentially maternal vitamin B_{12} intake during pregnancy and lactation.[7] The vitamin B_{12} content in breast milk declines during the first 4–6 months of lactation.[5,8,9] A declining infant vitamin B_{12} status from birth to 4–6 months of age has been observed.[5,10] In these studies,

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formula-fed or partially breastfed infants had a biomarker profile indicative of a more favorable vitamin B_{12} status, compared to exclusively breastfed.[5,10] Further, studies in both high- and low-income settings suggest that breastfed infants across different age groups appear to have lower cobalamin concentrations, accompanied by higher circulating concentrations of the functional biomarkers of vitamin B_{12} status, total homocysteine (tHcy), and methylmalonic acid (MMA), compared to non-breastfed infants.[11–14]

Typical symptoms of infant vitamin B_{12} deficiency are failure to thrive, lethargy, hypotonia, food refusal, and cognitive and motor developmental delays.[15] Low vitamin B_{12} status (often defined as serum cobalamin 148–221 pmol/L) is typically asymptomatic but may adversely affect long-term growth and development.[1] For instance, vitamin B_{12} status during infancy was associated with impaired growth and performance on neuropsychological tests in children at 5 years of age, even though only a small percentage of these children were classified as having vitamin B_{12} deficiency (< 148 pmol/L) during infancy.[16,17]

Various biomarkers, cut-offs, and algorithms have been used to define deficiency and low vitamin B_{12} status; however, limited age-specific reference intervals and cut-off values exist.[18] In this study, we describe maternal dietary intake of vitamin B12 and investigate the direct biomarkers serum folate and cobalamin in conjunction with the functional biomarkers, MMA and tHcy, in Norwegian infants. Interpretation of these markers remains challenging because of the relation between folate and tHcy, and other factors impacting the utility of MMA. For these reasons and in an effort to improve the biochemical assessment of vitamin B_{12} status, we also included the combined indicator of vitamin B_{12} status, $3cB_{12}$.[19] Furthermore, we aimed to evaluate the correlation between these biomarkers and explore their association with infant age and breastfeeding status.

Methods

Study procedure and participants

We examined data from a cross-sectional study investigating iodine status conducted at two public health clinics in Norway. Mothers, who had a scheduled visit to the clinics and whose infants were aged 0–12 months, were asked to participate. Mother–infant pairs were recruited from October to December 2018. The study design, including dietary assessment and eligibility criteria, have previously been described in detail.[20,21] In brief, information regarding the participants' background, breastfeeding status, and diet was collected during structured iodine-based 24 h dietary recalls and via online questionnaires that contained a food frequency questionnaire (FFQ). The FFQ was not validated. Prior to the start of the study, the mothers provided written informed consent. The trial was approved by the Regional Committees for Medical and Health Research Ethics South-East Norway (2018/1230). All study procedures were performed in accordance with the Declaration of Helsinki.

Dietary intake

Maternal habitual vitamin B_{12} was estimated from an FFQ containing 31 food items. The FFQ contained questions regarding the consumption of vitamin B_{12}-containing food groups such as meat (red, white, and game), fish (fat and lean), dairy products (milk, yoghurt, and cheese), and egg during the preceding 4 weeks. Each food group had seven possible responses: rarely/never, less than weekly, 1–3 times weekly, 4–6 times weekly, 1–2 times daily, 3–4 times daily, and 5 times daily or more. The frequencies were converted into daily amounts. The dietary intake of vitamin B_{12} was calculated using the Norwegian Food Composite Table[22] and standard portion sizes[23] or portion sizes as defined in the FFQ. We also calculated the intake of vitamin B_{12} from supplements using data from the structured iodine-based 24 h dietary recall. In this question, we captured the name and dosage of the supplements, in addition to how often the supplements were consumed per week. The same questions were used to capture infant's consumption of supplements. Information regarding the infant's consumption of formulas and cereals was collected from FFQs and 24 h dietary recalls. The nutrient content of reported supplements, formulas and cereals was based on information from the producers.

Biochemical analyses

Heel capillary blood samples were collected by a trained and dedicated staff in 1.5 mL micro tubes (Sarstedt AG & Co.) and left at room temperature for approximately 15 min before centrifuged (2000 G, 20°C, 10 min). The serum samples were stored at −80°C and transported on dry ice for analyses. We used microbiological assays based on a chloramphenicol-resistant strain of *Lactobacillus casei*[24] and colistin sulphate-resistant strain of *Lactobacillus leichmannii*,[25] to determine serum folate and cobalamin concentrations. Serum concentrations of MMA and tHcy were analysed by gas chromatography-tandem mass spectrometry (GC-MS/MS) based on methylchloroformate derivatisation.[26] The within- and between-day coefficient of variation was 4% and 5%, respectively, for both folate and cobalamin. For MMA and tHcy, within- and between-day coefficient of variation ranged from 1 to 5% and 1 to 3%, respectively.[26] All blood samples were analysed at Bevital Laboratory, Bergen, Norway (www.bevital.no).

Definitions and cut-off values

There is a lack of consensus on age-specific reference values for defining folate and cobalamin deficiency. Therefore, we used the World Health Organization (WHO) following cut-offs, previously defined for adults: serum folate concentrations of > 45.3 nmol/L, < 13.4 nmol/L, and < 6.8 nmol/L were considered as 'elevated', 'possible deficiency', and 'deficiency', respectively.[27] We also used the WHO-suggested cut-off of < 10 nmol/L to define folate deficiency.[27] Cobalamin concentrations of < 148 pmol/L, 148–221 pmol/L, and < 250 pmol/L were defined as 'deficiency', 'low vitamin B_{12}', and 'subclinical deficiency', respectively.[18] In addition to the direct

measures of folate and cobalamin status, we used the functional biomarkers MMA and tHcy. For adults, MMA concentrations above 0.26 μmol/L and tHcy concentrations above 13 μmol/L have been considered elevated.[18] In addition, we used a tHcy cut-off of > 6.5 μmol/L as a metabolic indicator of low vitamin B_{12} status in infants, as previously proposed.[28] This cut-off denoted the 97.5th percentile of infants following an intramuscular cobalamin injection[28] and has also been used in two RCTs evaluating the effect of intramuscular cobalamin injection on cognitive and motor function.[11,29] While vitamin B_{12} status is assessed predominantly by total serum cobalamin concentrations, both the sensitivity and specificity of the marker are questionable which is why the functional markers MMA and tHcy also tend to be measured. We also included the combined indicator of vitamin B_{12} status, $3cB_{12}$. In brief, the $3cB_{12}$ is calculated by the log-transformed cobalamin over the product of tHcy and MMA concentrations. In adults, a score of < -0.5 is regarded as low vitamin B_{12} status.[19] Self-reported maternal height and weight (at inclusion) were used to calculate the body mass index (BMI) in kg/m^2. We stratified maternal BMI into four categories: underweight (< 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (> 30 kg/m^2). The birth weight was stratified into three categories: low birth weight (< 2500 g), normal birth weight (2500–4500 g), and high birth weight (> 4500 g). Breastfeeding status was categorised as exclusively breastfed (only breast milk and vitamins/minerals), partially breastfed (defined as breast milk and other liquids, or breast milk and formulas, or breast milk and complementary food, or a combination of breast milk and liquids and complementary foods) and weaned infants (including 2 infants that were never breastfed). The infants were categorised based on current breastfeeding status as reported by the mothers in the 24 h dietary recalls and FFQs.

Statistical methods

Binary and categorical variables were reported as frequencies and percentages (infant gender, preterm delivery, birth weight, marital status, educational attainment, maternal BMI, maternal dietary preferences, breastfeeding status, and supplement use). Maternal and infant's age were reported as mean (SD). Biomarker concentrations were reported as median and interquartile range (IQR) due to the skewed distribution of the data. The biomarkers were also enumerated (frequency and percentages) according to suggested cut-off values. Multiple linear regression models were used to explore the possible associations between biomarker concentrations (log-transformed concentrations of folate, cobalamin, MMA, and tHcy and $3cB_{12}$), the infant's age, and breastfeeding status. Spearman's rank-order correlation coefficient was used to describe the monotonic correlations between the different biomarkers. We used fractional-polynomial prediction plots to explore non-linear relationships between the biomarkers. In these plots, we removed the lowest observed cobalamin concentrations. We also made a prediction plot of biomarker concentration as a function of age, according to breastfeeding status (exclusively or partially breastfed versus weaned/never breastfed). For interpretation of this plot, we included only infants above 6 weeks of age, so the curves (breastfed versus weaned) had the same starting point. Statistical analyses were performed using the statistical software Stata, version 16 (STATA Corp, Houston, TX, USA). P values < 0.05 were considered statistically significant.

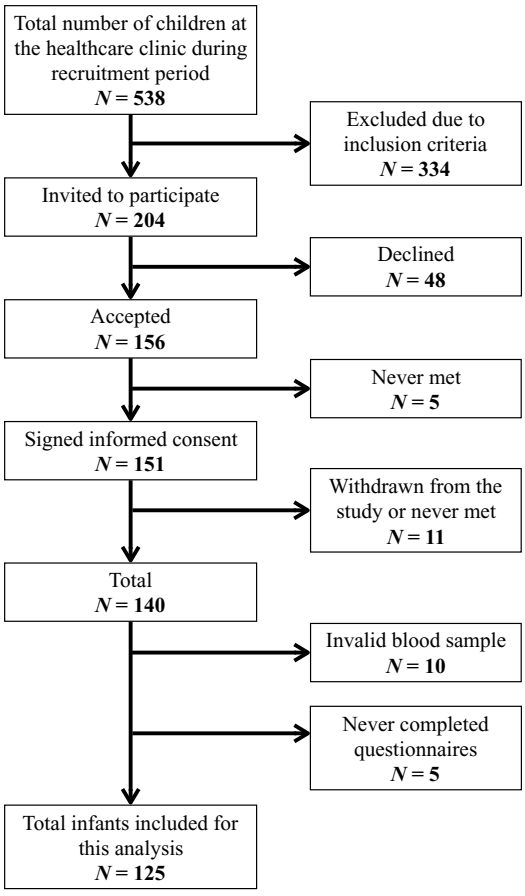

Fig. 1. Flow chart of participants.

Results

General characteristics

A total of 151 mother–infant pairs were recruited from the public health clinics. Eleven mother–infant pairs withdrew from the study or never met for sampling. Blood samples were available from 125 remaining infants (Fig. 1). The mother–infant pairs' background information is summarised in Table 1. Five infants had low birth weight (< 2500 g) and no infant had a birth weight under 2000 g.

Dietary intake

Fortified cereals were consumed by 60% of the partially breastfed infants and 73% of the weaned infants. All fortified cereals were enriched with folic acid, and 38% of partially breastfed and 54% of weaned infants consumed cereals

Table 1. Baseline characteristics of 125 Norwegian mother–infant pair[a]

Characteristic	Total ($n = 125$)	Exclusive ($n = 52$)	Partial ($n = 47$)	Weaned ($n = 26$)
Age child, week	24 ± 16	11 ± 6	31 ± 12	37 ± 15
Age mother, years	31 ± 5	31 ± 4	31 ± 5	31 ± 4
Girls	61 (49)	23 (44)	26 (55)	12 (46)
Preterm delivery[b]	17 (14)	5 (10)	8 (17)	4 (15)
Birth weight				
< 2500 g	5 (4.0)	1 (1.9)	2 (4.5)	2 (7.7)
2500–4500 g	116 (93)	49 (94)	44 (94)	23 (88)
> 4500 g	4 (3.2)	2 (3.8)	1 (2.1)	1 (3.8)
Marital status				
Single	1 (0.8)	0 (0)	1 (2.2)	0 (0)
Cohabitation	75 (61)	33 (65)	31 (69)	11 (42)
Married	46 (38)	18 (35)	13 (29)	15 (58)
Education, mother				
< 12 years	4 (3.2)	1 (1.9)	1 (2.2)	2 (7.7)
12 year	19 (15)	7 (13)	9 (20)	3 (12)
1–4 years college/university	47 (38)	20 (38)	14 (30)	13 (50)
> 4 years college/university	54 (43)	24 (46)	22 (48)	8 (31)
Maternal BMI at inclusion				
< 18.5 (underweight)	2 (1.7)	0 (0)	1 (2.3)	1 (4.3)
18.5–24.9 (normal weight)	77 (66)	34 (67)	30 (70)	13 (57)
25–29.9 (overweight)	25 (21)	13 (25)	7 (16)	5 (22)
> 30 (obese)	13 (11)	4 (7.8)	5 (12)	4 (17)
Maternal dietary variables				
Vegetarian/vegan	3 (2.4)	0 (0)	2 (4.4)	1 (3.8)
Vitamin B supplements[c]	38 (32)	17 (32)	12 (26)	9 (35)
Infant dietary intake[d]				
Formula	28 (22)	0 (0)	10 (21)	18 (69)
Fortified cereals	47 (38)	0 (0)	28 (60)	19 (73)
Vitamin B_{12} fortified cereals	32 (26)	0 (0)	18 (38)	14 (54)
Non-fortified cereals	8 (6)	0 (0)	5 (11)	3 (12)
Serum biomarkers				
Folate, nmol/L	47.2 (34.5, 65.8)	39.3 (30.0, 62.6)	48.9 (38.8, 65.6)	61.7 (46.4, 85.8)
Cobalamin, pmol/L	250 (178, 368)	223 (167, 277)	239 (190, 315)	391 (348, 461)
tHcy, µmol/L	6.99 (5.69, 9.27)	7.43 (6.19, 9.59)	7.22 (6.29, 9.34)	4.94 (4.52, 6.23)
MMA, µmol/L	0.35 (0.24, 0.83)	0.33 (0.26, 1.01)	0.67 (0.35, 1.20)	0.21 (0.17, 0.25)
3cB12	−0.32 (−1.05, 0.23)	−0.38 (−1.38, − 0.10)	−0.74 (−1.35, −0.11)	0.47 (0.23, 0.77)

tHcy, total homocysteine; MMA, methylmalonic acid; 3cB12, combined indicator of vitamin B_{12} status including 3 biomarkers (cobalamin, MMA, and tHcy). [a]Values are mean ± SD, median (IQR), or *n* (%). [b]Defined as birth < 37 weeks of gestation. [c]Reported as weekly intake from 24 h dietary recall. [d]Reported as daily intake from food frequency questionnaires.

additionally enriched with vitamin B_{12}. Among the partially breastfed infants, 17% of the infants had not been introduced to complementary foods and were only consuming formulas in addition to breast milk. For the weaned and partially breastfed infants, the mean age (SD) for introduction to complementary foods was 4.5 (0.74) months. Only one child consumed a multimicronutrient supplement and another child consumed a folic acid supplement. Among the mothers, 38 (32%) reported consuming supplements of folate, vitamin B_{12}, or a combination of these vitamins. Most of the reported supplements contained multiple micronutrients. The daily content of folic acid and vitamin B_{12} in these supplements ranged from 75 to 400 µg and 1 to 10 µg, respectively. Two mothers were consuming high-dose vitamin B_{12} supplements that contained 500 µg. One mother was vegetarian, and two mothers were vegans. Median (IQR) dietary intake of vitamin B_{12} was 4.2 (3.0–5.5) µg from food and 4.6 (3.5–6.3) µg from foods and supplements combined. Among the lactating women, 91% met the recommended intake (RI) from 2012 of 2.6 µg/d,[30] while 63% met the newly suggested adequate intake (AI) of 4.2 µg/d.[31] For the non-lactating women, 81% met the RI from 2012 of 2.0 µg/d,[30] while 58% met the newly suggested AI of 4.0 µg/d.[31]

Infant folate and vitamin B_{12} status

The median (IQR) concentrations of direct and functional biomarkers of folate and cobalamin status are reported in Table 1. Suggested cut-off values and the respective prevalence are shown in Table 2. Folate deficiency was not present among any of the infants (regardless of the cut-off values used). Vitamin B_{12} deficiency (cobalamin < 148 pmol/L) was identified in 10 exclusively breastfed, eight partially breastfed, and one weaned infant. None of the weaned infants had low vitamin B_{12} status (cobalamin 148–221 pmol/L). According to the $3cB_{12}$ cutoff used in adults ($3cB_{12} < -0.5$), 42.2% infants had low vitamin B_{12} status. Serum cobalamin was inversely correlated with serum concentrations of tHcy (rho = −0.67,

Table 2. Suggested cut-off values and corresponding prevalence of vitamin B_{12} and folate deficiency among 125 Norwegian infants[a]

Cut-off	Definition [ref]	*n* (%)
Folate < 13.4 nmol/L	Possible deficiency[27]	0 (0.0)
Folate < 10 nmol/L	Deficiency[27]	0 (0.0)
Folate < 6.8 nmol/L	Deficiency[27]	0 (0.0)
Folate > 45.7 nmol/L	Elevated Folate[27]	65 (52.0)
Cobalamin < 148 pmol/L	Deficiency[18]	19 (15.2)
Cobalamin 148–221 pmol/L	Low vitamin B_{12}[18]	29 (23.2)
Cobalamin < 250 pmol/L	Subclinical deficiency[18]	63 (50.4)
tHcy > 13 µmol/L	Elevated[18]	5 (4.0)
tHcy > 6.5 µmol/L	Elevated[28]	78 (62.4)
MMA > 0.26 µmol/L	Elevated[18]	87 (68.8)
3cB12 < −0.5	Low status[19]	53 (42.4)

tHcy, total homocysteine; MMA, methylmalonic acid; $3cB_{12}$, combined indicator of vitamin B_{12} status including 3 biomarkers (cobalamin, MMA, and tHcy). [a]Values are *n* (%). References for the cut-offs values and definitions included in parentheses.

$P < 0.001$) and MMA (rho = −0.51, $P < 0.001$). These correlations are depicted in Fig. 2.

Infant age and breastfeeding status and their association with folate and cobalamin status

Results from the regression analyses are reported in Table 3. Although none of the included infants were folate deficient, infants that were exclusively breastfed had 35% ($P = 0.004$) lower serum folate concentrations compared to weaned infants. The difference in folate concentration between partially breastfed and weaned infants did not reach statistical significance ($P = 0.08$). Compared to weaned infants, exclusively or partially breastfed infants had a 47% and 39% lower cobalamin concentration ($P < 0.001$), respectively. Both exclusively and partially breastfed infants also had a 46% higher serum tHcy concentration ($P < 0.001$). Serum MMA concentrations were more than two times higher in partially breastfed compared to weaned infants ($P < 0.001$). The mean difference in MMA concentration between exclusively breastfed and weaned infants did not reach statistical significance ($P = 0.186$). Compared to weaned infants, the 3cB12 scores were 0.84 and 1.17 units lower in exclusively and partially breastfed infants, respectively. The infant's age was not associated with biomarker concentrations. Figure 3 depicts biomarker concentrations in relation to infant age according to breastfeeding status.

Discussion

A key finding of the current study was the low vitamin B_{12} status in breastfed infants compared to weaned infants, independent of infant age (Fig. 3). Differences in vitamin B_{12} status according to infant's feeding practice have previously been reported worldwide[12–14,32] and among Norwegian infants[10,11] and are now replicated in the present study. Notably, the Norwegian studies have been conducted over the previous 10–25 years, and given the recent focus on and recommendation of more plant-based diets (typically with reduced vitamin B_{12} availability), our findings underscore the importance of evaluating vitamin B_{12} status in certain groups, such as infants.

Infant's feeding practice

The vitamin B_{12} concentration in breast milk depends on maternal biochemical status, both during pregnancy and after,[7] which unfortunately was not assessed in the present study. The partially breastfed infants also had lower serum cobalamin concentrations alongside higher serum tHcy compared to weaned infants. Similar findings have previously been reported among 6 and 12 months old Norwegian infants.[10] This may be related to low dietary cobalamin intake despite the introduction of complementary foods. Fruits and vegetables, which do not contain vitamin B_{12}, are common weaning foods,[33] while dairy, the food group that typically contributed the most to vitamin B_{12} intake among Norwegian toddlers,[34] is not recommended before 10–12 months of age.[35] Industrialised porridge is another very common complementary food.[33] Some, but not all, industrialised porridge on the Norwegian market is fortified with vitamin B_{12}. Other studies have found associations between infant age and vitamin B_{12} status;[36,37] however, we were not able to find such associations in the analyses taking feeding into account.

Maternal dietary vitamin B_{12} intake

Our estimation of maternal dietary vitamin B_{12} intake was lower than that reported in the Norwegian Mother and Child Cohort (Moba) where the mean (SD) dietary intake (including supplements) of vitamin B_{12} was 8.8 (23.2) µg/d.[38] A plausible explanation for the higher vitamin B_{12} estimations in Moba is the use of a more comprehensive FFQ containing a total of 250 foods, compared to our 31 foods. Thus, the estimated dietary intake of vitamin B_{12} in the present study might be too low, and the percentage of lactating women meeting the adequate intake of vitamin B_{12} is likely underestimated. The vitamin B_{12} concentration in breast milk is considered to depend on maternal status,[4] and in two randomised controlled trials (RCT), maternal vitamin B_{12} supplementation intake improved breast milk concentration and infant vitamin B_{12} status.[39,40] However, in these RCTs, the supplementation started in early pregnancy and continued until 6–12 weeks postpartum. In addition, the supplement contained high doses of vitamin B_{12} (50–250 µg/d). Unfortunately, we do not have information regarding maternal dietary intake and supplement use during pregnancy, which potentially could have an impact on the infants' vitamin B_{12} status, particularly in early infancy.

Cobalamin and tHcy concentration

In general, the infants' serum cobalamin concentrations in our study are reasonably comparable to other studies among newborns and infants in high-income settings,[5,9,37,41–43] as well as in some middle- and low-income settings.[36,44] Likewise, the tHcy concentrations observed in our study were comparable to other Scandinavian studies,[9,37,41,42,45] but not with studies from low-income settings. For instance, in Nepal, the infants' plasma tHcy concentrations were almost twice that of tHcy observed in our study, even though infants from these populations had comparable circulating cobalamin and folate measurements.[36,44] Other studies have shown that genetic variations,

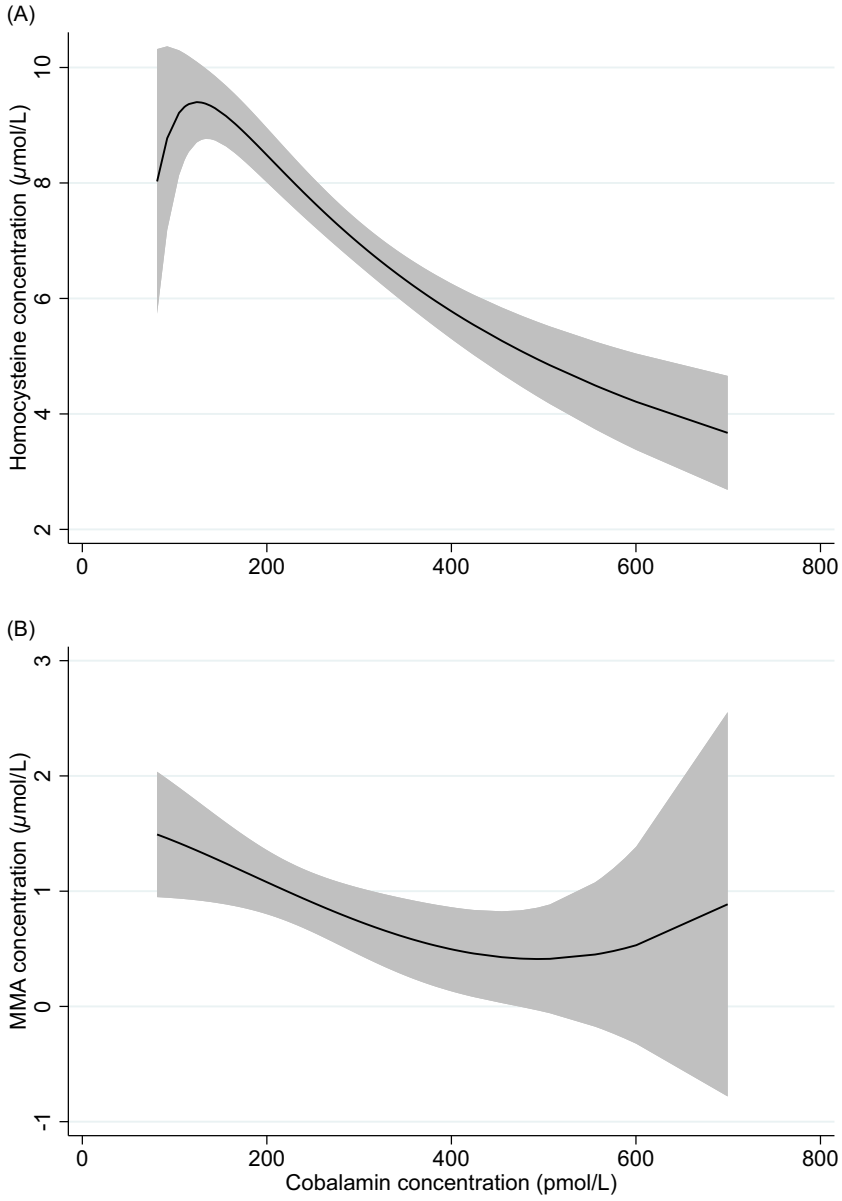

Fig. 2. Two-way fractional-polynomial prediction plot describing the correlation between serum concentrations of cobalamin and homocysteine (panel A) and cobalamin and methylmalonic acid (panel B).

BMI, and exposure to air pollution may influence the tHcy concentrations.[46,47] Despite these shortcomings, we and others have found that tHcy is a good marker of infant vitamin B_{12} status.[42,44,45]

MMA concentration and 3cB$_{12}$

The infants' cobalamin concentrations were also correlated to MMA, but less strongly. This finding is consistent with other studies among infants.[42,45] Even though serum cobalamin concentrations in exclusively and partially breastfed infants were similar, serum MMA concentrations were higher in partially breastfed infants. The higher MMA concentrations in partially breastfed infants may be related to factors other than cobalamin status, such as increased synthesis or dietary intake of MMA precursors. A number of metabolic pathways, including pyrimidine and propionic acid metabolism as well as branched amino acid (BCAA) degradation, converge upstream of MMA. It is therefore feasible that increased intake of BCAAs or synthesis of propionic acid (produced by the microbiome) stemming from ingestion of formula milk and/or introduction of complementary foods may affect the serum MMA concentrations observed among the partially breastfed infants in the current study. Compared to formula milk, breast milk has lower BCAA content and breastfed infants have lower plasma concentrations of BCAA compared to formula-fed infants.[48] Evolving microbiota represents another explanation and is likely modified by the introduction of complementary foods.[49] Colonisation of certain bacteria may increase the production of MMA precursors such as propionate or odd-chain fatty acids.[50] Such mechanisms potentially undermine the specificity and utility of MMA as a biomarker of vitamin B_{12} status, both alone

Table 3. Generalised linear models for the associations between breastfeeding status and infants' age in relation to biomarkers of vitamin B_{12} status[a]

	Folate[b]	Cobalamin[b]	tHcy[b]	MMA[b]	$3cB_{12}$[c]
Breastfeeding status					
Never/Weaned	Ref	Ref	Ref	Ref	Ref
Partially	0.82 (0.65, 1.02)	0.61 (0.50, 0.75)	1.46 (1.27, 1.69)	2.84 (1.88, 4.29)	−0.84 (−1.31, −0.37)
Exclusively	0.65 (0.49, 0.87)	0.53 (0.41, 0.69)	1.46 (1.21, 1.76)	1.43 (0.84, 2.44)	−1.17 (−1.53, −0.80)
Age of infant, week	1.00 (0.99, 1.01)	1.00 (0.99, 1.00)	1.00 (0.99, 1.00)	0.99 (0.97, 1.00)	0.008 (−0.005 to 0.02)

tHcy, total homocysteine; MMA, methylmalonic acid. $3cB_{12}$, combined indicator of vitamin B_{12} status including 3 biomarkers (cobalamin, MMA, and tHcy). [a]$n = 125$. [b]The values of the single biomarkers were log-transformed and the regression coefficients exponentiated (95% CIs). Thus, the effect measures represent the relative change from the reference group, i.e. the concentration of folate is 18% lower among the partially breastfed infants and 35% lower among the exclusively breastfed infants compared to the reference (never/weaned). [c]Regression coefficients (95% CIs) using untransformed $3cB_{12}$.

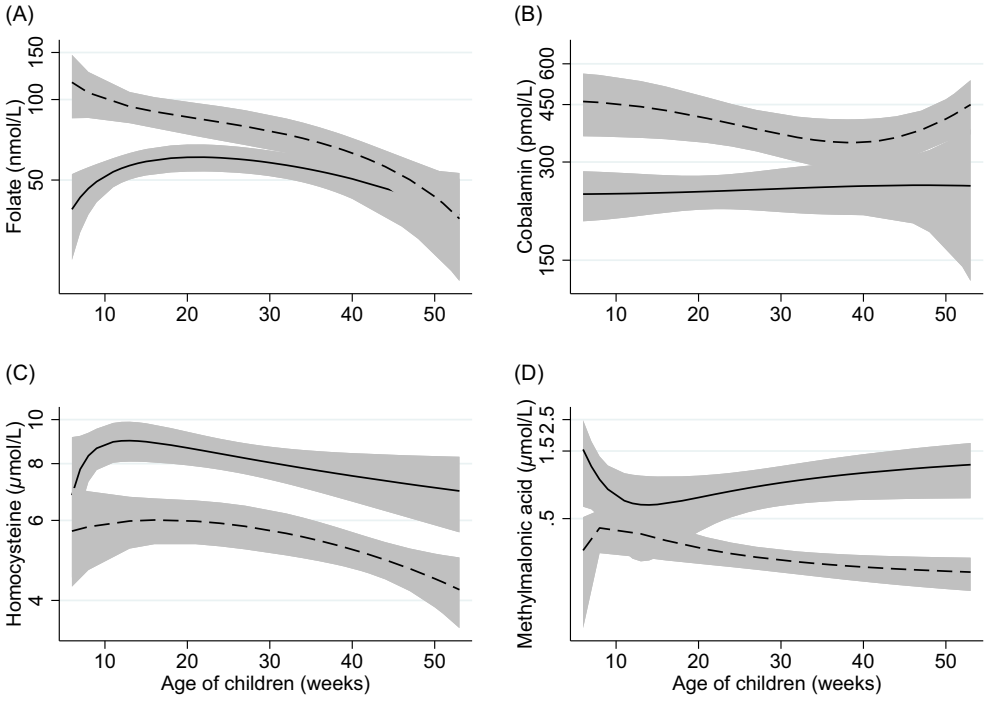

Fig. 3. Two-way fractional-polynomial prediction plot describing the correlation between serum concentrations of folate (panel A), cobalamin (panel B), homocysteine (panel C), methylmalonic acid (panel D), and age of infants (weeks), according to breastfeeding status. Footnotes: The solid line represents breastfed (exclusive and partially) infants ($n = 84$). The dashed line represents weaned and never breastfed infants ($n = 26$). The grey area shows the 95% CI.

and as a component of a combined indicator, especially for infants. For instance, the higher MMA concentrations in partially breastfed infants could also explain the lower $3cB_{12}$ observed in this group. Few, but some other studies have used $3cB_{12}$ in infants.[17,51] However, caution should be urged when evaluating $3cB_{12}$ in infants given that the algorithm was developed in adults over 18 years of age.[19] Moreover, we did not measure holotranscobalamin (holo-TC), which was originally included in the complete combined indicator of vitamin B_{12} status ($4cB_{12}$).[19] Our observations suggest the validity of the combined indicator may be limited by the number of, and which biomarkers are used when evaluating vitamin B_{12} status among infants. It is also worth noting that the use of a four-marker combined indicator tends to be precluded in both research and clinical settings which routinely assess only 2–3 vitamin B_{12}-related markers due to costs and other logistical considerations.

Folate concentration

None of the infants were observed to have deficient or low folate status, although exclusively breastfed infants had lower serum folate concentrations compared to weaned infants. This is surprising given that breast milk is considered a good source of folate.[4] One possible explanation could be a high dietary intake of folate (fruits, vegetables, and liver) or folic acid (formulas and industrialised cereals). Another explanation is that folic acid from formulas and enriched cereals has a higher bioavailability than folate.[52] The high serum folate concentration could also be due to low serum cobalamin

concentrations. This could be explained by a phenomenon commonly known as the 'methylfolate trap'.[53] This phenomenon is explained by poor function of the vitamin B_{12}-dependent enzyme methionine synthase, resulting in an artificially elevated 5-methyltetrahydrofolate concentration, masking true folate status. Moreover, we did not find any correlation between circulating folate and tHcy concentrations, which contrasts with findings among older Norwegian children.[54,55] Besides limited statistical power, another likely explanation for the absence of correlation between folate and tHcy is the adequate folate status among infants in the current study.

Cut-off values and consequences of low vitamin B_{12} status

As previously stated, there are no age-specific cobalamin cut-off values for assessing infant vitamin B_{12} status. If we use the cut-off values for adults, almost one quarter of the infants were categorised as having low vitamin B_{12} (148–221 pmol/L) and 50% of the infants would have been classified as subclinical vitamin B_{12} deficient (<250 pmol/L).[18] It is unclear whether the high prevalence of low vitamin B_{12} status in breastfed infants in our study is due to inappropriate cut-off values for this population or if breast milk is an insufficient source of vitamin B_{12}. It has been suggested that the adequate range of serum cobalamin concentration in infants is lower than what is recommended for adults,[1] while concerns have also been raised about vitamin B_{12} concentrations in breast milk and consequently low vitamin B_{12} status in breastfed infants.[5,6]

Plasma tHcy > 6.5 μmol/L, also denoted as the 'vitamin B_{12} optimised' tHcy cut-off, has been used in three studies among infants by the same authors.[9,11,29] Two Norwegian RCTs reported improvements in motor function after intramuscular injection of 400 μg cobalamin for infants with elevated tHcy (> 6.5 μmol/L).[11,29] However, the included infants had either low birth weight[11] or feeding difficulties, minor neurologic symptoms, or developmental delay,[29] limiting the generalisability of the findings. In addition, both studies had short follow-up periods, and the long-term effects could not be assessed. In a recently published study, 10% ($n = 25$) of 3–8-month-old infants were classified as suggestive vitamin B_{12} deficiency based on serum tHcy > 8 μmol/L, in combination with tremor or excessive sleep.[37] In this prospective study, infants that scored below the 5th percentile on fine motor skills for the Age and Stages Questionaries (ASQ) had, on average, higher circulating tHcy concentrations compared to infants with normal scores. Another important finding of the latter study was that in 78% of the cases with tHcy concentration > 8 μmol/L, the infants' parents did not report any vitamin B_{12} deficiency-related symptoms. Accordingly, the 'vitamin B_{12}-optimised' tHcy cut-off value may appear to have a high sensitivity yet low specificity for symptomatic vitamin B_{12} deficiency. The results from the abovementioned studies build a strong case underscoring the need for RCTs in healthy infants investigating the short- and long-term effects of vitamin B_{12} supplementation.

Strengths of the present study included the measurement of both direct and functional biomarkers of vitamin B_{12} status in a population-based study of healthy infants. It is also a strength that we included participants at different ages of infancy, allowing breastfeeding status and biomarker concentrations to be evaluated independently of age. However, residual confounding factors may limit the interpretation of our findings. Other weaknesses of this study are the age difference across the breastfeeding groups, the relatively small sample size, and we did not have information about breastfeeding duration and when the feeding regime changed for each infant. Thus, it is possible we may not have had sufficient statistical power to measure the association between the biomarkers, breastfeeding, and age. In addition, the participants were not selected at random, which limits the generalisability. Furthermore, we did not analyse total serum holo-transcobalamin, the bioactive form of vitamin B_{12} which is often utilised in combination with other biomarkers to evaluate vitamin B_{12} status.[18] Lastly, we did not measure maternal or breast milk vitamin status, nor did we assess infant dietary folate or vitamin B_{12} intake, which could have impacted the interpretation of the results.

Conclusion

We found that none of the infants were folate deficient; however, low vitamin B_{12} status was prevalent and appeared to be more common in the younger breastfed infants compared to older weaned infants. The implications of low vitamin B_{12} status in infancy are unknown and require further investigation.

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Competing interests

Beate S. Solvik: no conflicts of interest. Kjersti S. Bakken: no conflicts of interest. Adrian McCann: no conflicts of interest. Per M. Ueland: no conflicts of interest. Sigrun Henjum: no conflicts of interest. Tor A. Strand: no conflicts of interest.

Authorship

K.S.B, S.H, and T.A.S designed the study, B.S.S and K.S.B conducted research; P.M.U. and A.M. analysed the blood samples; B.S.S. and T.A.S. performed statistical analysis and had primary responsibility for the final content; All authors have read and approved the final manuscript.

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RESEARCH ARTICLE

Caregiver-reported barriers to engagement in a paediatric fresh fruit and vegetable prescription programme

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Abstract

Paediatric fruit and vegetable prescription programmes hold promise in improving food security and dietary patterns among youth. However, programme success is largely dependent upon caregiver and family engagement. The current study sought to gain a better understanding of environmental barriers to engagement in a paediatric fruit and vegetable prescription programme in one low-income, urban community (Flint, Michigan, USA). Following the implementation of a paediatric fruit and vegetable prescription programme, researchers conducted thirty-two semi-structured interviews with caregivers. Researchers explored caregivers' understanding of the fruit and vegetable prescription programme, barriers to programme engagement, and recommendations for improvement. Telephone interviews were transcribed for textual analysis. Researchers used thematic analysis to examine qualitative data, determine patterns across transcripts, and develop emerging themes. Researchers concluded interviews when data saturation was reached. The majority of participants were female (94%), African American (66%), and residents of Flint (72%). Five recurrent themes emerged: (1) nutrition security; (2) prescription distribution; (3) prescription redemption; (4) educational supports; and (5) programme modifications. Although caregivers indicated that the prescription programme addressed household food insecurity, environmental barriers to engagement were apparent. Caregivers provided suggestions, such as partnering with large grocery stores and developing digital prescriptions, to address programme engagement challenges. Fundamental to the success of fruit and vegetable prescription programmes is the understanding of barriers to engagement from the perspective of participants. This study explores challenges with one paediatric fruit and vegetable prescription programme and provides actionable solutions, from the viewpoint of caregivers, to address these challenges.

Key words: **Children: Farmers' market: Food access: Food security: Fruits and vegetables: Nutrition incentives: Prescription**

Introduction

Nutrients in fruits and vegetables are important for appropriate growth and development[1–8] as well as prevention of diet-related chronic health conditions.[9–12] Unfortunately, most children and adolescents fall short of meeting dietary recommendations for fruits and vegetables.[13–16] Because childhood represents a crucial period of growth when individuals establish enduring dietary habits,[17–19] it is imperative that youth have easy access to fresh, nutrient-dense foods, such as fruits and vegetables.

Fruit and vegetable prescription programmes (FVPPs) are one strategy to increase access to fruits and vegetables for young patients.[20–24] These programmes vary widely in scope and procedures; however, fruit and vegetable prescriptions are often written by primary care providers, distributed to patients, and exchanged for fresh produce at retailers, such as farmers' markets, mobile markets, and grocery or food stores.

In addition to supporting food equity, paediatric FVPPs hold promise as both a primary and secondary prevention strategy.[25] Preliminary research has shown that participation in paediatric FVPPs is associated with improvements in food security,[21,24] food shopping,[26] and youth dietary intake.[21,27–30] Thus far, however, there is a shortage of literature that explores environmental barriers to engagement in these programmes.[23]

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Because the effectiveness of paediatric FVPPs is largely dependent upon programme engagement among caregivers and their families, an investigation of barriers to participation is crucial. Moreover, as research continues to explore the short- and long-term implications of various models of paediatric FVPPs, qualitative research must simultaneously consider the limitations of differing programme designs.

In February 2016, Hurley Children's Center, a large paediatric clinic in Flint, Michigan, partnered with a local farmers' market to create Michigan's first fruit and vegetable prescription programme solely for children.[26,31] The programme provided one $15 prescription for fresh produce to every child, regardless of health status or income, at every office visit. Participants exchanged prescriptions for fresh produce at the Flint Farmers' Market or at Flint Fresh, a local mobile market and food hub. In August 2018, this identical programme was introduced at a second paediatric clinic in Flint as part of a study to examine replicability and preliminary effectiveness.[21,27] The current study sought to qualitatively explore caregiver understanding of this paediatric FVPP, barriers to programme engagement, and recommendations for improvement.

Experimental methods

Study setting and population

Flint, Michigan has approximately 100,000 residents and is home to General Motors. When the American automobile industry declined, Flint fell into an extreme recession.[32] In addition to a child poverty rate that is approximately 50%,[33] youth in Flint continue to struggle in the aftermath of a city-wide lead-contaminated water public health crisis.[34] Additionally, Flint lacks nutrition resources and healthy food options. Poor quality fresh foods in local food stores limit access to nutrient-rich items, such as fruits and vegetables,[31,35,36] and grocery store options in the city are scarce.[37]

Paediatric fruit and vegetable prescription programme (FVPP)

Hurley Children's Center, a residency-training paediatric clinic co-located within a farmers' market, introduced the first paediatric fresh fruit and vegetable prescription programme (FVPP) in Michigan in February 2016. This FVPP was established to actively address enduring challenges with accessing and purchasing fresh produce among youth and families living in Flint. The FVPP was thoughtfully designed to easily integrate into the busy paediatric office while highlighting the importance of daily fruit and vegetable consumption. Prescriptions were added to the clinic's electronic medical record (EMR) system and retained in patient records. Paediatricians ordered produce prescriptions using the EMR system and distributed the printed prescriptions to all paediatric patients at every office visit. The EMR generated monthly prescription distribution reports, which were used to track clinic-wide distribution rates.

The FVPP originally introduced at Hurley Children's Center[26,31] was expanded to one private-practice paediatric clinic in Flint in August 2018. This second clinic, Akpinar Children's Clinic, provides care to 3000 patients, most of whom live in Flint and receive public health insurance. Identical to the original programme, the paediatrician ordered $15 fruit and vegetable prescriptions using the EMR system and gave the prescriptions to all patients during office visits. FVPP vendors included Flint Farmers' Market, a year-round market located in downtown Flint, open Tuesday, Thursday, and Saturday from 9 AM until 5 PM, and Flint Fresh, a mobile market and food hub, that offered free delivery of participant-selected fresh produce boxes. Prescriptions were treated as vouchers that could only be exchanged for fresh fruits and vegetables and were valid for 90 days. Participants could not divide their prescription between multiple farmers' market vendors, and any remaining balance from a transaction totalling less than $15 was lost.

From August 2018 through March 2019, 365 caregiver–child dyads at Akpinar Children's Clinic provided written consent and assent to join a study assessing the feasibility of the FVPP,[21,27] including follow-up interviews to assess programme experiences.

Approach and theoretical framework

The study design and approach followed the theoretical framework of Bandura's Social Cognitive Theory (SCT). SCT explains behaviour through a 3-stage model connecting personal factors, environmental factors, and behaviour.[38] Since children's nutrition choices are typically guided by their caregivers,[39,40] a qualitative investigation of environmental factors that prevented caregivers from fully engaging in the programme with their children was of particular importance.

Participants and data collection

Researchers collected data via semi-structured telephone interviews between December 2022 and March 2023. Distribution data (EMR reports) and redemption data (redeemed paper prescriptions) were used to identify children who enrolled in the original study with their caregiver but failed to redeem fresh produce prescriptions during the approximate four-year study period. To assess barriers to engagement in the FVPP as well as caregiver experiences with the programme, an open-ended interview format was created.

Caregivers were eligible to participate in interviews if: (1) they enrolled in the original study and completed baseline surveys; (2) their enrolled child had received at least one fruit and vegetable prescription; (3) their enrolled child had not redeemed any $15 fruit and vegetable prescriptions during the study period; and (4) their enrolled child was an active patient at Akpinar Children's Clinic. A total of 98 caregivers met eligibility requirements. Researchers attempted to contact all 98 caregivers but were challenged with non-working or disconnected telephones. After 32 interviews were completed, researchers concluded that no new concepts were arising and that data saturation had been reached. Participants received one $50 electronic gift card after completing the interviews.

The open-ended interview format was used to detect caregiver understanding of the FVPP, barriers to programme engagement, and recommendations for improvement. One-on-one interviews were led by three research team members trained

in qualitative research methods. Questions such as *In your own words, can you explain the fruit and vegetable prescription program and how it works* invited conversation regarding caregiver understanding of the programme and redemption procedures, while more involved questions such as *What prevented you from redeeming all of your prescriptions* and *Do you have specific thoughts or ideas to change the program to address the barriers you have experienced redeeming your prescriptions* probed about specific challenges related to programme engagement. Researchers gathered additional information using an interview guide informed by previous literature,[31,41–44] research questions, and experiences with the subject matter and population.

Data analysis

All caregiver interviews were audio recorded and transcribed verbatim for textual data analysis. Researchers examined data by following a coding process informed by thematic analysis. During initial coding, four researchers individually analysed transcripts and identified notable patterns for thematic purposes. Researchers then met to collapse similar themes and determine final emerging themes. Lastly, three researchers selected illustrative direct quotes to represent the final themes and sub-themes.

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 Michigan State University Institutional Review Board – Study 00000666. Written informed consent was obtained from all subjects.

Results

A total of 32 interviews with caregivers were completed. Interview participants (mean age, 40.9 ± 9.4 years) were primarily female (94%), African American (66%), and residents of Flint (72%) (Table 1). These demographics are representative of the full sample of 365 caregivers who enrolled in the larger study (mean age 39.7 ± 9.9 years), most of whom were female (91%), African American (66%), and residents of Flint (72%).

Important to research findings was the general feedback from most caregivers that their household had redeemed at least one prescription during the study period. Although study records accurately tracked and recorded redemption rates for children enrolled in the study, records failed to capture prescription redemption for siblings living in the same household but not enrolled in the study. Only eight of the 32 caregivers interviewed (25%) acknowledged that no child in their household had ever redeemed a produce prescription. We present the following recurrent themes and associated findings, each centred around caregiver experiences with the FVPP: (1) nutrition security; (2) prescription distribution; (3) prescription redemption; (4) educational supports; (5) programme modifications. These themes can be found in Table 2, corresponding with the associated sub-themes.

Nutrition security

Nutrition security is defined as 'consistent access, availability, and affordability of foods and beverages that promote well-being, prevent disease, and, if needed, treat disease, particularly among racial/ethnic minority populations, lower income populations, and rural and remote populations'.[45] Most caregivers indicated that they had redeemed at least one produce prescription with a child in their household during the study period. These caregivers perceived the FVPP to have meaningful impacts on nutrition security.

Table 1. Characteristics of caregivers who completed interviews

	Caregiver characteristics	Frequency *n*	%
Age ± SD (Range)	40.88 ± 9.35 years (29–78 years)		
Gender	Female	30	94
	Male	2	6
Race	African American	21	66
	Caucasian	8	25
	Hispanic	1	3
	Native American/American Indian	1	3
	Other	1	3
Residence	Flint	23	72
	Non-Flint	9	28
Education	Some high school/High school	7	22
	Trade school	2	6
	Associate's degree	12	38
	Bachelor's degree	8	25
	Graduate or professional degree	3	9

A majority of caregivers expressed gratitude for the FVPP as a financial support for their families (Table 2, sub-theme 1.1). Many noted growing concerns regarding the cost of healthy foods, particularly fresh fruits and vegetables, and appreciated the FVPP for alleviating that burden. Some further noted the importance of paediatric office visits, accompanied by produce prescriptions, in directly combating the growing costs of fresh foods.

> *Sometimes it can be really expensive to get healthy fruits and vegetables. And it's $15 worth of fruits and vegetables that I don't have to pay out of pocket . . . It helps the pockets of low-income families.* (Participant 180, African American Female, Age 43)

> *Since COVID, vegetables are going up [in price]. I don't get food stamps, so I took the kids to their appointment today. At least I know I'll get some kind of help for vegetables, fruits and vegetables, by coming in [to the doctor].* (Participant 285, African American Female, Age 42)

Many caregivers recognised the value of the prescription programme in supporting the nutrition and health of their children (Table 2, sub-theme 1.2). Some noted their appreciation for paediatricians who offered the programme, rather than medicine or pills, to actively encourage disease prevention and healthy eating among young patients. Many felt the programme served as an incentive for families to bring their children to the paediatrician.

> *I think it's really great that the doctors care enough about our health to want to give us free fruits and vegetables and teach us about nutritional meals . . . I think that's definitely the best part, to go to the doctor and walk out with a prescription that's not a pill. It's like they are actually wanting you to eat healthy.* (Participant 129, Caucasian Female, Age 36)

Table 2. Illustrative quotes collected from caregivers of children enrolled in a produce prescription programme

Theme	Sub-theme	Illustrative quote
1. Nutrition Security	1.1 Financial Support	"My sister doesn't get any type of help from the state. So, she pays cash for everything. By you guys having this program, she gets some food and she loves going and spending those coupons on it." (Participant 176, African American Female, Age 43)
		"Right now [prescriptions] would be a huge help because my husband hasn't worked in a year and a half. So, I'm working seven days a week, two jobs, trying to keep food on the table, roof over our heads, electricity, all that stuff. I think [the prescription program is] fantastic." (Participant 267, Caucasian Female, Age 56)
		"I think it's great that they are doing it and helping people get fresh fruits and vegetables, especially people that can't afford it. They are expensive. It's more expensive to eat healthy than it is to eat unhealthy." (Participant 303, Caucasian Female, Age 36)
	1.2 Nutrition and Health	"I think it's an amazing program. It gives people an incentive. I work in the medical field, and not everyone takes their child to visit the doctor. A lot of times you see, like urgent cares or emergency rooms being overused for things they don't need to be used for. I think giving them an incentive like [produce prescriptions] brings people to bring their child in for a well visit." (Participant 198, Caucasian Female, Age 34)
		"I think it's a great program because it helps parents not only with their grocery bills, but it makes sure that their children are receiving fruits and vegetables that are vital to good health, and to help them grow." (Participant 267, Caucasian Female, Age 56)
		"I think [the prescription program is] a good way to introduce the importance of fruits and vegetables to the community. It is introducing it to children as young as infants, and parents to ensure that you know the importance of it." (Participant 302, Native American/American Indian Female, Age 33)
	1.3 Child Engagement	"I think the best [part of the] program is that the kids are involved! When I go with my daughter, she picks out the fruits that she wants or she likes to eat. It's kind of a bonding thing for us." (Participant 085, African American Female, Age 53)
		"When we see [pediatrician] and receive the prescription, [my son] knows that's for him to go pick out his own fruits and vegetables. I leave that on him, to pick out the ones he wants." (Participant 198, Caucasian Female, Age 34)
		"They [kids] are definitely helping because if I pick it, they don't want it." (Participant 302, Native American/American Indian Female, Age 33)
2. Prescription Distribution	2.1 Procedures	"I don't think it was every time I went. Like for a check-up or something . . . I thought [prescriptions] were given if [the clinic] wanted to give them to us. I didn't know it was for every time you went in there." (Participant 015, African American Female, Age 43)
		"I didn't know if [pediatrician] was still doing it or not. That's why I was asking my friends because I didn't get one [prescription] last time. I thought maybe the program ended." (Participant 090, Caucasian Female, Age 35)
		"[My son] just came to get a meningitis shot and a physical last week. He didn't get [a prescription]." (Participant 153, African American Female, Age 50)
	2.2 Program Understanding	"I'm not sure how it works. I know they give you a prescription, some for fruits and vegetables, but I didn't use it because I didn't know how to use it." (Participant 082, African American Female, Age 42)
		"When I've been grocery shopping, I've seen where it says you can get the fruit and vegetables, buy one get one free or something like that. Is that the same program?" (Participant 190, African American Female, Age 53)
		"I'm not all clear. It's a guy in like a food truck. I forget what it's called . . . . Sometimes I couldn't get to the farmers' market so Fresh Start [a different program] was the better choice." (Participant 037, African American Female, Age 39)
	2.3 Delivery Option	"I didn't realize that they have a delivery. I never find time to go to the famers' market because I live on the other side of town." (Participant 306, Caucasian Female, Age 36)
		"I didn't know they delivered, and I never really had time to go to the farmers' market." (Participant 347, African American Female, Age 36)
		"I didn't know that they delivered . . . a couple of months ago I heard about it but I'm like, 'Nah they wouldn't come all the way out here' but because I'm in [a different city]." (Participant 290, African American Female, Age 33)
3. Prescription Redemption	3.1 Vendor Site Navigation	"It's not labeled how much things are at the farmers' market. I was just worried about going over the amount and not having the money on me to cover it. I just didn't want to be in a weird position." (Participant 090, Caucasian Female, Age 35)
		"In the beginning we did not know [where to redeem at the farmers' market], and that's why we weren't using prescriptions as much." (Participant 085, African American Female, Age 53)
		"At first it's a little confusing trying to figure out where you go [on the Flint Fresh website] because the paperwork is completely different from the website." (Participant 306, Caucasian Female, Age 36)
	3.2 Paper Prescriptions	"When my kids go to the doctor, and they give me paperwork, I normally take that paperwork and file it away. Then, by the time I remember about the prescriptions and pull them out, they are already expired." (Participant 129, Caucasian Female, Age 36)
		"I lost the prescription. I'm not sure what I did with it. We had some things we had to do after the last visit, and I must have set it down somewhere. You know, out of sight, out of mind." (Participant 198, Caucasian Female, Age 34)
		"I just forget that I have them. And if they are not in my purse or wallet, I don't pay any attention." (Participant 273, African American Female, Age 45)

Continued

Table 2. Continued

Theme	Sub-theme	Illustrative quote
	3.3 Farmers' Market	"One thing was getting to the farmers' market. I worked a lot, and it was hard. It's hard to get to the farmers' market on the days they are open. And when I get there, either I have forgotten about [prescriptions] or it's so busy and so packed where I don't have time for them to explain to me how to go about using the prescription." (Participant 082, African American Female, Age 42) "The farmers' market is only open on certain days . . . And a day that we may have available as a family they may not have been open. And I do recall that, once or twice, a scenario where we just couldn't go that day." (Participant 312, Caucasian Male, Age 39) "I work a lot. And, by the time I get out of work, the farmers' market is closed." (Participant 362, African American Female, Age 37)
	3.4 Transportation	"I have had so many car issues the last couple of years. I just can only really make it to where I need to go and nowhere else." (Participant 129, Caucasian Female, Age 36) "Just living so far away . . . I have a vehicle and all that, but we're a low-income family. It's just hard to get out there and do it. We just shop locally." (Participant 303, Caucasian Female, Age 36) "Now, [a barrier to redeeming prescriptions] would be a transportation issue because I totaled my car out." (Participant 082, African American Female, Age 42)
4. Educational Supports	4.1 Culinary Programs	"Suggestions for healthy ways to get the kids to eat the vegetables. Something like that because I don't think the struggle is people wanting to feed their kids the thing. I think it's getting them to eat it." (Participant 128, African American Female, Age 43) "A cooking class where they taught you like how to take cauliflower and make bread crust, like a crust to a pizza out of cauliflower. Healthier options . . . a healthy dinner where you are getting your vegetables or your fruit." (Participant 198, Caucasian Female, Age 34) "I know we have COVID and stuff, but maybe once a week, a healthy cooking class?" (Participant 302, Native American/American Indian Female, Age 33)
	4.2 Recipes	"If they had pamphlets of recipes or something like that. At school they learn about a vegetable or a fruit, and they get sent home a little recipe card. My kids really enjoy those." (Participant 129, Caucasian Female, Age 36) "Maybe a digital link that will give different recipes to use fruits and vegetables that are available to us." (Participant 166, African American Female, Age 50) "I think a cookbook would be nice." (Participant 273, African American Female, Age 45)
	4.3 Virtual Format	"Maybe like a Facebook Live or YouTube video. Where they pull it up at their convenience. I think it would get a lot more people watching it. 'This is what we are going to make this week. This is what we are going to need.' So, everything is there, right in front of them." (Participant 267, Caucasian Female, Age 56) "I wouldn't be able to take part in the cooking, unless it's online or something . . . due to my work schedule." (Participant 285, African American Female, Age 42) "Most of the time it's just somebody finding the time . . . a lot of people aren't showing up to certain things because COVID and stuff is still around. So virtual is definitely a good thing." (Participant 306, Caucasian Female, Age 36)
5. Program Modifications	5.1 Prescription Format	"It would be nice if it was on a card or something. So, you can redeem everything on the card and don't have to keep up with the [prescription] paper. Just one [card] for the whole family . . . When you go to the market, you just have a card that tells you, 'You have this amount on there to buy fruits and vegetables.'" (Participant 033, African American Female, Age 30) "I think if there was a card, reloadable for the prescriptions, that would be a lot easier. Because everywhere, every store around, has their own cards and phone number to enter. If they had something like that, it would be easier to not lose." (Participant 198, Caucasian Female, Age 34) "If [the prescription] was available on an app, we as a family would have redeemed absolutely every single one, without question." (Participant 312, Caucasian Male, Age 39)
	5.2 Redemption Sites	"It would be nice if [the prescription] was good at local grocery stores as well . . . the main ones that's in my area would be very helpful." (Participant 033, African American Female, Age 30) "Could it be something that could expand to maybe Meijers or Krogers or I don't know Walmart? I don't know if it could be, but it's a suggestion." (Participant 085, African American Female, Age 53) "Some people live in those neighborhoods, so it's more convenient for them to walk down to the store and get those items if they can't make it to the farmers' market." (Participant 166, African American Female, Age 50)
	5.3 Expiration Date	"If maybe they took the expiration date away . . . I think that expiration date thing would help a lot." (Participant 037, African American Female, Age 39) "I wish they would let you go like maybe a year or so because I always file [prescriptions] away and then forget about it. By the time I find it or come across again, they have already expired." (Participant 129, Caucasian Female, Age 36) "They should set a reminder like, 'Hey, [the prescription is] about to expire.' Cause us as parents, we forget things. We get busy with our kids and forget things. So, a text reminder, like 'Hey, your benefits will expire by such and such date.' I think that would be helpful." (Participant 290, African American Female, Age 33)

Many caregivers talked about child engagement in the FVPP, particularly with regard to produce selection at the farmers' market (Table 2, sub-theme 1.3). Some further described how they planned trips to the farmers' market to use their prescriptions as an opportunity to educate and bond with their children.

> *The kids thoroughly enjoyed, and I do mean thoroughly enjoyed, going to the Flint Farmers' Market giving the vendors their [prescriptions], and picking out their own vegetables.* (Participant 312, Caucasian Male, Age 39)

Prescription distribution

Caregivers were asked to explain the FVPP in their own words. Although the majority of caregivers had a general understanding of the programme, key components were consistently unclear among interview participants. Most confusion occurred at the point of distribution (i.e. receipt of produce prescriptions at the paediatric clinic).

In contrast with programme distribution procedures that specified every child should receive one $15 prescription at each office visit, most caregivers indicated that prescriptions were not given during every office visit (Table 2, sub-theme 2.1). Some believed the prescriptions were reserved only for annual well-visits, while others simply explained that prescription distribution was inconsistent across office visits.

> *If I do a walk-in visit, I don't get nothing for that. It's only a scheduled appointment that I ever got [prescriptions].* (Participant 005, African American Female, Age 34)

> *I received it usually when we do their yearly check-ups. Any other time I was there, it would be an emergency call or something like that. I would receive them [only] when we do the check-up yearly.* (Participant 082, African American Female, Age 42)

Nearly every caregiver said that an introduction to the FVPP was provided at the paediatric office when their child received their first prescription. In spite of this programme introduction, many shared that their programme understanding was inadequate (Table 2, sub-theme 2.2). Some confused the FVPP with other food assistance and nutrition incentive programmes. Several admitted that their poor understanding of the FVPP influenced their decision not to redeem prescriptions.

> *I was trying to take everything in on how to do it. Honestly, I didn't fully understand how to [use prescriptions].* (Participant 036, Hispanic Female, Age 44)

> *I didn't redeem them because of the fact that I didn't understand that they were for fruits and vegetables. The fresh things at the market.* (Participant 085, African American Female, Age 53)

When describing the prescription programme in their own words, most caregivers talked only about the local farmers' market as the redemption site for prescriptions. A majority of caregivers were entirely unaware of the free produce delivery option through Flint Fresh (Table 2, sub-theme 2.3). Some, who were aware that Flint Fresh was a vendor for the FVPP, lacked general knowledge of this option, such as delivery procedures, radius, or associated costs.

> *I didn't even know they had delivery.* (Participant 166, African American Female, Age 50)

> *I was afraid with the delivery service that I was going to be charged. And money is tight, it's still tight, but I think the delivery service is a really good idea.* (Participant 267, Caucasian Female, Age 56)

Prescription redemption

When asked to explain specific barriers to engagement in the FVPP, most caregivers talked extensively about prescription redemption. Some were challenged when navigating redemption sites, both in-person at the farmers' market and online through Flint Fresh. Caregivers also discussed conflicts related to work hours and farmers' market hours, challenges with transportation, and management of paper prescriptions. Each of these, alone or in combination, acted as barriers to engagement in the FVPP.

When arriving at the farmers' market or placing an order on the Flint Fresh website, many caregivers shared their frustration with vendor site navigation (Table 2, sub-theme 3.1). Some were unsure which farmers' market vendors accepted prescriptions; while others were unclear regarding specifics of redemption, such as exceeding prescription value or splitting the prescription value between vendors. Some caregivers, who attempted to redeem prescriptions through the Flint Fresh website, described technical challenges.

> *When I got to the farmers' market and walked in, I didn't know where to go. So, that's where I got lost.* (Participant 290, African American Female, Age 33)

> *When I went on the website to try to order [a produce box], for some odd reason it wouldn't let me continue. I picked all my stuff, but it wouldn't let me submit it.* (Participant 176, African American Female, Age 43)

Although every caregiver expressed genuine appreciation for the prescription programme, many shared continual difficulties related to lost or forgotten paper prescriptions (Table 2, sub-theme 3.2). Some further indicated that when found, prescriptions were very often expired (beyond the 90-day expiration date). A majority of caregivers indicated that the format of prescriptions, small pieces of prescription paper, made tracking and management of the incentive difficult.

> *I lose them often because it's such a small piece of paper. I just misplace them. When I do find them again, they are expired. So, that's a big issue.* (Participant 090, Caucasian Female, Age 35)

Many caregivers discussed the exceptional quality of produce and generosity of farmers' market vendors. However, some commented on limitations of the farmers' market as the primary produce prescription programme redemption site (Table 2, sub-theme 3.3). Most indicated that the limited hours of operation of the farmers' market (Tuesday, Thursday, and Saturday from

9:00 AM to 5:00 PM) conflicted with work or family schedules. Some further shared that distance to the farmers' market or anticipated crowds discouraged prescription redemption there.

> *It is kind of hard, especially working nine to five . . . Really the only day I have available, if I'm available that day, is a Saturday.* (Participant 180, African American Female, Age 43)

Flint Farmers' Market is located in downtown Flint. It is easy to access through public transportation, specifically through bus routes. Still, many caregivers discussed challenges related to reliable transportation to the farmers' market (Table 2, sub-theme 3.4). Some shared problems specifically related to functioning cars, while others felt unsafe accessing public transportation.

> *I think over the years I've probably redeemed them [prescriptions] two or three times only because of lack of transportation.* (Participant 166, African American Female, Age 50)

> *There was one point in time when there was a transportation issue. Even though I did have access to the bus, it wasn't a safe area to get on the bus. Even though I had access to the bus line, where the bus line was, it wasn't safe to get on.* (Participant 302, Native American/American Indian Female, Age 33)

Educational supports

When asked to describe the type of education that caregivers felt would benefit their families, many indicated that they would like cooking classes or recipes to be offered alongside prescriptions. Some further suggested that educational programmes or sessions should be offered in a virtual format.

Most caregivers requested that culinary programmes be offered with the FVPP (Table 2, sub-theme 4.1). Some caregivers shared struggles encouraging their children to eat more fruits and vegetables and desired a class that would address this challenge. Others believed a youth-focused cooking class would be beneficial.

> *I have a 15-year-old daughter, and she absolutely would love some [culinary] education if it was presented to her. "Hey, go to the farmers' market, go buy these things and then you can use them to cook XYZ".* (Participant 312, Caucasian Male, Age 39)

Nearly all caregivers requested that recipes accompany produce prescriptions (Table 2, sub-theme 4.2). Some felt recipes should be distributed with prescriptions; while others suggested fruit and vegetable recipes be provided through a website, regular emails, or farmers' market kiosk.

> *Recipes would be awesome . . . Email or at the doctor. Just have the attendant print [recipes] off. Just hand recipes to you while you are at the doctor's office. Just like [pediatrician] does with the prescription.* (Participant 015, African American Female, Age 43)

> *A website or recipes you could download. For instance, when you get the prescription, maybe there would be a website for quick meals or quick recipes. Or when we go to the farmers' market, they give out free recipes or something.* (Participant 085, African American Female, Age 53)

Many caregivers suggested that if programme education, including nutrition or culinary instruction, were to be offered with the prescription programme, sessions should be presented in a virtual format (Table 2, sub-theme 4.3). Some noted busy schedules and competing priorities, while others pointed to struggles with transportation. Most felt that offering education virtually would address these barriers.

> *It's kind of inconvenient sitting in a class when you've got so much to do. I believe something on a QR code or app. So, [participants] can just go on and look it up and maybe even a video to help people and show them what to do.* (Participant 033, African American Female, Age 30)

> *A class or something through Zoom. So that even if a person doesn't have transportation, most people have cell phones, even the free government phones. So, they would be able to go to a Zoom meeting for the purpose of learning how this program works and things about nutrition.* (Participant 190, African American Female, Age 53)

Programme modifications

Throughout the interviews, caregivers were candid regarding modifications that should be made to the current programme to improve engagement. Most interview participants had redeemed at least one produce prescription, and many felt changes could be made to improve the programme experience for families.

The majority of caregivers felt strongly that the paper prescription format needed to be changed (Table 2, sub-theme 5.1). Caregivers offered thoughtful solutions to address the challenges with paper prescriptions. Most clearly indicated a desire for prescriptions to be available through a card or an application (i.e. app) for the entire household. Some further suggested that prescription cards or apps should automatically reload for members of the household following clinic appointments.

> *An app that's under an umbrella of all my kids versus individually and me having one for each kid at random times . . . Just having multiple kids and sending random papers [prescriptions] attached to check-out stuff. I think an app, where I know it's going to be there, already loaded and ready to go. I think that would be more helpful.* (Participant 005, African American Female, Age 34)

> *A card instead of paper. Because paper is something that can really easily get misplaced. But a card, you can put it inside of your wallet and you'll be able to see it and say, "Oh yeah, let me go use this." Even a reusable card that once you go in [to the pediatrician's office], they just refill it for you.* (Participant 015, African American Female, Age 43)

Although most caregivers expressed fondness for the farmers' market, many felt the programme should expand to include more redemption sites (Table 2, sub-theme 5.2). Specifically, caregivers felt that expansion to full-service grocery stores would increase programme engagement due to their broad

selection of food items that would allow caregivers to redeem prescriptions while grocery shopping. Some also mentioned that grocery stores were closer to their homes and more convenient to shop at than the farmers' market.

> *[Prescriptions] are only good at the farmers' market, and I don't always have the time to go to the farmers' market. I do a lot of my shopping at [grocery stores], and I can go straight there. I'm not big on going to multiple stores . . . I like to just go to where I am going and go back home. I have a lot of children, so I don't really have a lot of time to stop at the farmers' market as well as other grocery stores.* (Participant 033, African American Female, Age 30)

Produce prescriptions expired 90 days from the date of distribution. Many caregivers expressed frustration with these expiration dates (Table 2, sub-theme 5.3). Some asked that the expiration dates be removed from the prescriptions entirely. Others requested that reminder texts or emails be sent to caregivers when prescriptions were nearing their expiration date.

> *Just a small text message or an email . . . "Don't forget to use your coupon by this date." Like when the doctor reminds you that you have a doctor's appointment. Because sometimes, as parents, we get overwhelmed and sometimes forget. So, a reminder would definitely help.* (Participant 015, African American Female, Age 43)

> *I don't know why they have an expiration date. I did go one time, and I was excited to know that I had one of those prescriptions. But it was expired. So, maybe if they didn't have the expiration dates.* (Participant 036, Hispanic Female, Age 44)

Discussion

The current study is among the first to explore and describe caregiver-reported barriers to engagement in a paediatric FVPP. Most caregivers reported that they had redeemed at least one produce prescription with a child in their household and maintained deep gratitude for the programme that served as both a financial and nutritional support for their children. However, many were also forthcoming about the need for programme improvements to increase overall engagement. Central to our findings was a lack of awareness regarding specific features of the programme, such as prescription distribution schedules, vendor details, and redemption rules. Many caregivers further indicated that this lack of clarity influenced their decision not to redeem prescriptions. Additional environmental barriers to redemption included transportation challenges, management of small pieces of prescription paper, and limited farmers' market selection and hours. Caregivers offered practical suggestions, such as digital prescriptions and partnerships with full-service grocery stores, to address many of the challenges identified during interviews.

Similar to previous qualitative work, caregivers expressed a genuine appreciation for the FVPP, recognising that healthcare providers offered this programme out of concern for the health and well-being of their young patients.[31] Some even indicated that the programme served as a motivation for attending regular office visits or as a deterrent from utilising urgent or emergency care services. Although research examining health care utilisation among participants in a paediatric FVPP is unavailable, recent findings suggest that food prescription programmes may reduce emergency department utilisation among adults.[46] Given the importance of regular paediatric visits for immunizations and preventative care, research focused on the influence of paediatric FVPP on health care utilisation is needed. Additionally, feedback from caregivers also signalled a recognition of the impact of the FVPP on nutrition security. Many talked extensively about the growing cost of fresh, healthy foods, with some suggesting that it was more cost-effective to purchase convenience or packaged foods. The paediatric FVPP was viewed as a means for caregivers to provide fresh fruits and vegetables to their households, even as the cost of these items was increasing. This finding is consistent with previous research that indicates paediatric FVPPs have a positive influence on nutrition and food security among youth and their families.[21,24,31]

Similar to research with adults,[47] many caregivers shared a lack of understanding of the paediatric FVPP at the point of distribution (receipt of prescriptions). This finding illustrates the need for a consistent programme education plan and supporting materials at partnering clinics. Previous research has suggested that, in order for FVPPs to run effectively, a paid staff member should be responsible for coordinating the programme at clinics and managing education, referrals, and challenges.[48] Given the numerous demands on paediatric clinics and staff, it may be unreasonable to expect consistent in-person education regarding a paediatric FVPP without one dedicated staff member on-site. Although posters and pamphlets, which described vendor addresses, hours of operation, and redemption procedures, were available at the partnering clinic, many caregivers remained unclear about participating vendors and redemption procedures. Some admitted that this lack of programme understanding negatively influenced their engagement in the programme.

Studies among adults who participated in fruit and vegetable incentive programmes have highlighted several barriers to engagement, including insufficient redemption/vendor sites[49–51] and lack of transportation.[47,52] Caregivers in the current study reported similar challenges that prevented them from engaging in the paediatric FVPP with their children. The FVPP partnered with Flint Fresh (Flint Fresh | Fresh Vegetable & Fruit Delivery in Flint) in January 2018 to preserve produce selection capacity while directly addressing transportation barriers among participants. Flint Fresh is a food aggregation space for local farmers and provides free delivery of participant-selected $15 and $30 fresh produce boxes. Unfortunately, few participants were aware of this option which resulted in underutilisation of prescriptions at this redemption site. Additionally, some shared confusion when attempting to navigate the farmers' market and Flint Fresh website, highlighting a need for programme education at vendor sites and clinics. Future research will explore the impact of a community health worker, who specifically addresses challenges with programme education at both clinic and vendor sites, in improving engagement in the paediatric FVPP.

In February 2024, one major chain grocery store in Michigan partnered with the FVPP to accept fruit and vegetable prescriptions at seven of its stores in Flint and surrounding areas. Patients at Akpinar Children's Clinic may now select Flint Farmers' Market, Flint Fresh or Meijer grocery stores to redeem their prescriptions. Printed prescriptions may be redeemed at the pharmacy counter at Meijer stores in exchange for $15 fresh fruit and vegetable vouchers. Early results suggest this option has greatly improved engagement in the FVPP, and caregivers have expressed consistently positive experiences with the additional redemption site. The partnership was in direct response to caregiver feedback regarding the need for inclusion of at least one major chain grocery store where families may redeem prescriptions while shopping for other food items for their families.

Unlike most prescription programmes, participation in nutrition education activities was not required by the paediatric FVPP in the current study. Most caregivers indicated a desire for recipes or cooking classes to accompany prescriptions. Some requested cooking classes centred around children to teach culinary skills together with nutrition education. Others felt they would benefit from a culinary programme that focused on the fruits and vegetables that were purchased with prescriptions. Findings are consistent with earlier studies among adult and youth participants in FVPPs[31,49] and speak to a growing need for culinary support programmes. In direct response to early feedback from FVPP participants regarding a need for culinary programmes for youth,[31] Flint Kids Cook was developed in 2017.[53] Flint Kids Cook is a free six-week cooking and nutrition programme for youth (aged 8–18 years) taught by a professional chef and registered dietitian in a farmers' market kitchen. Results suggest that participation in Flint Kids Cook is associated with significant improvements in cooking attitudes, cooking self-efficacy, and health-related quality of life of participating youth.[54] A virtual version of the class, Flint Families Cook, launched in 2021 with results suggesting programme participation was associated with improvements in cooking self-efficacy, health-related quality of life, and dietary behaviours.[55,56] Over 500 youth have graduated from Flint Kids Cook with nearly 300 children waiting to participate. All paediatric clinics offering the FVPP advertise Flint Kids Cook through posters and brochures in waiting areas and patient rooms.

Finally, caregivers provided tangible recommendations to improve overall engagement in the paediatric FVPP. Some suggestions, including text or email reminders when prescriptions were set to expire, would be relatively easy to implement and have been reported in earlier qualitative studies with adults.[49] Other suggestions, including partnerships with large grocery store chains and digitising paper prescriptions, would require significant changes that may be costly to implement and require dedicated staff and procedural changes. Unfortunately, sustainable funding sources that allow produce prescription programmes to invest in these more costly initiatives to improve programme engagement are limited. Given the potential of paediatric FVPPs as a strategy to address inequities in food access in a broadly scalable manner,[25] substantial investment in continued programme improvements is warranted.

This current study has limitations. It was small and limited to one urban community in Michigan. Results may not be generalisable. As previously mentioned, because only one child and one caregiver enrolled into the original study, researchers were unable to accurately quantify the total number of prescriptions received and redeemed by each household. Finally, it is possible that views of the programme and challenges with redemption differed among participants researchers did not reach for interviews. However, participants were candid about their programme experiences and barriers to redemption. Each offered important feedback to improve overall engagement.

Conclusions

The potential impact of fruit and vegetable prescription programmes focused on paediatric patients is substantial. In addition to recent studies indicating a positive influence on caregiver- and child-reported food security,[21,24] food shopping,[26] and dietary behaviours of children,[21,27–30] these programmes offer notable benefits to all household members who consume produce purchased with prescriptions. Crucial to the success of these programmes, however, is the understanding of barriers to engagement among programme participants and their families. The current study elucidates challenges with one paediatric FVPP and provides actionable solutions, from the viewpoint of caregivers, to address these challenges. Future research will investigate whether and how expansion to full-service grocery stores and development of digital prescriptions impact programme engagement.

Abbreviations

FVPP: Fruit and vegetable prescription programme; **EMR:** Electronic medical record; **SCT:** Social Cognitive Theory.

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Competing interests

The authors declare none.

Authorship

A.S.-C. conceived the study, study design and analysis; led analysis of the data; and led all writing and drafting of the manuscript. S.E. participated in data collection and analysis; and contributed to drafting and revising of the manuscript. B.F. and K.P. participated in data collection and analysis; and participated in revising of the manuscript. A.S. assisted with data preparation and participated in drafting and revising of the manuscript. All authors have approved the final article.

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RESEARCH ARTICLE

Characterisation of complementary feeding practice and locally available climate-resilient crops for complementary food among agro-pastoralists of Ethiopia: a qualitative study

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Abstract

The current study aims to characterise the complementary feeding practice and identify locally available climate-resilient crops that can be used for complementary feeding among agro-pastoralists in Ethiopia. A phenomenological study in Benna-Tsemay district, comprising focused group discussions, key informant interviews, and household observations, was conducted. A pretested guide was used to capture information regarding types of complementary food, lists of food items, and ingredients included in their complementary formulation. A thematic analysis for emerging points of discussion was carried out. Three major themes, including infant and young child feeding practices, food items included in complementary food, and their consumption frequencies, as well as the incorporation of climate-resilient crops into complementary foods as coping mechanisms, emerged. Breastfeeding was common and regarded as essential. Gruel and porridge from grains, roots, and tubers were regular parts of complementary foods in the study area. Moringa and sorghum were dominantly identified as climate-resilient crops regularly grown and used in complementary foods. Growing these crops was regarded as a coping strategy for drought and seasonal constraints. The district is one of the most drought-prone areas in Ethiopia, compromising the quality of complementary food. Unlike the World Health Organization recommendation, the grains, roots, and tubers-based diet formed the basis of complementary food lacking flesh foods, eggs, pulses, and other fruits and vegetables. Thus, it is recommended to improve complementary food quality through value-addition using locally accessible crops.

Key words: **Agro-pastoralists: Children: Climate-resilient crops: Complementary food: Food group: Moringa: Sorghum**

Background

The World Health Organization (WHO) defines complementary feeding as the introduction of foods and liquids alongside breast milk to meet the evolving nutritional needs of infants exceeding those solely provided by breast milk.[1] The age between 6 and 23 months of infants and young children is a time when they reach a general and neurological stage of development that enables them to be fed on other foods in addition to breast milk.[1,2] While complementary feeding is a universal practice, its implementation across societies is demonstrably shaped by a complex interplay of cultural beliefs, individual caregiver characteristics, and socioeconomic factors.[3]

On a global scale, a significant disparity exists in access to minimally acceptable complementary feeding practices, with suboptimal practices prevalent even in high-income households. This highlights a critical concern—the low rates of minimum dietary diversity observed across the world. This is further compounded by insufficient consumption of fruits, vegetables, and animal-source foods in children's diets. Insufficient quantities and inadequate quality of complementary foods, often due to climate-induced factors, coupled with poor feeding practices, lead to adverse health and nutrition outcomes.[4] These factors particularly impact traditional cereal-based complementary foods, which often lack the

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required nutrients for growth and development in developing countries like Ethiopia.[4,5] Studies have shown a low prevalence of appropriate complementary feeding practices in Ethiopia, with only 10% of children aged 6–23 months receiving recommended foods and just 12% consuming diversified food groups.[6,7] Several factors contribute to suboptimal practices, including climate change, socioeconomic and demographic status, health service utilisation, individual and household food security, and livelihood status (i.e. pastoralism and agro-pastoralism).[6–8] Agro-pastoral areas in Ethiopia, which home for a significant portion of the population, are particularly vulnerable to climate change impacts.[9] Frequent droughts and high food insecurity prevalence in these communities pose major challenges to child nutrition.[10,11] As a coping mechanism, communities in southern Ethiopia have developed strategies utilising less preferred yet climate-resilient crops.[12]

Research in Benna-Tsemay district suggests that complementary feeding practices are heavily influenced by cultural beliefs, traditional knowledge held by women, and prevailing social norms. A study done by Anteneh (2018) highlights that the introduction of complementary foods is typically delayed until the infant reaches six months of age. This delay is often attributed to the fear of abdominal cramps, locally referred to as 'Gore' a term designated to the timeframe for the introduction of complementary food. It represents a culturally-embedded method for assessing an infant's readiness for complementary feeding. Traditionally, a thin, local stick is used to measure the circumference of the infant's neck and right arm. This practice serves as a marker for determining the appropriate time to introduce complementary foods.[13] Despite this good practice, cereals dominate homemade complementary foods, with sorghum being a common constituent in various forms like gruel, porridge, fetfet, kitta, and dabo.[14,15] Sorghum and moringa leaves are identified staple crops in Benna-Tsemay.[13]

However, a systematic investigation into their utilisation within complementary feeding practices is currently lacking. This study intends to characterise the complementary feeding practices among agro-pastoral communities and identify locally available climate-resilient crops as part of an effort to improve complementary foods based on locally available resources. In this regard, this research will contribute to the body of knowledge on complementary feeding practices and climate-resilient crops. Characterising both is crucial for several reasons. In Ethiopia, particularly the Benna-Tsemay district, exemplifies the importance of this research. Many traditional crops, with the potential to significantly enrich the nutrient profile of complementary foods, have been overlooked in favour of staple crops. Characterisation of these underutilised crops can unlock their hidden potential, promoting their use in complementary feeding interventions. Additionally, with climate change disrupting traditional growing seasons and weather patterns, characterisation becomes a vital tool for identifying crops that can adapt to these changes. This, in turn, can ensure food and nutrition security, thereby reducing the risk of malnutrition among children aged 6–23 months in agro-pastoralist communities.

Methods

Study setting

The study was conducted among agro-pastoralist communities of Goldia and Buneker sub-districts in the Benna-Tsemay district of South-Omo zone. The zone is one of the administrative units of the former Southern Nations, Nationalities, and Peoples' Region of Ethiopia and is currently structured under the South Ethiopia Regional State (Fig. 1). It is situated in the southwestern part of Ethiopia, bordering the Omo River to the east Bench Maji to the south and west, respectively. Benna-Tsemay is one of the five agro-pastoral districts in the South-Omo zone. It is located 739 kilometres in the south of Addis Ababa, the capital city of Ethiopia. It is composed of 35 sub-districts under six clusters, and its total population was 76,647.[16] The district is 1500 metres above sea level, and its average yearly temperature ranges from 26 °C in the winter to 40 °C in the summer. With an average annual rainfall of 800 mm, the distribution of rainfall is bimodal. The district has a lengthy dry season from December to the start of March, followed by a short dry season in June and July.[17] In this district, mixed farming is the main livelihood of the population, where agrarians and agro-pastoralists reside evenly across the clusters.[18] The major agricultural products include moringa tree (*Moringa stenopetala and Moringa oleifera*), sorghum, maize, pearl millet, teff, common bean, sesame, cowpea, sunflower, sweet potato, mango, papaya, avocado, banana, cabbages, tomato, pumpkin, and others.[19]

Study design and participants

Two agro-pastoral sub-districts were selected by stratified purposive sampling due to their larger population size and administrative clusters in the district and were included in an in-depth descriptive phenomenological study. Ten key informant interviews, nine overt types of direct household observations, and two focused group discussions were conducted from December 11 to 27, 2022. The participants for the focused group discussion and direct household observations comprised women with young children aged between six and twenty-three months. The district coordinator of the health office, public health expert, nutrition focal person, agriculture experts, agricultural researchers, health extension workers, development agents, and social and behaviour change communication (SBCC) experts in the district were among the key informants who were interviewed. The key informant profile is summarised and presented in the supplementary material.

Data collection procedures and techniques

A pretested guide was used for both focused group discussions and key informant interviews. The guides are included in the Appendix 2 of Supplementary Material. The guides focus on feeding practices, including breastfeeding and the introduction of complementary foods, types of foods available as complementary foods, types of locally available and climate-resistant seasonal crops, frequency of food items consumed by the children, and challenges associated with complementary feeding. Climate-resilient crops were defined as any crops

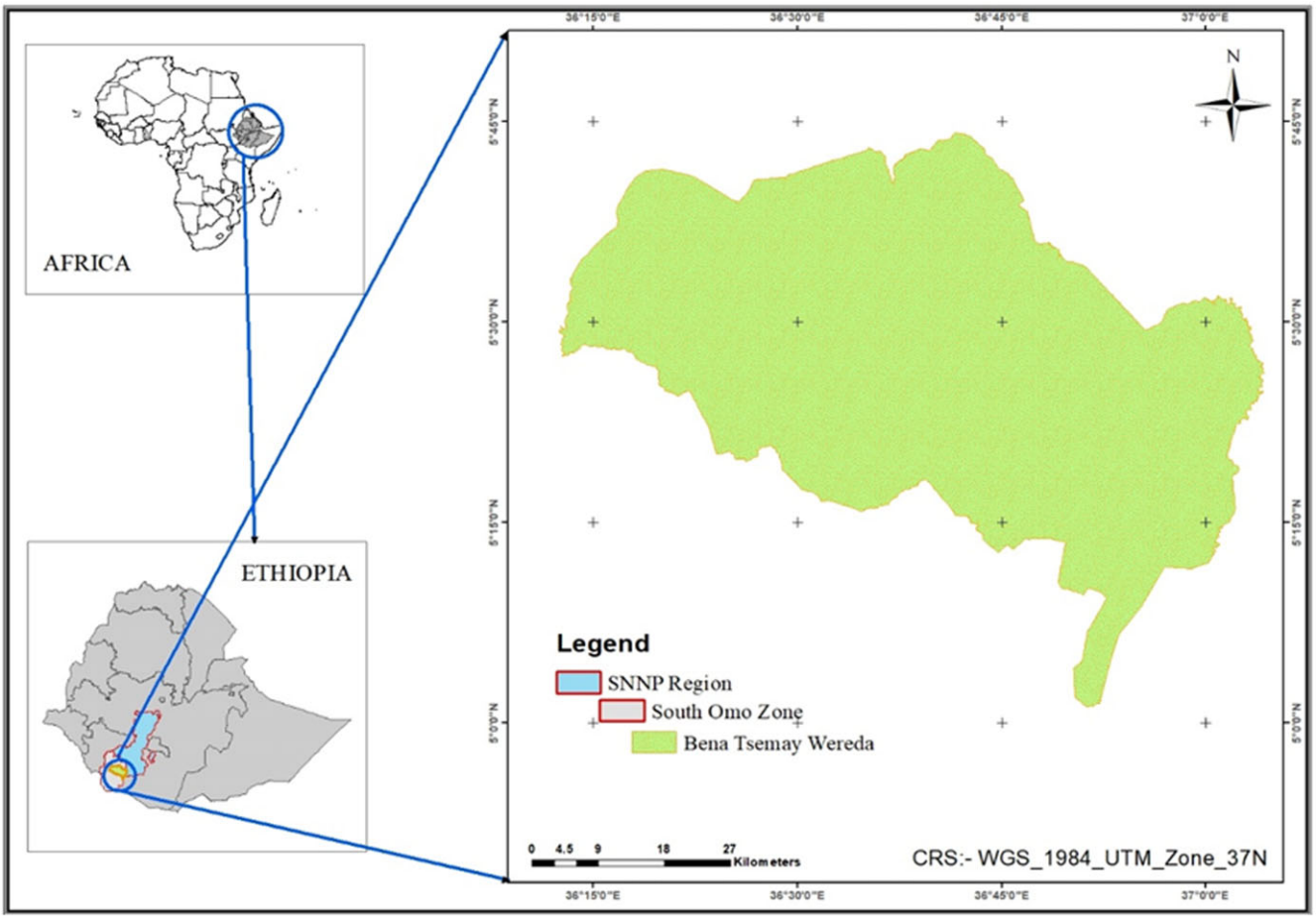

Fig. 1. Map of Benna-Tsemay District, South-Omo Zone, Ethiopia (2024).

mentioned by the study participants. Of the lists, crops were termed climate-resilient crops, which have enhanced tolerance to biotic and abiotic stresses. Specifically, crops that adapt to diminishing crop yields in the face of droughts, higher average temperatures, and other climatic conditions were termed climate-resilient crops.[20] The food groups considered in this study were the seven initially recommended by the WHO and the United Nations Children Fund (UNICEF). The food groups were grains, roots, and tubers (1); pulses (2); dairy (3); flesh (4); eggs (5); vitamin A-rich fruits and vegetables (6); and other fruit and vegetables (7).[1]

Furthermore, an observation checklist was developed to acquire information about complementary food preparation and feeding to triangulate the focused group discussion and key informant interviews. The direct observations were aimed at achieving triangulation of discussion points raised during the focused group discussions, and identification of commonly included food items in the complementary foods. The observation checklist includes crucial questions about complimentary food types, lists of food items, and ingredients.

The key informant interviews were done in a separate district health and agricultural office rooms to ensure the interviewee's privacy. The individual interviews with key informants lasted approximately forty minutes each. The two focused group discussions were held at the sub-district health posts. The first and second focused group discussions lasted for two and a half hours and two hours, respectively. Whereas, each direct observation session lasted for one and a half hours. The principal investigator led all data collection activities, including key informant interviews, household observations, and focused group discussions. To ensure effective communication with participants, the principal investigator was accompanied by an experienced nutritionist and public health expert fluent in Benna and Tsamai, the two local languages. Data saturation for each data collection method (focused group discussions, direct household observations, and key informant interviews) was achieved when point of discussion redundancy in the collected data. The redundancy was characterised by discussions reaching a point where new insights became scarce, and recurring discussion points dominated.

Data processing and analysis

Thematic analysis was performed in six steps.[21] During the initial phase, all interviews were audio- and video recorded. The second phase involved preparing and organising data for analysis using verbatim transcripts. Verbatim transcription and translation were done by a public health expert who speaks and listens in the local language. In the third phase, the transcriptions were reviewed for gaps and limits. In the fourth step, evolving data and potential biases were evaluated. In the fifth step, all evolving data was coded and categories were created. Manual

Table 1. Examples of themes and subthemes emerged with their respective codes and meanings

Themes	Subthemes	Codes	Meaning or definitions
Infant and young child feeding practices	Benefits of breastfeeding	Health and Immunity	Breastfeeding helps protect babies from illnesses and infections. *. . . .breastfeeding is good for my child's health and protects from repeated diseases.*
		Bonding and Development	Breastfeeding fosters a close emotional connection between mother and child. *I feel great happiness when I breastfeed and it is good for my child's physical strength.*
	Complementary foods introduction	Current Practices and perceived health benefit	Untimely introduction of complementary food. *Children who begin before or lately six months are frequently sick. They commonly have diarrhoea, vomiting, weight loss, and gastrointestinal distress, while children who start at six months are healthier and grow faster*
	Foods group	Food ingredients	Food used to prepare gruels, porridges, and bread for children. *I add a variety of foods to my child's diet, including grains like sorghum, corn, millet, wheat, oats, teff, and barley, as well as legumes such as Yergib ater pigeon pea, peas, lentils, and kidney beans.*
Climate-resilient crops in complementary foods	Climate-resilient crops as coping strategies	Seasonal Availability	Fluctuations in the availability of certain food items throughout the year. *Sorghum (locally called alaffa), millet, cassava, and sweet potatoes are accessible year-round. If it is not available, we can purchase it on the market or borrow it from someone.*
Existing challenges	Challenges associated with complementary feeding	Farming Activities	Tasks related to agricultural activities. *. . . women have to engage in agricultural activities, including clearing weeds, harvesting crops, cultivating vegetables, and selling cash crops.*
		Social Obligations	Participation in community events. *. . . even if women breastfeed, they are responsible for social events, such as burial rituals and wedding.*
		Food Availability	Fluctuations in food availability due to seasons. *The community is almost aid dependent for food shortages caused by drought and less agricultural productivity.*

coding was employed to analyse the qualitative data collected through the three methods: focus group discussions, key informant interviews, and direct household observations. To ensure reliability and consistency throughout the coding process, several strategies were implemented. Firstly, the coding outline was developed, aligning with the research question that centred on complementary feeding practices and the inclusion of climate-resilient crops into complementary foods. It encompassed specific codes such as breastfeeding practices, timing of complementary food introduction, types of food and ingredients used, seasonal availability of crops, and challenges encountered during complementary feeding (Table 1). Secondly, memoing served as a valuable tool. The documented detailed written notes during the focused group discussions, key informant interviews and direct household observation alongside assigned codes. These memos captured their reflections, interpretations, and emerging connections between various data segments. This practice enriched and deepen understanding of the emerged subthemes from the three of the qualitative data collection methods. Finally, any discrepancies or disagreements in coding decisions were addressed through ongoing refinement of the coding scheme. This process ensured consistency and reliability in the analysis.

Finally, during the six phases, comparable subthemes were organised into distinct themes. All related subthemes were categorised into three themes: infant and young child feeding practices, food items included in complementary food and their consumption frequencies, as well as the incorporation of climate-resilient crops into complementary foods as coping mechanisms.

Rigor and trustworthiness of the study

In order to establish the credibility and trustworthiness of the research, three qualitative methods, including focused group discussions, direct household observations, and key informant interviews, were utilised to gather data. Finally, the data collected from these diverse sources was then triangulated to ensure accuracy and validity. In addition, to strengthen the credibility of the study, the researcher employed a saturation sampling strategy. This involved prolonged engagement with participants across the various qualitative data collection methods. In that case, the data collection continued until data saturation level was achieved. To ensure the transferability of the study findings, the researcher conducted a comprehensive literature review of existing research on complementary foods in Ethiopia. This informed the development of all data collection tools, grounding them in established knowledge. The study site was purposefully chosen based on livelihood characteristics, and participants were selected to ensure they were representative of the district population. To strengthen the dependability of the research findings, a process of triangulation was employed. This involved comparing the results obtained through all the qualitative data collection methods with findings from previously published research on complementary foods in Benna-Tsemay district, and other agro-pastoralist communities in Ethiopia. This cross-checking aimed to identify agreement and disagreement between the current study and existing literature, enhancing the consistency and trustworthiness of the research.

The study also employed a triangulation approach, integrating data from focus group discussions (FGDs), key informant

interviews (KIIs), and direct household observations to gain a comprehensive understanding of the complementary feeding practices and inclusion of climate-resilient crops. A core finding emerged through convergence across all three data sources. For instance, all participants from the Focused Group Discussions (FGDs), key informants (KIIs), and household observations confirmed the use of cereals (maize, sorghum, wheat, millet, teff) and moringa leaf as primary components of complementary foods. This convergence strengthens the conclusion that cereals form the major focus of complementary feeding practices within the study area. On the other hand, the divergences between data sources provided further insights. For instance, compared to the KIIs' emphasis on key ingredients in porridge, the FGDs revealed a wider variety of foods used in complementary feeding. This suggests that while cereals dominate, some households might incorporate additional ingredients based on individual practices and preferences. In this regard, the additional ingredients were identified from the direct household observations. Based on convergence and divergence, sorghum, maize, and wheat can be identified as the staple grains used in most complementary foods, supported by all data sources. Household observations further revealed the incorporation of additional ingredients like teff, pigeon peas, eggs, vegetables, milk, oil, and salt.

While the first author conducted the majority of the data collection, he was assisted by a qualified nutritionist and public health expert. Both experts have more than ten years of experience in community outreach programmes. A half-day training on the overall objective and research questions of the qualitative data collection tools was given to the public health expert and nutritionist. In this regard, the first author as a researcher, mainly interested in infant and young child feeding, previous experience of reviewing research papers may have influenced the expectations of the interviews and discussions. Consequently, the first author expected that the infant and young child feeding practices in the study area would be similar to those in other Ethiopian districts. In the first focused group discussion, the researchers learned that moringa leaf was a part of complementary food, which was not well documented to their knowledge. The crop is well known for its health preferences, specifically for non-communicable diseases among adults. Thus, the researchers had to include probing questions about moringa and other underutilised crops. Then, more probing questions were added to the guides and other qualitative data collection approaches (i.e. household observation). Initially, the key informant interviews using the guide were prepared in English and translated into Amharic (the Ethiopian national language), making it easy to interact with the interviewees from the district health office. However, it was difficult to do the same with the other informants at the sub-district level (locally called kebele). Then the researchers were able to recruit a translator in addition to others to accompany and help translate all the questions and responses into the local languages. This helped to probe the questions and detail the responses. The focused group discussions were held in the local language. All the observations were also done with the help of translators. The role of the first author at the time of the discussions was to take a summary note at the end of the discussion and later triangulate during the narration of the transcribed data. Any thoughts that arose were noted down. Prior to the focused group discussions, the translator and the first author had a comprehensive conversation about what to do. All reported responses after the transcription were provided to the transcriber for any biases and crosschecked for additional thoughts. Several statements from the key informant interviews were checked by asking the interviewee on the phone. All other researchers took part in designing the guides and oversaw the whole data collection, analysis, and write-up.

Ethical clearance

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Hawassa University's Institutional Review Board (IRB/394/15). The study followed the essential requirements for working with human subjects. Prior to data collection, district administrators were approached with an explanation of the study and its objectives, and their agreement was obtained. The nature of the study was thoroughly described to the respondents. Participants provided written informed consent to willingly participate in the study, and they were assured that they may opt-out or quit at any moment. Furthermore, confidentiality of participant's information was assured and information was recorded and published anonymously. Those who are practicing inappropriate complementary feeding were advised to improve the complementary feeding practice.

Result

Socio-demographic characteristics of the study participants

In the current study, twenty mothers participated in the focused group discussion. The participants were all married, and half of them (50%) had never attended any kind of school. A total of ten key informants participated. These informants represented various governmental and non-governmental organisations (NGOs) engaged in diverse nutrition-related activities. The characteristics of the focus group discussion participants and key informants can be found in Tables 1 and 2 of the supplementary material.

Employing thematic analysis from the three qualitative data collection methods, the current study identified three core themes that captured the range of participant experiences: infant and young child feeding practices, the diversity of ingredients used in complementary foods and their feeding frequency, and lastly, the role of climate-resilient crops in complementary foods as coping strategies for addressing food security challenges.

Infant and young child feeding practices

Participants in the focused group discussion were asked about the key infant and young child feeding practices, such as breastfeeding experience and benefits, exclusive breastfeeding, and the introduction of complementary foods to their infant and young child, and emphasised the satisfaction it provided for

Fig. 2. Ingredients in the complementary foods. The larger size of the words indicates the higher frequency mentions during the focused group discussions and observed during direct household observations (December 2022).

them and their child, as well as the numerous health benefits of breastfeeding. In this sense, all of the participants stated that they had experienced breastfeeding. All participants viewed breastfeeding as beneficial to children because it avoids illness and brings pleasure to both infants and breastfeeding women.

> *'I feel great happiness when I breastfeed my child because it is good for my child's health and physical strength and protects him from repeated diseases',* says a 32-year-old mother from Buneker sub-district.

Additionally, breast milk was thought to be more contaminant-free than water, making it simple to feed and enjoy breast milk alone until the child reached six months.

> *'We are always advised to exclusively breastfeed for up to six months because breast milk is cleaner than water, easy to feed children, and does not require any additional knowledge to prepare',* said 28-year-old mothers from the Goldia sub-district.

Furthermore, in the focused group discussion, participants emphasised that exclusive breastfeeding for the first 6 months provides essential nutrients and antibodies that help strengthen the child's immune system.

> A 25-year-old mother from the Buneker sub-district stated, while comparing the early and late introduction of complementary food, that *'children who begin before or lately six months are frequently sick. They commonly have diarrhoea, vomiting, weight loss, and gastrointestinal distress, while children who start at six months are healthier and grow faster'*

The participants reflected on their experience with the introduction of complementary. They believed complementary food should be introduced at six months of age due to several reasons. They noted that giving complementary food too soon or late might raise the risk of infection in infants and young children.

> *'Now, we start complementary feeding at six months because we understand the benefits of breast milk',* said a 31-year-old mother from Buneker sub-district.

Diversity of ingredients in the complementary foods and feeding frequency

During the focused group discussion, participants conveyed that gruel and porridge are commonly regarded as complementary foods tailored for the nutritional needs of infants and young children. The primary components typically incorporated into these complementary foods encompass maize, sorghum, wheat, millet, moringa leaf (referred locally as Aleko), teff, pigeon pea (referred to locally as 'yergib ater'), eggs, cabbage, cow's milk, onions, oil, and table salt.

> A participant, a 33-year-old mother residing in the Buneker sub-district, listed a variety of commonly available food items incorporated into her child's diet. *'I add a variety of foods to my child's diet, including grains like sorghum, corn, millet, wheat, oats, teff, and barley, as well as legumes such as Yergib ater (pigeon pea), lentil, and kidney beans'*

During household observations, these foods were also used in complementary food. Lists of food items and ingredients observed are described in Fig. 2 and Table 3 of the supplementary material.

Maize, sorghum, teff, millet, and wheat were popular food items from the grains, roots, tubers, and plantains food group. Kidney beans, lentils, peas, and pigeon peas were from the pulse, nut, and seed food groups. Both of these food groups were the most often provided, as indicated by the participant. Only moringa leaf, carrot, tomato, papaya, and mango were identified as vitamin A-rich fruits and vegetables. According to the participants, the intake of this food group is determined by its availability in homes as well as the season, but moringa leaf, a vitamin A-rich fruit and vegetable, was readily available and incorporated into the complementary food.

> A 25-year-old mother from Buneker further elaborated on the food items that she incorporates into her child's diet. *'I also include vegetables in my child's diet, such as cabbage, onion, tomatoes, aleko (moringa leaf), and carrot'.*

Cow and goat milk were the dairy food categories accessible and given to infants and young children. According to the participants, these food products remain dependent on household availability. Yet again, the egg was available once a week, based on the family's capacity to purchase it. The flesh food group was the least given, with only once a month provided to those who could afford it. In addition to the aforementioned food groups, oil and table salt were frequently included in complementary foods.

> A 30-year-old mother in Goldia added animal-source foods, supplementing the previously mentioned items. However, she noted that consumption patterns were heavily influenced by availability. *'We do have access to some animal products like milk, egg,*

and oil. Between meat and butter, it depends. If we have cows and chickens available, we might get to have meat or eggs once a week, but that's only affordable for some families. As for meat and butter in general, those might only be consumed once a month, or even just once a year'

Climate-resilient crops in complementary foods, coping strategies, and challenges

Participants reported that they are using climate-resilient crops as coping mechanisms for food insecurity caused by seasonal variation. According to the participants in the focused group discussion, the most climate-resilient crops were sorghum, millet, and moringa leaf, all of which are common constituents of complementary foods. All focused group discussion participants and key informants stated that, compared with others, these crops are accessible throughout the year. During the household observations, the majority of women included boiled moringa leaf and sorghum flour in the complementary food. An example of blended maize, sorghum and moringa leaf as a complementary food for an eleven-month-old child is depicted in Fig. 1 of supplementary material.

In addition, during the focused group discussion, participants addressed a variety of problems linked to complementary food. The primary challenges are recurrent droughts and the seasonality of products used in complementary food. Workloads connected to home duties, social duties, and tough agricultural operations create time limits for preparing and serving complementary food for their respective infant and young children.

'In our community, women have to engage in agricultural activities, including clearing weeds, harvesting crops, cultivating vegetables, and selling cash crops. These activities are done outside the home, which may hinder us from timely preparation of complementary food and feeding our children'. A 28-year-old woman from Buneker sub-district mentioned, and all other focus group participants agreed.

A 30-year-old mother from Goldia sub-district added *"... even if women breastfeed, they are responsible for social events, such as burial rituals, which may occur outside of the house for an extended period of time and pose a difficulty"*

Furthermore, the key informants underscored many issues that the study district is experiencing. Low food production caused by drought and seasonal variation is posing challenges for the community. A monotonous diet consisting primarily of cereal, along with water shortages, is common.

"The community is almost aid dependent for food shortages caused by drought and less agricultural productivity. Mainly porridge from sorghum, maize, and wheat is the usual complementary food, which made the complementary feeding practice suboptimal in the study area" said the nutrition focal person from Health Office of Benna-Tsemay District.

Furthermore, both focused group discussion participants and key informants reported cultivating drought-resistant crops such as sorghum (locally known as Alaffa), cassava, and moringa trees (locally known as Aleko), with millet as the primary coping strategy for drought and seasonal food scarcity. Other coping mechanisms highlighted throughout the discussions included borrowing from friends and relatives and purchasing low-cost alternative food items from marketplaces.

"Sorghum (locally called alaffa), millet, cassava, and sweet potatoes are available year-round. If one of these becomes unavailable, we can buy it at the market or borrow it from someone" A 28-year-old mother from the Buneker sub-district.

Discussion

This research characterises the diversity of food groups in complementary foods for children aged 6–23 months and explores the use of climate-resilient crops. The thematic analysis revealed three core themes: infant and young child feeding practices, food items and their consumption frequencies of complementary foods, and the strategic use of climate-resilient crops in complementary foods as adaptation mechanisms. These themes underscore the need for quality improvement in moringa and sorghum-based complementary foods. Specifically, the inclusion of other food groups recommended by the WHO—such as flesh foods, eggs, pulses, vitamin A-rich fruits, and vegetables—is critical to address potential dietary inadequacies. Furthermore, the study highlights the ongoing challenges faced by the community, including frequent droughts, seasonal food insecurity, and the significant workload shouldered by women in both household and agricultural tasks.

The WHO recommends a variety of nutrient-rich, safe, and suitable complementary foods for young children, including five out of eight food groups along with breast milk, to ensure adequate macro- and micronutrient intake for healthy growth. The recommended food groups for complementary food include (i) grains, roots, and tubers; (ii) pulses; (iii) dairy products; (iv) meat; (v) eggs; (vi) vitamin A-rich fruits and vegetables; and (vii) other types of fruits and vegetables.[1] Despite the recommendations, the practice of complementary feeding in many developing countries, including Ethiopia, often falls short of these recommendations. For instance, the predominant use of grains, roots, and tubers in complementary foods has been observed in various studies.[4,15,22–24] The current study found that grains, roots, and tubers were the dominant or staple food groups used for complementary foods. This food group offers substantial health benefits since it contains calories, protein, fibre, and minerals.[1,25] This food group is recommended as a part of complementary foods for children aged 6–23 months but other food groups should be complemented. It is also important to choose better high digestibility varieties of cereal grains as the predominant one (sorghum) generally has proteins with poor digestibility and bioavailability, particularly in cooking.[26]

Flesh foods and eggs should also be eaten by infants and young children as often as possible. The consumption of these food groups is associated with increased intakes of energy, essential amino acids and fatty acids, vitamin B12, vitamin D, zinc, and other nutrients.[2,27,28] Livelihood contributes to high consumption of Animal Source Foods due to increased access specifically among the pastoralists in Ethiopia,[29] however, the

current study confirmed during the direct household observations that the complementary food lacks meat, fish, and eggs, requiring urgent attention.

Food items categorised as legumes and pulses are sources of dietary protein where consumption of animal protein is limited due to various factors. When combined with cereals, they provide well-balanced essential amino acids and are quite important for children.[30] However, none of the women included pulses in the complementary food during the direct household observations in the current study.

The current study further notes a significant gap in the inclusion of vitamin A-rich fruits and vegetables with exception of moringa leaf in complementary foods. The consumption of this food group is vital but is alarmingly low in Ethiopia, as corroborated by several studies.[6,8,15,23,24,31–35] This gap may pose a significant risk of inadequacy of micronutrients, including vitamin A and others. This in turn, potentially, may lead to increased susceptibility to communicable and non-communicable diseases.[36] The long-term impact of low consumption of micronutrient-rich food groups may also contribute to higher mortality risks in adulthood.[37]

The current study emphasises the utilisation of climate-resilient crops in complementary foods, a critical consideration in the context of global climate change. To mitigate the effects of climate change and extreme weather events, households in other parts of Ethiopia have adopted coping strategies, notably the consumption of climate-resilient crops.[25,38] The current study confirms utilisation of climate-resilient crops as an approach for complementary foods, particularly moringa and sorghum, which are the main ingredients in complementary food preparations.

Moringa tree is known for its adaptability to extreme weather conditions including high temperatures and rainfall.[19] It is nutritionally rich in vitamins, minerals, and amino acids, making it an accessible food source in developing countries.[39] Despite its benefits, it is sometimes considered a 'famine food' due to its year-round availability.[40] The current study also confirmed that moringa leaf is a year-round food source used during droughts and is easily accessible.

While the nutritional benefits of moringa, particularly in reducing micronutrient deficiencies in children, are well documented,[41–46] the methods of cultivation, processing, and storage significantly influence its nutrient content. Boiling was the primary method of processing moringa leaf for complementary food observed during the direct observation, demanding further investigation into its impact on nutrient composition and acceptance by children. Additionally, concerns about the potential reduction in nutrient bioavailability due to anti-nutritional factors present in moringa, especially when processed by boiling, have been raised.[47] On the other hand, moringa leaf-added complementary foods may contain higher levels of some micronutrients, including iron and zinc, which are associated with upper-tolerable intake among under five children,[42] necessitating careful intake monitoring.

Similarly, sorghum is recognised for its resilience to climate variability and water scarcity.[48] It is crucial for food security in arid and semi-arid regions globally[49] and is a staple in Ethiopia, used in various traditional food preparations.[50,51] Although a vital ingredient in traditional grain-based complementary foods for infants and young children,[14] the comprehensive nutritional profile of sorghum is subject to variations due to biotic and abiotic factors.[51] Its low digestibility, particularly in protein content, has led to its perception as a low-value crop for food uses compared to other cereals.[52] The study underscores the need for further research to investigate the impacts of including sorghum in complementary foods.

Implications of the study

This study highlights several key implications for improving complementary feeding practices within the study area. While the introduction of complementary foods is a positive step, the lack of recommended food groups from the WHO is concerning. These missing groups include flesh foods, eggs, pulses (legumes), vitamin A-rich fruits, and vegetables. This limited diversity suggests a need for interventions that promote the incorporation of these vital food groups into complementary foods. The study identifies sorghum and moringa leaf as core components of complementary foods. However, further research is needed to explore how the inclusion of the missing WHO food groups could improve the overall nutritional value of these moringa and sorghum-based complementary foods. Importantly, the focus on climate-resilient crops presents a valuable strategy for promoting sustainable complementary feeding practices. However, the implications of these crops on the overall nutritional and anti-nutritional content of local complementary foods require further analysis. The study also highlights challenges such as frequent droughts, seasonal food scarcity, and the heavy workload of women in agricultural tasks. These factors contribute to food insecurity and potentially hinder the consistent use of diverse food groups in complementary foods. Therefore, gender-sensitive agricultural interventions are crucial to address these challenges.

This study has a number of limitations and strengths. First, data collection during the non-harvesting season may not fully capture the impact of seasonal variations on food availability in the study area. This potentially limits the generalizability of the findings regarding seasonal food access and its influence on complementary feeding practices. Second, the study's focus on a single district, despite the region's diverse ethnic backgrounds, restricts its representativeness of all Ethiopian agro-pastoral communities. Further research across multiple districts with varying ethnic compositions could provide a more comprehensive understanding of complementary feeding practices in Ethiopia. At the same time, the strength of the current study is the inclusion of diverse respondents beyond mothers (key informants from governmental and NGOs) strengthens the study by enriching the data with a broader range of perspectives on complementary feeding practices within the community. This triangulation of viewpoints enhances the comprehensiveness and credibility of the research findings.

Conclusion

Complementary feeding in the study area primarily relied on gruel and porridge made from grains, roots, and tubers.

Notably, moringa and sorghum proved to be the dominant climate-resilient crops cultivated and incorporated into complementary foods. The participants identified the cultivation of these crops as a key coping strategy employed to manage drought and seasonal food limitations. Despite WHO recommendations, complementary food in the study area is still inappropriate in terms of food group composition. Thus current study highlights the need for interventions to promote the inclusion of additional food groups recommended by WHO (flesh foods, eggs, pulses, vitamin A-rich fruits and vegetables) in moringa and sorghum-based complementary foods. This diversification can address potential dietary inadequacies and improve the nutritional quality of complementary feeding practices. Droughts, seasonal food insecurity, and women's workload, agricultural, and social duties all make it difficult to prepare and feed complementary foods to children on time. This can help to optimise the use of these crops for improved child nutrition outcomes. On the other hand, the study underscores the challenges faced by women, who shoulder a significant workload in both household and agricultural tasks. Gender-sensitive agricultural interventions are crucial to support women and empower them to participate more effectively in food production for complementary food. Finally, the study suggests a need for further research to explore the impact of using climate-resilient crops on the nutritional and anti-nutritional content of complementary foods across multiple districts with varying ethnicities and ecological zones of Ethiopian agro-pastoral communities.

Abbreviations

CLIFOOD: Climate Change Effects on Food Security; **FGD:** Focus Group Discussion; **IRB:** Institutional Review Board; **KII:** Key Informant Interview; **NGO:** Non-Governmental Organization; **SBCC:** Social and Behavior Change Communication; **SDG:** Sustainable Development Goal; **UNICEF:** United Nations Children Fund; **UOHO:** University of Hohenheim; **WHO:** World Health Organization.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.53

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Competing interests

The authors declare that they have no competing interests.

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Authorship

Conceptualisation, D.T.D, T.F.T and S.G.; methodology, D.T.D, T.F.T and S.G.; formal analysis, D.T.D.; writing—original draft preparation, D.T.D.; writing—review and editing, D.T.D, T.F.T and S.G.; visualisation, D.T.D, T.F.T and S.G. All authors have read and agreed to the published version of the manuscript.

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RESEARCH ARTICLE

Coffee intake leads to preeclampsia-like syndromes in susceptible pregnant rats

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Abstract

Coffee is one of the most popular beverages worldwide, and there is an increasing concern of the health risk of coffee consumption in pregnancy. Preeclampsia (PE) is a serious pregnancy disease that causes elevated blood pressure and proteinuria in pregnant women and growth restriction of fetuses due to poorly developed placental vasculature. The aim of our study is to investigate the possible effect of coffee intake during pregnancy in rats with potential underlying vasculature conditions. The endothelial nitric oxide synthase inhibitor N(gamma)-nitro-L-arginine methyl ester (L-NAME) at a high dose (125 mg/kg/d) was used to induce PE in pregnant rats, which were used as the positive control group. In addition, low-dose L-NAME (10 mg/kg/d) was used to simulate the compromised placental vasculature function in pregnant rats. Coffee was given together with low-dose L-NAME to the pregnant rats from gestational day 10.5–18.5. Our results show that the pregnant rats treated with low-dose L-NAME + coffee, but not low-dose L-NAME alone, developed PE symptoms such as prominent fetal growth restriction, hypertension, and proteinuria. Therefore, our findings suggest that coffee intake during pregnancy may cause an increased risk of PE in susceptible women.

Key words: **Coffee: Fetus: Preeclampsia: Pregnancy**

Introduction

Preeclampsia (PE) is a pregnancy-specific disease with hypertension as the main maternal symptoms during the latter half of pregnancy and fetal growth restriction.[1] The mechanisms of PE include deficient trophoblast invasion and incomplete spiral artery remodelling in the placenta.[1] The incidence of PE has increased recently with about eight million new cases per year.[2,3] However, the causes of PE remain elusive, which makes it difficult to prevent the condition.[4,5]

Coffee, a popular beverage worldwide, contains numerous dietary compounds such as tannic acid, chlorogenic acid, *etc.*, which are generally thought to be beneficial for health.[6,7] However, coffee consumption during pregnancy, has been found to be associated with an increased risk of stillbirth, fetal abnormalities, and miscarriage as reported by several epidemiological studies.[8,9] These findings indicate that coffee consumption during pregnancy may have potential hazardous effect.

N(gamma)-nitro-L-arginine methyl ester (L-NAME) is an inhibitor of nitric oxide synthase (NOS), and L-NAME is one of the commonly used compounds to induce PE in experimental animals.[10,11] The reason is that endothelial nitric oxide synthase (eNOS) plays an important role in protecting blood vessels from hypoxia and ischemia, which are major causes of hypertension in PE.[10] Therefore, in this study, L-NAME (125 mg/kg per d)-treated Sprague-Dawley (SD) rats were used as the positive control group for inducing PE. This dose is similar to that used in the literature.[10] In addition, we used low-dose L-NAME (10 mg/kg per d) treatment in pregnant rats to simulate the pregnancy with predisposed underlying vascular condition caused by reduced NOS activity, and then we studied whether coffee could induce PE in these low-dose L-NAME treated rats. The results of this study provide experimental evidences on the potential health hazards of coffee intake during pregnancy.

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Materials and methods

Chemicals and reagents

Sinloy Coffee powder (Caffeine content 1.0–1.5%) was purchased from Baoshan Zhongka Food Co. Ltd (Baoshan, Yunnan, China). L-NAME was obtained from Macklin (Beijing, China). The enzyme-linked immunosorbent assay (ELISA) kit for soluble fms-like tyrosine kinase-1 (sFlt-1) was purchased from QSBIO (China). The kits for determination of urinary albumin, creatinine, serum uric acid were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Animal experiment

All the animal experiment details were approved by the Institutional Animal Care and Use Committee of the Chinese University of Hong Kong (Shenzhen) (the approval number is CUHKSZ-AE2021004) according to the national guidelines for the care and use of experimental animals and ARRIVE Guidelines. All animals were raised in the specific pathogen free environment with a light/dark cycle of 12 h, and given free access to food and water. SD rats were obtained from Vital River Laboratory Animal Technology (Beijing, China). Virgin female rats (12–14 weeks, with body weight 250–270 g) and male rats (20–24 weeks) were housed in the same cages at a ratio of 2:1. During the breeding, female rats were examined daily by vaginal smear and were judged to be pregnant when vaginal plugs or sperm were found (gestational day, GD 0.5). Then, at GD 9.5, pregnant SD rats were divided into four groups according to their body weight in order to make sure that the average body weight in each group (around 280–290 g) was not significantly different.

The four groups are as follows: control group, L-NAME high-dose group (L-H), L-NAME low-dose group (L-L), and L-NAME low-dose + Coffee group (L-L + Coffee). The treatment for the animals in different groups is as follows: Control group ($n = 8$): oral gavage: water, intraperitoneal injection: saline; L-H ($n = 11$) and L-L ($n = 7$) groups: oral gavage: water, intraperitoneal injection: L-NAME at a concentration of 125 mg/kg and 10 mg/kg, respectively; L-L + Coffee group ($n = 8$): oral gavage: coffee, intraperitoneal injection: L-NAME at a concentration of 10 mg/kg. Coffee and L-NAME treatment was performed daily from GD10.5 to GD18.5. The coffee was given by oral gavage because humans drink coffee orally. Intraperitoneal injection was chosen for L-NAME administration according to literature.[11]

Pregnant rats in L-NAME low-dose + Coffee group were orally administered with the fresh coffee solution (1.8 ml/200 g) daily from GD 10.5 to GD 18.5. The coffee solution was brewed with Sinloy coffee powder according to the manufacturer's instructions. The method we used to prepare coffee in this animal study is the same as that used to make coffee in daily life. The insoluble residues were filtered out. The coffee concentration was 3.86% (w/v), calculated by dividing the weight of the dried residue of brewed coffee solution by the volume of the coffee. The dose (1.8 ml/200 g body weight/d) given to rats is equivalent to 5 cups for humans according to the following calculation. The dose of 1 cup of coffee for one person per day in humans is approximately 125 mL/70 kg body weight/day, which is converted to 0.36 ml/200 g body weight/day in rats according to the body surface conversion. Therefore, the dose (1.8 mL/200g/d) given to rats in this study is approximately equivalent to 5 cups for humans. Although 5 cups of coffee per day are probably higher than average amount of coffee consumed by humans, due to the much shorter pregnancy time of rats compared to that of humans and the short exposure time in this study for only 8 d, this dose of coffee was used in the current study.

On GD 19.5, a cesarean section was performed on the pregnant rats, which were continuously anesthetised with isoflurane to minimise pain and discomfort. Non-pregnant female rats were excluded and only 34 female rats were left for analysis (Control group: $n = 8$, L-NAME high-dose group: $n = 11$, L-NAME low-dose group: $n = 7$, L-NAME low-dose + Coffee group: $n = 8$). The number, size and weight of fetuses and the number of stillbirths were recorded. The development of forelimbs and hind limbs and various organs were examined. The number and weight of placentas were recorded and measured.

Blood pressure measurement

Systolic blood pressure (SBP) was measured in each rat at GD 9.5 and GD 18.5 using the tail-cuff plethysmography with Medlab biological signal acquisition system (Bilead, Shenzhen, China). After the pulse was stabilised, SBP was measured 3–4 times and the mean SBP was calculated.

Urinary and serum parameters detection

Urine samples were collected by using metabolic cages on GD 9.5 and GD 18.5. The supernatant was collected after centrifugation at 3000 rpm for 10 min. Urinary albumin and creatinine were determined using a Thomas Brilliant Blue G-250 assay kit and a creatinine kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively using a microplate reader (BioTek Epoch, Vermont, USA). Serum isolated from blood samples was used to determine uric acid and creatinine (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Histology

The hematoxylin/eosin (HE) staining and immunohistochemical staining (IHC) were performed by Servicebio Technology Co., Ltd (Wuhan, China). The dissected tissue samples were fixed in formalin, and the fixed tissues were dehydrated and embedded with paraffin wax. The embedded placental tissues were cut into 5 μm thick wax slices in transverse and longitudinal directions, respectively. Staining was performed with HE. For IHC, sections were deparaffinised and rehydrated, and antigen was retrieved in 10 mM citrate buffer (pH 6.0). Sections were incubated with 3% hydrogen peroxide solution for inhibiting endogenous peroxidase. Then 3% BSA was used for blocking.

The primary eNOS antibody (Servicebio, GB12086, 1:200) was used for IHC staining.

Microscope inspection and image acquisition were completed by a light microscope (CKX53; Olympius, Tokyo, Japan). For the quantitative analysis, we randomly selected 5 sections from each animal and 5 fields from each section. The software ImageJ (National Institutes of Health, Bethesda, Maryland, USA) was used for quantitative analysis.

Statistical analysis

Each measurement was repeated three times. One-way ANOVA test was used. The unit of analysis is group of treatments. All data were presented as Mean ± Standard Deviation (SD). $P < 0.05$ was considered significant. Statistical analysis was performed using GraphPad Prism 8 software (GraphPad software, La Jolla, CA).

Results

Coffee causes defects in fetal development in pregnant rats treated with low-dose L-NAME

Treatment with high-dose L-NAME led to a significant reduction in placental and fetal weight and drastic increase in the number of malformed fetus relative to the control group (Fig. 1, Table 1). On the contrary, low-dose L-NAME caused only a modest decrease of fetus weight and no change in the placental weight and number of malformed fetus compared with the control group. However, when coffee was given together with low-dose L-NAME, the weight of fetus and placenta significantly decreased and the number of malformed fetus significantly increased compared to low-dose L-NAME group (Fig. 1, Table 1). The specific malformation of fetuses in L-NAME high-dose group and L-NAME low-dose + Coffee include subcutaneous stasis, hind limb hypoplasia and insufficient differentiation (Fig. S1).

Coffee causes hypertension and proteinuria in pregnant rats treated with low-dose L-NAME

SBP was measured on GD 9.5 (before treatment) and GD 18.5 (after treatment). On GD 9.5, SBP was not significantly different between each group. Compared with SBP at GD 9.5, SBP on GD18.5 in control group decreased by about 11 mmHg (Fig. 2a). On the contrast, SBP on GD18.5 in the high-dose L-NAME group increased by about 20 mmHg compared to that of GD9.5 (Fig. 2a). SBP on GD 18.5 in the low-dose L-NAME group remained unchanged compared to that of GD9.5 (Fig. 2a). The rats that received low-dose L-NAME + coffee had an increased SBP on GD18.5 by about 10 mmHg compared to that of GD9.5 (Fig. 2a). Overall, compared to control group, ΔSBP values in the high-dose L-NAME group and low-dose L-NAME + coffee group are significantly higher than that of control group (Fig. 2a). ΔSBP in the low-dose L-NAME + coffee group is higher than that of low-dose L-NAME group albeit without statistical significance due to large inter-individual variations (Fig. 2a).

Table 1. Comparison of fetus number, malformation rate, and death rate in each group

Group	Total number of fetuses	Malformation rate (%)	Death rate (%)
Control	107	1.87	0
L-NAME high dose	64	40.63	21.87
L-NAME low dose	96	4.17	1.04
L-NAME low dose + Coffee	92	6.52	9.78

The ratio of urinary albumin (U-Ab)/urinary creatinine (U-Crea) in the L-NAME high-dose group and the low-dose L-NAME + coffee group increased significantly after treatment compared with that of control group (Fig. 2b). On GD18.5, U-Ab/U-Crea in the low-dose L-NAME + coffee group increased significantly compared with that in the low-dose L-NAME group (Fig. 2b). In addition, there is a significant increase in creatinine content in serum in the L-NAME high-dose group and the low-dose L-NAME + coffee group (Fig. 2c). These results indicate that there is prominent kidney damage in the L-NAME high-dose group and the low-dose L-NAME + coffee group. The weight of the kidney and serum uric acid level was not significantly altered (Fig. S2).

Increased placental production of sFlt-1 is another important marker of PE. sFlt-1 level in the low-dose L-NAME + coffee group is significantly higher than that of control and low-dose L-NAME group (Fig. 2d). The high-dose L-NAME induced sFlt-1 level to some extent without statistical significance compared to control.

Coffee causes prominent histological change in placenta in pregnant rats treated with low-dose L-NAME

Histological analysis of the placental tissue was carried out to probe the tissue damage in the placenta. The placenta can be divided into the decidua layer, junctional zone, and labyrinth zone in the cross section (Fig. 3). There is a significantly thickened junctional zone in the placenta of L-NAME high-dose group and the low-dose L-NAME + coffee group compared to the control group (Fig. 3). The junctional zone in the placenta of the low-dose L-NAME + coffee group is significantly thicker compared to the low-dose L-NAME group (Fig. 3).

We next examined the labyrinth region of the placenta with longitudinal section (Fig. 4). Compared with control group, placentas from L-NAME high-dose group and the low-dose L-NAME + coffee group had poorly development vasculature and arterial diameters were significantly smaller (Fig. 4e). In the low-dose L-NAME group, vasculature structure in placenta was generally normal.

eNOS is important for synthesis of nitric oxide (NO), a vessel dilator. Expression of eNOS significantly decreased in the placenta in the L-NAME high-dose group and the low-dose L-NAME + coffee compared to control, but not in low-dose L-NAME group (Fig. 5).

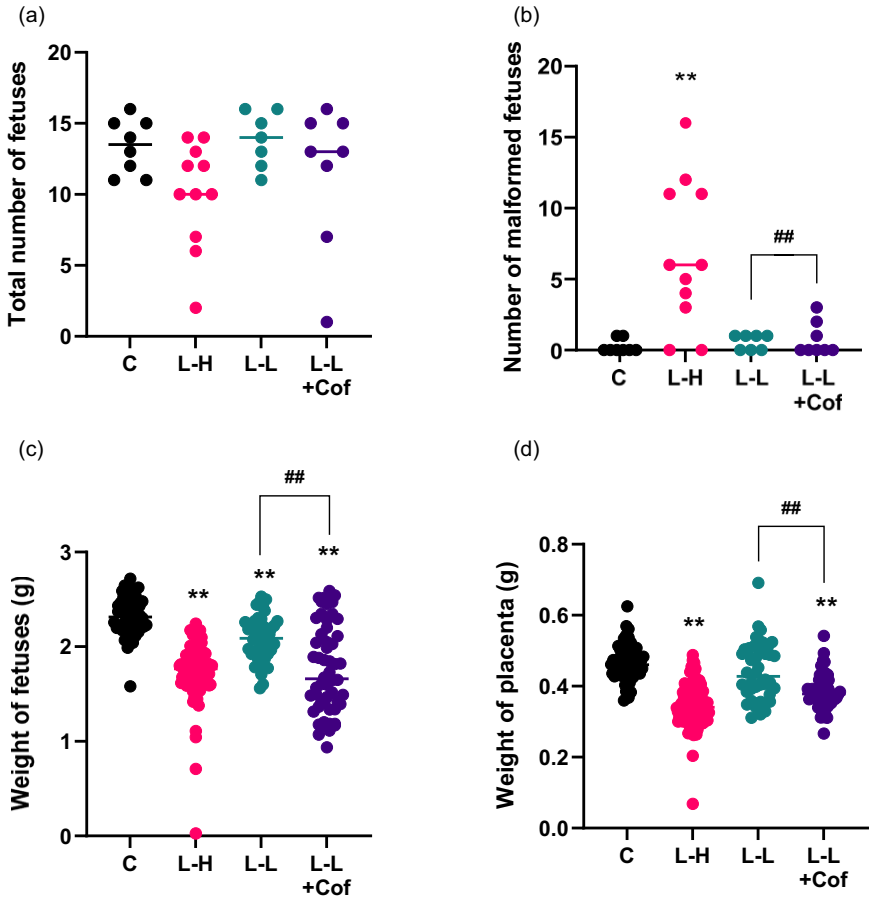

Fig. 1. Fetus development in pregnant rats treated with L-NAME and/or Coffee. (a) Total number of fetuses, (b) number of malformation fetuses, (c) Weight of fetuses, (d) weight of placenta. C: Control group, L-H: L-NAME high-dose group, L-L: L-NAME low-dose group, L-L + Cof: L-NAME low-dose + Coffee group. Results are shown as mean ± SD. For (a) and (b), Control group: n = 8, L-NAME high-dose group: n = 11, L-NAME low-dose group: n = 7, L-NAME low-dose + Coffee group: n = 8; For (c) and (d), Control group: n = 52, L-NAME high-dose group: n = 96, L-NAME low-dose group: n = 44, L-NAME low-dose + Coffee group: n = 54., *P < 0.05; **P < 0.01 compared to control group; ##P < 0.01 compared between the two indicated groups.

Discussion

There has been growing concerns in recent years about the potential health risk of coffee consumption during pregnancy.[12,13] L-NAME is a NOS inhibitor, which can inhibit the activity of NOS in human placental trophoblast cells and reduce the production of NO.[14,15] It is a widely used tool compound for inducing PE in animals,[10] because vascular endothelial NO has been reported to be a vital factor to protect vascular damage.[16] We used a commonly used dose of L-NAME (125 mg/kg) to establish a positive PE model and a low dose of L-NAME (10 mg/kg) to simulate some pregnant women who have a predisposed vascular problem. We studied whether coffee intake during pregnancy was sufficient to induce PE in these susceptible populations. Our results show that coffee intake during pregnancy can lead to the symptoms of PE such as fetal growth restriction, hypertension, proteinuria, and fetal growth in low-dose L-NAME treated animals.

Pregnancy induces extensive adaptations in cardiovascular physiology. NO production by eNOS has been found to be important for the maintenance of normal blood pressure.[17] We found that eNOS expression significantly decreased in the placenta in the L-NAME high-dose group and the low-dose L-NAME + coffee compared to control, which suggests that the reduced production of NO in placenta may contribute to the high blood pressure seen in the L-NAME high-dose group and the low-dose L-NAME + coffee group. Further investigations are merited to study the mechanism of the downregulation of eNOS by coffee.

Proteinuria is not essential to diagnosis but is related to disease severity and fetal outcomes in PE patients.[1] We found that urinary albumin levels increased in L-NAME high-dose group and L-NAME low-dose + coffee group (Fig. 2b). Multiple studies suggest that long-term administration of L-NAME alters various biochemical markers of the kidney by inhibiting NOS, which is consistent with our result.[18,19] However, at present, there are few clinical studies on the effect of coffee on the risk of kidney injury during pregnancy, which merits further investigation.

The histology of placenta in L-NAME high-dose group and L-NAME low-dose + coffee group showed that the thickness of the junctional zone increased compared to control and L-NAME low-dose group (Fig. 3), and the vasculature in the labyrinth region was poorly developed with smaller arterial diameters (Fig. 4). sFlt-1 is induced by hypoxia inducible factor-1 and is a marker of vascular endothelium dysfunction.[20] Elevated sFlt-1 level also may lead to the decrease of

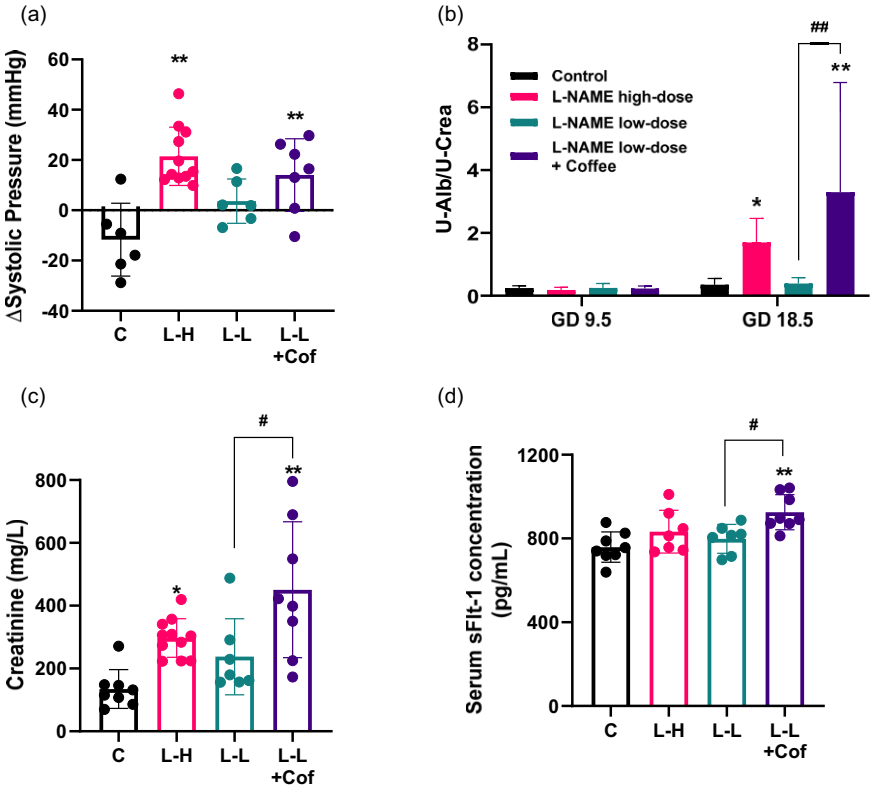

Fig. 2. Systolic blood pressure (SBP) and renal functions of pregnant rats treated with L-NAME and/or Coffee. (a) The change in SBP on GD18.5 compared to GD9.5, (b) Urinary protein level indicated by urinary albumin (U-Ab)/urinary creatinine (U-Crea), (c) Serum creatine, (d) serum sFlt-1 level in pregnant rats. C: Control group, L-H: L-NAME high-dose group, L-L: L-NAME low-dose group, L-L + Cof: L-NAME low-dose + Coffee group. Results are shown as mean ± SD. Control group: n = 8, L-NAME high-dose group: n = 11, L-NAME low-dose group: n = 7, L-NAME low-dose + Coffee group: n = 8. *P < 0.05; **P < 0.01 compared to control group; #P < 0.05; ##P < 0.01 compared between the two indicated groups.

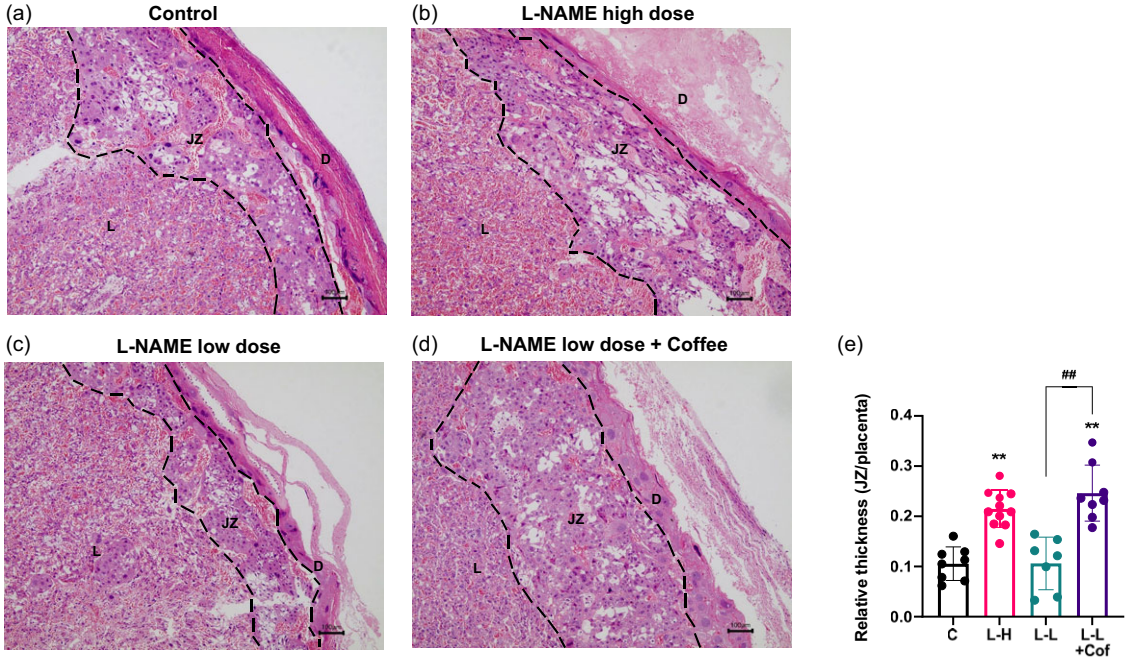

Fig. 3. Changes of placenta structure in pregnant rats. Placental histology in hematoxylin/eosin-stained sections from the control group (a), the L-NAME high-dose group (b), the L-NAME low-dose group (c), and the L-NAME low-dose + Coffee group (d). The dotted line divides the placental structure into three parts. D, decidua, JZ, junctional zone; L, labyrinth. Scale bars: 100 μm. (e) The relative thickness of the junctional zone (JZ). Results are shown as mean ± SD. Control group: n = 8, L-NAME high-dose group: n = 11, L-NAME low-dose group: n = 7, L-NAME low-dose + Coffee group: n = 8. **P < 0.01 compared to control group; ##P < 0.01 compared between the two indicated groups.

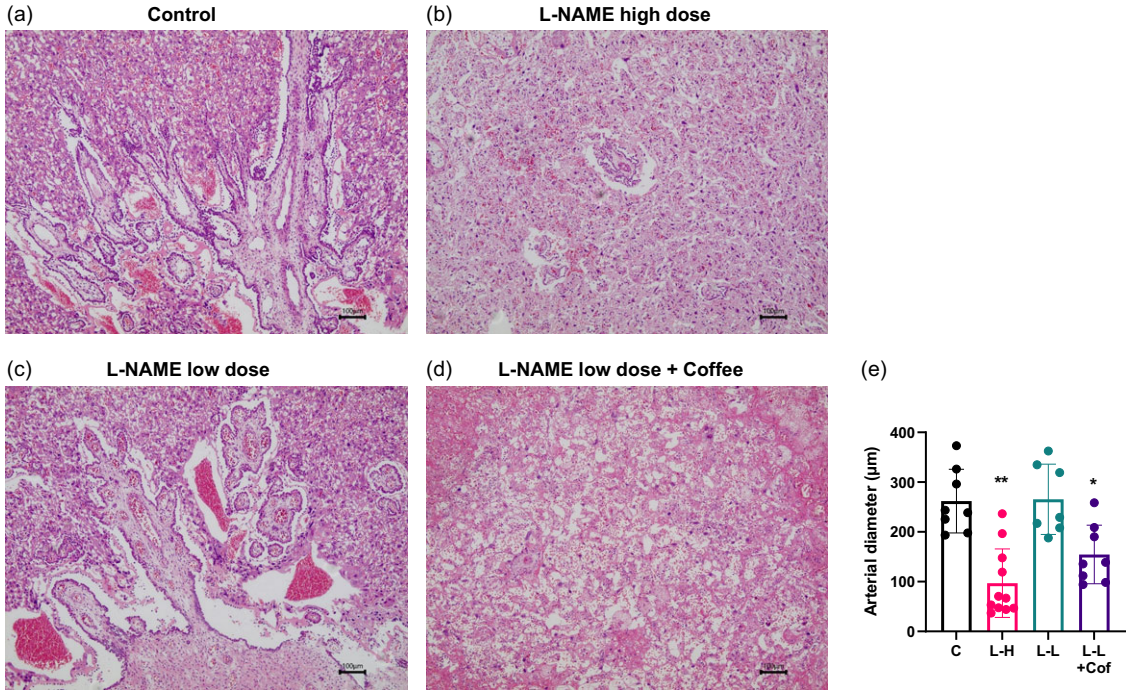

Fig. 4. Comparison of vascular network distribution in placenta. Cross sections of labyrinth region of placenta in the control group (a), the L-NAME high-dose group (b), the L-NAME low-dose group (c), and the L-NAME low-dose + Coffee group (d). Scale bars: 100 μm. (e) The arterial diameter in the labyrinth region. Results are shown as mean ± SD. Control group: n = 8, L-NAME high-dose group: n = 11, L-NAME low-dose group: n = 7, L-NAME low-dose + Coffee group: n = 8. *P < 0.05; **P < 0.01 compared to control group.

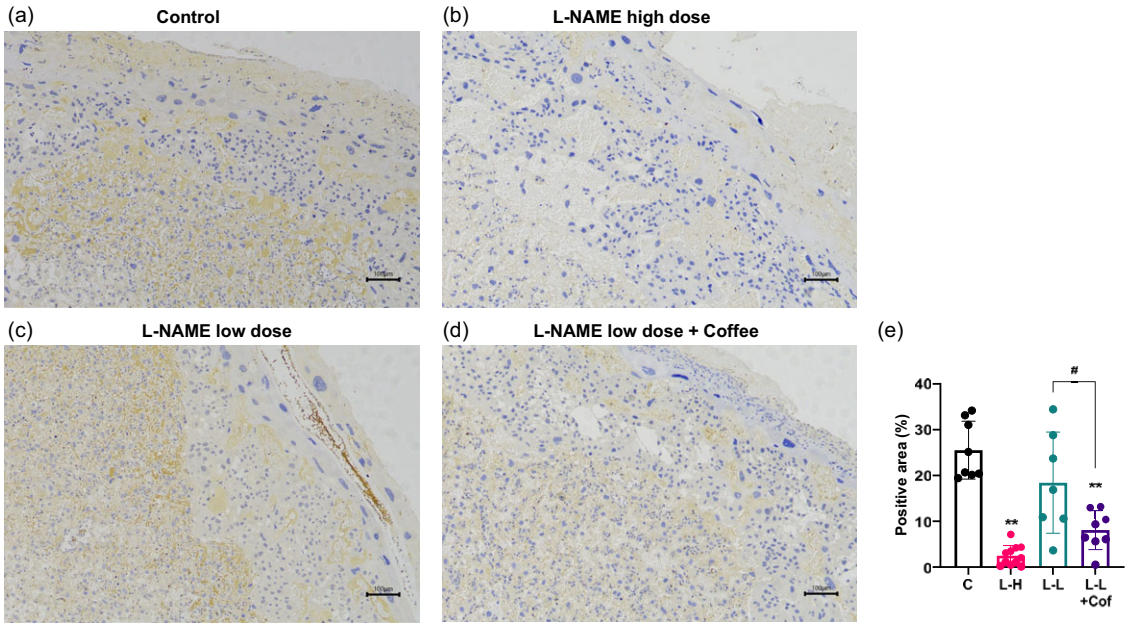

Fig. 5. Comparison of eNOS expression in placenta. Immunohistochemical staining of placenta with eNOS antibody in the control group (a), the L-NAME high-dose group (b), the L-NAME low-dose group (c), and the L-NAME low-dose + Coffee group (d), Scale bar =100 μm. (e) The quantitative analysis of the positive area (%). Results are shown as mean ± SD. Control group: n = 8, L-NAME high-dose group: n = 11, L-NAME low-dose group: n = 7, L-NAME low-dose + Coffee group: n = 8. *P < 0.05; **P < 0.01 compared to control group, #P < 0.05 compared between the two indicated groups.

placental vascular development, thus affecting fetal nutrition and oxygen supply, and causing fetal growth restriction.[21] In addition, NO and eNOS play important roles in placental vasculature maturation.[22] Therefore, elevated sFlt-1 and decreased eNOS induced by L-NAME high-dose treatment and L-NAME low-dose + coffee treatment may contribute to the impaired placenta structure and function in these two groups.

It is of note that the malformations of fetuses were slightly different between the high-dose L-NAME group and the L-NAME low-dose + coffee group. In the high-dose L-NAME

group, hind limb developmental defects characterised by severe hemorrhage were dominant, while subcutaneous stasis was the dominant malformations in the L-NAME low-dose + coffee group. This may be due to their different mechanisms of inducing PE symptoms. Reports have revealed NO production controls late embryonic hind limb development.[15] Therefore, high-dose L-NAME treatment caused hind limb defects. Subcutaneous bleeding in the L-NAME low-dose + coffee group may be related to hormonal disruption caused by coffee. There were also cases of forelimb defect in the L-NAME low-dose + coffee group, which should have been differentiated on GD12.5.[23] One plausible hypothesis is that it could be attributed to the impaired ectodermal cristae and active polarisation centre, which play a crucial role in the development of limb buds.[24] The mechanisms for these differences in fetus development merit further investigation.

In this study, we have found that coffee consumption during pregnancy with underlying PE risk can induce symptoms of PE in animals. Further clinical studies are merited to validate this finding in humans. In addition, there are lots of components in coffee such as various coffee polyphenols. Further studies are merits to determine whether caffeine or a particular coffee polyphenol could induce PE in pregnant rats and the underlying mechanisms.

In conclusion, our findings reveal the potential for the increased PE risk by coffee intake during pregnancy in pregnant individuals with underlying vasculature problems. Therefore, coffee intake during pregnancy could be potentially hazardous for the health of both mother and fetus.

Abbreviations

ELISA: enzyme-linked immunosorbent assay; **eNOS:** endothelial nitric oxide synthase; **GD:** gestational day; **HE:** hematoxylin/eosin; **IHC:** immunohistochemical; **L-NAME:** NG-nitro-L-arginine methyl ester; **NO:** nitric oxide; **NOS:** nitric oxide synthase; **PE:** Preeclampsia; **SBP:** Systolic blood pressure; **SD:** Sprague-Dawley; **sFlt-1:** soluble fms-like tyrosine kinase-1; **U-Ab:** urinary albumin; **U-Crea:** urinary creatinine.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.36

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Declaration of interests

The authors declare no conflicts of interest.

Authorship

P. W. designed the research; L. C. performed research; L. C. analysed data; Y. D., L. C., and P. W. wrote the paper.

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RESEARCH ARTICLE

Effectiveness of a nutrition education intervention using simulation videos and encouragement of parental involvement for elementary school students

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Abstract

This study aims to develop a nutrition education intervention to promote healthy eating, and to evaluate the effectiveness of this intervention on healthy eating knowledge, attitude and behaviour among elementary students. A quasi-experimental study was conducted in two elementary schools in Taiwan. The intervention course design included simulation videos, lectures, and the after-school learning worksheet designed for parental involvement. A total of 4 courses along with 4 simulation videos were given to the intervention group. The four course themes were Sugar patrol, Balanced Diet during the Mid-Autumn Festival, Rainbow Fruit and Vegetables, and Smart Dine Out. The study recruited 35 3rd grade students for the intervention group and 30 for the control group. Data were collected from the pre- and post-test questionnaires. The nutrition intervention had significant effects on improving participants' knowledge about tips for making healthy choices and the necessity of balanced diet, and on attitude toward healthy eating. There was no significant improvement in participants' healthy eating behaviours. This nutrition education intervention, which utilized simulation videos and encouraged parental involvement, could be recommended for teaching practice in elementary schools to improve healthy eating knowledge and attitude among students.

Key words: **Healthy eating: Nutrition education intervention: Parental involvement: Simulation videos**

Introduction

Based on the results of Nutrition and Health Surveys in Taiwan, the consumption of sugar-sweetened beverages, insufficient consumption of vegetables and fruits, and insufficient intake of dairy products among schoolchildren constitute pressing nutritional issues. Dietary intake has a profound impact on children's development and health and may further affect their health conditions in adulthood.[1] Imbalanced nutrition intake is considered to be closely related to childhood obesity which can lead to an increased incidence of CVD, diabetes, asthma, and sleep apnoea.[2–6] The necessity and benefits of preventing and controlling childhood obesity has been suggested in literatures.[7] A survey study in Taiwan revealed that the frequency of vegetable consumption in overweight schoolchildren was lower than that of students with normal body weight.[8] Excessive consumption of sugar-sweetened beverages[9–11] and skipping breakfast in schoolchildren are also unhealthy lifestyles which have been linked to increased risk of obesity and CVD.[12–14]

The school-based health interventions have demonstrated results of developing healthy behaviours among elementary students.[15]A systematic literature review indicated that childhood obesity and subsequent health problems could be improved through nutrition education.[16] Nutrition education courses can improve participants' nutritional knowledge and help improve badly balanced eating habits.[17] Those with better nutritional knowledge tended to demonstrate healthy dietary behaviours, such as increased consumption of vegetables and fruits, and decreased consumption of sugar.[18] In the childhood obesity prevention guideline compiled by the Taiwan Pediatric Association, it was strongly recommended that nutrition education be incorporated into the core curriculum of schools.[19] Presently, nutrition-related topics are included in

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health education curriculum for schoolchildren in Taiwan. However, in practice, health education teachers in elementary schools do not necessarily have expertise in health education, and they often face challenges when guiding students in obtaining health-related knowledge and skills.[20] Consequently, elementary school children may not be able to acquire knowledge on healthy eating in schools, which may affect the development of healthy dietary behaviour.

To promote nutrition education on campus and improve the dietary habits of schoolchildren, the WHO has put forward specific teaching recommendations based on empirical research. Selected recommendations were adopted to develop intervention materials of this study: (1) Designing professional nutrition courses; (2) Parental participation; (3) Experiential learning such as cooking and food preparation activities; (4) Using media for teaching; (5) Establishing a learning community to provide resources and feedback.[21] These teaching strategies should be implemented and evaluated by empirical research. Scholars have proposed the concept of parental influences on shaping the children's preferences and eating behaviours.[22] In addition to nutrition educators working in schools, parents may also play a similar and active role in teaching their children about nutrition.[23–25] Increasing nutrition interventions attempted to incorporate family involvement and take-home activities to encourage good nutrition practices at home[26,27]; however, a systematic review of nutrition interventions with parental involvement showed the inconclusive effectiveness.[28] The possible explanations for the inconclusive results might be that ensuring parental involvement in school-based interventions was challenging, and the information on parental attendance was often lacking.[29] Further approaches to encourage parental engagement need to be explored and tested. The present study referred to the above recommendations, considered common dietary problems of Taiwanese schoolchildren, and designed a nutrition education course module that combined simulation teaching videos and parental involvement. Simulation is considered an effective educational technique in nursing education. Either virtual patients or scenarios are commonly created for nursing students to practice and acquire skills.[30–32] Simulation video is also recommended as a teaching media to enhance student engagement in the learning process.[33] Its possible application in nutritional education should also be examined. The objective of the study was to evaluate the effectiveness of the intervention and to observe whether this curriculum could improve schoolchildren's knowledge, positive attitudes, and behavioural practices about healthy eating.

Methods

Study participants

This study adopted a quasi-experimental design. The experimental group and the control group were recruited from two elementary schools located in different districts in Taipei City in Taiwan. Study participants were third-grade students aged 9–10. The experimental group consisted of 35 students who received nutrition education module instruction. The control group consisted of 30 students who received health courses that were not related to nutrition education (e.g. physical activity and personal hygiene) during the intervention period. Before the participants were recruited, the school teachers confirmed that neither of the two groups had taken relevant nutrition courses. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving study participants s were approved by the Tri-Service General Hospital in Taiwan (#1-106-05-065). Written informed consent was obtained from the guardians of all participants and their parents.

Course module planning and preparation

The course module consisted of four lessons of 40 minutes each, which were implemented from September to October 2018. The four lessons conducted weekly for four weeks. Course topics were formulated based on schoolchildren's nutritional issues as identified in the literature review, prior understanding of learners' needs, and taking into the consideration the folk festivals occurring during the semester. The four themes were Sugar patrol, Balanced Diet during the Mid-Autumn Festival, Rainbow Fruit and Vegetables, and Smart Dine Out. Table 1 presents details about the topic contents and course flow.

The course began with a five-minute simulation video depicting the daily life and dietary experience of a fictional character 'Student Nutritionist Mimiko', such as going to the market with family to buy groceries and hanging out with classmates for a meal in a restaurant. Teachers used these simulation videos to stimulate students' learning motivation and guide students to connect the scenario in the video with life experiences. After watching the videos, the teaching was then extended to guide students to realize the importance of healthy eating and to discuss how to implement healthy eating behaviours in daily life. Each lesson ended with a specially designed homework assignment, 'Little Nutritionist Learning Worksheet', allowing students to assume the role of nutritionists at home and fill out the worksheet with their parents, thereby extending the effectiveness of classroom learning. To encourage parental participation, the instructor prepared a 'letter to parents' before the intervention started, explaining course objectives and establishing an online communication group to provide resources and feedback.

Evaluation of intervention effectiveness

Intervention effectiveness was evaluated using a self-administered questionnaire which was compiled according to the study objectives and with reference to the questionnaires used in the Nutrition and Health Survey in Taiwan (NAHSIT) which was a series of surveys conducted by the government. The target population of the surveys included elementary school students, and the specialized questionnaires were planned.[34] A total of 43 questions were used to assess 'Healthy Eating Knowledge', including the functions of foods, nutrients and disease, nutritional contents in food, tips for making healthy food choices, the necessity of balanced diet, and the concept of healthy eating. A higher score indicates better knowledge about

Table 1. Content of the nutrition education course modules for the experimental group

Lesson topic	Learning objective	Course flow
Sugar patrol	Raising self-awareness of healthy body weight and understanding the health problems caused by foods high in sugar	1. Watch a video: Student Nutritionist Mimiko's Journey Through Space and Time 2. Explain the course content and conduct prize Q&A 3. Explain the homework assignment
Balanced diet over the mid-autumn festival	Understanding the concept of food classification and balanced dietary intake	1. Watch a video: Mimiko's Market Mission 2. Explain the course content and conduct prize Q&A 3. Explain the homework assignment
Rainbow fruit and vegetables	Knowing the recommended amount of fruit and vegetables the schoolchildren need.	1. Watch a video: Mimiko's Dinner Table Password 2. Explain the course content and conduct prize Q&A 3. Explain the homework assignment
Smart dine out	Choosing healthy meals when dining out	1. Watch a video: Mimiko's Gathering with Her Classmates 2. Explain the course content and conduct prize Q&A 3. Explain the homework assignment

healthy eating (Cronbach $\alpha = 0.72$). Fifteen questions were used to assess 'Healthy Eating Attitudes', referring to the attitude towards making healthy eating choices. A five-point scale was used for scoring, with a higher score indicating a more positive attitude towards healthy eating (Cronbach $\alpha = 0.68$). Finally, 16 questions were used to evaluate 'Healthy Eating Behaviour', concerning the recommended daily intake of six food groups, healthy breakfast intake, sugar-sweetened beverage consumption, fried food consumption, and low-nutrient-density food consumption. A higher score in this question set indicated a better implementation of healthy eating behaviours (Cronbach $\alpha = 0.63$).

Data collection and statistical analysis

Both the experimental group and the control group took a questionnaire survey before and after the nutrition education course. The collected data were analysed using SPSS 23 (SPSS, IBM, Armonk, NY). Generalized Estimating Equations (GEE) were used to assess the effectiveness of curriculum intervention on healthy eating knowledge, attitude, and behaviour. The GEE method can be used to analyse repeated measures with non-normal response variables, and it can take into account the correlation of within-subject data.

Results

The pre- and post-test questionnaires

All the participants of two groups completed the intervention and the pre- and post-test questionnaires. There were no statistically significant differences between the experimental and control groups in terms of gender distribution, self-perceived body size satisfaction, and BMI (Table 2). The two-sample *t*-test showed that there was also no significant difference in the pre-test scores of healthy eating knowledge, attitude, and behaviour between the two groups (Table 3). The results of the GEE analysis showed that the intervention had no significant effect on the mean overall score for healthy eating knowledge ($\beta = 2.452$, $P = 0.147$). However, there was a significant improvement in 'tips for making healthy choices' and 'the necessity of balanced diet' after intervention ($\beta = 0.81$, $P = 0.06$; $\beta = 1.257$, $P = 0.007$) (Table 4). The intervention also had a significant effect on the attitude towards healthy eating ($\beta = 4.705$, $P = 0.048$) (Table 5), but not on healthy eating behaviour (Table 6).

After-school learning worksheets and online group interactions

Besides questionnaire data, the review of after-school learning worksheets showed that those distributed in the first lesson had the highest completion rate, with 34 copies returned. As the course progressed, only 28 worksheets filled out fully by both parents and students and signed by parents were returned for the fourth lesson. In addition, the online group interactions among parents and intervention instructor were limited. During the intervention process, only four parents frequently and consecutively interacted in the online group to discuss the course content and after-school learning worksheets.

Discussion

The features of this intervention study were to utilize simulation videos as the primary teaching medium in designing the curriculum and to use after-school learning worksheets as assignments to encourage parental involvement. The intervention helped participants make significant improvements in their scores on the knowledge and attitude question sets, which could be attributed to the use of simulation videos in the curriculum which raised participants' learning motives and concentration. However, there was no significant change in participants' healthy eating behaviour. Changes in nutrition behaviours might not be observed after a short-term intervention, which emphasizes the need to conduct long-term interventions measuring effects on nutrition behaviour outcomes.[20] Besides, although previous studies suggested that parental involvement and take-home activities should be effective strategies to help children develop healthy eating practices,[21,26] it was observed in the present study that the level of parental involvement was unable to sustained during the entire intervention, which might be associated to the unsatisfied outcomes in changing schoolchildren's eating behaviour.

Table 2. Study participant characteristics, BMI, and pre-test scores

Variable		Experimental group (n = 35)		Control group (n = 30)		P value
		n	%	n	%	
Gender						0.900
	Male	11	31.4	9	30	
	Female	24	68.6	21	70	
Semester grade						0.909
	A or A+	18	51.4	15	50	
	Below A	17	48.6	15	50	
Satisfaction with body size						0.128
	Satisfied	11	31.4	15	50	
	Dissatisfied	24	68.6	15	50	
		Mean	Standard deviation	Mean	Standard deviation	
BMI(kg/m^2)	Boys	16.2	2.8	16.9	3.0	0.393
	Girls	16.0	3.0	17.4	3.2	0.147

Table 3. Mean scores of healthy eating knowledge, attitude and behaviour before and after the intervention

Variable	Experimental group (n = 35)		Control group (n = 30)		P value[a]
	pre-test	post-test	pre-test	post-test	
Mean overall knowledge score	32.1 ± 5.1	33.5 ± 5.8	29.6 ± 7.4	27 ± 7.6	0.109
Function of foods	3.2 ± 1.0	3.5 ± 1.1	2.6 ± 1.5	2.3 ± 1.4	
Nutrients and disease	3.5 ± 1.2	3.7 ± 1.4	2.7 ± 1.4	2.5 ± 1.5	
Nutritional contents in food	10.1 ± 2.0	9.6 ± 2.7	9.3 ± 3.6	7.6 ± 2.9	
Tips for making healthy food choices	4.6 ± 1.5	4.9 ± 1.4	4.7 ± 1.6	4.2 ± 1.4	
Necessity of balanced diet	5.1 ± 1.4	6.5 ± 1.5	5.6 ± 1.3	5.8 ± 1.5	
Concept of healthy eating	5.7 ± 0.5	5.4 ± 1.7	4.63 ± 1.4	4.56 ± 1.5	
Mean attitude score	61.3 ± 13.2	65.5 ± 8.2	61.8 ± 6.8	61.3 ± 11.6	0.839
Mean behaviour score	34.7 ± 3.4	35.0 ± 3.7	33.4 ± 3.1	33.2 ± 3.0	0.687

[a]Comparison of pre-test scores between the two groups using the two-sample *t*-test.

Simulation video has been widely used in nursing education because of its advantages in creating real-life situations for students to engage in the learning process.[35,36] This study extended its application to nutrition education for elementary students, and found its effectiveness in improving participants' knowledge and attitude regarding healthy eating. In order to motivate participants' learning interests and guide them to reflect real-life experiences, the short simulation videos showed the daily life of a fictional character who is at the same age as the study participants. Participants watched the simulation video and learned how the fictional character completed various dietary-related missions. After watching the video, participants were asked to imitate the fictional character's missions and complete homework assignments. The use of simulation videos allows for the demonstrations of situations that learners are yet to experience and provides them with a contextualized opportunity for learning.[37]

Based on observing students' reactions in class and analysing the learning worksheets, it was found that some students and parents had difficulties in grasping numerical concepts such as the daily 'recommended upper limit of added sugar' and 'calories contained in snacks'. Teaching nutritional knowledge often involves computational skills. For future intervention material design, it is suggested that numerical concepts be translated into specific food types and servings to facilitate comprehension. For example, if a schoolchild eats one slice of red bean bread for breakfast, sugar-free beverages shall be recommended when choosing pared drinks. Additionally, instructors might illustrate the abstract concept by analogy with concrete cases, for example, by using the traffic light system to help students identify foods with high energy density and learn to be cautious about intaking these foods.

Several limitations of this study and suggestions for future research are addressed. This intervention was implemented for small sample size. In terms of implementation process, an issue should be mentioned was about the imperfect completion of 'Little Nutritionist' after-school learning worksheets designed to increase parental involvement. The worksheets were designed to be completed by children and parents jointly, but the handwriting on some returned worksheets appeared to be completed by children alone. Future intervention should consider improving the after-school worksheet design or planning other parents-child activities to encourage parental engagement. Moreover, the intervention period coincided with the Mid-Autumn Festival in Taiwan, and the topic of choosing healthy festival foods was purposely integrated into the curriculum to conform participants' real-life experience. However, during actual implementation, it was found that schoolchildren's dietary

Table 4. GEE analysis results of the effectiveness of nutrition education intervention on healthy eating knowledge

Variable		β	SE	P
Overall score	Intercept	29.567	1.3364	0.000
	Group	2.548	1.5839	0.108
	Pre- and post-test	–2.567	1.2389	0.038
	Pre- and post-test × Group	2.452	1.6912	0.147
Functions of foods	Intercept	3.000	0.4738	0.000
	Group	–0.086	0.5741	0.881
	Pre- and post-test	–0.367	0.2728	0.811
	Pre- and post-test × Group	0.652	0.3384	0.054
Nutrients and disease	Intercept	2.933	0.4495	0.000
	Group	0.381	0.6028	0.527
	Pre- and post-test	–0.200	0.2724	1.000
	Pre- and post-test × Group	0.400	0.3731	0.284
Nutritional contents in food	Intercept	10.967	1.1338	0.000
	Group	–0.395	1.3599	0.771
	Pre- and post-test	–1.667	0.5988	0.007
	Pre- and post-test × Group	1.181	0.7959	0.138
Tips for making healthy food choices	Intercept	5.167	0.4271	0.000
	Group	–0.938	0.5811	0.106
	Pre- and post-test	–0.467	0.2094	0.673
	Pre- and post-test × Group	0.810	0.2936	0.006
Necessity of balanced diet	Intercept	5.367	0.5153	0.000
	Group	–1.738	0.7236	0.016
	Pre- and post-test	0.200	0.3347	0.000
	Pre- and post-test × Group	1.257	0.4641	0.007
Concept of healthy eating	Intercept	4.700	0.5167	0.000
	Group	1.243	0.5503	0.024
	Pre- and post-test	–0.067	0.3298	0.333
	Pre- and post-test × Group	–0.219	0.3640	0.547

Note: Y = β0 + β1 (pre- and post-test) + β2 (group) + β3 (pre- and post-test × group); Pre- and post-test: post-test = 1, pre-test = 0, Group: experimental group = 1, control group = 0.

Table 5. GEE analysis of the effectiveness of nutrition education intervention on healthy eating attitude

Variable		β	SE	P
Healthy eating attitude	Intercept	61.833	1.2303	0.000
	Group	–0.548	2.5203	0.828
	Pre- and post-test	–0.533	1.9042	0.770
	Pre- and post-test × Group	4.705	2.3834	0.048

Note: Y = β0 + β1 (pre- and post-test) + β2 (group) + β3 (pre- and post-test × group); pre- and post-test: post-test = 1, pre-test = 0, group: experimental group = 1, control group = 0.

Table 6. GEE Analysis of the effectiveness of nutrition education intervention on healthy eating behaviour

Variable		β	SE	P
Healthy eating behaviour	Intercept	33.367	0.5587	0.000
	Group	1.376	0.7955	0.084
	Pre- and post-test	–0.167	0.1639	0.309
	Pre- and post-test × Group	0.424	0.7579	0.576

Note: Y = β0 + β1 (pre- and post-test) + β2 (group) + β3 (pre- and post-test × group); Pre- and post-test: post-test = 1, pre-test = 0, Group: experimental group = 1, control group = 0.

preferences in cultural festivals tend to be shaped by sociocultural environment, rather than being easily altered through a short-term intervention. Furthermore, peer influences on schoolchildren's eating behaviours were not included in this intervention. Peer effects on children's eating behaviour were observed to link with the consumption of energy-dense and low-nutrition value foods,[38,39] which should be taken into account in future nutrition interventions for schoolchildren.

Abbreviations

GEE: Generalized Estimating Equations

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Conflict of interest

None.

Authorship

SR and YC designed the study, and SR implemented the intervention. Both SR and YC contributed to data analysis and manuscript writing. Both authors read and approved the final manuscript.

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RESEARCH ARTICLE

Food insecurity and its association with socio-demographic characteristics in Cyprus

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Abstract

The prevalence of food insecurity in Cyprus and the socio-demographic factors that are related to this public health problem are unknown. Data used in this cross-sectional study were collected between 1 June 2022 and 21 May 2023 using a self-reported method. Food insecurity was evaluated using the Greek version of Adult Food Security Survey Module. The data regarding socio-demographic and socio-economic characteristics along with body weight and height measurements were collected through self-administered questionnaire. A representative sample of n=1255 adults, ≥18 years old living in the five different districts of the Republic of Cyprus, was recruited. Prevalence of food insecurity in Cypriot population was 12.6%. Prevalence was higher in females, in older adults, in adults living in Paphos, in individuals who were separated, divorced, or widowed, in retired people, in people living with children, and in people with low income and education. Based on multivariable analysis, income was the strongest socio-demographic factor independently associated with food security (€ 6,500–€ 19,500: AOR: 0.49, 95% CI 0.28, 0.86 and >€ 19,500: AOR: 0.15, 95% CI 0.73, 0.31). Food insecurity is a global problem that need further examination. The association between food insecurity and socio-demographic characteristics needs to be highlighted in order for each country to develop specific public health policies (e.g. financial support to low income people) to decrease food insecurity and improve people's overall health and quality of life.

Key words: **Cross-sectional study: Food insecurity: Prevalence: Socio-demographic factors**

Introduction

Food security exists when all people, regardless of season, have physical, social and economic access to safe and nutritious food to meet their needs for an active and healthy life.[1] Based on the above, food insecurity can be defined as limited or uncertain availability of nutritionally adequate and safe foods, or inability to acquire acceptable food in a socially acceptable way.[2]

According to the World Food Program, 349 million people in 79 countries are experience acute food insecurity, an increase of 200 million people compared to 2019, before the pandemic of Covid 19.[3] Although food insecurity is generally associated with lower income[3] and it is more prevalent in lower and middle income countries,[4] recent studies revealed that food insecurity is also a concerning public health problem in high income countries.[5,6] In the USA, in 2021, 10.2% of households experienced food insecurity, with 38% of them experiencing severe food insecurity.[7] In regards to Canada, in 2019, 14.2% of women and 12.1% of men experienced food insecurity.[8] As for the European countries, in Denmark, in 2015, the prevalence of low and very low food security was 6% and 2.4% respectively.[9] A recent study conducted in Finland concluded that 65% of private sector service workers were food insecure with over a third (36%) reposting severe food insecure.[10] The data regarding the prevalence of food insecurity in the Mediterranean

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countries are scarce. Bocquier et al, reported that, in 2015, 12.2% of French adults citizens were food insecure.[11] As for Greece, two cross-sectional studies were conducted in 2016 among Greek university students[12] and in 2019 among older adults.[13] Theodoridis et al, concluded that a significant high proportion of Greek university students were severe food insecure (45.3%) and 22.5% and 14.8% of the sample were moderate and low food insecure.[12] The study by Gkiouras et al highlights the high prevalence (50.4%) of food insecurity in older adults.[13] The only study occurred in Cyprus aiming to examine the risk of experiencing food stress, defined as the product of the cost of healthy food relative to the income of the household, among the low-income Cypriot population was conducted by Chrysostomou et al, in 2017.[14] The researchers concluded that there is a significant proportion of low income Cypriots who experience food stress. There are no available data in regards to the prevalence of food insecurity in the general population in Cyprus.

Socio-demographic factors such as age, sex, province and territory, education, household structure and socio-economic factor such as income are a group of variables that affect food insecurity.[15–17] It has been found that, there is an inverse association between income and food insecurity. Specifically, for every decrease in a family income unit, the food insecurity increases by 12.5%.[18] Baxter et al, stated that people who receive less than €20 000 annually are at greater risk for food insecurity compared with those who receive more than €20 000 annually.[16] Moreover, a recent cross-sectional study conducted in USA, by Cai et al, analyzed data from the «National Health Interview Survey- NHIS» (2020–2021) and they concluded that young people, female sex, certain nationalities, being unmarried, divorced or widowed, having low education and no health insurance were significantly associated with food insecurity.[17] In addition, the odds of being food insecure were higher for unmarried volunteers and for households with children in the house.[15]

In a context of continuously rising food costs, there is a general perception that healthy eating is more expensive compared to unhealthy eating.[18] People experiencing food insecurity, due to the lack of money, are forced to consume energy-dense foods and foods high in sugar and fat[19] which are cheaper compared to foods that promote a healthy nutritional behavior[20] such as fruits, vegetables, and dairy products.[21] A recent study, conducted in Cyprus, concluded that low-income people tend to spent almost two third of their monthly income in order to buy nutritious foods and therefore, low-income people in Cyprus are food insecure.[14] Due to the consumption of unhealthy foods, food insecure people are more likely to suffer from chronic diseases such as diabetes, cardiovascular diseases and hypertension[22] compared with food secure people. Other chronic health conditions associated with food insecurity are anemia, especially in women,[20] depression,[23] and coeliac disease.[24]

Notably, the prevalence of food insecurity among the whole population in Cyprus has not yet been evaluated. Moreover, the factors affecting food insecurity have not been well established considering that the risk of poverty in Cyprus, even though it records a slight download course, it nevertheless remains at high levels compared with other European countries such as Slovakia, Finland, Poland, Slovenia, and Czechia.[25] Based on the latest data, in 2021, the risk of poverty in Cyprus was 13.8%, compared to 15.9% in 2008.[26] Considering that poverty is only one factor associated with food insecurity, other factors may also play an important role in this phenomenon.[15–17] Therefore, the aim of the study was to assess the prevalence of food insecurity among Cypriot population and its relationship with socio-demographic factors.

Methods

This study used a community-based cross-sectional study design. A total of 1255 men and women >18 years' old living in the five different districts of the Republic of Cyprus participated in the study. Data used in this cross sectional study were collected between 1 June 2022 and 21 May 2023 to assess the prevalence of food insecurity and associated factors including socio-demographic and socio-economic factors in a nationally representative sample of the Cypriot population in terms of region, age and sex.

The required sample size for the estimation of the prevalence of food insecurity was 1255 individuals with a precision of 2%, using a 95% confidence interval (CI) and assuming a true prevalence of food insecurity to be 12%.

In order to achieve a representative sample of the Cypriot population, using the quote sampling method, the sample was divided into the five municipalities of Cyprus (Nicosia, Larnaca, Limassol, Ammochostos, and Paphos) based on the latest available data (2011). Afterwards, the sample was further divided in regards to sex and age creating four age groups (18–24, 25–44, 45–64, and ≥65 years old).[22] The sample nationally representativeness was confirmed using the Chi-square goodness of fit test with statistical significant to be <0.05. Recruitment occurred in public areas (e.g. malls, supermarkets, and churches).

After obtaining the consent form, participants completed a standardised questionnaire[27] and a questionnaire that has been used in previous studies in the Cypriot population.[22,28] In case of participant's inability to complete the questionnaire, two trained researchers were present to offer any help needed. The first questionnaire included close ended questions about socio-demographic characteristics (i.e. age, sex, body weight, height, body mass index-BMI, marital status, employment, income, education, and family composition) where the second questionnaire was about the assessment of food security.

In regards to socio-demographic characteristics, age, height, and body weight were self-reported. BMI was derived from a ratio of weight in kilograms divided by height in meter squared. The three BMI groups were defined based on the National Heart, Lung and Blood Institute terminology (underweight<18.5, normal weight: 18.5–<25, overweight: 25–<30 and obese:≥30).[29] Marital status was recorded as never married, married, or living with partner and separated, divorced, or widowed. Employment was classified into three categories: employed, unemployed, and retired. In regards to income, as a socio-economic factor, three categories were used: low (€6,500), moderate, (€6,500–19,500), and high (>€19,500). Primary

(<7 years of schooling), secondary (7–12 years of schooling), and higher (>2 years of schooling) education were used to evaluated participant's education. For the evaluation of family composition, participants reported the number of people living in the same house with them and how many of them are under 18 or over 75 years old. The same questionnaire had been used in previous studies conducted in Cyprus.[22,28]

The outcome variable for this study was food insecurity which was evaluated using the Greek version of Adult Food Security Survey Module (AFSSM).[27] The above-mentioned questionnaire is a self-reported questionnaire which evaluates adult's food insecurity status over the past 12 months. It consists of 10 close ended questions and can be used for households with or without children. The questions are regarding food-related behaviors, experiences, and conditions that are common in people experiencing food insecurity. Affirmative responses to three or more items indicated that that the participant experienced food insecurity.[30] Based on the study conducted in Greece, by Michalis et al, the Cronbach's alpha coefficient of reliability for the Greek AFSSM was good (0.763 and 0.783, respectively, for the 2 measurements).[27]

During the study, participants had the opportunity to be informed regarding the purpose of the study as well as the method of data collection and processing by two skilled researchers in public areas, including but not limited to shopping centres, malls, churches, and universities. The participants were also informed that the above study was conducted anonymously and that the latter was also approved by the local Bioethics Committee. Lastly, it is noted that all participants provided relevant consent for their participation prior the provision of the questionnaire.

Kolmogorov–Smirnoff test of normality was used to assess the distribution of age and BMI Descriptive analysis was done to assess the prevalence of food insecurity. The continuous variables (e.g. age and BMI) are presented as mean ± standard deviation (SD) and categorical variables (e.g. age group, sex, BMI group, marital status, employment, income, and education) as absolute and relative (%) frequencies. Baseline characteristics of participants by food insecurity group were examined using Pearson's chi-square test for categorical variables and Independent Sample t-test for continuous variables. At first, univariate analysis was performed in order to assess the relationship between food insecurity (which was defined as the outcome of measure) and each socio-demographic and socio-economic factor separately (which were defined as the exposures) (model 1). Afterwards, we performed a multivariate analysis (model 2) in order to assess the association between food insecurity and each socio-demographic factor controlling for the other socio-demographic factors (such as age, employment, marital status living with persons < 18 years old, income, and education). Statistical analysis was conducted using SPSS v.29.0 with statistical significance level set at $P < 0.05$.

Results

Table 1 shows the socio-demographic and socio-economic characteristics of participants by food security status. The study consisted of 1255 people, men and women, with mean age 43.43 (SD ± 17.07) years. More than a half of the volunteers were females (54.4%), 48.7% were living with a child and 10.4% were living with an older adult (>75 years). Furthermore, the majority of the sample (26.3%) was living in a household with 3 persons. Moreover, 46.0% had a normal body weight, according to their BMI, whereas 33.8% and 17.1 % were overweight and obese respectively. In regards to region, most of the participants were living in Nicosia (34.7%) and Larnaca (27.5%) and the rest of them were living in Limassol (23.6%), Paphos (8.9%) and Ammochostos (5.3%). Over a third of the sample were married (66.0%), 70.6% were employed and 17.7% of the participants had an annual income < €6,500. As for education, the majority of the sample (68.7%) reported higher education.

Our results indicate that 12.6% of the volunteers are food insecure. Regarding the socio-demographic and socio-economic characteristics our findings, show that age, BMI, region, marital status, living with persons < 18 years old, employment status, income, and education were all significantly associated with food insecurity ($P < 0.05$). Moreover, among individuals with food insecurity, participants were older (21.3%), living in Paphos (21.3%), separated, divorced or widowed (26.7%), retired (22.9%), living with no children (15.1%), having an income less than €6,500, and report low education (26.0%) compared to food secure participants. Also, participants with food insecurity had a higher BMI compared to those without experiencing food insecurity (27.00 ± 5.64 vs 25.63 ± 5.39) (Table 1).

Logistic regression analysis was conducted in order to examine the relation between food insecurity and socio-demographic and socio-economic characteristics (Table 2). The main finding from the adjusted model indicates that people with income € 6,500–€ 19,500 and >€ 19,500 had 50.6% (AOR: 0.49, 95% CI 0.28,0.86) and 85.1% (AOR: 0.15, 95% CI 0.73,0.31) lower risk for food insecurity compared to participants with income < € 6,500. As for the unadjusted model, being a separated, divorced or widowed increased the risk for food insecurity by 2.5 times (OR: 2.51, 95% CI 1.41, 4.47) compared to people who never got married. Furthermore, individuals who are unemployed or retired had 1.97 (OR: 1.97 [95%CI: 1.28, 3.04]) and 2.83 (OR: 2.83 [95%CI: 1.06, 1.32]) higher risk to present food insecurity, respectively, compared to those who are employed. Moreover, it seems that participants who had higher education presented lower risk for food insecurity OR: 0.30 [95% CI: 0.17, 0.53] compared with people having a primary education. The same relationship seems to apply for participants who were living with no children. More specifically, participants living with no children presented lower risk for food insecurity OR: 0.63 [95%CI: 0.05, 0.88] compared with participants who were living in the same house with children.

Discussion

To the best of our knowledge, this is the first study in Cyprus to assess the prevalence of food insecurity and examine the effect of socio-demographic characteristics on food insecurity in a representative sample of adult population. The prevalence of food insecurity in Cyprus is 12.6%. Among socio-demographic

Table 1. Socio-demographic characteristics overall and by food insecurity group

	Total[a] (n = 1255)	Food insecurity[b] (n = 229)	Food security[b] (n = 1026)	*P* value
Age (years)*	43.43 ± 17.07	46.46 ± 19.48	42.99 ± 16.66	**0.017**[c]
Age group				**<0.001**[d]
18–24	194 (15.5)	27 (13.9)	167 (86.1)	
25–44	507 (40.4)	47 (9.3)	460 (90.7)	
45–64	357 (28.4)	42 (11.8)	315 (88.2)	
≥65	197 (15.7)	42 (21.3)	155 (78.7)	
Gender				0.348[d]
Male	572 (45.6)	78 (13.6)	494 (86.4)	
Female	683 (54.4)	80 (11.7)	603 (88.3)	
BMI (kg/m^2)	25.86 ± 5.29	27.00 ± 5.64	25.63 ± 5.39	**0.03**[c]
BMI group (kg/m^2)				0.082[d]
Underweight (<18.5)	40 (3.2)	5 (12.5)	35 (87.5)	
Normal weight (18.5–24.9)	577 (46.0)	63 (10.9)	514 (89.1)	
Overweight (25–29.9)	424 (33.8)	52 (12.3)	372 (87.7)	
Obese (≥30)	214 (17.1)	38 (17.8)	176 (82.2)	
Region				**0.031**[d]
Ammochostos	66 (5.3)	8 (12.1)	58(87.9)	
Larnaca	345 (27.5)	35 (10.1)	310(89.9)	
Limassol	296 (27.5)	33 (11.1)	263(88.9)	
Nicosia	436 (27.5)	58 (13.3)	378(86.7)	
Paphos	112 (27.5)	24 (21.4)	88 (78.6)	
Marital status				**<0.001**[d]
Single	339 (27.1)	43 (12.7)	296 (87.3)	
Married or living with partner	825 (66.0)	92 (11.2)	733 (88.8)	
Separated, divorced, or widowed	86 (6.9)	23 (26.7)	63 (73.3)	
Individuals in the family				0.442[d]
1	107 (8.5)	18 (16.8)	89 (83.2)	
2	325 (25.9)	45 (13.8)	280 (86.2)	
3	255 (20.3)	27 (10.6)	228 (89.4)	
4	330 (26.3)	42 (10.9)	288 (87.3)	
≥5	238 (19.0)	26 (10.9)	212 (89.1)	
Persons < 18 years old				**0.009**[d]
0	644 (51.3)	97 (10.9)	547 (84.9)	
≥1	611 (48.7)	64 (10.9)	550 (90.0)	
Persons ≥ 75 years old				0.779 [d]
0	1124 (89.6)	140 (12.5)	984 (87.5)	
≥1	131 (10.4)	18 (13.7)	113 (86.3)	
Employment				**<0.001**[d]
Employed	883 (70.6)	84 (9.5)	799 (90.5)	
Unemployed	198 (15.8)	34 (17.2)	164 (82.8)	
Retired	170 (13.6)	39 (22.9)	131 (77.1)	
Income				**<0.001**[d]
<€ 6,500	206 (17.7)	51 (24.8)	155 (75.2)	
€ 6,500–€ 19,500	517 (44.3)	74 (14.3)	443 (85.7)	
>€ 19,500	444 (38.0)	20 (4.5)	424 (95.5)	
Education				**<0.001**[d]
Primary	77 (6.2)	20 (26.0)	57 (74.0)	
Secondary	314 (25.1)	56 (17.8)	258 (82.2)	
Higher	859 (68.7)	82 (9.5)	777 (90.5)	

Abbreviations: BMI, body mass index.
[a]Means ± sd, [b]n (%), [c]Independent sample t-test, [d]Pearson's chi-squared test.
*Bold values represent statistically significant associations P < 0.005.

and socio-economic characteristics, income has the strongest relationship with food insecurity whereas all the other characteristics were not significantly related.

In regards to the prevalence of food insecurity in Cyprus, we found that it's similar to other European countries such as France (12.2%)[11] and Portugal (16.5%).[31] In the present study, income was the strongest socio-economic factor independently associated with food security. Similar results were shown from other researchers,[31] were annual income was found to be an independent factor affecting food insecurity. A recent systematic review, about food insecurity and related contributing factors demonstrated that various socio-demographic characteristics including income influence food insecurity in older adults.[32] Furthermore, Nagata et al, performed an analysis using data from U.S. Census Household Pulse Survey and concluded that income below the federal line is associated with food insecurity.[33] No other socio-demographic factor has been found to have statistically significant effect on food insecurity after controlling for the rest of socio-demographic factors (i.e. age, BMI, region, marital status, living with persons < 18 years old, employment status, and education). A finding which agrees with ours. Another

Table 2. Logistic regression analysis to evaluate the association of socio-demographic characteristics with food insecurity

	Total ($n = 1255$)	
	Model 1[a]	Model 2[b]
Age group		
18–24	1.00	1.00
25–44	0.63 (0.381, 1.05)	1.18 (0.59, 2.37)
45–64	0.823 (0.491, 1.39)	1.38 (0.63, 3.15)
≥65	1.68 (0.986, 2.85)	0.81 (0.26, 2.50)
Persons < 18 years old (0/≥ 1)	**0.63 (0.45, 0.88)***	0.92 (0.61, 1.39)
Employment		
Employed	1.00	1.00
Unemployed	**1.97 (1.28, 3.04)***	1.09 (0.55, 2.19)
Retired	**2.83 (1.06, 1.32)***	1.69 (0.67, 4.17)
Marital status		
Never married	1.00	1.00
Married or living with partner	0.86 (0.59, 1.27)	0.93 (0.51, 1.67)
Separated, divorced, or Widowed	**2.51 (1.41, 4.47)***	1.93 (0.85, 4.35)
Income		
<€ 6,500	1.00	1.00
€ 6,500–€ 19,500	**0.51 (0.34, 0.76)***	**0.49 (0.28, 0.86)***
>€ 19,500	**0.14 (0.08, 0.25)***	**0.15 (0.73, 0.31)***
Education		
Primary	1.00	1.00
Secondary	0.62 (0.34, 1.11)	1.37 (0.65, 2.91)
Higher	**0.30 (0.17, 0.53)**	1.002 (0.44, 2.29)

[a]univariate analysis, [b]multivariate analysis.
*Bold values represent statistically significant associations $P < 0.001$.

study in USA indicated that from a group of socio-demographic characteristics (i.e. age, sex, race, household income, employment, children in home, and married) only income, marriage and living with children were significantly associated with food insecurity.[15] In contrast, previous studies conducted in USA,[16,17] indicated that individuals with low education are more common to present food insecurity compared with individuals without food insecurity. Similarly, other studies also found that the prevalence of food insecurity increase with low education, living with children under 18, low income, pension, female sex, larger family size, being widowed.[18,32] Therefore, it seems that for other countries characteristics other than income are associated with food insecurity.

Social, cultural and economic differences across countries may explain the above findings. The FAO worldwide study of 147 countries[34] aimed to compare food insecurity in different subpopulations across countries and to assess which factors affect food insecurity and concluded that different socio-demographic may affect food insecurity in each country. For example, in developed countries, key determinant of food insecurity is having a lower level of education which is often related to having no decent job. However, among less developed countries, sex appears to have a significant impact on food insecurity and more particular, women are in a much higher risk of developing food insecurity compared to men[35] which comes in line with other studies.[18,32]

Food insecurity has been known to have characteristics of complexity and dynamic capacity. No income due to unemployment and reduce income because of sadden dismissal is increasing the risk for food insecurity.[35] Based on previous studies, food insecure people, due to low income, consume high-fat, high-calorie food products which costs less than healthy foods.[35] The increase energy and fat consumption may have a negative impact on an individual's body weight leading to obesity[36] and increasing the risk of non-communicable diseases such as cardiovascular diseases[37] and type 2 diabetes.[38] Moreover, food insecure people face an additional barrier in managing their chronic disease progression because of their inability to purchase foods indicated for their illness. For example, food insecure people with diabetes, reported poor glycemic control, as indicated by hemoglobin a(1c) ≥8.5%, due to the increase consumption of fat-rich foods and low intake of fruits and vegetables.[38] In addition, low-income people with coeliac disease reported reduce adherence to gluten free diet due to its high cost and their low affordability in purchasing such a diet.[24] As a result of food insecurity, multimorbidity may increase expenses for medications, transportation to doctors, and physiotherapy sessions.[39] Based on the above, it seems that low income people with multimorbidity are in a high risk of being food insecure. However, the relationship between multimorbidity and food insecurity is vice versa, and more studies are needed for the identification of the real association and the effect of other confounding factors such as the socio-demographic characteristics.

Our results should be interpreted in the context of several limitations. First, the cross-sectional design allows us to report associations, which, however, have not casual nature. Furthermore, data collection through self-reporting increases the probability of recall bias. Moreover, the number of people that denied participation in the study was not considered. Also, using other sampling methods such as PPS (Probability-Proportional-to-Size sampling) we may have concluded to more accurate results. Lastly, food insecurity seasonal variations were not considered in the methodology. At the same time, the study has several strengths as it is a large population-based study using a representative sample of Cypriot population.

In conclusion, the prevalence of food insecurity in Cyprus is similar to that of other European countries. Moreover, our findings suggest that income is the strongest socio-demographic factor associated with food insecurity. Food insecurity is a global problem, affecting both developed and non-developed countries. The association between food insecurity and socio-demographic characteristics needs further examination in order for each country to develop specific public health policies targeted in specific population (e.g. financial support to low income people) so as to decrease food insecurity and improve people's overall health and quality of life.

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Competing interests

None.

Authorship

Maria Kantilafti design the study, performed the data collection, performed the statistical analysis, and wrote the original manuscript. Mary Yannakoulia contributed to the interpretation of results. Konstantinos Giannakou designed the study and contributed to the methodology used for the data analysis, Maria Kyprianidou contributed to the methodology used for the data analysis, and Stavri Chrysostomou supervised the study design and data collection, design the study, and performed the original manuscript editing.

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JNS
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REVIEW

Impact of gluten-free diet (GFD) on some of cardiovascular risk factors: a systematic review and meta-analysis

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Abstract

A gluten-free diet (GFD) may have a stronger potential impact on reducing cardiovascular (CV) risk factors, according to research evidence. We investigated the impact of GFD on CV risk variables by doing a systematic review and meta-analysis for this reason. We conducted a thorough database search starting on January 1, 2000, and ending on July 12, 2022. We used random-effects models to pool the data. Totally 19 articles met the eligible criteria and were included. Pooled findings indicated that intervention with GFD has a significantly beneficial effect on high-density lipoprotein (HDL) (WMD: 4.80 mg/dl, 95% CI: 2.09, 7.51, $P = 0.001$), systolic blood pressure (SBP) (WMD: –2.96 mmHg; 95% CI: –4.11, –1.81, $P < 0.001$), and C-reactive protein (CRP) (WMD: –0.40, mg/l, 95% CI: –0.67, –0.14, $P = 0.002$) levels. In celiac patients as well as with an intervention duration of more than 48 weeks, GFD increased TC and HDL compared to non-celiac patients and with an intervention duration lower than 48 weeks, respectively. The results of the present study showed that GFD can have a significant and beneficial effect on HDL, SBP, and CRP.

Keyword: **Cardiovascular: Gluten-free diet: Glycaemic: Inflammation: Lipid profile: Meta-analysis**

Introduction

Cardiovascular (CV) diseases, the main type of non-communicable diseases, were responsible for about 17.8 million deaths in 2017, and it is predicted to increase the number of deaths to 23.6 million by 2030.[1,2] Apart from CVD mortality, imposing the heavy costs of the disease on individuals and society as well as reducing the quality of life are two serious challenges not only at the individual levels, but also at health system and macroeconomic levels.[1,3] Therefore, due to the important and widespread role of CVD as a major health problem, it is necessary to identify and follow appropriate guidelines for the prevention and treatment of this disease. Glycaemic, insulin, and lipid disorders as metabolic risk factors, play a vital role in the onset and development of CVD.[4–7] In addition to genetic factors, several environmental factors including, smoking, sedentary lifestyle and imbalanced dietary intake are important factors contributing to increase CVD risk factors.[8,9] Therefore, interventions targeting modification of this risk factors such as

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hyperglycaemia, impaired insulin secretion, hyperlipidaemia and hypertension, are of significant importance in prevention and treatment of CVD.[10]

In this context, modifying the diet and using some special diets or micronutrients has attracted special attention.[11] In fact, studies show that special diets, in addition to having fewer side effects than chemical drugs, are better predictors of various CVD risk factors and all causes related to its death.[12] So that the reports of meta-analysis articles show the beneficial effects of several special diets including DASH, Palaeolithic diet, Mediterranean diet, and healthy Nordic diet on these risk factors.[13–15] Another one of these diets that has recently attracted the attention of researchers and people is the gluten-free diet (GFD). According to the evidence, the number of people using GFD is increasing every day, and it seems that the value of the global industry of this diet has reached more than 6 billion dollars.[16] Wheat, barley, and oats are rich sources of the complex protein known as gluten, which is made up of glutenin and prolamin.[16] Recent research demonstrates the potential positive effects of GFD in a number of illnesses, including type I diabetes, obesity, and insulin resistance. Although GFD is acknowledged as the primary and prospective therapy for celiac disease (CD).[17–19] Through the decrease of peripheral adipose tissue and fat cell size, GFD appears to have the potential to have positive impacts on CVD risk factors such insulin resistance, lipid profile, inflammatory variables, and blood pressure.[17] Although, the role of GFD on blood glucose and lipid level have been investigated in several human studies, the finding are equivocal. In the several studies, people without CD had a significant reduction in insulin resistance factors and higher levels of HDL-C after receiving a GFD compared to people who consumed a normal diet.[20–22] However, another study was observed no significant effects on CVD risk factors.[20,23] Therefore, given these contradictory results, this study was conducted to estimate a more precise effect of GFD on CVD risk factors.

Methods

Search strategy

The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) criteria were followed for conducting this study.[24] The study protocol has been previously registered with the PROSPERO database (registration number CRD42022365144). Without regard to language or time restrictions, a thorough search was carried out in the PubMed/MEDLINE, Web of Science, SCOPUS, and Embase databases from the beginning until July 12, 2022. The following medical subject were chosen to search the online databases: ("diet, gluten free " OR "Gluten-Free Diet") AND ("Glycated Hemoglobin A" OR HbA1c OR "Insulin Resistance" OR Insulin OR Glucose OR "Glucose Intolerance" OR Triglycerides OR Cholesterol OR "Cholesterol, HDL" OR "Cholesterol, LDL" OR "High-density lipoprotein" OR "Low-density lipoprotein" OR "Blood Pressure" OR "Arterial Pressure" OR "Hypertension" OR SBP OR DBP OR "C-reactive protein" OR CRP). To locate potentially overlooked qualifying trials, the reference lists of the papers that were collected and associated review articles were also manually searched.

Eligibility criteria

Using titles, abstracts, or the complete texts of the studies, the authors separately removed duplicate articles, found and reviewed related articles. Following criteria were used to extract the articles, which was the final step: (1) articles with follow-up studies of one week or more, including prospective or retrospective single arm, (2) performed on paediatric and adult participants who underwent GFD (Individuals with and without CD), (3) reported the primary and secondary outcomes (HbA1c, fasting glucose sugar (FBS), insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), total cholesterol (TC), triglyceride (TG), High-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), systolic blood pressure (SBP), diastolic blood pressure (DBP), and C-reactive protein (CRP)) at the baseline and after GFD. When a research reported the amount of a factor over several follow-up times, the longest or most recent follow-up period was taken into account. Exclusion criteria for our investigation included papers with duplicate or unclear data, systematic publications, other observational studies, studies with less than one week of follow-up, and studies with no response after contacting relevant authors.

Data extraction and quality assessment

Information required for the article included mean and standard deviation (SD) HbA1c, FBS, insulin, HOMA-IR, TC, TG, HDL-C, LDL-C, SBP, DBP, and CRP at the baseline and after GFD, the name of the authors, year of publication, country, number of participants, percentage of male participants, type of population, mean age (year), studies design, and follow-up duration of the intervention. Two independent researchers reviewed relevant articles before extracting this data. Additionally, using the Newcastle-Ottawa Quality Assessment Scale, two writers independently evaluated the included publications' quality.[25]

Data synthesis and statistical analysis

The statistical analysis was done using RevMan 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark) software and STATA version 12.0 (Stata Corp, College Station, TX, USA). The mean and SD of variables at the baseline and after GFD were taken from each research to determine the weight mean differences (WMD), and the WMD was then calculated for each article using a random-effects models. Standard calculations were carried out to determine the mean and SDs when data were provided in a different manner.[26,27] Furthermore, for studies that only reported standard error of the mean (SEM), SDs were obtained using the following formula: $SD = SEM \times \sqrt{n}$, where 'n' is the number of subjects. Q Statistics and I-squared (I^2) were used to evaluate the heterogeneity status between studies. Insignificant, low, moderate, and high heterogeneity were identified with an I^2 values of 0–25%, 26–50%, 5–75%, and

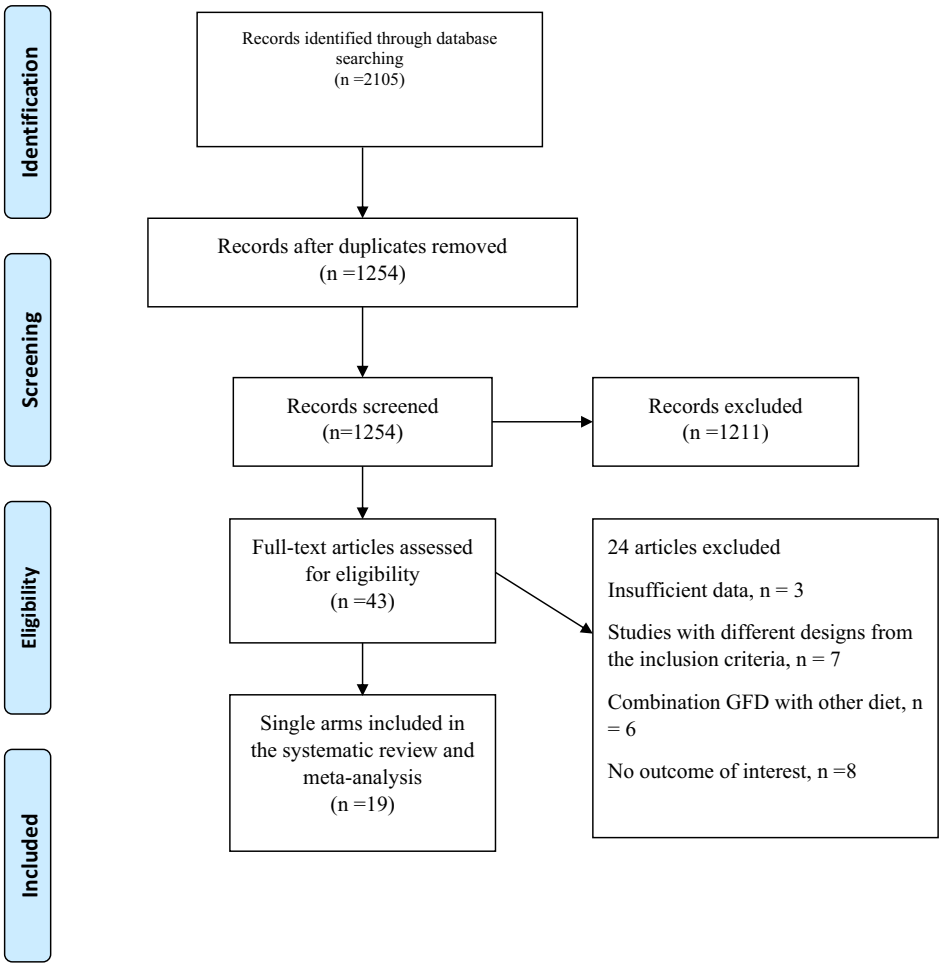

Fig. 1. Flow chart of the included studies, including identification, screening, eligibility and the final sample included.

76–100%, respectively.[28] Pre-defined subgroup analysis based on participant type (celiac/non-celiac) and duration study follow-up was carried out to find possible causes of heterogeneity. To determine the contribution of each research to the total mean difference, a sensitivity analysis was used. Additionally, we used the Begg tests to assess the publication bias. To determine if publication bias influenced the stability of the total estimate when there was severe publication bias, we employed the trim and fill approach.[29]

Results

Process of selection is shown in Fig. 1. After searching the systematic databases, 2105 results were selected (one additional articles identified through other sources), with 1254 articles remaining after the elimination of duplicate studies. Then, after reviewing the abstract or title, 1211 studies did not meet the inclusion criteria. After retrieving the full text of the remaining 24 articles, nine articles were deleted due to insufficient data (absence of investigated variables or non-reporting of mean or standard deviation data and other transformable data) ($n = 3$), study design differing from the inclusion criteria ($n = 7$), combination GFD with other diet ($n = 6$), and absence of outcome of interest ($n = 8$). Finally, 19[21,22,30–45] studies with 21 treatment arms met the eligibility and were included in our analysis.

Study characteristics

Table 1 reveals the characteristics of the pooled articles. According to our studies, two studies have been conducted in the Americas, two studies in Asia and the rest in Europe. All studies were published between the years 1996–2021. In addition, according to our information, all studies were performed on both sexes and the percentage of males in the studies varied from 0% to 56%. The follow-up intervention of the studies was between 4 and 114 weeks. The mean age at the baseline varied between 7.6 and 58.5 years. In addition, according to the findings, 8 studies have been conducted as a prospective cohort, and the rest as a retrospective cohort. On the other hand, 4 studies on patients with type 1 diabetes mellitus (T1DM) and CD at the same time, 10 studies on patients with CD alone, 2 articles on T1DM, and finally 3 studies on people with rheumatoid arthritis, metabolic syndrome, and healthy individual have been done. Five studies for HbA1c and insulin, 7 articles for glucose, 3 studies for HOMA-IR, as well as, 11 articles for TC, TG and HDL, and 9 study with 11 arms for LDL provided data on the comparison of mean changes. Four studies reported the effect of GFD on SBP, DBP, and CRP level.

Table 1. Characteristics of eligible studies

Number	Author (y)	Years	Country	Study design	Population	Mean age year	Male (%)	Sample size	Duration of study (wk)	Outcomes
1	Kaur *et al.*	2020	India	PC	T1DM and CD	25.7	56.0	15	48	HbA1c.
2	Bakker *et al.*	2012	Netherlands	RC	T1DM and CD	47	42.1	31	N/R	HbA1c.
3	Kaukinen *et al.*	1999	Finland	RC	T1DM and CD	N/R	N/R	22	48	HbA1c.
4	Neuman *et al.*	2020	Prague	PC	T1DM	10.2	50	20	48	HbA1c.
5	Marchi *et al.*	2013	Italy	RC	CD	N/R	45	20	28	TC, LDL, HDL, TG, SBP, DBP, CRP.
6	Zanini *et al.*	2013	Italy	RC	CD	35	29/5	547	144	FBS, Insulin, HOMA-IR, TC, LDL, HDL, TG.
7	Riezzo *et al.*	2014	Italy	PC	CD	34	25	20	48	TC, LDL, HDL, TG, SBP, DBP, CRP.
8	Salardi *et al.*	2017	Italy	RC	T1DM and CD	7/6	N/R	129	48	HbA1c, TC, LDL, HDL, TG.
9	Remes-Troche *et al.*	2019	Mexico	PC	CD/NCD	30	10	22	24	FBS, TC, LDL, HDL, TG.
10	Ehteshami *et al.*	2018	Iran	RC	Metabolic syndrome	58/55	26	23	8	FBS, Insulin, HOMA-IR, TC, LDL, HDL, TG, SBP,DBP.
11	Brar *et al.*	2006	USA	RC	CD	44/4	34	132	82	TC, LDL, HDL, TG.
12	Ciacci *et al.*	2002	Italy	RC	CD	27/9	23/3	390	7	TC.
13	Elkan *et al.*	2008	Sweden	RC	Rheumatoid arthritis	50	10/3	22	48	TC, LDL, HDL, TG, CRP.
14	Lewis *et al.*	2009	UK	PC	CD	51	N/R	100	48	FBS, TC, HDL, CRP.
15	Rea *et al.*	1996	Italy	PC	CD	N/R	N/R	23	48	TC, TG.
16	Pastore *et al.*	2003	Italy	RC	T1DM	16	N/R	14	24	FBS, Insulin, HOMA-IR.
17	Goddard *et al.*	2021	UK	PC	Health	29	9	11	4	FBS, Insulin, SBP, DBP.
18	Tortora *et al.*	2014	Italy	PC	CD	N/R	N/R	254	48	FBS, HDL.
19	Capristo *et al.*	2005	Italy	RC	CD	31.4	0	18	104	Insulin.

N/R, not reported; PC, prospective cohort; RC, retrospective cohort; T1DM, Type 1 diabetes mellitus; CD, celiac diseases; NCD, none celiac diseases; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; FBS, fasting blood sugar; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein.

In addition, the quality results of the articles are shown in Table 2.

Meta-analysis

The effect of GFD on HbA1c, fasting glucose, insulin, and HOMA-IR. Pooled results from the random-effects model indicated that the GFD has no significant effect on any of the factors of glucose metabolism, including fasting glucose (WMD: –0.50 mg/dl, 95% CI: –1.22, 0.21, $P = 0.343$), insulin (WMD: –0.04 μU/ml, 95% CI: –0.68, 0.61, $P = 0.906$), HbA1c (WMD: 1.45, 95% CI: –1.55, 4.46, $P = 0.169$), and HOMA-IR (WMD: 0.26, 95% CI: –0.40, 0.92, P= 0.437). Furthermore, significant heterogeneity was noted for HbA1c (Cochran Q test, $P < 0.001$, $I^2 = 94.7\%$), HOMA-IR (Cochran Q test, $P < 0.001$, $I^2 = 97.1\%$), and fasting glucose (Cochran Q test, $P < 0.001$, $I^2 = 88.9\%$) among the studies. However, there was no evidence of significant between-study heterogeneity for insulin (Cochran Q test, $P = 0.337$, $I^2 = 12.2\%$) (Fig. 2). Also, subgroup analyses for these outcomes did not show significant results (Supplementary Fig. 1–3).

The effect of GFD on lipid profile. Pooled data indicated a significant efficacy in increasing serum HDL (WMD: 4.80 mg/dl, 95% CI: 2.09, 7.51, $P = 0.001$) following adherence to a GFD. However, no significant beneficial effects on TC (WMD: 6.22 mg/dl, 95% CI: –4.02, 16.47, $P = 0.232$), LDL-C (WMD: –2.68 mg/dl, 95% CI: –11.95, 6.59, $P = 0.571$), and TG levels (WMD: –4.05 mg/dl, 95% CI: –8.64, 0.54, $P = 0.084$) concentration was reported after consumption of GFD. Significant heterogeneity was observed between these articles for TC (Cochran Q test, $P < 0.001$, $I^2 = 96.8\%$), TG (Cochran Q test, $P < 0.001$, $I^2 = 77.6\%$), LDL-C (Cochran Q test, $P < 0.001$, $I^2 = 93.5\%$), and HDL levels (Cochran Q test, $P < 0.001$, $I^2 = 88.9\%$) (Fig. 3).

The subgroup results reported that following a GFD increased TC and HDL in celiac patients compared to non-celiac patients and with an intervention duration of more than

Table 2. Risk of bias assessment according to the Newcastle-Ottawa Quality Assessment Scale tool

Study	Selection	Comparability	Outcome/exposure
Kaur *et al.*	***	*	***
Bakker*et al.*	****	**	**
Kaukinen *et al.*	**	**	**
Neuman *et al.*	***	–	***
Marchi *et al.*	**	**	***
Zanini *et al.*	****	*	**
Riezzo *et al.*	**	**	***
Salardi *et al.*	**	**	***
Remes-Troche *et al.*	****	–	**
Ehteshami *et al.*	***	*	***
Brar *et al.*	**	***	***
Ciacci *et al.*			
Elkan *et al.*	**	***	***
Lewis *et al.*	**	***	***
Francesco *et al.*	**	**	***
Pastore *et al.*	**	**	***
Goddard *et al.*	****	–	**
Tortora *et al.*	***	*	***
Capristo *et al.*	**	***	***

Quality assessment of articles were classified into low (< 5 stars), moderate (5–7 stars) and high quality (> 7 stars).

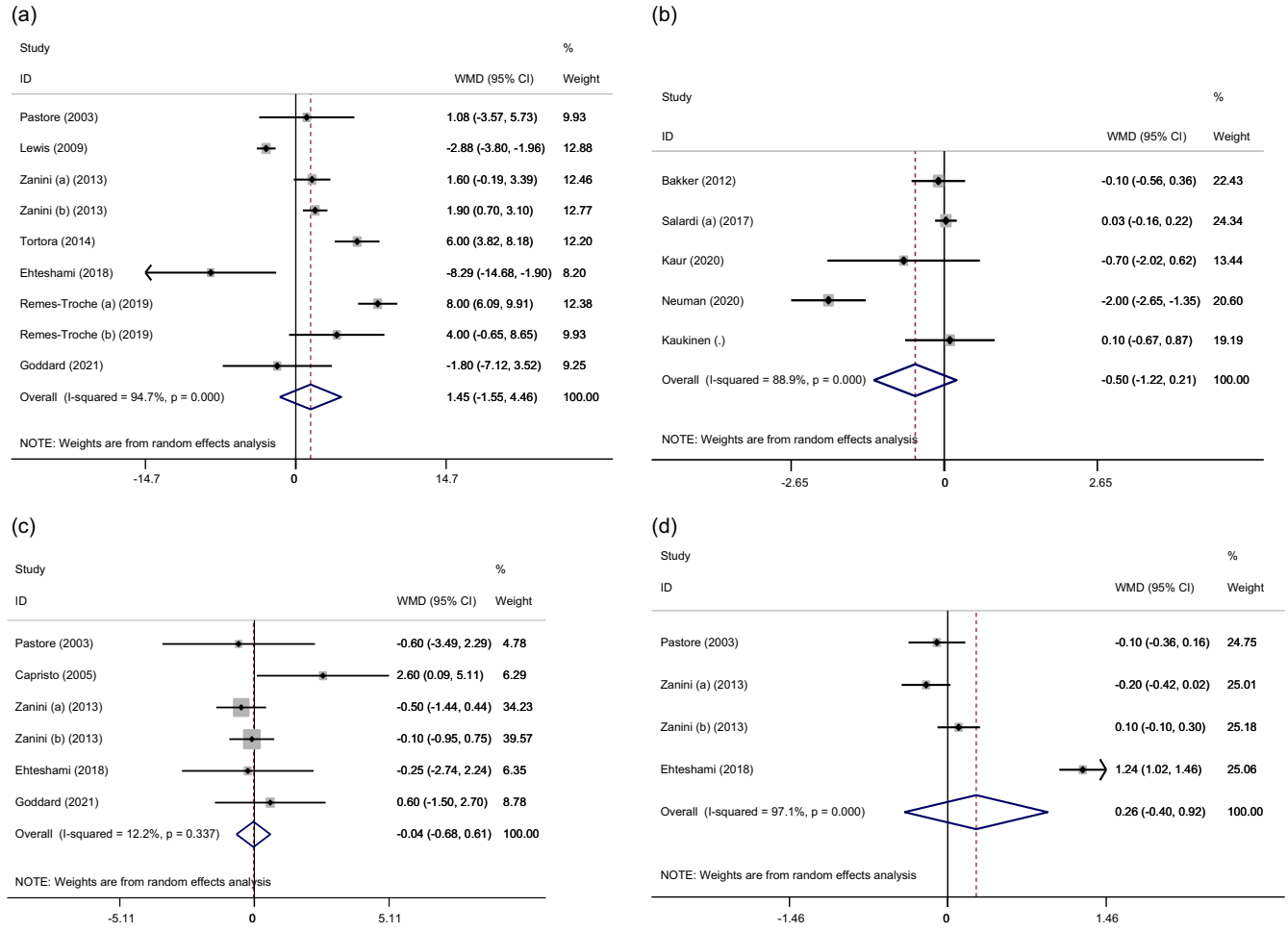

Fig. 2. Forest plots from the meta-analysis of investigating the effects of gluten-free diet on (a) HbA1c, (b) glucose, (c) insulin, and (d) HOMA-IR. WMD: weighted mean.

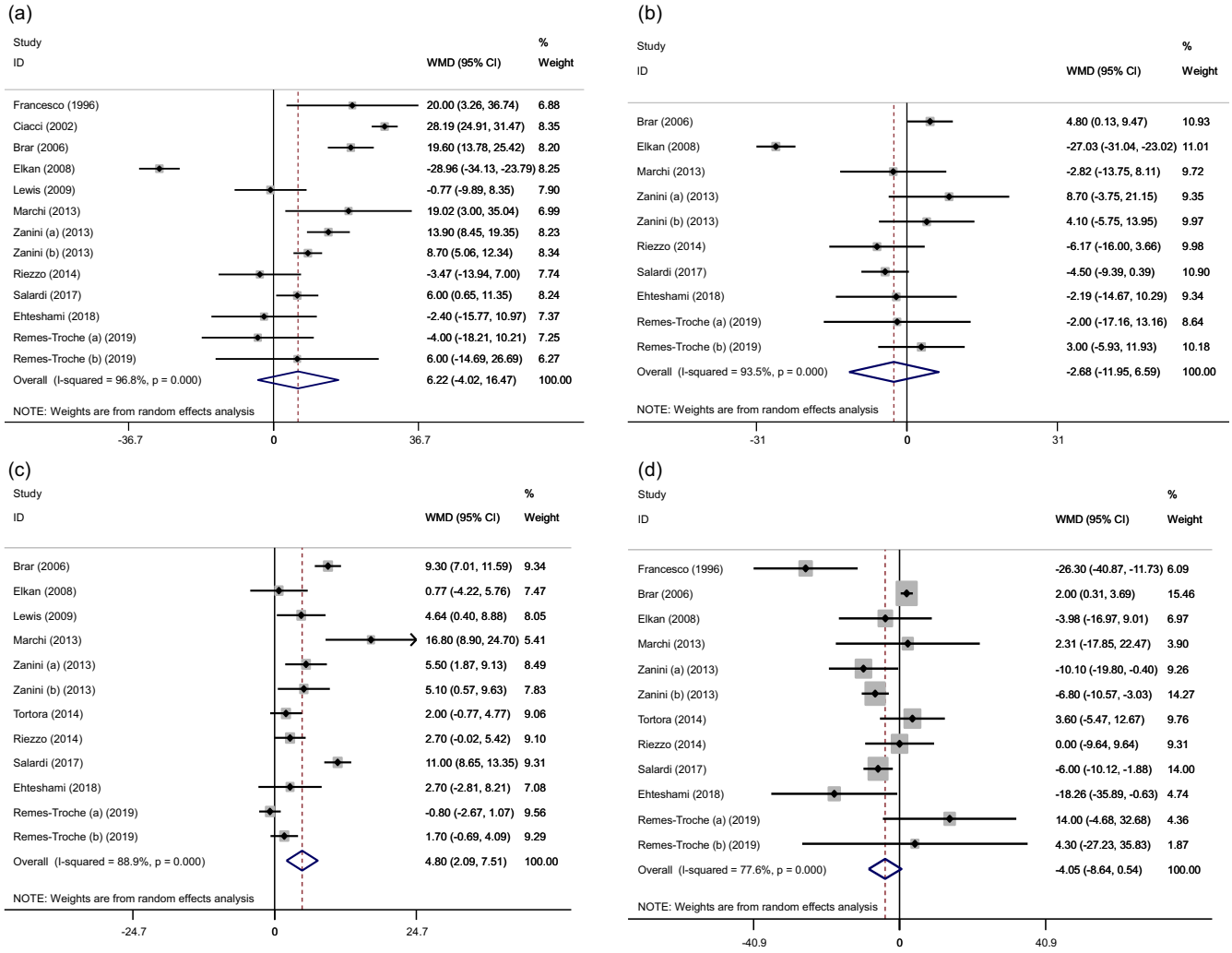

Fig. 3. Forest plots from the meta-analysis of investigating the effects of gluten-free diet on (a) cholesterol, (b) LDL, (c) HDL and (d) TG. WMD: weighted mean.

48 weeks. Also, the effect of this diet on the increase of LDL was greater during the intervention of more than 48 weeks (Supplementary Figs. 4–7).

The effect of GFD on SBP and DBP. The pooled effect sizes from three studies indicated that GFD had a significant effect on SBP (WMD: –2.96 mmHg; 95% CI: –4.11, –1.81, $P < 0.001$). But no significant effect was observed on DBP (WMD: 1.17 mmHg; 95% CI: –0.87, 3.20, $P = 0.262$). However, for SBP and DBP, the detected heterogeneity was not significantly high (Cochran Q test, $P = 0.331$, $I^2 = 12.4\%$ for SBP and Cochran Q test, $P = 0.111$, $I^2 = 50.1\%$ for DBP), respectively (Fig. 4).

Effect of GFD on CRP. Three studies reported data for serum as outcome measures. The results from our meta-analysis indicated a significant reduction of CRP (WMD: –0.40, mg/l, 95% CI: –0.67, –0.14, $P = 0.002$) levels following GFD consumption. In addition, significant heterogeneity was noted among the analysed studies for CRP (Cochran Q test, $P < 0.001$, $I^2 = 84.3\%$) (Fig. 4).

Sensitivity analysis

In order to discover the effect of each article on the pooled effect size for TC, LDL-C and HDL-C, TG, HbA1c, glucose, insulin, CRP, SBP, and DBP levels, we step by step discarded each trial from the analysis. The leave-one-out sensitivity analysis indicated the robustness of the results (Supplementary Figs. 8–10).

Publication bias

Evaluation of publication bias by visual inspection of funnel plot and Egger's test demonstrated no evidence for publication bias in the meta-analysis of GFD on CRP ($P = 0.497$), SBP ($P = 0.174$), DBP ($P = 1.00$), TC ($P = 0.903$), LDL-C ($P = 0.655$) and HDL-C ($P = 0.493$), TG ($P = 0.681$), glucose ($P = 0.297$), insulin ($P = 0.851$), HOMA-IR ($P = 0.497$), and HbA1C ($P = 0.327$) levels (Supplementary Figs. 11–13).

Discussion

The findings of this meta-analysis indicated that GFD has no significant effect on HbA1c, fasting glucose, insulin, and

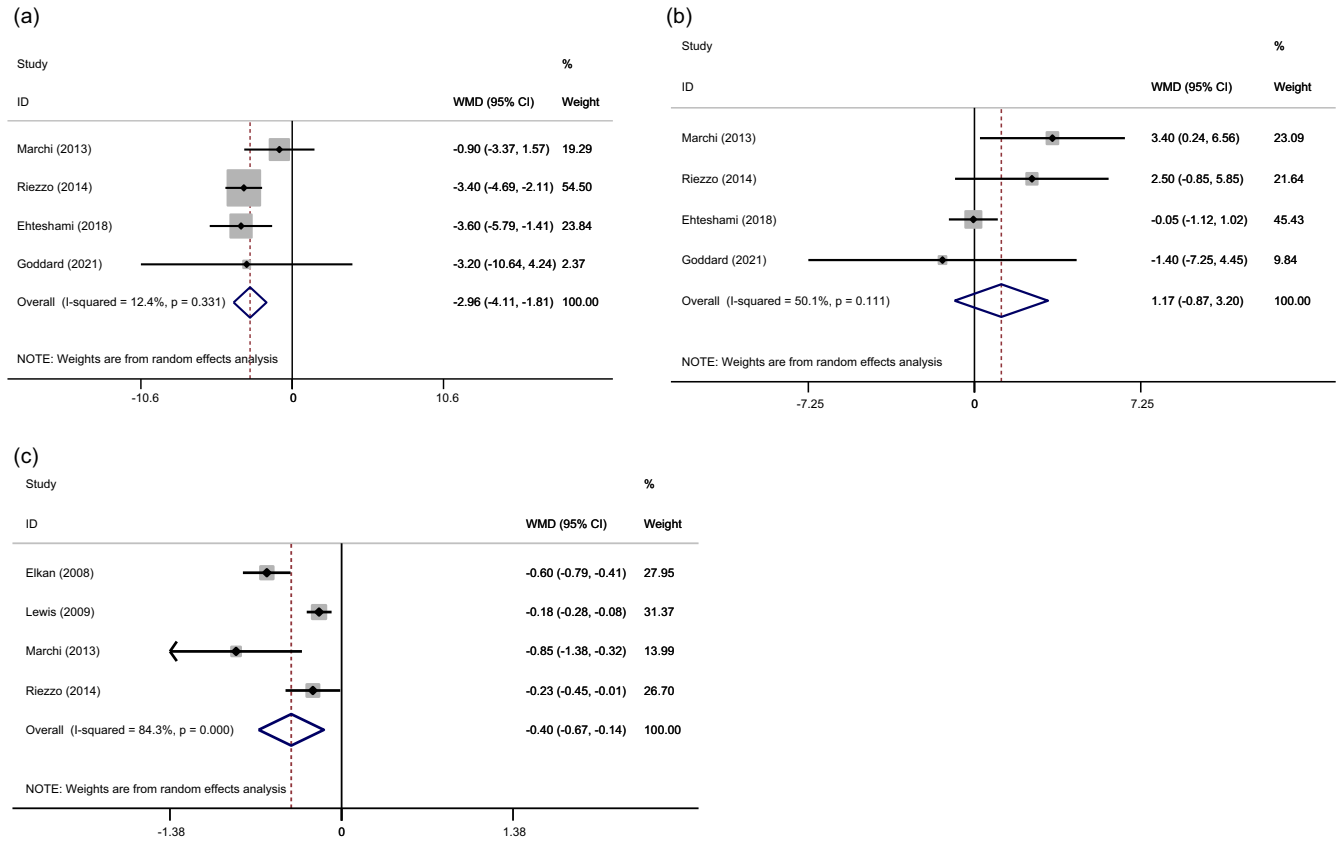

Fig. 4. Forest plots from the meta-analysis of investigating the effects of gluten-free diet on (a) SBP, (b) DBP, (c) CRP.WMD: weighted mean.

HOMA-IR. Thus, these findings do not support the recommendation to exclusion of the gluten of the feeding in clinical practice to the improvement of parameters of glucose metabolism.

On the other hand, regarding lipid profile, results of the meta-analysis showed significant effect of the GFD in increasing serum HDL. In celiac patients as well as with an intervention duration of more than 48 weeks, GFD increased TC and HDL compared to non-celiac patients and with an intervention duration lower than 48 weeks, respectively. The effect of this diet on the increase of LDL also was greater with a higher duration of the intervention.

Regarding HDL cholesterol, it is proposed that the elevated blood HDL levels following GFD are reasonably attributed to mucosal healing and, as a result, to an improvement in intestinal absorption of HDL and apo-A1), the primary apo-protein of circulating HDL cholesterol.[23,46] Additionally, given that Apo-A1 secretion and HDL cholesterol synthesis are decreased in the small intestinal mucosa of CD patients who are not receiving treatment, the significant increase in serum HDL levels seen in celiac patients with the longer intervention duration compared to non-celiac patients with the shorter intervention duration is also plausible.[46]

As for the increase in serum levels of total and LDL cholesterol after intervention with a GFD, this may be a result of the concomitant increase in serum HDL levels as an effect of the GFD.[22] It is noted that the serum HDL level has greater relevance as a cardiovascular risk factor than total cholesterol and is independent of LDL levels, despite evidence that a GFD also increases serum levels of total and LDL cholesterol, which may increase the risk of cardiovascular disease.[47,48] This might not be true necessary given that genetic studies and clinical trials generally show that the relationship between HDL-C and CVD is not causal.[49]

Concerning blood pressure, results of the meta-analysis showed that GFD had a significant effect only on SBP. The possible mechanisms to explain the reduction in blood pressure following GFD are still unknown. The possible mechanisms to explain the reduction in blood pressure after the GFD are still unknown. Contrarily, it has been shown that the angiotensin I-converting enzyme (ACE) is inhibited by gliadin (a protein found in gluten). Because ACE destroys bradykinin, a vasodilator, and transforms angiotensin I into angiotensin II, a vasoconstrictor, its suppression may help lower blood pressure.[50] Therefore, additional research is required to learn more about the mechanisms that may underlie the benefits of a GFD on blood pressure.

Regarding CRP, results of the meta-analysis showed a significant reduction of CRP levels following GFD. This finding suggests possible decreased inflammatory response secondary to gluten abstinence indicated by a significant decrease in inflammatory markers such CRP levels serum.[51]

In a systematic study in 2018 by Potter et al to investigate the effect of this diet on cardiovascular risk factors, the results showed that GFD increases TC, HDL-C, FBS, and BMI. However, no significant effect was reported on LDL-C, TG, and BP. In general, most of its findings were different from our

findings. However, this study differed in design from ours in several ways. In Potter's study, unlike our study, the study of the effect of this diet was only limited to patients with CD and only systematically reviewed the results without conducting a meta-analysis, and also the overall quality of the study was reported to be low, which ultimately all these factors can affect the final results and cause contradictory results between two studies.

Our study has a number of advantages. The current study is the first meta-analysis to look into how GFD might affect patients with and without CD in terms of their cardiometabolic risk factors. We made an effort to incorporate every study that would have met the inclusion criteria in our meta-analysis so that the large number of studies would boost the reliability of the findings. In addition, we conducted a subgroup analysis to identify the causes of high heterogeneity in study results. The subgroups were divided according to the population type and the length of follow-up.

The inclusion of studies without an appropriate control group, different inclusion criteria for study participants, the absence of other common treatments, prior medical history, various and variable levels of disease activity, and ultimately different essential characteristics, such as age, sex, BMI, duration of CD and other diseases that these may contribute to population heterogeneity and eventually have an impact on the outcomes, are all limitations of the current study.

In summary, the results of this meta-analysis showed that the GFD showed a beneficial impact on some cardiometabolic risk factors such as an increase in serum HDL cholesterol levels, a reduction in SBP and serum CRP levels. However, no significant effect was observed on parameters of glucose metabolism. It is important to take into account also the differences in the magnitudes of the effects of the GFD when comparing their effect in celiac and non-celiac patients, as well as the duration of the intervention period.

A GFD does not automatically mean a better diet. If care is made to choose whole-grain goods, include more vegetables, and choose items with reduced energy density, a GFD may be a well-balanced diet.[52] Therefore, other dietary strategies to reduce cardiometabolic risk factors may be taken into consideration in the absence of gluten-related illnesses.

Abbreviations

GFD: Gluten-free diet; **CV:** Cardiovascular; **HDL:** High-density lipoprotein; **SBP:** Systolic blood pressure; **CRP:** C-reactive protein; **CD:** Celiac disease; **FBS:** Fasting glucose sugar; **HOMA-IR:** Homeostatic model assessment for insulin resistance (HOMA-IR); **TC:** Total cholesterol; **TG:** Triglyceride; **LDL:** Low-density lipoprotein; **DBP:** Diastolic blood pressure

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.39

Data Availability

Data available on request due to privacy/ethical restrictions.

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Authors' contributions

RP, SMH: conception, design, statistical analysis, data collection, writing-original draft, supervision. FS: data collection and writing-original draft. All authors approved the final version of the manuscript.

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Conflict of interest

No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Ethics approval

This study was approved by the research council and Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

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RESEARCH ARTICLE

Perceived benefits and challenges of school feeding program in Addis Ababa, Ethiopia: a qualitative study

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Abstract

Addis Ababa initiated a universal Home-Grown School Feeding Program (HGSFP) in February 2019 to address hunger and improve the educational outcomes of schoolchildren. This study aimed to document the perceived benefits and challenges of the HGSFP in Addis Ababa, where such information was lacking. In May 2023, a qualitative phenomenological study was conducted to collect data from 20 schools participating in the HGSFP. Data were collected through key informant interviews and focus group discussions (FGDs) involving 98 purposively selected participants. The study encompassed 48 student mothers in 5 FGDs, 20 student interviews, 20 school principals, and 10 experts from the Ministry of Education, Sub-cities, and the School Feeding Agency for in-depth interviews. Data collected in the local language were transcribed, translated into English, and thematically analysed using ATLAS-TI software. The study's findings unveiled the transformative impact of the HGSFP in Addis Ababa, Ethiopia. It demonstrated remarkable improvements in attendance, concentration, academic performance, reduced dropout rates, financial relief, enhanced behaviour, and a safer learning environment. However, urgent measures are imperative to tackle pressing challenges such as underpaid kitchen workers, operational issues, reduced reading time, rising food costs, limited market access, inadequate infrastructure, and growing dependency. To ensure the enduring sustainability of HGSFP, addressing challenges like workload reduction, kitchen infrastructure enhancement, government guideline implementation, promoting self-reliance, overcoming budget limitations, and addressing school gardening obstacles is vital.

Key words: **Addis Ababa: Benefits: Challenges: Ethiopia: Home-Grown School Feeding Program**

Introduction

The school feeding program is one of the world's largest and most widespread social safety net programs, benefiting nearly half a billion school children worldwide.[1] An estimated 66 million primary school children, of which 23 million are in Africa, attend school hungry, struggle to learn, have poor concentration, and have little interest in learning.[2–6] Furthermore, approximately 67 million children do not attend school at all.[7] Attending classes while hungry hurts children's and adolescents' ability to learn, thrive, and reach their full potential.[6,8]

School feeding programs (SFPs) are widely regarded as a game-changing option for improving food availability and education, as well as a prominent and innovative vehicle for addressing multiple Sustainable Development Goals (SDGs) outcomes.[1,9] SFPs help school-age children and adolescents develop physically, mentally, and emotionally, especially in low- and middle-income nations.[6] The World Bank and the World Food Program (WFP) published a joint review of SFPs in 2009[10] reinforcing the rationale and objectives of SFPs. The three main goals identified were to provide safety nets for

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families to absorb social and economic shocks, to improve school-aged children's education and scholastic performance, and to improve children's nutrition and health status.[10]

Malnutrition impairs the academic performance of schoolchildren from low-income families.[11] A 2015 study in Ethiopia found that malnutrition affected 31% of schoolchildren (19.6% stunted, 15.9% underweight, and 14.0% wasted).[12] Furthermore, studies have shown that adolescent girls and boys aged 15–19 years are prone to chronic energy deficiency.[11,13]

School feeding in Ethiopia began in 1994 as a pilot project by the WFP in war-affected zones in Tigray Region.[14–16] Later, the aid was expanded to the remaining five food-insecure regions in the Somali Regional States: Afar, Amhara, Oromia, and SNNPR.[16] Currently, in Ethiopia, homegrown SFPs are run by locally produced food products.[17] From the perspective of the WFP, HGSFPs aim to both increase children's well-being and promote local agricultural production and development by providing an ongoing market for small landholders.[18]

The program is implemented through three approaches: government-owned and financed homegrown school feeding programs (HGSFPs), NGO-run SFPs like the WFP, and community-initiated and owned SFPs. It is supported by national school feeding policy, food and nutrition policy, and strategy.[19,20] The program also receives political attention and support from various stakeholders.

In Addis Ababa, the HGSFP was initiated on a small scale by charitable societies, NGOs, and other stakeholders,[11,21] and now the city administration has expanded the program and assumed full ownership. Earlier, economically disadvantaged students used to be targeted by the program; however, since February 2019, the program has covered all public pre-primary and primary schools (grades 1–8) in the city.[11,21] Ethiopia's government has made every effort to ensure that all of the country's children have access to education. In order to increase learning achievement, reduce temporary hunger, and enhance the health and nutritional status of schoolchildren, the Addis Ababa City Administration School Feeding Agency is currently implementing HGSFPs.[11,21]

Furthermore, little is understood about the benefits and challenges of HGSFPs in Ethiopia. Research conducted in the Sidama Region, Southern Ethiopia, and the Somali Regional State revealed several challenges.[14,22,23] These include the absence of a consistent supply of clean water, the delay in the delivery of rations, poor quality food provision, the insufficient amount of food allotted for the academic year, the lack of program infrastructure, the lack of sanitation and hygiene training for cooks, inadequate funding for schools and independent structures, inconsistent resource mobilisation, ineffective monitoring and evaluation, and inappropriate use of the allotted food.[14,22,23] Another study carried out in the Jigjiga Zone, Somali regional area, Ethiopia, revealed that the main program challenges were water supply, storage facility shortages, kitchen utensil issues, and delays in ration delivery.[23] The benefits include raising students' academic performance and attendance in class. Additionally, the program helped the parents save money and time.[23]

Several donors and national governments from both developed and developing countries have also invested millions of dollars in SFPs.[24,25] Despite the attention and resources devoted to SFPs, little rigorous evidence exists to support these investments,[25] and no adequate research on HGSFPs has been conducted in our country to date,[24,26] and there is a lack of studies that directly address the unknown perceptions of parents, school principals, students, and other stakeholders towards HGSFPs.[27]

Our study focuses on the HGSFPs in Addis Ababa, the capital city of Ethiopia. This urban setting presents unique characteristics that contribute to our understanding of HGSFP. By examining the perceived benefits and challenges of urban HGSFP, our research informs policy frameworks and implementation strategies not only in Addis Ababa but also in other urban areas. Through qualitative methods like interviews and focus groups, we gather diverse perspectives on the perceived benefits and challenges of HGSFP in public primary schools. Our findings have broad applicability, filling a crucial literature gap and contributing to the fields of nutrition and education.

Materials and method

Study setting and period

The study was conducted in twenty primary schools in five selected sub-cities in Addis Ababa, the capital city of Ethiopia,[28] from 10 April 10 to 26 May 2023. Addis Ababa is the country's largest city and plays an important political, economic, and symbolic role in the country.[28] By 2036, the population is expected to exceed 5 million, as reported by the Central Statistical Agency.[28] In 2019, the City Administration School Feeding Agency employed 10,000 mothers from various unions to participate in the school feeding program. Each mother was responsible for preparing meals for a group of 30–50 children.[15] The HGSFP has now been implemented in all 264 public primary schools located in all 11 sub-cities, and the city government provides a school feeding program for about 638,857 students.[29]

Study population

- Mothers of beneficiary students from 20 schools participating in the HGSFP.
- Grades 6 and 7 students enrolled in the HGSFP participating schools.
- Public primary school directors directly involved in implementing, coordinating, or managing the HGSFP in the selected schools.
- Experts from the Ministry of Education, Sub-cities, and the School Feeding Agency were responsible for overseeing, coordinating, planning, executing, supervising, and managing various aspects of the HGSFP.

Inclusion criteria

- Mothers of beneficiary students actively participating in the HGSFP.
- Grades 6 and 7 students in HGSFP-participating schools, who are capable of conducting independent interviews.

- School administrators or directors directly involved in the implementation, coordination, or management of the HGSFP.
- Ministry of Education experts, sub-city experts, and School Feeding Agency experts involved in oversight, coordination, planning, execution, supervision, and operational aspects of the HGSFP.

Exclusion criteria

- Individuals who are not mothers of beneficiary children in the HGSFP.
- Individuals who are not actively engaged in the HGSFP or do not have direct responsibilities related to its implementation.
- Students who have opted out or declined to participate, and those with significant physical or mental health conditions affecting interview accuracy.

Study design

This study employed a quantitative phenomenological study with a case study approach to examine the challenges and perceived benefits of SFPs in the public primary schools of Addis Ababa city. A case study is a qualitative research method that enables an in-depth examination of a phenomenon or a program using a variety of data sources in its natural context.[30,31]

Theme selection

The selection of themes for our study on the HGSFP was a meticulous process guided by multiple criteria. We considered our research objectives, feasibility, relevance, significance, stakeholder input, ethical guidelines, and resource availability. These considerations ensured a comprehensive exploration of the program within the limitations of our study. By addressing these criteria and considerations, we conducted a rigorous analysis and generated meaningful insights into the HGSFP.

Obtaining parental consent

To obtain parental consent, we employed strategies such as parent-teacher meetings, existing platforms, and in-person discussions led by school directors. We explained the study's purpose, procedures, risks, benefits, and confidentiality measures in detail. Consent forms were carefully explained, and parents were encouraged to ask questions and express concerns. Our procedures followed institutional and ethical guidelines, guaranteeing data security, anonymity, and confidentiality. These strategies successfully obtained parental consent while honoring their autonomy.

Sampling procedure

A multi-stage sampling procedure was employed to select the participants for the study. Three rounds of sampling were used to choose the final research subjects. In the first step, five of the eleven sub-cities (or 50% of the sub-cities) were selected using a lottery technique. Arada, Bole, Kirkose, Ledeta, and Yeka Sub-city were the five sub-cities that were selected. In the second phase, after identifying each sub-city's public primary school, four were selected at random from each sub-city. In the study, twenty primary schools with HGSF programs were included.

In consultation with the school principals, all study participants who had major roles in the HG-SFPs (parents, students, SFP coordinators, and school directors) were specifically chosen. Furthermore, the current study purposefully included experts from the sub-cities, the Addis Ababa City Administration School Feeding Agency, and the Ministry of Education.

The study involved five parent focus groups, twenty key informant interviews with SFP coordinators and school directors, and interviews with twenty beneficiary students. Moreover, in-depth interviews were conducted with three experts each from the Ministry of Education and the Ababa City Administration School Feeding Agency, along with four experts from the sub-city.

We decided the number of study participants based on the study's design, the population's diversity, and the depth of the data. Many academics advise using a range of research participant sizes. For example, Lincoln and Guba advise 12–20 participants for studies based on interviews.[32] Considering these factors, the key informants in this study were purposefully selected. There were 48 participants in all for the focus group discussions (FGDs); each FGD had an average of ten participants. According to data saturation principles, which defined the point at which additional interviews produced no new data, the study's final sample size was 98 participants.[33]

Sampling strategy

To comprehensively analyse the HGSFP, we employed a sampling strategy by selecting four schools from each of Addis Ababa's five sub-cities. This approach captures variations in program implementation, contextual factors, and stakeholder perspectives, enhancing the reliability and generalisability of our findings. We considered practical factors like socioeconomic, demographic, and geographic characteristics to understand the factors influencing the HGSFP's success or limitations within each sub-city. Our strategy encompasses diverse school characteristics, stakeholder perspectives, and potential variations in challenges and commitment-related issues across sub-cities. It acknowledges the unique challenges and commitment levels faced by schools in different sub-cities due to socioeconomic factors, population density, and infrastructure. Including multiple schools from each sub-city provides a comprehensive understanding of challenges and commitment-related issues from various stakeholders, including students, parents, school directors, program administrators, and experts. This strategy also enables us to explore intra-sub-city variations resulting from differences in school demographics, resources, and community involvement.

Participant selection

To achieve a comprehensive understanding of our research topic, we selected participants from diverse households,

including individuals with varying family backgrounds, socio-economic statuses, and living conditions. This approach enhances the representativeness of our sample and improves the validity and reliability of our data by incorporating independent perspectives from students and parents. Furthermore, the inclusion of participants from diverse households increases the generalisability of our findings to a broader population. Although establishing a direct link between specific family characteristics and student experiences or outcomes may have limitations, we employ statistical techniques and control for relevant variables to extract valuable insights. This approach aligns with our research objectives, capturing a wide range of perspectives and facilitating a comprehensive understanding of the factors under study.

Data collection tools and procedure

Semi-structured interviews and FGDs guides were developed to assess the challenges and perceived benefits of the homegrown school feeding program in the study area. Before being used for the actual data collection, these guides were developed by reviewing various literature and evaluated by three experts in the field. The guide was prepared in the English language following the review of literature.[31,34–36]

Data for this exploratory qualitative study was collected from twenty primary schools that implemented HGSFPs. The data collection involved key informant interviews, FGDs, beneficiary student interviews, in-depth interviews with school principals, and interviews with experts from the Ministry of Education, Addis Ababa City Administration, School Feeding Agency, and sub-cities. The interviews took place in private settings, and audio recordings were made in Amharic (the national language) with the participants' consent.

Data were audio-taped and transferred to a personal computer to which only investigators had access. Audio-taped files were transcribed verbatim using the local language and translated into English. Each FGD and KII was transcribed before the next data collection, which enabled the capture of emerging insights into the semi-structured guide to enhance the credibility and comprehensiveness of the conversations. The principal investigator conducted a triangulation of the data generated from FGDs and KIIs. The principal investigator has experience gathering qualitative data and has taken advanced qualitative research methodologies.

Data was collected using pre-tested, semi-structured, in-depth interview guides. These guides included predetermined questions and prompts, allowing flexibility in participants' responses. The interviews occurred in relevant locations like schools and government offices, chosen for participant convenience and a comfortable environment.

The data collection team comprised five individuals with a master's degree in public health and nutrition, experienced in qualitative inquiries. After a three-day training session covering study objectives, data collection techniques, and ethical principles, the trained data collectors conducted in-depth interviews. FGDs were moderated by the principal investigator and a note-taker. Interviews with school principals and stakeholders from the Ministry of Education, sub-cities, and the school feeding agency took place in the mornings at their respective offices.

To address the potential influence of work-related exhaustion on data quality, interviews with principals were scheduled in the morning hours, taking into account our natural productivity rhythms. All respondents engaged in face-to-face interviews, while in-depth interviews with principals were conducted in their respective offices, lasting approximately forty to sixty minutes. Furthermore, five FGDs with parents of students were held in a private conference room within the school, with each session spanning between 60 and 90 minutes.

To reduce socially desirable responses to interview questions, all of the student interviews took place in a separate room with just the interviewee present. After reaching a saturation level, interviews with students were stopped. An average of ten parents of students participated in each focus group discussion (n = 48; 10, 10, 9, 9, and 10 participants) and ranging from 6 to 12 participants.[31]

Data quality assurance

Appropriate note-taking and abstraction were carried out to maintain the quality of the data. The data's credibility and dependability were maintained through continuous follow-up and data triangulation in time, person, and place.

Reflexivity

Reflexivity was employed to increase the data collection's rigor. This made it possible to conduct interviews with better probing, fewer assumptions, avoidance of early interpretation, and an amplified feeling of curiosity. To increase engagement and trust, share interview control, and ultimately increase the richness of the interview content, we also used reciprocity between the interviewer and interviewee as a technique.

Data analysis

Data collection and analysis commenced simultaneously. The verbatim Amharic transcriptions of the audio files were translated into English following their initial translation into Amharic for the analysis. The theme analysis method recommended by Braun and Clark[37] was employed, which involved a thorough examination of the transcripts using the theoretical lens of the resilience framework.[37,38]

The transcripts were independently read and reread by two authors to gain a comprehensive understanding of the data and generate initial codes. Thematic analysis was conducted by grouping related codes into categories and categories into themes. The software Atlas IT was utilized to analyse the data and develop a final coding scheme based on emergent themes. As new themes emerged, existing themes were modified and added. Through multiple discussions, the research team refined, mapped, and organized the codes into themes. These themes underwent further refinement until a consensus was reached on their interpretation and meaning. The draft findings were shared with culturally competent academics and stakeholders for validation and deeper contextual insights. The analysis concluded after thorough discussions between the authors.

Table 1. The four-dimensions criteria (credibility, dependability, confirmability, and transferability) strategies adapted from Lincoln and Guba[42,43]

Rigour criteria	Purpose	Original strategies	Strategies applied in our study to achieve rigor
Credibility	To establish confidence that the results (from the perspective of the participants) are true, credible and believable	• Interviewing process and techniques	• Interview protocol was tested before application and pilot interviews were conducted
		• Establishing investigators' authority	• We ensured the investigators had the required knowledge and research skills to perform their roles
		• Collection of referential adequacy materials	• We asked interviewers to send all the field notes to the principal investigator for analysis and storage.
		• Peer debriefing	• We had regular debriefing sessions with multi-disciplinary co-authors
Dependability	To ensure the findings of this qualitative inquiry is repeatable if the inquiry occurred within the same cohort of participants, coders, and context.	• Rich description of the study methods	• We prepared detailed drafts of the study protocol throughout the study.
		• Establishing an audit trail	• We developed a detailed track record of the data collection process. Keeping records of the raw data, field notes, transcripts
		• Stepwise replication of the data	• We measured the coding accuracy and inter-coders' reliability of the research team.
			• ensure the research process is logical, traceable, and documented
Confirmability	To extend the confidence that the results would be confirmed or corroborated by other researchers	• Reflexivity	• Periodic investigators and coauthors meetings.
		• Triangulation	• We applied several triangulation techniques (methodological, data source, investigators, and theoretical).
		• Establishing that the researcher's interpretations and findings	• Reasons for theoretical, methodological, and analytical choices throughout the entire study, so that others can understand how and why decisions were made.
Transferability	To extend the degree to which the results can be generalised or transferred to other contexts or settings	• Purposeful sampling to form a nominated sample	• We used a combination of three purposive sampling techniques.
		• Data saturation	• We quantified operational and theoretical data saturation.

Trustworthiness and rigor

Lincoln and Guba developed strict standards for determining trustworthiness in qualitative research, known as credibility, dependability, confirmability, and transferability.[39–41] Different measures were taken to ensure the trustworthiness of these findings, including participant triangulation (data were gathered from students, parents, and teachers), a method of triangulation (in-depth interviews, FGDs, and document reviews), and extended engagement to build rapport and trust among participants. Expert-reviewed interviews and FGD guides were employed.

Credibility was addressed using a variety of approaches, such as prolonged involvement, persistent observation, data collection, and researcher triangulation. Frequent peer debriefing was carried out to assess referential adequacy, check preliminary findings and interpretations against the raw data, and conduct an external check on the study process to boost credibility. The study sites for the findings' transmission were unknown to the principal investigator and researchers. All research processes were logical, traceable, and documented to ensure dependability. Furthermore, in this study, the research's interpretations and conclusions relied heavily on the collected data as a reliable source. To ensure the dependability, credibility, transferability, and confirmability of the findings, all necessary measures were implemented in the research[42] (see Table 1).

Study procedure

In this study, we used and applied Lincoln and Guba's established strict standards known as credibility, dependability, confirmability, and transferability for determining and enhancing the trustworthiness of the research. To assess and ensure the robustness of the study, we carefully organized and carried out a series of semi-structured interviews and FGDs based on the above-mentioned criteria. These standards have been applied in numerous qualitative studies on health in the past.[44]

Ethical considerations

The research study obtained ethical approval (Ref. No. CNCSDO/623/15/2023) from the CNS-IRB of Addis Ababa University, aligning with the Declaration of Helsinki. Ethical practices with student participants included using age-appropriate consent forms, obtaining parental permission and student assent, ensuring confidentiality, emphasising voluntary participation, and obtaining ethical approval. Participants were informed of their right to withdraw without consequences. Written consent and assent were obtained from all participants, adhering to confidentiality requirements, and all individuals participated voluntarily. Permission was granted by the Addis Ababa Education Bureau.

Table 2. Socio-demographic characteristics of study participants

Participants	Gender	Age range	Number of study participants
Mother	Female	25–45	48
School director	Mixed (F/M)	25–52	20
Students	Mixed (F/M)	12–19	20
Ministry of education expert	Mixed (F/M)	35–55	3
Sub-cities experts	Mixed (F/M)	30–42	4
School feeding agency experts	Mixed (F/M)	32–46	3
Total study participants			98

Table 3. Summary of themes and their respective sub-themes

	Themes		Sub-themes
1	Perceived benefits of school feeding program	1	Improved academic performance, class attendance, attention and, and reduced dropout rates and class repetition
		2	Reduces the socioeconomic burden of the family
		3	Improved student behaviour and reduced disruptive behaviour
		4	Reducing psychosocial stress and increasing social integrity
2	Perceived barriers and challenges to homegrown school feeding program	5	Underpayment of workers
		6	The poor market linkage between fostering mothers and consumer cooperatives
		7	Poor infrastructure
		8	Increased sense of dependency
		9	Increase workload for school staff
		10	Provision of poor-quality food
		11	Lack of adequate collaboration between the government and stakeholders
		12	Lack of linkage between SFP and school gardening

Results

Socio-demographic characteristics

The study included a diverse range of participants: mothers (aged 25–45), school directors (aged 25–52), and students (aged 12–19). Interviews were conducted with 20 students, 20 school administrators, 3 Ministry of Education experts, 4 sub-city experts, and 3 School Feeding Agency experts. FGDs involved 48 mothers of beneficiary children, with an average of 10 participants per group (6–12 mothers per group). In total, the study had 98 participants and aimed to examine the benefits and challenges of homegrown SFPs in Addis Ababa. See Table 2 for more information.

Themes and sub-themes

Although there have been many challenges to the implementation of homegrown SFPs in Addis Ababa, this study only identified two main themes and twelve sub-themes. Perceived benefits of SFPs and perceived challenges to homegrown SFPs emerged as the two main themes. For a detailed description of the themes and subthemes, please refer to Table 3.

Theme 1: perceived benefits of the home grown school feeding program

The significance of the HGSFPs in preventing hunger among students from low-income households was attested to by all FGD participants, students, and key informants. Additionally, the mothers of the students have acknowledged this. According to the key informants from schools, the program has helped to cut dropout and class repetition, and improved school attendance, academic performance, and concentration of students, Reportedly, before the initiation of the program, some students used to come to schools without lunch boxes and sometimes students collapsed in classes due to hunger. Arriving late for class, which used to be a big problem for the schools, has been solved since the program started. The perceived benefits of SFPs in Addis Ababa, Ethiopia, were found to be categorized under the following themes.

Sub theme 1.1: improvements in student attendance, concentration, academic performance, school dropout, and class repetition reduction. Most of the participants of this study explained that the school feeding program has improved students' attendance, academic performance, and completion through reduced hunger. They also believed that providing school meals incentivizes households to send their children to school through a transfer (the daily meal) that is intended to help offset the financial and opportunity costs of schooling.

One of the key informants explained that before the provision of school meals, students who arrived at school on an empty stomach had trouble focusing on their education. Now, school meals can provide immediate relief from hunger, reducing distraction and increasing attention span among students. One of the FGD participants explained that the program also prevents students from missing school due to hunger, which saves parents time and money by reducing the amount of meal preparation required.

> *"Imagine the severity of the issue that caused mothers to send their kids to school with empty lunch boxes . . . . . I observed students carrying empty lunch boxes even before the program started."* SFP monitoring expert, sub-city education office

> *"Previously, we used to conduct action research to reduce students late coming to school. Yet, there is no longer any worry following the SFP's implementation."* SFP improvement and monitoring team leader, sub-city education office

> *"Most of the time go to school with an empty stomach, so I feel tired and sleepy. However, since the feeding program started in our school, I get food every day, and feel energetic; I don't feel hungry anymore. Now I am happy and enjoy the class"* (An interview from an SFP beneficiary student)

> *"Had poor academic performance and no interest in learning before the school feeding program, but now he is much more interested in learning and his academic performance has considerably improved."* (An interview from SFP beneficiary student)

Sub theme 1.2: reducing burdens on the family. In addition to positive effects on education, the school feeding program offers several socio-psychological benefits. According to FGD participants, parents are relieved from the financial and physical strain of cooking meals for their school-age children every day. Additionally, this has indirectly increased food security in the home through the phenomenon known as spillover effects, in which the family's usage of one resource by one child may help another. Children from poor households are no longer suffering from the psychological pressure of being unable to bring lunch to school.

The program has also made kids happy and focused. The majority of participants concurred that the school feeding program lessened the financial strain on students' families as well as the stress placed on them associated with their poor economic status. One of the FGD participants said the following "I work at this elementary school, where my daughter and son attend. Because of my low monthly income, I find it difficult to feed my entire family. Before the feeding program, I was unable to give my school-age children breakfast and lunch, but now, thanks to the government, the problem has been rectified."

> *"I had a lot of difficulty deciding what my children should eat for breakfast and lunch before the school feeding program started, as well as what I should cook for them. Most of the time, they went to school without eating breakfast or holding their lunch. Now, thanks to God, my stress has been resolved."* (FGDs participants).

In Addis Ababa, the homegrown school feeding program has so far only covered elementary schools, and the program has not reached high school students. As reported by sub-city education offices, poor students get challenged as they advance to high school. Many high school students from economically disadvantaged families stay the whole day at school without lunch boxes, while others share the lunch with other students.

> *"High schools have to benefit from the program as well. After entering high schools, underprivileged students who were previously served by the program are having difficulty, and some high school students have illegally entered the nearby elementary school to get school meals."* (A school director, at a primary school)

Sub theme 1.3: change in students' behaviour. The majority of key informants believed that students had improved behaviour and safety at school as a result of the school feeding program. Besides, respondents said it increased the student's motivation to avoid harm, go to unnecessary places, and skip school compounds. Before the implementation of HGSFP, students reported used to climb over the school fence to look for food when they became hungry. Additionally, they explained that when students go looking for food, they get exposed to crime or threats, sexual abuse, harm, or harassment, and get victimized. Student violence is supported and influenced by out-of-school gangs, juvenile delinquents, street drug dealers, and drug addicts.

According to one of the key informant explanations, before the implementation of the homegrown school feeding program, many students were subjected to unnecessary behaviours such as chewing khat and shisha. Since the introduction of the school meal program, they have stayed at the school the whole day, and as a result, they are safer than ever and focusing only on their education.

> *"Various misconduct offenses, such as those that disrupt the learning environment and those that involve aggressive behavior, such as fighting, bullying, and student assault, were observed on students before the implementation of the school feeding program, but these disciplinary issues have now significantly decreased."* (A school director, at a primary school)

The majority of participants in focus groups and key informant interviews acknowledged that food insecurity and hunger among children have a detrimental impact on their academic focus, performance, and behavioural patterns. Moreover, the majority of research participants reported that the HGSFP has led to a reduction in student misconduct, including conflicts with school security guards, class skipping, and financial theft from parents. Notably, access to free school meals, especially, has been associated with a decrease in disciplinary infractions.

> *"Before the school feeding program started, some students used to steal money from their parents to buy bread and various sweets and spend the stolen money. It was causing them to grow up with inappropriate behavior, but after the school feeding program was started, the problem was significantly reduced."* (FGD participants)

> *"Before the implementation of the school food program, there were numerous behavioral issues among students, and occasionally, students would fight with the school security staff for various reasons. However, now these issues have significantly decreased."* (A school director, at a primary school)

Sub theme 1.4: decreased psychological stress and increased social integrity. Before the start of the school feeding program, only students from low-income families were eligible for the program, which subjected students to discrimination, as was commonly mentioned by some FGD and key informant participants. Furthermore, the majority of participants also described that the SFP increased unity and social integrity and decreased discrimination among students when they ate together, regardless of their families' economic status.

> *"Students are protected from psychological harm and shame by the school meal program. Many students did not bring food to school prior to the school feeding program's implementation, and those from families with comparatively higher incomes brought lunch boxes. This caused psychological trauma for the students, but these issues have since been resolved thanks to the government, and all students now share the same meal."* (A school director, at a primary school)

> *"The school feeding program helps alleviate perceived discrimination, instead fosters strong relationships with students through eating together and enabling them to concentrate on their studies by providing equal access to services for all students."* (FGDs participant)

Theme 2: perceived challenges to the HGSFP in Addis Ababa city

The Addis Ababa City Administration School Feeding Agency recently assigned 1-2 nutrition experts to each school, which improved program quality and encouraged ownership. The experts oversee the program's execution and offer technical support. Although assigning nutrition experts at the school level has been taken as a positive step, sub-city and district education offices continue to lack the staff needed to consistently offer schools supportive supervision.

The women groups receive 20 birr per student payment to prepare two meals per day for each student. With this modest budget and given the ongoing food price inflation, the women are struggling to prepare the meals according to the standard menu. Due to budget shortages, the women are losing interest, and sometimes they provide substandard meals.

Sub theme 2.1: underpayment of workers. The majority of key informant interviews and focus group discussion participants agreed that the salary currently provided to women who prepare meals for students is insufficient to cover inflation or the actual cost of living. During the focus group discussion, a study participant mentioned that food handlers face a lot of pressure due to their low salaries, which makes them demoralized at work and forces them to take on other jobs like housecleaning and washing clothes to support their families. As a result, there might be a detrimental effect on the quality of services provided. According to one of the key informants, the government must assess the food handlers' payment, and before determining how much to pay for them, a market evaluation study that takes into account the challenges imposed by the current cost of living must be conducted.

According to one of the key informant's opinions, with the existing budget, the quality of school meals may deteriorate to the extent that it threatens the very significance of the program. The current food price inflation is even pushing HGSFPs run by NGOs to compromise their meal plans.

> *"A daily budget of 20 birr is set aside for breakfast and lunch for each student. It is challenging to feed children on this budget in the current market."* SFP monitoring expert, sub-city education office

> *"An egg currently costs 12 or 13 birrs. So, how can you feed a student for two meals with a budget of 20 birr a day?"* SFP improvement and monitoring team leader, sub-city education office

> *"A kilogram of bananas cost 25 birr when our school feeding program first began; the price has now doubled. Thus, the banana must be eliminated from the menu."* Respondents from an NGO implementing SFP

The majority of FGD participants explained that they were unhappy with the refusal of the government not to pay for food handlers during maternity leave and the summer when schools are closed. Food handlers lack alternate means of income, which makes it difficult for them to support their families and lead during this time as a result of these issues.

> *"The problem of not paying the mothers who prepare food for the students after June 30 when the school is closed. Along with this, breadwinner mothers are exposed to problems as they will not have monthly income until the school opens."* (FGD participant)

> *"During maternity leave, the government is not paid for food handlers like other workers, and due to this feel discrimination and dissatisfied with their work."* (FGD participant)

Sub theme 2.2: the poor market linkage between fostering mothers and producers. The major challenge explained by FGD participants and key informant participants was the lack of market connections or linkage between foster mothers and suppliers of market items and agricultural products. Some participants indicated that the HGSFP's inability to obtain essential supplies like tef, flour, oil, sugar, and bread sufficiently, as vegetables and fruits did not get.

The women groups providing school meal service have some market linkage with consumer cooperatives (specifically for sugar, flour, and oil) and Sheger Bakery. However, according to the key informants, the linkage is not adequate to financially sustain the program. Schools also do not have any established linkage for other supplies like vegetables and fruits. As reported by the key informants, the cost of grains and vegetables might have been lower if there had been direct market connections established with farmers or agricultural cooperatives. Despite the government's previous promises, there has been no development of this type of market linkage.

Thus, women's unions that organize food for public school children in Addis Ababa face a threat to their sustainability due to the current inflation and rising prices of supplies and commodities in the city. The mothers who participated in the FGDs explained that they were unhappy working as cooks in the school feeding program because the payment they received did not consider the high workload and the current market inflation.

> *"There is a link between sugar, oil, flour, and the sheger bread association, but it is insufficient because it does not account for the quantity and number of students."* (FGDs participant)

Sub theme 2.3: poor infrastructure. Most participants in focus groups and key informants agreed that schools did not have the necessary infrastructure for a successful school food program. Many schools also do not have adequate dining halls to accommodate students; therefore, classrooms and libraries have to be used for the same purpose. Also, there is a lack of standard kitchens and standard stores; instead, they use the floor or improvised areas for food storage. As a result, the school feeding program's food safety is at risk due to the aforementioned issues.

Even though every school in the city has access to safe drinking water, the program has been impacted by the regular outages of water supplies and electricity, as well as the absence of conventional water storage facilities. Since most schools currently lack access to three-phase electricity, which is necessary for mass cooking, biomass fuel is used instead.

> *"Among the public primary schools under the Addis Ababa City Administration, only a few have standard kitchens. It is difficult to talk about food safety issues while the kitchen is substandard"* SFP improvement and monitoring team leader, sub-city education office

> *"Frequent water and electricity outages in our schools had a major challenge on the implementation of the school feeding program."* (A school director, at primary schools)

> *"Most schools' kitchens for preparing meals for students are made of tin, which prevents sufficient airflow. So it is very difficult for the mothers to do their work and risk for their health."* (FGDs participant)

Sub theme 2.4: increased sense of dependency. One of the school directors explained during a key informant interview that the school feeding program causes families of children to feel dependent on the program. Another school director stated during a key informant interview that the school feeding program forces students to refocus their attention on school meals rather than their schoolwork and causes them to spend too much time eating at the nearby dining room, not providing enough time for studying.

> *"School feeding programs are beneficial for students. However, it would be preferable if it just applied to low-income parents of students. The school feeding program has unnecessarily raised dependence on the parents of students. They are no longer able to take on responsibility or handle problems alone, and as a result, they develop a dependency mentality."* (A school director, at primary schools)

Sub theme 2.5: increased work burden. The initiation of the HGSFP has also caused a burden for the school in terms of managing operational issues and facilitating the finances needed for the program. The HGSFP has increased the work burden on principals, teachers, and admin staff. The feeding program is also compromising the time allotted for learning activities. However, considering the benefits of the program, the school community has so far taken on the burden of positivity.

> *"SFP has increased the work burden on school directors, teachers, and administrative staff. But they are aware of the benefit of the program as well."* SFP improvement and monitoring team leader, sub-city education office

> *"Some students give a lower priority to their educational activities, including study time, rather than spending much time eating breakfast and lunch."* (A school director, at primary schools).

Sub theme 2.6: provide poor meal quality. The Addis Ababa School Feeding Agency (SFA) prepared the current school menu to diversify diets, standardize meals, and ensure that children's nutrient needs are satisfied. The SFA has introduced standardized recipes based on locally available ingredients.

Despite adhering to a set menu, some key informants have expressed concerns about the nutritional value of school meals. Financial constraints prevent the inclusion of animal-based products like milk and eggs, while limited resources make it challenging to provide sufficient fruits and vegetables. As a result, the quality of the meals served to students may be compromised. Key informants from sub-city education offices have reported that bulk preparation sometimes leads to unappealing school lunches, affecting student satisfaction.

During a key informant interview, one of the school directors indicated that the lack of adequate funding for the school feeding program made it impossible to include animal products, fruits, and vegetables in the food menus. A student who participated in this study expressed dissatisfaction with the current food quantity and quality provided in the school feeding program. Another student also provided the following explanation: "We are unhappy because the food they received throughout the week was not varied and had no animal products, like eggs, milk, and meat."

> *"Sometimes students bring lunch boxes from home complaining that the school meals are not palatable"* SFP improvement and monitoring team leader, sub-city education office

Sub theme 2.7: engaging diverse stakeholders in HGSFP. According to a key informant from the Ministry of Education (MoE), with the recent food price inflation, it is not possible to fully finance SFP with the government budget alone. So, efforts have to be made to mobilize resources from the community and other partners. There is also increasing interest from NGOs to support the HGSFP. However, the collaboration between the School Feeding Agency (SFA) and NGOs engaged in HGSFPs is far from ideal. Effort to engage individual local contributors is also low.

Although the Addis Ababa City Administration greatly needs multi-stakeholder engagement, there is currently no clear government directive on how to involve NGOs in school food programs. Instead, the School Feeding Agency is pressuring NGOs and contracting out the food preparation to a local women's union, which has lowered the standard of services offered to students.

Sub theme 2.8: linkage between HGSFP and school garden. According to a key informant from the Ministry of Education (MoE), some schools in the Amhara, Oromia, and SNNP regions have started school gardening to strengthen their HGSFP. The respective regional structures of the agriculture sector are supporting the initiative by providing technical and material support, including agricultural inputs like improved seeds. Experience from elsewhere outside Addis Ababa indicated that school gardening has improved access for schools to a fresh supply of vegetables and fruits. Further, it has also excluded middlemen from the market chain and reduced the expenses of the program.

> *"One major approach to ensuring students access to fresh and nutritious vegetables and fruits is the initiation of school gardens."* Health and Nutrition Expert, MoE

In contrast, the achievement of school gardening is modest in Addis Ababa because, unlike rural schools, schools in the city do not have adequate space to initiate gardening at scale. The Addis Ababa City Administration (AACA) still has not initiated urban

agriculture within the school environment. High schools in Addis Ababa are comparatively bigger than elementary schools. However, since high schools are not currently enrolled in the HGSFPs, this cannot be directly linked to the HGSFP.

School gardening has the potential to improve the accessibility of the SFP and to get vegetables at better prices and quality. This has also been observed in schools that started gardening. So far, most schools that run SFP in Addis Ababa have not started gardening. Many of the schools have no adequate space to establish meaningful urban agriculture, while others have not received technical support for doing so. Those had experience with school gardening, scarcity of water, lack of personnel to take care of the farm, and limited production. Many key informants assumed urban agriculture may help students understand how agriculture works. Furthermore, it will serve as a demonstration site for the agriculture course that will be included in formal education starting in the coming year.

One major challenge that hinders the linkage between urban agriculture and SFP is the low productivity of school gardens. Experience from the schools that implemented urban agriculture at different levels suggests that the scale of production is too small to have a meaningful effect on the meals delivered to students.

> *"The existing space in schools does not allow for large-scale production. However, school gardening is used as a demonstration center so that students will apply the experience at home."* SFP monitoring expert, sub-city education department

> *"We are implementing school gardens and we have been acknowledged for that. However, the demand of the HGSFP is large and cannot be met by the gardens alone."* A SFP coordinator

Discussion

Our study aimed to address gaps and limitations in previous research on HGSFP. To fill these gaps, we conducted a qualitative phenomenological study in Addis Ababa, Ethiopia, comprehensively exploring the perceived benefits and challenges of HGSFP. Our findings indicate improved student nutrition and education outcomes but persistent challenges in infrastructure, logistics, operational, and financial issues. These findings align with previous research emphasising the importance of contextual factors in HGSFP implementation.

In terms of benefits, our study identified increments in school enrollment, attention span, academic performance, and attendance, along with reductions in dropout rates and class repetition. The program also alleviated the socio-economic burden on students' families, decreased social psychological pressure, improved social integrity among students, and prompted changes in students' behaviour. However, challenges such as poor-quality meal provision, lack of market linkage, poor infrastructure, underpayment of workers, dependency on the program, lack of collaboration between stakeholders, and inadequate linkage between HGSFPs and school gardening were identified as key barriers in the effective and efficient implementation of HGSFPs in Addis Ababa City, Ethiopia.

Our research enhances the theoretical understanding of homegrown school feeding programs (HGSFP) by revealing the intricate interplay between local contexts and program effectiveness. These findings can serve as a foundation for future studies to explore long-term impacts and formulate scaling strategies specifically for urban environments. Offering school meals has multiple benefits, including improved academic performance, increased enrollment, attendance, retention, and completion rates for pre-primary and primary school students. It also reduces hunger, enhances students' health and nutritional status, and helps break the generational cycle of malnutrition. Additionally, providing daily meals lowers the opportunity and financial costs of sending children to school, encouraging families to prioritize their education.

According to the results of the current study, The feeding program has improved enrollments, decreased the dropout rate, improved academic achievement and concentration, and decreased absenteeism and dropout rates. Thus, the HG-SFPs may have enhanced educational and nutritional outcomes by reducing short-term hunger. This keeps students from having to leave school to get food and makes them more interested in class. This result is consistent with a quantitative study conducted in Addis Ababa, Ethiopia,[14,45] which revealed a favorable relationship between academic achievement and SFP.

This result is in line with a review[46] of qualitative studies that examined the consequence of SFP on the education outcomes of students' such as enrollment, completion, and academic success and were conducted in Nigeria and Niger.[47,48] Therefore, strengthening the SFP could be a crucial intervention to enhance students' academic performance and improve the quality of their education.

The lessened socioeconomic strain on student families was the other perceived advantage of HGSFPs in Addis Ababa. It was difficult for low-income families to feed their kids breakfast and lunch, and when students went hungry and looked for food, the families experienced worry and anxiety. However, the HGSFPs helped them reduce the pressure that society's socioeconomic system placed on families, especially those with lower incomes.

This finding is supported by a systematic review report,[46] and a study conducted in the Sidama region of Ethiopia.[14] This conclusion was further supported by a Tennessee, USA study that found school lunch programs decreased family stress regarding time and money spent on food shopping, cooking, and packing for their kids.[49] Therefore, the Homegrown School Feeding Program (HGSFP) has the potential to be a crucial strategy for enhancing the quality of life for families, particularly those with low incomes.

According to the study, the School Feeding Program (SFP) led to a reduction in social psychological pressure and an increase in social integrity among students. Previously, there was a school feeding program exclusively for students from the poorest families, which placed psychological pressure on the students. However, with the endorsement of the Homegrown School Feeding Program (HGSFP) in all public primary schools in Addis Ababa, this pressure has been alleviated.

Currently, there are no eligibility requirements for students to receive breakfast and lunch at school, irrespective of their parents' financial situation. This inclusive approach has led to a decrease in psychological stress and an enhancement of social integrity among students. This finding is supported by evidence from a qualitative study conducted in India[50] and a systematic review that reported an increase in social interaction and integrity among students through the School Feeding Program (SFP).[46]

The HGSFPs contribute to reducing poverty both directly and indirectly through improving community wellbeing.[47] The study found that implementing the Homegrown School Feeding Program (HGSFP) resulted in a decrease in inappropriate student behaviours, including class absenteeism, substance use, and exploitation. This finding is consistent with a study conducted in Niger, which also highlighted the role of SFPs in reducing student misconduct such as theft, drug use, sexual assault, and delinquency.[47] This suggests that HGSFP could be applied as a strategy for generating a disciplined and productive generation in the future.

Ethiopian school feeding policy made clear direction that the government is responsible for providing schools with basic facilities that are appropriate for the local environment.[19] However, this study indicated that inadequate facilities, such as those in the kitchen, food storage, dining room, water supply, and electric supply, were among the main obstacles to the implementation of HGSFP. When there was no water in the school, the food handlers paid laborers to fetch water, and when the electricity went out, the food handlers used a wood fire to cook the food instead, which exposed them to hazardous smoke. Qualitative research in the Sidama region of Ethiopia,[14] Nigeria,[48] and Uganda[51] as well as a qualitative systematic review undertaken globally,[46] all provide credence to this finding. Therefore, building basic facilities as well as improving the water and electric supply could positively impact the success and sustainability of HGSFPs in settings with scarce resources, such as Ethiopia.

This study also indicated that underpayment of workers under HGSFP, absence of payment for workers in the summer season, maternity leave, and budget that doesn't consider inflation were other challenges to the effective implementation of HGSFPs. This finding is supported by evidence from a qualitative systematic review in a global context[46] and a qualitative study conducted in Addis Ababa.[52] These studies reported that the absence of a budget that considers inflation was a challenge for the smooth implementation of HGSFPs. Therefore, this program should give due emphasis on adjusting the budget according to the living cost of the country.

In this study, a key obstacle to effective Homegrown School Feeding Programs (HGSFPs) was the lack of networking among producers, suppliers, and the program. This resulted in shortages of essential supplies and commodities for the HGSFP, a finding supported by a global review of SFPs.[46] Thus, it is crucial to prioritize the improvement of the food supply chain, as emphasized in the school feeding policy.[19]

According to Ethiopian school feeding policy,[19] effective and sustainable HGSFPs need cross-sectoral cooperation, and the local community is expected to oversee the implementation and allocation of resources with a sense of ownership. The HGSFPs were, however, found to be developing a sense of dependency among the parents of the students. Thus, to maximize the sense of ownership and minimize the sense of dependency on it, as already mentioned in the national school feeding policy, program implementers should pay appropriate attention to engaging parents of students in particular and the local community in general in the process of planning, implementing, and evaluating this program.[19]

Strengths and limitations

One of the strengths of this study is the inclusion of diversified potential study participants who have in-depth information and rich insight into the homegrown school feeding program. Furthermore, the interview facilitators were proficient in the local language, well-versed in the community, and had prior experience with qualitative research. Relationships were established before data collection by approaching the primary school directors for prior permission. The analysis was conducted as a team with the help of multiple researchers. Considering the study's limitations, its qualitative nature limits the generalisation of its findings due to the lack of a representative sample. This study would have benefited more from a mixed-methods approach to quantifying the size of each perceived benefit and challenge associated with HGSFPs so that planners and policymakers may more easily prioritize problems.

Conclusions and recommendations

Awareness-raising efforts should be started to address the parent's incorrect perception of their dependence on the school feeding program. To lessen problems and enhance the quality of HGSFP's, all primary schools must fulfill the necessary infrastructure for the program, like constructing standard dining halls that can accommodate the number of students as well as standard food storage and cooking areas. They also must have ready-to-use water storage facilities and private generators.

In addition, the government should establish market links for fruits and vegetables, which will contribute to the HGSFPs and meet the nutritional needs of the students. Furthermore, starting and supporting urban agriculture and school gardening should be the alternative strategy. Besides, direct market connections with agricultural cooperatives are essential for decreasing the current inflation of food prices and securing food supplies at lower costs.

To improve the efficiency of the HGSFP, lessen the workload on administrators, teachers, and other staff members, and streamline the handling of program-related operational and financial issues. It is better to entirely outsource to the appropriate body. In addition, it is important to assign the right people to each position, clearly define roles and responsibilities, and allocate funds at all levels.

According to the researchers, future studies should be needed, to quantify the perceived benefits and drawbacks of HGSFP to make it straightforward for policymakers and program designers to prioritize problems based on their magnitude. The HGSFPs are not stand-alone interventions;

therefore, they need to be strengthening the network of local private and public partners and NGOs working in HGSFP-related fields by developing a coordinated partnership strategy with well-defined roles, management, financial responsibilities, and implementation guidelines.

In order to overcome the operational and strategic obstacles to the program and improve the effectiveness of the domestic school feeding program, a framework for coordination between the various sector ministries and task forces must be established.

Abbreviations:

FGDs: Focus group discussions; **HGSFP:** Home-Grown School Feeding Program; **KII:** Key informant interview

Supplementary material

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Authorship

Y.T. conceptualized the study, curated the data, performed formal analysis, acquired funding, conducted investigations, developed the methodology, administered the project, created visualisations, and wrote the original draft. S.G. contributed to data curation, formal analysis, methodology, project administration, supervision, reviewed and edited the manuscript, and approved it. A.M. contributed to methodology, formal analysis, project administration, supervision, and reviewing and editing the manuscript. A.A. was involved in data curation, methodology, project administration, supervision, and reviewing and editing the manuscript. D.J.B. and E.M. contributed to data curation, provided resources, and reviewed and edited the manuscript. All authors critically reviewed and approved the final article.

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RESEARCH ARTICLE

Acceptability and feasibility of integrating psychosocial stimulation interventions in the inpatient care of children with severe acute malnutrition in resource-poor settings: a qualitative study

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Abstract

Children with Severe Acute Malnutrition (SAM) are at risk of developmental problems. Psychosocial stimulation can improve the developmental outcomes of hospitalised children with SAM. However, the intervention has remained underutilised in health facilities in resource-poor settings. Moreover, there is limited evidence on the acceptability and feasibility of the intervention. We conducted a qualitative study to explore the acceptability and feasibility of integrating psychosocial stimulation interventions in the inpatient care of children with SAM in selected areas in the Silti Zone, Central Ethiopia. Nine focus group discussions and 15 key informant interviews were conducted with parents, health workers, and other stakeholders. The data were transcribed, translated, and analysed using a thematic approach. Caregivers and health workers had positive attitudes toward the intervention and perceived it beneficial for the children's development, recovery, and bonding with the mothers. Health workers reported barriers such as lack of materials, time, and space, capacity building training, and supervision for the effective implementation of the intervention. At the household level, gendered factors such as the housework burden of mothers and the inadequate engagement of fathers in childcare were the main challenges to the implementation of the intervention. Overall, psychosocial stimulation intervention was found to be acceptable and feasible for routine implementation with inpatient care provided for children with SAM. The study recommends supporting health facilities, health workers, and partners with the necessary resources and skills to promote the implementation of stimulation interventions along with the existing care provided in health facilities in resource-poor settings.

Key words: **Psychosocial stimulation: Malnourished children: Acceptability: Feasibility**

Introduction

Severe acute malnutrition (SAM) is a global life-threatening condition that affects millions of under-five children[(1)]. Ethiopia is one of the countries with the highest burden of SAM in the world, with an estimated 400,000 children suffering from SAM in 2020[(2)]. Based on the recommendation of the World Health Organization (WHO) and the Ethiopian Federal Ministry of Health (FMoH), the management of SAM involves both inpatient and outpatient care, depending on the severity and the associated complications of the condition. A SAM child with medical complications or failed appetite tests or referred from the Outpatient Therapeutic Program should be admitted to a dedicated nutrition unit and given medico-nutritional care and support[(3,4)]. The integration of psychosocial stimulation interventions into the inpatient management of SAM children is also recommended to reduce the risk of intellectual disability

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and emotional impairment and promote development and growth in the rehabilitation phase[3–5].

Psychosocial stimulation intervention refers to the provision of responsive care, play, communication, and learning opportunities that enhance children's cognitive, social, and emotional development[6]. The intervention is particularly important for children hospitalised for severe malnutrition in early childhood. According to some scholars, these children were found to have long-term deficits in cognitive development and school achievement up to adolescence[7], owing to the effect of undernutrition on the structural and functional impairment of the brain and central nervous system[5].

Studies conducted in Jamaica, Bangladesh, and Ethiopia confirmed the positive contribution of psychosocial stimulation interventions in improving the development outcome of children hospitalised with SAM[8–10]. However, psychosocial stimulation intervention has not been adequately implemented as part of the inpatient management of children with SAM, particularly in health facilities in resource-poor settings including in Ethiopia. A study conducted in South Africa reported that play and stimulation were not implemented in the inpatient management of severe malnutrition in under-resourced rural hospitals[11]. Moreover, evidence on the perceptions of parents and health workers towards the stimulation intervention and the feasibility of implementing the intervention is limited globally. A qualitative study conducted in Malawi to understand the perceptions and experiences of caregivers who participated in the Kusamala Program, a 4-day hospital-based counselling programme for caregivers of children with SAM that integrates nutrition, water, sanitation, hygiene, and psychosocial stimulation, reported the positive sentiments and experiences of the parents towards the programme[12]. A feasibility study of the Kusamala Program identified caregivers' perceived value as a potential barrier to the implementation of the programme. Prioritisation of other ward activities and shortages of staff were also identified as constraints for the health workers[13]. In the studies that tested the stimulation intervention among hospitalised SAM children, the WHO recommendations on stimulation interventions were not followed[8–10,12]. Therefore, the implementation practice and the feasibility of the WHO recommendations on psychosocial stimulation interventions for children hospitalised with SAM were unclear, especially in low-resource contexts[14]. A systematic review published in 2017 has reported feasibility concerns on the stimulation interventions used in studies conducted in Jamaica and Bangladesh[14].

Therefore, we have been conducting a Cluster Randomized Control Trial to examine the effects of psychosocial stimulation on the development, nutrition, and treatment outcomes of hospitalised SAM children In Ethiopia (EPSoSAMC Study), following the recommendations of WHO and FMoH[3,4]. This qualitative study was included in the trial to explore the acceptability of the stimulation intervention among parents and health workers who participated in the implementation of the intervention. Another aim of the qualitative study was to understand the feasibility of implementing the WHO recommendations on psychosocial stimulation interventions for children hospitalised with SAM in resource-poor health facilities. Our findings could generate relevant information on how best the intervention would be integrated, the financial, material and human resources required, the training needs, and how best to ensure the follow up of children and their families[15]. Moreover, it could enable the identification of the barriers and facilitators that affect the routine implementation of the stimulation intervention in the care and support of hospitalised SAM children.

Methods

Study area

Based on the new administrative establishment, the Silti Zone is one of the administrative divisions of the Central Ethiopia Regional State. It has a population of 1,020,337[16] and agriculture is the main source of livelihood for about nine out of ten people in the Silti Zone[17]. Malnutrition is one of the major causes of childhood deaths in Ethiopia. The prevalence of stunting, wasting, and underweight in the country was 37%, 7%, and 21%, respectively[18]. The present study was carried out among health facilities located in Hulbareg Woreda, Silti Woreda, and Worabe Town Administration, which represent various socio-demographic disparities in the Zone.

Psychosocial stimulation interventions

The study is part of a Parallel-Group Cluster-Randomized Controlled Trial (EPSoSAMC Study) that has been underway in the Siliti Zone, Central Ethiopia. The trial aimed to examine the effect of psychosocial stimulation interventions on the development, growth, and treatment outcome of hospitalised SAM children aged 6–59 months [Pan African Clinical Trials Registry: PACTR201901730324304]. The trial involved health facilities (clusters) that provide inpatient care for children with SAM. The health facilities were randomly assigned into intervention or control groups, and trained health workers were responsible for the recruitment of eligible SAM children admitted to the facilities. In the intervention facilities, dedicated play corners were established and equipped with age and developmentally appropriate play materials. Therefore, children were given psychosocial stimulation interventions following the recommendations of the WHO and FMoH. In these facilities, trained intervention workers (Play guides) recruited from the local areas facilitate a half-hour stimulation session daily after the initial phase of treatment for children. In the six-month follow up period, intervention workers visit children five times (at the end of the 1st week, 2nd week, 1st month, 3rd month, and 6th month) based on the WHO's recommended follow up schedules. In each visit, intervention workers engage children in a half-hour stimulation session. In the control health facilities, children were given routine inpatient care and home visits without psychosocial stimulation. All primary caregivers of SAM children were also given counselling on health, nutrition, development, and related topics during the inpatient and follow up periods. Further details of the trail have been presented in previous publications[19].

Participants and data collection

We conducted a qualitative study using Key Informant Interviews (KIIs) and Focus Group Discussions (FGDs) between November and December 2022. Nine FGDs that lasted 1:15—1: 45 hours were conducted among a homogenous group of 6-9 mothers and fathers of children who received psychosocial stimulation intervention as part of their inpatient care. Fifteen Key Informant Interviews (KIIs) lasting 30-45 minutes were also conducted with health workers and intervention workers that deliver the stimulation intervention, Health Extension Workers (HEWs), Woreda and Zonal Health Office staff, and the staff of Worabe University. The saturation of information was used to judge the adequacy of the KIIs and FGDs. A purposive sampling technique was employed to select KII respondents and a convenience-sampling technique was used for the selection of FGD participants. Semi-structured KII and FGD Guides were used to guide the data collection where probing questions were included in the tools to allow getting detailed information. Data on the perception of the parents about the benefit of the stimulation intervention, their adherence to the recommended stimulation practices, and the barriers and facilitators for implementing the interventions including family support were collected. Moreover, the experience of those directly and indirectly involved in the implementation of the intervention, and the potential challenges and opportunities in the implementation of the stimulation interventions with the routine care given in the nutrition units were also explored. The data were collected in the local language (Amharic). All interviews and discussions were tape-recorded with the consent of participants and notes were also captured during the data collection. To ensure the research participants' privacy, data were collected in private areas such as in the compounds of health facilities and offices and no one else was present in the interviews and discussions except the study subjects and the data collection personnel. Two public health researchers (TT and AA) who are members of the main trial team collected the data. TT was responsible for facilitating the interviews and discussions, while AA was in charge of taking notes and recording. They have MSc. level training in public health with additional training in qualitative research methods. They also have demonstrated experiences in qualitative data collection and knowledge of local context. They maintained neutrality and consistency throughout the data collection process to enhance the validity and reliability of the data. Although they have an interest in the subject matter, their background does not influence the collected data. The involvement of other authors in different stages of the study could have also minimised the potential introduction of biases owing to the background of the researchers engaged in the data collection. Moreover, the finding of the main trail was not known during the collection and analysis of the data. The whole process of the study followed the recommendations included in the Consolidated Criteria for Reporting Qualitative Research[20].

Data management and analysis

Before the actual analysis, the data collected in a digital recorder were transcribed into written form and translated into English. The thematic approach was used to analyse the data following the six steps namely: familiarisation, coding, generating themes, reviewing themes, defining and naming themes, and writing up. Themes or patterns within the data were identified using a combination of inductive and deductive approaches[21]. Two researchers (TT and AA) independently read the transcripts and assigned initial codes. Once all codes were identified, they were then discussed, and similar codes were collated together. The codes were then sorted and collated into potential themes, which were reviewed in relation to the research objective to ensure the identified themes represented the data. Finally, the themes were defined and named as presented in the below section. In the analysis process, no specific software was used. The five main themes derived from the data include 1) The perception of parents about SAM and its impact on child health, growth, and development: 2) The concerns of parents about the future of their children: 3) The perception of psychosocial stimulation intervention and its benefits: 4) The acceptability of psychosocial stimulation interventions: and 5) The feasibility of incorporating psychosocial stimulation interventions into the inpatient care of children with SAM.

Result

A total of 63 parents (Mothers-43 and Fathers- 21) participated in the FGDs. Fifteen interviews were also facilitated with health workers involved in psychosocial stimulation interventions (6), HEWs (2), intervention workers (2), Woreda Health Office staff (2), Zonal Health Office staff (2), and the staff of Worabe University (1). In the section below, the key findings of the study are presented based on the five identified themes, and illustrative quotes stated by the participants are presented to elaborate on issues in more detail.

The perception of parents on SAM and its impact on child health, growth, and development

Parents were asked several questions to explore their understanding of SAM. Issues such as the causes of SAM, the consequences of SAM on the health, growth, and development of children, and their understanding of the required treatment were explored. Some parents showed an adequate understanding of SAM and mentioned details about its effects on the health, growth, and development of children. They voiced inappropriate feeding practices as the major cause of the problem and cited special milk and medicine as the key elements for treating their children in the health facilities. However, there were still parents who had a limited understanding of SAM and how to prevent it.

> It is a condition that affects the health of children making them very thin or swollen. It is caused by a lack of food. These children are treated with special food and medicine in the health facilities. Feeding children in a better way prevents SAM from happening (FGD, Mother).

Nutrition and health education were provided to all parents whose children were admitted for inpatient care in the

stabilisation centre (SC). Therefore, we attempted to explore the parents' understanding of SAM before their children were admitted to the SC to understand the improvement in their knowledge that could be attributed to the education interventions implemented in the health facilities. Accordingly, nearly all parents, including those with an adequate level of understanding at the time of data collection, universally acknowledged that they did not know the details of SAM until their children were admitted to the health facility.

> I did not know what SAM was until health workers told me in this hospital. They measured my child and said he has SAM. I thought my child was just thin." (FGD, Mother)

Health workers have also expressed similar sentiments, mentioning that most parents lack a basic understanding of the problem when their children are first diagnosed with SAM. Therefore, it could be sound to consider the valuable contribution of health education intervention in terms of improving the understanding of SAM among parents who participated in the study.

This study also attempted to explore community-based beliefs and practices related to SAM in children. The participants shared various beliefs and practices related to SAM that could have influenced community wide behaviours and decisions, including health-seeking practices for children affected by SAM. Attributing SAM to supernatural causes, such as evil spirits, curses, or God's /Allah's will was among the issues identified in the community. Traditional and spiritual remedies from traditional healers and spiritual people were also mentioned among practices often considered before or instead of seeking medical care for children with SAM.

> My child suffers from SAM, which I believe was caused by a curse. He used to be healthy, but then he began to lose weight and fall ill frequently. I took him to a traditional healer who prescribed him some herbs [FGD, Mother]

In addition to the community-based beliefs and practices, some participants also expressed dissatisfaction with the health system and the health workers when explaining why the community prefers going to the traditional and/or spiritual people rather than seeking help from the available health facilities.

> We are not getting good service in the health facilities. They just give them some medicine and food and send us home. They do not explain anything to us except to tell us the child has experienced a food shortage [FGD, Mother].

The concerns of parents about the future of children with SAM

The study revealed that most parents have various concerns about the future of their children with SAM. They expressed concerns about their children's survival, recovery, growth, and health. The fact that their children had difficulties in speaking, playing, and socialising compared with other children of their age was mentioned to have exacerbated their concerns about their children's future. Moreover, some participants mentioned their concern that their children would face stigma and discrimination owing to their physical conditions in their community.

> I worry that my child will be behind the development of other children. He is more than one year old but he has difficulty walking properly [FGD, Father].

The perception of psychosocial stimulation intervention and its benefits

This theme explores how parents whose children received psychosocial stimulation interventions as part of inpatient care and health workers perceived psychosocial stimulation interventions and the benefits of the intervention. Accordingly, most parents showed a good understanding of the interventions. They described psychosocial stimulation as the provision of care, love, and attention to children through activities such as talking, singing, and playing with children. They further expressed positive attitudes toward psychosocial stimulation interventions and recognised the importance of the intervention in terms of improving the condition of their children, enhancing the interaction of the children with their mothers, and increasing the knowledge and skills of the parents. Improvement in the appetite, weight gain, and health status of children, their skill and activity level, and the reduction of stress among parents and children were further mentioned among the benefits of the intervention.

> Psychosocial stimulation is giving love and care to children. It is talking to them, playing with them, and making them feel happy and safe. It helps me to bond with my child and better understand him to meet his needs [FGD, Mother].

> I see differences in my child after stimulation interventions. He is now interacting more with other children and staff [FGD, Mother].

A health worker added

> The psychosocial stimulation intervention is very beneficial for the relationship between children and caregivers. It encourages them to play together, talk to each other, and share their feelings. It also helps caregivers to better understand their children and show love and care [KII, Health Worker, Male].

The observed good level of understanding of the intervention and the benefit among most parents could be attributed to the educational activities that were integrated into the psychosocial stimulation interventions. Health workers have also stated that the intervention has improved the understanding of parents about the problem of SAM children and their treatment process because of their engagement in the delivery of the intervention. They further reported the benefit of the interventions in terms of enhancing treatment outcomes for children. According to the respondents, such interventions would have a long-lasting

benefit of improving the care given to children, thereby preventing the re-occurrence of SAM.

> Psychosocial stimulation intervention helps children to recover faster. It helps them to eat more, gain weight, and get healthier [KII, Health Worker, Male].

The acceptability of psychosocial stimulation interventions

This study attempted to explore the experience of parents who were engaged in psychosocial stimulation intervention of their SAM children as part of the medico-nutritional care provided in the SC. Similarly, health workers were also consulted to share their experience of delivering psychosocial stimulation interventions for children with SAM. It was revealed that parents and health workers shared both positive and negative experiences in the implementation of the interventions.

Most parents expressed positive experiences with participating in psychosocial stimulation interventions for children with SAM, although few of them also shared unpleasant experiences. Because play was the major intervention introduced into the routine medico-nutritional care of SAM children, parents described the intervention as fun, interesting, and rewarding activities that they looked forward to doing with their children. The positive progress, such as positive changes in the children's behaviour, mood, and performance the parents have seen in their children and their enhanced interactions with their children were mentioned to have contributed to the positive experience reported by parents

> I enjoy doing psychosocial stimulation with my child. It is fun and interesting. We play games and sing songs. We laugh and have a good time together [FGD, Mother].

However, similar to most of the parents, nearly all of the health workers and intervention workers who facilitated the interventions also reported their positive experiences in delivering the intervention. One of the health workers mentioned the following underlining the motivation brought by the intervention and the associated better outcome seen in SAM children among his team members.

> . . . The intervention is, I think, rewarding. Compared to the time before, I have seen changes in SAM children. I see them improving their skills and becoming more active and engaged, which seems encouraging [KII, Health worker, Female]

The parents and health workers perceived the intervention to be relevant in terms of benefiting children with SAM. This possibly indicates the acceptability of the psychosocial stimulation interventions among the parents of children with SAM and health workers who have a crucial role in caring, feeding, stimulating, and supporting children during and after hospitalisation.

The feasibility of incorporating stimulation interventions into inpatient care of children with SAM

In this section, how psychosocial stimulation interventions could be adapted to fit the local context and the key barriers and opportunities that influence the effective implementation of the interventions are presented with due emphasis on the existing capacity of the health facilities operating in resource-limited settings. Health workers identified several factors that confer the opportunity for providing the intervention to hospitalised SAM children in the area. The alignment of stimulation interventions with the national guidelines for SAM management, the availability of functional SC at the level of health facilities with health workers and community health workers to deliver interventions at the health facility and community level, and the relative cost-effectiveness of the intervention were reported. In the same manner, challenges such as the lack of standards to guide the implementation of the intervention, material resources, human resources, time, tools, and the limited priority given to the intervention by the health system were repeatedly reported among the major challenges. Health workers mentioned that, except for information available in the few national documents, there were no documents such as guidelines or manuals to guide the standardisation and appropriate implementation of the intervention.

> We do not have enough information about the psychosocial stimulation intervention. No protocol or manual is available to guide the health workers and the health facilities in setting up and implementing the intervention [KII, Health Worker, Male].

Moreover, the lack of adequate resources, including play materials, space/ play area, and other essentials needed to administer the intervention was also mentioned among the challenges.

> The intervention needs resources and toys that are suitable for different ages and abilities of children. In most of the health facilities in our area, we do not have the required materials to do psychosocial stimulation with the children [KII, Woreda Heath Office Staff, Male].

> We do not have enough space to facilitate psychosocial stimulation with the children. As you can see, we are using this room, which is very small [KII, Heath Worker, Female].

The shortage of health workers in most health facilities was also mentioned as a challenge for the successful implementation of the intervention. Moreover, the lack of capacity-building training on psychosocial stimulation interventions, except for the very basic elements included in the training of SAM management was also stated.

> In our Woreda, we have a staff shortage. We only have a few health workers who provide nutrition services who are busy and overwhelmed making it challenging to implement the intervention regularly [KII, Woreda Heath Office Staff, Male].

> . . . To my knowledge, no capacity-building training was provided to the health workers on the psychosocial stimulation intervention except those trained through the research project implemented in our Zone [KII, Health Worker, Male].

Time was also found to be the major limitation on behalf of the health workers. The fact that the interventions demand dedicating several hours daily to administer the intervention and the additional time needed to prepare play materials, educate mothers about how to stimulate their children, and the related issues were mentioned as challenging. A health worker who participated in the delivery of the interventions reported the following when asked to share his thoughts about difficulties in delivering psychosocial stimulation interventions for children hospitalised with SAM.

> Psychosocial stimulation is a time-intensive intervention. We have too many other tasks and responsibilities to attend to daily [KII, Health Worker, Female]

The lack of adequate supervision and support to enable health workers to practice psychosocial stimulation interventions consistently and the lack of accountability mechanisms or incentives were also reported as challenges. Some health workers linked the lack of tools, capacity building initiatives, and accountability and supervision mechanisms toward psychosocial stimulation interventions with the inadequate priority given to the intervention at different levels of the health system. Lastly, the resistance or reluctance from some caregivers was also reported as a challenge in implementing psychosocial stimulation interventions.

The study has attempted to explore whether the interventions would be feasible to the parents, particularly mothers of the children, who could play a crucial role in providing a nurturing and stimulating environment for their children, and about the potential challenges that could hinder their ability to implement the interventions effectively and consistently. Among others, some social and gendered norms, such as the disproportionate household burden of women were reported to influence the intervention. Women in the area spend most of their time doing multiple household chores such as fetching water and firewood, cooking, cleaning, washing clothes, and caring for children. Hence, owing to their competing priorities, they may not have adequate time to stimulate their children at home. The lack of support from husbands and the inadequate engagement of men in the care of children were further mentioned to further limit the effective implementation of the stimulation intervention at the household level.

> Here, I am doing everything based on the direction of the health workers, as I have nothing to do except care for my sick child. This is not the case at home. I have many household responsibilities. How would I spend this much time playing with my kid and preparing his play materials? [KII, Mother].

Some respondents have also mentioned community-based misconceptions such as associating SAM with spiritual beliefs such as the will of God/ Allah or a curse to influence the mothers' intention, behaviour, and confidence in implementing the psychosocial stimulation interventions in a home setting. Lastly, health workers and those who participated from Woreda and Zonal Health Offices were asked about the potential feasibility of integrating the interventions with the routine medico-nutritional care given to hospitalised SAM children in the SC. According to most of the respondents, the intervention was viewed as feasible. However, a well-tailored mechanism to address the potential demand and supply side challenges relevant to the intervention should be designed to ensure the feasibility of the intervention in the area.

Discussion

The study has shown that the parents of children hospitalised for SAM lack adequate understanding about the problem of their children and its medical aspects until their children are diagnosed in the health facilities. Parents expressed concerns about the future health and growth of their children mainly because of the difficulties they experienced. Parents also stated community-based beliefs that attributed SAM to supernatural causes and expressed concerns that their children would face stigma or discrimination in the community. The community-based practice of using traditional and spiritual measures to manage SAM has also been reported.

Parental knowledge and practices are crucial factors in preventing and managing SAM among children as they have a key role in providing adequate nutrition, care, and health services to their children[22–24]. The understanding of parents about SAM and its impact on child health, growth, and development in Ethiopia is not well documented in the literature. However, the available studies suggest that many parents lack sufficient knowledge or skills to recognise the signs and symptoms of SAM, initiate appropriate feeding practices, seek timely medical attention, or follow up with treatment and rehabilitation programmes[25,26].

All parents reported that they had never heard of psychosocial stimulation interventions before the admission of their SAM children to the SC. However, most parents showed a good understanding of the interventions and expressed positive attitudes. The health education provided in integration with the interventions and the active engagement of parents in the delivery of the stimulation intervention were repeatedly mentioned by the health workers to have contributed to the observed level of understanding among parents. Health workers involved in the feasibility study of the Kusamala Program expressed similar thoughts and the counselling was mentioned to have improved the knowledge of the parents about the intervention[12]. A recent meta-analysis that reviewed 13 randomised controlled trials of psychosocial stimulation interventions in Low- and Middle- Income Countries also reported similar findings, where the intervention improved maternal knowledge about the development needs of children[27]. Similarly, health workers also reported the benefit of the interventions in terms of enhancing the outcome of hospitalised children and expressed their positive attitude.

Moreover, most parents expressed positive experiences with participating in psychosocial stimulation interventions for children with SAM, although some of them also shared their unpleasant experiences. The positive experiences of the parents were mainly attributed to the improvements in the behaviour, mood, and performance of children and enhanced interactions between the child and parents. In line with this, health and

intervention workers who facilitated the intervention reported positive experiences with the implementation of the intervention. The finding highlighted the acceptability of psychosocial stimulation interventions among both parents and health workers. A study conducted in Jimma, Ethiopia reported a similar finding where psychomotor/ psychosocial stimulation was well accepted by most caregivers. The positive changes seen in children's development and behaviour as well as improved parent-child interactions were reported as the prime factors for the acceptance of the intervention by mothers/caregivers[10]. Another qualitative study conducted to understand the perspectives of primary caregivers who participated in the Kusamala Program reported that the programme was viewed positively by caregivers, with many participants outlining concepts they learned and aimed to apply at home[12]. A pilot study conducted in rehabilitation centres across India has also reported the positive feedback of the mothers. The improvement in the children's development after the sensory/ psychosocial stimulation and structured play activities was reported as key motivator for the mothers to implement the interventions[28]. Health workers identified several opportunities and challenges to the effective integration of the intervention for the long-term benefits of children hospitalised with SAM. The fact that psychosocial stimulation interventions were already included in the national guidelines for SAM management, and the availability of functional SC in most public health facilities with health workers and community health workers were identified as key factors that confer the opportunity for the integration of the intervention with routine medico-nutritional care. Given the limited capacity of the health system in Ethiopia to afford the cost of resource-intensive interventions, health workers mentioned the relative cost-effectiveness of the intervention as the key contributor to the feasibility of the intervention.

Challenges such as the lack of an operational manual that guides the setting up and implementation of the intervention were reported as major challenges. Health workers mentioned that, except for little information available in the national documents, there was no document such as a guideline or manual that guides the standardisation and effective implementation of the intervention. Moreover, the lack of adequate resources, including play materials, space/play area, and other essentials needed to administer the intervention, was also mentioned as a major challenge. The shortage of health workers in most of the health facilities and the lack of capacity building training, except for the very basic elements included in the training of SAM management, were also identified. Moreover, time was also found to be the major limitation on behalf of the health workers. Lack of adequate supervision and support for service providers and lack of accountability mechanisms or incentives were also reported as challenges. Some health workers linked the lack of tools, capacity building initiatives, and accountability and supervision mechanisms toward psychosocial stimulation interventions with the inadequate priority given to the intervention at different levels of the health system.

A study conducted to assess the feasibility of implementing and sustaining the WHO guidelines for inpatient management of SAM identified that stimulation and an effective system of follow up were not implemented in under-resourced hospitals in rural South Africa. Among others, understaffing, inadequate training, high doctor turnover, nurses' inattentiveness, and insufficient interaction with carers were reported as the constraints[11]. A study conducted in Malawi reported prioritisation of other activities among health workers and staff shortages as the barriers to the implementation of the Kusamala Program that integrated psychosocial stimulation with nutrition, feeding; water, sanitation, and hygiene counselling[13]. Another pilot study conducted in India has also reported lack of adequate resource and space in Nutrition Rehabilitation Centers for execution of the age-appropriate activities[28].

Social and gendered norms such as the disproportionate household burden of women, the lack of support from husbands, and their inadequate engagement were reported to influence the effective implementation of the intervention. Despite the challenges, health workers, parents, and other participants reported the feasibility of integrating the interventions with the routine medico-nutritional care provided to hospitalised SAM children in the SC.

The study has some limitations that should be acknowledged. First, the study was conducted in one zone in Central Ethiopia, which may limit the generalizability of the findings to other settings. Second, the study used a qualitative approach that relied on self-reported data from the participants, which may introduce some biases in the data. Third, the study used a purposive sampling strategy that may not capture the diversity of the population of interest.

Conclusion

Psychosocial stimulation intervention was found to be acceptable by parents and health workers. The intervention was also feasible for implementation in health facilities operating in resource-poor settings. However, challenges that could potentially influence the effective implementation of the interventions were identified. The findings suggest the need to allocate more resources and support for the effective implementation of psychosocial stimulation interventions with the existing care provided for children with hospitalised SAM. This may include developing and implementing guidelines, standards, and appropriate monitoring mechanisms. At the health facility level, adequate resources, including play materials, space, and other essentials, should be available to regularly implement the intervention. Training programmes should also be designed for health workers. Education and awareness programmes targeting parents and the community could also be among the relevant interventions. Finally, more studies should be conducted to understand the perspectives of key stakeholders, including policymakers, civil society organisations, donors, and others.

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TT was the principal investigator of the study and contributed to the conceptual design, tool development, and manuscript preparation. TT and AA collected and analysed the data. AA, EM, FD, and TY contributed to tool development, data analysis, manuscript preparation, and finalisation. TA and TB provided technical support and contributed to conceptual design, manuscript preparation, and finalisation. All authors have read and approved the final version of the manuscript.

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 St. Paul's Hospital Millennium Medical College (Reference No–PM 23/144), Jimma University (Reference No-JHRPGO 380) and the Ethiopian National Research Ethics Review Committee (Reference No–MoSHE//RD/141/098/14). Verbal informed consent was obtained from all subjects where the consent was witnessed and formally recorded.

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RESEARCH ARTICLE

Association between diet quality during pregnancy of mothers and that of 3-year-old offspring: a prospective hospital-based birth cohort study

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Abstract

Children's eating habits are established early in life and parents play a major role therein. Pregnancy is a teachable moment for the promotion of healthy eating because many women are concerned about their babies' health and have frequent contact with healthcare providers. We aimed to investigate the association between diet quality during pregnancy and the offspring. A total of 691 sets of data on pregnant mothers and their 3-year-old offspring were obtained from the Seiiku Boshi cohort study. Dietary intake was assessed using a validated food frequency questionnaire for mothers in mid-to-late pregnancy and a validated diet history questionnaire for Japanese preschool children at the 36-month checkup. Diet quality was scored using the Japanese Food Guide Spinning Top. Maternal diet quality score was categorised into quartiles, and the association between offspring and maternal diet quality score, adjusted for socioeconomic factors, was assessed using multiple linear regression. The total offspring diet quality score showed a linear trend with the maternal diet quality score (the mean increments (confidential intervals) for each quartile were –0.12 (–1.32–1.08), 1.54 (0.34–2.73), and 1.22 (0.03–2.42)). In particular, offspring vegetable dishes scored higher in all quartiles of maternal vegetable dish scores than in the lowest quartile (0.69 (0.21–1.17), 0.97 (0.50–1.45), and 1.36 (0.88–1.83)). A high diet quality score during pregnancy was positively associated with that of offspring, suggesting the importance of nutritional education in pregnant women to improve their diet quality.

Key words: **Birth cohort: Diet quality: Japan: Preschool children: Pregnant women**

Introduction

Dietary habits and quality are important for healthy eating and growth to promote good health and prevent illness in adulthood.[1] Food behaviour and specific food choices are established early in life and become stable in the long term.[2] Interventions in school-age children are not always successful, with a systematic review of interventions to increase fruit and/or vegetable consumption in obese or overweight children showing inconsistent results, suggesting that they are unlikely to achieve long-term changes in dietary behaviour.[3]

The critical time for dietary change occurs between the ages of 1.5 years and 3 years, wherein the diet tends to move decisively toward an increased intake of added sugars, and lower fruit and vegetable intake tendencies are established.[4] Toddlers as small as 1–2 years old may not consume enough vegetables, cereals, and grains and instead consume discretionary foods.[5]

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Health behaviours are influenced by social environment and personal factors, as suggested by the model of triadic reciprocal causation[6]; social environmental factors play a major role in food choices. The mother's health behaviours and parenting strategies mediate the relationship between the effects of family-based interventions on the child's dietary behaviours and diet.[7] Furthermore, parental intake is positively associated with children's fruit and/or vegetable consumption,[8] especially among those who are conscious of eating all their vegetables.[9] Thus, encouraging mothers to maintain favourable dietary habits for children in early childhood may lead to the formulation of favourable dietary habits.

It is difficult for many mothers to improve healthy eating habits during the busy postpartum child-rearing period. First, mothers raising small children have limited access to advice on improving their dietary quality. Although health centres provide dietary advice to parents when their children undergo health checkups, this advice tends to focus on food and feeding practices.[10] Second, mothers of older preschool children may have less time to prepare food and spend time eating with their children due to work.[11,12] However, improving dietary habits during pregnancy may be more effective than providing nutritional education during the busy period after birth. Pregnancy may be a teachable period to promote healthy eating because many women are concerned about the health of their babies during pregnancy and are in frequent contact with their healthcare providers.[13,14] Indeed, antenatal nutrition education improves nutritional knowledge and dietary diversity.[15] Furthermore, a varied diet during pregnancy may lead to broader taste acceptance in offspring, and prenatal and early postnatal exposure to flavours enhance the infant's enjoyment of that flavour in solid foods during weaning.[16]

However, evidence regarding the diet quality of mothers during pregnancy being associated with the diet quality of offspring is scarce. Additionally, examining which food group intake increases in mothers may be effective to improve the quality of the offspring's diet would provide useful information for nutrition education; however, the number of studies is limited. Maternal diet quality during pregnancy is associated with that of the offspring at 14 years of age,[17] and maternal dietary variety is positively associated with higher dietary scores in children aged 2–4 years.[18] However, these studies did not examine the relationship between the dietary components of mothers and their children in detail. In a small sample study, diet quality during pregnancy was associated with a 2-year-old child's diet quality, with postpartum diet quality acting as a mediating factor.[19] However, in addition to overall diet quality, only vegetables and fruits were selected to examine the association with the child's diet.[19]

Studies have analysed the relationship between maternal dietary patterns during pregnancy and the child's diet; however, the relationship between maternal and offspring food group intakes has not been evaluated.[20,21] In a study of infants, maternal sugary beverage consumption during pregnancy was associated with higher sugary beverage intake among their offspring at 2 years of age; however, this study did not evaluate the overall diet quality.[22] We, therefore, aimed to investigate the association between diet quality during pregnancy in mothers and offspring at 3 years of age. We also examined the relationship between the components of the maternal and offspring diet to improve nutritional education for pregnant women to improve dietary health and choices in their offspring.

Participants and methods

The Seiiku Boshi cohort

Our study was based on the Seiiku Boshi cohort study, a prospective hospital-based birth cohort study conducted at the National Center for Child Health and Development (NCCHD) in Tokyo. The study design, recruitment, and data collection have been described in detail elsewhere.[23,24] We invited 4,164 pregnant women to participate in the study, of whom 2,309 pregnant women submitted written consent forms at their first prenatal visit before 14 weeks of gestation between May 2010 and November 2013. A baseline survey was conducted at recruitment, and subsequent surveys were conducted in mid-to-late pregnancy and after delivery annually. Including cases with multiple births, 2,403 children were born to these pregnant women at NCCHD. From July 2014 to October 2016, a 36-month follow-up, scheduled according to each child's birth month, was conducted at the NCCHD.

Ethical approvals

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the Institutional Review Board of the National Center for Child Health and Development (project number 417). Written informed consent was obtained from all participants.

Dietary intake

Maternal dietary intake was assessed using a self-administered food frequency questionnaire (FFQ) as part of a mid-to-late pregnancy survey. The FFQ consisted of 167 food and beverage items, with nine frequency categories and three portion size categories, which assessed the habitual consumption of the listed foods within the previous 2 months. Information on dietary supplements was not used to calculate the nutrient intake because of the lack of a reliable composition table in Japan. This questionnaire has been validated in adults[25] as well as in women in early and late pregnancy based on 3-day dietary records.[26–28] The intakes of energy, nutrients, and food groups were calculated using a food composition table developed for the FFQ based on the Standard Tables of Food Composition in Japan.[29]

Offspring dietary intake was assessed using the Brief-type self-administered diet history questionnaire for Japanese preschool children (BDHQ3y), which was developed to assess dietary intake during the preceding month for Japanese children aged 3–6 years.[30] This is a four-page, structured questionnaire with four sections: (1) intake frequency of 57 selected foods commonly consumed in Japan and non-alcoholic beverage items; (2) daily intake of rice, including the type of rice (refined, unrefined, or rice boiled with barley) and miso soup; (3) usual cooking methods; and (4) general dietary behaviour.

The BDHQ3y also collects information about the frequency of consumption of fortified food/supplements in the previous month but does not collect the names of the nutrients in the fortified food/supplement. Additionally, the questionnaire does not cover portion sizes of food consumed by the participants but rather assumes age-specific portion sizes of food and beverages, which have been calculated based on previous studies.[30,31] Estimates of the daily intake of food, energy, and selected nutrients were calculated using an ad hoc computer algorithm for the BDHQ3y,[30,31] using the nutrient composition chart of food included in the Standard Tables of Food Composition in Japan.[32]

Diet quality score

The Japanese Food Guide Spinning Top (JFGST) is based on the Dietary Guidelines for Japanese people and guides people on the types and amounts of food they should eat daily to promote health.[33] The Food Guide comprises five categories of dishes: grain dishes (rice, bread, and noodles); vegetable dishes (vegetables, mushrooms, potatoes, and seaweed); fish and meat dishes (meat, fish, eggs, and soybeans); milk (milk and milk products); fruits (fruit and 100% fruit juice); and snacks and beverages.[33]

JFGST reference values were created for the general population. Reference values for pregnant or nursing mothers have been proposed by the Japanese Ministry of Health, Labour and Welfare[34] by considering the additional energy and nutrients for pregnant and nursing mothers proposed in the Dietary Reference Intakes for Japanese. The JFGST reference values provided the recommended number of servings for each dish category for children under six years old, as created by the Tokyo Metropolitan Government.[35]

The diet quality score of adherence to the JFGST for the general population has been developed[36] and is associated with a lower risk of death from all causes and cardiovascular disease[36,37] among middle-aged to early elderly men and women. However, we applied several modifications which have been proposed to this method. First, we replaced the subscore for energy intake with the subscore for sodium intake. This was based on a previous amendment suggested for young women in Japan to identify more areas for dietary improvement among the weight-conscious age[38] in a population where excessive sodium intake,[39] as well as underweight and weight gain restrictions during pregnancy,[40] are important public health issues. The 10% tile value of sodium intake, adjusted for energy intake using the residual method, was used as the cut-off value for the sodium intake subscore. Second, as the intake levels of most micronutrients were below the dietary reference intake among pregnant women in Japan,[41] we did not penalise for exceeding servings of grains, meat and fish, and dairy products; thus, participants who exceeded the lower recommendation value of not only vegetables and fruits but also grains, meat, fish, and dairy products received a score of 10 for that category.[38] This method has been previously validated by comparing it to other methods of calculating diet quality[42] and has shown that a maternal balanced diet score is negatively associated with low birth weight risk.[43] The same amendments were conducted on the children's scores since current Japanese guidelines do not recommend using energy intake to evaluate the adequacy of energy intake among children (evaluation using a growth chart is recommended), and it is difficult to scientifically determine the upper limit for each food category for a growing child. Finally, the scores (0–10) of the seven components were summed to provide a modified JFGST score for pregnant mothers (JFGST-PM score) and children (JFGST-3y score) (Supplementary Table 1). The JFGST-PM and JFGST-3y scores range from 0 to 70, with higher scores indicating higher adherence to the current dietary guidelines. The summed score for the five food groups (grain dishes, vegetable dishes, fish and meat dishes, milk, and fruits), excluding snacks, beverages, and sodium, was calculated as a subscore ranging from 0 to 50.[44]

Covariates

Self-reported pre-pregnancy height and weight, weight gain during pregnancy, maternal age, sex of offspring, birth height and weight, smoking and drinking status during pregnancy, and history of childbirth were retrieved from medical records. The BDHQ3y also asks about current height, weight, and response date. If the child's data were missing, they were complemented with measurements obtained at the 36-month visit. We calculated the body mass index (BMI) from self-reported height and weight. Children were defined as overweight according to the criteria of the International Task Force on Obesity in Childhood using population reference data specific for age and sex for BMI.[45] Maternal sociodemographic data were obtained using questionnaires administered at enrolment and were categorised as follows: annual household income (<4 million yen, 4–6 million yen, 6–8 million yen, 8–10 million yen, 10–15 million yen, or >15 million yen, missing), maternal education (university/graduate school, technical college/two-year junior college, high school or less, missing), and employment status during pregnancy (housewife, part-time employee, self-employed, full-time employee, or other).

Statistical analysis

We analysed children, excluding those with either extremely low or high energy intake (< 1 percentile or over the 99th percentile), of the pairs that responded during both pregnancy and at 3 years of age. Our main focus was not only to determine whether maternal diet during pregnancy correlates with the diet of 3-year-old children, but also to provide information that could be referenced in nutrition education for mothers. For both of the new scoring methods, JFGST-PM and JFGST-3y, there is no clinically valid cut-off point for determining any risk. Therefore, participant characteristics and dietary intakes were compared to the JFGST-PM score categorised into quartiles (Q1, 13.93–40.35; Q2, 40.41–45.01; Q3, 45.05–49.82; Q4, 49.83–66.04) using a linear-by-linear test for trend.

Next, associations between the JFGST-PM and JFGST-3y total scores, as well as between the JFGST-PM five-dish group score and the JFGST-3y five-dish group score, and each component score of the mother and offspring, were tested using linear regressions with offspring score as the dependent variable and maternal score categorised in quartiles as independent

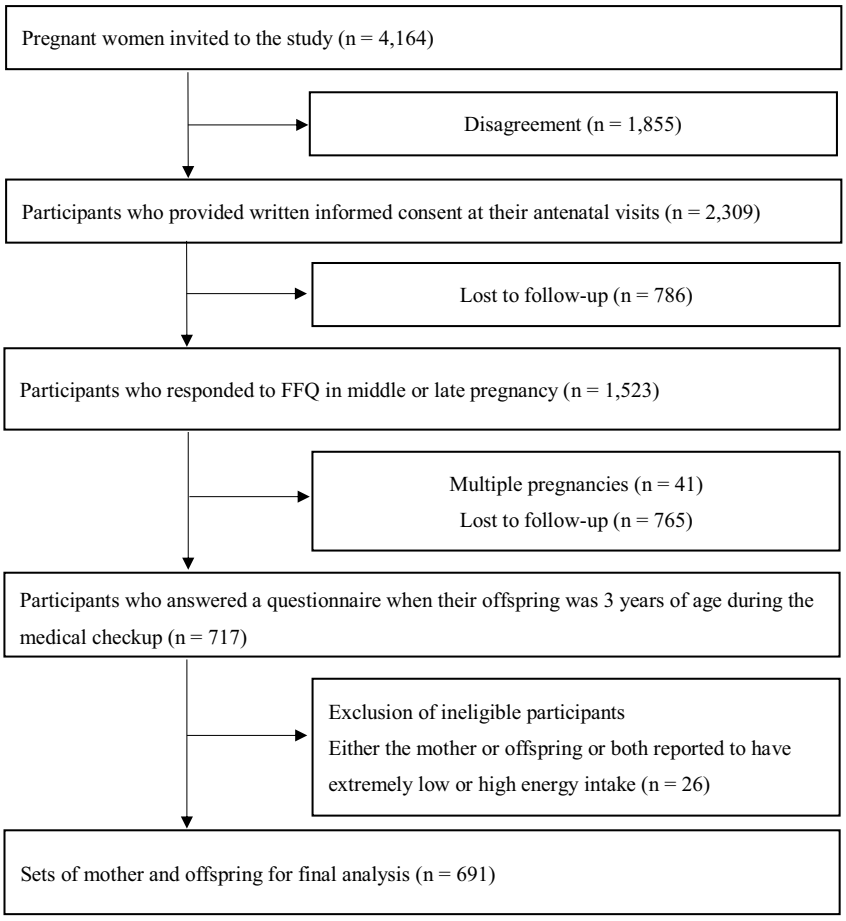

Fig. 1. Participants flow chart for the analytic sample.

variables. Indicators that could not be divided into quartiles due to the distribution of scores were divided as follows: second quantile for fish and meat dishes, tertiles for milk, and snacks and beverages. The adjusted models included the following potential confounders: sex of offspring, maternal age at birth, mother's educational history, and annual household income. Missing values were assigned to a "missing" category for each of these covariate variables. All statistical analyses were performed using Stata version 18.0 (Stata Corp. College Station, Texas). Two-sided P-values were presented.

Results

Of the 4,164 women who were invited to the study, 2,309 women provided written informed consent at their antenatal visits, 1,523 responded to the FFQ in middle or late pregnancy. Of these, 43 were multiple pregnancies, and 717 mothers of singletons answered the questionnaire when their offspring were 3 years of age during the medical checkup. Twenty-six results where the child, mother, or both reported extremely low or high energy intake (< 1 percentile, below 553 kcal in pregnant mothers or 803 kcal in offspring; or over the 99th percentile, more than 3,632 kcal in pregnant mothers or 2,705 kcal in offspring) were excluded.[23] In total, 691 dyads of mothers and 3-year-old offspring were included in the analysis (Fig. 1).

A comparison of mothers included in the analysis (n = 691) with those lost to follow-up, excluding extremely low or high energy intake (excluding under the first percentile or over the 99th percentile, n = 749) among responders to the FFQ during pregnancy (n = 1,523), showed that more of those included were older (36.5 (SD 4.2) years vs. 35.2 (SD 4.4) years, $P < 0.001$) and had their first child (65.8% vs. 56.0%, $P < 0.001$). However, fewer women were underweight before pregnancy (18.2% vs. 24.5%, $P = 0.034$) and had less weight gain during pregnancy (9.6 (SD 3.6) kg vs. 10.1 (SD 3.7) kg, $P = 0.019$). The prevalence was similar for both annual household income and educational history. There were no significant differences in the intake of dish categories other than vegetable dishes (3.8 (SD 1.9) servings vs. 3.5 (SD 2.1) servings, $P = 0.045$) (Supplementary Table 2 and Table 1).

The overweight rate among offspring was 8.6%, whereas the rate for maternal pre-pregnancy overweight was 4.8%. Sixty-two percent of mothers held a university/graduate school degree (Table 1).

The mean JFGST scores for mothers and offspring were 44.9 (SD 7.4) and 56.3 (SD 5.8), respectively. Of the maternal intakes of dish categories, the average number of servings in the grain dishes, vegetable dishes, and fruits were below the recommended number of servings for pregnancy, and the energy intake from snacks and beverages was above the recommended amount (Supplementary Table 3). Offspring diet quality score and each dish category score of vegetable dishes, fish and meat dishes, milk, and fruits were higher for higher quartiles of the maternal diet quality score, whereas the score of sodium was

Table 1. Characteristics of the study population according to the maternal diet quality score in quartiles

			JFGST-PM score in quartiles							
	All		Q1 (n = 173)		Q2 (n = 173)		Q3 (n = 173)		Q4 (n = 172)	
	Mean or No.	SD or %	Mean or No.	SD or %	Mean or No.	SD or %	Mean or No.	SD or %	Mean or No.	SD or %
Offspring characteristic										
Gender										
Boys	371	53.7	106	61.3	83	48.0	89	51.4	93	54.1
Girls	320	46.3	67	38.7	90	52.0	84	48.6	79	45.9
Height (cm)	92.2	3.8	92.4	3.7	92.1	3.7	92.3	4.3	92.2	3.6
BMI (kg/m^2)	15.9	1.4	15.9	1.3	15.8	1.3	16.1	1.6	15.8	1.3
Overweight[a]	59.0	8.6	18.0	10.5	13.0	7.5	18.0	10.4	10.0	5.8
Birth height (cm)	49.3	2.6	49.2	2.5	49.3	2.7	49.8	2.0	49.1	3.1
Birth weight (g)	3006	435	3008	464	3008	412	3050	394	2960	465
Maternal characteristic										
Age at birth (y)	36.5	4.2	36.6	4.4	36.2	4.1	36.7	3.9	36.7	4.3
Pre-pregnancy BMI (kg/m^2)	20.5	2.6	20.8	2.8	20.6	2.4	20.4	2.6	20.4	2.6
Pre-pregnancy BMI category										
<18.5 kg/m^2	125	18.2	28	16.5	31	18.0	28	16.4	38	22.1
18.5–24.9 kg/m^2	527	76.9	129	75.9	137	79.7	134	78.4	127	73.8
≥25 kg/m^2	33	4.8	13	7.6	4	2.3	9	5.3	7	4.1
Missing	6		3		1		2		0	
Gestational weight gain (kg)	9.6	3.6	9.4	3.8	9.6	3.8	10.0	3.4	9.5	3.4
History of childbirth										
First	455	65.8	98	56.6	117	67.6	121	69.9	119	69.2
Parity	236	34.2	75	43.4	56	32.4	52	30.1	53	30.8
Educational history										
High school or less	44	6.6	11	6.8	13	7.7	9	5.4	11	6.6
Technical college/two-year junior college	208	31.3	50	30.7	51	30.0	54	32.5	53	31.9
University/graduate school	413	62.1	102	62.6	106	62.4	103	62.1	102	61.5
Missing	26		10		3		7		6	
Employment status during pregnancy										
Housewife	273	41.1	66	40.7	84	49.7	60	35.7	63	38.2
Part-time employee	93	14.0	22	13.6	24	14.2	20	11.9	27	16.4
Self-employed	29	4.4	6	3.7	7	4.1	11	6.5	5	3.0
Full-time employee	247	37.2	62	38.3	49	29.0	71	42.3	65	39.4
Other	22	3.3	6	3.7	5	3.0	6	3.6	5	3.0
Missing	27		11		4		5		7	
Annual household income (yen/year)										
<4 million	48	7.6	15	9.6	14	8.7	13	8.2	6	3.8
4 million to less than 6 million	93	14.6	22	14.1	26	16.1	28	17.7	17	10.6
6 million to less than 8 million	129	20.3	36	23.1	32	19.9	26	16.5	35	21.9
8 million to less than 10 million	123	19.4	30	19.2	31	19.3	29	18.4	33	20.6
10 million to less than 15 million	173	27.2	33	21.2	42	26.1	47	29.7	51	31.9
>15 million	69	10.9	20	12.8	16	9.9	15	9.5	18	11.3
Missing	56		17		12		15		12	
Smoking status during pregnancy (yes)	6	0.9	1	0.6	3	1.7	0	0.0	2	1.2
Drinking status during pregnancy (yes)	11	1.6	3	1.7	2	1.2	6	3.5	0	0.0

JFGST-PM score; scores on the adherence to the Japanese Food Guide Spinning Top for pregnant mothers; BMI, body mass index.
[a]Classification by gender and age-specific cut-off points advocated by The International Obesity Task Force.

lower for higher quartiles of the maternal diet quality score (Table 2). For the intake of dish categories, the number of servings of vegetable dishes and fruits was higher in the higher quartiles of maternal diet quality scores (Table 2).

The mean energy intake for mothers and offspring was 1,619 (SD 507) kcal and 1,403 (SD 337) kcal, respectively. Maternal intake of energy and nutrients other than carbohydrates and dietary fibre was higher in the higher quartiles of the maternal diet quality score (Supplementary Table 4). Offspring energy, protein, retinol equivalent, vitamin B1, vitamin B2, vitamin C, calcium, ferric, dietary fibre, and potassium intakes were higher in the higher quartiles of maternal diet quality scores (Table 3).

Table 4 shows an increase in the average offspring diet quality total score and five-dish group scores across the high quartiles of maternal diet quality scores (P for trend <0.001). After adjustment for confounding variables, the mean increments of diet quality score of offspring for total score in the third and fourth quartile categories were 1.54 (0.34–2.73) and 1.22 (0.03–2.42) compared to the first category of maternal diet quality score. Similarly, adjusted mean increments of diet quality score of offspring for five-dish groups score in the third and fourth quartiles were 1.45 (0.39–2.52) and 1.65 (0.58–2.72) compared to the first category of maternal diet quality score. Maternal vegetable dishes, fish and meat dishes, milk, fruits, and

Table 2. Distribution of the scores on the adherence to the Japanese Food Guide Spinning Top for 3-year-old offspring and the intakes of each dish category according to the maternal diet quality score in quartiles

				JFGST-PM score in quartiles								
	Recommended No. of servings in the JFGST	All		Q1 (n = 173)		Q2 (n = 173)		Q3 (n = 173)		Q4 (n = 172)		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P^a
JFGST-3y score												
Total (0–70)		56.3	5.8	55.8	6.6	55.4	6.0	57.2	5.0	57.0	5.1	0.005
Five-dish groups (0–50)		40.0	5.1	39.2	5.8	39.1	5.4	40.7	4.5	40.9	4.5	<0.001
Grain dishes (0–10)		8.2	1.6	8.3	1.6	8.2	1.7	8.1	1.6	8.1	1.6	0.107
Vegetable dishes (0–10)		5.2	2.3	4.9	2.4	5.1	2.4	5.2	2.3	5.4	2.1	0.023
Fish and meat dishes (0–10)		9.2	1.5	9.0	1.7	9.1	1.5	9.3	1.3	9.3	1.3	0.028
Milk (0–10)		8.9	2.3	8.8	2.3	8.3	2.8	9.2	2.1	9.2	1.9	0.006
Fruits (0–10)		8.6	2.5	8.2	2.9	8.4	2.7	8.9	2.1	8.9	2.2	0.004
Snacks and beverages (0–10)		9.4	1.6	9.4	1.6	9.1	2.0	9.6	1.3	9.4	1.5	0.368
Sodium (0–10)		7.0	2.1	7.1	2.3	7.2	2.1	6.9	2.2	6.7	2.1	0.020
Intakes of dish categories (/day)												
Grain dishes (serving)	3–4	2.1	0.6	2.2	0.6	2.2	0.6	2.1	0.6	2.1	0.5	0.147
Vegetable dishes (serving)	4	1.8	0.9	1.7	0.9	1.8	0.9	1.9	0.8	2.0	1.0	0.018
Fish and meat dishes (serving)	3	2.9	1.0	2.8	1.0	2.8	1.0	2.9	0.9	2.9	0.9	0.204
Milk (serving)	2	2.4	1.4	2.4	1.5	2.3	1.5	2.5	1.2	2.6	1.4	0.145
Fruits (serving)	1–2	0.7	0.5	0.6	0.5	0.7	0.5	0.7	0.4	0.8	0.5	0.002
Energy intake from snacks and beverages (kcal)	≤200	142	79	146	76	150	87	136	69	137	83	0.146
Energy-adjusted Sodium intake (mg)	–	3635	680	3599	708	3575	673	3650	688	3717	647	0.065

JFGST, Japanese Food Guide Spinning Top; JFGST-PM score, scores on the adherence to the JFGST for pregnant mothers; JFGST-3y score, scores on the adherence to the JFGST for 3-year-old.
[a]P values are based on linear-by-linear test for trend.

Table 3. Energy and nutritional intakes of 3-year-old offspring according to the maternal diet quality score in quartiles

			JFGST-PM score in quartiles								
	All		Q1 (n = 173)		Q2 (n = 173)		Q3 (n = 173)		Q4 (n = 172)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P^a
Energy (kcal)	1403	337	1337	314	1406	331	1445	352	1423	344	0.010
Protein (%E)	15.0	2.1	14.8	2.0	14.7	2.3	15.1	2.0	15.3	2.0	0.011
Fat (%E)	28.6	4.8	28.7	4.6	28.6	5.2	28.7	4.9	28.5	4.6	0.773
Carbohydrate (%E)	55.3	6.0	55.3	5.8	55.6	6.5	55.2	6.0	55.2	5.7	0.723
Retinol equivalent (μg/1000kcal)	357	144	332	127	348	140	369	149	380	155	0.001
Vitamin B1 (mg/1000kcal)	0.41	0.07	0.40	0.06	0.41	0.07	0.41	0.06	0.42	0.07	0.013
Vitamin B2 (mg/1000kcal)	0.73	0.16	0.72	0.16	0.70	0.17	0.74	0.15	0.75	0.15	0.012
Vitamin C (mg/1000kcal)	53.6	22.2	49.7	22.9	52.8	23.4	54.5	20.4	57.5	21.4	0.001
Calcium (mg/1000kcal)	429	125	419	130	411	129	437	117	449	122	0.006
Ferric (mg/1000kcal)	3.9	0.8	3.8	0.8	3.9	0.8	3.9	0.7	4.0	0.8	0.002
Dietary fibre (g/1000kcal)	6.4	1.6	6.1	1.5	6.3	1.6	6.4	1.4	6.7	1.7	0.002
Saturated fatty acids (%E)	9.7	2.4	9.8	2.5	9.6	2.6	9.7	2.1	9.7	2.3	0.761
Sodium (g/1000kcal)	6.6	1.3	6.6	1.4	6.5	1.2	6.6	1.2	6.8	1.2	0.218
Potassium (mg/1000kcal)	1272	262	1231	254	1245	270	1288	249	1323	267	<0.001

JFGST-PM score; scores on the adherence to the Japanese Food Guide Spinning Top for pregnant mothers.
[a]P values are based on linear-by-linear test for trend.

sodium scores correlated with offspring scores (P for trend <0.05, respectively). In particular, vegetable dishes scored higher in all quartiles compared to the lowest quartile (2nd, 0.69 (0.21–1.17); 3rd, 0.97 (0.50–1.45); and 4th, 1.36 (0.88–1.83)).

Discussion

Analyses based on 691 mother–offspring pairs who participated in the Seiiku Boshi cohort study showed that a high diet quality score during pregnancy was associated with that of 3-year-old offspring. Of the components of the diet quality score, maternal vegetable dishes, fish and meat dishes, milk, fruits, and sodium were associated with the offspring components of the diet quality score, with vegetable dishes being the most strongly associated. These results suggest that improving the overall quality of the maternal diet during pregnancy, especially by increasing vegetable intake, can be one of the ways to improve the quality of the offspring's diet.

Table 4. Unadjusted and adjusted differences in mean increments in the diet quality score of offspring according to the maternal diet quality score in quartiles[a]

	Unadjusted				Adjusted for covariables			
	Increment	95% CI		*P*	Increment	95% CI		*P*[b]
JFGST-3y for total score (0–70) in quartiles of JFGST-PM for total score								
Quartile 1 (13.93–40.35)	Ref			–	Ref			–
Quartile 2 (40.41–45.01)	−0.37	−1.58	0.83	0.544	−0.12	−1.32	1.08	0.843
Quartile 3 (45.05–49.82)	1.44	0.23	2.64	0.020	1.54	0.34	2.73	0.012
Quartile 4 (49.83–66.04)	1.21	0.01	2.42	0.049	1.22	0.03	2.42	0.045
P for trend				<0.001				0.006
JFGST-3y for five-dish groups score (0–50) in quartiles of JFGST-PM for five-dish groups score								
Quartile 1 (3.93–27.70)	Ref			–	Ref			–
Quartile 2 (27.72–33.26)	1.11	0.03	2.19	0.044	1.06	0.00	2.13	0.051
Quartile 3 (33.27–37.82)	1.46	0.38	2.53	0.008	1.45	0.39	2.52	0.008
Quartile 4 (37.83–50.00)	1.87	0.79	2.95	0.001	1.65	0.58	2.72	0.002
P for trend				0.001				0.002
Grain dishes (0–10) in quartiles of maternal grain dishes score								
Quartile 1 (1.05–4.245)	Ref			–	Ref			–
Quartile 2 (4.25–5.642)	0.14	−0.20	0.48	0.428	0.18	−0.17	0.52	0.313
Quartile 3 (5.643–6.908)	0.02	−0.32	0.36	0.918	0.02	−0.32	0.36	0.893
Quartile 4 (6.909–10.00)	−0.18	−0.52	0.16	0.306	−0.14	−0.49	0.20	0.410
P for trend				0.235				0.291
Vegetable dishes score (0–10) in quartiles of maternal vegetable dishes score								
Quartile 1 (0–4.11)	Ref			–	Ref			–
Quartile 2 (4.12–5.81)	0.70	0.23	1.18	0.004	0.69	0.21	1.17	0.005
Quartile 3 (5.82–7.81)	0.96	0.49	1.44	<0.001	0.97	0.50	1.45	<0.001
Quartile 4 (7.88–10.00)	1.37	0.90	1.85	<0.001	1.36	0.88	1.83	<0.001
P for trend				<0.001				<0.001
Fish and meat dishes (0–10) in 2-quantile of maternal fish and meat dishes score								
Two-quantile 1 (0–7.58)	Ref			–	Ref			–
Two-quantile 2 (7.63–10.00)	0.35	0.10	0.60	0.007	0.36	0.10	0.61	0.007
Milk score (0–10) in tertiles of maternal milk score								
Tertile 1 (0–5.08)	Ref			–	Ref			–
Tertile 2 (5.13–8.73)	0.44	−0.05	0.93	0.079	0.41	−0.08	0.90	0.100
Tertile 3 (8.75–10.00)	0.55	0.13	0.98	0.010	0.49	0.07	0.91	0.022
P for trend				0.013				0.028
Fruits score (0–10) in quartiles of maternal fruits score								
Quartile 1 (0–2.439)	Ref			–	Ref			–
Quartile 2 (2.442–4.26)	0.50	−0.02	1.02	0.058	0.48	−0.04	1.00	0.068
Quartile 3 (4.27–6.868)	0.85	0.33	1.37	0.001	0.71	0.18	1.23	0.008
Quartile 4 (6.874–10)	0.95	0.43	1.47	<0.001	0.93	0.41	1.45	<0.001
P for trend				<0.001				<0.001
Snacks and beverages score (0–10) in tertiles of maternal snacks and beverages score								
Tertile 1 (0–3.92)	Ref			–	Ref			–
Tertile 2 (3.96–9.39)	−0.04	−0.39	0.30	0.806	−0.09	−0.44	0.26	0.613
Tertile 3 (9.40–10)	0.15	−0.14	0.45	0.310	0.13	−0.17	0.43	0.402
P for trend				0.240				0.300
Sodium score (0–10) in quartiles of maternal sodium score								
Quartile 1 (0–3.37)	Ref			–	Ref			–
Quartile 2 (3.39–5.7491)	0.23	−0.22	0.68	0.317	0.18	−0.28	0.63	0.452
Quartile 3 (5.7494–7.78)	0.37	−0.08	0.82	0.110	0.39	−0.07	0.84	0.098
Quartile 4 (7.81–10.00)	0.51	0.05	0.96	0.028	0.48	0.03	0.94	0.038
P for trend				0.023				0.024

JFGST, Japanese Food Guide Spinning Top; JFGST-PM score, scores on the adherence to the JFGST for pregnant mothers; JFGST-3y score, scores on the adherence to the JFGST for 3-year-old; CI, confidence interval.
[a]The group of the lowest quartile was used as a reference for each pairwise comparison.
[b]Adjusted for offspring sex, gestational age, mother's educational history, and annual household income.

Previous studies in other countries and at different offspring ages have confirmed that diet quality during pregnancy has a long-term effect on both mother and offspring. A large national birth cohort in Norway showed that maternal diet quality during pregnancy is associated with the diet quality of offspring at 14 years of age.[17] A European birth cohort study showed that maternal dietary variety, consisting of five food group scores, was positively associated with that of a higher score of children aged 2–4 years.[18] A prospective study of 52 mother–child dyads in Australia found an association between pregnancy and

a 2-year-old child's diet quality.[19] A study of 1,171 pairs in Canada showed that maternal sugary beverage consumption during pregnancy was associated with higher sugary beverage intake among offspring at age two.[22] The novelty of our study lies in that we showed, in a prospective design with a large population, that the quality of the mother's diet during pregnancy affects the diet quality of their 3-year-old offspring, which is the formative period of eating habits.

We examined which dish categories mothers could increase to affect the dish categories of their offspring's diets. We showed that the establishment of fish and meat, milk, fruits, and especially vegetable intake and decreasing sodium intake during the gestational period may contribute to improving offspring diet quality. Women do not appear to consume a wide variety of nutritious foods during pregnancy[41,46]; therefore, expert guidance to build an optimal diet during pregnancy is important.

We also observed that vegetables showed the lowest scores among all categories for offspring and that many mothers, even those with the highest overall diet quality score, did not meet the recommended values for vegetables. Thus, increasing vegetable intake among mothers may be a promising intervention to improve maternal and offspring diet quality. The strong association observed between maternal and offspring vegetable intake is likely due to two factors: either maternal vegetable intake directly influences the offspring's personal preference, or indirectly, the offspring are exposed to vegetables through the mother's postnatal diet. As shown in a previous small study of 52 mother-infant dyads, vegetable intake during pregnancy is strongly correlated with postnatal vegetable intake, which acts as a mediator of the association with offspring intake, suggesting that the indirect effect is stronger than the direct effect.[19] Additionally, limited but consistent evidence has shown that foetal exposure to flavours of certain vegetables, such as carrots and garlic, contained in the maternal diet and transmitted through the amniotic fluid increases the acceptance of the exposed flavour during infancy and potentially during childhood.[16] Similar findings have been reported for maternal fruit and vegetable variety.[19]

In this study, maternal sodium score positively correlated with offspring sodium score, whereas the group with a higher quartile of overall maternal diet quality score had lower offspring sodium scores. Our findings are consistent with a systematic review of Japanese studies in which a higher frequency of consumption of meals consisting of grain, fish, meat, and vegetable dishes was related to not only a higher intake of energy, protein, vitamins, and minerals, and higher odds of meeting the Dietary Reference Intakes for Japanese,[47] but also a higher intake of sodium. This may be a unique cultural problem in Japan because Japanese diets tend to contain high levels of sodium.[39] Thus, when nutritional education is provided to mothers to increase their intake of fish and meat dishes, and vegetable dishes, it is also important to educate them on low-sodium cooking methods.

Unlike in previous studies,[22] the maternal scores for snacks and beverages during pregnancy were not associated with the offspring scores in our study. This may be due to the current criteria for snacks being less strict in Japan compared to other countries where the evaluation criteria are set at "less than 200 kcal" for both parents and children, leading to many children getting a score of 10 for snacks in this study. The average intakes of snacks and beverages were 252 kcal for mothers and 142 kcal for children. Thus, using stricter criteria such as portion size may better differentiate children and lead to an association with maternal intake.

In the present study, no association was found between the maternal diet quality score and children's energy intake from fat, saturated fatty acids, and carbohydrates. This may be partly because the categories strongly associated with the maternal diet quality score were vegetable dishes and fruits, which had a low impact on fat and carbohydrate intake. In contrast, according to the studies that assessed adherence to the JFGST in young women, higher dietary adherence was associated with higher intake of protein, carbohydrates, vitamins, and minerals and lower intake of total and saturated fat.[38,48] Since the JFGST expresses diet quality in terms of dish units per category and does not consider food choices within food groups, the dietary characteristics reflected in scores may vary according to the participants' food choices. The mean energy percentages of fat and saturated fatty acids for the children in this study were 28.6% and 9.7%, respectively, which were lower than the target amounts in the Dietary Reference Intakes (20–30% and <10%, respectively)[49]; therefore, these were not high on the priority list as restriction targets. However, to avoid excessive intake of fat and saturated fatty acids, it is advisable to avoid meat as the only main dish and choose low-fat dairy products.

In our study, the absolute mean difference in JFGST-3y scores among the quartile groups of JFGST-PM scores was relatively small compared with that in previous studies on diet quality scores conducted among adults and older children. This may be attributed to the fact that the proportion size was small in Preschool children. As it has been reported that unfavourable eating habits such as increased intake of free sugars, and lower fruit and vegetable intakes tend to be acquired around 1.5–3 years of age,[4] and as portion size increases with age, we believe the absolute difference in scores observed in this study may underestimate its true long-term effect.

Our study suggests that improving the dietary habits of pregnant women can have benefits not only for the women themselves but also for their offspring. Currently, individualised nutrition education for pregnant women is mainly provided to those who have developed specific nutritional problems, such as gestational hypertension, obesity, or anaemia, which are eligible for medical reimbursement in Japan. However, considering that a mother's eating habits can influence the long-term dietary choices of her children, it may be beneficial to provide nutrition education even to those without existing nutritional problems. Since pregnant women have regular healthcare visits during their pregnancy, there is an opportunity to incorporate nutrition education from a registered dietitian alongside medical advice from an obstetrician. Specifically, our study highlights the importance of increasing intake of fish and meat, milk, fruits, and especially vegetables during pregnancy while also emphasising the need for education on reducing sodium in cooking. Given that adopting these dietary changes may require more than a single education session, it would be beneficial to provide

ongoing instruction during antenatal visits and set short-term goals through regular follow-ups until these habits become established.

The strengths of our study include its prospective design, use of diet quality rather than focusing on single nutrients or foods, and utilisation of dietary and food frequency questionnaires validated for pregnant women[26–28] and children.[30,31] However, this study had some limitations. First, our diet quality index did not consider energy intake or the upper limit of each food group[38] and could have led to an overestimation of diet quality if high energy intake and overeating were prevalent. However, the prevalence of such participants was likely low, as the average number of servings in all categories was below the recommended number, with the exception of snacks and beverages for pregnant women and milk for preschool children. The proportion of overweight and obese children was also very low among both pregnant women and preschool children in our population.[49] In the previous studies, the methods for calculating diet quality scores were adjusted to align with the characteristics of the participants.[36–38,44] Further research is needed to assess the suitability of the calculation method employed in this study for pregnant women and their offspring. Second, we utilised a diet quality score that did not consider the quality of foods within a food group, such as red meat among meats, or whole grains among cereals. Since the JFGST itself does not consider the quality of foods, it is unclear whether the same results would be obtained if dietary quality were assessed using the methods used in other countries. Third, the dietary questionnaires used for the offspring diet survey and maternal dietary questionnaire during pregnancy were created independently and had different question formats, which may have underestimated parent-offspring correlations. Fourth, we did not make adjustments for physical activity, smoking, alcohol intake during pregnancy, or the introduction of foods, factors that have been considered in other studies.[17,18] However, even in these studies, the effects of these covariates were found to be relatively small. Fifth, this study was conducted at a single perinatal centre in an urban area that generally had more pregnant women of advanced age,[50] higher socioeconomic status, and more pregnant women with complications. These differences may have led to a higher diet quality than in the general population but may also have contributed to the higher accuracy of responses to both maternal and offspring dietary questionnaires. Although a nationwide survey reported a positive correlation between annual household income and vegetable intake,[51] the number of servings of vegetable dishes in this study was similar to that observed in another nationwide birth cohort study.[43] Lastly, because pre-pregnancy intake data was not collected in this study, we cannot demonstrate changes in dietary intake specifically due to pregnancy. However, according to a nationwide birth cohort study in Japan, food intake did not significantly differ from pre-pregnancy to pregnancy, with no notable improvement in diet observed after pregnancy.[41]

In conclusion, a high score of diet quality during pregnancy in mothers affects that of their 3-year-old offspring. Increasing maternal fish and meat, milk, fruits, and especially vegetable intake and decreasing sodium intake from the gestational period can be one of the ways to improve the quality of the offspring's diet. These results suggest the usefulness of nutrition education to improve maternal diet quality during pregnancy when there are more opportunities to interact with healthcare providers.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.24

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Competing interests

The authors declare none.

Authorship

The authors' contributions were as follows: Y.T. contributed to the conceptualisation of the study question, conducted the statistical analysis, interpreted the data, prepared the first draft of the manuscript, and had primary responsibility for the final content of the manuscript; K.I. contributed to the conceptualisation of the study question, interpreted the data, and provided input into the final draft of the manuscript; A.P. contributed to the statistical analysis, interpreted the data, and provided input into the final draft of the manuscript; H.T., K.O., M.K., R.H., and T.F. contributed to the concept and design of the survey, coordination of the fieldwork, data collection and management, and provided input into the final draft of the manuscript; N.M. contributed to the concept and design of the survey, conceptualised the study question, interpreted the data, and provided input into the final draft of the manuscript. All authors were involved in writing the paper and provided their final approval for the publication of the submitted version.

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OBITUARY

Michael J. Gibney (1948–2024): a man of his time who had the time of his life

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Professor Mike Gibney, who died on 23 February 2024, was born in 1948 into a family of six children living on the north side of Dublin, to a father trained as a carpenter, who became a trade union leader, and a mother, formerly a nurse, who, like many mothers of that era, was highly ambitious for her children. That ambition proved to be very well-founded as Ireland would soon be entering a period of rapid economic, social and cultural change. As well as the post-war developments that were benefiting many developed countries of Western Europe, the loosening of ties with the Catholic Church and the yet-to-be-realised impact of joining the European Union in 1973 would prove to be transformative for the Irish population. Mike Gibney, a child of that time, would progress to become one of the most outstanding academics and science communicators of his generation. For many of his colleagues, students and collaborators, his gift for friendship, love of conversation and conviviality and the importance he gave to mentoring and developing other people will live long in their memories and in their lives.

Today, it might seem inevitable that a bright, articulate and focused young man was more than likely to succeed in whatever career he chose. But Mike's story of how he came to follow an academic career in human nutrition, from an undergraduate degree in agricultural chemistry to founding director of the Institute of Food and Health at University College Dublin, did not follow a linear process. Nevertheless, in his autobiography, 'A Life in Food: A Grain of Salt and Some Humble Pie'[1], he lays down some pointers, which suggest his subsequent career was at least in part determined by his undergraduate preference to study animal rather than plant biology, and a master's project involving the analysis of the fatty acid composition of adipose tissue of growing lambs, indicators of interests that foreshadowed a significant part of his research in later years.

This paper has been published in the *British Journal of Nutrition* as well as *Public Health Nutrition*, *Journal of Nutritional Science*, *Proceedings of the Nutrition Society*, *Nutrition Research Reviews*, and *Gut Microbiome*.
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Mike was offered several PhD posts and, with his new wife Jo, chose to leave Dublin in 1973 to study for his PhD in Sydney, Australia under Dr Don Walker, a leading researcher in the nutrition of neonatal lambs. His experimental research was technically demanding, but the outcomes were successful[2], and Mike made relative light of his heavy workload despite the additional responsibilities he held as a teaching fellow and later an assistant lecturer. Mike and Jo left Sydney in 1976 with Mike's PhD in hand and two children (plus one on the way). This was a formative and productive time, with Mike learning the tools of his trade as well as founding a family that would prove to be the core of his life for the next 50 years.

His next appointment back in Dublin was less auspicious but ultimately determined his migration towards human nutrition research. His post was funded by a government post-doctoral fellowship for returning Irish PhD students who had studied abroad. So limited were the facilities at the Agricultural Institute which hosted him and the supervision non-existent that Mike was left pretty much to his own devices and in his own words 'began to dabble in human nutrition'. Mike and Dr Pat Upton (Veterinary College of Ireland) began to compute nutrient intakes for the Irish population using household budget surveys and retail price data. This led to a presentation on patterns of fatty acid intake in Ireland at the Nutrition Society in London for which he was complimented by Professor Robert McCance[3]. This unexpected support from such an eminent scientist buoyed him up for months, and the successful dabbling into population food intake data foreshadowed another road he would take in his later work.

Mike decided that his present post was a lost cause and started seeking an opportunity that could strengthen his credentials as an aspiring human nutrition researcher. He was initially offered a 2-year post in nutrition at the University of Southampton which appeared ideal except for the short-term nature of the contract. He wavered and decided it was too risky for the family, but Jo intervened knowing that his heart was in the Southampton post. In 1977, Mike joined the Medical School of Southampton University, where he would stay for 7 years enjoying the 'majestic labs' of Southampton and the company of academics and researchers from globally leading universities. He immersed himself in the world of mechanistic nutrition, including working with an animal model of human atherosclerosis which would give him his first paper in experimental biology (his 'badge of honour')[4]. But he also began to work with human volunteers investigating the effects of fibre on blood pressure, post-prandial lipids and measurement of human antibodies to dietary proteins. At Southampton, he became fully involved in the Nutrition Society, working with Bob Grimble as assistant programme secretary and immersing himself in the joys of regular science meetings around the UK.

Around this time, Mike started providing science articles to the *Guardian* newspaper, some of which included challenges to conventional wisdom on topics such as the role of antioxidants in CVD, which provoked a 'snooty response from Sir Richard Doll' but later proved to be a pretty accurate assessment. And so the scene was set for the full emergence of Mike Gibney – experimental scientist, energetic collaborator, scientific communicator and enthusiastic myth buster.

It was time to go home.

In 1984, Mike was offered and accepted a post at Trinity College, Dublin, in the Department of Clinical Medicine, where his work flourished, gaining the title of Professor of Nutrition and leading many successful collaborations, including his work on post-prandial lipid metabolism. Over the next few years, his persuasive charm, leadership and strong networks contributed to major developments in nutrition at the national level in Ireland. A meeting convened by Professor Gerry McKenna (University of Ulster) formalised a relationship between universities in Dublin, Ulster and Cork – the Irish Universities Nutrition Alliance, which has continued to be highly successful with strong research and teaching programmes funded nationally and via the European Union. The three leaders of the Irish Universities Nutrition Alliance, Mike Gibney, Sean Strain and Albert Flynn, worked together in great harmony forming not only a powerful scientific collaboration but a friendship that would last their lives. Mike called them the 'Three Amigos' who, to be sure, would be seen in great conviviality at most meetings of the Nutrition Society. The formation of the Irish Section of the Nutrition Society, which many had considered would take years to achieve, was established in 1988, propelled by the work of Dr Fred Andrews with Mike as co-plotter, and soon-to-be first chair of the Irish Section of the Nutrition Society. Mike's membership in the Nutrition Society Council and his drive and leadership skills inevitably led to an invitation for him to become the next president of the Society. He readily accepted and served from 1995 to 1998 establishing working groups to carry out several strategic initiatives, which included developing stronger links with Europe, the formation of a Register of Nutritionists (now the Association for Nutrition) and the launch of a suite of nutrition textbooks aimed at undergraduate and post-graduate students. The latter he had described as 'the craziest project I have ever undertaken' proved to be very successful, generating income for the Society and a highly regarded set of textbooks now under the second editor-in-chief, Professor Sue Lanham-New.

At Trinity College, Mike launched himself into Europe, becoming a member of the European Scientific Committee on Food and of a collaboration known as Euronut led by Professor Jo Hautvast of the University of Wageningen, which aimed to encourage pan-European collaboration. Mike became a huge admirer of Jo Hautvast and vowed to emulate Jo's style of leadership – positivity, vision and generosity. I was a beneficiary of Mike's leadership with my own first big Framework grant in 1992, in which Mike was a collaborator. His support for me and enthusiasm for the work we were doing gave me confidence in making tough decisions and embedded my life-long respect and affection for this generous man. We collaborated again on the LIPGENE project, a much larger consortium with Mike as coordinator and Professor Julie Lovegrove as lead at the University of Reading. Mike's wife Jo became the administrator on LIPGENE, proving to be an outstanding appointment as the go-to person for all the issues that arose during these complex and lengthy projects. Mike and Jo came as a pair, always succeeding in hitting deadlines, no matter how gruelling the barriers, bureaucracy and frustrations that work of this size can generate.

Mike's move from Trinity College to University College Dublin in 2006, although controversial at the time, was part of his vision to advance the discipline of human nutrition – first, to exploit the development of metabolomics and data analytics to advance understanding of the effects of diet on human metabolism[5] and, second, to bring nutrition into an academic environment where integration between relevant centres of excellence could allow solutions to big global challenges, such as climate change and obesity, to be developed. This bold and unexpected move to University College Dublin with his team to form the virtual Institute of Food and Health can be logically traced through his scientific career and his journey as a decisive leader, informed risk-taker and integrative nutrition scientist[1].

Since 'retiring' Mike further developed his skills and inclination as a myth buster, offering a challenge to conventional thinking such as the benefits of a sugar tax on obesity[6] and criticising the concept of ultraprocessed foods[7]. Mike did not direct his views to any particular vested interest – all have been the object of his active brain and incisive analyses. Wherever he saw sloppy thinking, lack of an evidence base, insufficient safety or impact data and, at times, the absence of sheer common sense, he would be inclined to 'stick his head above the parapet'. He understood that some thought him wrong and excessively opinionated, but he remained an iconoclast to the last.

Mike collaborated with and provided advice to parts of the food industry. His repost to those who claim this could be a conflict of interest and a potential source of bias was brisk, 'Scientific integrity is a necessary virtue of any researcher, and the only adjudicator of your compliance is your own conscience. Working with industry does not diminish science. Only you can do that, with whomsoever you collaborate'.

Mike's ferocious work ethic, ambition and drive might suggest his attention to family matters could have been restricted. But he married Jo, and she made sure this could never be the case. They parented three children through the journey from Dublin to Sydney and back, then Southampton and back to Dublin, using the same love of conversation and conviviality that was evident in his working life. Their home was an open house to their friends and their children's friends and to extended family, as well as to the many colleagues who became part of their lives. Eileen, Sinead and Michael are talented, successful, independent people with families of their own, whose love for their father shone through the extraordinary day of mourning and celebration of the life of their beloved father on 28 February 2024. The family were comforted in their loss by the hundreds of people who joined the congregation at St. John the Baptist's Church, Blackrock, Dublin.

The three amigos have become two, and none of us will see his like again.

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LETTER TO THE EDITOR

Vitamin K supplementation and cardiovascular risk factors: a critical appraisal

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Key words: **Bias: Cardiovascular risk factors: Certainty of evidence: Vitamin K**

We read with pleasure the systematic review and meta-analysis by Zhao *et al.*[1] which provided valuable insight into the role of vitamin K supplementation in risk factors associated with cardiovascular disease. The authors did a great effort in their meta-analysis to clearly exhibit any effect, or lack thereof, of vitamin K supplementation on blood glucose levels, HbA1c, insulin resistance, homeostatic model assessment insulin resistance (HOMA-IR), body weight, body mass index (BMI), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, C-reactive protein (CRP), and blood pressure. The findings are certainly interesting, but we find it necessary to point out a few inconsistencies in the methods and results that could potentially mislead or confuse the readers.

In the results, the authors have mentioned that 'HOMA-IR was significantly reduced following vitamin K supplementation compared (WMD: –0.24, 95% confidence interval (CI): –0.49, –0.02, $P = 0.047$) with placebo", but the forest plot displays the upper bound of the 95% CI as 0.02. This crosses the line of no effect, which is 0 in the case of continuous outcomes.[2] We tried to resolve this ambiguity by pooling the data provided by the authors and obtained the same result. The authors seem to have misinterpreted the forest plot for HOMA-IR.

Additionally, the authors used the NutriGrade scoring system to evaluate the certainty of evidence.[3] However, we found some oversight in its application. First, this scoring system is based on seven items, one of which is publication bias, as mentioned by the authors in their methods section. However, they have not described the methods employed to assess the publication bias and no such assessment is presented in the results. We sought to identify publication bias by constructing funnel plots for outcomes with more than ten studies[4] and Doi plots with the associated Luis Furuya-Kanamori (LFK) index for articles with less than ten studies[5] using the data provided. While we found no publication bias in the outcomes of glucose, total and LDL cholesterol, and systolic blood pressure, we observed sufficient evidence for publication bias in the remaining outcomes: insulin (LFK index = –1.72, minor asymmetry), HbA1c (LFK index = –4.06, major asymmetry), HOMA-IR (LFK index = –2.87, major asymmetry), weight (LFK index = 8.14 major asymmetry), BMI (LFK index = 4.5, major asymmetry), HDL cholesterol (LFK index = –1.5, minor asymmetry), triglycerides (LFK index = –1.02, minor asymmetry), CRP (LFK index = –1.44, minor asymmetry), and diastolic blood pressure (LFK index = –1.31, minor asymmetry). Second, the NutriGrade system is a tool to judge the certainty of evidence with regard to individual outcomes, classifying a particular outcome as high-, moderate-, low-, or very-low-quality evidence.[3] The authors seem to have misunderstood the scoring system; while assessing the quality of a meta-analysis is useful in certain circumstances, NutriGrade is unsuitable for the task.

We commend Zhao *et al.*[1] for their valuable contribution and invite them to clarify the misinterpreted outcome. Moreover, we request that the authors reassess the certainty of evidence, keeping the potential publication bias in view and evaluate each outcome separately to help the readers better comprehend their results. Lastly, we urge the readers to exercise diligence when interpreting the findings.

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Competing interests

The author(s) declare none.

Author contributions

AN: writing — original draft, and writing — review and editing, ZAN: conceptualisation, methodology, formal analysis, and writing — review and editing, UA: writing — review and editing.

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RESEARCH ARTICLE

Social jetlag and diet quality among US young adults: interactions with race/ethnicity

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Abstract

The objective was to examine associations between social jetlag and diet quality among young adults in the US using nationally representative data from the 2017–2018 NHANES survey, and evaluate effect modification by gender and race/ethnicity. Social jetlag was considered ≥2-hour difference in sleep midpoint (median of bedtime and wake time) between weekends and weekdays. Diet quality was assessed with the Healthy Eating Index (HEI)-2015 and its 13 dietary components. Ordinal logistic models were run with diet scores binned into tertiles as the outcome. Models accounted for potential confounders and survey weights. Effect modification by gender and race/ethnicity was examined. The study sample included 1,356 adults aged 20–39 years. 31% of young adults had social jetlag. Overall, there were no associations between social jetlag and diet quality. However, interaction analysis revealed several associations were race-specific (P, interaction<0.05). Among Black adults, social jetlag was associated with lower overall diet quality (OR = 0.4, 95% CI 0.2, 0.8; i.e. less likely to be in higher diet quality tertiles) and more unfavourable scores on Total Vegetables (OR = 0.6, 95% CI 0.3, 1.0) and Added Sugar (i.e. OR = 0.6, 95% CI 0.4, 0.9). For Hispanic adults, social jetlag was associated with worse scores for Sodium (OR = 0.6, 95% CI 0.4, 0.9) However, White adults with social jetlag had better scores of Greens and Beans (OR = 1.9, 95% CI 1.1, 3.2). Within a nationally representative sample of US young adults, social jetlag was related to certain indicators of lower diet quality among Black and Hispanic Americans.

Key words: **Circadian: Disparities: 24-hour recalls: Sleep**

Abbreviations: US, United States; NHANES, National Health and Nutrition Examination Study; MEC, Mobile Examination Center; HEI, Healthy Eating Index; NCI, National Cancer Institute; FPED, Food Patterns Equivalents Database; WTDR2D, dietary two-day sample weights.

Introduction

In the US, poor diet is a contributing factor to most leading causes of death[1] and morbidity, including cardiovascular disease,[2] type 2 diabetes,[3] cancer,[4] and stroke.[5] It is also related to weight gain[6] and inflammation,[7] risk factors for COVID-19 severity. Across all age groups, young adults are among the populations with the lowest quality diets in the US.[8] This is alarming, since young adulthood diets tend to track over time and may be much harder to modify later in life.

There are multiple reasons for poor diet quality in young adulthood. First, young adulthood is a transitional life-stage with newfound independence and responsibility, which may include new jobs and careers, continuing education, marriage/partnerships, and children. These transitions and responsibilities could be associated with lower-quality diets for a number of reasons, including lack of time or knowledge of food preparation,[9,10] lower access to healthy food due to financial insecurity,[11,12] or higher preference for unhealthy foods due to stress and uncertainty.[13,14]

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Sleep is emerging as an important predictor of dietary behaviour among young adults. Short sleep duration and poor quality sleep have each been related to lower diet quality among young adults.[15] Consistency of sleep may also affect dietary habits,[16] although it has not been examined as thoroughly. Moreover, the consistency of sleep timing from weekdays to weekends ('social jetlag'), could be particularly relevant for young adults. It has been documented that young adults are more likely to have social jetlag,[17] often defined as >2 hour difference in timing of weekend and weekday sleep.[18] This association may be explained by the fact that adults in their early twenties typically have a circadian drive for eveningness[19] and thus may be more likely to stay up late on weekends and shift to an earlier sleep weekday schedule to align with school or work. Moreover, social jetlag could be related to poor diet quality for several reasons. Late weekend bed and wake times could be directly tied to less-healthy diet patterns on weekends, including breakfast skipping, irregular meal-times, and late-night eating.[20–22] In support, although sleep was not examined, nationally representative US data show that weekend eating patterns are less healthy overall than during the week.[23,24] In addition, the constant shift in sleep schedules from weekends to weekdays could impact overall sleep quality and duration, which in turn relate to lower-quality diets. Indeed, studies among Spanish,[25] Japanese,[26] and Brazilian populations[20] have reported associations between social jetlag and lower diet quality among young adults. Yet, to our knowledge no studies have been conducted with nationally representative data from the US. Further, it is important to consider potential interactions by sex and race/ethnicity, since there may be different contexts and norms regarding sleep and eating behaviours by these characteristics.[27] Furthermore, there are known disparities in both diet quality and sleep health, with underrepresented populations often experiencing worse diet quality and sleep.[28,29]

Thus, in order to address the current gaps in the literature, we sought to examine the role of social jetlag in diet quality among young adults aged 20–39 years in the US. Because there is not a clear consensus on the ages that encompass young adulthood, we selected a wide range. We hypothesized that relationships with diet quality would differ by both sex and race/ethnicity.

Participants and methods

Study population: the national health and nutrition examination survey

First conducted during the early 1960s, the National Health and Nutrition Examination Survey (NHANES) aims to evaluate health and nutritional status of US children and adults. Since 1999, the NHANES programme has become a continuous annual survey that provides a nationally representative sample to study the US population of all ages. Each year, around 5000 randomly selected noninstitutionalized US residents participate in the survey, where they receive interviews and physical examinations. Deidentified data is then released to the public in 2-years cycles. The present analysis utilized 2017–2018 NHANES data to examine the relationship between social jetlag and diet behaviour among a restricted sample of adults of age 20–39 years (N = 1,308).

Exposure: social jetlag status

In 2017–2018 NHANES, questions on sleep timing were adapted from the Munich ChronoType Questionnaire.[30] Participants of age 16 and above were asked to report usual sleep and wake times on weekdays (or workdays) and on weekends (or non-workdays), respectively. These questions allowed us to assess individual social jetlag status, defined as the discrepancy in sleep timing between work and free days.[18] Specifically, for each study participant, we calculated and compared weekday and weekend sleep midpoints (i.e. the median of sleep and wake times). Those with at least a 2-hour difference in sleep midpoints were categorized as having social jetlag. Since there is no standardized cut-off for defining social jetlag, we also considered a cut-off of 1 hour in post hoc analysis. However, we used the more stringent definition for our primary analysis since social jetlag is so prevalent in young adult populations.

Outcome: healthy eating index

Two 24-hour dietary recall interviews were conducted for study participants to estimate intakes of energy and nutrients from consumed foods and beverages. The first interview was administered in person in the Mobile Examination Center (MEC), and the second one was over telephone 3–10 d later. We utilized the Healthy Eating Index (HEI-2015), a measure evaluating diet quality based on key recommendations of the *2015–2020 Dietary Guidelines for Americans,* to quantify participants' diet behaviour.[31] Rated with a scoring system, the HEI-2015 includes a total score that indicates the overall diet quality, and 13 component scores (i.e. total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids, refined grains, sodium, added sugars, and saturated fats) with each assessing one aspect of the *Dietary Guidelines.* All scores have a minimum of 0 points. The maximum is 100 points for the total score, which varies from 5 to 10 for component scores. Higher scores suggest better alignment between diet quality of food intakes and key recommendations by the *Dietary Guidelines.* Utilizing the 'simple HEI scoring algorithm – per person' method by National Cancer Institute (NCI),[32] participant-specific HEI-2015 scores were calculated using the average of the first day and second day total nutrient intakes from the NHANES data and the Food Patterns Equivalents Database (FPED). Scores were then binned into three groups, using tertile values as cut-offs.

Potential confounders

The following demographic and health characteristics were considered as potential confounders – age, gender (female/male), race/ethnicity (White, Black, Hispanic, Asian, other), educational attainment (below high school/high school degree and above), tobacco use in the last 5 d (yes/no), and typical engagement in work or recreational physical activity at least at the moderate level (yes/no).

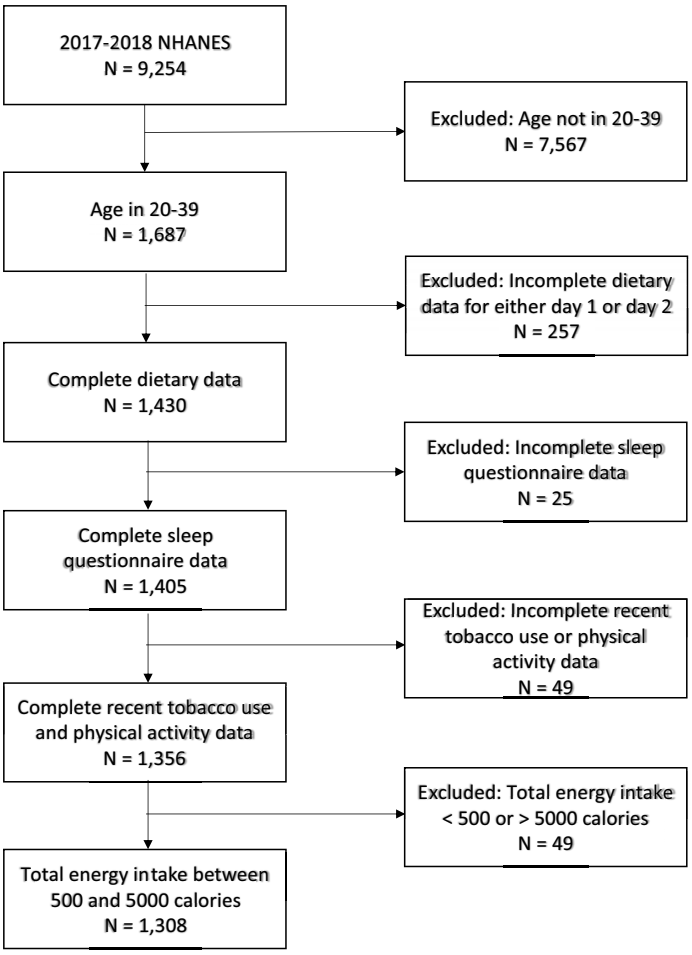

Fig. 1. Flowchart of the data included in the final analytic sample.

Statistical analysis

A total of 9,254 individuals participated in the 2017–2018 NHANES survey. Participants with incomplete dietary recall, sleep questionnaire, or potential confounders (recent tobacco use and physical activity were the only confounders with missing data) were excluded from the study. Furthermore, the analysis focused on young adults of age 20–39 years with total energy intake between 500 and 5000 energies (considered as plausible energy intake), resulting in an analytic sample of 1,308 participants (Fig. 1). Using complex survey analytic methods, demographic characteristics and personal habits were evaluated for all participants and by social jetlag status. Adjusted by the dietary two-day survey weight, bar charts were also generated to present gender-specific proportions of participants with social jetlag, for overall and race/ethnicity-stratified samples.

Associations between social jetlag and diet behaviour were examined with ordinal logistic regression models, with the tertiles of diet scores (overall and components) as categorical outcomes. Through incorporating the dietary two-day sample weight (WTDR2D) in the analysis, effect sizes and corresponding 95% CIs were estimated (R statistical software version 4.2.1, with the *survey* package); models included age, gender, race/ethnicity, educational attainment, recent tobacco use and physical activity as covariates. We considered BMI and sleep duration as potential mediators and therefore did not adjust for them.

We further performed interaction analyses by including an interaction term for social jetlag by gender and social jetlag by race/ethnicity. Because we saw evidence for effect modification (P, interaction<0.05) by race/ethnicity, we finally ran race/ethnicity-stratified analyses. Race/ethnicity-stratified analyses are shown only for Whites, Blacks, and Hispanics due to insufficient sample sizes in the other race/ethnicity categories (Asian and other).

Results

Demographic and health characteristics of survey participants are presented in Table 1. 31% of young adults in the overall sample had social jetlag. In addition, social jetlag was more prevalent among males than females (e.g. 34% males had social jetlag vs. 28% among females). Compared to those of other racial/ethnic groups, Black Americans were more likely to experience social jetlag, with more than 40% being affected (Fig. 2).

Table 1. Demographic and health characteristics of young adults (20–39yr) by social jetlag status

	Overall[a] N = 1308 (%)	Social jetlag[a,b] N = 421 (%)	No social jetlag[b] N = 887 (%)
Sex			
Female	719 (51)	212 (28)	493 (72)
Male	637 (49)	209 (34)	394 (66)
Race/ethnicity			
White	437 (55)	114 (28)	323 (72)
Black	290 (13)	115 (41)	175 (59)
Hispanic	316 (20)	118 (33)	198 (67)
Asian	191 (7)	47 (25)	144 (75)
Other	74 (5)	27 (33)	47 (67)
Education			
More than high school	802 (65)	235 (30)	567 (70)
High school and below	506 (35)	186 (31)	320 (69)
Age (mean ± SD)	29.4 ± 5.7	29.8 ± 5.4	28.4 ± 5.8
Physical activity			
Any moderate/vigorous activity	1048 (84)	346 (32)	702 (68)
No moderate/vigorous activity	260 (16)	75 (23)	185 (77)
Tobacco use in the last 5 d			
Yes	347 (23)	134 (38)	213 (62)
No	961 (77)	287 (29)	674 (71)

[a]Only counts for categorical variables are unweighted. Proportions, means, and standard deviations are weighted according to the dietary two-day sample weight (WTDR2D).
[b]Social jetlag, a binary indicator, is defined as having at least 2-hr difference in weekday/workday and weekend sleep midpoints.

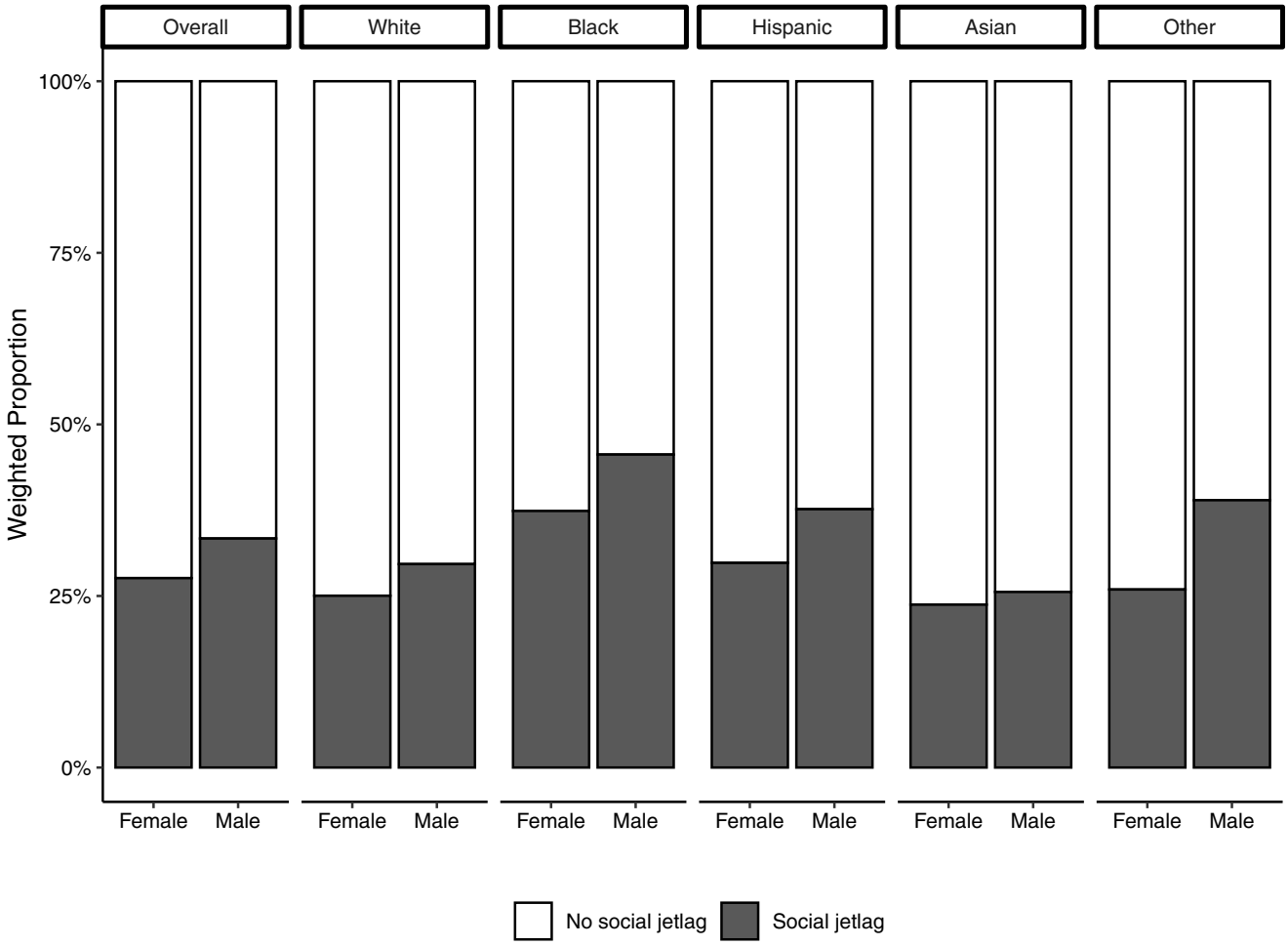

Fig. 2. Survey-weighted proportions of social jetlag participants by gender.

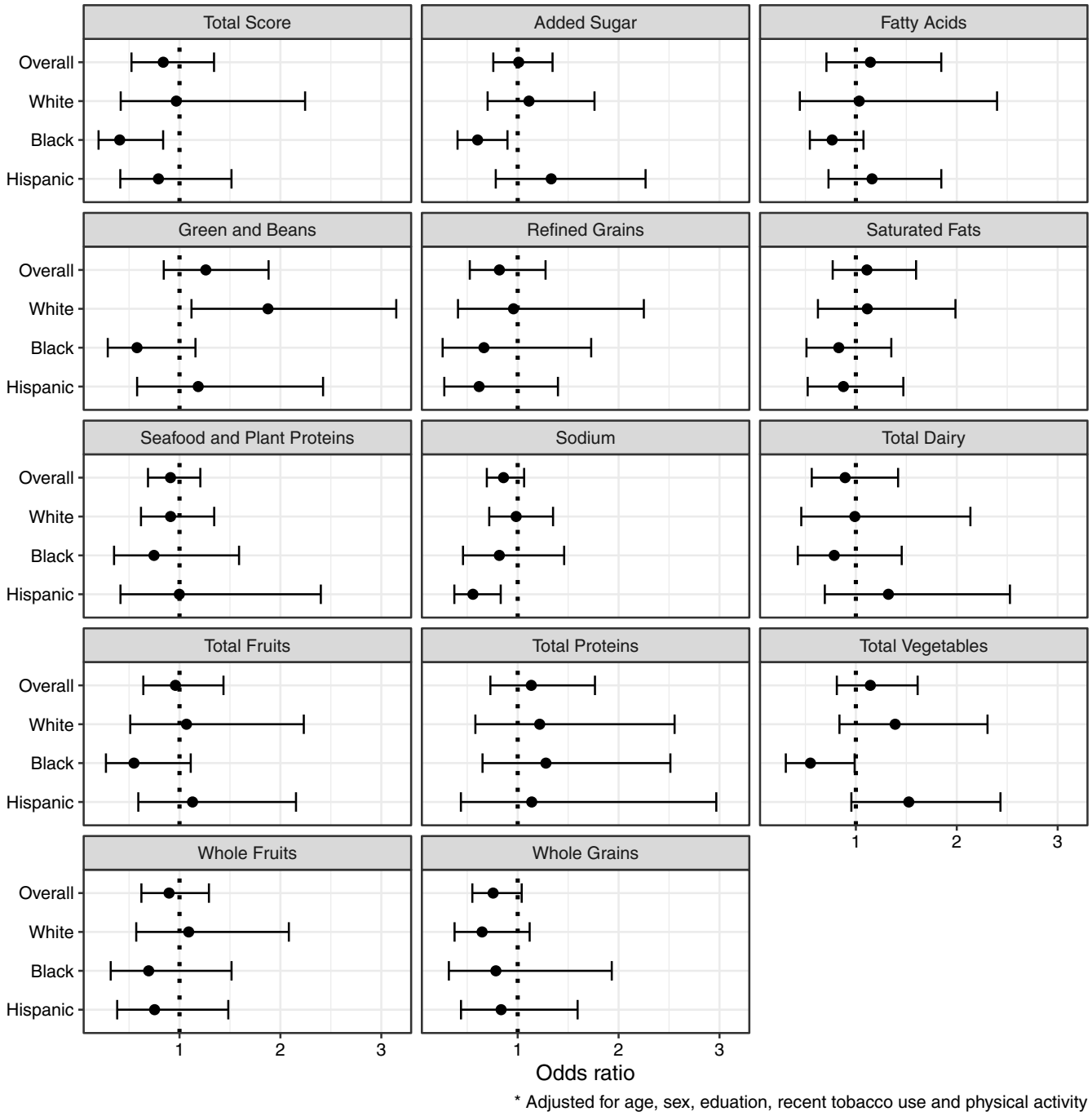

Fig. 3. Forest plots for overall HEI score and component scores, unstratified and stratified by race/ethnicity. [1]Odds ratios are from adjusted ordinal logistic regression models with tertiles of diet scores as the categorical outcome and social jetlag as the exposure, adjusted for gender, race/ethnicity (only in the unstratified models), educational attainment, recent tobacco use and physical activity. Odds ratios<1 indicate lower diet quality (i.e. lower odds of being in the upper tertiles of diet quality).

For the overall sample, no significant relationships between social jetlag status and HEI-2015 scores were identified (Fig. 3).

However, there was effect modification of the relationship between social jetlag and diet quality according to race/ethnicity (Fig. 3). In particular, social jetlag was associated with lower overall diet quality scores for Black individuals (OR of being in higher-quality tertiles = 0.4 with 95% CI 0.2, 0.8). Regarding individual diet component scores, Black young adults with social jetlag had lower scores for Total Vegetables and Added Sugar (i.e. lower intake of vegetables and higher consumption of added sugar; OR = 0.6 with 95% CI 0.3, 1.0 and OR = 0.6 with 95% CI 0.4, 0.9, respectively). Among Hispanic young adults, social jetlag was associated with lower scores in the Sodium component (i.e. higher sodium intake; OR = –1.1, 95% CI –2.0,

–0.2). Conversely, White young adults with social jetlag had higher scores in the Greens and Beans component (OR = 1.9, 95% CI 1.1, 3.2). There was no evidence for effect modification by gender (all P for interaction > 0.05). In post hoc analysis when social jetlag was defined as ≥1 hour different between weekday and weekend sleep timing, social jetlag was associated with higher intake of sodium (OR = 0.7, 95% CI 0.5, 0.9).

Discussion

Within this nationally representative sample of US young adults, social jetlag was not significantly associated with overall diet quality. However, there was effect modification by race/ethnicity. Specifically, Black Americans with social jetlag had lower overall diet quality scores and lower scores of the total vegetables and added sugar components (i.e. higher added sugar intake), while Hispanic Americans with social jetlag had lower scores on the sodium component (i.e. higher sodium intake). In contrast, White young adults with social jetlag had higher scores for the greens and beans component.

Overall, social jetlag was not an important determinant of diet quality, which contrasts with some young adult studies in Spanish,[25] Japanese,[26] and Brazilian populations.[20] Nevertheless, a recent review[33] of social jetlag and dietary intake that took into account findings of 17 studies provided a more cautious conclusion that social jetlag is *possibly* related to diet, given mixed findings especially for individual foods and nutrients. There could be a few reasons for the lack of an association in this study. First, diet quality is meant to capture total diet, and it could be that social jetlag primarily affects diet quality during the weekends. Furthermore, there could be compensatory mechanisms such that young adults consciously improve their diets and sleep during the week to 'make up' for poor diet and sleep on the weekends. Second, it is possible that we did not find strong associations between social jetlag and diet quality because there was insufficient variability in either diet quality or social jetlag in the young adult population as a whole.

Among racial/ethnic minorities, namely Black and Hispanic Americans, there was stronger evidence of associations between social jetlag and diet. Furthermore, these associations were in the expected directions, given that social jetlag has been previously linked with lower diet quality and higher consumption of individual foods/drinks such as sugar-sweetened beverages.[20,25,26,34] Of note, social jetlag was overall higher in Black and Hispanic Americans than in other groups. There could be several reasons for this. Black and Hispanic Americans are more likely to live in neighbourhoods that may not be conducive to healthy sleep.[35] Furthermore, racial/ethnic minorities suffer disproportionately from food insecurity[36] and economic uncertainty,[37] which could result in stress and changing circumstances (job, housing, etc.) that make it difficult or impossible to maintain consistent sleep schedules. In populations of US young adults not facing economic or job uncertainty, social jetlag could instead reflect different social or lifestyle decisions, e.g. choosing to stay up late on the weekends to socialize with friends. Thus, the reasons underlying social jetlag may differ between racial/ethnic subgroups of young adult populations in ways that modify relationships between sleep and diet. Indeed, we found that among White non-Hispanic young adults, social jetlag was unexpectedly associated with higher diet quality scores on the Greens and Beans component. While the reasons underlying this association are unclear, it is worth noting that at least one other study among US adolescents reported healthier behaviours among adolescents with higher social jetlag (although the study was conducted among Hispanic adolescents).[38] In addition, our dataset showed that more physically active individuals were also more likely to have social jetlag which was not in line with expectation.[39] Further research is needed to understand the drivers of social jetlag, especially within different subpopulations of young adults within the US.

There are both strengths and limitations to consider. This sample is large and reflective of the young adult population in the US, increasing the generalizability of findings. Second, the examination of effect modification by ethnic and racial minority groups uncovered potential disparities in sleep health and diet. Third, the comprehensive diet information allowed examination of specific dietary domains in relation to social jetlag. Some limitations include the self-report sleep timing that could be over- or under-estimated and the cross-sectional study design which prohibits the evaluation of the temporality of the associations. Furthermore, the two dietary recall days were not restricted to weekdays or weekends (i.e. it was not required that one recall day occur on a weekday and the other on a weekend), and the two-day survey weights were constructed to minimize differences in diet across the week. Given this design, we could not readily examine weekday-weekend differences.

In conclusion, within a nationally representative sample of US young adults, we found that social jetlag was related to certain indicators of lower diet quality among Black Americans and Hispanic Americans. These findings demonstrate that consistency of sleep timing from weekdays to weekends could constitute another barrier for consuming a healthy diet in the US, especially among Black and Hispanic Americans.

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None.

Authorship

XL conducted statistical analysis and contributed to the original draft of the manuscript; GLD contributed to conceptualization of the research question, interpretation of results, and critically revised the manuscript; CWL contributed to the methodology and interpretation of results and critically revised the manuscript; ECJ contributed to the conceptualization of the research question and original draft of the manuscript, as well as the interpretation of results and final editing of the manuscript.

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Conflict of interest

None.

Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. As this was a secondary data analysis of publicly available deidentified information, institutional review was not necessary.

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RESEARCH ARTICLE

Validity and reproducibility of the Prime Diet Quality Score (PDQS) against a four-day food diary in adults at risk of cardiovascular disease on the island of Ireland

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Abstract

There is an increasing need for valid, rapid diet screening tools. A significant association between the Prime Diet Quality Score (PDQS) and reduced risk of cardiovascular disease (CVD) has been demonstrated in the US but evidence of its use in Europe is lacking. The aim of this study was to amend the PDQS for a UK/Irish population and determine validity and reliability in those at risk of CVD. Participants were recruited via online adverts across the island of Ireland. The PDQS was amended for a UK/Irish population and participants completed PDQS and reference measure (4-day food diary (FD)) on two occasions. PDQS score was calculated directly from PDQS and indirectly from FDs. Validity was determined using Spearman correlation coefficients (SCCs) (r), intraclass correlation coefficients (ICCs) and weighted kappa. Reliability was determined using SCCs (r), ICCs, weighted kappa and coefficient of variation.

'Data were available for n = 115 (Month 0) and n = 108 (Month 3) participants for validity and n = 110 for reliability assessment (PDQS completed at both timepoints)'. PDQS score from PDQS was significantly correlated with PDQS score from FDs at months 0 ($r = 0.59$, $P < 0.01$) and 3 ($r = 0.65$, $P < 0.01$), with similar associations observed via ICCs. Weighted kappa indicated moderate agreement. PDQS score at month 0 was significantly correlated with PDQS score at month 3 ($r = 0.78$, $P < 0.01$), with similar associations observed via ICCs. Weighted kappa indicated moderate agreement. Results indicate that the amended PDQS is a valid and reliable tool to determine diet quality in a UK/Irish population at risk of CVD.

Key words: **Diet quality: Diet quality scores: Diet score validation**

Introduction

It is thought that up to 80% of cardiovascular diseases (CVD) and over one-third of cancers could be prevented by modifying behaviours such as diet, smoking, alcohol consumption and physical activity.[1] Despite this, the global burden of non-communicable diseases (NCDs) has continued to rise in recent decades, with 71% of worldwide deaths attributable to NCDs.[1]

A healthy dietary pattern is known to reduce the risk of NCDs.[2–6] However, accurate assessment of dietary intake is vital to determine how best to identify those with suboptimal dietary intake. Commonly utilised methods of dietary assessment, including food frequency questionnaires (FFQs), 24 h recalls and food diaries (FD), are burdensome to both research participants and researchers[7] and the American Heart Association has called for widespread adoption of valid, rapid diet screener tools in primary care and relevant prevention settings to help easily identify suboptimal dietary intake and reduce incidence and improve management of NCDs.[8]

A systematic review of brief (<35 items) dietary questionnaires suitable for clinical use in the prevention and management of obesity, CVD and type 2 diabetes was published in 2015 and reported on 35 tools, 20 of which had been developed for use in the United States (US).[9] Authors concluded that the tools evaluated are suitable for guiding clinicians but must be adapted and evaluated locally to ensure acceptable levels of relative validity and reliability for the population under study.[9]

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Since the publication of this systematic review, several other US studies have developed/amended such tools.[10–12] In a study that prospectively examined the association between diet quality assessed using three different diet quality indexes and CVD risk in three US cohorts, the 21-item Prime Diet Quality Score (PDQS), which included both 'healthy' and 'unhealthy' food groups, was inversely associated with CVD risk in all three cohorts. In contrast, the Food Group Index and the Minimal Diet Diversity Score for Women, which only accounted for 'healthy' food groups, had only a limited association with CVD risk across the cohorts.[11] It has been suggested that the PDQS has a simpler scoring system than other diet quality scores despite its greater gradation of scoring, which enables rapid administration and better categorisation of diet quality. Furthermore, it was better able to predict CVD than the other scores in both young and older men and women.[11] As such, it has been suggested that a tool such as the PDQS could be utilised as an efficient field tool to screen diet quality in place of more burdensome dietary assessment methods.[11] Several more recent studies have adapted the PDQS, firstly adapting a 22-item PDQS to be administered either as a 24-hour recall or with a 30-day reference period,[13] and secondly developing a rapid 13-item rPDQS[14]; each of these has been validated against food group equivalents and Healthy Eating Index-2015 (HEI-2015) scores estimated from Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tools in US populations.[13,14] These versions of the PDQS have also been associated with several NCD outcomes including coronary artery disease, all-cause mortality and hypertension in pregnancy and gestational diabetes.[11,15–18] The Global Diet Quality Score (GDQS), a further diet screening tool which was also based on the PDQS, has also been associated with NCD-related outcomes in nonpregnant, nonlactating US women of reproductive age.[19] These studies have therefore highlighted the broad applicability of the PDQS in terms of its association with various non-communicable disease health outcomes.

Research into CVD-specific diet screening tools in European populations is lacking. Various adaptations of the PDQS have demonstrated its ability to predict CVD risk in multiple population groups. It has been suggested that future studies should investigate if this efficient field tool is associated with a wider range of health outcomes relevant to diverse populations in both high- and low-income countries,[11] so it is of interest to adapt and test this tool for a European population, in order to account for regional differences in dietary patterns and food preparation habits, prior to use in future research. This study aimed to adapt the original PDQS dietary screening tool for a UK/Irish population and determine its validity and reliability against a 4-day FD in a population at risk of CVD.'

Methods

This was a dual-centre study completed at both Queen's University Belfast (QUB), Northern Ireland and University College Dublin (UCD), Republic of Ireland. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the both the Faculty of Medicine, Life and Health Sciences QUB (Reference number: MHLA 21_92) and the UCD Sciences Human Research Ethics Committee (Reference number: LS-20-02-Brennan).

Amendment of PDQS for population on the island of Ireland

The US version of the PDQS is a 21-item dietary screening tool previously described by Fung *et al.*[11] and based on the PrimeScreen.[10] Food groups are classified as 'healthy' or 'unhealthy' based on the scientific evidence with regards to their direction of association with the risk of NCDs, and their nutrient contribution across various global regions.[11] The food groups considered 'healthy' in the original PDQS dietary screening tool were: dark green leafy vegetables, cruciferous vegetables, carrots, other vegetables, whole citrus fruits, other whole fruits, legumes, nuts, poultry, fish, eggs, whole grains, and liquid vegetable oils. The food groups considered 'unhealthy' were: red meat, potatoes, processed meat, whole milk dairy, refined grains, baked goods, sugar-sweetened beverages, fried foods obtained away from home, desserts and ice cream. Points are assigned according to the following criteria for 'healthy' food groups: 0–1 serving per week (0 point), 2–3 servings per week (1 point) and ≥4 servings per week (2 points) and scoring is reversed for 'unhealthy' food groups.

In order to amend the original PDQS dietary screening tool for a UK/Irish population, researchers based at both sites reviewed original PDQS food groups and amended these, where appropriate, to reflect any differences in consumption habits or dietary recommendations between the US and UK/Ireland regions.[20,21] All decisions regarding amendments were reached by consensus by the research team who are all research nutritionists or dietitians, are based in the UK and Ireland and have extensive experience in utilisation and review of dietary assessment tools for various population groups in these regions.

The amendments are as follows: the food category 'potatoes' were categorised as 'unhealthy' in the original version of the PDQS but were re-classified as 'healthy' in the amended PDQS with accompanying examples listed as 'boiled, baked, mashed' rather than 'chips/fries or roast potatoes' as listed in the original PDQS to reflect UK/Ireland cooking methods. Unhealthy potato products such as chips, roast potatoes and crisps listed in the original PDQS under the 'potatoes' group were then incorporated into the 'high-fat foods' group examples within the amended PDQS. The categories 'whole eggs' and 'nuts' were classified as 'healthy' within the original US PDQS, but the highest consumption frequency option of 'twice or more per day' was given 0 points rather than 2 within amended PDQS, so that the frequency of 'nearly daily or daily category' received the highest number of points. This was because consuming nuts or eggs twice or more per day was considered excessive according to current regional dietary recommendations.[20,21] The amended PDQS dietary screening tool utilised in this validation and reliability assessment is presented in Table 1 with colour coding to indicate scoring guidance and will be referred to as PDQS from this point forward.

Table 1. Amended Prime Diet Quality Score Dietary Screening Tool for UK/Irish Population

	Prime Diet Quality Score					
	For each question, mark the column indicating how often on average you have used the item(s) during the past 3 months	Less than once per week	Once per week	2–4 per week	Nearly daily or daily	Twice or more per day
1	Red Meat e.g. minced beef (lasagne, bolognaise, Cottage Pie), beef stew/ casserole, steak, pork chop, lamb.	☐	☐	☐	☐	☐
2	Processed Meats e.g. sausages, bacon, ham, corned beef, tinned meat.	☐	☐	☐	☐	☐
3	Poultry e.g. chicken or turkey breast/fillet, slices (no batter/crumbs).	☐	☐	☐	☐	☐
4	Fish e.g. cod, haddock, salmon, tuna, mackerel (no batter/crumbs).	☐	☐	☐	☐	☐
5	Whole Eggs e.g. boiled, scrambled, poached (not fried).	☐	☐	☐	☐	☐
6	Whole Milk Dairy Foods e.g. whole/full-fat milk, hard cheese, butter, full-fat yoghurts.	☐	☐	☐	☐	☐
7	High-Fat Foods e.g. fast food takeaways (chips, fried chicken/fish/burgers), fried breakfasts (e.g. fried breads, eggs), roast potatoes, crisps.	☐	☐	☐	☐	☐
8	Whole-Grain Foods e.g. wholegrain breads, wholegrain breakfast cereals (porridge, Weetabix, Shredded Wheat), brown pasta/rice.	☐	☐	☐	☐	☐
9	Sweet Baked Foods e.g. cakes, buns, muffins, cookies, scones.	☐	☐	☐	☐	☐
10	Potatoes e.g. boiled, baked, mashed (not chips, roast).	☐	☐	☐	☐	☐
11	Dark Green Leafy Vegetables e.g. spinach, lettuce, kale, spring greens (includes frozen, tinned).	☐	☐	☐	☐	☐
12	Cruciferous Vegetables e.g. broccoli, cauliflower, cabbage, brussels sprouts (includes frozen, tinned).	☐	☐	☐	☐	☐
13	Carrots e.g. raw, boiled, steamed, mashed, microwaved, frozen, tinned.	☐	☐	☐	☐	☐
14	Other vegetables e.g. mushrooms, corn, turnip, cucumber, tomatoes (includes frozen, tinned).	☐	☐	☐	☐	☐
15	Legumes e.g. peas, baked beans, kidney beans, lentils, chickpeas (includes frozen, tinned).	☐	☐	☐	☐	☐
16	Whole Citrus Fruit e.g. oranges, grapefruit, lemons (not fruit juices).	☐	☐	☐	☐	☐
17	Other fruits e.g. apples, pears, bananas, strawberries, raspberries, grapes, melon (includes frozen, tinned).	☐	☐	☐	☐	☐
18	Liquid Vegetable Oils e.g. olive oil, rapeseed oil (not palm oil or coconut oil).	☐	☐	☐	☐	☐
19	Nuts e.g. almonds, peanuts, cashew, hazelnuts, brazil nuts (unsalted only).	☐	☐	☐	☐	☐
20	Desserts, Puddings and Confectionery e.g. ice cream, custard, sponge puddings, crème brulee, fruit pies, cheesecakes, chocolate, sweets.	☐	☐	☐	☐	☐
21	Sugar Sweetened Beverages e.g. cola, sodas, lemonade, flavoured juices, energy drinks (not diet/sugar-free varieties).	☐	☐	☐	☐	☐

Scoring system: red = 0, yellow = 1, green = 2. Score range 0–42.

Study population and recruitment

Study eligibility criteria at both sites were as follows: participants were eligible if they were aged 45 years or over and had one or more of the following risk factors for CVD: current Body Mass Index (BMI) of 25 kg/m^2 or over; a current smoker, diagnosed hypertension (elevated blood pressure of 140/90 mmHg or above diagnosed by a medical professional) or diagnosed hypercholesterolaemia (elevated total cholesterol of 5 mmol/l or above diagnosed by a medical professional). Those being pharmacologically managed for hypertension or hypercholesterolaemia were also considered at risk of CVD. Participants were excluded if they had a medical condition which required significant dietary management (e.g. diet-only management of type 2 diabetes; active inflammatory bowel disease), dietary restriction(s) that would substantially limit their ability to complete the study requirements (e.g. food allergy, Coeliac Disease), excessive alcohol consumption (>28 units/week for men or >21 units/week for women) or if they were unable to provide informed consent.

Standardised recruitment protocols were implemented across both sites. Study recruitment commenced just before the onset of the COVID-19 pandemic in February 2020 and consequently paused between March and June 2020 during national lockdowns. The original intention of the validation and reliability assessment was to validate the PDQS against a 4-day FD and to assess agreement of the PDQS with urinary dietary biomarker levels. Due to requirement to progress with the research remotely during the pandemic, the study protocol was amended to remove collection of biological samples and analysis of urinary biomarkers and, after ethical amendment approval, recruitment re-commenced remotely in June 2020. Recruitment posters were distributed online via online community notices, social media posts and some workplace email distribution lists. Contact details of the research team were provided on the poster and interested participants made contact with the research team via phone or email. Eligibility was determined via telephone with a study researcher. Participant Information Sheets were distributed to those eligible and researchers answered any queries via telephone. Consent was obtained via Qualtrics online survey software (Qualtrics, Provo, UT).

The study aimed to examine the level of agreement between two methods of measurement, so a sample size of 100 was considered appropriate, giving a 95% CI ± 0.34 SD approximately.[22] This sample size calculation was also supported by a

comprehensive dietary questionnaire validation review by Cade *et al.*[23] which recommended that a sample size of at least 50–100 participants is desirable for FFQ validation.[23,24] The QUB site aimed to recruit n = 70 participants, whilst the UCD site aimed to recruit n = 30 participants.

Study design

To determine criterion-related validity, the test measure (PDQS) was administered to participants followed immediately by the reference measure (4-day FD) on two occasions (month 0 and month 3) to enable comparison between methods. Reliability was determined by comparing the test measure (PDQS) administered at both timepoints (month 0 and month 3). The 3-month interval time frame was intended to account for any seasonal variations in dietary intake, but a short enough time frame to avoid capturing major changes in dietary habits that are more likely to occur years apart.[23]

Administration of the test and reference measures

The PDQS was administered to participants via online survey software. Participants were asked to complete the PDQS with reference to their dietary intake over the previous 3-month period and researchers discussed the PDQS over the phone with participant prior to administration to ensure as accurate an estimation of dietary intake as possible and offered to assist the participant via telephone during completion if necessary. A 4-day FD was used as the reference measure for assessment of criterion-related validity and was posted to participants at home at months 0 and 3 after completion of the PDQS. Participants were asked to record everything they ate and drank over 4 consecutive days (3 weekdays and 1 weekend day) and to include brand names of foods, cooking methods and whether fat/sauces were added to foods. Participants were asked to weigh foods or use household measures to estimate intake and leftovers and provide ingredients and amounts used in preparing a composite dish. Portion-size information and guidance were given to participants via telephone and portion-size photo guides and advice were included within the FD. Participants were asked to contact the researchers if they had any questions during the recording period and were given a pre-paid envelope to return their completed FD. Completed FDs were reviewed by researchers who followed up with a phone call if further details were necessary.

Feedback

As this study aimed to adapt the PDQS for a UK/Irish population at risk of CVD, comments and feedback in relation to the format and clarity of the questionnaire were informally collected from participants during the study to indicate whether further amendments were necessary.

Data management

Calculating PDQS score from PDQS and food diary data

PDQS (test measure). The PDQS score was calculated directly from PDQS responses completed by participants at month 0 and month 3 using the criteria previously detailed and outlined in Table 1 (score range 0–42).

Food diary data (reference measure). FDs were coded using the Nutritics online dietary survey software (Nutritics. (2019). Research Edition (v5.09) [Computer software]. Dublin). In order to ensure standardisation of data entry across sites, 10% of FDs completed at each site were entered in duplicate, reviewed and compared. Any discrepancies identified between sites in the coding of dietary data were discussed and resolved with the wider research team.

Researchers reviewed food files obtained from Nutritics and coded all PDQS-relevant food items so that they corresponded with the food groups 1–21 listed within the PDQS. A list of more ambiguous food items was identified by researchers at both sites and a coding guide was developed to assist with coding these food items (Supplementary Files Table 1).

Intake of each of the coded food items was quantified by calculating total weight in grams (g) consumed for each of the PDQS coded food items (1–21) over the course of the 4-day FD recording period for each participant at each timepoint, e.g. all food items which were coded within the 'red meat' category were collated under PDQS group 1 and total amount in grams was summed. The research team estimated an average portion size for each of the 21 PDQS food groups by listing published portion sizes[25] for each example food listed within each PDQS category and calculating an average portion size for each of the 21 food groups (Supplementary Files Table 2). Guidance regarding portions of food typically consumed in multiple units at a time (e.g. 2 sausages, 2 slices of bread, 2 eggs) was obtained from the British Nutrition Foundation 'Find Your Balance' Full Portion Size List.[26] Contributing weights within composite dishes listed as examples within the 'red meat', 'poultry', 'fish' or 'legumes' food groups, were capped e.g. contributing weight of red meat within Bolognese was capped at 140 g of minced beef, the published portion size.[19]

Total weight (g) consumed for each of the 21 PDQS groups was converted to total servings per day over the 4-day recording period by dividing by the average portion size calculated for each of the 21 food groups as described above and converting to a weekly serving estimate by multiplying by 1.75. The weekly serving estimate was converted to the corresponding PDQS frequency categories as follows: 0–0.49 servings per week corresponded with 'less than once per week', 0.5–1.49 servings per week corresponded with 'once per week', 1.5–4.49 servings per week corresponded with '2–4 per week', 4.5–13.49 servings per week corresponded with 'nearly daily or daily' and 14+ servings per week corresponded with 'twice or more per day'. This enabled the research team to calculate a PDQS score for each participant at each timepoint.

Additional outcomes

A short lifestyle questionnaire was administered to participants at month 3 via online survey software to capture any notable changes in lifestyle or health over the 3-month study period.

Statistical analyses

To compare PDQS score derived from PDQS and PDQS score from food diaries, Wilcoxon Signed Rank Test, Mann-Whitney *U*-Test and Kruskal-Wallis *H* Tests were used.

To determine validity of the PDQS, total PDQS scores and individual PDQS food group scores derived from both the PDQS and the 4-day FD at both timepoints were compared. Spearman correlation coefficients (*r*) and intraclass correlation coefficients were obtained for total PDQS scores and individual PDQS food groups derived from both the test and reference measures. The ability of the PDQS to categorise participants into equal thirds of total PDQS from FD data was assessed by weighted kappa, with values of K > 0.8 considered to indicate almost perfect agreement, 0.61–0.80 substantial agreement, 0.41–0.60 moderate agreement, 0.21–0.40 fair agreement, 0.0–0.20 slight agreement, and 0 poor or disagreement.[27] Statistical analyses were performed using SPSS version 20.0 (SPSS Inc, Chicago, IL).

To determine the reliability of the PDQS, Spearman correlation coefficients (*r*) and intraclass correlation coefficients were obtained for total PDQS score derived from the PDQS test measure at both timepoints. Weighted kappa was performed to determine ability of the PDQS to categorise participants into equal thirds of total PDQS score at month 0 and month 3. Coefficient of variations was also obtained to assess reliability of PDQS administered at month 0 and month 3.

Subgroup analyses were performed by gender. Any dietary intakes from the food diary data which were considered implausible using the <500 kcal and >3500 kcal/d energy intake criteria, as described in previous dietary research,[28–31] were identified and analysed separately, where relevant.

Kruskal-Wallis *H* Tests were used to compare average daily nutrient intake by tertile of PDQS total score from amended PDQS.

Data from the changes in lifestyle questionnaire were limited in terms of changes reported but were analysed in terms of changes reported in frequencies and percentages.

Results

In total, n = 130 participants were screened for study eligibility across both sites. Of these, n = 120 were considered eligible and were recruited. As per study protocol, n = 10 of the screened participants were ineligible to take part for the following reasons: n = 4 had a dietary restriction that would substantially limit their ability to complete study requirements, n = 3 had been diagnosed with Type 2 diabetes and were either on dietary management and/or medication, n = 1 had a history of a previous cardiac event, n = 1 wasn't able to fulfil collection of data at Month 3 due to relocation and n = 1 reported excessive alcohol consumption.

Numbers included in validity analysis were the number of participants who completed both the PDQS and the food diary at each timepoint to allow validity assessment, whereas the numbers included in the reliability analysis were the numbers of participants who completed the PDQS at both timepoints to allow reliability assessment. For validity assessment, data were complete for n = 115 participants at month 0 and n = 108 participants at month 3; for reliability assessment, data were complete for n = 110 participants (Fig. 1). No implausible energy intakes were reported according to the <500 kcal and >3500 kcal/d criteria[28–31] so it was not necessary to exclude any participants from the analyses.

Sample demographics are presented in Table 2. Mean age of participants was 59.0 years (SD: 9.7), the majority of participants were female (78.3%), 38.3% of participants were classified as overweight and 51.3% with obesity according to BMI (kg/m^2), 43.5% of participants reported hypertension, 40.9% reported hypercholesterolaemia and 15.7% currently smoked.

Average total PDQS scores derived from both the PDQS and the food diaries at both month 0 and month 3 are presented in Table 3. Wilcoxon Signed Rank Test indicated that average total PDQS score derived from the PDQS was significantly higher, indicating a better diet quality, at both month 0 (20.4; SD: 5.7) and month 3 (21.0; SD: 5.6) than average total PDQS score derived from food diaries at month 0 (18.2; SD: 4.6) and month 3 (18.3; SD: 4.7; $P < 0.01$ at both timepoints). Mann-Whitney *U*-Test indicated that average total PDQS score from the PDQS was significantly higher in females (21.0; SD: 5.6) than in males (18.2; SD 5.7, $P < 0.05$) at month 0; whilst for PDQS score derived from food diaries, females scored higher (19.0; SD: 4.2) than males (16.1; SD: 5.5) at month 3 ($P < 0.05$). Kruskal-Wallis *H* Test indicated that average total PDQS score obtained from the PDQS was significantly lower, indicating a poorer diet quality, in participants with overweight and obesity compared with participants who were underweight/healthy weight at both month 0 ($P < 0.01$) and month 3 ($P = 0.01$). Average PDQS score derived from food diaries was also significantly lower in participants with overweight and obesity compared with participants who were underweight or healthy weight participants at month 0 ($P < 0.01$), but no significant difference was observed between weight categories at month 3.

Spearman correlation coefficients (*r*), weighted kappa (K) and ICCs for average total PDQS score from PDQS and food diaries at months 0 and 3 are presented in Table 4. Average total PDQS score from PDQS was significantly correlated with average total PDQS score derived from food diaries at month 0 ($r = 0.59$; $P < 0.01$) and month 3 (0.65; $P < 0.01$). Individual food group scores from PDQS were also significantly correlated with those derived from food diary data for 16 of the 21 food groups at month 0 (all $P < 0.01$); and 20 of the 21 food groups at month 3 ($P < 0.05$ for 2 food groups, $P < 0.01$ for 18 food groups). Similar intraclass correlation coefficients were observed between average total PDQS score from the PDQS and average total PDQS score derived from food diaries at month 0 (0.70; 95% CI: 0.49–0.81) and month 3 (0.42; 95% CI: 0.41–0.86). Weighted kappa indicated moderate agreement between the two PDQS scores at month 0 (0.40; SE: 0.07) and month 3 (0.42; SE: 0.07). Bland-Altman plots (Fig. 2) indicated that, for 95% of participants, average PDQS scores were within the limits of agreement at month 0 (mean difference: 2.3), whereas at month 3, 90.5% of participants were within limits of agreement (mean difference 2.8).

Reliability assessment indicated that average total PDQS scores obtained directly from PDQS at months 0 and 3 were statistically significantly correlated ($r = 0.78$; $P < 0.01$) and similar intraclass correlation coefficients were observed (0.88; 95% CI: 0.82–0.92). Weighted kappa indicated moderate agreement between PDQS score at the different timepoints (0.54; SE: 0.04). Coefficient of variation for average total PDQS

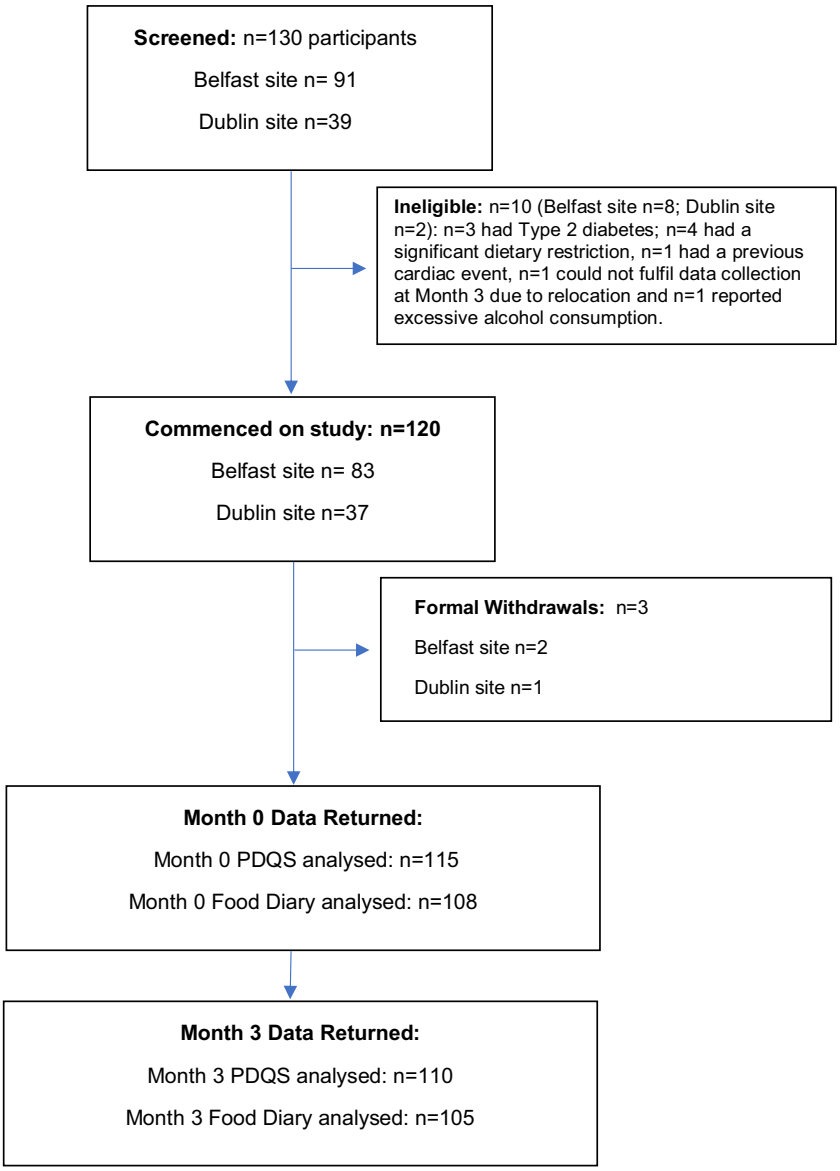

Fig. 1. Flow diagram illustrating study recruitment.

obtained at both timepoints was 0.11, indicating low variation between the means.

Subgroup analyses were performed by gender (Supplementary Files Table 3 and 4), although 78.3% of the sample were female. Agreement between measures was statistically significant in both females and males at both months 0 and 3; whilst stronger agreement was observed at month 0 in females compared with males and stronger agreement seen in males compared with females at month 3. However, overall validity and reliability results were consistent with primary analysis. As the majority of the sample was classified either as overweight (38.3%) or obese (51.3%), subgroup analyses were not conducted by BMI category.

Average daily nutritional intake from food-only sources, assessed using food diary data, is presented in Table 5 alongside nutritional intake by tertile of total PDQS score from PDQS at month 0 and month 3. Kruskal-Wallis *H* Test was utilised to determine if there were any statistically significant differences between groups. At month 0, average daily fibre (g) and folate (µg) intakes were significantly higher in those with total PDQS score in highest tertile of PDQS score compared with those who scored in the middle and lowest tertiles (both $P < 0.01$). Average daily potassium, thiamine and riboflavin intakes (mg) were significantly higher in those that scored in the highest tertile of PDQS score compared with those in the lowest tertile of PDQS score ($P < 0.01$; 0.03 and 0.02, respectively), whilst average daily iodine (µg), vitamin C (mg) and vitamin D (µg) intakes were significantly higher in those who scored in the middle and highest tertile of PDQS score compared with those who scored in the lowest tertile of PDQS intake ($P < 0.01$; <0.01 and 0.04, respectively).

At month 3, average daily fibre (g) and folate intakes (µg) were significantly higher in those with a total PDQS score in the highest tertile compared with those in the lowest and middle

Table 2. Sample demographics

N = 115	Mean (range)
Age	59.0 (45–89)
BMI (kg/m^2)	31.2 (18.4–68.8)
	N (%)
Male	25 (21.7)
Female	90 (78.3)
Underweight	1 (0.9)
Normal Weight	11 (9.6)
Overweight	44 (38.3)
Obese	59 (51.3)
Current smoker	18 (15.7)
Hypertension	50 (43.5)
Hypercholesterolaemia	47 (40.9)

tertile of total PDQS score (both $P < 0.01$). Average daily potassium intake (mg) was significantly higher in those who scored in the highest tertile of PDQS score compared with those in the lowest tertile ($P = 0.01$). Average daily vitamin C intake increased significantly across each tertile of PDQS score ($P < 0.01$).

Average daily nutritional intake from both food and dietary supplement sources was also analysed by tertile of total PDQS score (Supplementary Files 5). Similar trends were observed when including both foods and supplements as when considering foods-only data, with the exception that average intake of vitamin A (total retinol equivalents µg/d) was significantly higher in those that scored in the highest PDQS tertile compared with the lowest and middle tertile ($P = 0.01$) and there was no significant difference in average folate/folic acid intake between tertiles at month 3 when supplement data were also included.

Change in lifestyle data collected at month 3 were analysed to determine any changes in health or lifestyle over the 3-month study period and are presented in Supplementary Files (Table 6). 'Only a small percentage of participants reported any changes (14.2% reported improvement in dietary intake; 19.2% reported increased physical activity; all other lifestyle changes reported were minimal (0.83–10.8%) at Month 3). As such, these data were too limited to perform further analysis with.'

A number of minor further amendments to the amended PDQS were made after the validation and reliability assessment based on feedback received from participants when completing the PDQS, and some individual food group results. Firstly, additional example foods were added to some food groups to clarify the diversity/breadth of particular food groups when making an estimate of frequency of consumption. Secondly, as the 21 PDQS food groups were presented individually to participants via the online questionnaire in the current study, the study team hypothesised that estimates may be improved by presenting related food groups together i.e. vegetable food groups (e.g. 'other vegetables', 'cruciferous vegetables', 'dark green leafy vegetables', 'carrots' and 'legumes') and also fruit groups ('other fruits', 'whole citrus fruits') and also ensuring 'other vegetables' and 'other fruits' groups are presented after the other specific fruit and vegetables category to assist with estimation of overall intake. The revised and final version is available in Supplementary Files Table 7.

Discussion

This paper reports the amendment of the PDQS and its validity and reliability against a 4-day food diary in a UK/Irish population at risk of CVD. The original PDQS questionnaire was designed for a US population and, in order to adapt it for a UK/Irish population, food groups and descriptions were amended to reflect regional eating and cooking habits. Results indicate that the amended PDQS is a valid and reliable dietary screening tool for a UK/Irish population at risk of CVD.

Validity analyses indicated that total PDQS score from the PDQS was strongly positively correlated with total PDQS score obtained from FDs at both timepoints, with stronger correlations observed at month 3. In total, 76.2% of individual food group scores from the PDQS were strongly positively correlated with individual food scores derived from FDs at month 0. At month 3, 95.2% of the individual food scores from the PDQS were strongly positively correlated with individual food scores derived from food diaries with the exception of 'legumes'. Intraclass correlation coefficients and weighted kappa both indicated a moderate level of agreement between measures for total PDQS, whilst Bland-Altman plots demonstrated a good level of validity between measures; providing further evidence of the validity of the PDQS. These results are similar to the level of agreement seen for energy and nutrient intake in validation studies that used food diaries as the reference measure[12,23,24,32] and validations of different versions of the PDQS that used HEI-2015 derived from the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool as a reference measure.[13,14]

The stronger validity correlations observed at month 3 compared with month 0 may be due to familiarity with the questionnaire and food diary completion process at the second administration, and therefore more accurate estimation of dietary intake. Stronger associations have also been observed at the second administration of the test and reference measures in an FFQ validity and reliability study in a childhood population that used a dietary record as a reference measure.[33]

In general, those who had a better diet quality, as determined via PDQS, had significantly higher intakes of micronutrients and fibre assessed via FD compared with those in the lowest tertile of PDQS score, which further supports the validity of the amended PDQS in terms of appropriately identifying those with suboptimal diet quality with a poorer intake of particular nutrients and micronutrients, and is a trend also observed in the validation of the rPDQS and the 24-hour and 30 d PDQS validation in US populations.[13,14]

Reliability analyses indicated that total PDQS score from the PDQS was strongly positively correlated with total PDQS score from PDQS at Month 3, with intraclass correlation coefficients and coefficient of variation indicating good reliability. The time interval between administration of the PDQS is similar to many reliability studies, which typically range between 1 and 12 months.[17] Similar correlations were observed compared in other reliability studies, including the reliability of the rapid 13-item PDQS (rPDQS),[14] the Diet Risk Score (DRS)[29] and the nutrition component of the Rapid Eating and Activity Assessment for Patients (REAP) tool.[31]

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Table 3. Total Prime Diet Quality Score (PDQS) scores obtained from both questionnaire and food diaries at month 0 and month 3

	Total PDQS (PDQS)			Total PDQS (Food Diary)			Difference between total PDQS derived from PDQS and food diaries	
	Month 0 N Mean (SD)	Month 3 N Mean (SD)	Difference between PDQS between M0 and M3 P[1]	Month 0 N Mean (SD)	Month 3 N Mean (SD)	Difference between PDQS from FD between M0–M3 P[1]	Month 0 P[1]	Month 3 P[1]
All sample	115 20.4 (5.7)	110 21.0 (5.6)	0.11	108 18.2 (4.6)	105 18.3 (4.7)	0.62	<0.01	<0.01
Males	25 18.2 (5.7)	24 19.2 (5.5)	0.38	24 17.6 (5.2)	24 16.1 (5.5)	0.16		
Females	90 21.0 (5.6)	86 21.6 (5.5)	0.21	84 18.4 (4.4)	81 19.0 (4.2)	0.16		
P[2]	0.04	0.06		0.36	0.01			
Underweight/normal weight	12 25.4 (3.7)[a]	12 25.6 (3.6)[a]	0.46	11 21.5 (3.2)[a]	11 20.0 (2.9)	0.21		
Overweight	44 21.1 (5.2)[b]	42 21.5 (5.4)[b]	0.30	42 17.4 (4.3)[b]	42 18.6 (4.9)	0.04		
Obese	59 18.8 (5.8)[b]	56 20.0 (5.5)[b]	0.29	55 18.1 (4.8)[b]	52 17.8 (4.8)	0.53		
P[3]	<0.01	0.01		<0.01	0.29			

Superscript letters denote groups that are significantly different from one another. PDQS score range 0–42; higher score indicates better diet quality.
[1]Wilcoxon Signed Rank Test.
[2]Mann-Whitney *U*-Test.
[3]Kruskal-Wallis *H* Test.

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Table 4. Spearman correlation coefficients (*r*), weighted kappa (K) (where appropriate) and intraclass correlation coefficients (ICCs) for Prime Diet Quality Score (PDQS) questionnaire and PDQS scores derived from 4-day food diaries at month 0 and month 3; and Spearman correlation coefficients, ICCs and coefficient of variation (where appropriate) for PDQS questionnaire at month 0 and month 3

	Month 0 (n = 108)		Month 3 (n = 105)		Reliability (n = 110)		
PDQS Food Groupings	Validity (*r*) K (SE)	ICC (95% CI)	Validity (*r*) K (SE)	ICC (95% CI)	Reliability (*r*) K (SE)	ICC (95% CI)	Coeff. of variation
Total PDQS score	0.59** 0.40 (0.07)	0.70 (0.49–0.81)	0.65** 0.42 (0.07)	0.73 (0.41–0.86)	0.78** 0.54 (0.04)	0.88 (0.82–0.92)	0.11
Red meat	0.37**	0.55 (0.34–0.69)	0.34**	0.53 (0.30–0.68)	0.67**	0.79 (0.69–0.86)	–
Processed meat	0.30**	0.46 (0.22–0.63)	0.21*	0.33 (0.02–0.54)	0.56**	0.72 (0.59–0.81)	–
Poultry	0.32**	0.44 (0.18–0.61)	0.35**	0.49 (0.25–0.65)	0.55**	0.70 (0.57–0.80)	–
Fish	0.25**	0.36 (0.08–0.56)	0.43**	0.54 (0.33–0.69)	0.69**	0.81 (0.73–0.87)	–
Whole eggs	0.27**	0.47 (0.23–0.63)	0.49**	0.68 (0.54–0.79)	0.63**	0.79 (0.69–0.85)	–
Whole milk dairy foods	0.13	0.24 (–0.09–0.47)	0.36**	0.52 (0.30–0.67)	0.42**	0.63 (0.46–0.75)	–
High-fat foods	0.17	0.18 (–0.11–0.41)	0.21**	0.21 (–0.10–0.44)	0.22*	0.42 (0.16–0.61)	–
Wholegrain foods	0.50**	0.69 (0.54–0.79)	0.42**	0.67 (0.51–0.77)	0.61**	0.77 (0.66–0.84)	–
Sweet baked foods	0.30**	0.46 (0.20–0.63)	0.34**	0.49 (0.25–0.65)	0.49*	0.66 (0.51–0.77)	–
Potatoes	0.33**	0.48 (0.23–0.65)	0.46**	0.62 (0.43–0.74)	0.64**	0.78 (0.68–0.85)	–
Dark green leafy vegetables	0.16	0.18 (–0.11–0.40)	0.46**	0.46 (0.01–0.69)	0.61**	0.76 (0.65–0.84)	–
Cruciferous vegetables	0.30**	0.46 (0.20–0.63)	0.20*	0.31 (0.02–0.53)	0.64**	0.77 (0.67–0.84)	–
Carrots	0.27**	0.38 (0.11–0.58)	0.32**	0.50 (0.27–0.66)	0.64**	0.77 (0.67–0.85)	–
Other vegetables	0.38**	0.45 (0.08–0.66)	0.38**	0.49 (0.12–0.69)	0.60**	0.75 (0.63–0.83)	–
Legumes	0.37**	0.51 (0.29–0.67)	0.13	0.19 (–0.19–0.45)	0.55**	0.72 (0.59–0.81)	–
Whole citrus fruit	0.64**	0.75 (0.64–0.83)	0.52**	0.64 (0.43–0.76)	0.71**	0.82 (0.74–0.88)	–
Other fruits	0.59**	0.77 (0.67–0.84)	0.60**	0.77 (0.66–0.85)	0.67**	0.82 (0.74–0.88)	–
Liquid vegetable oils	0.18	0.22 (–0.08–0.45)	0.26**	0.32 (–0.05–0.55)	0.67**	0.81 (0.72–0.87)	–
Nuts	0.38**	0.60 (0.41–0.73)	0.48**	0.69 (0.54–0.79)	0.69**	0.85 (0.78–0.89)	–
Desserts, pudding, confectionery	0.09	0.18 (–0.19–0.43)	0.25**	0.42 (0.15–0.61)	0.44**	0.59 (0.41–0.72)	–
Sugar sweetened beverages	0.37**	0.53 (0.31–0.68)	0.39**	0.67 (0.52–0.78)	0.64**	0.78 (0.67–0.85)	–

R = Spearman correlation coefficient; K = weighted kappa statistics, SE, standard error; ICC, intraclass correlation coefficient. ICC (two-way mixed; absolute agreement).
*Significant at the 0.05 level (2-tailed).
**Significant at the 0.01 level (2-tailed).

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Table 5. Average daily nutrient intake by tertile of Prime Diet Quality Score (PDQS) total score from amended PDQS (food only)

	Month 0					Month 3					
		PDQS total score					PDQS total score				
Nutrient	Average daily intake Mean (SD)	Tertile 1 (n = 36) Mean (SD)	Tertile 2 (n = 38) Mean (SD)	Tertile 3 (n = 41) Mean (SD)	Difference between tertiles P*	Average daily intake Mean (SD)	Tertile 1 (n = 31) Mean (SD)	Tertile 2 (n = 40) Mean (SD)	Tertile 3 (n = 39) Mean (SD)	Difference between tertiles P*	Difference in nutrient intake Month 0–Month 3 P**
Energy (kcal)	1943.1 (492.1)	1944.8 (555.8)	1981.2 (538.8)	1904.6 (384.3)	0.96	1790.3 (490.6)	1886.5 (603.3)	1761.9 (457.8)	1752.8 (438.1)	0.79	<0.01
Carbohydrate (g)	205.1 (52.6)	207.7 (50.7)	210.0 (62.5)	198.2 (43.6)	0.56	189.3 (61.9)	201.3 (81.7)	184.9 (57.6)	185.4 (49.7)	0.76	<0.01
Protein (g)	81.0 (24.0)	77.7 (30.0)	84.0 (21.1)	80.6 (19.8)	0.23	76.6 (19.8)	77.5 (23.2)	74.7 (17.8)	78.0 (19.6)	0.95	0.02
Total Fat (g)	80.2 (26.2)	79.8 (26.1)	82.1 (29.4)	78.9 (23.4)	0.98	73.8 (24.3)	79.2 (24.2)	72.0 (22.6)	71.8 (26.0)	0.40	0.01
Saturated Fat (g)	29.4 (11.4)	30.5 (11.6)	31.4 (13.6)	26.5 (8.0)	0.13	27.8 (10.3)	31.6 (10.8)	27.3 (8.0)	25.7 (11.4)	0.05	0.19
Free Sugars (g)	40.0 (26.1)	44.6 (28.6)	41.6 (30.3)	34.5 (18.1)	0.34	35.8 (23.8)	44.8 (32.4)	34.4 (22.4)	31.0 (15.7)	0.34	0.13
Alcohol (g)	25.3 (17.6)	23.9 (17.4)	25.9 (19.1)	25.8 (17.4)	0.95	20.9 (17.0)	19.9 (17.5)	21.8 (20.0)	20.6 (13.8)	0.84	0.15
NSP Englyst Fibre (g)	15.3 (5.0)	12.7 (4.0)[a]	15.0 (4.9)[a]	17.8 (5.0)[b]	<0.01	14.4 (5.4)	11.6 (5.0)[a]	13.6 (4.3)[a]	17.1 (5.4)[b]	<0.01	0.03
Fibre (g)	20.8 (6.7)	17.4 (5.6)[a]	20.4 (5.7)[b]	24.3 (7.0)[c]	<0.01	19.0 (6.6)	15.4 (5.8)[a]	18.2 (5.5)[b]	22.4 (6.6)[c]	<0.01	<0.01
Iron (mg)	11.6 (3.9)	11.0 (4.4)	11.0 (2.8)	12.6 (4.3)	0.11	11.1 (4.1)	10.5 (4.2)	10.5 (3.2)	12.2 (4.7)	0.12	0.15
Sodium (mg)	2115.6 (687.5)	2139.2 (624.0)	2220.9 (805.3)	1992.5 (610.0)	0.41	2078.3 (758.7)	2210.1 (822.1)	2037.4 (676.9)	2029.2 (800.9)	0.57	0.64
Potassium (mg)	3245.5 (831.6)	2923.0 (923.2)[a]	3252.4 (720.5)[a,b]	3518.7 (766.9)[b]	<0.01	3119.3 (824.7)	2830.7 (799.4)[a]	3023.4 (725.7)[a,b]	3417.3 (861.0)[b]	0.01	0.01
Calcium (mg)	824.9 (258.0)	763.4 (269.2)	864.0 (279.4)	840.3 (220.8)	0.24	781.2 (247.8)	819.7 (225.3)	748.9 (264.7)	788.0 (246.6)	0.29	0.09
Iodine (ug)	145.8 (61.7)	116.3 (35.3)[a]	150.7 (64.6)[b]	166.6 (68.0)[b]	<0.01	144.7 (74.3)	129.8 (67.6)	10.5 (3.2)	12.2 (4.7)	0.12	0.70
Thiamine (mg)	1.6 (0.6)	1.4 (0.6)[a]	1.6 (0.5)[a,b]	1.7 (0.6)[b]	0.03	1.5 (0.5)	1.4 (0.5)	1.4 (0.5)	1.6 (0.5)	0.06	0.02
Riboflavin (mg)	1.7 (0.6)	1.5 (0.6)[a]	1.7 (0.5)[a,b]	1.9 (0.6)[b]	0.02	1.6 (0.6)	1.6 (0.5)	1.6 (0.6)	1.7 (0.6)	0.28	0.01
Niacin (mg)	38.0 (13.2)	38.0 (16.9)	38.7 (11.4)	37.3 (11.5)	0.82	35.9 (0.6)	37.7 (16.3)	35.2 (9.7)	35.3 (11.6)	0.86	0.04
Folate	275.0 (121.0)	230.2 (118.2)[a]	258.7 (82.3)[a]	329.9 (136.1)[b]	<0.01	254.2 (111.0)	227.3 (112.8)[a]	232.5 (69.9)[a]	294.9 (132.8)[b]	<0.01	0.02
Vitamin A (Total Retinol Equivalents) (µg)	843.5 (552.0)	712.9 (403.0)	874.6 (552.9)	926.7 (648.1)	0.40	867.8 (499.5)	714.3 (295.1)[a]	843.2 (437.3)[b]	999.4 (631.3)[b]	0.09	0.76
Vitamin B12 (Cobalamin) (µg)	5.7 (2.8)	5.3 (3.5)	6.0 (2.9)	5.7 (2.1)	0.11	5.1 (2.7)	5.4 (4.1)	4.8 (2.0)	5.2 (2.2)	0.66	0.01
Vitamin C (mg)	95.0 (57.7)	56.8 (32.6)[a]	98.1 (49.7)[b]	125.3 (63.7)[b]	<0.01	84.1 (58.7)	50.6 (39.3)[a]	74.8 (45.5)[b]	116.8 (66.3)[c]	<0.01	0.02
Vitamin D (µg)	3.6 (2.2)	2.8 (2.1)[a]	3.9 (2.4)[b]	3.8 (2.0)[b]	0.04	3.0 (2.0)	2.6 (1.6)	2.9 (2.1)	3.3 (2.1)	0.32	0.02
Omega-3 (g)	1.5 (1.2)	1.3 (0.9)[a]	1.6 (1.3)[b]	1.8 (1.3)[b]	0.16	1.2 (1.0)	1.0 (1.0)	1.2 (0.8)	1.3 (1.1)	0.25	0.01
Omega-6 (g)	6.5 (4.7)	6.5 (5.6)	6.2 (3.8)	6.9 (4.8)	0.44	5.5 (3.6)	4.6 (2.3)	5.8 (4.2)	5.8 (3.7)	0.62	<0.01

Superscript letters denote groups that are significantly different from one another.
*Kruskal-Wallis *H* Test.
**Paired sample t-test.

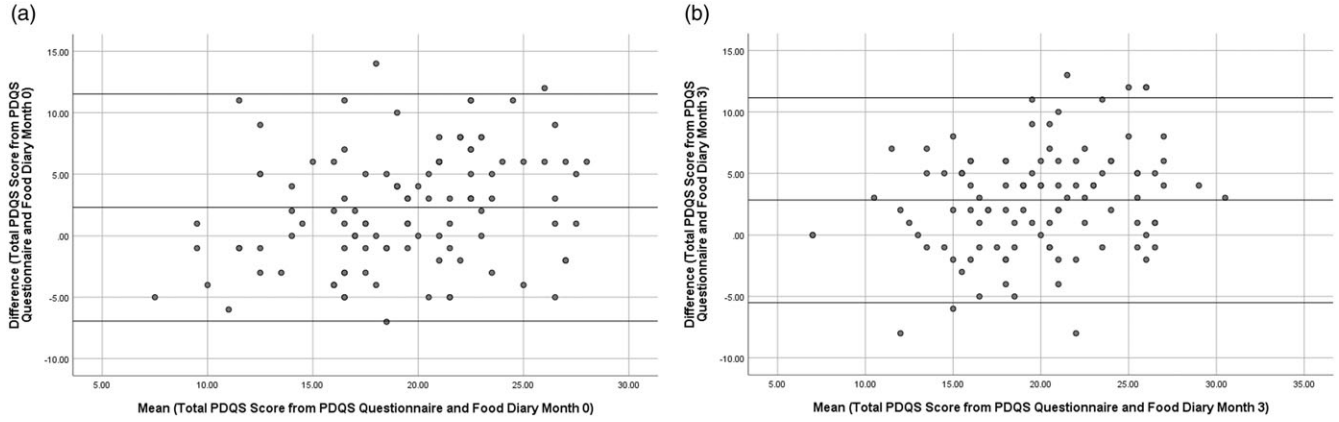

Fig. 2. (a) Bland-Altman Plot showing difference between total Prime Diet Quality Score (PDQS) score from PDQS Questionnaire and Food Diary against mean of total PDQS score from PDQS Questionnaire and Food Diary at Month 0 (n = 108). Mean difference: 2.29. (b) Bland-Altman Plot showing difference between total PDQS score from PDQS Questionnaire and Food Diary against mean of total PDQS score from PDQS Questionnaire and Food Diary at Month 3 (n = 105). Mean difference: 2.82.

Average total PDQS score derived from PDQS was significantly higher, indicating a better diet quality, at both month 0 and month 3, than average total PDQS score derived from FDs at month 0 and month 3. The difference in reference time frame may help to explain this observation, as PDQS referred to average intake over the previous 3 months, whilst the 4-day FDs captured a shorter 4-day period which may have been less representative of habitual intake. In the systematic review of dietary validation studies by Cade *et al.*,[23] it was stated that, if FFQs are compared with weighed records, any lack of agreement can be attributed in part to within-subject variance that is inherent in the weighted record measure due to the shorter time frame and the more accurate record of dietary intake. Furthermore, a review of dietary validation and reliability studies[23] discussed that individuals often have difficulty in estimating portion sizes of foods, both when examining displayed foods and when reporting about foods previously consumed and how if the individual cannot assign portion size, it can influence estimation of absolute nutrient intake[23] So the ability to estimate portion size may have differed between the food diary where participants were asked to weigh or use portion size photos as guidance and the PDQS which was based on standard servings. Regardless of this, Cade *et al.*[23] recommend that weighed or dietary records are the preferred reference measure for FFQ validation rather than a 24-hour recall as the sources of error of a dietary record are less likely to be correlated with the error of the FFQ.[23]

In a systematic review of gender analysis in the development and validation of FFQs it was noted that, although gender can impact food choice and portions, gender differences are often not analysed during FFQ development and validation,[34] and studies assessing the validity of the DRS and REAP scores did not present results by gender.[29,31] Subgroup analyses were performed by gender in the present study despite the majority of the participants being female (78.3%), and agreement between measures was statistically significant in both females and males at both month 0 and 3; whilst stronger agreement was observed at month 0 in females compared with males and stronger agreement seen in males compared with females at month 3. In terms of reliability, stronger agreement was observed in females compared with males, but both were statistically significant. The consideration of gender is a notable aspect of the present study and indicates that the PDQS can be used as a diet screening tool for both males and females at risk of CVD.

As the vast majority of the sample (89.6%) were participants with overweight or obesity, it was not possible to perform subgroup analysis by BMI category (kg/m^2). Data on changes to lifestyle were presented within Supplementary Files because only a small percentage of participants reported any improvement in dietary intake or physical activity at Month 3. As such, results were not presented in main paper as unlikely to have had an impact on PDQS validity and reliability assessment as both the test and reference measures were administered at both timepoints.

Strengths of the present study include the use of 4-day FDs as the reference measure. Seven-day FDs are considered to be one of the most robust 'gold standard' methods of dietary assessment available[35,36] but 4-day FDs are widely considered a good practical alternative for many populations and study designs.[37,38] Another strength of the current study is the sample size, which is larger than other validation studies[24,26,27] and is at the high end of the sample size range recommended in a dietary validation study review by Cade *et al.*.[23] Furthermore, this validation and reliability assessment was conducted in a well-defined population at risk of CVD on the island of Ireland and subgroup analyses was performed by gender. Furthermore, as the PDQS is designed to be a rapid diet quality screening tool, in that it is able to used in a time-sensitive clinical setting taking <10 minutes to complete (previously established as no more than 35 items),[39] and comprised of food groups rather than specific food items, it is likely to be appropriate for use in more diverse populations than those studied, including regions other than UK and Ireland, and this is one of the strengths of its design and administration. In order to improve the validity and reliability of the PDQS further and informed by the results of this study, the authors recommend the following minor further amendments to the finalised PDQS (Supplementary Table 8) based on feedback received from participants when completing the PDQS, as described in Results section. These amendments are likely to improve the accuracy of the PDQS in determining diet quality by assisting participants with their estimations of

intake as they better describe some food groups, give more extensive lists of example foods within some food groups to help participants categorise their intake correctly, and present related food groups together such as all vegetable groups and all fruit groups, to avoid errors is miscategorising some vegetables/fruits.

Limitations of the current study include the comparison of two self-reported methods of dietary assessment. It is well recognised that there is no objective and practical 'gold standard' method for directly assessing the validity of FFQs or dietary questionnaires[23] and a dietary validation study can therefore only indicate whether both assessment methods obtain similar responses rather than accurate responses.[23] However, as 7-day FDs are often considered to be one of the most robust and practical methods of dietary assessment, the choice of a 4-day FD was considered appropriate. Further to this, the method of administering the food diary over 4 consecutive days has been widely utilised in the literature[40–42] although there is some evidence to suggest that administration on random days may obtain more representative data. As such, this may be a potential limitation but was considered appropriate and practical for the current study which was conducted during national lockdowns.[43] In terms of reliability, the 3-month interval time frame chosen for the present study was intended to help account for any seasonal variations in dietary intake, but a short enough time frame to avoid capturing major changes in dietary habits that are more likely to occur years apart. Some literature suggests shorter intervals between assessments may make it more likely that latter responses are influenced by earlier responses, whilst too long an interval may reduce reproducibility as assessments are less able to detect true changes in dietary intake.[23] It has been recommended that the time interval between repeat administrations of the test measures should minimise potential changes in dietary intake.[23] In terms of sample population, the assessment of validity and reliability was conducted in a UK/Irish population at risk of CVD, but the majority of the sample recruited in the present sample were female (78.3%). No consistent differences in agreement were observed in the present study between genders so it is likely that this did not have a significant impact and would indicate generalisability to an adult population. The PDQS tool validated here has, however, not been designed for or validated in a population that follows an exclusively plant-based diet so it would be of interest to explore its application to more diverse population groups in future.

In conclusion, the amended PDQS demonstrated good validity and reproducibility in the current study and is appropriate for assessment of diet quality in a UK or Irish population at risk of CVD in place of more burdensome dietary assessment methods. The next step for this validated PDQS, or similar dietary scores, is to determine their association with NCD related-outcomes in European population cohorts, in a similar way as has been done with the variations of the PDQS in US cohorts,[11,15–19] to further support is use in both the healthcare and research settings.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.23

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Competing interests

None.

Authorship

JVW and LB formulated the research question, designed the study and directed the research activities. SFB and RF recruited the participants and collected, cleaned and analysed all study data with input from LB and JVW. SFB drafted the manuscript, with input from RF, MF, CC, LB, and JVW. All authors read and approved the final manuscript.

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BRIEF REPORT

Effects of short-term, high-dose cocoa-derived flavanol supplementation on gut microbiota composition: secondary findings from a randomized, double-blind, placebo-controlled crossover study

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Abstract

Cocoa-derived flavanols (CDF) may act as prebiotics. However, evidence is inconsistent, and the duration and dose of CDF intake needed to elicit any prebiotic effect are undefined. This randomized, double-blind, crossover study determined the effects of short-term, high-dose dietary supplementation with CDF versus matched placebo on gut microbiota composition in 8 healthy adults. A single faecal sample was collected 8 d after supplementation with 900 mg/d CDF or placebo. Gut microbiota composition and *Bifidobacterium* spp. and *Lactobacillus* spp. abundance were measured as secondary outcomes by 16S ribosomal ribonucleic acid (rRNA) amplicon sequencing and quantitative polymerase chain reaction, respectively. No between-treatment differences in the relative or absolute abundance of *Bifidobacterium* spp. (Cohen's $d = 0.89$, $P = 0.22$) or *Lactobacillus* spp. (Cohen's $d = 0.42$, $P = 0.65$) were detected. Shannon diversity (Cohen's $d = 0.38$, $P = 0.04$) and overall community richness (Cohen's $d = 0.34$, $P = 0.06$) were lower following CDF supplementation versus placebo, but no between-treatment differences in β-diversity or taxa relative abundances were observed. Findings are not consistent with a clear prebiotic effect of this short-term, high-dose CDF supplementation strategy relative to placebo.

Key words: **Chocolate: High-throughput Sequencing: Microbiome: Polyphenol**

Introduction

The gut microbiota is increasingly recognized as an important mediator of human health.[1] One approach to favourably modulating the gut microbiota is by consuming prebiotics, defined as substrates that are selectively utilized by host microbes conferring a health benefit.[2] Although some controversy surrounds what substrates can be classified as prebiotics, several have promising but incomplete supporting evidence.[2] These "candidate prebiotics" include polyphenols.[2] Polyphenols are secondary plant metabolites that are poorly absorbed in the small intestine, but undergo enzymatic/bacterial metabolism in the colon.[3] Certain polyphenols may favourably modulate the gut microbiota by promoting the growth of beneficial bacteria including *Bifidobacterium* and *Lactobacillus* spp.,[4,5] while reducing abundance of potentially harmful microbes such as *Clostridium perfringens*,[6] which is consistent with a prebiotic effect.[2] However, over eight-thousand polyphenolic compounds have been identified in plant species, and the effects of these compounds on the gut microbiota vary.[3]

Flavonoids are the most studied group of polyphenols. The subclass flavanols, specifically the monomeric flavanols catechin, epicatechin, epigallocatechin, gallocatechin, and their gallate derivatives, are found in abundance in tea and cocoa.[3]

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Initial studies conducted in in vitro models, animals, and healthy humans reported that cocoa-derived flavanols (CDF) favourably impacted the gut microbiota by selectively increasing absolute abundance of beneficial genera *Lactobacillus* and *Bifidobacterium*.[4,5,7,8] In contrast, results of more recent human trials utilizing modern high-throughput sequencing that relies on compositional analysis (i.e. relative abundance), rather than absolute quantification, have failed to observe increases in the relative abundance of *Lactobacillus* or *Bifidobacterium*. Instead, they reported changes in other taxa that are not consistently recognized as beneficial.[8,9] Reasons for the inconsistency across studies are unclear but could be attributed to differences in the microbiota measurement methods, study population, or supplementation strategy, including CDF source, dose, and duration of supplementation. For example, several studies used whole chocolate or cocoa supplementation which contain other compounds known to modulate the gut microbiome, like fibre, caffeine, and theobromine,[1,10] rather than isolated CDF. Additionally, recent studies have not measured absolute abundance of gut microbes, including *Lactobacillus* or *Bifidobacterium*, as is necessary to support prebiotic effects.[2] Thus, more research is needed to substantiate any prebiotic effect of CDF.

Notably, previous CDF or cocoa supplementation trials relied on long duration pre and post supplementation measurements, ranging in duration from 3 to 10 weeks, with no assessment of the minimal time to achieve an effect.[5,8,9] Other candidate prebiotics are known to alter the gut microbiota within a week of starting consumption.[11] Determining whether short-duration (e.g. ~1 week) supplementation strategies have prebiotic effects may impact cost:benefit decisions within certain populations. These include military personnel, in whom constrained diets may be consumed for only short periods of time.[12] Prebiotics may also be most cost effective before exposure to environments wherein prebiotic health benefits may be maximized.[13] Therefore, this study aimed to determine the effects of short-term dietary supplementation with CDF at a higher dose (900 mg/d) than used in previous studies on gut microbiota composition and abundance of *Lactobacillus* or *Bifidobacterium* in generally healthy adults. We hypothesized this regimen would not result in substantive differences in gut microbiota community composition but would lead to significantly higher absolute and relative abundances of *Lactobacillus* and *Bifidobacterium* following the CDF treatment compared to following the placebo treatment.

Methods

Participants

Participants 17–49 years were recruited from the Natick, MA area between January 2020 and October 2021. Study exclusion criteria included antibiotic use within three months; gastrointestinal disease; <4 bowel movements weekly; regular use of medications impacting gastrointestinal function; colonoscopy within three months; inability to avoid non-steroidal anti-inflammatory medications; vegetarian diet; inability or unwillingness to not consume fermented products for 2 weeks prior and through study participation; and actively trying to lose or gain weight. Participants were instructed to discontinue use of any probiotic, prebiotic, or other dietary supplements (excepting multi-vitamins) and refrain from consuming cocoa-based products and flavanol-rich foods for 2 week prior to and throughout the study. If participants routinely ingested tea or coffee, instruction was given to maintain the usual intake throughout the study.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the US Army Medical Research and Development Command Institutional Review Board (approval number M-10762). Written informed consent was obtained from all subjects/patients. The trial was registered on www.clinicaltrials.gov as NCT04359082.

Study design

The data reported herein were included as secondary outcomes in a trial designed to determine the effects of CDF on cold-induced vasodilation and thermoregulatory responses.[14] The study followed a randomized, double-blind crossover design consisting of two eight-day phases with a minimum two-week wash out. Upon enrollment, participants were randomized using computer-generated randomization. Medical staff otherwise unaffiliated with the study then assigned each participant to receive daily supplementation of CDF (225 mg/pill, CocoaVia, Mars, Inc.) and then placebo (100 mg dextrose/pill, Compounded Solutions in Pharmacy, LLC, Monroe, CT) or vice versa, administered in capsule form. The capsules were matched on both caffeine (10 mg/pill) and theobromine (30 mg/pill) content. Participants consumed four pills every morning approximately one hour after breakfast every day throughout each eight-day study phase. When feasible, pills were consumed under the direct supervision of medical staff not involved in data collection. When visual confirmation was not feasible, verbal confirmation of adherence was obtained daily. The dose and duration of CDF supplementation were based on primary study outcomes relating to the effects of CDF on manual dexterity during cold exposure. Study staff and participants were all blinded.

Participants maintained a 4-day food record during days 4–7 of each phase and were asked to maintain a similar diet during both study phases. Research dietitians provided instructions to participants, reviewed all food records to ensure compliance with study food restrictions, and entered data into Food Processor (ESHA Research, Salem, OR) for analysis of nutrient intakes.

Faecal sample collection and sequencing

A single faecal sample was collected during a 48-hr period between days 6 and 8 of each experimental period. All samples were collected into plastic collection containers, transported at room temperature, and aliquoted within 12 hr of collection. Aliquots were immediately frozen and stored at −80°C until analysis.

16S rRNA amplicon sequencing. Samples were processed and analysed with the ZymoBIOMICS Targeted Sequencing Service (Zymo Research, Irvine, CA). Deoxyribonucleic acid (DNA) was extracted using the ZymoBIOMICS®-96 MagBead DNA Kit. Both positive and negative controls were included in sequence runs. Primers designed to amplify the V3–V4 region of the 16S rRNA gene were used for PCR amplification, and all samples were sequenced in triplicate on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA). Deconvoluted sequences were processed using the DADA2 pipeline with default parameters to obtain amplicon sequence variants (ASVs).[15] Potential sequencing errors and chimeric sequences were removed. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1 using the Zymo Research Database.[16]

The median read count for each sample was 33,510 reads (range: 24,722–38,981 reads/sample). Possible sequencing errors were filtered to remove ASV with fewer <2 counts in ≥10% of samples. Remaining reads were classified into 405 unique ASVs assigned to 89 unique genera. For diversity analyses, samples were rarified to 20,000 reads/sample. Within-sample diversity was calculated in R 4.2.1 by Shannon, Simpson and observed ASVs. Absolute total bacteria abundance was determined using a standard curve technique as part of the ZymoBIOMICS Targeted Sequencing Service. Briefly, a standard curve was generated via quantitative polymerase chain reaction (qPCR) from plasmid DNA containing the 16S gene. The equation generated by the standard curve was used to transform Cq values to a number of gene copies/sample. Genome copies/ul were calculated by dividing the gene copy number by the assumed number of gene copies per genome, which is four. Lastly, the amount of DNA/ul of the sample was calculated using the assumed genome size of 4.64×10^6 (*Escherichia coli*).

Quantitative polymerase chain reaction DNA from faecal samples and isolated bacterial cultures were extracted using the DNeasy PowerSoil Pro Kit (QIAGEN, Inc., Germantown, MD). DNA concentrations were then quantified using a Nanodrop (ThermoFisher Scientific, Waltham, MA). To calculate absolute abundance from qPCR, standard curves using serial dilutions were constructed using DNA from isolated cultures of *Bifidobacterium longum* subsp. *longum* (ATCC-55813) and *Lactobacillus acidophilus* (ATCC-4356). DNA was further amplified using 3 µl of the template with previously published primers[17] and Luna Universal qPCR Kits (New England Biolabs, Ipswich, MA). Amplification was performed for 45 cycles at 95°C for 15s and 60°C for 60s, followed by a melt curve from 60°C to 95°C, using a QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific, Waltham, MA). Genome size for each bacterium was used to calculate qPCR copy number (http://cels.uri.edu/gsc/cndna.html).

Statistical analysis

Sample size calculations for primary study outcomes determined that 10 participants would be necessary for detecting meaningful and expected mean ± standard deviation (SD) between-treatment differences in skin temperature at the finger (2°C ± 2.3°C)[18] and finger blood flow response (25 units ± 10 units)[19] to cold exposure at $\alpha = 0.05$ and power = 0.80.[14] Sample size calculations for the secondary outcomes presented here were based on data reported by Tzounis *et al.*[5] wherein cocoa flavanol supplementation increased abundance of *Bifidobacterium* spp. and *Lactobacillus/Enterococcus* spp. by Cohen's *d* effect sizes >3. Based on those data, four participants would be needed to detect similar effect sizes at $\alpha = 0.05$ and power = 0.80.[14] The eight participants included in the present analysis were sufficient to detect a minimum effect size of 1.2 at $\alpha = 0.05$ and power = 0.80.

Between-treatment differences in α-diversity, absolute abundance, and qPCR copy number were determined using a linear mixed model with a supplement, treatment sequence, their interaction, age, and body mass index (BMI) included as fixed factors, and subject as a random effect, using R packages afex v 1.3.0. Normal distribution and homoscedasticity of residuals were verified for all models. β-diversity was measured using Bray–Curtis dissimilarity, and weighted/unweighted UniFrac distances and were analysed by nested PERMANOVA with the R package microbiome association with linear models (MaAsLin2)[21] (total sum scaling normalization, minimum abundance set to 0.0001, and prevalence set to 0.2) with a supplement, treatment sequence, study phase age, and BMI included as fixed factors. The subject was included as a random effect in the MaAsLin2 model. The Benjamini–Hochberg procedure was used to adjust *P*-values. Data are presented as mean ± SD or median (interquartile range) unless otherwise noted. Statistical significance was defined as $P \leq 0.05$ and $Q \leq 0.20$.

Results and discussion

Previous studies reporting the effects of CDF and cocoa supplementation on the human gut microbiota are inconsistent, with some, but not all, reporting potential prebiotic effects.[4,5,7,8] This randomized, double-blind, placebo-controlled crossover study aimed to extend that evidence by determining the effects of short-term (8 d) and high-dose (900 mg/d) dietary supplementation with CDF versus placebo on the gut microbiota of healthy adults. Eleven volunteers participated in the study, but two were withdrawn from the study and faecal samples could not be collected from a third (Supplemental Fig. 1). Thus, the present analysis includes the seven male and one female volunteer who provided faecal samples following both study phases. Dietary energy and macronutrient intakes were similar between phases (Table 1), and adherence to the intervention was 100% during both phases.

Faecal samples were analysed using both targeted quantitative and compositional approaches to better substantiate selective growth of the health-promoting genera *Lactobacillus* and *Bifidobacterium*. However, neither higher absolute nor relative abundance of *Lactobacillus* or *Bifidobacterium* were observed following CDF supplementation relative to placebo (Fig. 1, Table 2). Results contrast with previous studies that have reported increases in the absolute abundance of both genera following supplementation with 494 mg cocoa flavanols for

Table 1. Baseline demographics and dietary intake

	Placebo	Cocoa flavanol	*P*-value[a]
Male/Female	7/1		
Age (year)	25 ± 5		
BMI (kg/m^2)	25 ± 4		
Energy (kcal/d)	2481 ± 723	2309 ± 685	0.48
Fat (g/d)	105 ± 46	102 ± 36	0.39
Protein (g/d)	118 ± 44	105 ± 32	0.18
Carbohydrate (g/d)	262 ± 79	239 ± 69	0.84
Fibre (g/d)	20 ± 8	17 ± 6	0.16

[a]Paired *t*-test (n = 8).

4 weeks in humans and ~410 mg/d for 4 weeks in pigs.[4,5] Of note, in the present study, *Bifidobacteria* spp. relative and absolute abundance were higher following CDF supplementation relative to placebo in 6/8 volunteers and the mean between-treatment difference in absolute abundance was 0.26 $\log_{10}$ copy numbers (95% confidence inrerval (CI): 0.25, 0.78); Fig. 1c). The latter result is similar to that reported by Tzounis *et al.*, wherein CDF supplementation increased the growth of *Bifidobacterium* by ~0.2 $\log_{10}$ units more than the placebo group when compared to baseline samples.[5] Comparisons between that study and this study are complicated by Tzounis *et al.*'s use of change from baseline in the analysis whereas this study relied solely on post-treatment values. Nonetheless, the similarities in effect sizes suggest that an effect of CDF on *Bifidobacterium* spp. in this study should not be ruled out and may have been detected with a larger sample size, higher dosage, or longer duration supplementation period.

Most participants had no *Lactobacillus* detected by 16S rRNA amplicon sequencing (Fig. 1d), which may be due to the inherent bias in the V3–V4 primers used.[22] All participants did have *Lactobacillus* counts detected via qPCR. That discrepancy highlights one limitation of relying solely on 16s rRNA sequencing when assessing candidate prebiotics. The observed differences between relative and absolute abundance of *Lactobacillus* may also be due to the qPCR primers amplifying low abundance, food-related bacteria within the genera *Pediococcus*, *Leuconostoc*, and *Weissella*.[17] Although none of those taxa were identified with via amplicon sequencing. Thus, low baseline abundance may be one reason why the effects of CDF on *Lactobacillus* were not detected. A 700% increase in faecal *Lactobacillus* (current taxonomy: *Lacticaseibacills*) *casei* abundance was reported by Jang *et al.* following CDF supplementation in a pig model, but no difference in the abundance of other *Lactobacillus* species were detected.[4] It is possible that *L. casei* was not present within our cohort or other *Lactobacillus* species were detected, leaving *L. casei*-specific growth undetectable. Differences in the primer or nucleic targets used may also explain differences in the present results relative to previous studies. Specifically, the oligonucleotides used by Tzounis *et al.* in their study of CDF supplementation simultaneously detected both *Lactobacillus* and *Enterococcus* spp.[5] The primers used in this study are not reported to amplify *Enterococcus* spp. Alternately, the shorter duration of CDF supplementation in this study compared to the 4 weeks duration used by Jang *et al.* and Tzounis *et al.* may not have been long enough to promote growth of *Lactobacillus* spp. Whether CDF do indeed promote the growth of multiple *Lactobacillus* spp. or only a select subset requires further research. Future research should also consider the revised taxonomic groupings that have reclassified *Lactobacillus* into 25 separate genera.[17]

When diversity was examined, the presence of rare taxa in the community did appear to be lower following CDF supplementation relative to placebo. Specifically, Shannon diversity (mean difference (95% CI) = –0.08 (0.003, 0.15), Cohen's $d = 0.38$; $P_{supplementation} = 0.04$), and overall community richness (–9 (–19, 2), Cohen's $d = 0.34$; $P_{supplementation} = 0.06$) were lower. Total DNA content (mean difference (95% CI) = –86 (21, 192) and Cohen's $d = 0.35$; $P_{supplementation} = 0.10$) demonstrated a tendency to be lower following CDF supplementation compared to placebo (Fig. 2d–g). Shannon's index is more sensitive to the number of rare species in a community than the Simpson index, which is influenced more by dominant taxa, and which did not differ between CDF supplementation and placebo (Fig. 2f). Additionally, although no significant between-treatment differences were observed for any of the β-diversity metrics, dissimilarity between samples collected from the same individuals was visually apparent in the unweighted UniFrac analysis ($P = 0.09$) which relies on the presence and absence of taxa rather than their relative abundance which are used by the other metrics (Fig. 2a–c; $P_{supplementation} \geq 0.20$). Of note, broad-spectrum antibiotics are known to decrease overall diversity, and polyphenols are known antimicrobials that have been added to food products to increase shelf life.[1,7] Specifically, flavonoids have been shown to inhibit several pathogenic bacteria in both gram stain groups, including *Staphylococcus aureus, Vibrio cholerae, Streptococcus mutans, Clostridium perfringens, Clostridium difficile, Streptococcus pyogenes, and Escherichia coli*.[6] Studies in rats have also reported decreases in the genera *Clostridium* and *Staphylococcus* following cocoa-flavanol supplementation.[23]

Differences in α-diversity metrics following CDF supplementation relative to placebo contrasted with an absence of any observed differences in genus relative abundances (Table 2). No differences in genus relative abundances between CDF supplementation and placebo were observed in either DeSeq2 or MaAsLin2 models ($Q > 0.2$) (Supplemental Tables 1 and 2). When considered along with the diversity results, these findings suggest that specific rare species were decreased following the CDF supplementation relative to placebo. However, those differences were not detected in differential abundance analyses likely due to limitations of 16S rRNA sequencing or the filtering approach/statistical models used for analysis.

Two previous studies using lower doses of CDF-containing products (132–425 mg/d CDF) for longer durations (3–10 weeks) and 16S rRNA amplicon sequencing reported either no effect or an increase in alpha-diversity and variable effects on the relative abundance of several taxa including *Blautia, Lachnospira,* and *Faecalibaterium* (Table 2).[8,9] Reasons for the inconsistencies across these studies could be due to not having baseline gut microbiota comparisons in the present study, the dose or duration of CDF supplementation, or the type of supplement used. Regarding the latter, previous studies supplemented with

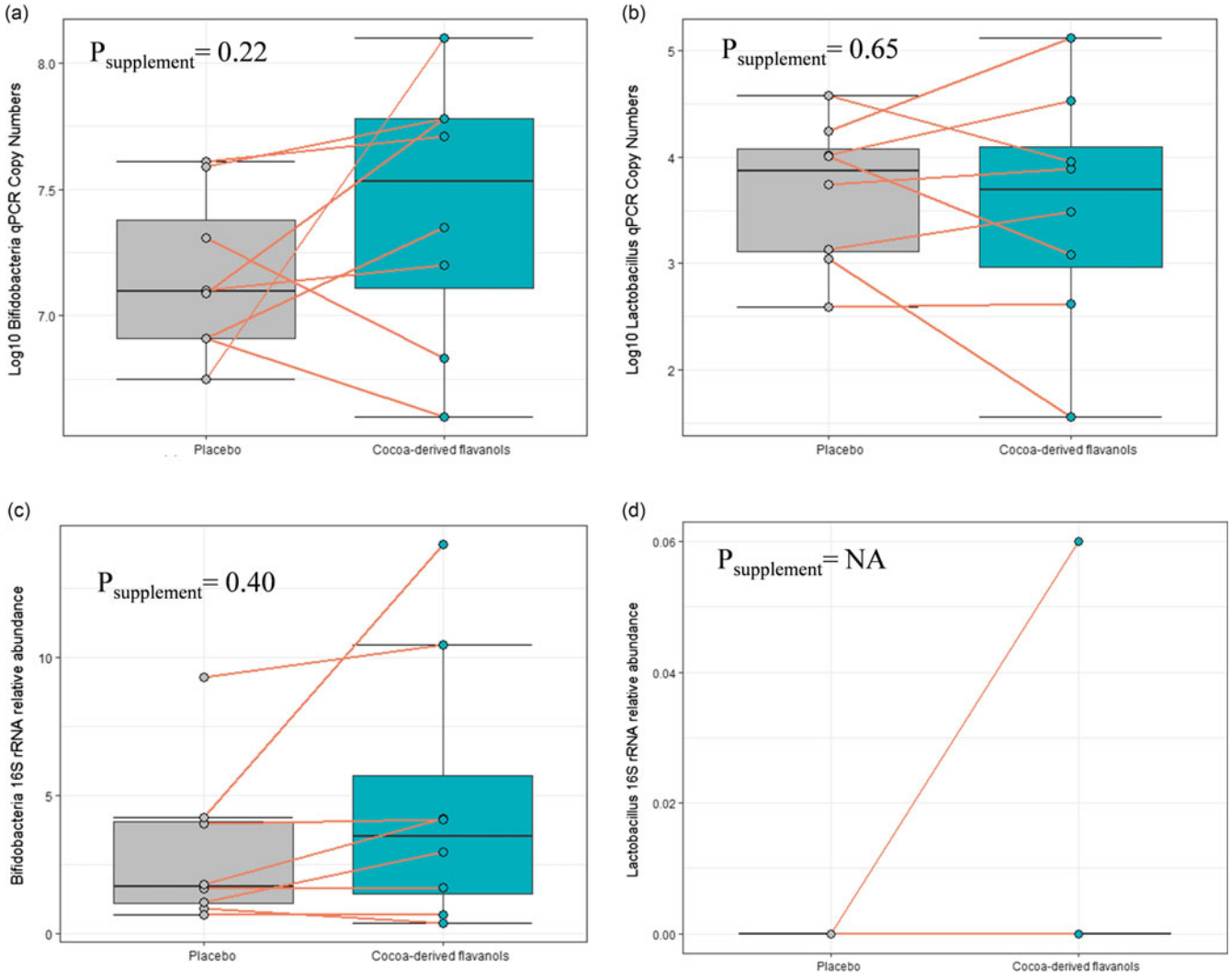

Fig. 1. Comparison between log10 qPCR copy numbers (a–b, absolute abundance) and 16S rRNA sequencing (c–d, relative abundance) for Bifidobacteria and Lactobacillus spp. following eight days of cocoa-derived flavanol supplementation. Bar plots show individual data after placebo and cocoa-derived flavanol supplementation. Individual data are shown. Box plots display median, interquartile range, and range. Lactobacillus was only detected in one volunteer via 16S rRNA sequencing.

85% dark chocolate[9] or cocoa-powder[8] contain not only CDF but also other microbiota-modulating compounds such as caffeine, theobromine, and fibre. Importantly, the provided placebos did not contain any of those compounds. Thus, whether CDF were solely responsible for observed effects in those studies is unclear.

Strengths of this study included the double-blind crossover design, an attempt to isolate the effects of CDF by matching of theobromine and caffeine content in the placebo and intervention products, and the use of both compositional and quantitative microbiota measures. Several limitations warrant consideration. First, this study did not include a baseline time point. Rather, all comparisons were made between samples collected at the end of the supplementation phases. This precludes comparing changes in gut microbiota composition during each phase or assessing whether changes during the first phase returned to baseline prior to starting the second phase. As such, results may not be directly comparable to previous studies assessing changes from baseline. Second, the small sample size, although adequate to detect previously reported effect sizes,[5] reduced the power to detect effect sizes measured herein as variability was higher than in previous reports. The higher variability was likely due in part to collecting only one sample during each study period, preventing an assessment of change in composition within each period. Third, the predominantly male cohort reduces generalizability. Finally, the supplementation period may not have been sufficiently long enough to elicit measurable changes within the gut microbiota. However, studying this brief supplementation period is warranted as previous studies have reported diet and diet supplement-induced shifts in the gut microbiota within days.[11] Importantly, this intervention duration has practical implications for certain populations such as military personnel.

In summary, this study investigated the effects of dietary supplementation with a high dose (900 mg/d) of CDF for 8 days on gut microbiota composition compared with a placebo. Within that context, a lower diversity of the gut microbiota was observed following CDF supplementation compared to

Table 2. Differential abundance analysis for select genera[a]

Genus	Group	Relative abundance (%)[b]	*P*-value, *Q*-value	Cohen's *d*
Lactobacillus[c](5)	CDF	0.00 (0.00)	DeSeq2- N/A	–
	Placebo	0.00 (0.00)	MaAsLin2- N/A	
Bifidobacterium(5)	CDF	3.55 (4.30)	DeSeq2- 0.11, 1.0	0.46
	Placebo	1.72 (2.95)	MaAsLin2- 0.24, 0.82	
Blautia(8,9)	CDF	12.64 (4.08)	DeSeq2- 0.89, 1.0	0.28
	Placebo	13.38 (4.72)	MaAsLin2- 0.56, 0.87	
Lachnospira(8)	CDF	0.33 (2.64)	DeSeq2- 0.28, 1.0	0.60
	Placebo	0.61 (2.85)	MaAsLin2- 0.75, 0.94	
Clostridium(5)	CDF	0.02 (0.07)	DeSeq2- 0.68, 1.0	0.39
	Placebo	0.00 (0.08)	MaAsLin2- 0.73, 0.94	
Faecalibacterium(9)	CDF	12.41 (2.35)	DeSeq2- 0.17, 1.0	0.55
	Placebo	10.31 (4.27)	MaAsLin2- 0.22, 0.82	

n = 8. CDF, cocoa-derived flavanols.
[a]Genera selected are those that have been reported to be altered by cocoa-derived flavanol supplementation or cocoa products in clinical trials. See Supplemental Tables 1 and 2 for full results.
[b]Reported as median (interquartile range).
[c]Detected in only one volunteer via 16S rRNA amplicon sequencing throughout the study.

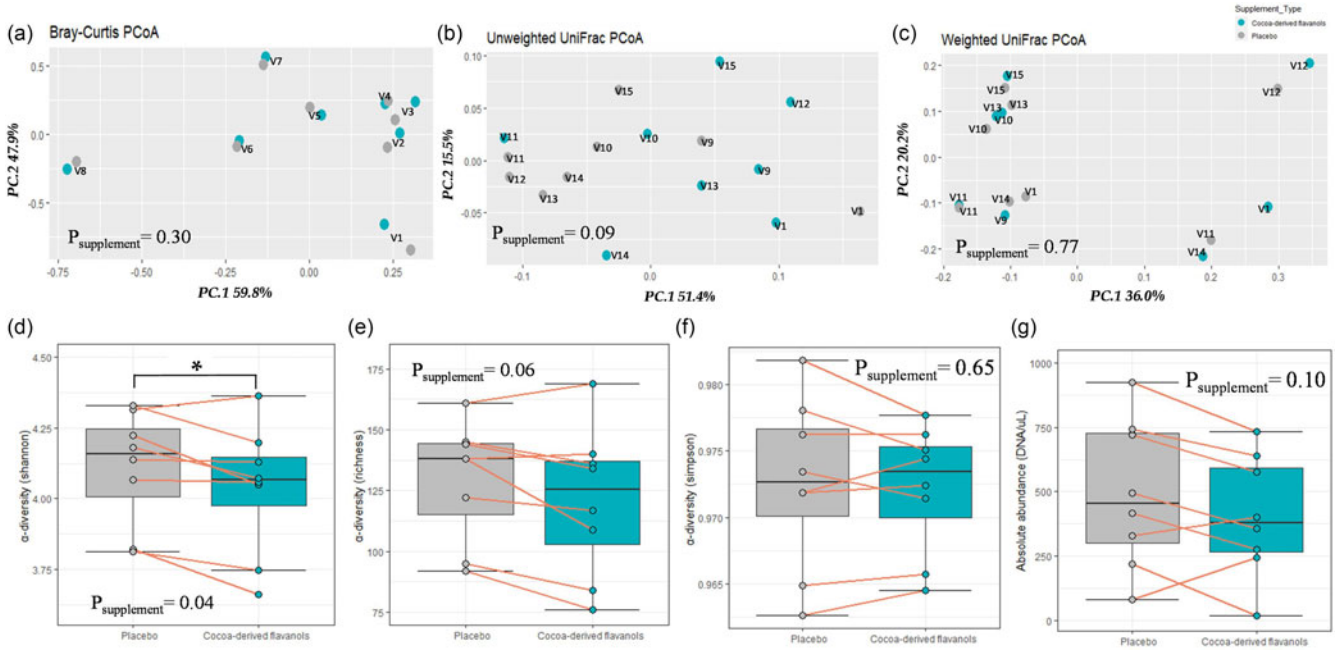

Fig. 2. Ecology diversity, richness, and absolute abundance following eight days of cocoa-derived flavanol supplementation. Diversity measures (a) Bray–Curtis, (b) unweighted UniFrac, (c) weighted UniFrac, (d) Shannon, (e) Simpson, (f) richness, and (g) absolute abundance. Individual data are shown. Box plots display median, interquartile range, and range. Data analysed by nested permutational analysis of variance (PERMANOVA) (a–c) or linear mixed model (d–g) with supplementation, sequence, their interaction, age and BMI as fixed effects, and subject as a random intercept/restricted permutation.

placebo, which suggested a reduction in rare taxa. No between-group differences in the differential abundance of any taxa, including those previously reported to be affected by CDF, dark chocolate, or cocoa powder, were observed. However, an effect of CDF on *Bifidobacterium* could not be ruled out and may have been detected with a larger sample size or longer duration supplementation period. Findings therefore indicate that additional investigations into the prebiotic potential of CDF and the dose and duration of supplementation required to elicit any prebiotic effect are warranted.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2024.17

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Conflict of interest

The authors declare no conflicts of interest.

Authorship

B.K.A., J.W.C., and J.P.K designed the study. B.K.A, B.R.Y., P.N.R., and H.S.F. collected the data. C.S performed the data analysis and drafted the manuscript. C.S. and J.P.K interpreted the data. All authors reviewed and edited the manuscript.

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REVIEW

Mitochondrial protective potential of fucoxanthin in brain disorders

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Abstract

Mitochondrial dysfunction is a common feature of brain disorders. Mitochondria play a central role in oxidative phosphorylation; thus changes in energy metabolism in the brain have been reported in conditions such as Alzheimer's disease, Parkinson's disease, and stroke. In addition, mitochondria regulate cellular responses associated with neuronal damage such as the production of reactive oxygen species (ROS), opening of the mitochondrial permeability transition pore (mPTP), and apoptosis. Therefore, interventions that aim to protect mitochondria may be effective against brain disorders. Fucoxanthin is a marine carotenoid that has recently gained recognition for its neuroprotective properties. However, the cellular mechanisms of fucoxanthin in brain disorders, particularly its role in mitochondrial function, have not been thoroughly discussed. This review summarises the current literature on the effects of fucoxanthin on oxidative stress, neuroinflammation, and apoptosis using *in vitro* and *in vivo* models of brain disorders. We further present the potential mechanisms by which fucoxanthin protects mitochondria, with the objective of developing dietary interventions for a spectrum of brain disorders. Although the studies reviewed are predominantly preclinical studies, they provide important insights into understanding the cellular and molecular functions of fucoxanthin in the brain. Future studies investigating the mechanisms of action and the molecular targets of fucoxanthin are warranted to develop translational approaches to brain disorders.

Key words: **Antioxidant: Brain: Fucoxanthin: Mitochondria**

Introduction

Brain disorders include a wide range of conditions such as neurodegenerative diseases, stroke, mental illness, epilepsy, traumatic brain injury (TBI), and cancer, and changes in cognition, movement, sense, and personality are commonly associated with these disorders. Data extracted from the Global Burden of Disease 2019 show that stroke is the leading cause of death and disability worldwide and that neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) have increased considerably over the last 20 years.[1] Neurodegenerative diseases are generally correlated with aging. However, a recent study has reported an alarming increase in anxiety and depressive disorders among children and adolescents.[2] Furthermore, researchers predict an increase in the incidence rate of ischaemic stroke in all age groups over the next decade.[3]

Patients with brain disorders often live with symptoms or disabilities for an extended period. In patients with neurodegenerative diseases, structural or functional loss of brain cells can begin years or decades before clinical manifestations are exhibited,[4,5] and this process continues until death. Therefore, long-term interventions that are applicable to a wide range of the population may aid in the prevention or alleviation of the severity of brain disorders.[6] Research suggests that diet is an important factor in the maintenance of brain function.[7,8] Numerous studies have demonstrated the benefits of diets rich in vegetables, fruits, whole grains, and seafood for brain health. However, these dietary pattern studies generally do not include seaweed data.

The major objectives of this review are to introduce the neuroprotective properties of fucoxanthin, a bioactive compound found abundantly in brown seaweed, and to suggest

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potential cellular mechanisms based on recently published articles. In particular, we highlight the role of fucoxanthin in mitochondrial protection since mitochondrial dysfunction is a prominent and recurrent feature of various brain diseases, including AD, PD, and stroke. Here, we synthesise and analyse the existing scientific literature to determine the potential role of fucoxanthin in mitochondrial protection in the context of brain disorders.

Mitochondrial dysfunction in brain disorders

The brain requires a substantial amount of energy to maintain electrophysiological activities; therefore, the generation of ATP through oxidative phosphorylation by mitochondria is critical for brain health. Since the brain lacks sufficient long-term energy storage capacity, neurones are vulnerable to impaired energy metabolism,[9] which commonly occurs in brain disorders that are associated with mitochondrial dysfunction. For example, the lack of blood flow to the brain disrupts the function of the electron transport chain (ETC), leading to energy failure in cases of cerebral ischaemia.[10] Furthermore, impaired energy metabolism has been reported in neurodegeneration models. The aggregation of α-synuclein inhibits the function of complex I in the ETC, which causes ATP depletion.[11] Impaired ETC functioning has been reported in the brains of AD patients.[12]

ATP depletion impairs the ATP-dependent Na^+/K^+ and Ca^{2+} pumps, which leads to ionic imbalance in the cytosol, where increased Ca^{2+} ions activate cellular responses that cause neuronal damage. Ca^{2+} triggers the release of neurotransmitters into the extracellular space, including glutamate, which then activates N-methyl-D-aspartate receptors causing a large influx of Ca^{2+} and excitotoxicity. In addition, Ca^{2+} activates the opening of the mitochondrial permeability transition pore (mPTP),[13,14] which is a large non-selective channel that triggers cell death. Once opened, mitochondrial membrane depolarization, ATP depletion, and mitochondrial swelling occur, ultimately leading to cell death. Research has shown that excitotoxicity triggers the dissociation of the F_1 subcomplex from the Fo component of F_1Fo ATP synthase, which results in the opening of a leak channel.[15,16] A rodent model of focal cerebral ischaemia showed a decreased mitochondrial membrane potential in isolated mitochondria when compared with the control model.[17] An ischaemia-reperfusion model revealed that the loss of mitochondrial membrane potential was prevented by treatment with cyclosporine A, which inhibits mPTP.[18] The neuroprotective properties of cyclosporine A were also observed in rodent TBI models.[19] In addition, amyloid-β (Aβ) peptide can trigger mPTP opening by causing the translocation of cyclophilin D.[20] A deficiency in cyclophilin D could maintain mitochondrial membrane potential and improve cognitive function in AD mice, thereby indicating the involvement of mPTP in AD pathology.[21] In a PD model, α-synuclein aggregates caused the loss of mitochondrial membrane potential and induced the activity of a leak channel similar to mPTP.[22]

Mitochondria are critical for the functioning of apoptotic pathways. Oligomerization of pro-apoptotic Bcl-2 family proteins such as Bax and Bak in the mitochondrial membrane promotes the release of cytochrome c from the mitochondria. This cytochrome c then binds to apoptotic protease-activating factor-1 and activates caspases,[23] which causes the proteolysis of structural and functional proteins, leading to cell death. This apoptotic pathway can be inhibited by anti-apoptotic Bcl-2 family members, such as Bcl-2 and Bcl-xL, which sequester pro-apoptotic proteins. Maintaining a balance between pro- and anti-apoptotic Bcl-2 family proteins is critical for cell survival, and alterations in these protein levels are evident in brain tissues of humans and animals with brain disorders. Postmortem studies have identified increased levels of pro-apoptotic Bax proteins in the brain tissues of PD patients.[24] Similarly, mouse PD models have shown increased pro-apoptotic Bax protein and mRNA levels and decreased anti-apoptotic Bcl-2 protein levels in dopaminergic neurones.[25] In addition, genetically modified Bax-deficient PD mice were revealed to be resistant to the loss of dopaminergic neurones.[25] The Aβ peptide, which is a hallmark of AD, can decrease Bcl-2 and increase Bax, thereby potentially contributing to neuronal apoptosis and neurodegeneration.[26] Increased levels of Bax and decreased levels of Bcl-2 and Bcl-xL have been observed in a rodent model of cerebral ischaemia,[27] and treatment with pro-apoptotic protein inhibitors reversed excitotoxicity-associated neuronal death.[28]

Mitochondria are major contributors to cellular ROS, which are by-products of oxidative phosphorylation.[29] ETC impairment can cause excessive ROS production, which overwhelms the antioxidant defence system and promotes oxidative stress. Increases in oxidative stress markers that indicate lipid peroxidation and DNA oxidation have been observed in the brain during neurodegeneration[30,31] and the depletion of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (SOD) has been identified in neurodegenerative diseases. In AD, Aβ plaques accumulate in the brain and disrupt the activities of ETC complexes, which causes an overproduction of ROS.[12] Moreover, oligomeric or fibrillar α-synuclein inhibits the function of the mitochondrial respiratory chain, resulting in increased ROS production in PD cases.[11] Oxidative stress can activate the NLRP3 inflammasome, which is a multiprotein complex that triggers the processing and secretion of pro-inflammatory cytokines.[32] Elevated levels of ROS and ROS-mediated cellular damage in neurodegenerative diseases trigger inflammatory responses that cause immune cell recruitment and additional ROS production. Extracellular accumulation of Aβ plaques can activate microglia, which release pro-inflammatory cytokines.[33] In cerebral ischaemia, both hypoxia and reperfusion trigger inflammatory responses that activate macrophages and release pro-inflammatory cytokines, including TNF alpha (TNF-α), IL-1, IL-6, and IL-8.[10] These inflammatory cytokines induce the production of adhesion molecules on endothelial cells that promote neutrophil adhesion; these activated neutrophils then generate additional ROS.[34]

Mitochondrial DNA has been implicated in the pathogenesis of neurodegenerative disorders because of its predisposition to oxidative damage due to its proximity to the ETC and the lack of a proper DNA repair mechanism.[35] Furthermore, mutations in genes associated with mitochondrial function

have been linked to neurodegeneration, such as the PARK genes, which are critical for mitochondrial function and have been implicated in the pathogenesis of PD. Mutations in the PINK1 and Parkin genes have been associated with decreased ETC enzyme activity and ATP depletion.[35] These mutations disrupt the balance of mitochondrial fission, fusion, and mitophagy, leading to an increased number of fragmented mitochondria. Mutations in the DJ1 and LRRK2 genes are associated with decreased mitochondrial membrane potential, increased oxidative stress, decreased complex I function, and increased mitochondrial fragmentation.[36,37]

Neuroprotective effects of fucoxanthin

Fucoxanthin ($C_{42}H_{58}O_6$) is a carotenoid marine xanthophyll that is found in brown seaweed such as *Undaria pinnatifida* and *Laminaria japonica*.[38,39] It has a strong antioxidant capacity because of the presence of multiple double bonds, including a conjugated carbonyl group. Fucoxanthin has a radical scavenging effect when treated with 1,1-diphenyl-2-picrylhydrazyl and 2,2′-Azinobis-3-ethylbenzo thiazoline-6-sulfonate[40–43] and exhibits a stronger hydroxyl radical quenching activity than other antioxidants.[43] In addition to its direct scavenging effect, fucoxanthin regulates the gene expression of antioxidant enzymes such as SOD and catalase to help support cellular redox homeostasis.[44]

Although epidemiological data demonstrating the direct effect of fucoxanthin on brain disorders are limited, a high intake of seaweed has been reported to lower the risk of PD[45] and stroke-mediated mortality in humans.[46] Pharmacokinetic studies using human plasma detected fucoxanthinol, a metabolite of fucoxanthin, after a single oral administration of kombu (*Laminaria japonica*) extract containing fucoxanthin (31 mg)[47] or a 1-week dose of dried wakame (*Undaria pinnatifida*) containing 6.1 mg of fucoxanthin.[48] Quantitative analyses of fucoxanthin in human tissues other than blood have not been conducted; however, Hashimoto *et al.* observed its distribution and that of its metabolites such as fucoxanthinol and amarouciaxanthin A in tissues after oral administration in a mouse model.[49] Although the brain was not analysed in that study, fucoxanthin was present in other lipid-rich organs such as the adipose and liver. While the neuroprotective potential of fucoxanthin has been documented in *in vivo* animal models, its ability to cross the blood-brain barrier (BBB) remains unconfirmed. However, since other carotenoids from the xanthophyll family, such as astaxanthin, lutein, and zeaxanthin, can permeate the BBB, fucoxanthin has the potential for uptake into brain tissue.[50,51]

Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder that is characterised by cognitive impairment, memory decline, and behavioral and personality changes. It is the most common cause of dementia in older adults and affects one in nine US residents aged 65 and older.[52] The complex neuropathology of AD involves the extracellular aggregation of Aβ into plaques and the intracellular accumulation of hyperphosphorylated tau protein into neurofibrillary tangles. These processes accompany and affect significant mitochondrial dysfunction in AD models. Aβ can impair the functioning of ETC, cause the loss of mitochondrial membrane potential, increase ROS production, and disrupt Ca^{2+} homeostasis.[53–56] In addition to mitochondrial dysfunction, Aβ induces neuroinflammation and impairs neurotransmission.

Fucoxanthin has been suggested to protect mitochondria against AD-associated pathologies.[44,57,58] Treatment with fucoxanthin prevents the loss of cell viability against Aβ-induced cytotoxicity.[57,58] In particular, fucoxanthin-treated cells were resistant to apoptotic death, thereby indicating mitochondrial protection,[57,58] and fucoxanthin-modified Aβ1-42 oligomers have been reported to be less toxic than Aβ1-42 oligomers to SH-SY5Y cells.[44] Furthermore, fucoxanthin may directly prevent the formation of Aβ plaques and neurofibrillary tangles. Xiang *et al.* showed the binding of fucoxanthin to Aβ1-42 peptides, where it inhibited the formation of Aβ fibrils and oligomers[44] and prevented Aβ-mediated mitochondrial dysfunction.[59–62] Similarly, Jung *et al.* reported the binding of fucoxanthin and inhibition of β-site amyloid precursor protein cleaving enzyme 1, which cleaves the amyloid precursor protein to produce Aβ.[63] Fucoxanthin may inhibit the production and aggregation of Aβ through interaction with two hydroxyl groups.[63] In addition, co-incubation of Aβ monomers with fucoxanthin resulted in a dose-dependent decrease in Aβ oligomer formation through hydrophobic interactions. Lee *et al.* reported that fucoxanthin reversed the loss of mitochondrial membrane potential in Aβ-treated PC12 cells.[58] In that study, 5 μM fucoxanthin was as effective as 50 μM resveratrol, an antioxidant with neuroprotective function, indicating a strong mitochondrial protection capacity. Fucoxanthin inhibits Aβ-mediated upregulation of Bax, thereby helping to maintain the integrity of the mitochondrial membrane.[58] Those authors also showed that treatment with fucoxanthin prevented the increase of intracellular Ca^{2+}, as measured by fluo3-AM. Although mPTP was not primarily discussed in that study, the data suggests that fucoxanthin has a role in inhibiting the opening of the mPTP.

Fucoxanthin protects cells from oxidative damage associated with AD.[57,58] Treatment with fucoxanthin was shown to increase nuclear Nrf2 expression, whereas co-treatment with a PI3K inhibitor attenuated this increase, suggesting that fucoxanthin mediated this increased expression through the Akt/GS3K signalling pathway.[58] Similarly, treatment with fucoxanthin increased the activation of the pro-survival PI3K/Akt pathway in Aβ SH-SY5Y cells.[57] Nrf2 is an important regulator of cellular antioxidants that is normally sequestered in the cytosol by Keap1.[59] Oxidative stress induces the translocation of Nrf2 to the nucleus, where pFyn eventually stimulates its export. Fucoxanthin may increase nuclear Nrf2 by preventing the phosphorylation of Fyn and Nrf2 by GS3K, thereby decreasing the degradation and export of Nrf2 from the nucleus.[58] Similarly, fucoxanthin regulated redox homeostasis in *in vivo* models of AD and was shown to attenuate the decrease in SOD, catalase, and glutathione in AD mice.[44] The BV2 cells treated with the poly lactic-*co*-glycolic acid-*block*-polyethylene

glycol (PLGA-PEG)-fucoxanthin nanoparticle prevented Aβ-induced NF-κB, TNF-α, and IL-1β induction compared with the BV2 cells treated with only Aβ oligomers, indicating that fucoxanthin prevented Aβ-induced neuroinflammation.[60] Furthermore, fucoxanthin has anticholinesterase and antibutyrylcholinesterasic activity,[61] and since acetylcholine is considerably depleted in the AD pathology, fucoxanthin may help maintain acetylcholine levels in the brain.[61]

Parkinson's disease

Parkinson's disease is a progressive neurodegenerative disorder caused by the loss of dopaminergic neurones in the *substantia nigra* that leads to a deficiency of dopamine, which is a neurotransmitter that controls movement, pleasure, and motivation.[62] The common symptoms of PD include tremors, rigidity, akinesia, and postural instability,[62] while non-motor symptoms such as depression, anxiety, and cognitive impairments can also occur.[62] In 2019, over 8.5 million individuals worldwide were estimated to be living with PD.[64] A 2022 study stated that close to 90,000 people are diagnosed with PD every year in the United States, representing a 50% increase from the previously estimated number of 60,000 annually.[65] Currently, patients with PD are treated with levodopa (L-DA) to alleviate symptoms, although this treatment may cause neurotoxicity in the long term.[66]

The degradation of dopaminergic neurones is associated with the accumulation of misfolded α-synuclein proteins. Oligomeric and fibrillar α-synucleins inhibit the function of ETC and impair ATP production. Postmortem studies have shown decreases in complexes I and II in the PD brain.[67] Furthermore, the α-synuclein aggregate interacts with F_1Fo ATP synthase in the respiratory chain, which causes the opening of the mPTP,[22] and disruption of the respiratory chain leads to the overproduction of ROS that damages the mitochondria. Increased oxidative stress also impairs the function of the ubiquitin-proteasome system, thereby inhibiting the clearance of these misfolded proteins and leading to their accumulation in neurones.[68] Degradation of dysfunctional mitochondria is important for maintaining a healthy pool of neuronal mitochondria. Mutations in the PINK1 and Parkin genes in the PD brain impair the mitophagy process resulting in the accumulation of dysfunctional mitochondria.[69] Similarly, DJ1 and LRRK2 gene mutations are associated with loss of mitochondrial membrane potential and morphology.[36,37] DJ1 binds to the β-subunit of the F1Fo ATP synthase enhancing ATP production. In contrast, a DJ1 mutant fails to close the mitochondrial inner membrane leak, thereby altering energy metabolism.[70] The aggregation of α-synuclein can also affect mitochondrial fragmentation[71] and movement, which alters the mitochondrial distribution in axonal regions that have high energy demands.[72]

In vitro and *in vivo* studies have suggested the mitochondrial protective effects of fucoxanthin in PD pathology. Treatment with fucoxanthin prevented the loss of mitochondrial membrane potential in PC12 cells challenged with 6-hydroxydopamine (6-OHDA) or a combination of 6-OHDA and L-DA.[73,74] Although the levels of ATP production and oxygen consumption were not directly measured in those models, maintaining mitochondrial membrane potential is critically important to power F_1Fo ATP synthase. Thus, fucoxanthin may help maintain mitochondrial energy metabolism. In addition, annexin V and propidium iodide co-staining showed that fucoxanthin prevented PD-associated apoptotic cell death,[73,74] suggesting that fucoxanthin is involved in the protection of mitochondrial membrane integrity. The direct role of fucoxanthin on mitochondrial quality control in the PD brain is unknown. Lian *et al.* showed that treatment with fucoxanthin increased the ratio of LC3-II to LC3-I, the protein level of Parkin, and the number of autophagosomes and mitophagosomes in retinal ganglion cells challenged with excitotoxicity,[75] suggesting that fucoxanthin has a role in regulating mitophagy. Those authors further showed that fucoxanthin prevented the loss of mitochondrial membrane potential during excitotoxicity and helped protect from apoptotic death by lowering Bax and increasing Bcl-2.

The loss of mitochondrial membrane integrity and inefficient operation of ETC increases ROS production. PC12 cells challenged with 6-OHDA or a combination of 6-OHDA and L-DA showed increased DCF signals indicating increased intracellular ROS,[73,74] whereas fucoxanthin-treated PC12 cells were resistant to ROS production in a dose-dependent manner. In addition to its radical scavenging properties,[40–42] fucoxanthin increased antioxidant enzyme expression in PD models.[73,74] Fucoxanthin binds to a hydrophobic site on Keap1 where it decreases the affinity of Keap1 to Nrf2-binding in a dose-dependent manner.[73] Therefore, fucoxanthin increased the nuclear expression of Nrf2 and the downstream genes that encode antioxidant enzymes such as haem oxygenase-1, the glutamate-cysteine ligase modifier subunit, and the glutamate-cysteine ligase catalytic subunit in 6-OHDA-treated PC12 cells.[73] In addition, PD mice that underwent intragastric administration of fucoxanthin (50, 100, or 200 mg/kg/day) for 28 d improved pole climbing, swimming, and suspension experiment scores, indicating improved motor function.[74] Similarly, zebrafish larvae treated with different concentrations of fucoxanthin for 4 d showed improved swimming abilities after exposure to 6-OHDA.[73]

Cerebral ischaemia

Cerebral ischaemia is a condition that commonly occurs during cardiovascular events such as stroke or cardiac arrest and involves reduced blood flow to the brain. This condition impairs mitochondrial energy metabolism through the deprivation of oxygen and essential nutrients to the brain.[76,77] Dysfunctional mitochondria cause the production of excessive ROS, which damage various cellular components and trigger apoptotic pathways, including the oligomerization of pro-apoptotic proteins in the mitochondrial membrane and the release of cytochrome c, leading to programmed cell death.[78] In addition, ischaemia-mediated energy depletion causes the failure of the ATP-dependent ionic pump, thereby altering intracellular ionic homeostasis. Furthermore, excessive cellular Ca^{2+} concentrations cause the opening of mPTP,[79,80] which exacerbates the energy crisis and leads to neuronal death.

Ikeda *et al.* showed that treatment with fucoxanthin isolated from wakame (*Undaria pinnatifida*) promoted the release of

lactate dehydrogenase in hypoxia-exposed primary cortical neurones,[81] which suggests that fucoxanthin reduces cytotoxicity during oxygen depletion. Those authors performed an *in vivo* study using stroke-prone spontaneously hypertensive rats, which were characterised by severe spontaneous hypertension and the development of cerebrovascular diseases. Supplementation with 5% wakame powder delayed the development of stroke and increased the lifespan of the rats. Hu *et al.* further investigated the cellular mechanisms of fucoxanthin-mediated neuroprotection[82] by intragastrically administering 30, 60, and 90 mg/kg of fucoxanthin to Wistar rats 1 h before middle cerebral artery occlusion (MCAO). The results showed that rats treated with fucoxanthin exhibited a dose-dependent reduction of MCAO-induced brain injury. Treatment with fucoxanthin increased the ratio of Bcl-2/Bax and decreased the cleaved caspase 3 protein level, indicating inhibition of mitochondria-mediated apoptosis during cerebral ischaemia. Consistent with *in vivo* data, rat cortical neurones treated with fucoxanthin showed anti-apoptotic properties in response to oxygen-glucose deprivation and reoxygenation challenges.[82] Furthermore, the study suggested that fucoxanthin increased antioxidant proteins such as SOD[82] via the activation of Nrf2,[83] thereby protecting neurones from oxidative stress. Wang *et al.* used PLGA-PEG nanoparticles to increase fucoxanthin bioavailability in the brains of MCAO-induced rats.[83] PLGA-PEG encapsulation improves fucoxanthin stability in the body and allows for its extended release and enhanced penetration into the central nervous system. The results of that study showed that the intravenous administration of PLGA-PEG fucoxanthin nanoparticles (20 and 40 mg/kg) half an hour before MCAO reduced the behavioural deficits associated with cerebral ischaemia in the rats. In addition, the infarct volumes and brain oedema extents were decreased in rats receiving the nanoparticle treatment.[83] Furthermore, PLGA-PEG fucoxanthin nanoparticles prevented the loss of glutathione peroxidase, SOD, and catalase activity in the ischaemic brain indicating the roles of these compounds in regulating antioxidant defence. Fucoxanthin nanoparticles exhibit anti-inflammatory properties through the inactivation of the NF-κB pathway.

Depression and anxiety

Depression is characterised by a pervasive lack of interest in daily activities that can lead to a profound sense of hopelessness or self-harm, while anxiety involves excessive worry and enduring fear. Depression and anxiety frequently co-occur, possibly due to overlapping cellular mechanisms, with 41.6% of individuals diagnosed with a depressive episode presenting with anxiety within 12 months of the diagnosis,[84] demonstrating a substantial comorbidity between the two conditions. Although the neurological pathways that affect these disorders are not fully understood, a growing body of studies suggests an association with mitochondrial dysfunction.[85,86] Cellular energy metabolism is dysregulated in patients with depression.[87] Similarly, a proteomic analysis of mice challenged with chronic corticosterone, a stress hormone associated with depression and anxiety, showed that oxidative phosphorylation-related protein expression was decreased in these mice.[88] Treatment with ATP reversed the impaired synaptic transmission and excitability in neurones in depression mouse models.[89] Moreover, repeated unpredictable stress downregulated anti-apoptotic genes such as Bcl-2 and Bcl-xL in rat brains,[90] whereas approaches that improved the anti-apoptotic/pro-apoptotic Bcl-2 protein ratio alleviated the depression-associated behaviours.[91]

Although the risk factors for behavioural disorders are complex, studies have demonstrated that the consumption of a healthy diet lowers the risk of depression.[92,93] A prospective cohort study with Japanese adults, which was adjusted for biological, socio-economic, and dietary factors, reported that high seaweed intake was negatively associated with depressive symptoms.[94] In animal models, treatment with extract from the brown seaweed *Sargassum horneri* (500 mg/kg) for 3 weeks prevented the loss of neurotransmitters such as serotonin, dopamine, and norepinephrine in the mouse brain, as well as improvements in depressive-live behaviours caused by an intraperitoneal injection of corticosterone.[95] The authors performed a quantitative analysis and verified the presence of fucoxanthin in the extract. That study further revealed that the underlying mechanism involved the activation of the ERK-CREB-BDNF pathways by the brown seaweed extract. Although the role of *Sargassum horneri* in mitochondrial protection was not intensively featured in that study, the BDNF has been previously shown to increase anti-apoptotic Bcl-xL[96] where the depletion of Bcl-xL impairs the maturity of BDNF.[97] Thus, treatment with fucoxanthin may promote the development and growth of neurones by preventing the mitochondrial dysfunction associated with depression. In addition, the intragastric administration of fucoxanthin (0, 50, 100, and 200 mg/kg) improved Lipopolysaccharide (LPS)-induced anxiety behaviours in mice.[98] Treatment with fucoxanthin regulates the AMPK-NF-κB pathways and prevents the accumulation of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, and iNOS and COX-2 in the hippocampus, cortex, and hypothalamus.

Brain aging

The cumulative impact of oxidative damage on neurones contributes to the process of brain aging.[99,100] This oxidative damage results from the generation of free radicals through environmental exposure, inflammation, immune responses, and infection.[99,100] Postmortem analyses of aged human brain tissues, particularly in the hippocampus and cortical regions, revealed elevated ROS levels and a decrease in antioxidant enzymes such as SOD and catalase.[101] The accumulation of dysfunctional mitochondria in aged brains is associated with brain atrophy and neuronal apoptosis,[102] and the mitochondria isolated from the brains and other tissues of aged rodents demonstrated decreased ETC activity. Specifically, ETC complexes I and IV exhibit reduced activity in the aging brain, which can be attributed to increased oxidative stress.[102]

The diet patterns in Okinawa, a blue zone region with a high life expectancy, include nutrient-dense foods such as lean meat, fish, and vegetables, including seaweed.[103] Fucoxanthin is abundant in brown seaweed, which is a common part of the Okinawan diet. In the 1990s, Okinawan people had the lowest

mortality rate from age-associated diseases in the Japanese and US populations.[104] Research investigating the direct role of fucoxanthin in brain aging is still limited; however, oxidative stress and inflammation are the main risk factors for age-associated cognitive decline,[105,106] and the antioxidant effects of fucoxanthin have been determined.[40–42] Chen *et al.* reported that the administration of 100–200 mg/kg of fucoxanthin for 7 d post-laparotomy surgery improved cognitive function in 12–14-month-old mice.[107] This treatment also decreased neuroinflammation and oxidative stress by activating the Akt and Nrf2 pathways, decreasing the levels of pro-inflammatory cytokines such as TNF-α and IL-1β, and increasing the antioxidant enzymes in the hippocampal tissue.[107]

Fucoxanthin increased the lifespan and resilience of *Caenorhabditis elegans (C. elegans)* and *Drosophila melanogaster* models to starvation and thermal and oxidative stress. Two carotenoids, fucoxanthin and β-carotene, were tested in that study, and lifespan extensions in *C. elegans* were only observed in the fucoxanthin-treated group.[108] Additionally, transcriptome analysis showed that fucoxanthin regulates the pathways involved in longevity, Wnt, and autophagy.[109] In particular, the Wnt signalling pathway controls mitochondrial function, including metabolism, biogenesis, and dynamics in cancer and non-transformed cells. Increased Wnt signalling activates mitophagy by increasing ROS production, which in turn decreases the number of damaged mitochondria and increases the quantity of working mitochondria through biogenesis. The Wnt signalling pathway is important for maintaining mitochondrial homeostasis; therefore, fucoxanthin-mediated upregulation of genes associated with the Wnt pathway could lead to mitochondrial protection. In addition, autophagy is an important quality control mechanism to remove damaged organelles such as dysfunctional mitochondria. Dysregulated autophagy promotes protein aggregation, which is associated with neurodegeneration such as that of PD. Furthermore, this study also showed that oxidative phosphorylation and apoptosis were one of the major targets of fucoxanthin, suggesting its role in regulating mitochondrial function.

Discussion

Mitochondrial protection is critically important to prevent the ATP depletion that occurs as an effect of brain disorders. In addition to cellular energy metabolism, targeting mitochondria can prevent pathological cell damage associated with ROS, apoptosis, and mPTP opening.[110] Fucoxanthin demonstrates neuroprotective effects towards a range of brain disorders including AD, PD, cerebral ischaemia, depression, and anxiety (Table 1). Although limited studies have focused on the direct mechanisms of fucoxanthin-mediated mitochondrial function, fucoxanthin has been implicated in mitochondrial protection in several reports (Fig. 1). In particular, mitochondria-mediated apoptosis under fucoxanthin treatment has been tested in various brain disorder models. It was found that this treatment prevented the loss of the Bcl-2/Bax ratio and the cleavage of caspase-3 in ischaemia-induced brain infarct.[82] Similar results have been reported in cells challenged with Aβ and 6-OHDA, in which the anti-apoptotic Bcl-2 family protein was maintained while pro-apoptotic proteins were suppressed.[58,74] Treatment with fucoxanthin has also been shown to prevent Aβ-induced intracellular Ca^{+2} increase in PC12 cells,[58] suggesting the potential role of this compound in halting the opening of the mPTP. Despite promising data regarding the mitochondrial protective roles of fucoxanthin, the measurements that show the effects on cellular energy metabolism, such as those of ATP production and oxygen consumption, have not been performed in brain disorder models. However, cells treated with fucoxanthin have been shown to maintain rhodamine 123 and JC-1 fluorescent signals in neurodegenerative disease models, indicating the presence of the mitochondrial membrane potential.[58,74] Since the mitochondrial membrane potential drives the operation of F_1Fo ATP synthase, fucoxanthin may help alleviate the neuronal dysfunction that is associated with energy depletion in brain disorders.

Oxidative stress and inflammation, which commonly occur in brain disorders during pathological processes, can damage intracellular organelles including mitochondria; therefore, the antioxidant and anti-inflammatory effects of fucoxanthin (Fig. 1) have been studied with various models. In particular, treatment with fucoxanthin has been shown to activate Nrf-2 signalling and upregulate genes that encode antioxidant enzymes such as SOD, catalase, and haem oxygenase-1 in conditions with neurotoxic challenges induced by Aβ, 6-OHDA, and ischaemia.[58,60,73] Mitochondria are the major sources of ROS, and the fucoxanthin-mediated transcriptional regulation of antioxidants may protect mitochondria from oxidative stress. In addition, fucoxanthin has been shown to alleviate neuroinflammation in the hippocampus and cortex of Aβ-treated mice.[60] This study showed that fucoxanthin decreased the NF-κB activity and its downstream targets such as TNF-α and IL-1β. Similarly, treatment with fucoxanthin prevented NF-κB p65 expression in the hippocampus, cortex, and hypothalamus of LPS-injected depressive mice[98] and the production of TNF-α in MCAO-induced rats.[83]

In this review, we discuss the existing literature on the potential of fucoxanthin to protect mitochondria in brain disorders. However, we acknowledge limitations. First, the existing body of literature investigating the mechanism of fucoxanthin on mitochondrial function in the context of brain diseases is limited. This scarcity of dedicated studies hinders a comprehensive understanding of the specific effects of fucoxanthin on mitochondrial function in the brain. In addition, the majority of the discussed evidence was derived from preclinical studies. While the results from these experimental studies provide valuable insights into the cellular and molecular aspects, the findings must be adaptable to clinical applications in humans. Moreover, the bioavailability of fucoxanthin could pose a challenge since the absorption, distribution, metabolism, and excretion in the human body may influence its efficacy; therefore, these aspects should be explored.

Conclusion

Fucoxanthin has been shown to protect the brain against challenges associated with brain disorders. Here, we discuss the potential roles of fucoxanthin in cellular responses associated

Table 1. The effects of fucoxanthin on *in vitro* and *in vivo* brain disorder models

Brain Disorder	Model	Pathological challenge	Effect of fucoxanthin	Ref.
Alzheimer's Disease	SH-SY5Y cells	Treatment with Aβ1-42	-Inhibits the formation of Aβ1-42 fibril and oligomer -Prevents Aβ1-42 -mediated cytotoxicity	(44)
	ICR mice	Hippocampal injection of Aβ1-42 oligomers	-Improves recognition performance, spatial learning, and memory -Prevents the reduction of Aβ1-42-mediated antioxidant activities and the downregulation of BDNF and ChAT	
Alzheimer's Disease	SH-SY5Y cells	Treatment with Aβ1-42	-Attenuates Aβ-mediated cell death -Attenuates Aβ-mediated ROS production -Prevents the alteration of PI3K/Akt and ERK pathway	(57)
Alzheimer's Disease	PC12 cells	Treatment with Aβ25-35	-Attenuates Aβ-mediated ROS production -Prevents Aβ-mediated apoptosis -Protects the mitochondrial membrane potential -Prevents Aβ-mediated Ca^{2+} production -Increases nuclear translocation of Nrf2 -Regulates Akt/GSK-3β/Fyn signalling	(58)
Alzheimer's Disease	SH-SY5Y cells BV2 microglial cells	Treatment with Aβ oligomers Treatment with Aβ oligomers	-Prevents Aβ-mediated cell death and ROS production -Prevents Aβ-mediated neuroinflammation	(60)
	ICR mice	Hippocampal injection of Aβ1-42 oligomers	-Improves cognitive performance -Attenuates neuroinflammation and oxidative stress -Activates Nrf2 pathway -Reduces NF-κB pathway	
Parkinson's Disease	PC12 cells	Treatment with 6-OHDA	-Binds to Keap1 and activates Nrf2 pathway -Prevents 6-OHDA-mediated decrease in antioxidant enzymes -Prevents 6-OHDA-mediated cell damage	(73)
	Zebrafish	Treatment with 6-OHDA	-Improves 6-OHDA-mediated locomotor dysfunction -Prevents 6-OHDA-mediated ROS production and cell damage -Upregulates antioxidant genes	
Parkinson's Disease	PC12 cells	Treatment with 6-OHDA and L-DA	-Prevents 6-OHDA and L-DA-mediated apoptosis -Prevents 6-OHDA and L-DA-mediated mitochondrial damage -Inhibits the ERK/JNK-c-Jun pathway	(74)
	C57BL/6 mice	6-OHDA injection	-Improves 6-OHDA-mediated motility dysfunction -Prevents 6-OHDA-mediated dopaminergic neuronal loss	
Cerebral Ischaemia	Primary cortical neurones	Hypoxia	-Attenuates hypoxia and re-oxygenation-mediated cell damage	(81)
	SHRSP rats		-Prevents the incidence of stroke and increases the mean lifespan in SHRSP	
Cerebral Ischaemia	Primary cortical neurones	Oxygen-glucose deprivation and reperfusion (OGD/R)	-Prevents OGD/R-mediated apoptosis -Activates Nrf2/HO-1 signalling	(82)
	Wistar rats	Middle cerebral artery occlusion (MCAO)	-Decreases ischaemia-mediated infarction and neurological deficits -Prevents ischaemia-mediated apoptosis and ROS production	
Cerebral Ischaemia	HT22 cells	Treatment with iodoacetic acid (IAA)	-Prevents IAA-mediated ROS production -Prevents IAA-mediated cell death	(83)
	Sprague-Dawley rats	MCAO	-Prevents MCAO-mediated brain infarct and behavioural deficits -Attenuates MCAO-mediated neuroinflammation and oxidative stress	
Depression and Anxiety	ICR mice	Intraperitoneal injection of corticosterone	-Improves corticosterone-mediated depressive-like behaviours -Prevents corticosterone-mediated dysfunction of HPA axis and neurotransmitters -Activates the ERK-CREB-BDNF pathway	(95)
Depression and Anxiety	ICR mice	Intraperitoneal injection of LPS	-Improves depressive and anxiety-like behaviours -Increases AMPK activation -Prevents LPS-induced inflammation	(98)
Cognitive Impairment	Aged ICR mice	Laparotomy	-Prevents surgery-mediated cognitive impairment in aged mice -Activates the Akt pathway and prevents inflammation -Activates the ERK pathway and increases antioxidant enzymes	(107)
Aging	*Drosophila*	Aging, thermal stress, and paraquat	-Increases lifespan and stress resistance -Regulates the expression of stress resistance genes	(108)
	C. elegans	Aging	-Increases lifespan	
Aging	*Drosophila*	Aging, thermal stress, paraquat, and starvation	-Increases lifespan -Regulates the genes related to longevity, autophagy, apoptosis, and neurogenesis	(109)

HPA, hypothalamic-pituitary-adrenal; ICR, Institute of Cancer Research; ROS, reactive oxygen species; SHRSP, Stroke-prone spontaneously hypertensive.

with mitochondrial dysfunction. The antioxidant and anti-apoptotic effects of fucoxanthin suggesting mitochondrial protection have been evaluated in various brain disorder models. However, future mechanistic studies focusing on the role of fucoxanthin in mitochondrial function, along with clinical studies on its efficacy in alleviating neuronal damage, can aid in the development of dietary recommendations to offset the burden of brain disorders.

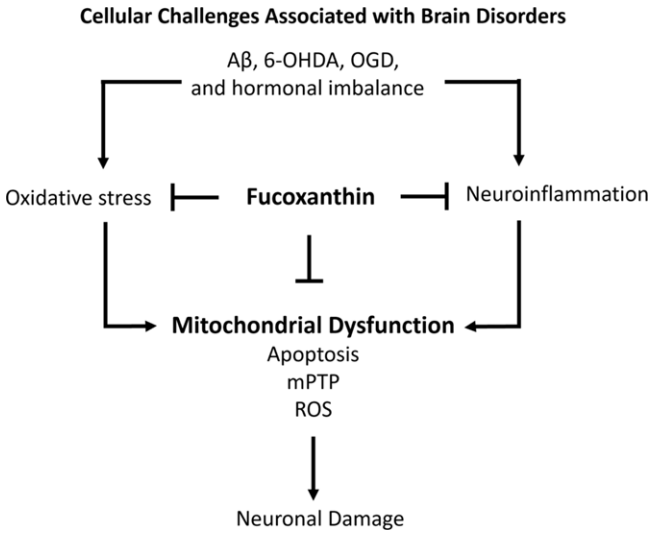

Fig. 1. Mechanism of mitochondrial protection by fucoxanthin in brain disorders.

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Authorship

KAF was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript. JJ was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript. EA was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript. EP was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript. RB was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript. AB was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript. MC was involved in drafting the original manuscript. HP was involved in drafting the original manuscript, interpretation of findings, and editing of the manuscript.

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RESEARCH ARTICLE

The level and determinants of knowledge and attitude towards nutrition among pregnant women in Minjar Shenkora district, Ethiopia

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Abstract

Maternal knowledge and attitudes towards nutrition are essential for achieving healthy dietary behaviours for a positive pregnancy outcome. Therefore, this study aimed to assess the level and determinants of knowledge and attitudes towards nutrition among pregnant women in the Minjar Shenkora district, Ethiopia. Institution-based cross-sectional study was conducted between June and August 2021 among 334 pregnant women who were selected using a systematic random sampling technique. Data were collected using a semi-structured questionnaire and analysed using SPSS version 21. Bivariate and multivariate logistic regression analyses were used to identify the determinants of nutritional knowledge and attitudes. $P < 0.05$, with a 95% CI was used to declare statistical significance. Overall, 69.2% and 44.0% of the pregnant women were not knowledgeable and had an unfavourable attitude toward nutrition, respectively. The educational status of pregnant women ($P < 0.01$) and access to nutritional information ($P < 0.01$) were significantly associated with nutritional knowledge, while the educational status of pregnant women ($P < 0.01$) was associated with nutritional attitude. Respondents' knowledge of and attitudes toward nutrition were low. Pregnant women's educational levels and access to nutritional information are strongly related to their nutritional knowledge. Likewise, the educational status of the pregnant women was significantly associated with their nutritional attitudes. Therefore, education and counselling on maternal nutrition are needed to improve healthy maternal nutrition tips in the study area.

Key words: **Attitude: Ethiopia: Knowledge: Minjar Shenkora: Nutrition: Pregnant women**

Introduction

Pregnancy is an anabolic process, and pregnant women have a higher demand than non-pregnant women, possibly because of the growing number of uterine cells.[1,2] It is a critical period in the life cycle when additional nutrients are required to meet the metabolic and physiological demands as well as the increased needs of the growing foetus. Pregnant women are more vulnerable to malnutrition owing to the high demand for nutrients to deposit energy in the form of new tissue, the growth of existing maternal tissues such as the breast and uterus, and the increased energy requirements for tissue synthesis.[3,4] Adequate nutrition is essential for the health and reproductive performance of women as well as the health, survival, and development of children.[5–7] However, maternal nutritional deficiency is a global public health problem that contributes to the death of 3.5 million women annually, mainly in low- and middle-income countries.[8,9] Africa has significantly higher undernutrition in pregnancy, and sub-Saharan Africa is the most affected region.[10–12] Ethiopia is the country where the undernutrition problem is still among the major health problems, especially among children and mothers, with a higher prevalence.[13–17]

In Ethiopia, nearly one in five pregnant women are malnourished in rural areas and are approximately 68% more likely to be malnourished than urban women.[18] To ensure positive pregnancy outcomes, pregnant women should have adequate nutritional knowledge and attitude. Malnutrition and other nutrition-related complications are immediately caused by

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a lack of knowledge and attitude.[19,20] Nutritional knowledge is predictive of changes in dietary habits, and health advice encourages pregnant women to increase their food intake.[21,22] Nutritional knowledge during pregnancy is also important for increasing maternal awareness of proper nutrition.[23] Nutritional attitude also refers to the affective feelings of expectant mothers who like or dislike prenatal services. As a result, pregnant women's personal experiences with prenatal services can be favourable or unfavourable.[24,25] Women should be made aware of the effects of healthy nutritional attitudes, either before conception or as early as possible during pregnancy. Good knowledge and a favourable attitude toward optimal nutrition are essential elements to ensure a positive pregnancy outcome and are integral to achieving healthy dietary behaviours, which then improve the quality of the diet.[26,27] This is true for pregnant women, as good maternal nutrition is one of the best ways to ensure maternal and foetal well-being.

Good knowledge of maternal nutrition and a favourable attitude toward pregnancy is necessary to ensure positive pregnancy outcomes.[22,28] Women's nutrition knowledge and attitudes before pregnancy are also important in raising maternal awareness about the importance of good nutrition for both the mother and healthy foetal development. Limited knowledge and an unfavourable attitude towards nutrition influence dietary practices and are, thus, potential determinants of pregnancy outcomes, including maternal mortality, infant mortality, and low birth weight in infants.[22,27,28] The World Health Organization (WHO) has approved antenatal nutrition education for women to reduce the birth of underweight infants and prevent maternal complications. The WHO also implements a nutrition education and counselling strategy focusing on increasing the variety and quantity of foods consumed, promoting sufficient and balanced dietary intake, and encouraging the regular use of fortified foods.[29] Ethiopia's Ministry of Health (MoH) has used different strategies, such as nutrition education via social media.[30] Similarly, health extension workers have been working to address women's lack of dietary knowledge and attitude. However, approximately 47% of women lack knowledge of balanced and diversified diets during pregnancy.[31] Similarly, only 27% and 48.4% of pregnant women were knowledgeable and had a favourable attitude toward the need to eat a varied and balanced diet, respectively.[32] Pregnant women's knowledge of and attitudes toward nutrition are influenced by several factors. These include the occupational status of respondents, the mother's educational level, her monthly income, her ANC visits, the size of her family, and her marital status among pregnant women in Ethiopia.[31,33–35]

Even though few studies on nutrition knowledge and attitudes have been done in Ethiopia, the studies indicate inconsistency in the proportion and contributing variables of pregnant women's knowledge and attitudes toward nutrition.[31,32,36,37] Although factors such as educational status, occupation, family size, and monthly income have been associated with maternal nutrition knowledge and attitude, some local and environmental determinants such as place of residence, access to nutrition information, and pregnancy conditions are variables that were not statistically identified. In addition, previous studies on maternal nutritional knowledge and attitude are not sufficient to improve and provide recommendations on maternal nutrition counselling and education programmes because the studies focused on communities' urban areas, regions, and zone levels and did not consider rural settings. Accordingly, additional findings are needed, especially in rural communities, to identify nutritional knowledge and attitude, as well as the contributing factors to implementing evidence-based maternal nutrition counselling and education programmes, and to strengthen the few initiated programmes in the country. Despite research efforts, no study has documented the level and determinants of knowledge and attitudes towards nutrition among pregnant women in the study area. Therefore, this study aimed to assess the level and factors of knowledge and attitude towards nutrition among pregnant women in the Minjar Shenkora district, Ethiopia.

Methods

Study area, design, and period

This institutional-based cross-sectional study was conducted from June to August 2021 in public health centres in the Minjar Shenkora district of Ethiopia. The district is located at the southern end of the North Shewa Zone and is bordered to the east, south, and west by the Oromia region, to the northwest by Hegere Mariam, and to the northeast by Berehet Woreda. It is an administrative woreda of the North Shewa Zone, Amhara National Regional State. Arerti is the administrative centre of Minjar Shenkora Woreda, which is located 135 km from Addis Abeba, Ethiopia's capital city. According to the 2007 population census report, the district has a total population of 128879 people, comprising 66918 men and 61.961 women. Six public health centres in the district provide antenatal services to the public, including Arerti Hospital. According to a health centre report, an average of 515 pregnant women follow antenatal care services within two months in the district.

Experimental methods

The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Wollega University Ethical Review Committee (WU 486/2021). Written informed consent was obtained from all subjects.

Source and study population. The source population of this study was pregnant women attending antenatal care services in the public health centres of the Minjar Shenkora District. The study subjects consisted of systematically selected pregnant women who were attending ANC follow ups in public health centres in Minjar Shenkora District during the study period.

Inclusion criteria and exclusion criteria. Pregnant women who visited public health centres for antenatal care service follow up and lived in the district for at least six months in the district were included in the study. Pregnant women who were seriously ill during the data collection period, lived below six months in the study area, could not listen or speak, and were referred from other health centres were excluded from the study.

Sample size determination

The required sample size for this study was calculated using a single population proportion formula, considering a previous finding of 27% with a 95% confidence and 5 % margin error.[32] The number of samples was calculated using the following formula: $\boldsymbol{n} = \frac{(\boldsymbol{Z\alpha/2})^2.\boldsymbol{p.q}}{\boldsymbol{d^2}}$

N = the sample size. **P** = proportion of nutritional knowledge (27%) and **d** = margin of error (required precision), which was assumed to be 5%. **Z** = upper percentile of the normal distribution. Considering a nonresponse rate of 10%, the final study sample size was 334.

Sampling techniques

The calculated sample size was proportionally allocated to six public health centres, Bolo Health Center, Balchi Health Center, Dire Health Center, Kristos Semra Health Center, and Arerti Health Center, including Arerti Hospital, located in Minjar Shenkora District, based on the average number of clients in the respective antenatal care units before the study period. The average number of participants who visited the ANC health facility daily through data collection was predicted primarily based on the previous two months' daily client flow in the clinic, which was obtained by referring to client registration books or records before data collection. The average number of flow records for ANC service in health centres for the previous two months total of those who visited was 515. The sampling interval was calculated using the equation (k = N/n = 515/334 = 2). The first participant women were randomly selected using the lottery method, and every second woman was selected using a simple random sampling technique until the required sample size was attained.

Data collection tools and procedure

Data were collected using a semi-structured questionnaire adapted from different previous literature.[31,33,35] The questionnaire consisted of questions on sociodemographic characteristics, obstetric and health-related characteristics, nutritional knowledge, and nutritional attitude. Data were collected by three trained diploma-holding enumerators and one bachelor's holder assigned as a supervisor.

Data quality control

The questionnaire was carefully translated into Amharic and then back to English to ensure consistency. To preserve data quality, the questionnaires were pre-tested on 5% of pregnant women, and corrections and modifications were made based on the gaps identified during the interview. Data collectors and supervisors were trained in the study's background, objectives, tools, and ethical procedures. Training for data collectors emphasised the importance of keeping the study participant information confidential. Furthermore, supervisors had to check how the data collectors performed their tasks, including ethical procedures, and verify the completion of questionnaires during the data collection process.

Data processing and analysis

The collected data were edited, coded, cleaned, and checked for consistency and completeness. The data were put *epi* data Version 3.1 software and then exported to the Statistical Package for Social Science (SPSS) Version 21 for analysis. The data were changed to analyse the use of SPSS Model 21 software. Descriptive statistics was used to describe the characteristics of the study population. Bivariate logistic regression at a 95 % confidence interval (CI) was used to assess the predict association between the dependent and independent variables. Variables with a $P < 0.2$ in the bivariate logistic regression were inserted at the same time in the multivariate logistic regression to control for possible confounding. Multivariate logistic regression analysis with an adjusted odds ratio at 95 %CI was performed to assess the determinants of knowledge and attitude toward nutrition, and associations were declared significant at $P < 0.05$ to declare the results statistically significant. Hosmer-Lemeshow examined what was used to test the model's fitness.

Operational definitions

Knowledge refers to an individual's understanding of nutrition, including their intellectual ability to remember and recall food- and nutrition-related terminology, specific pieces of information, and facts.[38,39]

Knowledgeable: The respondent answers ≥ 70% (out of 100%) of the knowledge questions.[36]

Not knowledgeable: The respondent answered < 70% (out of 100%) to the knowledge questions.[40]

Attitudes are emotional, motivational, perceptive, and cognitive beliefs that positively or negatively influence an individual's behaviour or practice.[41,42]

Favourable attitude: if respondents score attitude questions ≥ mean[32,37]

Unfavourable Attitude: if respondents score for attitude questions < mean[32,37]

Ethical approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki guidelines. The Wollega University Ethical Review Committee approved this study (WU /453/2021). A formal permission letter was sent to the administrators of the Minjar Woreda Health Office and to each of the health centres that also approved the study. Informed consent was obtained from each participant at the time of the data collection. All procedures were performed according to the relevant rules and regulations described in the methods section. The data collectors informed clients that they had the right to cancel or refuse participation in the study, and client responses were treated confidentially to uphold this.

Results

Socio-demographic characteristics of pregnant women

A total of 334 women were interviewed, resulting in a response rate of 100 %. More than half of the 190 respondents (56.9 %)

Table 1. Socio-demographic characteristics of pregnant women in Minjar Shenkora district, Ethiopia, 2021 (n = 334)

Variables	Categories	Frequency (%)
Age	18–24	107 (32)
	25–34	190 (56.9)
	35–44	37 (11.1)
Marital status	Single	29 (8.7)
	Married	267 (79.9)
	Divorced	24 (7.2)
	Widowed	14 (4.2)
Religion	Christian	247 (74)
	Muslim	65 (19.5)
	Others[a]	22 (6.6)
Ethnicity	Amhara	281 (84.1)
	Others[b]	53 (15.9)
Place of residence	Rural	121 (36.2)
	Urban	213 (63.8)
Family size	One	12 (3.6)
	Two	90 (26.9)
	Three	111 (33.2)
	Four	85 (25.4)
	Five and above	36 (10.8)
Educational women	No formal education and primary school	185 (55.4)
	9–12 class	90 (26.6)
	College and above	59 (17.7)
Educational of husband	No formal education & primary school	116 (43.4)
	9–12 class	77 (28.8)
	College and above	74 (27.7)
Occupation women	Housewife	169 (50.6)
	Private sector	96 (28.7)
	Governmental	45 (13.5)
	Others[c]	24 (7.2)
Occupation of husband	Private sector	78 (29.2)
	Governmental	68 (25.5)
	Farmer	98 (36.7)
	Others[c]	23 (8.6)
Monthly income	<2000 ETB	183 (54.8)
	≥ 2000 ETB	151 (45.2)

[a]Protestants and Catholics.
[b]Oromo, Argoba, and Tigre.
[c]Daily labour and Merchant.

were aged 25–34 years. The majority of the respondents, 267 (79.9%), were married. A total of 247 (74%) respondents were followers of the Orthodox Tewhido Church. Approximately 281 (84.1%) participants were Amhara in their ethnic group (Table 1).

Obstetrics and health characteristics of pregnant women

The majority of the respondents, 215 (64.4%), were multigravida and 186 (55.7%) were multiparous. More than half (55.4%) of the participants were in their third trimester of pregnancy. One-third, 120 (34.9%) of the women had four or more antenatal visits during their current pregnancy, and 137 (41%) accessed pregnancy-related nutritional information from different sources (Table 2).

Level of knowledge towards nutrition among pregnant women

In this study, only 103 (30.8%) respondents were knowledgeable, while the remaining 231 (69.2%) were not. About 222 (66.5%), 140 (41.9%), and 164 (49.1%) pregnant women were aware of a balanced diet, the importance of iron, and how pregnant women's diets differed from those of non-pregnant women (Table 3).

Table 2. Obstetrics and health characteristics of pregnant women in Minjar Shenkora district, Ethiopia, 2021 (n = 334)

Variables	Categories	Frequency (%)
Gravida	Primigravida	119 (35.6)
Multigravida	Multigravida	215 (64.4)
Parity	Primipara	148 (44.3)
Multipara	Multipara	186 (55.7)
Trimester	First trimester	40 (12.0)
Second trimester	Second trimester	109 (32.6)
Third trimester	Third trimester	185 (55.4)
Previous delivery	Normal	293 (87.7)
	Abnormal	41 (12.2)
Associated disease	Yes	97 (29.0)
No	No	237 (71.0)
Intake of iron folate supplement	Yes	190 (56.9)
	No	144 (43.1)
Antenatal follow up	Once	64 (19.2)
	Twice	68 (20.4)
	Three times	82 (24.6)
	Four and above	120 (35.9)
Nutritional information	Yes	137 (41)
	No	197 (59)

Determinant factors of knowledge towards nutrition among pregnant women

In the bivariate analysis, pregnant women's education, place of residence, monthly average income, and inaccessible nutritional information predicted associations with nutritional knowledge. However, in a multivariate analysis, educational status and access to nutritional information were significantly associated with nutritional knowledge during pregnancy. Pregnant women with an educational level of college or above were six times more likely to be knowledgeable about nutrition during pregnancy than those with no formal education ($P < 0.01$). Likewise, pregnant women with access to nutrition information were twice as likely to be knowledgeable as women without access to nutrition education during their pregnancy (Table 4).

Level of attitude towards nutrition among pregnant women

One hundred eighty-seven (56%) respondents had a favourable attitude, and the remaining 147 (44%) had an unfavourable attitude towards nutrition. Approximately 188 (56.3%), 179 (53.6%), and 196 (58.7%) participants favoured eating more protein, testing milk and milk products, and testing meat and other iron-rich foods during pregnancy, respectively. However, 115 (34.4%), 107 (32.1%), and 110 (32.9%) pregnant women were unsure whether to eat more food during pregnancy, drink milk during pregnancy, or prepare meals with iron-rich foods during pregnancy, respectively (Table 5).

Determinant factors of attitude towards nutrition among pregnant women

The results of bivariate analysis showed that the educational status of pregnant women, husbands' educational status,

Table 3. Level of knowledge towards nutrition among pregnant women in Minjar Shenkora district, Ethiopia, 2021 (n = 334)

Variables	Know frequency (%)	Don't know frequency (%)
Balanced diet	222 (66.5)	112 (33.5)
Pregnant diet differs from non-pregnant diet	164 (49.1)	170 (50.9)
Component of balanced diet	183 (54.8)	151 (45.2)
Source of protein	141 (42.2)	193 (57.8)
Important of protein for mother and foetus	132 (39.5)	202 (60.5)
Source of vitamin	115 (34.4)	219 (65.6)
Source of calcium	102 (30.5)	232 (69.5)
Important of milk and milk product	119 (35.6)	215 (64.4)
Micronutrient supplements for pregnant women	94 (28.1)	240 (71.9)
Health benefit of folic acid for baby	88 (26.3)	246 (73.7)
Source of iron	161(48.2)	173 (51.8)
Important of iron	140 (41.9)	194 (58.1)
Important of birth spacing	205 (61.4)	129 (38.6)
The danger of malnutrition for pregnant	147 (44)	187 (56)
The danger of malnutrition for babies	156 (46.7)	178 (53.3)
Level of nutritional knowledge		
Knowledgeable	103 (30.8%)	
Not knowledge	231 (69.2%)	

Table 4. Bivariate and multivariate analysis of associated with knowledge towards nutrition among pregnant women in Minjar Shenkora district, Ethiopia, 2021 (n = 334)

		Nutritional knowledge		95% CI		
Variables		Knowledgeable	Not knowledgeable	COR	AOR	P value
Residence	Rural[a]	21 (17.5%)	99 (82.5%)	1		
	Urban	82 (38.3%)	132 (61.7%)	2.92 (1.69, 5.05)	1.11 (0.46, 2.67)	ns
Women's education	No formal and primary school	21 (11.4%)	164 (88.6%)	1	1	
	9–12 class	48 (53.3%)	42 (47.7%)	8.92 (4.82, 16.50)	0.78 (0.29, 2.14)	
	College and above	34 (57.6%)	25 (42.4%)	10.62 (5.33, 21.12)	6.92 (2.13, 22.42)[b]	0.001
Husband education	No formal and primary school	20 (17.3%)	96 (82.7%)	1	1	
	9–12 class	30 (38.9%	47 (61.1%)	3.06 (1.57, 5.95)	0.85 (0.33, 2.19)	
	College and above	35 (47.3%)	39 (53.7%)	4.30 (2.21, 8.36)	1.06 (0.36, 3.11)	
Women's occupation	Housewife[a]	36 (21.3%)	133 (78.7%)	1	1	
	Private sector	31 (32.3%)	65 (57.7%)	1.76 (1.00, 3.09)	1.02 (0.15, 6.75)	
	Government sector	31 (68.9%)	14 (31.1%)	8.18 (3.94, 16.98)	0.89 (0.12, 6.37)	
	Others	5 (20.8%)	19 (79.2%)	0.97 (0.34, 2.78)	0.63 (0.08, 4.94)	
Husband occupation	Private[a] sector	27(34.6%)	51(65.4%)	1	1	
	Governmental	38 (55.9%)	30 (41.1%)	2.39 (1.22, 4.66)	0.92 (0.20, 4.21)	
	Farmer	17 (17.4%)	81 (82.6%)	0.39 (0.19, 0.79)	0.36(0.07, 1.74)	
	Others	3 (13%)	20 (87%)	0.28 (0.77, 1.04)	0.92 (0.02, 4.21)	
Average monthly income	<2000 ETB[a]	35 (19.1%)	148 (80.9%)		1	
	≥ 2000 ETB	68 (45%)	83 (55%)	3.46 (2.12, 5.64)	0.85 (0.39, 1.86)	ns
Gravidity	Primigravida[a]	50 (42%)	69 (58%)	1	1	
	Multigravida	53 (24.7%)	162 (75.3%)	0.45 (0.28, 0.72)	0.64 (0.19, 2.15)	
Parity	Primipara	43 (23.1%)	143 (76.9 %)		1	
	Multipara	60 (40.5%)	88 (59.5%)	0.44 (0.27, 0.70)	0.62 (0.19, 2.04)	
Nutritional information	No[a]	36 (18%)	163 (82%)	1	1	
	Yes	67 (49.6%)	68 (50.4%)	4.46 (2.72, 7.31)	2.73 (1.39, 5.37)[b]	<0.001

AOR, adjusted odds ratio; COR, crude odds ratio; ETB, Ethiopian Birr; ns, No significance.
[a]Reference categories.
[b]Significant.

pregnant women's occupation, and inaccessibility of nutrition information predicted nutritional attitudes. However, in the multivariate analysis, the educational level of women was significantly associated with their attitude towards nutrition during pregnancy. Pregnant women whose educational level was college and above were four times more likely to have a favourable attitude towards nutrition than those who had no formal education during pregnancy (Table 6).

Table 5. Level of attitude towards nutrition among pregnant women in Minjar Shenkora district, Ethiopia, 2021 (n = 334)

Variables	Good Frequency (%)	Not sure Frequency (%)	Not good Frequency (%)
Eat more food during pregnancy	131 (39.2)	115 (34.4)	88 (26.3)
Eat more carbohydrate during pregnancy	116 (34.4)	106 (31.7)	112 33.5)
Eat more protein or beans during pregnancy	188 (56.3)	77 (23)	69 (20.7)
Eat more milk during pregnancy	140 (41.7)	107 (32.1)	87 (26)
To prepare meals with iron-rich food	137 (41)	110 (32.9)	87 (26)
Test of meat and other iron-rich food	196 (58.7)	59 (17.6)	79 (23.7)
Test of omega3-rich food; olive oil, fish	103 (28.5)	101 (30.2)	130 (38.9)
Test of milk and milk product	179 (53.6)	52 (15.6)	103 (30.8)
Prepare meals with iodine salt	215 (64.4)	102 (30.5)	17 (5.1)
Level of nutritional attitude			
Favourable attitude	187 (56 %)		
Unfavourable attitude	147 (44%)		

Table 6. Bivariate and multivariate analysis of associated attitudes towards nutrition among pregnant women in Minjar Shenkora district, Ethiopia, 2021 (n = 334)

		Nutritional attitude		95%CI		
Variables		favourable	unfavourable	COR	AOR	P value
Women's education	No formal and primary school[a]	71 (33%)	144 (67%)	1	1	
	9–12 class	69 (76.7%)	21 (23.3%)	5.27 (2.98, 9.34)	0.81 (0.27, 2.44)	
	College and above	47 (79.7%)	12 (20.3%)	6.28 (3.12, 12.66)	4.26 (1.47, 12.34)[b]	<0.001
Husband education	No formal and primary school[a]	53(45.7%)	63 (54.3%)	1	1	
	9–12 class	51 (62.2%)	26 (37.8%)	2.19 (1.20, 4.00)	0.91 (0.37, 2.20)	
	College and above	48 (64.9%)	26 (35.1%)	2.33(1.28–4.23)	1.44 (0.58, 3.55)	
Women's occupation	Housewife[a]	84 (49.7%)	85 (50.3%)	1	1	
	Private sector	54 (56.3%)	42 (43.7%)	1.30 (0.78, 2.15)	1.56 (0.42, 6.29)	
	Governmental	38 (84.4%)	7 (15.6%)	5.49 (2.32–12.99)	1.63 (0.37, 6.48)	
	Others	11 (45.8%)	13 (54.2%)	0.85 (0.36, 2.01)	0.36 (0.05, 2.52)	ns
Husband occupation	Private sector[a]	48 (61.5%)	30 (38.5%)	1	1	
	Governmental	46 (67.6%)	22 (32.4%)	1.30 (0.66, 2.58)	1.18 (0.35, 3.94)	ns
	Farmer	48 (49%)	50 (51%)	0.60 (0.32, 1.09)	0.76 (0.27, 2.13)	
	Others	10 (43.5%)	13 (56.5)	0.48 (0.18, 1.23)	0.76 (0.35, 2.21)	
Nutritional information	No[a]	96 (48.2%)	103 (51.8%)	1	1	
	Yes	91 (67.4%)	44 (32.6%)	2.22 (1.40, 3.49)	1.32 (0.73, 2.41)	ns

AOR, adjusted odds ratio; COR, crude odds ratio; ns, No significance.
[a]Reference categories.
[b]Significant.

Discussion

Nutritional knowledge and attitudes influence the quality of food consumed and healthy food choices. Limited nutrition knowledge and unfavourable attitudes influence eating habits and are thus possible predictors of pregnancy outcomes such as maternal mortality, infant mortality, and low newborn weight in infants. It is critical to identify the level and determinants of knowledge of and attitudes toward nutrition. Therefore, this study assessed the levels and determinants of knowledge of and attitudes toward nutrition during pregnancy. Overall, 69.2% of the participants were unknowledgeable about nutrition. This finding is consistent with a study conducted in the Ambo District, West Shewa Zone, Ethiopia (66.7%).[35] However, this study was higher than the studies conducted in Addis Ababa (26.1%), Southern Ethiopia (38.1%), and East Wollega (35.6).[33,36,37] The results of this study were lower than those of a previous study conducted in Ethiopia, in which 73% of the participants were not knowledgeable about nutrition during pregnancy.[32] This dissimilarity might be due to variations in study years and settings, sociodemographic factors, an individual's lifestyle, and the accessibility of nutritional information.

In this study, the educational status of pregnant women and accessibility of nutritional information were associated with nutritional knowledge. Accordingly, pregnant women with educational levels of college and above and secondary school were six times more likely to be knowledgeable than those with no formal education. This result is supported by previous findings conducted in Southern Ethiopia, East Wollega, Addis Ababa, and West Shoa Zone, Ethiopia.[32,35,37] This might be because educated women are aware of nutrition and can utilise information delivered through different channels and stakeholders regarding nutrition and health. Similarly, pregnant women who had access to nutritional information were more likely to be knowledgeable than those who had no nutritional information during pregnancy. This result is supported by research conducted in the West Shoa Zone of Ethiopia.[35] This may be because the accessibility of nutritional information has an immediate effect on improving women's knowledge of nutrition.

Regarding nutritional attitude, 44% of pregnant women had an unfavourable attitude towards nutrition during pregnancy, which was consistent with a previous study conducted in Southern Ethiopia (40.5%).[37] This finding is lower than those of studies conducted in other parts of Ethiopia (68.7%, 66.8%).[31,37] These differences may be due to variations in the study period, sociodemographic characteristics, and individual and social perspectives of the respondents. Educational status was associated with pregnant women's attitudes toward nutrition; thus, pregnant women with secondary education, college education, and above were more likely to have a favourable attitude regarding nutrition during pregnancy. The finding is supported by the studies conducted in West Shoa and other parts of Ethiopia.[31,32,37] This may be because educated women can understand and utilise nutritional information during their pregnancies. Unlike the findings of previous studies,[33,34] in our study, variables such as pregnant women and husbands' occupational status, monthly average income of respondents, and family size were not associated with knowledge and attitude towards nutrition. This may be due to the similarity of the population in those variables, and the work environment may not play a role in changing knowledge and attitude unless nutritional information is delivered to the stakeholders.

Overall, this study assessed the knowledge and attitude towards nutrition as well as its determinant factors. These data are essential for providing information on the topic to policymakers, government authorities, and other stakeholders investing in maternal nutrition. This study was conducted in all health centres in the district, and most of the pregnant women in these districts were found in rural areas. This is different from previous studies conducted in urban areas of Ethiopia, which addressed all health centres found in the district, most of which are in rural settings. However, this study has limitations that need to be considered and addressed in further investigations, such as the institution-based cross-sectional study design, which does not address pregnant women not attending antennal services, and the nutritional practices of the respondents, which were not assessed.

Conclusion and recommendation

In the present study, the knowledge and attitude of the respondents towards nutrition in pregnancy were low compared with previous findings conducted in Ethiopia, especially knowledge about nutrition. The educational level of pregnant women and their access to nutritional information during pregnancy were significantly associated with their knowledge of nutrition. Similarly, the educational level of pregnant women was associated with their attitudes toward nutrition in the study area. Therefore, nutrition education and counselling programmes regarding maternal nutrition are needed to increase maternal nutritional knowledge and attitude, which will improve healthy maternal nutrition tips in the study area through continuous training, home visits, health centre counselling, and other behavioural change communication programmes for nutrition.

Abbreviations

AOR: Adjusted odds ratio; **ANC**: Antenatal care; **CI**: Confidence interval; **COR**: Crude odds ratio; **MoH**: Ministry of Health Ethiopia; **SPSS**: Statistical Package for Social Science; **WHO**: World Health Organization; **WU**: Wollega University

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Competing interests

The authors declare that they have no competing interests.

Authorship

MD and KA created the tool, oversaw data collection, performed the statistical analysis, and wrote the manuscript. HF contributed to the study's design and statistical analysis. All authors read and approved the final manuscript.

Data availability

Data will be made available upon request from the corresponding authors.

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The Wollega University Ethical Review Committee granted ethical approval *(WU RD/453/2021)*. A formal permission letter was sent to the administrators of the Minjar Shenkora Woreda Health Office and each of the health centres, who also approved. At the time of data collection, informed consent was obtained from every participant. All procedures were carried out by the relevant rules and regulations described in the method section. Data collectors informed clients that they had the right to cancel or refuse participation in the study, and client responses were treated confidentially to uphold that.

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