

Healthy foods and dietary patterns in modern consumer

Edited by

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Healthy foods and dietary patterns in modern consumer

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Editorial: Healthy foods and dietary patterns in modern consumer

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KEYWORDS

foods, health, label, Mediterranean diet, Nutriscore, Med Index, Planeterranean diet

Editorial on the Research Topic

Healthy foods and dietary patterns in modern consumer

According to the ancient Greek philosophers, “*we are what we eat*.” Nowadays, obesity and overweight, even in young people and children, represent a serious public health problem, reaching epidemic proportions all over the world (the “new pandemic of the twenty first century,” as defined by the World Health Organization). There is a clear link between nutritional habits and body weight, as well as with natural mortality, type II diabetes, cognitive function, and other conditions. These topics have been addressed in the Research Topic by [Almoraie et al.](#) (obesity), [Brayner et al.](#) (diabetes), [Gou et al.](#) (cognitive function), [Goulart et al.](#) (cognitive performance), [Kim et al.](#) (metabolic syndrome), [Liu et al.](#) (nutritional patterns and mortality), and [Xi et al.](#) (sodium intake), while [Trabelsi et al.](#) gave an original insight into temporary fasting.

On the other issue, the Research Topic of nutritional deficiencies in relation to locally available foods and their ability to meet nutrient requirements has been recently highlighted by the scientific community as a threat to low-income countries. As highlighted by [Foroozanfar et al.](#) in their article about social determinants of nutritional behaviors, it is noteworthy that food and the way we eat are a major driver of our health. This explains the increasing interest of the general public toward the health properties of food and labeling regulations that are able to foster informed nutritional choices from the perspective of modern consumer-friendly policies, as highlighted in the article by [Badji et al.](#). The issue is so crucial that some international institutions—such as the European Union—are assessing the possibility of adopting unified labeling systems to foster appropriate food choices by consumers. For this purpose, the so called “Nutriscore” (1) has been proposed as a front-of-pack labeling system (FOPL), which was created by the French Nutritional Epidemiology Research Team (EREN) on the basis of nutritional scores elaborated by the Food Standards Agency in the UK. More recently, a new FOPL based on the “One Health” approach named “Med Index” (2) has been released, which was created by the University of Bari in Italy along with the Italian Society of Environmental Medicine SIMA and officially presented at the 1st Yale Gastronomy and Culture Symposium in Crete, 3–5 May 2023. Med Index has been designed to provide information not only about calories and fats (as the Nutriscore does) but also to summarize on a simple label the healthy nutritional properties of the food, the physical activity needed to burn the energy gained by eating it, the sustainability of production processes that are behind the food, and the social responsibility of the companies. Interestingly, Med Index does not require food producers to perform new efforts, but it is based on the certifications already acquired by the companies concerning both the dimensions of sustainable production and social responsibility. These two scores

might possibly integrate different kinds of information if used in combination, thus avoiding possible confounding messages for the consumers. In particular, the 4th International Yale Symposium on Olive Oil and Health (held in Rome, September 15–18, 2022) had already endorsed a position statement on the issue of the NutriScore use and front-of-package label (FOPL) for olive oil, recommending that—based on the constantly accumulating evidence of its health benefits (3)—olive oil should either be labeled as a Green-A-grade food by NutriScore or be included in the highest category within any FOPL system (or as an alternative recommending its exclusion from the NutriScore). The reason for that is mainly related to the labeling of olive oil with the NutriScore level B/C, which would undoubtedly create confusion and dampen the trust of consumers in European guidance and regulations, as it is in clear contrast to the EFSA health claim [Commission Regulation (EU) 432/2012]. This health claim unequivocally emphasizes the health benefits of olive oil: “olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. The claim may be used only for olive oil, containing at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil. Replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels. The claim may be used only for food, which is high in unsaturated fatty acids, as referred to in the claim HIGH UNSATURATED FAT as listed in the Annex to Regulation (EC) No 1924/2006.”

Olive oil is a healthy superfood, and this is one of the primary reasons that consumers purchase it. The use and application of the NutriScore algorithm on olive oil, without consideration of its numerous health benefits, would substantially negatively impact its use and consequently primary prevention through a healthy nutrition. Currently, Mediterranean nutrition, of which olive oil is the central food, has over the decades stood the test of time as the healthiest nutritional paradigm. A lot of scientific evidence has accumulated over the last 50 years about the direct and indirect health benefits of olive oil. Such well-recognized health benefits are universally accepted by physicians, nutritionists, and dietitians (3). The evidence concerning the health benefits of olive oil have been demonstrated for cardiovascular and metabolic systems, cancer prevention, high blood pressure, cholesterol levels, cognitive/neurological conditions, diabetes, inflammatory process, oxidative stress, and coagulation, etc., (3).

Specific foods and nutritional models or diets have been even directly associated to health benefits in the frame of individual commitment toward health—the path to “personalized nutrition” described by Renna et al.—and to environmental sustainability within a “One Health” perspective (as addressed by the Med Index). This broad topic has been proposed by Kim et al., describing the traditional Korean diet composed of a multigrain rice-containing meal with fruits and nuts, and by Trichopoulou in her paper about the healthy properties of and sustainable production granted by the Greek Mediterranean Diet, with a specific focus on extra-virgin olive oil (EVO), as recently presented at the 1st International Symposium on Gastronomy and Culture, which was organized in Crete by the Yale School of Public Health. The issue of the transferability of Mediterranean nutritional habits to the non-Mediterranean is the challenge addressed by Vetrani et al. in their

paper “*Planeterranea: an attempt to broaden the beneficial effects of the Mediterranean diet worldwide*,” which is aimed at developing country-specific nutritional pyramids based on the foods locally available that present the same nutritional properties and health benefits of the Mediterranean Diet (MD).

This Research Topic is very interesting and has been addressed during the “Dean’s Lecture” held by Trichopoulou at the Yale School of Public Health on April 18th 2023. On the one hand, the scientific community has recognized the health benefits granted by the nutritional profile of the nutritional habits typical of Mediterranean areas and the sustainability of food production (produced close to the place where they are consumed) that preserves biodiversity and natural resources, as well as cultures and traditions. This also means that it cannot be hypothesized to export Mediterranean products and culture to other areas of the world, but the same effects in terms of the reduced prevalence of cardiovascular, metabolic, or neurodegenerative diseases as well as for cancer prevention can be obtained by proposing the consumption of local products (and healthy cooking methods) that present the same nutritional properties of foods that are typical of Mediterranean tradition. This challenge is currently being addressed by the UNESCO Chair on Health Education and Sustainable Development at the University of Naples. “Planeterranean” is the name of this newly proposed dietary model, which is also consistent with the United Nations Sustainable Development Goals (SDGs, Agenda 2030) from the perspective of the circular economy. The “Planeterranean” model could be a solution to overcome the problem of the poor nutritional model (both in term of food quality and variety) that characterizes the majority of people across the world, who receive most of their energy intake from foods with high glycemic index (such as white rice and potatoes) and sugar-rich and fatty ultra-processed foods (ready-to-eat foods, sugar-sweetened beverages, candies, chips, and pastries, etc.). These are the nutritional habits—unfortunately now frequent also in Mediterranean areas—that are responsible for the current obesity epidemic affecting both adults and children, resulting in metabolic and cardiovascular diseases or premature mortality. On the other hand, in every place of the world, it is possible to find specific fruits, vegetables, legumes, wholegrain, and sources of unsaturated fats that are consistent with the nutritional principles found to produce health benefits in the studies carried out on the Mediterranean Diet. On this basis, the UNESCO Chair has defined multiple “nutritional pyramids”—based on the foods available at the local level in different parts of the world—presenting the same nutritional properties and health benefits (and also environmentally friendly production processes) observed in the Mediterranean Diet. The research is open, as is the call for fostering healthier eating habits worldwide.

Author contributions

All authors conceived, wrote, and approved the manuscript.

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“Planeterranea”: An attempt to broaden the beneficial effects of the Mediterranean diet worldwide

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Non-communicable diseases (NCDs) lead to a dramatic burden on morbidity and mortality worldwide. Diet is a modifiable risk factor for NCDs, with Mediterranean Diet (MD) being one of the most effective dietary strategies to reduce diabetes, cardiovascular diseases, and cancer. Nevertheless, MD transferability to non-Mediterranean is challenging and requires a shared path between the scientific community and stakeholders. Therefore, the UNESCO Chair on Health Education and Sustainable Development is fostering a research project—“Planeterranea”—aiming to identify a healthy dietary pattern based on food products available in the different areas of the world with the nutritional properties of MD. This review aimed to collect information about eating habits and native crops in 5 macro-areas (North America, Latin America, Africa, Asia, and Australia). The information was used to develop specific “nutritional pyramids” based on the foods available in the macro-areas presenting the same nutritional properties and health benefits of MD.

KEYWORDS

Mediterranean diet, health, sustainability, local foods, nutritional properties, bioactive compounds, nutritional pyramid

Introduction

Non-communicable diseases (NCDs) account for more than 70% of global mortality (1). A recent report from the Global Burden Disease Study reported that almost 400 million people are suffering from diabetes (2) and the presence of diabetes is associated with increased mortality from infections, cardiovascular disease (CVD), and cancer (2, 3). Other to diabetes, high systolic blood pressure and high low-density lipoprotein cholesterol levels have been appointed as major contributors to the onset of CVD (54.6 and 46.6%, respectively) (4). Noteworthy, a 26.3% increase in new cases of cancer (mainly breast and gastrointestinal cancers) has been reported recently (5).

Given the epidemic of NCDs with increasing trends in high and middle-income countries and in adolescents/young adults (6, 7), the World Health Organization demanded for effective strategies to treat and prevent NCDs. In addition to lifestyle factors such as physical inactivity, smoking, alcohol intake, also diet has been established as highly modifiable risk factors for NCDs. Indeed, more than 9.1 million premature deaths from CVDs worldwide are attributable to dietary risks, regardless of age, sex, and sociodemographic development of the native country (8). As for cancer (breast and gastrointestinal cancers), nutritional inadequacy, and excess body weight (overweight and obesity) lead to a significant increase in cancer incidence during the last decades (9, 10).

Different dietary approaches have been proposed for the prevention and treatment of NCDs. Among this, Mediterranean diet (MD) have been associated with body weight control (11, 12) and reduced risk for chronic diseases, including CVD (13, 14), type 2 diabetes (T2D) (15), and some cancers (16–20).

Moreover, MD has shown a protective role also for immune-related diseases such as atopy and asthma (21, 22).

These beneficial effects are related to the nutritional composition of MD that can be obtained through the combination of some foods with a specific frequency of consumption during the week (23): (a) regular consumption of plant-based foods (fruits, vegetables, wholegrain, legumes, and nuts), and extra-virgin olive oil as the primary source of fat; (b) moderate amount of animal protein and fat, with fish and low-fat dairies as the preferred sources, respectively; (c) limited intake of sweets and processed foods. More in detail, most of MD energy intake is provided by non-refined carbohydrate (55–60%), 30–35% from fat, and ~15% from protein (24). Carbohydrate is provided by low-glycemic index foods (i.e., wholegrain-based products and legumes) while sugar intake is <10% by limiting the consumption of sweets and sugar-sweetened beverages. Fat is mainly represented by monounsaturated fatty acids (MUFA, 19%), followed by saturated fatty acids (SFA, 9%) and polyunsaturated fatty acids (PUFA, 5%), and cholesterol is 300 mg/day (24). According to MD, plant protein should be preferred, while animal protein (fish, lean cuts of meat, eggs, and dairies) should be used as alternative options during the week (25). MD can provide relevant amounts of vitamins, minerals, and other phytochemicals (23). Over the recommendations for the frequency of food consumption, MD relies on cultural, social, and lifestyle features. In brief, a moderate intake of wine and other fermented beverages (women: one glass/day; men two glasses/day) is advised while respecting religious and social beliefs. A daily intake of 1.5–2 l of water (6–8 glasses) is recommended for proper hydration. Regular physical activity (i.e., sports, fitness, or leisure activities outdoors) should complement diet in order to sustain adequate body weight and other health benefits. Finally, seasonality of foods, biodiversity preservation, conviviality, and socialization are advocated to preserve MD cultural heritage (23).

Given the beneficial effects of MD, several efforts have been made to transfer this dietary pattern to non-Mediterranean populations for the prevention of NCDs. Nevertheless, several barriers toward adherence to MD in non-Mediterranean countries exist. Firstly, it is known that changing individual dietary habits is challenging, as it requires long-lasting modifications in behavior (26). Furthermore, there are practical, cultural, and economic factors that affect dietary changes (27). In brief, the main issues related with poor compliance to MD are affordability (high cost for transportation and commercialization), lack of knowledge (health benefits, cooking methods, recipes), and cultural differences (traditions, native crops, and environment protection vs. globalization).

On the other hand, in recent years there is a growing recognition that “healthy nutrition” should be integrated with food systems, i.e., “all the factors (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities that relate to the production, processing, distribution, preparation and consumption of food, and the outcomes of these activities” (28). This would lead to a more sustainable diet, with relevant benefits on the health of people and the planet (29).

In line with this, the UNESCO Chair of “Health Education and Sustainable Development” of the University of Naples “Federico II” has focused part of its activities on the implementation of MD-based dietary patterns as a pivotal approach to prevent and manage NCDs. This specific task of the Chair represents a response to the call of the World Health Organization to reduce the pandemic of NCD and their risk factors (30). Indeed, the long-term objective of the UNESCO Chair of “Health Education and Sustainable Development” is to enhance the health status of populations by facilitating a “Knowledge Transfer Exchange” about the effects on human health of major cultural, nutritional, and environmental factors (30).

Against this complex background, the aim of the present review was to collect the available evidence on native crops and dietary habits worldwide, grouping the countries into 5 macro-areas (North America, Latin America, Africa, Asia, and Australia). Then, the information retrieved was used to propose feasible alternatives with similar composition of foods characterizing MD. Finally, we used all the information to develop specific “nutritional pyramids” based on the foods available in the 5 macro-areas presenting the same nutritional properties and health benefits (as well as environmental-friendly production processes) observed for MD.

Methods

We used the UNESCO network to retrieve information about dietary habits of different countries. In brief, we interviewed native people from the 5 macro-areas to collect information about local crops, eating habits, traditional recipes,

etc. In addition, we performed complementary research by selecting national and international websites providing information about crop production and eating habits (i.e., National Department of Agriculture, USDA, Google Scholar, SciELO—Scientific Electronic Library Online, etc). Then, a 20-years literature search for this narrative review was conducted until December 2021 by searching PubMed database for articles published in the English language. Each specific food identified in the preliminary search was used as keyword “AND” (Boolean operator) combined with “health OR plasma glucose OR glucose metabolism OR plasma lipid OR lipid metabolism OR plasma insulin OR insulin resistance OR inflammation OR oxidative stress OR type 2 diabetes OR cardiovascular disease OR cancer OR risk factors OR effects OR composition OR intake OR consumption.”

Overall, our search retrieved a total of 860 studies suitable for our review. The citation pool of publications was further supplemented by analyzing the reference list of the selected articles.

Articles considered in this narrative review were (a) meta-analyses of prospective studies or randomized clinical trials; (b) observational studies and clinical trials not included in the meta-analyses that added significant information; (c) literature reviews providing the nutritional composition of the specific foods identified. Only articles published in journals in the highest impact factor quartile in the “Public Health,” “Endocrinology, Diabetes and Metabolism” or “Nutrition and Dietetics” areas were included. Moreover, we excluded notes, book chapters, letters, editorials, conference papers, articles published in languages other than English and those not specifically related to each issue of interest.

Results

North America

North America has been appointed as the cradle of the so-called Western diet, characterized by high intakes of refined grains, red and processed meat, pizza and fast food, potato, sweets, sugar, and high-energy beverages. It translates in increased amount of SFA (12%), refined carbohydrates (21% refined grains, fruit juice, and potatoes), and added sugars (14.4%) (31). These dietary features have been associated with poor diet quality and are a primary cause for chronic diseases and mortality in USA (32). Therefore, new approaches for promoting healthy dietary choices are advocated. Although massive changes are required to increase diet quality, the introduction of some local products could represent a first step to improve dietary habits.

Canola oil is a vegetable oil derived from a variety of rapeseed *Brassica napus* and *Brassica rapa* that originated in Canada. It contains MUFA (54% oleic acid), PUFA (21% linoleic acid and 11% α -linolenic acid), and high concentrations of phytosterols (769 mg/100 mg canola oil) (33, 34).

Mounting evidence supports the hypocholesterolaemic effect of canola oil. Indeed, two meta-analyses of randomized controlled trials have shown that replacing SFA or sunflower oils with canola oil significantly reduces total and LDL-cholesterol concentrations (35, 36). Moreover, a non-linear dose-response curve suggested that replacing ~15% of total caloric intake with canola oil might provide the greatest benefits on lipid profile (36). Besides, a recent meta-analysis demonstrated that canola oil can positively affect body weight, with no major changes of other anthropometric parameters (37). The effect is greater in studies in women, patients with T2D, and when canola oil is compared to saturated fatty acids.

Pecans (*Carya illinoensis* (Wangenh.) K.Koch) are considered as the traditional tree nut in North America. They are an excellent source of MUFA, having 12 g/standard serving (28 g), and γ -tocopherol (24.4 mg/100 g) (38). Moreover, they present the highest total flavonoid content among nuts (34 mg/100 g), consisting mostly of flavan-3-ols and anthocyanins (39).

Human studies with pecans supplementation have addressed mainly to lipid profile. In a 4-week trial, 23 middle aged healthy volunteers were randomized to pecan supplementation (72 g/day) in addition to the habitual diet or habitual diet alone. At the end of the study, participant following pecan supplementation presented a significant reduction of total and LDL-cholesterol, and increased HDL-cholesterol concentrations, with no changes of body weight. In addition, a significant reduction of triglyceride, lipoprotein (a), and apolipoprotein B concentrations was detected (40).

Conversely, a 12-week healthy diet enriched with pecans (30 g/day) did not improve lipid profile in patients with stable coronary artery disease suggesting that the threshold of pecan doses might be higher than the recommended portion for other nuts (41).

Over the hypolipidemic effect, a 4 week incorporation of 42 g/day of pecan in a typical American diet has shown to improve glucose metabolism in middle-aged volunteers with central adiposity. In particular, the pecan-enriched diet significantly reduced fasting insulin and insulin resistance (evaluated as homeostatic model assessment, HOMA), while improving β -cell function (assessed as HOMA- β) (42).

Okra (*Abelmoschus Esculentus*) is a popular vegetable crop cultivated throughout the world mostly in tropical and subtropical regions. It is the main ingredient of the “Gumbo,” a popular American dish, particularly in Louisiana.

Okra is rich in fibers (8.16 g/100 g), both insoluble (4.73 g/100 g) and soluble (3.43 g/100 g), and it has been suggested that 100 g of okra could cover a 33% of the recommended daily fiber intake. It has 2% protein, 7% carbohydrates and traces of fat (43).

To the best of our knowledge, no clinical studies with okra supplementation are available so far.

However, several *in vitro* and animal studies suggested potential antidiabetic and lipid-lowering effects by virtue of the high fiber content (44, 45).

Pinto Beans (*Phaseolus vulgaris* L.) is the major dry beans in terms of production (31%) and consumption (20%) in North America (46). They provide 8–10% of the daily recommended amount of protein *per* 100 g serving and have a great fiber content (16 g/100 g) (47).

A randomized controlled crossover trial with a 3 × 3 block design tested the effect of Pinto beans incorporation into the diet for 8 weeks as compared to black-eyed peas and carrot used as control (1/2 cup/day for all tested foods). Sixteen mildly insulin resistant adults completed the trial, and an 8% reduction of LDL-cholesterol was detected after the consumption of Pinto beans (48). Interestingly, it has been reported that 1% reduction in LDL-cholesterol reduces risk for coronary heart disease (CHD) by ~1% (49). Therefore, the reduction observed after Pinto beans consumption may translate in an 8% reduction of CHD risk. Similar results were obtained in a longer-term study (12 weeks) where the same amount of Pinto beans was consumed by individual with pre-Metabolic Syndrome (MS) (increased central adiposity and one clinical feature considered in the diagnosis of MS) (50). In this study, complementary experiments in an *in vitro* colonic fermentation model showed a significant increase of propionate with Pinto beans incubation. Therefore, it can be hypothesized that the LDL-cholesterol lowering effects of Pinto beans may be linked to the microbial products (mainly propionate) derived from fiber fermentation.

As reported above, massive changes are required to improve the overall quality of the actual North American diet. The Harvard School of Public Health and other American Institution, both have proposed practical guidelines to enhance the transferability of the MD in real-life settings (32). However, to increase the feasibility of these dietary changes, the incorporation of local foods rich in fiber, phytochemical and other bioactive compounds may represent a complementary strategy. Therefore, over the main principles of the MD for animal protein-sources and sweets, the following changes are proposed:

- use canola oil as the main daily fat source (the amount should be according to individual energy needs)
- increase the intake of vegetables (at least 2 servings/day) and legumes (at least 3 serving/week), preferring local varieties (i.e., okra and pinto beans)
- use nuts for snacking, in particular pecans (42 g/day)

These changes are summarized in the new food pyramid for North America (Figure 1).

Latin America

The traditional Latin American diet is mainly characterized by the consumption of plant-based foods, i.e., fruit, vegetables, legumes, nuts, and seeds. Starchy foods habitually consumed

are corn, potatoes, and rice. Red meat is the main protein source while small portion of animal protein are provided by poultry, eggs, fish, seafood, and dairy. Therefore, the overall daily macronutrients distribution is balanced, with 54% carbohydrate, 30% fat, and 16% protein intakes (51).

Nevertheless, many nutritional surveys have reported that Latin Americans, particularly those living in urban areas or in low-socioeconomic conditions, presented low diet quality and variety. More in details, over 25% of total energy intake was provided by high glycemic index (i.e., white rice and potato), and sugar and fat-rich ultra-processed foods (i.e., ready-to-eat foods, sugar-sweetened beverages, pastries, chips, and candies). In addition, the consumption of foods rich in fiber, micronutrients, and phytochemicals (i.e., whole grains, fruits, vegetables, beans, and nuts) represented only 17.7% of total energy intake (51, 52).

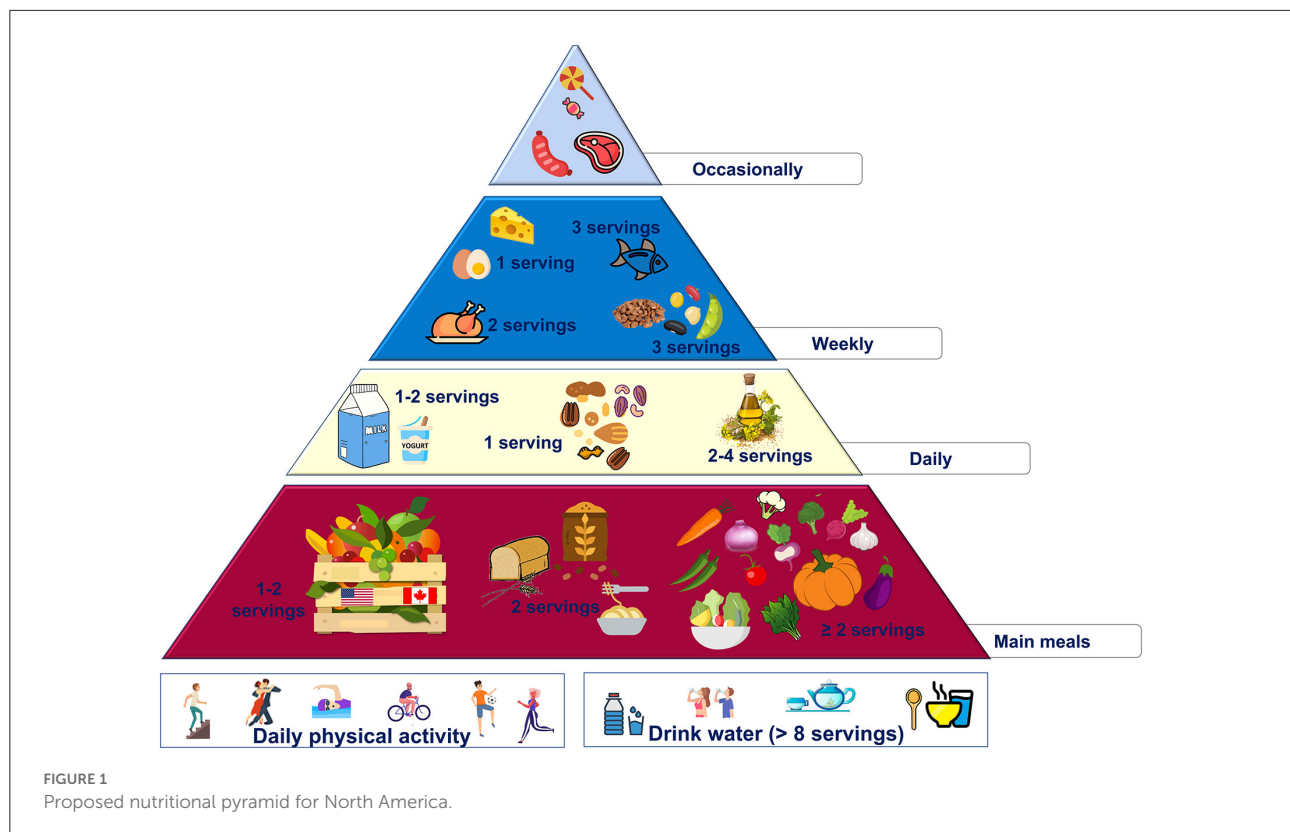
These nutritional changes have unfavorable effects on blood glucose homeostasis and lipid profile, and possibly explain the high prevalence of obesity and cardiometabolic diseases in Latin American populations (53).

Therefore, preferring the consumption of low-glycemic index starchy foods and foodstuffs with hypolipidemic and antioxidant properties is an essential shift to accomplish. In addition, promoting the consumption of diverse local foods may improve the overall diet quality in these populations.

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal native to the Andean region. Due to its small size its botanical parts can not be separated during the milling process. Therefore, quinoa is considered as a whole grain. It has a higher total protein content (12.9–16.5%) compared to other cereals (but equal to wheat) and provides all essential amino acids (54). Fat content ranged 5–9%, which is higher than other cereals, and mainly consists in oleic acids (19.7–29.5%), linoleic (49.0–56.4%), and α -linolenic (8.7–11.7%) acids. It exhibits a low glycemic index (ranging 35–53) by virtue of its small granules (particles <2 μ m in diameter), and 10% total dietary fibre, mainly soluble types (55). As compared to other cereals, 100 g of quinoa also contains higher amount of calcium (149 mg), iron (13.2 mg), and potassium (927 mg) (56).

It is also a good source of vitamins and other phytochemicals, such as polyphenols (251.5 mg/g ferulic acid, 0.8 mg/g *p*-coumaric acid, and 6.31 mg/g caffeic acid) and phytosterols (118 mg/100 g) (57, 58). Notably, it was reported that one serving of quinoa consumption (40 g raw) meets an important part of daily recommendations for essential nutrients (i.e., zinc, folic acid, magnesium, copper, and phosphorus) (59).

As for the health benefits of quinoa consumption, few human studies have been carried out so far. Recently, a meta-analysis of randomized controlled trials has reported that quinoa supplementation (20–65 g/day) may represent a useful strategy to manage cardiovascular risk factors in general adult population (60). Indeed, the addition of quinoa to the habitual diet significantly affected anthropometric parameters (body weight, waist circumference, and fat mass) as well as fasting insulin



and blood lipid concentrations in individuals with overweight obesity or pre-diabetes. The mechanisms behind these effects are still unclear (61, 62). Nevertheless, it can be speculated that fiber can be fermented by colonic microbiota with the production of short chain fatty acids (SCFAs), as occur for other wholegrains. SCFAs has been proven to modulate appetite feeling, and to improve insulin sensitivity and lipid metabolism (63).

Plátanos (*Musa sp.* also known as “green bananas”) are starchy foods used in Caribbean and Northern Latin American cooking. They contain elevated total carbohydrates amount (73.5%), but they are mainly represented by indigestible carbohydrates, i.e., resistant starch and other fibers (cellulose, hemicelluloses, and lignin) (64). Resistant starch has shown to reduce postprandial glucose response in individuals with overweight/obesity (65) as well as in patients with T2D (66). In addition, it promotes colonic fermentation and improve the gut microbiota composition, possibly influencing insulin sensitivity and other cardiovascular risk factors (67, 68).

Human studies with platano and its derived products (starch) provided promising results. A short-term trial (45-day) in 25 postmenopausal women with MS tested the effects of plátanos addition (20 g/day) to the habitual diet. At the end of the study, a significant improvement of fasting glucose concentrations and systolic blood pressure were observed, with no changes in body weight or composition. These findings have been related to the low glycemic index of platano (15.3) assessed

during the study, although the possible effects of other bioactive compounds can not be excluded (i.e., polyphenols, vitamins, and minerals) (69).

Platano starch supplementation was used in trials carried out in patients with T2D. In 4-week crossover trial 28 middle aged patients with T2D underwent a nutritional intervention either with starch (24 g/day) or soy milk (24 ml/day) dissolved in 240 mL of water. As compared to soy milk, starch supplementation induced a greater body weight loss, with no major changes in blood glucose control and lipid concentrations. Moreover, a reduction of insulin resistance (measured as HOMA-IR) was observed after starch supplementation, driven by decreased fasting insulin levels (70). In a more recent trial with longer duration (6 months), the supplementation with 4.5 g/day was tested in a group of patients with prediabetes or T2D ($n = 61$ starch vs $n = 52$ control). Starch significantly affected anthropometric parameters (body weight, waist, and hip circumferences, body fat) and blood pressure compared to control (71). Moreover, a significant improvement of fasting glucose and glycated hemoglobin (HbA1c) concentrations was observed after starch supplementation. These effects were obtained with no changes of the overall dietary composition, thus suggesting an independent role of starch in the improvement of metabolic control and body composition.

Avocado (*Persea americana*) is one of the main fat sources in Latin American diet and it might be preferred to olive oil due its lower cost, particularly among low-income individuals.

Avocados are rich in monounsaturated fatty acids (9.8/100 g), vitamin E (1.97 mg/100 g), and polyphenols (almost 200 mg/100 g), which can contribute to reduction of CVD risk (72). It has been reported that one avocado (136 g) has nutrient and phytochemical profiles similar to 40 g of nuts (72) and presents the same MUFA amount contained in almost 18 ml of olive oil (73).

The main health benefits of avocado consumption are related to the improvement of blood lipid concentrations. Indeed, several meta-analyses (74–76) have reported a moderate to large effect on LDL cholesterol (−3.54 mg/dl; 95% CI: −9.66, 2.58 mg/dl) even great heterogeneity was observed among studies. Moreover, avocado consumption (1 to 3 avocado/day) has shown to significantly increase HDL-cholesterol concentrations (3.90 mg/dl; 95% CI: 0.44, 7.36 mg/dl) (75). In addition, the consumption of one avocado/day (almost 136 g) has shown to improve oxidative stress (77) and cognitive function (78) in individuals with overweight/obesity by virtue of its bioactive compounds with antioxidant properties, in particular polyphenols (almost 200 mg), lutein and zeaxanthin (370 µg) (72).

Açaí berries (*Euterpe oleracea* Mart.) are the fruit of a typical palm tree found in the floodplains along the Amazon River estuary. They can be green (white açaí) or purple (dark açaí) according to the variety with no difference in nutritional composition. They are good sources of fat (32–48 g/100 g, mainly omega 6 and 9), fiber (44 g/100 g) polyphenols (424.9 mg/100 g), particularly anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, and peonidin-3-rutinoside), proanthocyanidins, phenolic acids (gallic, protocatechuic, p-coumaric, ellagic, vanillic, and syringic acids), stilben (resveratrol), and other flavonoids (epicatechin, quercetin, catechin, velutin, homoorientin, orientin, isovitexin, and taxifolin deoxyhexosein) (79).

Few human trials evaluating the effects of Açaí berries on health are available. In a short-term trial (4 weeks), 10 overweight volunteers were asked to consume 200 g/day of açaí pulp. At the end of the study, an improvement of fasting glucose, total and LDL-cholesterol concentrations was observed (80). The same amount of açaí pulp was used in a study focusing on the potential antioxidant activity of açaí berries in 35 young women (81). After 4-week, açaí pulp increased the activity of antioxidant enzymes, total antioxidant capacity, and reduced the production of reactive oxygen species and protein carbonyl concentrations. In a more recent randomized, double-blind, placebo-controlled clinical trial, 69 overweight dyslipidemic patients were assigned to a hypocaloric diet + 200 g/day of açaí pulp or hypocaloric diet alone. The hypocaloric diet was designed to achieve a 6 kg-weight loss with a balance macronutrient distribution (protein 15%, 45–60% carbohydrate,

and 25–30% fat). After 8 weeks, in the group consuming açaí pulp, a significant improvement of oxidative stress (measured as plasma 8-isoprostane concentrations) and inflammation (evaluated as IL-6 and IFN-γ) was observed (82).

As reported above, the traditional Latin American diet is a plant-based dietary pattern and provides adequate amount of the main macro- and micronutrients. However, globalization has induced a shift toward Western dietary habits. In addition, some traditional foodstuffs should be limited and substituted with other having more beneficial characteristics. Therefore, over the main principles of the MD for animal protein-sources and sweets, the following changes are proposed:

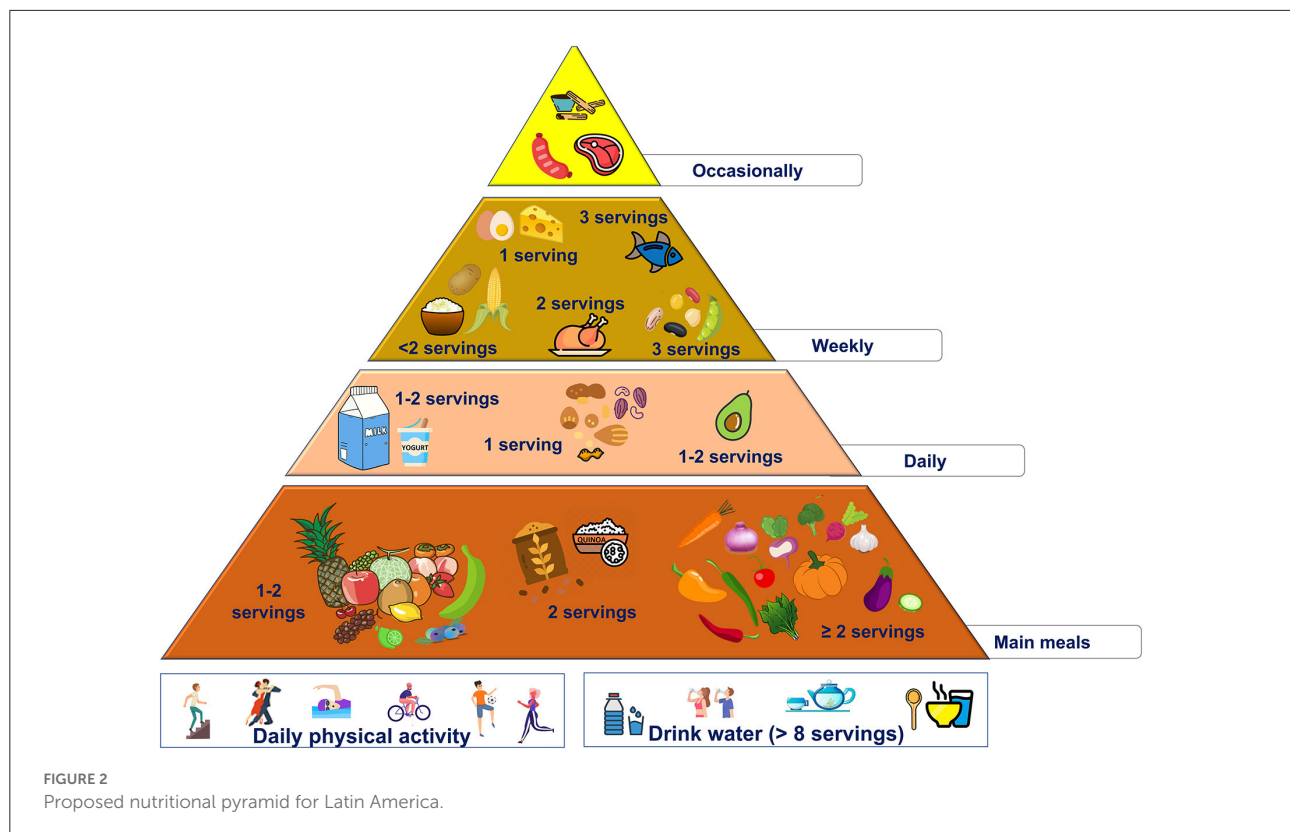
- 2 servings/day of starchy foods with low glycemic index (quinoa, plátanos, and wholegrain)
- no more than 2 servings/week of starchy foods with high glycemic index (rice, corn, and potato)
- use avocado as the main daily fat source (the amount should be according to individual energy needs)
- increase the intake of vegetables (at least 2 servings/day) and fruits (1 or 2 servings/day), preferring fruit with high antioxidant activities (like açaí and other berries).
- These changes are summarized in the new food pyramid for Latin America (Figure 2).

Africa

Although malnutrition and food shortages are still prevalent in this continent, many African countries underwent the “nutrition transition” during the last years. Indeed, very recently the profound changes that have taken place in Africa have been reported (83). Overall, these dietary changes consist of a shift from the consumption of natural foods with marginal handling—mainly fruit, vegetables, and legumes—to highly processed foods (refined carbohydrates, SFA and trans fats, added sugars, salt, and food additives). Notably, this phenomenon occurred also in African Mediterranean countries (Egypt, Libya, Tunisia, Algeria, and Morocco) (84). Unfortunately, to the best of our knowledge, no clear information on the actual nutritional habits of African populations is available so far. This lack of information is mainly due to methodological limitations in the assessment of dietary intake as reported in previous studies (85).

Nevertheless, as for other continents, it could be hypothesized that promoting the consumption of local foods could improve the diet quality in African populations.

Teff (*Eragrostis tef*) is a grain native to Ethiopia and Eritrea (Eastern Africa). Teff is the smallest of all grains and is considered a minor cereal due to its meager use, limited to a few regions of the world (86). Teff is a good source of dietary fiber (8 g/100 g), and it has higher amount of protein (11–13%) as compared to other grains (87). As for micronutrients, teff



contains the highest iron and calcium contents (11–33 mg and 100–150 mg, respectively) among all grains (86). Therefore, a cross-sectional study in 592 pregnant women suggested that the lower risk of anemia in Ethiopia might be explained by the daily consumption of this grain (88).

Moringa (*Moringa Adans*) is a plant that can grow in extreme climatic conditions (hot dry and less fertile soils) and is widespread in Ethiopia and Kenya. All botanical parts of Moringa (flowers, pods, leaves, and seeds) have been traditionally used for their potential beneficial effects. Indeed, Moringa is a good source of phytochemicals and micronutrients (89). As for vitamins, Moringa has shown a content of vitamin A and β -carotene (11,300–23,000 IU and 6.6–6.8 mg per 100 g), as well as vitamin C (200 mg/100 g), which are even higher than other plant foods (i.e., carrots and pumpkins, and oranges and kiwi, respectively). In addition, the amount of Vitamin E is similar to nuts (9.0 mg/100 g) whereas Moringa contains more polyphenols than fruit and vegetables (88). Pods and seeds contain unsaturated and essential fatty acids, especially oleic acid as well as omega 3, that make Moringa a source of healthy fat (90).

This nutritional profile might contribute to the antioxidant, antimicrobial, and anti-inflammatory activities associated with its consumption. Nevertheless, most of the evidence is provided by *in vitro* experiments and studies in animal models (91). Besides, a recent meta-analysis of 46 studies in animal models

suggested a potential role of Moringa-derived products in the reduction of glucose and lipid levels (92).

Native fruit and vegetable, particularly from South Africa, have been reported as relevant contributors to the survival of local communities. It is worth mentioning that almost 119 species of plant-derived products grow in Africa (93). Undoubtedly, fruit and vegetable provide huge amounts of vitamins, minerals, and other phytochemicals with beneficial effects on health.

Recently, Nkosi et al. (94) evaluated the nutritional composition of ten African fruit [*Ficus capensis* Thunb (Cape fig), *Landolphia kirkii* (Sand apricot vine), *Engelerothymum magalismsontanum* (Transvaal milkplum), *Parinari curatellifolia* (Mobola plum), *Sclerocarya birrea* (Marula), *Strychnos spinosa* (Green monkey orange), *Strychnos madagascariensis* (Black monkey orange), *Syzgium cordatum* (Water berry), *Ximenia caffra* (Sour plum), and *Vangueria infausta* (Wild medlar)]. They detected different profile of phenolic compounds thus suggesting a potential involvement in different biological activities. Indeed, *in vitro* experiments showed that Mobola plum, Water berry, and Marula presented the strongest antioxidant activity. Conversely, Sour plum and Sand apricot vine induced the highest inhibition of carbohydrate hydrolysing enzymes, thus suggesting a potential role in carbohydrates digestion.

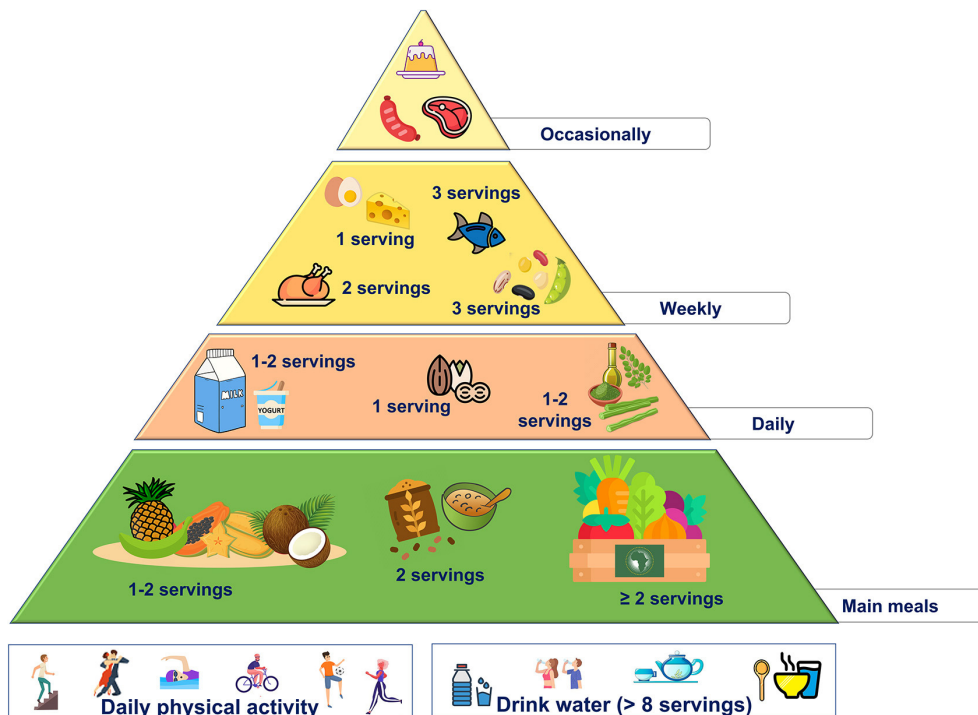


FIGURE 3
Proposed nutritional pyramid for Africa.

Unfortunately, scientific evidence in humans or large information on food composition are lacking (93). Nevertheless, the consumption of local fruit and vegetable should be encouraged to improve the diet quality and sustainability of food systems.

In conclusion, to improve diet quality in African populations the proposed changes are:

- favor the consumption of native grains like teff by consuming at least 2 servings/day of teff-derived products
- use Moringa oil as daily fat source (the amount should be according to individual energy needs)
- increase the intake of plant-based foods according to African traditional diet and endorse the use of native fruits (1 or 2 servings/day) and vegetables (at least 2 servings/day).

These changes are summarized in the new food pyramid for Africa (Figure 3).

Asia

The Asian continent is very extended and, consequently, dietary habits might be more heterogeneous across the different

countries than in other continents. Although each Asian region has its distinct traditions, there are many unifying characteristics that allows us to perform a critical appraisal of the nutritional profile of population living in Asia.

Grains account for the main part of crops in Asian countries this continent (95–97). Indeed, carbohydrates intake is high (65% to >80% of daily energy intake), with rice and its derivatives being the main dietary sources (98). Although different rice varieties are consumed across Asian countries, white rice is the main contributor to carbohydrate intake. In addition, the higher consumption of rice, the lower intake of fat sources and wholegrain (almost 15 and 5%, respectively) (99). This translates in a high-carbohydrate-low-fat diet characterized by the consumption of food with high glycemic index and glycemic load (99). Several studies have shown that both carbohydrate amount and quality associated with MS and metabolic diseases in Asian countries (100, 101) as well as in other populations (102–104).

Besides, the prevalence of micronutrient deficiencies (mainly vitamin A, vitamin B12, iron, iodine, and folic acid) is widespread in different Asian countries (105).

Therefore, the main dietary changes that should be encouraged are related to the consumption of low-glycemic index starchy foods, adequate carbohydrate intake (<60%), and foodstuffs containing vitamins, minerals, and phytochemicals.

Barley (*Hordeum vulgare* L.) is a major food crop in Middle East (Iran, Iraq, and Syria) and Turkey, but also in Central and South Asia (mainly Kazakhstan, Afghanistan, India, and Pakistan) (46). It is classified as wholegrain, and it received the health claim from its lowering effects on glucose and cholesterol levels by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) (106, 107). These metabolic effects are triggered by β -glucan, a viscous soluble dietary fiber, which can form a viscous gel that can reduce carbohydrate and fat absorption in the gut (108). On the other hand, it is more prone to fermentation by gut microbiota than other type of fibers, thus inducing a greater production of beneficial microbial metabolites (i.e., SCFAs) (109).

Sesame seeds (*Sesamum indicum*) are traditional food in East Asia, but its oil is widely used in the whole continent (110). Sesame is richer in lignans (sesamin, sesamol, sesamol, episesamin) and γ -tocopherol than other foods (i.e., flaxseeds, nuts, grains, and legumes) (111). In addition, sesame oil contains a beneficial fatty acids profile, consisting of linoleic acid (41%), oleic acid (39%), palmitic acid (8%), stearic acid (5%) (112).

Studies in humans have investigated the possible effects of daily sesame oil consumption on cardiovascular risk factors (113, 114). A meta-analysis of 8 randomized controlled trials ($n = 843$ participants) showed that sesame consumption (35 g of sesame oil) can reduce systolic as well as diastolic blood pressure (-7.83 and -5.83 mmHg, respectively) (113). More recently, Yargholi et al. evaluated the effect of sesame consumption on blood glucose control in a meta-analysis of 8 randomized controlled trials ($n = 382$ participants with T2D). A significant reduction of fasting blood glucose (-28 mg/dl) and HbA1c (-1.00%) levels were observed with the consumption of 30 g of sesame-derived products (114).

Marine macroalgae i.e., seaweeds, have been consumed for centuries in Far East Asia, mainly Japan and Korea (115, 116). They are a great source of complex polysaccharides, minerals, proteins, and vitamins, and well as of several phytochemicals (117, 118). In addition, seaweeds have a significant amount of essential PUFA, namely eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), and their precursors α -linolenic acid (ALA; 18:3 n-3) and docosapentaenoic acid (DPA; 22:5 n-3) (119).

The regular consumption of seaweeds (15 g/day) has been associated with a reduced risk of CVD risk (119, 120). This effect is triggered mainly by the lowering-triglyceride effect of DHA and EPA, as stated also by a health claim by EFSA (121).

Nevertheless, some seaweeds have shown to influence other CVD risk factors. Spirulina (*Spirulina platensis*), is a blue-green algae which grows naturally in high salt alkaline water reservoirs in subtropical and tropical areas of Asia. It is a good source of high-quality protein (60–70%/dry weight) and contains an iron with high bioavailability as compared to other foods (i.e., grains) (122). Interestingly, in a meta-analysis of randomized controlled

trials the spirulina supplementation decrease total and LDL-cholesterol (-47 and -41 mg/dl, respectively) while increase HDL-cholesterol concentrations (6 mg/dl) (123). Besides, a recent meta-analysis has shown that spirulina consumption (1–8 g) decrease systolic and diastolic blood pressure (-4.59 and -7.02 mmHg, respectively), with greater reduction in patients with hypertension (124).

Wakame (*Undaria pinnatifida*) is one of the most consumed macroalgae worldwide. It contains higher amounts of alginate, a polysaccharide with high viscosity (125). Therefore, its potential role in the modulation of glucose and lipid metabolism has been postulated.

Izaola et al. (126) investigated the effects of a wakame-enriched snack on CVD risk factors in 40 individuals with MS. After 8 weeks, a significant reduction of total and LDL-cholesterol (-10 and -8.9 mg/dl, respectively) observed with no effects on glucose metabolism. More recently, in an acute cross-over trial, wakame intake (5 g) significantly reduced glucose and insulin response to a mixed meal compared to the control (127). However, it is worth mentioning that seaweeds contain iodine their overconsumption may pose a risk of thyroid diseases in susceptible individuals (128). In addition, due to pollution contamination in the aquatic system, seaweeds might contain heavy metals and metalloids (129). Therefore, regular consumption of seaweeds should be monitored due to the potential health risk in the long term.

Soy (*Glycine max*) and its derivatives are largely used in Asian countries. Soy is an East Asian native leguminous plant rich in proteins (36–46%, depending on the variety), lipids (18%), soluble carbohydrates (15%), and fiber (15%). In addition, soy also contains several bioactive compounds such as lecithin (0.5%), sterols (0.3%), isoflavones (0.1%), tocopherols and tocotrienols (0.02%) (130).

Epidemiological studies have shown an inverse association between soy-based foods and the incidence of CVD, T2D, and certain types of cancer (breast and stomach) (131–137). These effects are related to its isoflavone content. Indeed, isoflavones are phytoestrogens which can bind the estrogen receptor thus having an estrogen-like activity (130). Moreover, soy proteins (β -conglycinin and glycinin) and peptides obtained by their intestinal hydrolysis have repeatedly shown a cholesterol-lowering effects by promoting LDL-receptor expression (138). Notably, soy intake (25 g/day) has been granted by FDA for its beneficial effects on cardiovascular health (139).

As reported above, the traditional Asian diet is particularly rich in starchy foods with high glycemic index, and lower amount of other nutrients and possibly micronutrients. Therefore, some traditional foodstuffs should be limited favoring other foodstuff with more beneficial effects on health. Accordingly, the following changes are proposed to improve diet quality:

- 2 servings/day of starchy foods with low glycemic index (barley and wholegrain)
- no more than 2 servings/week of starchy foods with high glycemic index (rice, and noodles)
- use sesame oil as the main daily fat source (the amount should be according to individual energy needs), and use sesame seeds to enrich soups
- increase the intake of vegetables (at least 2 servings/day) and fruits (1–2 servings/day), preferring fruit with high antioxidant activities.
- 2 servings/week of plant protein sources (soy-derived foods)
- 1 serving/day of seaweeds (in particular, spirulina and wakame)

These changes are summarized in the new food pyramid for Asia (Figure 4).

Australia

The actual Australian diet resembles a Western dietary pattern, with plant-based foods replaced by high-fat, energy-dense, and greater animal-derived foodstuffs (140). Therefore, due to the “nutrition transition,” dietary changes favoring healthier food choices of local products could represent a first step to improve diet quality also in Australia.

Macadamia nut (*Macadamia integrifolia*), a tree nut native to Australia, contains ~75% fat, higher levels of MUFA than any other food sources (more than 60 g/100 g), and phenolic compounds (141).

As for the potential health benefits of macadamia nuts intake, few studies in humans are available. In the study by Garg et al. 17 hypercholesterolemic male were given macadamia nuts (40–90 g/day, accounting for 15% of total energy intake) for 4 weeks. At the end of the intervention, plasma markers of inflammation (leukotriene) and oxidative stress (8-isoprostane) were significantly lower in the group consuming macadamia nuts than control (leukotriene: -323 ± 96 pg/ml and 8-isoprostane: 197 ± 19 pg/ml) (142). In addition, in a 5-week clinical trial with macadamia nut supplementation (40–90 g/day, accounting for 15% of total energy intake) significantly reduced total and LDL-cholesterol as compared to control (-20 ± 7 and -12 ± 5 mg/dl, respectively) in a group of 25 hypercholesterolemic men and women (143).

Atlantic salmon (*Salmo salar*) and Barramundi (*Lates calcarifer*) are two major Australian farmed fish species. They are rich in omega-3 PUFA with 980 and 790 mg/100 g, respectively (144). As reported above, omega 3 fatty acids (namely DHA and EPA) bear a health claim for their triglyceride-lowering effects (121), possibly contributing to the reduction of CVD risk.

Native fruits, i.e., Davidson’s plum (*Davidsonia spp.*), pepper berry (*Tasmannia lanceolata*), finger lime (*Citrus australasica*

var. sanguinea), are great contributors for dietary intake of vitamins, minerals, and other phytochemicals with beneficial effects on health (145).

Preliminary studies suggested that the extracts of these fruits might inhibit cancer cells growth in several *in vitro* models (pancreas, breast, lung, brain, skin, colon, and ovary cancers) (146, 147). Unfortunately, to the best of our knowledge, no clinical studies in humans are available so far.

As reported above, important changes are required to improve the overall quality of the actual Australian diet. However, to increase the feasibility of these dietary changes, the incorporation of local foods rich in fiber, phytochemical and other bioactive compounds may represent a complementary strategy. Therefore, over the main principles of the MD for animal protein-sources and sweets, the following changes are proposed:

- use macadamia oil as the main daily fat source (the amount should be according to individual energy needs)
- increase the intake of vegetables (at least 2 servings/day), preferring local varieties
- increase the intake of fruits (>2 servings/day), preferring local varieties (i.e., Davidson’s plum, native pepper berry, and finger lime)
- increase the intake of fish rich in omega-3 PUFA (2–3 servings/week), preferring local varieties (i.e., Atlantic salmon, barramundi)
- use macadamia nuts for snacking, in particular (40–90 g/day, accounting for 15% of total energy intake).

These changes are summarized in the new food pyramid for Australia (Figure 5).

Conclusion and future perspectives

Although the pivotal role of the MD in the prevention and management of NCDs, it is not easy to transfer this dietary pattern to other populations. Indeed, the adoption of the real MD implies to shift from local traditions that have been in those territories for centuries to new and unknown habits. Therefore, it seems more reliable—and also desirable— that each country rediscovers its own heritage to develop a healthier nutrition pattern based on traditional and local foods. This would be aligned with UNESCO advocacy to preserve cultural identity, continuity of communities, and environment.

Some non-Mediterranean dietary patterns, as Nordic (148) and Okinawan (149) diets, have been shown to reduce disease risk and mortality. These beneficial effects are linked to a nutritional profile that is superimposable to MD (150, 151). Therefore, it would be possible to obtain healthful effects by combining different foodstuff belonging to the own country

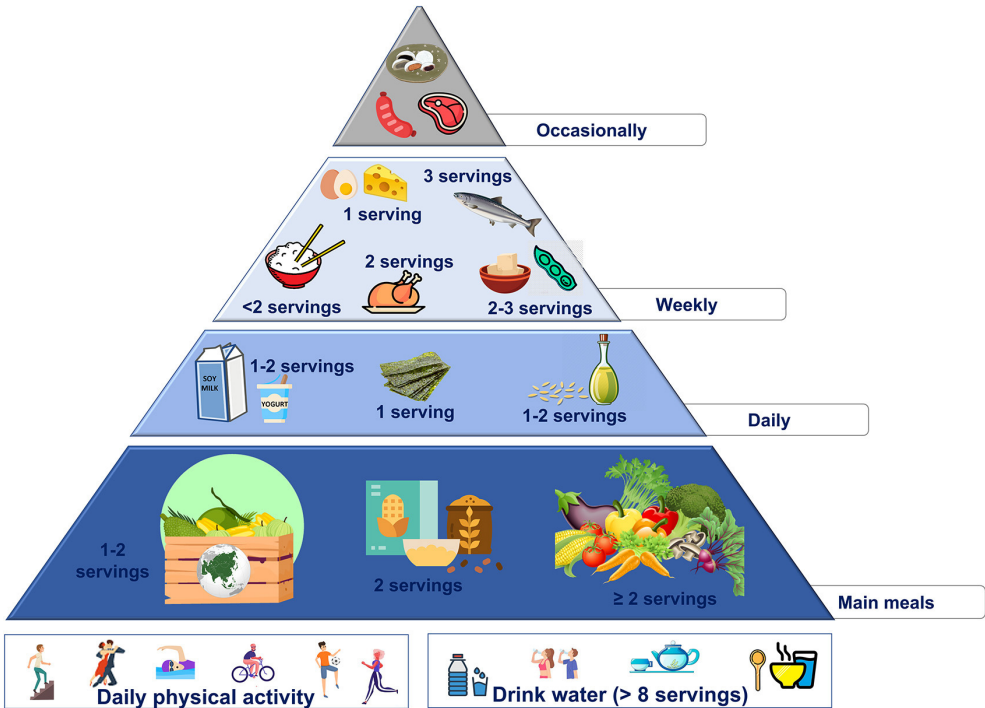


FIGURE 4
Proposed nutritional pyramid for Asia.



FIGURE 5
Proposed nutritional pyramid for Australia.

with significant advantages not only for people but also for the environment.

The present review is an attempt to promote a healthy and sustainable dietary model based on the nutritional properties of MD but implemented at the local level by using the food products available in different areas of the world. Nevertheless, as the first release of the research project “Planeterranea,” this manuscript provides an overview of the rationale, aims, and main results, and the limitations will be addressed in future publications. Indeed, some of the analyzed macro-areas (Asia, Africa) include many heterogeneous countries with specific eating habits. Therefore, it is necessary to evaluate whether a macro-area might require multiple nutritional pyramids to endorse the compliance of the different populations.

On the other hand, the sustainability of the proposed pyramids should be evaluated in *ad hoc* investigations that should consider (a) affordability in terms of commercialization, costs, farming, etc.; (b) impact on local economies that should meet the needs of growing populations; (c) greenhouse gas emissions of local crops as compared to long-traveling food products.

Finally, more studies are needed to evaluate the bioavailability of micronutrients and bioactive compounds contained in the identified foods. In addition, the safety of local foods with potential health benefits might be investigated to assess whether they might have detrimental side effects in the long term.

In conclusion, it is important to increase global knowledge about healthy and sustainable dietary patterns rather than force people to a useless change. Therefore, nutritional research should focus not only on the amount and frequency of food consumed but also on cultural behaviors, socio-economic conditions, food quality and processing. This would lead to a

sharply increased adherence to nutritional recommendations with a relevant impact on public health and clinical practice.

Author contributions

CV, PP, and AC: conceptualization and validation. CV and PP: writing—original draft preparation. GM, LB, DL, CG, and FM: review and editing. AC: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Serum creatinine as an indicator of lean body mass in vegetarians and omnivores

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Growing numbers of Americans are adopting vegetarian or vegan diets. While risk for some chronic conditions may be lower when following these diets, concern remains over the ability to consume adequate amounts of various nutrients, notably, protein. Knowing that serum creatinine is a reliable marker of muscle mass, this study examined the relationships between serum creatinine, lean body mass (LBM), handgrip strength, and protein intake in healthy vegetarian ($n = 55$) and omnivorous ($n = 27$) adults. Significantly higher protein intakes (+31%), LBM (+7%), serum creatinine (+12%) and handgrip strength (+14%) were observed for the omnivore participants compared to vegetarian participants. Positive correlations ($p < 0.001$) were noted between creatinine and LBM ($R^2 = 0.42$), creatinine and handgrip strength ($R^2 = 0.41$), protein intake and LBM ($R^2 = 0.29$), and handgrip strength and LBM ($R^2 = 0.69$). These data show that serum creatinine concentrations were lower in vegetarian women and men in comparison to their omnivorous counterparts and that serum creatinine concentrations correlate with LBM and strength in healthy adults, regardless of diet.

KEYWORDS

vegetarian, creatinine, lean body mass, muscle, grip strength

Introduction

In recent years vegetarian diets have increased in popularity. A 2020 Harris Poll reported that 4% of men and 7% of women surveyed over 18 years of age in the United States identified as vegetarians, with half of those identifying as vegan (1). While these diets are often, but not exclusively, associated with decreased risk for cancer (2–4), cardiometabolic diseases (3–5), diabetes (6), and obesity (7–9), the possibility of micronutrient inadequacies, including iron, vitamin B12, and vitamin D remain a concern. Furthermore, although energy intakes tend to be similar, protein intake is often significantly lower for vegetarians when compared to omnivores (10–14). This can be concerning as protein intakes are directly linked to muscle mass and strength (15–17). Following ingestion, protein is digested to component amino acids, which are rapidly absorbed. The increase in plasma amino acid concentrations, particularly the essential amino acid leucine, stimulates muscle protein synthesis (18, 19). In comparison to animal proteins, plant proteins contain lower amounts of leucine (20); moreover, plant protein is 10–20% less bioavailable due to antinutrient factors present in these foods (11, 21). Clinical trials have demonstrated that the rate of muscle protein synthesis is less for

plant-based versus animal-based protein (22, 23). Indeed, decreased lean body mass (LBM) and reduced handgrip strength has been reported in vegetarians (24, 25). Since handgrip strength is an indicator of health-related quality of life irrespective of age and gender (26–28), it is important to consider this quality in vegetarians.

Serum creatinine, a normal waste product of muscle metabolism, has been shown to be a reliable marker of muscle mass since it is continually produced and filtered through the kidney (29, 30). However, many factors can impact serum creatinine levels, most notably declining kidney function. At the nephron, creatinine is filtered through the glomerulus and is not reabsorbed by the tubule; hence, elevations in serum creatinine can be utilized as a proxy for renal function. Since serum creatinine is generated from muscle, concentrations are impacted by age, sex, and body size. Equations have been developed using serum creatinine to estimate the glomerulus filtration rate (GFR) while controlling for these common confounding factors, and currently, the CKD–EPI Collaboration (Chronic Kidney Disease Epidemiology Collaboration) equation is considered the most accurate GFR measurement for clinical use (29), and GFR values <90 ml/min/1.73 m² indicate declining kidney function. However, serum creatinine is influenced by other factors as well including medications, chronic illness, nutritional status and diet (31), all of which need to be considered when utilizing this measure.

Several investigators have reported lower serum creatinine concentrations in vegetarian populations in comparison to omnivores (32, 33). However, a comparison between serum creatinine, LBM, and strength based on diet adherence in a healthy adult population has not been reported. Therefore, the purpose of this investigation was to examine the relationship between serum creatinine, LBM, and handgrip strength in healthy, non-athlete vegetarian and omnivore women and men. We hypothesized that serum creatinine would be positively associated with LBM and handgrip strength in both genders, with omnivores exhibiting higher levels of creatinine, LBM, and handgrip strength than vegetarians.

Subjects and methods

This study is a secondary analysis of a cross-sectional data set gathered from healthy, non-obese adults (body mass index [BMI] >18.5 and <30 kg/m²) to examine associations between indicators of bone health, bone mineral density, and diet adherence (vegetarian vs. omnivore) (34). Participants (27 omnivores and 55 vegetarians), aged 19–50 y, were recruited from the Phoenix metropolitan area using university list serves, connections with local vegetarian groups and farmers markets, and social media. Participants were healthy and free of chronic diseases by self-report; not taking prescription medications with the exception of oral contraceptives; not

competitive athletes or training for an endurance event; and, if female, not recently pregnant. These exclusion criteria were designed to control covariables known to impact bone mineral density, but they were also relevant to this secondary analysis with a focus on lean body mass. Participants were classified as “vegetarian” if they reported never eating meat, fish, or poultry over the preceding year. “Omnivores” were classified as eating at least 3 servings of meat, fish, and/or poultry per week over the preceding year. Omnivores who reported less than 3 servings of flesh foods weekly [e.g., flexitarians or “meat-avoiders” (35)] were excluded from participation. All participants provided written consent and the study was approved by the Institutional Review Board at Arizona State University.

A 24-h diet recall was conducted by a trained nutrition professional using the multiple pass method, and diet data were analyzed using the Food Processor software (version 7.71; ESHA Research, Salem, OR, USA). Physical activity levels were estimated by validated questionnaire and reported as metabolic equivalents (METs) (36). A venous blood sample was collected following a 12-h fast, and serum was extracted for the creatinine analyses (Jaffé method, COBAS C311, Roche Diagnostics International Ltd, Switzerland). Glomerular filtration rate (GFR) was calculated using the CKD–EPI equation (29). Height was measured using a stadiometer, and body mass was recorded using a calibrated scale (model TBF-300A, Tanita Corporation, Tokyo, Japan). Dual energy X-ray absorptiometry (DEXA) (GE Lunar iDXA, Chicago, IL, USA) was used to measure LBM by a trained X-ray technician. Dominant arm handgrip strength was measured in triplicate while seated with the elbow flexed at 90 degrees and a neutral wrist position using a handheld dynamometer (Takei Scientific Instruments, Niigata-City, Japan). Three consecutive measures were taken, and the mean score was used for analyses.

Statistical analyses

For this secondary analysis, relationships between dietary protein, serum creatinine, strength, and LBM in omnivore vs. vegetarian participants were examined by gender. Data not normally distributed based on the Kolmogorov–Smirnov test were transformed prior to analyses (LBM only). Following a significant multivariate analysis, univariate analyses were used to assess differences between means while controlling for covariates. Pearson correlations were used to identify relationship between variables, and multiple regression analyses were utilized to determine the predictive value of variables for LBM and handgrip strength. Statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY, USA), and $p \leq 0.05$ was considered significant. Data are reported as mean \pm SD.

Results

The study sample ($n = 82$) was composed of 55 vegetarians (67%) and 58 women (71%) (Table 1). Gender, BMI, energy intake, and physical activity did not vary between the diet groups; however, the vegetarian group was older on average than the omnivorous group (32.5 ± 8.8 and 27.2 ± 6.7 y respectively; $p = 0.008$). In subgroup analyses, the vegetarian men were less active than their omnivorous counterparts (41.1 ± 25.6 and 74.9 ± 49.0 METS respectively; $p = 0.036$); hence, age and physical activity were controlled in the remaining analyses.

All variables tested were significantly impacted by diet type (Table 2). Significantly higher protein intakes (+31%), LBM (+7%), serum creatinine (+12%) and handgrip strength (+14%) were observed for the omnivore participants compared to vegetarian participants ($p > 0.05$; Table 2). Additionally, the diet related differences for serum creatinine and handgrip strength were observed within gender groups. There were no significant relationships between serum creatinine and energy intake ($r = -0.131$; $p = 0.289$) or alcohol intake ($r = -0.010$; $p = 0.934$) among the participants. The average GFR measure was raised for the vegetarian participants relative to the omnivorous participants (+8%; $p = 0.002$), and this diet related difference was also observed within genders.

With LBM as the criterion variable, multiple regression analyses identified gender and serum creatinine as significant predictor variables ($p < 0.05$) in a model including age ($p = 0.396$), diet group ($p = 0.316$) and dietary protein ($p = 0.111$) [$F_{(5,65)} = 32.094$, $p < 0.001$, $R^2 = 0.712$] (Table 3). With handgrip strength as the criterion variable, gender, diet group, and LBM, were predictor variables ($p < 0.05$) in a model also including age ($p = 0.614$) and dietary protein ($p = 0.423$) [$F_{(5,65)} = 44.816$, $p < 0.001$, $R^2 = 0.775$] (Table 3). Correlations between serum creatinine, LBM and handgrip strength, dietary protein and LBM, and handgrip strength and LBM were significant in the study sample ($p \leq 0.001$; Figure 1), and these significant results were retained after controlling for GFR.

Discussion

This investigation revealed that serum creatinine is significantly reduced in vegetarian women and men compared to their meat-eating counterparts. Moreover, serum creatinine was a strong, independent predictor of LBM. The vegetarian men and women tended to consume less protein than the omnivore men and women and, importantly, protein intake and LBM were directly correlated. Moreover, vegetarians in this study had significantly lower handgrip strength than their omnivore counterparts. These findings may have important physiological relevance, as LBM and handgrip strength are related to quality of life (26–28). Moreover, Srikanthan et al. reported a negative association between muscle mass and

all-cause mortality in older Americans (37). Kang et al. found a direct correlation between decreased handgrip strength and impaired mobility, increased pain and greater discomfort in both Korean men and women, with exacerbated effects as age increased, thus leading to a poorer quality of life (26). Lower levels of physical activity are associated with both a lower quality of life and with reduced handgrip strength; hence, physical activity needs to be controlled in these types of investigations (38). Herein, adults who participated in competitive sports were excluded, and the diet related differences in handgrip strength by gender remained significant controlling for age and physical activity level.

In several large cross-sectional trials, serum creatinine concentrations were significantly reduced in vegetarian populations in comparison to omnivores (32, 33). Utilizing a randomized controlled study design, Dinu et al. reported a significant reduction in serum creatinine in adults at medium-to-low risk for cardiovascular disease randomized to a hypocaloric vegetarian diet vs. a hypocaloric Mediterranean diet for 3 months (-0.04 and $+0.01$ mg/dL respectively, $p < 0.001$) (39). The only dietary differences between groups were for protein and fiber; however, correlation analyses showed no significant associations between these dietary changes and change in serum creatinine. Losses in total body mass was similar between groups; however, 26% more fat mass was lost in the omnivore group in comparison to the vegetarian group suggesting a greater degree of LBM loss in the latter group, which may have contributed to the significant reduction in serum creatinine noted for this group (38). In a case report, a 65-y old male with type 2 diabetes and stage 3 kidney disease, adopted a vegetarian diet, and after four months serum creatinine concentrations fell 38% from 1.6 to 1.03 mg/dL, a reduction attributed to the loss of lean body mass (40). Kochlik et al. followed serum creatinine change in adults adhering to a meatless diet for 6 days but fed 160 g cooked chicken (e.g., approximately two servings meat equating to 35 g protein) on day 4 (41). Creatinine concentrations did not fluctuate on trial days 3, 4, 5 and 6, suggesting the dependence of plasma creatinine on muscle metabolism and not meat ingestion (41). However, Mayersohn et al. reported a 52% rise in serum creatinine 2 h after consumption of the equivalent of 225 g cooked meat (42). Animal products, primarily meat, fish, and poultry, are the sole dietary source of creatine, which is partially converted to creatinine based on the cooking method (43).

Creatinine is generated at a constant rate from the spontaneous, non-enzymatic cyclization of creatine in muscle cells (30). Due to its steady production, and the fact it is freely filtered through the glomerulus, serum creatinine is used clinically as an indicator of kidney function, and high serum concentrations of creatinine (>1.4 mg/dL) are a marker of compromised kidney function (44). In fact, acute elevations in serum creatinine are considered a potent risk factor for adverse outcomes in general in the inpatient setting (43). However, less

TABLE 1 Age, body mass index, energy intake, and physical activity by diet adherence^a.

	OMN	VEG	P value
<i>n</i> (M/F)	8/19	16/39	0.267*
Age (y)	27.2 ± 6.7	32.5 ± 8.8	0.008
Male	24.9 ± 3.7	34.8 ± 7.9	0.003
Female	28.2 ± 7.5	31.6 ± 9.2	0.167
Body mass (kg)	66.8 ± 12.0	63.8 ± 11.0	0.054
Male	76.4 ± 11.6	72.5 ± 11.0	0.043
Female	62.8 ± 9.8	60.2 ± 8.9	0.205
Body mass index (kg/m ²)	23.5 ± 3.1	22.4 ± 2.6	0.095
Male	23.6 ± 2.4	23.3 ± 2.4	0.844
Female	23.4 ± 3.4	22.0 ± 2.6	0.076
Energy intake (kcal)	2,153 ± 736	2,109 ± 613	0.746
Male	2,689 ± 860	2,461 ± 518	0.433
Female	1,901 ± 528	1,938 ± 589	0.828
METS (min/wk)	50.1 ± 39.0	38.8 ± 27.9	0.131
Male	74.9 ± 49.0	41.1 ± 25.6	0.036
Female	39.7 ± 29.6	37.8 ± 29.0	0.816

^aData are mean ± SD; p value for univariate analysis (gender controlled in total sample analysis); *indicates chi square analysis; METS, metabolic equivalents.

TABLE 2 Characteristics by diet adherence^a.

	OMN	VEG	P value
<i>n</i> (M/F)	8/19	16/39	0.267*
Dietary protein (g/kg)	1.48 ± 0.63	1.13 ± 0.46	0.046
Male	1.95 ± 0.74	1.47 ± 0.67	0.466
Female	1.26 ± 0.45	1.09 ± 0.45	0.262
Lean body mass (kg)	47.5 ± 11.2	44.2 ± 7.8	0.029
Male	61.5 ± 7.9	54.2 ± 5.7	0.010
Female	41.6 ± 5.6	40.1 ± 3.6	0.278
Serum creatinine (mg/dL)	0.86 ± 0.17	0.77 ± 0.12	0.002
Male	1.04 ± 0.12	0.89 ± 0.09	0.003
Female	0.79 ± 0.14	0.72 ± 0.09	0.034
GFR (mL/min/1.73 m)	104.2 ± 14.9	112.1 ± 11.8	0.002
Male	104.0 ± 14.1	113.1 ± 8.5	0.002
Female	104.3 ± 15.6	111.7 ± 13.0	0.022
Hand grip strength (kg)	29.9 ± 9.6	26.3 ± 7.7	0.005
Male	41.8 ± 8.4	35.8 ± 4.8	0.009
Female	24.9 ± 4.1	22.3 ± 4.6	0.047

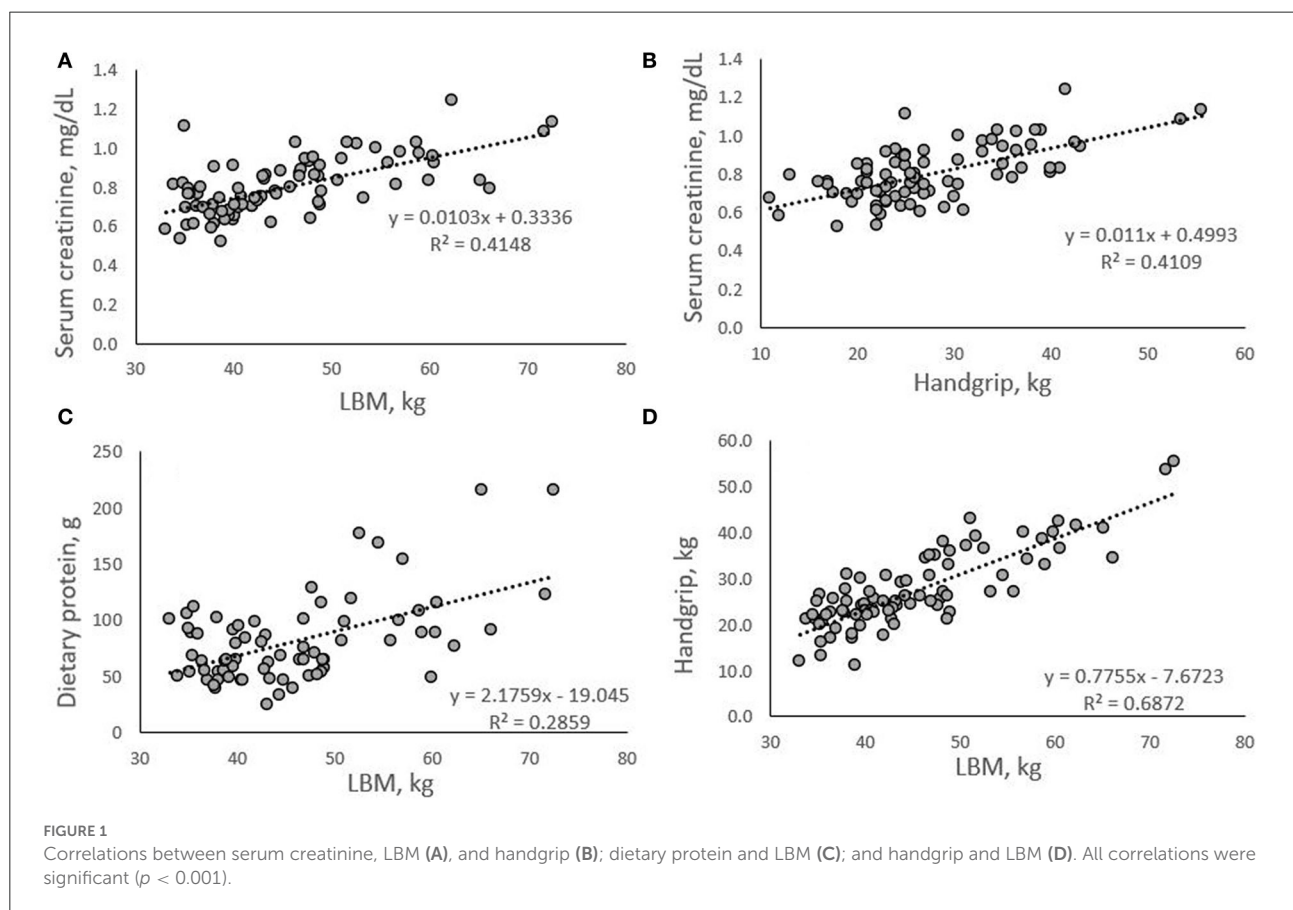
^aData are mean ± SD; p value for univariate analysis (gender controlled in total sample analysis; age and physical activity controlled in all analyses); *indicates chi square analysis. Serum creatinine reference ranges: adult men, 0.7 to 1.2 mg/dL; adult women, 0.5 to 1.0 mg/dL. GFR (glomerular filtration rate) reference range: >90 mL/min.

attention is directed at creatinine concentrations in the low-normal range. Low serum creatinine concentrations can indicate acute illness, severe liver disease, and the loss of muscle mass, such as in malnutrition, muscular dystrophy, or sarcopenia in older adults (45–49). Also, serum creatinine is linked to hydration status, and concentrations are lower in dehydrated states (50, 51).

In the present report, participants were young, healthy adults, screened for chronic disease, underweight and medical conditions. Thus, this report suggests that low-normal serum creatinine concentrations were linked to low LBM, reduced strength, and vegetarian diet adherence in healthy, young adults. Participants were likely well-hydrated when tested since less than 5% of the adults had serum sodium concentrations >146

TABLE 3 Multiple regression results predicting lean body mass and handgrip strength.

	Lean body mass					Handgrip strength				
	<i>B</i>	SE	Beta	<i>t</i>	<i>p</i>	<i>B</i>	SE	Beta	<i>t</i>	<i>p</i>
Gender	−12.423	1.975	−0.623	−6.290	0.000	−8.809	1.999	−0.465	−4.407	0.000
Age	0.067	0.078	0.062	0.855	0.396	−0.033	0.064	−0.032	−0.507	0.614
Diet group	−1.543	1.526	−0.079	−1.011	0.316	−2.686	1.247	−0.145	−2.154	0.035
Dietary Protein	0.034	0.021	0.138	1.615	0.111	−0.014	0.018	−0.062	−0.806	0.423
LBM						0.448	0.101	0.472	4.446	0.000
Serum creatinine	11.900	5.875	0.187	2.026	0.047					
Constant	54.766	9.862				28.655	8.634			
<i>R</i> ²	0.712					0.775				
Adjusted <i>R</i> ²	0.690					0.758				
	<i>F</i> _(5,65) = 32.094***					<i>F</i> _(5,65) = 44.816***				

****p* < 0.001.

mEq/L. The vegetarian men and women averaged creatinine concentrations below the mid-point of the reference ranges of 0.7–1.2 mg/dL for men and 0.5–1.0 mg/dL for women (52). In the men, serum creatinine was below 0.95 mg/dL in 81% of vegetarians vs. 13% of omnivores ($p = 0.002$). Although not statistically significant, serum creatinine was below 0.75 mg/dL

in 67% of vegetarian women vs. 42% of omnivorous women ($p = 0.094$).

Previous research indicates that creatinine can be used reliably to estimate muscle mass (30, 53) and data from the present analysis support this contention (Figure 1A). Additionally, we show a positive relationship with large effect

size between serum creatinine and handgrip strength, with serum creatinine explaining 41% of variance in handgrip strength ($R^2 = 0.4109$; Figure 1B). It is known that dietary protein, and specifically the amino acid leucine, leads to the upregulation and activation of anabolic signaling systems responsible for muscle protein synthesis (18, 19). We have previously shown that in vegetarians and vegans who averaged less than the protein recommended dietary allowance (RDA) value of 0.8 g/kg/d, an increase in protein intake by 18 g per day significantly increased muscular strength in the absence of a training program (54). This novel finding suggested that increasing protein intake above the RDA when intake is low has beneficial effects on strength levels in vegetarians. It is well documented that inadequate protein intakes are associated with muscle protein breakdown, leading to catabolism and functional decline (55).

Some factors presented limitations in this study. Data were analyzed from a cross-sectional trial; therefore, causation cannot be determined. Additionally, this was a secondary data analysis, thus the study and data collection procedures were not initially designed for this specific outcome variable. There are many confounding variables that need to be considered as discussed, and although many of these variables were measured and/or controlled in this investigation, others were not. Gender significantly impacted the outcome variables and must be carefully considered when interpreting these data. Furthermore, flexitarians (e.g., meat-avoiders) were excluded from the study; hence, it is not known how occasional meat consumption may impact the outcome measures. This is an important consideration for a future trial. The renal function of participants was estimated using the calculated GFR; however, the gold standard measurement of renal function involves the injection of a tracer and its clearance by the kidneys (56). A single 24-h recall was used to estimate protein intakes. Research suggests that two to three 24-h records are needed for reasonable accuracy in estimating dietary protein (57). Future studies might include randomized controlled trials examining the impact of dietary protein on the outcome measures herein while addressing the limitations encountered in this report.

Conclusion

This study shows that both men and women who follow an omnivore diet have significantly greater dietary protein intake, serum creatinine levels, and grip strength compared to vegetarian men and women. Additionally, the data show that creatinine is positively correlated to LBM and handgrip strength, and that dietary protein is positively correlated to LBM. These

data offer the possibility that in healthy adults, serum creatinine may be a complementary indicator of muscle mass and strength and can be utilized by practitioners and coaches, particularly for advising vegetarian clients. However, more work is warranted regarding the relevance of serum creatinine concentrations in the low-normal range in terms of LBM and functional strength, and it is important to consider gender, altered kidney function and physical activity in these assessments.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board at Arizona State University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EB and CJ designed the secondary research, analyzed the data, wrote the manuscript, and had primary responsibility for the final content. JK and CJ designed the original research. EB conducted the secondary research and drafted the first version of the manuscript. All authors read and approved the final manuscript and provided critical comments on the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Socioeconomic determinants of nutritional behaviors of households in Fars Province, Iran, 2018

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Introduction: Households' dietary habits are affected by their environment and socioeconomic status (SES). This study aims to investigate eating behaviors and determine the factors affecting nutritional status in households in Fars Province in 2018.

Method: In this cross-sectional study, urban and rural households were selected using the multistage sampling method. A questionnaire was employed to interview the mother or householder to record the demographic, SES, and dietary habits of the family for major food items commonly used. A logistic regression model was used to analyze the data. The *p*-value less than 0.05 was considered significant.

Results: In total, 6,429 households participated in the study. The majority of households use traditional flatbread, low-fat milk, and liquid/cooking oil. Frying was the most prevalent method of cooking. Parents' level of education and SES were associated with type of consumed bread, milk and dairy, methods of food preparation, adding salt at the table, eating out, and fast-food usage. Parents' higher level of education was significantly associated with salt storage in optimal conditions and not using salt before tasting the meal.

Conclusion: Most of the households had healthy practices, especially in consumption of certain oils and methods of preparing their food as well as keeping salt in an optimal condition and using iodized salt. The most important unhealthy nutritional behavior was high consumption of fast food and outdoor food, especially in urban regions. Unhealthy dietary habits were more prevalent in households with low household and regional SES. Both households and regions with higher SES had better dietary habits.

KEYWORDS

nutrition behaviors, socio-economic status, level of education, Iran, households

Introduction

Recently, changes in lifestyle have raised the prevalence of chronic diseases such as cardiovascular disease, hypertension, diabetes, and cancers (1). The most important lifestyle determinants of non-communicable diseases (NCDs) are reduced physical activity and poor eating habits (2).

A healthy dietary habit includes various approaches to the consumption of different food groups, food items, and the preparation of daily meals. Several recommendations are available in order to stick to a healthy dietary habit and, consequently, to prevent NCDs, such as choosing vegetable oils over animal-based oils, consuming fruits and vegetables on a daily basis, and using low-fat dairy products (3). Moreover, the food preparation method is also a major factor affecting health (4). According to the nutritional transitions, fast-food consumption has been increased alarmingly, with its health consequences emerging (3).

In addition, the dietary habits of individuals and households are affected by their environment and socioeconomic status (SES). The association between SES and poorer health has been recognized, and inequalities in nutrition have been associated with inequalities in health. Higher SES environment, education, and income are linked to changing dietary habits, but not always in a desirable way (3). Populations in low SES probably are at a higher risk of unhealthy conditions due to the lack of access to healthcare and poor nutrient intake. Thus, given public health policies, the assessment of eating habits in each population is essential for preventing diseases and nutrient deficiencies (5).

A few studies have been conducted in Iran to comprehensively assess nutritional behavior and its possible determinants for different geographical areas in rural and urban populations. Therefore, we aimed to assess the nutritional behavior of households in rural and urban populations of Fars Province, Iran.

Materials and methods

A cross-sectional study was designed to investigate the nutritional behavior of urban and rural populations of Fars Province, Iran. The study protocol was approved by the Shiraz University of Medical Sciences (SUMS), Fars, Iran, under the registration code: IR.SUMS.REC.13940598.

Sampling

Households residing in the urban and rural areas of Fars Province, covered by the Health Department of SUMS, were included in the study.

Fars Province, a populated province, is located in the southwest area of Iran. SUMS is one of the major universities of

medical sciences in Iran, which is placed in Shiraz, the capital of Fars. SUMS Health Department covers 29 out of 36 towns in Fars Province (Figure 1). In this study, a multistage sampling method was used. In the first phase, all 29 towns were considered 29 stages (28 affiliated towns and Shiraz as the center). In the second phase, the towns were divided into two urban and rural communities. In addition, Shiraz was divided into 7 areas, including 5 urban communities (3 districts of Shiraz—northern, central, and southern, and 2 northern and southern suburbs of Shiraz) and 2 rural communities. Subsequently, by means of the maps, urban and rural areas were separated into three clusters of north, center, and south with a maximum spatial accuracy. Finally, we obtained 168 clusters from affiliated towns and 21 clusters from Shiraz. Then, 34 households in each cluster were included using a systematic random sampling method. In this stage, the first household was selected by random selection and sampling was continued from the right side of the house until reaching the predetermined sample size. Thus, a total of 6,426 households were included in this study. The mother of each household was considered the representative in order to respond to the interviewer, or, in the absence of the mother, the householder (the person in the household who is responsible for making decisions and earning money) was interviewed. Figure 1 depicts the sampling procedure of the study.

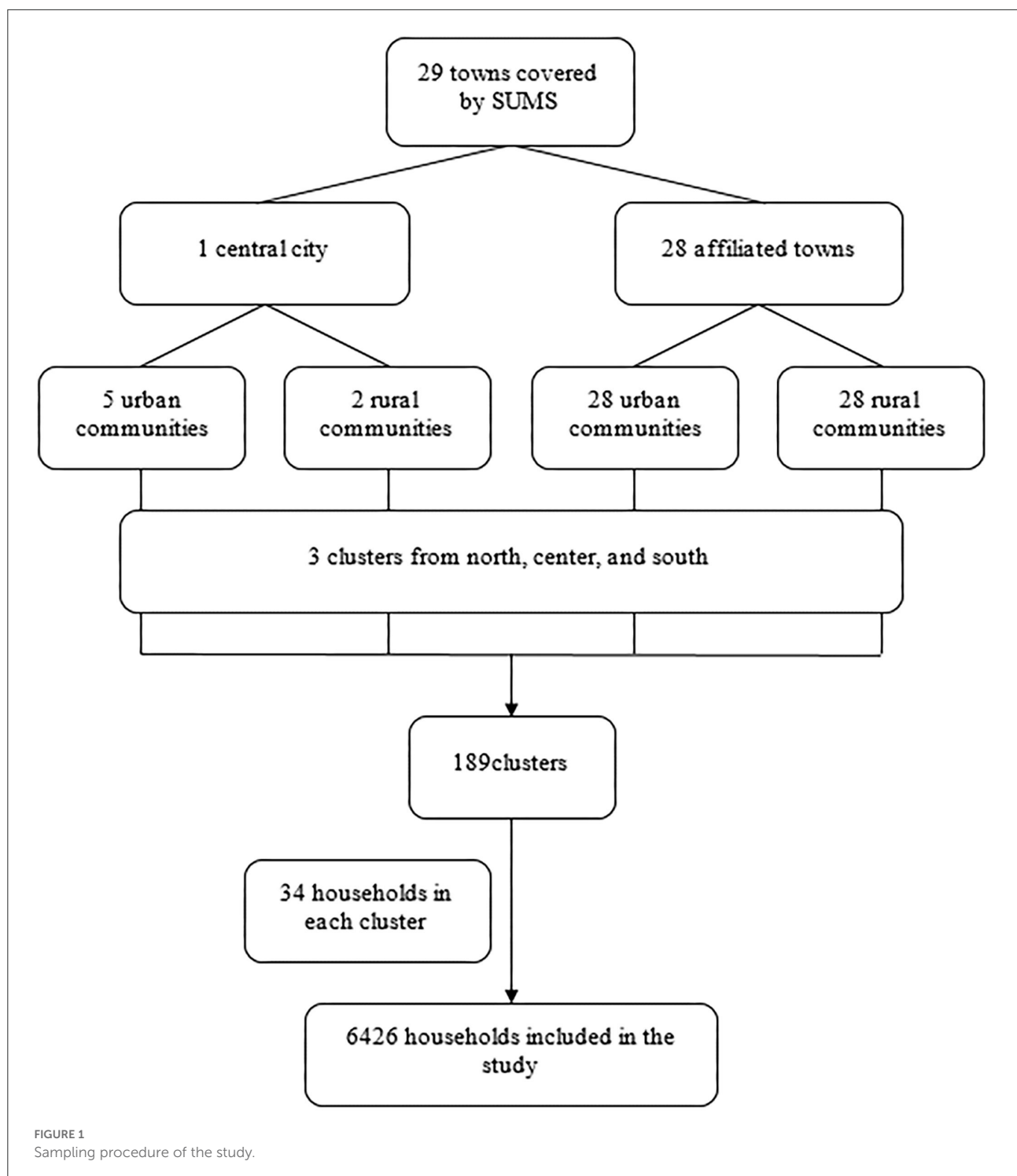
Household assessment

A questionnaire was compiled to assess general and sociodemographic characteristics of the households. The questionnaire asked about the persons who were present in the household for the last 7 days continuously, head of the household, and his/her spouse's level of education (illiterate, elementary level, high school, diploma, graduate, and postgraduate), and job status (unemployed, farmer, full-time worker, daily worker, employed, self-employed, and retired for father and housewife, employed, and self-employed for mother).

The sociodemographic status of the household was calculated by considering the possession of 9 specific items, including home, personal vehicle, washing machine, LCD TV, dishwasher, refrigerator, handmade rug, laptop, and microwave. Based on the number of items possessed by households, the SES was categorized into three groups, namely, low (3 items or less), moderate (4–6 items), and high (more than 7 items) (6).

Dietary assessment

A nutritional behavior questionnaire was used to investigate the overall food consumption habits of households (1, 7). Interviewers asked about types of consumed milk and dairy (low fat vs. high fat), bread (traditional flatbreads or homemade breads), and oil (different types of vegetable oils or animal



oils), in addition to their consumed amount. The frequency of the consumption of vegetables and salad (weekly) and salt consumption habits were assessed by the questionnaire. Interviewers asked about iodized salt consumption, and in case of a positive response, its storage method within the household was investigated to indicate whether the storage is optimal or

undesirable. In addition, the habit toward salt was recorded based on using salt at the table and adding salt to the meal before tasting it. Moreover, using dietary habit questionnaires, data were gathered about food preparation methods, including boiling, grilling, steaming, roasting, frying, or, other traditional methods, and consuming fast-food and packaged food.

Statistical analysis

To describe the quantitative and qualitative characteristics (ordinal and nominal variables) of the households, descriptive statistical procedures including mean \pm standard deviation (SD), frequency, and percentage were performed, respectively. To determine the contribution of food habits or consumption and place of residence, the chi-square test was used. In addition, the logistic regression model was used to estimate the association between sociodemographic characteristics and food habits or consumption. All statistical analyses were performed using the SPSS software version 22 (IBM, USA). A *p*-value of less than 0.05 was considered significant.

Results

Household general characteristics

A total of 6,429 households were included in the study, of which 5,826 (91.4%) were headed by a father. The mean household size was 3.91 ± 1.38 , which was 4.01 ± 1.48 in rural areas and 3.80 ± 1.27 in urban areas. The characteristics of households, householders, and spouse's level of education and job based on place of residence are reported in Table 1.

Nutritional behavior and consumption

The majority of households (65.7%) used traditional flatbreads, more than half of the households (53.9%) consumed low-fat milk and dairy products, and liquid/cooking oil was the most consumed oil within households (65.9%). Frying was the most prevalent method of cooking (54.2%). In addition, the iodized salt was used in 97.2% of households in comparison with non-iodized salt (2.8%), and storage of salt was in optimal condition in 72.0% of households for total population. Table 2 represents household nutritional behavior in total population based on the place of residence.

As shown in Table 2, there was a significant difference between rural and urban areas in terms of food habits, such as consumed bread, milk and dairy, and oil type ($p < 0.001$). Food preparation methods, eating out, and fast-food consumption were significantly different between urban and rural communities ($p < 0.001$).

Factors associated with nutritional behavior and consumption

Higher levels of education of the head of the household and his/her spouse as well as higher SES were significantly associated with reduced consumption of home-made bread, consumption

of high-fat dairy products, and frying food. In addition, the larger family size was associated with increased consumption of high-fat dairy products and frying food ($p < 0.05$). According to our results, a higher level of education was significantly associated with household iodized salt intake (odds ratio [OR] = 1.23, 95% CI: 1.08, 1.39). Moreover, a higher level of education of the head of the household and his/her spouse was significantly associated with salt storage in optimal conditions as well as not using a salt shaker at the table and not using salt before tasting food. Larger family size was associated with salt storage in undesirable conditions (OR = 0.95, 95% CI: 0.91, 0.98), using a salt shaker at the table (OR = 1.09, 95% CI: 1.05, 1.14), and using salt before tasting food (OR = 1.13, 95% CI: 1.08, 1.18). Higher SES was associated with not using a salt shaker at the table (OR = 0.89, 95% CI: 0.82, 0.98). Moreover, higher levels of education of the head of the household and his/her spouse as well as higher SES were significantly associated with increased consumption of outdoor food and fast foods. In addition, a larger family size was associated with increased consumption of fast foods (OR = 1.10, 95% CI: 1.06, 1.15). Table 3 represents the association between dietary habits and related factors.

The association between sociodemographic characteristics of households and food habits in urban and rural areas is reported in Tables 4, 5, respectively. According to the results, in rural areas, a higher level of education was associated with household iodized salt intake, but there was no association between education and iodized salt intake in urban areas. Larger family size was associated with increased consumption of home-made bread in urban areas, but there was no association between size of family and consumption of home-made bread in rural areas.

Discussion

In this study, liquid/cooking oils were the most consumed oil type among households (65.9%). In addition, olive and canola oils were consumed by 11.5 and 0.87% of households, respectively. However, consumption of liquid and vegetable oils in Sanandaj Province, Iran, was 26.75% (8). These vegetable oils have shown beneficial properties toward cardiovascular diseases due to their fatty acid composition. Consuming more than 2.5 times mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) present in vegetable oils (9) than saturated fatty acids (SFAs) is considered a healthy behavior in the Healthy Eating Index 2015 (10).

In contrast to liquid and vegetable oils, animal meat and high-fat dairy products contain high amounts of SFA. SFA consumption is responsible for various chronic conditions, including cardio heart disease (CHD) (11); thus, it is recommended by the Dietary Guideline for Americans (DGA) to use <10% of daily calorie intake from SFA and replace it with PUFA (11).

TABLE 1 Socio-demographic characteristics of households by place of residence.

		Total (n = 6,429)	Urban areas (n = 3,261)	Rural areas (n = 3,168)
Household size (mean ± SD)		3.91 ± 1.38	3.80 ± 1.27	4.01 ± 1.48
Householder	Father	5,826 (91.4)	2,975 (91.8)	2,826 (91.0)
	Mother	501 (7.9)	246 (7.6)	255 (8.0)
	Other	45 (0.7)	19 (0.6)	26 (0.8)
Mother's level of education	Illiterate	1,214 (19.0)	428 (13.2)	786 (24.9)
	Elementary	2,083 (32.6)	794 (24.5)	1,289 (40.9)
	High school	1,241 (19.4)	660 (20.4)	581 (18.4)
	Diploma	1,156 (18.1)	782 (24.1)	374 (11.9)
	Graduate	655 (10.2)	532 (16.4)	123 (3.9)
	Post graduate	44 (0.7)	43 (1.3)	1 (0.0)
Father's level of education	Illiterate	772 (12.8)	269 (8.8)	503 (16.9)
	Elementary	1,665 (27.5)	594 (19.3)	1,071 (35.9)
	High school	1,589 (26.3)	714 (23.3)	875 (29.4)
	Diploma	1,197 (19.8)	799 (26.0)	398 (13.4)
	Graduate	700 (11.6)	583 (19.0)	117 (3.9)
	Post graduate	127 (2.1)	111 (3.6)	16 (0.5)
Father's job	Unemployed	400 (6.6)	143 (4.7)	257 (8.7)
	Farmer	1,238 (21.3)	192 (6.3)	1,091 (36.8)
	Fulltime worker	348 (5.8)	165 (5.4)	183 (6.2)
	Daily worker	655 (10.9)	226 (7.4)	429 (14.5)
	Employee	688 (11.4)	555 (18.1)	133 (4.5)
	Self-employed	2,094 (34.7)	1,305 (42.6)	789 (26.6)
	Retired	565 (9.4)	479 (15.6)	86 (2.9)
Mother's job	Housewife	5,920 (92.8)	2,897 (89.7)	3,023 (95.9)
	Self-employed	158 (2.5)	99 (3.1)	59 (1.9)
	Employee	303 (4.7)	233 (7.1)	70 (2.2)
Socio-economic status	Low	2,569 (40.1)	1,011 (31.1)	1,558 (49.3)
	Middle	3,513 (54.8)	1,965 (60.4)	1,548 (49.0)
	High	328 (5.1)	275 (8.5)	53 (1.7)

Qualitative variables (ordinal and nominal variables) are reported as N (%) and quantitative variables as mean ± SD.

Dietary guidelines suggest reducing the consumption of high-fat dairy products (12). In the present survey, almost nearly half of the households (53.9%) consumed low-fat milk and dairy. Although low-fat milk and dairy products were significantly higher, it was observed that a significant difference could be due to the large sample size. As the American meta-analysis reported, high-quality evidence supports favorable associations (i.e., decreased risk) between total dairy intake and hypertension risk and between low-fat dairy and yogurt intake and reduced risk of type 2 diabetes (T2D) and the consequent massive burden of health economics in this area (13). Thus, proper methods of awareness are required to amend dairy product consumption patterns in this area.

In this study, the most common methods of cooking were fried cooking (54.2%). Deep frying with oil negatively changes the fatty acid composition of oil. Frying increases energy density and decreases the water content of the meal. In a case-control

study in India, patients with coronary heart disease, when compared with the control group, reported a greater intake of shallow fried food. Data from a case-control study in China showed that the frequency of fried food intake was significantly higher in patients with acute myocardial infarction. Data from the Nurses' Health Study and the Health Professional Follow-Up Study showed that frequent fried food consumption was significantly associated with a higher risk of coronary artery disease (CAD) (14). Thus, health and nutrition education as well as the improvement of healthy snacks and physical activity, especially at schools and kindergarten can change nutritional behaviors in Iranian households.

Iodine plays an essential role in the functions of thyroid hormones. The prevention of iodine deficiency disorders (IDDs) is one of the most important health programs in Iran. Based on our results, 97.2% of households used iodized salt. The World Health Organization (WHO) and International Council

TABLE 2 Household Food habits in total population as well as place of residence.

		Total (<i>n</i> = 6,429)	Urban areas (<i>n</i> = 3,261)	Rural areas (<i>n</i> = 3,168)	<i>p</i> -value
Consumed bread	Traditional flat breads	44,224 (65.7)	2,790 (85.6)	1,434 (45.3)	0.001*
	Homemade bread	2,205 (34.3)	471 (14.4)	1,734 (54.7)	
Milk and dairy	Low fat	3,454 (53.9)	2,066 (63.4)	1,388 (44.0)	0.001*
	High fat	2,960 (46.1)	1,191 (36.6)	1,769 (56.0)	
Consumption of salad, lettuce and vegetable (weakly)	Never	223 (3.5)	96 (2.9)	127 (4.0)	0.001*
	1–2 times	1,749 (27.3)	792 (24.3)	957 (30.3)	
	3–4 times	1,884 (29.4)	964 (29.6)	920 (29.1)	
	5–6 times	575 (9.0)	310 (9.5)	26 (8.4)	
	Daily	1,983 (30.9)	1,095 (33.6)	888 (28.1)	
Oil type	Liquid/cooking oil	4,234 (65.9)	2,273 (69.7)	1,961 (61.9)	0.001*
	Frying oil	3,818 (59.4)	2,075 (63.3)	1,743 (55.0)	
	Solid/semi-solid vegetable oil	3,241 (50.4)	1,283 (39.3)	1,958 (61.8)	
	Animal oil/animal butter	763 (11.9)	294 (0.9)	469 (14.8)	
	Olive oil	738 (11.5)	565 (17.3)	173 (5.5)	
	Canola oil	56 (0.87)	45 (1.4)	11 (0.3)	
	Other	5 (0.1)	2 (0.1)	3 (0.1)	
Methods of food preparation	Boiled	968 (15.1)	564 (17.3)	404 (12.8)	0.001*
	Grilled	84 (1.3)	46 (1.4)	38 (1.2)	
	Steamer / oven / microwave	123 (1.9)	86 (2.6)	37 (1.2)	
	Roasted	1,762 (27.4)	994 (30.5)	768 (24.3)	
	Fried	3,477 (54.2)	1,564 (48.0)	1,913 (60.5)	
	Other	5 (0.1)	2 (0.1)	3 (0.1)	
	Other	5 (0.1)	2 (0.1)	3 (0.1)	
Iodized salt	No	182 (2.8)	96 (2.9)	86 (2.7)	0.580
	Yes	6,247 (97.2)	3,165 (97.1)	3,082 (97.3)	
Salt storage method	Optimal	4,623 (72.0)	2,350 (72.2)	2,273 (71.8)	0.357
	Undesirable	1,795 (28.0)	904 (27.8)	891 (28.2)	
Adding salt at the table	No	4,281 (66.7)	2,194 (67.5)	2,087 (66.0)	0.207
	Yes	2,134 (33.3)	1,058 (32.5)	1,076 (34.0)	
Add salt before tasting food	No	5,472 (85.6)	2,763 (85.3)	2,709 (86.0)	0.464
	Yes	917 (14.4)	475 (14.7)	442 (14.0)	
Eating out	Yes	2,418 (37.6)	1,540 (47.2)	878 (27.7)	0.001*
	No	4,011 (62.4)	1,721 (52.8)	2,290 (72.3)	
Fast food usage frequency	Never	4,327 (67.5)	1,913 (58.5)	2,414 (76.4)	0.001*
	Weekly	258 (4.0)	197 (6.1)	61 (1.9)	
	Monthly	1,169 (18.2)	784 (24.1)	385 (12.2)	
	Annually	658 (10.3)	358 (11.0)	300 (9.5)	
Use of industrial foods Packaged food	Never	4,714 (73.5)	2,324 (71.5)	2,390 (75.6)	0.200
	Weekly	270 (4.2)	153 (4.7)	117 (3.7)	
	Monthly	969 (15.1)	529 (16.3)	440 (13.9)	
	Annually	460 (7.2)	246 (7.6)	214 (6.8)	

Data reported as n (%).

* Significant at 0.05 level.

for Iodine Deficiency Disorders (ICCIDD) standards state that the elimination of IDD will be possible if more than 90% of households consume adequately iodized salt (15). The

Iodized Salt Coverage Study 2010 shows that the availability of adequately iodized salt in the households in Orissa has almost doubled from 32.4%, conducted by the National Food

TABLE 3 Factors associated with food habits in total population.

		Family size			Mother's education level			Father's education level			Socio-economic status		
		OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value
Consumed bread	Traditional flat breads	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Home-made	1.02	0.98–1.06	0.222	0.68	0.65–0.71	0.001*	0.68	0.65–0.71	0.001*	0.68	0.62–0.75	0.001*
Milk and dairy	Low fat	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	High fat	1.13	1.09–1.18	0.001*	0.86	0.83–0.90	0.001*	0.84	0.81–0.87	0.001*	0.79	0.72–0.68	0.001*
Methods of food preparation	Not fried	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Fried	1.02	1.15–1.24	0.001*	0.90	0.87–0.94	0.001*	0.91	0.88–0.95	0.001*	0.74	0.68–0.80	0.001*
Iodized salt	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	0.98	0.88–1.09	0.798	1.15	0.98–1.24	0.098	1.23	1.08–1.39	0.001*	1.07	0.83–1.39	0.592
Salt storage method	Undesirable	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Optimal	0.95	0.91–0.98	0.011*	1.08	1.03–1.13	0.001*	1.06	1.02–1.16	0.004*	1.04	0.94–1.14	0.432
Adding salt at the table	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.09	1.05–1.14	0.001*	0.87	0.84–0.91	0.001*	0.86	0.83–0.91	0.001*	0.89	0.82–0.98	0.017*
Add salt before tasting food	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.13	1.08–1.18	0.001*	0.94	0.89–0.99	0.034*	0.94	0.86–0.96	0.002*	1.07	0.95–1.21	0.250
Eating out	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.03	0.99–1.07	0.084	1.53	1.47–1.60	0.001*	1.43	1.38–1.50	0.001*	1.93	1.76–2.12	0.001*
Fast food usage	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.10	1.06–1.15	0.001*	1.41	1.35–1.48	0.001*	1.34	1.28–1.41	0.001*	1.72	1.55–1.90	0.001*

* Significant at 0.05 level; CI: confidence interval.

TABLE 4 Factors associated with food habits in urban population.

		Family size			Mother's education level			Father's education level			Socio-economic status		
		OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value
Consumed bread	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	
	Traditional flat breads	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Home-made	0.88	0.82–0.96	0.003*	0.81	0.76–0.88	0.001*	0.83	0.77–0.89	0.001*	1.07	0.91–1.23	0.398
Milk and dairy	Low fat	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	High fat	1.18	1.11–1.25	0.001*	0.91	0.86–0.96	0.001*	0.91	0.86–0.96	0.001*	0.88	0.78–0.99	0.048*
Methods of food preparation	Not fried	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Fried	1.25	1.18–1.32	0.001*	0.92	0.87–0.96	0.001*	0.94	0.89–0.99	0.030*	0.80	0.71–0.90	0.001*
Iodized salt	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.03	0.88–1.22	0.673	1.02	0.87–1.18	0.843	1.14	0.97–1.35	0.100	0.93	0.65–1.32	0.684
Salt storage method	Undesirable	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Optimal	0.94	0.88–0.99	0.043*	1.07	1.01–1.14	0.014*	1.07	1.01–1.14	1.015*	1.06	0.93–1.21	0.365
Adding salt at the table	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.15	1.08–1.21	0.001*	0.84	0.80–0.89	0.001*	0.84	0.79–0.89	0.001*	0.82	0.72–0.92	0.002*
Add salt before tasting food	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.21	1.13–1.31	0.001*	0.91	0.85–0.98	0.020*	0.87	0.81–0.94	0.001*	1.02	0.86–1.19	0.849
Eating out	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.01	0.95–1.04	0.759	1.46	1.38–1.54	0.001*	1.34	1.27–1.42	0.001*	1.67	1.48–1.89	0.001*
Fast food usage	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.10	1.04–1.17	0.001*	1.31	1.23–1.39	0.001*	1.23	1.16–1.30	0.001*	1.49	1.31–1.69	0.001*

* Significant at 0.05 level; *CI*, confidence interval.

TABLE 5 Factors associated with food habits in rural population.

		Family size			Mother's education level			Father's education level			Socio-economic status		
		OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value
Consumed bread	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	
	Traditional flat breads	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Home-made	1.01	0.95–1.05	0.916	0.83	0.77–0.88	0.001*	0.82	0.77–0.88	0.001*	0.88	0.72–1.01	0.058
Milk and dairy	Low fat	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	High fat	1.07	1.02–1.12	0.005*	1.00	0.94–1.06	0.998	0.94	0.87–1.02	0.057	0.94	0.82–1.07	0.367
Methods of food preparation	Not fried	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Fried	1.14	1.09–1.20	0.001*	1.02	0.96–1.09	0.487	1.04	0.97–1.11	0.283	0.80	0.70–0.91	0.001*
Iodized salt	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	0.94	0.82–1.08	0.427	1.39	1.11–1.75	0.004*	1.56	1.23–1.98	0.001*	1.35	0.89–2.05	0.150
Salt storage method	Undesirable	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Optimal	0.95	0.91–1.01	0.113	1.12	1.03–1.19	0.006*	1.06	0.99–1.15	0.083	1.01	0.87–1.17	0.916
Adding salt at the table	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.05	1.01–1.11	0.034*	0.90	0.84–0.96	0.003*	0.89	0.84–0.96	0.003*	1.02	0.88–1.17	0.824
Add salt before tasting food	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.07	1.01–1.15	0.033*	0.95	0.86–1.04	0.264	0.93	0.85–1.02	0.146	1.13	0.93–1.36	0.208
Eating out	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.11	1.05–1.17	0.001*	1.41	1.31–1.51	0.001*	1.31	1.22–1.41	0.001*	1.76	1.51–2.04	0.001*
Fast food usage	No	Ref.	–	–	Ref.	–	–	Ref.	–	–	Ref.	–	–
	Yes	1.20	1.13–1.28	0.001*	1.26	1.15–1.37	0.001*	1.19	1.08–1.31	0.001*	1.44	1.19–1.73	0.001*

* Significant at 0.05 level; *CI* confidence interval.

and Health Survey (NFHS) 3 in 2005–2006 to 59% in 2010. Consumption of iodized salt has increased, but it is still way behind the universal salt iodization (USI) target of 90% of households consuming adequately iodized salt (16, 17). In a study by Srivastava et al., two-thirds (65.2%) of the households were adequately consuming iodized salt, while about one-fifth (21.5%) of the households were consuming iodized salt inadequately (18).

The association between nutritional behavior and level of education

In this study, a higher level of education was significantly associated with reduced consumption of fried food and high-fat dairy products, although, in such families, traditional flatbreads and fast food were consumed more.

In addition, a large-scale meta-analysis investigated 15 European countries and determined that the parents' higher level of education was associated with healthy diet behaviors. A similar result was reported in a Danish survey too (19). The analysis of demographic and socioeconomic data determined that education is usually the strongest component of socioeconomic differences (20). In contrast, Aslam et al. reported that consumption of all groups, healthy and unhealthy foods such as sugar and fast foods, was highly associated with the level of education (21).

In this study, a higher level of education of the head of the household and his/her spouse was significantly associated with iodized salt storage in optimal conditions as well as not using a salt shaker and not using salt before tasting food. Valexi et al. showed that women and men with a high level of education knew how to store iodized salt (22). However, Azizi et al. showed that the level of education was not significantly associated with the storage of salt (23). Higher levels of education may also improve the ability to believe or understand health-related information, in general, or dietary practice, in particular, needed to develop health-promoting skills and beliefs in the field of food habits. It is believed that with a higher level of education, there is more knowledge about healthy food items (20, 24).

The association between nutritional behavior with socioeconomic status and family size

In this study, higher SES was significantly associated with reduced consumption of fried food and high-fat dairy products but fast food/outdoor food was increased. A similar result was found in the Kelishadi's study, suggesting that families with higher SES had healthier dietary practices (2). Esghinia et al. (23) and Rezazadeh et al. (25) found a rising trend toward healthy and nutritive behaviors with the increase in SES.

According to previous studies, SES is one of the most important determinants of diet quality in children and adolescents. This can be due to higher nutritional knowledge in high SES regions and limited access and affordability for some fast foods in low SES regions (26).

In this study, SES was not significantly associated with iodized salt intake. However, a study in Tehran showed that the SES influenced iodized salt consumption (27). In studies conducted by Kouhi et al. (28), Sharifirad et al. (29), and Yarmohammadi et al. (30) on students, there was a direct relationship between economic status and fast-food consumption (31). It can be concluded that good income acts as a double-edged sword. Although a favorable economic status can cause the consumption of healthy snacks and healthy nutrition, it can increase their purchasing power for junk foods.

Based on our findings, an unhealthier dietary pattern may exist among the households who are in lower socioeconomic level in Iran. The relationship between SES and nutritional performance has been studied (32). Many studies have determined that eating patterns of people in low SES groups threaten public health (33–37). People in the low SES group, due to a lack of accessibility to healthcare, improper living conditions, less education, and greater psychological stress, may be at a greater risk of poorer health status than others (38). Thus, appropriate policies, interventions, and efforts aimed at improving nutrition-related health, especially in high-risk groups, are necessary.

In this study, daily consumption of vegetables was close to 30%. A healthier dietary pattern is believed to include a higher consumption of fruits and vegetables and a lower consumption of fat and meat. Thus, people with a higher socioeconomic level tend to show a higher consumption of vegetables, fruits, and fiber products, and a lower consumption of meat, meat products, and fats in comparison with people with a lower socioeconomic level (20). Abdollahi et al. showed that with the increase in knowledge and occupational level of the heads of households, consumption of high-dense calories and lower healthy groups decreased. A large-scale meta-analysis from 15 European countries also showed that higher levels of literacy were associated with higher consumption of vegetables and fruits (1, 20, 39). One of the main principles of healthy diet is the daily intake of vegetables that are effective in preventing NCDs. Therefore, certain interventions are necessary to increase vegetable consumption per capita, including increasing access to this group of food and promoting vegetable consumption from childhood.

In this study, family size was associated with increasing consumption of fast food, high-fat dairy products, and fried food, while larger family size was significantly associated with iodized salt storage in undesirable condition as well as using a salt shaker and using salt before tasting food. It is obvious that family size can affect dietary behaviors in the household. When family size increases, the eating habits of a person in the family

can affect all members of the family and show itself as the eating behavior of the family. It is to be expected that family members have different eating habits, but unhealthy eating habits are usually more pronounced than other eating habits.

The association between food habits and place of residence

In this study, almost all eating practices were significantly different between rural and urban areas. According to the results, in rural areas, higher levels of knowledge of the head of the household and his/her spouse were associated with household iodized salt intake, but in urban areas, there was no association between knowledge of the head of the household and his/her spouse and iodized salt intake. A larger family size was associated with decreased consumption of home-made bread in urban areas, but there was no association between size of family and consumption of home-made bread in rural areas. Consumption of fast food was significant in both urban and rural areas in this study.

Kelishadi et al. did not report any significant differences between urban and rural areas for salt intake. This finding is probably because of the main sources of salt intake, i.e., bread, cheese, and many junk foods such as cheese puffs and potato chips, which are regularly consumed by all Iranian people and are not limited to urban areas or a kind of socioeconomic categories. On the contrary, a survey in Ethiopia and Sudan showed that individuals in urban areas were 9 times more likely to be aware of iodized salt consumption than those who lived in rural areas (40).

Participants in regions with higher SES had healthier nutritional behaviors, but some nutritional behaviors, such as consumption of fast food less often, were similar in areas with the lowest and highest SES. This can be due to higher health knowledge in high SES regions, and inaccessibility and limited affordability for some fast foods in low SES regions (2).

Despite the expectation that increased knowledge of the head of the household and his/her spouse may create a negative attitude toward eating unhealthy food by being more aware of the importance of healthy nutrition for families, the tendency toward consuming more unhealthy diets and fast food, especially among the adolescents, has increased in households in today's urban communities as a result of longer working hours, more busy educated parents in outdoor work environments, as well as time limitations.

Study strengths and limitations

One of the strengths of this study is that it was a comprehensive study with a large sample size, so that the

samples represent the whole community. In addition, this study examined all aspects of household dietary behaviors and considered all factors affecting eating habits. The main limitation of this study was that we did not assess the disease and anthropometric data of the households. Therefore, future studies are suggested to obtain more information, including disease and anthropometric data.

Conclusion

Most households follow healthy practices, especially types of oils consumed, methods of preparing their food as well as keeping salt in an optimal condition and consuming iodized salt. The most important unhealthy nutritional behavior was the high consumption of fast food and outdoor food, especially in urban regions. Unhealthy nutritional behaviors were more prevalent in households with low household and regional SES. It is suggested to consider community nutrition education and socioeconomic disparities should be considered for public health interventions aiming to improve food habits.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Shiraz University of Medical Sciences (SUMS), Fars, Iran, under the registration code: IR.SUMS.REC.13940598. The consent form was pre-designed and for all interviewed households, before the research questions, the consent form was completed and if the head of the household or the interviewee was satisfied, the study questionnaire was completed. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZF: analysis and interpretation of data and writing the original draft. MM: writing the original draft. MAM: research idea and study design. AH: questionnaire design, analysis interpretation of data, and review and editing. AF: collect and clear data and review editing. MA: research idea and study

design. RS: research idea and study design, patient enrolment, and writing the original draft. All authors have read the final version of the manuscript and approved it.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Soilless biofortification, bioaccessibility, and bioavailability: Signposts on the path to personalized nutrition

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Propelled by an ever-growing awareness about the importance of following dietary recommendations meeting specific biological requirements linked to a person health status, interest in personalized nutrition is on the rise. Soilless biofortification of vegetables has opened the door to the potential for adapting vegetable production to specific dietary requirements. The evolution of vegetables biofortification toward tailored food is examined focusing on some specific categories of people in a context of personalized nutrition instead to simple describe developments in vegetables biofortification with reference to the single element or compound not adequately present in the daily diet. The concepts of bioavailability and bioaccessibility as a useful support tool for the precision biofortification were detailed. Key prospects for challenges ahead aiming to combine product quality and sustainable are also highlighted. Hydroponically cultivation of vegetables with low potassium content may be effective to obtain tailored leafy and fruit vegetable products for people with impaired kidney function. Simultaneous biofortification of calcium, silicon, and boron in the same vegetable to obtain vegetable products useful for bone health deserve further attention. The right dosage of the lithium in the nutrient solution appears essential to obtain tailored vegetables able to positively influence mental health in groups of people susceptible to mental illness. Modulate nitrogen fertilization may reduce or enhance nitrate in vegetables to obtain tailored products, respectively, for children and athletes. Future research are needed to produce nickel-free vegetable products for individuals sensitized to nickel. The multidisciplinary approach toward tailored foods is a winning one and must increasingly include a synergy between agronomic, biological, and medical skills.

KEYWORDS

in vitro digestion model, bioavailability, bone health, impaired kidney function, mental illnesses, modulated nutrition

Introduction

According to some investigators (1), Personalized Nutrition (PN) “tailors dietary recommendations to specific biological requirements on the basis of a person’s health status and goals.” PN could be also described as a “field that leverages human individuality to drive nutrition strategies that prevent, manage and treat disease and optimize health” (2). At the same time, the term “personalization” can be interpreted both as “individualization” and “categorization,” since the two meanings can indeed coexist. In fact, although individuals can be considered unique, some can be regarded as similar enough to make up a category (3).

Generalizable nutrition recommendations can guide public policy independently of one-on-one nutrition counseling sessions. For example, the well-known Mediterranean Diet (MD) is a dietary model characterized by a high intake of vegetables and fruits leading to a reduction in blood pressure, insulin resistance, and inflammatory markers thus contributing to healthy lifestyles and practically eliminating inadequate ones (4, 5).

Not only the MD, but many other dietary patterns for human health and wellbeing have highlighted the positive role of large quantities of fresh fruits and vegetables in the daily diet (6). Nevertheless, indicating generally to eat standard portions of fruits and vegetables results inadequate to address human individuality (2). Therefore, it is important that studies in this field identify specific dietary patterns producing the most favorable impacts on health and wellbeing in similar groups of people. For example, allergen-free diets, such as nickel-free one can be tailored for specific group of people (7, 8). Adopting these types of diets can nevertheless entail limiting or eliminating multiple food products, resulting in a decrease in the quality of life. Therefore, a diet that includes vegetables with a lower nickel content than their common counterpart could be very useful for people affected by nickel hypersensitivity, since it would avoid a decrease in the quality of life.

At the same time people affected by some diseases or with a health status requiring higher quantities of some nutrients with respect to standard Dietary Reference Values (DRV) need to follow specific dietary guidelines. For example, the malabsorption of folic acid is a common complication of celiac disease (9). Thus, the average daily intake of folate in celiac patients is often lower with respect to that in the general population (10). Therefore, eating vegetables high in folate content could help to supplement the diet and improve the health of celiac patients. Biofortification is usually defined as the practice of deliberately increasing or decreasing the content of an essential micronutrient (i.e., vitamins and minerals) in plants (11, 12). Independently of definitions, the primary aim of biofortification is to improve the nutritional quality of fruits and vegetables for a healthier diet. Biofortification differs from conventional fortification since it is applied to crops

during their growth phase. Fortification, instead, refers to the practice of adding micronutrients to food products during the processing phase of food production (13). Biofortification is a process that can be applied to fresh, uncooked vegetables. Increasing research activities have been directed toward the biofortification of vegetables in the last few years (Figure 1). Moreover, some mineral-enriched vegetables are already present on the market (14, 15). But although biofortified vegetables are becoming more and more popular, there are only limited data regarding their potential role in personalized nutrition. In the light of these considerations, this review aims to provide up-to-date information regarding biofortified vegetables for specific categories of people whose health could be enhanced by personalized nutrition plans.

From vegetable biofortification to tailored food

Although biofortification strategies can include agronomic practices, conventional plant breeding and genetic engineering methods, the first is considered the most promising because it is the least expensive and requires only simple tools and techniques to modify the content of specific compounds in plants (16).

Agronomic practices can be considered the “starting point” of biofortification strategies in light of the fact as they were first used in the “Finland case.” In fact, since 1984 agricultural fertilizers in Finland have been supplemented with sodium selenate in an attempt to improve the nutritional quality of local foodstuffs known to be exceptionally low in selenium. This agronomic strategy affecting several crops and producing higher selenium concentrations in different food items has been proving effective since 1985, and, in fact, the selenium intake in the Finn population has increased significantly (17). We must nevertheless bear in mind that prolonged fertilization application using an enriched fertilizer may modify the soil chemical characteristics and may have a potentially negative environmental impact. It goes without saying that more agronomic practices for improving the soil’s health and the nutritional value of crops need to be identified.

When soilless cultivation systems are used, the soil is replaced by a substrate; plants are grown in liquid culture and are fed through a nutrient solution containing all needed elements (Figure 2). Soilless cultivation is considered an advanced, environmentally friendly agriculture practice for enhancing the quality of fresh vegetables (18).

In fact, although soilless cultivation systems have been developed primarily to address the challenge of excessive soil pathogens, it is nonetheless true that they also favor optimal control of plant growth, high productivity, and an efficient use of water and fertilizers (19). Furthermore, soilless systems represent an opportunity to modulate the nutrient

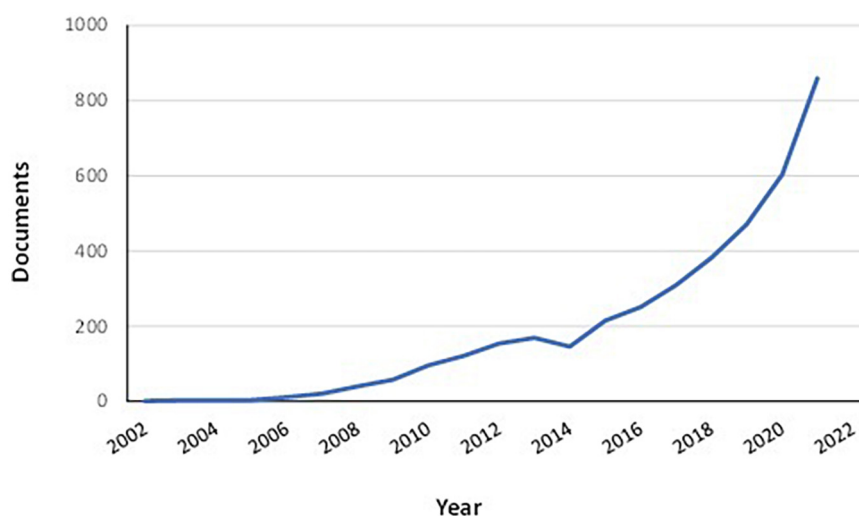


FIGURE 1

Documents regarding vegetable biofortification published from 2002 to 2021. Documents by type: article (69.3%); review (18.7%); book chapter (8.1%); conference paper (2.2%); book (1.0%); other (0.7%). Data retrieved from Scopus[®] database (on 15 February 2022) using “biofortification” AND “vegetables” as key search terms.

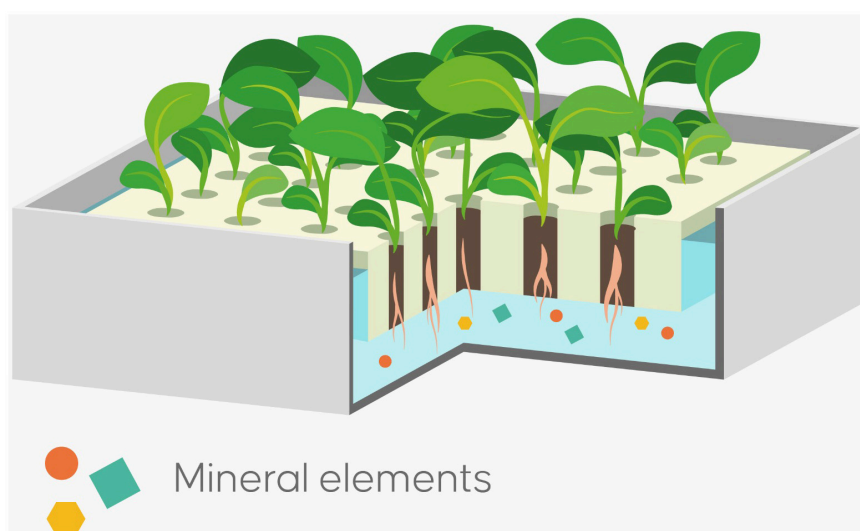


FIGURE 2

Floating hydroponic system; plants are grown in liquid culture and are fed through a nutrient solution with macro and micro-nutrient essential for plant growth.

solution precisely and efficaciously in the effort to improve both its organoleptic and bioactive quality traits (20). Soilless cultivation systems are being used for all of these reasons to monitor nutrient content and to enhance the quality of fresh vegetables. In fact, the constant exposure of the roots to the nutrient solution without soil interaction can maximize their uptake, translocation, and accumulation in the vegetable edible parts (20). At the same time, these novel cultivation systems, especially hydroponic ones, enhance the nutrient

content of some vegetables, and thus promote the production of biofortified vegetables for personalized nutrition (21, 22).

The idea of personalized nutrition is based on the widely accepted concept of medically tailored meal programs that are prescribed to patients with specific diseases, such as diabetes (23). The idea of using some foods with specific nutritional traits for a personalized nutrition can be considered advantageous from both clinical and quality of life viewpoints (24). The soilless production of biofortified vegetables could thus represent

an important strategy for directly obtaining tailored fresh vegetables without any processing steps in between.

Biofortified vegetable products for specific categories of people

This section presents current up-to-date knowledge about the use of soilless systems to produce biofortified vegetables focusing on three categories of individuals who might benefit the most from their distinctive peculiarities: (a) patients with impaired kidney function; (b) individuals diagnosed with or at risk of osteopenia/osteoporosis; (c) persons suffering from mental illnesses.

(a) Impaired kidney function

Although potassium (K) is an essential nutrient of the human body, groups of people with reduced renal potassium excretion are sensitive to the intake of K recommended by the World Health Organization (at least 3,510 mg day⁻¹ in the adult population) (25). The first group on this list is that of subjects with chronic kidney disease (CKD). Together with other so-called “lifestyle-related diseases,” the condition represents a global problem (26). It is estimated that about 10% of the worldwide population is affected by CKD, and millions die each year because they do not have access to affordable treatments. As opposed to that of the healthy population, the K intake for people affected by CKD is generally restricted to 1,500 mg day⁻¹ (27) in the effort to avoid adverse effects on heart function due to disturbances in plasma potassium concentrations, commonly known as hyperkalemia (28). It is important to note that in addition to patients with CKD, individuals taking medicines such as mineralocorticoid receptor antagonists affecting the renal excretion of potassium, need a lower K intake with respect to the healthy population (28).

Since vegetables contain high concentrations of potassium (29), individuals with impaired kidney function are generally told to avoid raw vegetables and to eat only small quantities of cooked ones given that K content is reduced by leaching and boiling (30). While it is true that boiling vegetables reduces their K content, it should also be remembered other important compounds for human health, such as hydrophilic vitamins, are also lost (31). Another important consideration is that people who are accustomed to frequently eating raw vegetables will almost certainly find it difficult to change their eating habits. Clearly, people with impaired kidney function would benefit if vegetables containing lower K content become available. Experimental results regarding vegetables production with low K content are summarized in Table 1.

Some investigators used a hydroponic system to produce micro chichory and micro spinach without K or with 29.1 mg of K L⁻¹ (24). They reported that 100 g of low K content microgreens provided only 7.7–8.6% of the K daily intake recommended for people affected by CKD, while the same serving size of their

mature counterparts provided approximately a fourfold higher K intake (24).

A hydroponic floating system of spinach and Swiss chard using a nutrient solution with 50 mg of K L⁻¹ led to a significant decrease in K in baby-leaves for both (on average 27 and 39%, respectively, for spinach and Swiss chard) with respect to the K concentration (200 mg L⁻¹) usually used to grow baby leaf vegetables in hydroponic conditions (21). Since both of these leafy greens are commonly considered potassium-rich vegetables, these experimental results confirm that the commercial production of baby-leaves of spinach and Swiss chard is possible.

When the K content in the nutrient solution was reduced from 156 to 0, the decrease in K in hydroponically grown melons was between 20 and 40% (32). Likewise, melon plants grown with 50% of the required K, produced fruits with a K content about 53% lower with respect to a standard solution of 156 mg of K L⁻¹, excluding other differences among the cultivars (33). These results highlight that 100 g of low-K content melons would provide about 9.3–14.7% of the K daily intake recommended for people affected by CKD, while the same serving size of conventionally grown melons would provide an approximate threefold higher intake of K (32, 33).

The hydroponic production of tomatoes using a nutrient solution of 39 mg of K L⁻¹ and completely removing K from the nutrient solution after anthesis led to a cultivar-dependent decrease of K (between 40 and 60%) in the tomatoes with respect to using a standard solution containing 156 mg of K L⁻¹ (34). These low-K content tomatoes would provide about 6.3–9.1% of the K daily intake recommended for people affected by CKD, while the same serving size of conventionally grown tomatoes would provide approximately a threefold higher K intake (34).

Overall, these experimental results demonstrate that hydroponic production technologies to produce vegetables with a low potassium content can obtain low-K products for people with impaired kidney function. Reducing K content in fruit appears nevertheless to be a more complex process as the growing cycle is longer. A transition from the vegetative to the reproductive phase is also involved and it is difficult to decide the right time to drastically reduce or entirely remove K from the nutrient solution without negatively affecting yield and/or the quality of the vegetable products. In fact, in Japan a standard solution of 156 mg L⁻¹ of K, which was used in the studies described above, is widely utilized for the hydroponic cultivation of tomatoes and melons (33, 34). In other countries, however, 150 mg L⁻¹ is already considered a low K concentration for tomatoes grown hydroponically, while quantities of 300 and 450 mg of K L⁻¹ are considered, respectively, as medium and high (35).

(b) Bone health and osteoporosis

The human skeletal system is a complex organ in constant equilibrium with the rest of the body. In addition to providing structural support for the body, bone is the major

TABLE 1 Reduced-potassium vegetable products for people with impaired kidney function.

Genotype	Vegetable type	Treatments	Effect	References
Chicory (<i>Cichorium intybus</i> L.)	Microgreens	Hydroponic system using polyethylene terephthalate fiber pads as growing medium and 0, 29.1, 58.4, and 117 mg of K L ⁻¹ in the nutrient solution.	In microgreens grown using a nutrient solution without K or with 29.1 mg of K L ⁻¹ the K content was between 103 and 129 mg 100 g ⁻¹ FW. Whereas, by using a nutrient solution with 58.4 or 117 mg of K L ⁻¹ the K content in microgreens was between 225 and 250 mg 100 g ⁻¹ FW.	(24)
Lettuce (<i>Lactuca sativa</i> L. Group <i>crispa</i>)	Baby leaf	Floating hydroponic system using a nutrient solution with 50 and 200 mg of K L ⁻¹ (K ₂₀₀). The lower K concentration in the nutrient solution was used over the entire growing cycle (K ₅₀) or only during the 7 days before harvest (K _{50-7d}).	For spinach the K content in baby leaf was of 670, 624, and 490 mg 100 g ⁻¹ FW, respectively, for K ₂₀₀ , K _{50-7d} and K ₅₀ . For Swiss chard the K content in baby leaf was of 459, 387, and 280 mg 100 g ⁻¹ FW, respectively, for K ₂₀₀ , K _{50-7d} and K ₅₀ .	(21)
Spinach (<i>Spinacia oleracea</i> L.)				
Swiss chard (<i>Beta vulgaris</i> L. ssp. <i>vulgaris</i>)				
Melon (<i>Cucumis melo</i> L.)	Fruit	Three experimental trials using a hydroponic system and nutrient solutions with K content as follow: (i) 39 and 156 mg of K L ⁻¹ ; (ii) 10, 19.5, 39, 78 and 156, mg of K L ⁻¹ ; (iii) 0, 10, 19.5 and 156 mg of K L ⁻¹ . In all trials the amount of 156 mg of K L ⁻¹ was the standard, while all other lower treatments were applied from anthesis to harvest.	In the first trial the K content in melon fruits was of 175 and 287 mg 100 g ⁻¹ FW, respectively, for low and standard K content in the nutrient solution. In the second trial the K content in melon fruits was between 250 and 360 mg 100 g ⁻¹ FW, passing from 10 to 156 mg of K L ⁻¹ in the nutrient solution. In the third trial the K content in melon fruits was between 220 and 360 mg 100 g ⁻¹ FW, passing from 0 to 156 mg of K L ⁻¹ in the nutrient solution.	(32)
Melon (<i>Cucumis melo</i> L.)	Fruit	In the first trial one melon cultivar (Panna) was hydroponically grown. All plants were fertigated with a standard nutrient solution (156 mg of K L ⁻¹) during first 2 weeks of growing cycle; in the following 2 weeks, applied potassium was 50, 75, 100, and 125% of required potassium, while the standard solution was still used for the control. In the second trial the same experimental protocol was applied on four cultivars: Panna, Miyabi shunjuukei, Miyabi akifuyu 412, and Miyabi soushun banshun 309.	In the first trial the K content in melon fruits was of about 195 and 410 mg 100 g ⁻¹ FW, respectively, for plants grown with 50% of its required potassium and the control. In the second trial the average K content in melon grown under limited K supply was of about 140 mg 100 g ⁻¹ FW without difference among cultivars.	(33)
Tomato (<i>Solanum lycopersicum</i> L.)	Fruit	In the first trial one tomato cultivar Cindy Sweet was hydroponically grown using a nutrient solution with 39 (low) and 156 (standard) mg of K L ⁻¹ . For each treatment, K was entirely removed from the nutrient solution either just after anthesis of the first flower (a third of the plants) or after set of the sixth fruit in the first truss (a third of the plants). In the second trial four cultivars (Aichan, Yellow Olle, Frutica, and Cindy Sweet) were hydroponically grown using a nutrient solution with 39 (low) and 156 (standard) mg of K L ⁻¹ . For plants treated with low-K solution, K was entirely removed from the solution after the first flower of the third truss reached anthesis.	In the first trial fruits K content was highest (202 mg 100 g ⁻¹ FW) in plants grown with standard nutrient solution and without K withdrawal and lowest (152 mg 100 g ⁻¹ FW) in plants grow with low nutrient solution and withdrawal at anthesis. In the second trial fruit K content in plants grown with standard nutrient solution was on average 242, 250, 193, and 185 mg 100 g ⁻¹ FW, respectively, for Aichan, Yellow Olle, Frutica, and Cindy Sweet; fruits K content in plants grown with low nutrient solution was of (on average) 95, 134, 133, and 136 mg 100 g ⁻¹ FW, respectively, for Aichan, Yellow Olle, Frutica, and Cindy Sweet.	(34)

FW, fresh weight.

reservoir for many minerals and compounds essential for maintaining a healthy pH balance (36). Bone health is the resultant of bone mass, bone architecture, and body mechanics. Illnesses like osteoporosis, characterized by low bone mass and microarchitectural deterioration of the bone tissue lead to decreased bone strength and increased risk of low-energy fractures, or so-called fragility fractures (37). Bone Mineral Density (BMD) is the measure that is commonly used to quantify bone health. A lower BMD value indicates an increased risk of osteoporosis or fractures. Osteoporotic fractures are a major cause of morbidity and disability in the elderly population and, in the case of hip fractures, can lead to premature death. It is important to remember nevertheless that although the deterioration of the body during the aging process renders the older adult particularly susceptible to poor bone health, osteoporosis should not be considered a disease exclusively pertaining to elderly individuals as it globally affects millions of men and women of all ages and ethnicities (38, 39).

Bone mass is influenced by factors such as sex, hormones, as well as genetic and environmental variables and last, but not least, nutrition. From a nutritional viewpoint, it is well known that the optimal intake of calcium (Ca) and vitamins D and K are important factors in the primary as well as secondary prevention of osteoporosis (38). It is also well known that the daily recommended intake of Ca for individuals between 19 and 50 is 1,000 mg, while it is 1,200 mg for those over 50 (40). Silicon is another element that has been associated with promoting bone formation and increasing BMD in men and premenopausal women (41, 42). It would seem that an intake between 10 and 25 mg per day is able to improve bone health (43, 44). Furthermore, some data indicate that between 1.0 and 3.0 mg per day of Boron (B) has beneficial effects on bone health (43–45). It is important to remember nevertheless, that there is no single food or nutrient capable of ensuring bone health on its own. Instead, a balanced diet with appropriate quantities of fruits and vegetables containing vitamins, minerals, and alkalizing substrates is thought to be the best approach (38, 39). In the light of these remarks, eating vegetables with a high content of Ca, Si, and B appears to be an efficacious way of promoting bone health particularly in people who are susceptible to osteoporosis. Examples of biofortified vegetables with Ca, Si, and B are reported in [Table 2](#).

Silicon in the range of 50–100 mg L⁻¹ in the nutrient solution was used to biofortify a series of baby-leafy vegetables (chicory, basil, purslane, Swiss chard, tatsoi, and mizuna). The biofortified vegetables showed, on average, more bioaccessible Si with respect to the unbiofortified ones (12). It was found that 100 g of these biofortified vegetables could provide approximately 13, 20, 17, 55, 14, and 14% of silicon intake (25 mg/day), respectively, for tatsoi, mizuna, purslane, basil, Swiss chard and chicory. All of these vegetables can be eaten raw or cooked; silicon-biofortified-basil can also be used to make a

pesto sauce (46), a particularly healthy dish for persons with risk factors for osteoporosis.

Silicon-enriched green beans through soilless cultivation has produced a Si concentration about 192% higher than that in controls (47). Therefore, the full daily intake of Si (25 mg Si per day) could be satisfied by consuming about 96 g of biofortified green beans. Some investigators reported that the Si content of biofortified pods is higher than that in unbiofortified ones even after cooking, regardless of the cooking method used. Furthermore, Si bioaccessibility in the cooked pods was more than tripled following biofortification (47).

In a study evaluating silicon biofortification of strawberries, the Si content was increased with respect to that in controls, obtaining 3.7–12.2-fold higher values by using, respectively, 50 and 100 mg of Si L⁻¹ in the nutrient solution (48). These results suggest that it is possible to satisfy the full daily intake of Si (25 mg of Si/day) by consuming 83 or 29 g of biofortified strawberries, respectively, for plants grown by adding 50 and 100 mg of Si L⁻¹ in the nutrient solution.

Carosello, an Italian melon consumed ripe or immature, has been biofortified by adding 100 mg of Si L⁻¹ in the nutrient solution (49). Some investigators reported identifying a Si concentration in these landraces about 95% higher than that in plants grown using a nutrient solution without added Si. These results suggest that it is possible to satisfy the full daily intake of silicon (25 mg/day) by consuming only 57 g of biofortified Carosello fruits.

Quantities higher than 100 mg of Si L⁻¹ were added to the nutrient solution in a study evaluating the Si biofortification of baby leaf spinach (50). The investigators reported a Si content about 288% higher in spinach grown using 100 mg Si L⁻¹ in the nutrient solution with respect to that in the controls (2 mg of Si L⁻¹); no further increase in Si content in the spinach was found when addition in the nutrient solution went from 100 to 200 mg of Si L⁻¹ (50). According to that study, 100 g of biofortified spinach provided 17% of the Si intake (25 mg per day), independently of the quantity of Si (100 or 200 mg L⁻¹) added to the nutrient solution.

Other investigators (51) found that the biofortification of chicory plants with Si in combination with NaCl supplementation enhanced the Si tissue enrichment more with respect to Si biofortification alone. Moreover, bioaccessible Si in chicory under “Si + NaCl” treatment was found to be the highest (51). According to these results 100 g of chicory grown using a nutrient solution enriched with Si in combination with NaCl could supply about 46% of the Si intake (25 mg per day). It is important to remember that eating 100 g of silicon-biofortified chicory under salinity stress conditions would mean consuming 190 mg of Na, a value that could be considered negligible as far as the recommended limits are concerned (51).

As far as Ca is concerned, some investigators (52) found that the average Ca content increased by 9.5% in four types

TABLE 2 Biofortified vegetables with calcium, silicon and boron indicated to people for whom it is desirable to promote bone health.

Element	Genotype	Vegetable type	Treatments	Effect	References
Silicon	Chicory (<i>Cichorium intybus</i> L.)	Baby leaf	Floating hydroponic system by adding 0, 50, and 100 mg of Si L ⁻¹ in the nutrient solution.	The added silicon in nutrient solution caused a species-related accumulation of Si: from 8 to 32 mg kg ⁻¹ FW in tatsoi, from 9 to 50 mg kg FW in mizuna, from 7 to 43 mg kg ⁻¹ FW in purslane, from 19 to 137 mg kg ⁻¹ FW in basil, from 8 to 36 mg kg ⁻¹ FW in Swiss chard, and from 11 to 36 mg kg ⁻¹ FW in chicory.	(12)
	Basil (<i>Ocimum basilicum</i> L.)				
	Swiss chard (<i>Beta vulgaris</i> L. ssp. <i>vulgaris</i>)				
	Purslane (<i>Portulaca oleracea</i> L.)				
	Tatsoi (<i>Brassica rapa</i> L., Tatsoi group)				
	Mizuna (<i>Brassica rapa</i> L., Mizuna group)				
Silicon	Green bean (<i>Phaseolus vulgaris</i> L.)	Fruit	Hydroponic system using perlite as growing medium and adding 0 (unbiofortified) and 100 mg of Si L ⁻¹ (biofortified) in the nutrient solution.	Silicon biofortification allowed to increase silicon content in pods from 8.9 (unbiofortified) to 26.0 (biofortified) mg 100 g ⁻¹ FW.	(47)
Silicon	Strawberry (<i>Fragaria × ananassa</i>)	Fruit	Hydroponic system by adding 0 (control), 50 (Si-50), and 100 (Si-100) mg of Si L ⁻¹ in the nutrient solution.	Silicon content in strawberry was 6.4, 30.0, and 85.0 mg 100 g ⁻¹ FW, respectively, in “Control,” “Si-50” and “Si-100.”	(48)
Silicon	Melon (<i>Cucumis melo</i> L.)	Fruit	Hydroponic system using a mixture of perlite-peat as growing medium and adding 0 and 100 mg of Si L ⁻¹ in the nutrient solution. Two Italian landraces of melon (Carosello and Barattiere) were used in the experiment.	Only for the Carosello the Si concentration in fruits increased from about 22.5 to 43.9 mg 100 g ⁻¹ FW, passing from 0 to 100 mg Si L ⁻¹ added in the nutrient solution.	(49)
Silicon	Spinach (<i>Spinacia oleracea</i> L.)	Baby leaf	Floating hydroponic system with three Si level in the nutrient solution: 2 (control), 100 (Si-100), and 200 (Si-200) mg L ⁻¹ .	Silicon content in spinach was of 1.13, 4.38, and 4.30 mg 100 g ⁻¹ FW, respectively, in “Control,” “Si-100” and “Si-200.”	(50)
Silicon	Chicory (<i>Cichorium intybus</i> L.)	Baby leaf	Floating hydroponic system using a nutrient solution with four combination of added Si and NaCl levels: (i) 0 mg of Si L ⁻¹ —0 mg NaCl L ⁻¹ (“Control”); (ii) 100 mg Si L ⁻¹ - 0 mg of NaCl L ⁻¹ (“Si”); (iii) 0 mg Si L ⁻¹ —2,922 mg of NaCl L ⁻¹ (“NaCl”); and iv) 100 mg of Si L ⁻¹ Si - 2,922 mg of NaCl L ⁻¹ (“Si + NaCl”).	Silicon content in baby leaf was of 1.14, 1.97, 3.06 and 11.4 mg 100 g ⁻¹ FW, respectively, in “Control,” “NaCl,” “Si” and “Si + NaCl”	(51)
Calcium	Endive (<i>Cichorium endivia</i> L.)	Baby leaf	Floating hydroponic system using 100 (unbiofortified) and 200 (biofortified) mg of Ca L ⁻¹ in the nutrient solution.	Calcium biofortification (200 mg L ⁻¹) allowed to significantly increase Ca content in all genotypes. On average, calcium content in baby leaf increased from 109 mg 100 g ⁻¹ FW (unbiofortified) to 120 mg 100 g ⁻¹ FW (biofortified).	(52)

(Continued)

TABLE 2 (Continued)

Element	Genotype	Vegetable type	Treatments	Effect	References
Calcium	Basil (<i>Ocimum basilicum</i> L.)	Leaf	Floating hydroponic system using a nutrient solution with six different concentrations of added calcium: 0, 100, 200, 400, 600, 800 mg of Ca L ⁻¹ .	The highest Ca content (204 mg 100 g ⁻¹ FW) was found in lettuce grown by using 800 mg of Ca L ⁻¹ in the nutrient solution. No differences were found among all other treatments with an average Ca content in lettuce of about 35 mg 100 g ⁻¹ FW.	(53)
	Tatsoi (<i>Brassica rapa</i> L., Tatsoi group)				
	Mizuna (<i>Brassica rapa</i> L., Mizuna group)				
Calcium	Lettuce (<i>Lactuca sativa</i> L.)	Leaf	Floating hydroponic system using a nutrient solution with four different concentrations of added Ca: 50, 100, 150, and 300 mg L ⁻¹ . The experiment was conducted in growth chambers set at 21°C and 28°C under a 16 h photoperiod.	Only at 28°C the Ca concentration in the lettuce leaves increased from about 175 to about 220 mg 100 g ⁻¹ FW, passing from 50 to 300 mg Ca L ⁻¹ in the nutrient solution.	(54)
Boron	Purslane (<i>Portulaca oleracea</i> L.)	Baby leaf	Floating hydroponic system using a nutrient solution with 0.3, 3, and 6 mg of B L ⁻¹ .	In the first trial the B content in purslane was of 0.5, 3.1, and 5.1 mg 100 g ⁻¹ FW, respectively, for 0.3, 3, and 6 mg of B L ⁻¹ in the nutrient solution. In the second trial the B content in purslane was of 1.2, 2.3, and 3.4 mg 100 g ⁻¹ FW, respectively, for 0.3, 3, and 6 mg of B L ⁻¹ in the nutrient solution.	(22)

FW, fresh weight.

of baby leaves (endive, basil, tatsoi, and mizuna) when the addition in the nutrient solution went from 100 to 200 mg of Ca L⁻¹. Moreover, Ca bioaccessibility ranged from 25% (basil) to 40% (endive), and the biofortified vegetables showed more bioaccessible Ca. On average, the consumption of 100 g of Ca biofortified baby leaf vegetables would provide an intake of 119 mg of Ca, equivalent to 10–12% of the daily intake.

Adding 800 mg of Ca L⁻¹ to the nutrient solution led to a Ca concentration in lettuce about fivefold higher with respect to that for other treatments (53). It is interesting to note that 100 g of lettuce biofortified using 800 mg of Ca L⁻¹ in the nutrient solution can supply about 20% of the Ca daily intake; the same serving size of lettuce after lower quantities of Ca biofortification supplies only 3.5% of the Ca daily intake.

Another study aiming to evaluate the increase in calcium content in lettuce leaves grown at different temperatures uncovered that the Ca concentration increased by about 26% rising from 50 to 300 mg of Ca L⁻¹ in the nutrient solution, but only at 28°C (54). In fact, the same Ca concentration used at 21°C did not affect the Ca content in the lettuce (54). The results of this study indicate that 100 g of Ca biofortified lettuce can supply about 18–22% of the recommended daily intake.

When they evaluated boron-biofortification in purslane, a wild edible plant, some investigators found that the B content was increased with respect to that in the controls, obtaining 1.8–10.7-fold higher values by using, respectively, 3 and 6 mg L⁻¹ of B in the nutrient solution. The average daily intake of B (2 mg) could thus be satisfied by consuming between 48 and 75 or 48 g of biofortified purslane (22).

Overall, these experimental results reviewed in the present manuscript show that the hydroponic cultivation of biofortified vegetables for Ca, Si, and B could be an effective way to obtain vegetable products promoting bone health. It should also be remembered that a serving size of less than 100 g of biofortified fruit or vegetables could satisfy the full recommended daily intake of Si, while a serving size of 100 g of leafy vegetables could supply only a part, generally between 13 and 55%. The Ca biofortification of vegetables would mean that 100 g of a vegetable could contain 20% of the recommended daily intake. As far as B biofortification is concerned, consuming a serving size of vegetables less than 100 g would satisfy its recommended daily intake.

Experimental results also demonstrated that progressively increasing amounts of a mineral elements in a nutrient solution

does not necessarily correspond to the increase of this element in the edible part of vegetables. Moreover, it should also be remembered that a salt must be introduced into the nutrient solution to increase the content of a specific cation (such as Ca). As a consequence, even the content of undesirable anions, such as nitrate and chloride, may be higher in biofortified vegetables.

From a nutritional point of view, if a vegetable can be enriched with Ca, Si, and B simultaneously this would facilitate the production of bone-healthy vegetables in particular for individuals at risk of osteopenia/osteoporosis.

(c) Mental illnesses

Mood disorders, including bipolar disorder, represent an important category of mental illnesses, whose prevalence is generally increasing in developed countries. Lithium (Li) compounds seem to be among the most promising and effective drugs used to treat this disorder, in particular with regard to bipolar affective disorder (55). There has also been some evidence that Li may be useful in preventing neurodegenerative diseases such as Alzheimer's disease (56) in treating depression, and in stabilizing moods due to its antimanic, antisocial, and prophylactic properties (57).

Lithium, a naturally occurring metal in the earth's crust, is used in the form of carbonate (Li_2CO_3) as a treatment for psychiatric disorders. Between 600 and 1,200 mg Li_2CO_3 per day, containing 113–226 mg of elemental Li (58), are the usual doses prescribed for these types of disorders. It should in any case be remembered that there is some evidence that although lithium is not officially considered a micronutrient, a daily intake of 1,000 μg Li for a 70-kg adult (14.3 μg kg^{-1} body weight) could effectively prevent mood disorders and reduce impulsiveness and nervousness especially in subjects at risk (59). According to these studies, lithium-enriched foods could help to stabilize moods in those subjects. The main sources of Li in the diet are nuts, cereals, fish, and vegetables, but their percentages are negligible in many geographic regions (59).

In a study aiming to evaluate the Li intake in foods served to students, some Authors (60) found that the Li amount supplied daily via the diet was 10.7 μg , an intake that can be considered low with respect to the proposed amount of 1,000 μg Li per day. Thus, vegetables enriched with lithium could have a positive effect on the mental health of individuals susceptible to mental illness. Some vegetables biofortified with lithium are reported in Table 3.

A study carried out some time ago evaluated Li concentrations in tomatoes, which was increased with respect to that in controls, obtaining a value 70-fold higher by using 34.5 mg of Li L^{-1} in the nutrient solution (61). The investigators reported a content of 21.8 μg of Li g^{-1} of dry weight, but they did not indicate the dry weight values. Thus, hypothesizing an average dry weight of 5.5 g 100 g fresh weight (62), a serving size (100 g) of lithium-biofortified tomatoes can supply about 12% of the recommended daily intake (1,000 μg Li per day), while

the same serving size of unbiofortified tomatoes can supply less than 0.2% of the daily intake.

Another study aiming to evaluate the increase in Li content of lettuce grown using five different foliar spray concentrations (0—control, 10, 20, 30, and 40 mg of Li L^{-1}) and to compare two mineral Li sources (lithium sulfate— Li_2SO_4 —and lithium hydroxide— LiOH) (63) reported that the Li concentration in the lettuce leaves was directly proportional to the concentrations of this element sprayed on the leaves regardless of the chemical form used. The Li concentration, which was low in the control plants (61 μg 100 g^{-1} dry weight), resulted 84-fold higher when 40 mg of LiOH L^{-1} was used and 61-fold higher when 40 mg of $\text{Li}_2\text{SO}_4 \text{ L}^{-1}$ was used, although for both sources the highest Li levels caused about a 15% reduction in plant height with respect to that in the controls (63). Nevertheless, considering an average dry weight of 9.5 g 100 g, a serving size (100 g) of Li biofortified lettuce can supply up to 484 μg of Li (using 40 mg of LiOH L^{-1}), which is about 50% of the Li daily intake.

Several studies have been carried out evaluating the biofortification of mushrooms with Li as a means to increase the daily intake of the mineral. Lithium chloride (LiCl) and lithium acetate (CH_3COOLi) at concentrations of 0 (control) 0.25, 0.5, 0.75, and 1.0 mM were used to enrich the substrate in which *Agrocybe cylindracea* (known as poplar mushroom, velver pioppin or Yanagi-matsutake) and *Hericium erinaceus* (traditionally called lion's mane, bearded tooth, satyr's beard, bearded hedgehog or pom pom) (64) were grown. Although the authors of the study did not indicate the mushrooms' dry weight, they reported that a concentration of 1.0 mM determined a Li content in the mushrooms hundreds of times higher than that in the controls. One study reported an average dry weight of 9.3 g 100 g^{-1} fresh weight for *A. cylindracea* (65) and 11.4 g 100 g^{-1} fresh weight *H. erinaceus* (66). Thus, a serving size (100 g) of biofortified *A. cylindracea* and *H. erinaceus* would supply, respectively, up to 2.3 and 7.8% of the lithium daily intake (64).

Other Authors used LiCl and CH_3COOLi at concentrations of 0 (control) 0.25, 0.5, 0.75, and 1.0 mM to enrich the cultivation substrate in which *Ganoderma lucidum* (known as Reishi mushroom), *Pleurotus eryngii* (also known as king trumpet mushroom) and *P. ostreatus* (also known as oyster mushroom, "hiratake," "shimeji," or "houbitak") (67). Similarly to the previous study, these Authors found an increase in Li hundreds of times higher with respect to that in the control when 1.0 mM was used; again, even in this study the authors did not report the dry weight of the mushrooms. An average dry weight of 11.8, 8.7, and 10.8 g 100 g^{-1} fresh weight, respectively, for *G. lucidum*, *P. eryngii* and *P. ostreatus* has been reported by other authors (68–70). It would seem then that a serving size (100 g) of biofortified *G. lucidum*, *P. eryngii* and *P. ostreatus* would supply, respectively, up to 83, 13.1, and 17.8% of the recommended Li daily intake (67).

TABLE 3 Lithium biofortified vegetable products indicated to people for whom it is desirable to promote mental health.

Genotype	Vegetable type	Treatments	Effect	References
Tomato (<i>Solanum lycopersicum</i> L.)	Fruit	Hydroponic system by adding 0 (control), 0.69, 6.89 e 34.47 mg of L ⁻¹ Li in the nutrient solution.	Lithium biofortification allowed to increase Li content in fruits from < 0.3 (control) to 21.8 (34.5 mg L ⁻¹) μg g ⁻¹ DW.	(61)
Lettuce (<i>Lactuca sativa</i> L. Group <i>crispa</i>)	Leaf	Foliar application by adding 0 (control), 10, 20, 30, and 40 mg of Li L ⁻¹ , comparing two mineral sources of Li (lithium sulfate—Li ₂ SO ₄ —and lithium hydroxide—LiOH).	The Li content in leaves ranged from 61 μg 100 g ⁻¹ DW (control) to 3,770 (40 mg of Li ₂ SO ₄ L ⁻¹) and 5,100 (40 mg LiOH L ⁻¹) μg 100 g ⁻¹ DW.	(63)
<i>Agrocybe cylindracea</i> <i>Hericium erinaceus</i>	Mushrooms	Growing media enrichment by adding 0 (control), 0.25, 0.5, 0.75, and 1.0 mM Li, comparing two Li salts (lithium chloride—LiCl—and lithium acetate—CH ₃ COOLi).	The added lithium in growing media caused a species-related accumulation of Li in mushrooms: from about 0 to 2.44 mg kg ⁻¹ DW in <i>A. cylindracea</i> and from about 0.1 to 6.87 mg kg ⁻¹ DW in <i>H. erinaceus</i> .	(64)
<i>Ganoderma lucidum</i> <i>Pleurotus eryngii</i> <i>Pleurotus ostreatus</i>	Mushrooms	Growing media enrichment by adding 0 (control), 0.25, 0.5, 0.75 and 1.0 mM Li, comparing two Li salts (lithium chloride - LiCl - and lithium acetate - CH ₃ COOLi).	The added lithium in growing media caused a species-related accumulation of Li in mushrooms: from about 0 to over 70 mg kg ⁻¹ DW in <i>G. lucidum</i> , from about 0 to 16.5 mg kg ⁻¹ DW in <i>P. ostreatus</i> and from about 0 to 15.1 mg kg ⁻¹ DW in <i>P. eryngii</i> .	(67)
<i>Lentinus crinitus</i>	Mushrooms	Growing media enrichment by adding 0 (control), 5, 10, 15, 20, 25, 30, 40, 50 or 100 mg of Li L ⁻¹ , comparing two Li salts (lithium carbonate—Li ₂ CO ₃ —and lithium chloride—LiCl).	The lithium content in mushrooms ranged from 0 (control) to 267 mg kg ⁻¹ by using 30 mg of LiCl L ⁻¹ and from 0 (control) to 574 mg kg ⁻¹ by using 25 mg of Li ₂ CO ₃ L ⁻¹ .	(71)

DW, dry weight.

By using LiCl and lithium carbonate (Li₂CO₃) at concentrations of 0 (control), 5, 10, 15, 20, 25, 30, 40, 50, or 100 mg L⁻¹ to enrich the substrate for growing *Lentinus crinitus*, other Authors obtained biofortified mushrooms containing up to 26.7 and 57.4 mg Li 100 g⁻¹ dry weight, respectively, with 30 mg of LiCl L⁻¹ and 25 mg of Li₂CO₃ L⁻¹ (71). The Li concentrations in the mushrooms were higher than those reported by the previous studies. In this case the authors did not provide data concerning the dry weight of the mushrooms and there is no data in the literature specifying the dry weight of *L. crinitus*. We can assume that the average dry weight is about 11 g 100 g⁻¹ fresh weight given the weight of a mushroom of the same genus, namely *L. edodes* (also known as “shiitake” mushroom) (72). Based on these assumptions, the daily intake of lithium can be satisfied by consuming about 34 g of *L. crinitus* biofortified using lithium chloride or about 18 g of mushrooms biofortified using lithium carbonate (71).

Overall, the experimental results show that plants can be biofortified with Li by adding an enriched nutrient solution to the culture substrates or *via* foliar applications. They also demonstrate that progressively increasing quantities of Li in the nutrient solution corresponds to increasing quantities of the element in the edible part of the vegetables (61, 63, 67, 71). The appropriate dosage of Li in the nutrient solution to positively affect the mental health of people susceptible to mental illness is an important consideration. It would seem that a too low Li

level in the nutrient solution translates into vegetable products which supply low daily intakes of Li per serving size. On the other hand, too high Li levels in the nutrient solution should be avoided, since it cannot be excluded that vegetable products with high concentrations may be harmful to human health.

Bioavailability and bioaccessibility: A useful support tool for the precision biofortification

Whatever the biofortification approach used, obtaining biofortified vegetables represents only the first step toward achieving tailored food for personalized nutrition. The next step is determining whether the increase or reduction in a specific nutrient in the edible parts of the plant changes its bioaccessibility and bioavailability parameters. The evaluation of the benefits and/or risks associated with absorbing a particular element from biofortified vegetables must, in fact, take into consideration their bioaccessibility and bioavailability (Figure 3), which refer to the processes involved in extracting mineral elements and absorbing them; food components do not in fact exactly correspond to their functional value.

When plant foods are consumed, the nutrients (organic and inorganic) and bioactive compounds are released from

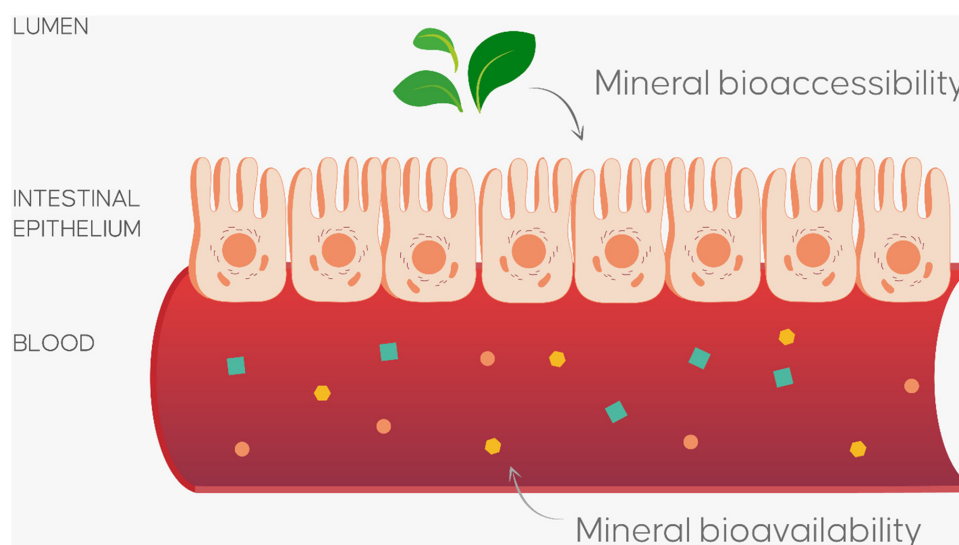


FIGURE 3

In vitro model for evaluate bioaccessibility (percentage of nutrient release from food matrix during gastro-intestinal digestion process) and bioavailability (percentage of nutrient adsorbed in intestinal tract after gastro-intestinal digestion process) of nutrients and/or bioactive compounds.

the plant tissue and modified into absorbable units which can subsequently be absorbed by the epithelial cells in the gastrointestinal tract and transported to their respective target tissues (73) through the blood (74). But not all nutrients in the edible parts of a food can carry out a biological activity. Some *in vitro* models have been developed to simulate the physiological conditions of the human gastrointestinal digestion. The biochemical, physiological and dynamic conditions of the gastrointestinal system (the mouth, stomach, and intestines) such as temperature, mechanical forces, pH, the concentration of some enzymes (pepsin, pancreatin, and lipase) and the presence of bile (75) have been artificially reproduced in the gastrointestinal digestion model. The *in vitro* digestion model provides information about the amount of nutrients released from the food matrix (basically its bioaccessibility), their biochemical transformation and chemical degradation, the nutrient-nutrient and nutrient-antinutrient interactions and the effect of the matrix and food processing (75).

The release of nutrients (the bioaccessible fraction) in the intestinal tract during the digestive process of plant materials depends on a variety of factors, such as the species and the type of plant materials, the localization of the nutrient, and it is affected by a host of variables such as the concentrations of other nutrients and/or anti-nutritional compounds, the cooking method used, if and how the food has been processed, and the interaction of other nutrients. The concentration of the nutrient in the edible parts is important, but even the biofortification process can modify the release of nutrients, as has been reported

by a number of studies regarding different mineral elements (12, 21, 47, 51, 52, 76).

The gastrointestinal digestion model has been used to identify the biofortified species or treatments (agronomic and/or food processing) that are able to release high quantities of silicon (12), Ca (52), zinc (76, 77), selenium (78), iodine (79), iron (80) in the intestinal tract. Thus, bioaccessibility assessment methodologies can be used to select the species, cultivars and/or genotypes that are able to release high quantities of nutrients during the digestion process in order to maximize the health effects of the biofortification process.

The bioavailability fraction can be used to evaluate bioavailability *via* an established bioassay (81); this parameter has been defined as the quantity of nutrient/s adsorbed, in the intestinal tract during the gastrointestinal digestive process (82). In general, mineral bioavailability needs to be evaluated *via in vivo* human studies. Some *in vitro* models have been proposed as an alternative to using animal models to evaluate mineral bioavailability to estimate nutritional efficiency (bioefficacy) and to determine the potential health effects of the biofortification process (79, 83–85). Currently, there are several *in vitro* models capable of simulating the intestinal mucosa with phenotypic characteristics comparable to *in vivo* conditions. The most widely used cell model is represented by the Caco-2 with applications in the study of active transport of mineral nutrients (84, 86).

Only a few studies, aiming to evaluate biofortified vegetables have been conducted to assess bioaccessibility and bioavailability as part of the biofortification process. In general, these studies used a multidisciplinary workflow based on an evaluation of

the efficiency of the biofortification process from a nutritional point of view, taking into consideration bioaccessibility, bioavailability and biological activity as well as agronomic efficiency (12, 80, 84, 86, 87). The approach is summarized in **Figure 4**. Bioavailability and bioaccessibility assessment provides nutritional and biological information regarding biofortified products generally not furnished by agronomic biofortification studies. In addition, it can be used to improve the development of biofortified foods with regard to the choice of the species or of the type of food processing to use to produce plant foods that satisfy consumer needs (76, 80, 86, 88).

Ongoing trends and the challenges ahead

In addition to the three specific categories of individuals discussed above, other groups may be interested in switching over to the consumption of soilless produced vegetables containing higher quantities of essential micronutrients/compounds and low concentrations of undesired elements or compounds.

Nickel (Ni) is an ubiquitous trace element and the commonest cause of metal allergy. Individuals sensitized to Ni through dermal contact and who have allergic contact dermatitis (some have estimated to that it affects up to 15% of women, but it is an undiagnosed entity) develop hand eczema from oral, as well as dermal, exposure to Ni salts. Oral intakes of Ni as low as approximately 500 μg per day have been reported to aggravate hand eczema in Ni sensitized subjects (89). Nickel in soil and water is taken up by living organisms, plants and animals that are food sources for humans; it is therefore present in most of the constituents of a normal diet. The Ni content in fruits and vegetables is on average fourfold higher with respect to food of animal origin (90). At the same time, Ni content in vegetable food products can vary widely, depending on the Ni content in both soil (ranging between 5 and 500 $\mu\text{g g}^{-1}$) and water (between 5 and 100 $\mu\text{g L}^{-1}$). For these reasons, the Ni content in individual foods appears to vary widely depending on a number of variables (90). But independently of the Ni content of the soil, some vegetable products, such as legumes, whole wheat, and cocoa and derivatives are known to have a high Ni content. With regard to other types of vegetable food products it is difficult to define what is “high in nickel” because different thresholds have been used by many authors and institutions. In fact, the threshold can range from 0.5 to 0.03 mg kg^{-1} . Using the latter threshold, a host of vegetable products including tomatoes and carrots should be considered high in Ni (90). More reliable data about the Ni content in foods are therefore needed. Currently, some hydroponic companies are producing certified vegetables that comply with the requirements of the “Product Technical Specification” regulating the vegetable supply chain and guaranteeing that nickel is absent in food products (91, 92).

For the time being there are no studies in the literature regarding the soilless production of vegetables with undetected (verified analytically) nickel. Future studies should thus aim to develop soilless vegetable production methods experimenting with the growing media, irrigation water, fertilizers and cultivars in order to reduce the uptake of Ni and restrict its translocation to the edible parts of the plant and therefore to produce practically Ni-free vegetables for individuals sensitized to Ni.

Nitrate (NO_3), which is a naturally occurring form of nitrogen, is an integral part of the nitrogen cycle in the environment. Approximately 80% of the nitrates in the daily diet come from the consumption of vegetables, mainly through green-leaf vegetables (93). For the most part NO_3 -accumulating vegetables belong to the *Brassicaceae* (rocket, radish, mustard), *Chenopodiaceae* (beetroot, Swiss chard, spinach) and *Amarantaceae* families. The *Asteraceae* (lettuce) and *Apiaceae* (celery, parsley) include species that are characterized by a high content of NO_3 (94). Nitrate *per se* is relatively non-toxic; nevertheless once ingested NO_3 is converted to nitric oxide. It can react with hemoglobin (oxyHb) to form methaemoglobin (metHb), which may impair oxygen delivery to human tissue causing methaemoglobinaemia, or blue baby syndrome. Infants are more susceptible to a syndrome characterized by clinical symptoms such as the blue discoloration of the skin due to the presence of deoxygenated blood and asphyxia. This occurs because young infants have less of the reductase needed to reconvert the metHb back to oxyHb and have low NO_3 -reducing activity due to low gastric acidity (94). Given these considerations, the European Food Safety Authority (EFSA) released a statement on possible public health risks for infants and young children linked to the presence of nitrates in leafy vegetables, and established that the maximum NO_3 concentration for baby foods is 200 mg kg^{-1} fresh weight, including vegetables (95). Some of the strategies that can be used to reduce the NO_3 content in vegetables grown using hydroponic systems are: (i) removing part or all of the nitrate nitrogen from the nutrient solution a few days before harvesting; (ii) using nutrient solutions with $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ rather than nitrate nitrogen only (96); (iii) growing vegetables under high light intensity conditions (97).

Beyond the possible acute health effects of nitrates in infants and young children who consume spinach and lettuce, it should be remembered that NO_3 supplementation enhances nitric oxide (NO) bioavailability *via* the NO_3 -nitrite-NO pathway, which is involved in several physiological processes that could potentially improve skeletal muscle function. In fact, there is evidence that dietary NO_3 supplementation has ergogenic effects during endurance and sprint-type exercises and others types of physical activities such as weightlifting (93, 98). The limited number of studies as well as the diversity of the results published impede us from drawing any clear conclusions about NO_3 supplementation in athletes. As far as endurance sports are concerned, the dose necessary for a significant effect continues

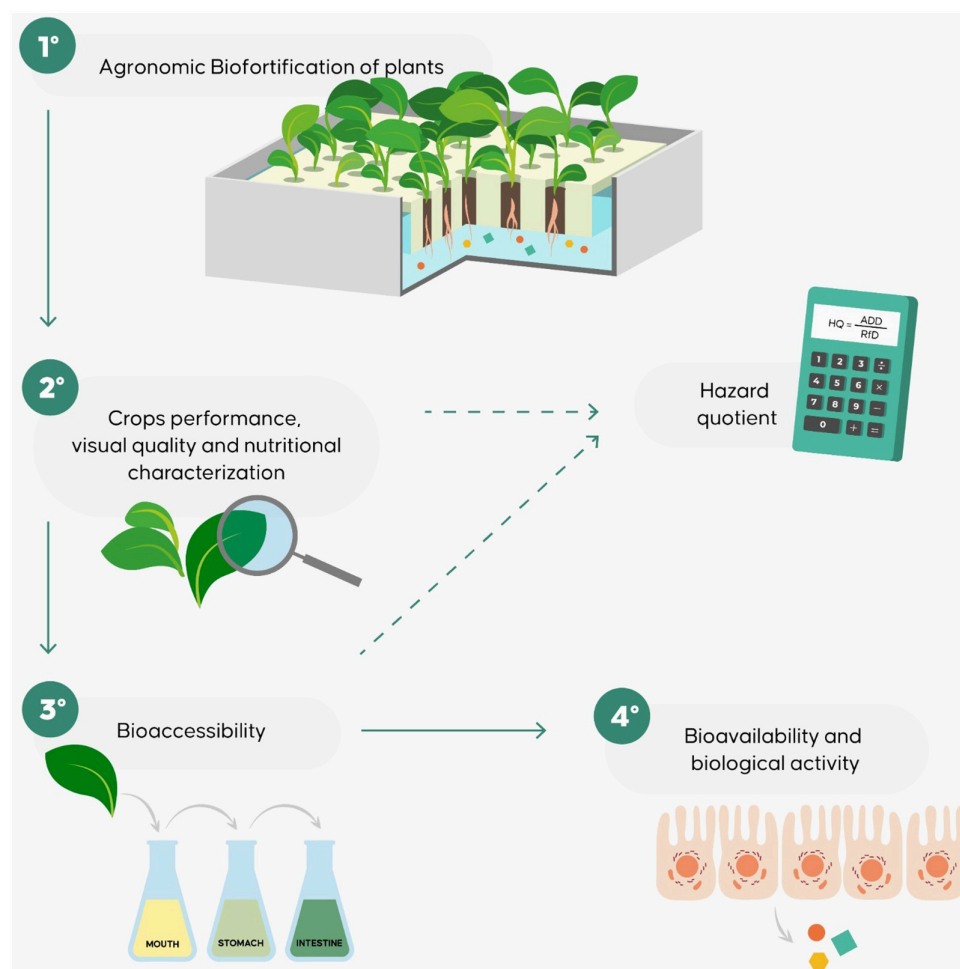


FIGURE 4

The workflow proposed to evaluate the agronomic, nutritional, and bioefficiency of biofortified products.

to be unclear since in some trials acute doses of 12 mmol of NO_3 were used while in others smaller doses (up to 6 mmol day^{-1} of NO_3) were utilized (93). Despite the differences in the supplementation dosage (from 32.5 mg NO_3 to 6.4 mmol of NO_3) used in the trials and in the periods of supplementation (from acute to chronic over 6 days), the limited data existing in the literature suggest that dietary nitrate supplementation could potentially enhance weightlifting performance (98). Further research should attempt to analyze the ergogenic effect of nitrate supplementation on athletes as well as to study the optimal sources and the most suitable species and doses of nitrates and the best hydroponic systems to produce nitrate-enriched tailored vegetables. The challenges ahead include those of identifying the categories of people who would most benefit from vegetable biofortification and determining the sustainability of the production processes. According to the “Farm to Fork” strategy, which is at the heart of the European Green Deal, food systems cannot be resilient to crises if they

are not sustainable. Our food systems need therefore to be set on a sustainable path which will also create new opportunities for operators in the food value chain (99). While it may seem counterintuitive, high-tech soilless cultivation systems and organic agriculture have several converging points in view of a sustainable use of the planet’s natural resources (100). In the future those working in the sector should aim to verify that soilless cultivation systems have a low environmental impact and that biofortified vegetables are high quality products.

Conclusion

Enhancing the precision biofortification processes through soilless systems and understanding the aspects related to bioaccessibility and bioavailability of a particular element from a biofortified vegetables are launching horticultural science into the era of personalized nutrition. Consequently, it is clear that the multidisciplinary approach toward tailored foods is a

winning one and must increasingly include a synergy between agronomic, biological and medical skills. Therefore, for further goals vegetable biofortification trials could be joined to clinical studies for assessing the potential additional benefits of the emerging biofortified vegetables for specific categories of people.

Author contributions

MR, MD'I, and FS contributed to the study concept and design of the manuscript, tables and figure preparation, and edition. MR, MD'I, FS, and SM critically reviewed the article. MR and MD'I contributed to the acquisition and analysis of data and drafted the manuscript. All authors gave final approval for all aspects of the work, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Inverse association of a traditional Korean diet composed of a multigrain rice-containing meal with fruits and nuts with metabolic syndrome risk: The KoGES

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Background: Hansik, a traditional Korean diet, may have a beneficial impact on metabolic syndrome (MetS) risk as dietary westernization increases its prevalence. We examined the hypothesis that adherence to the hansik diet may be inversely associated with the risk of MetS and its components and sought to understand the gender differences in 58,701 men and women aged over 40.

Materials and methods: Hansik was defined using 14 components from which the Korean dietary pattern index (K_{diet} -index) was generated by summing their scores. Low-hansik intake was defined as the K_{diet} -index with <8 . MetS was categorized based on the 2005 revised NCEP-ATP III criteria modified for Asians.

Results: The K_{diet} -index score was negatively associated with the dietary inflammation index and showed that the high intake of a meal with multigrain rice, fruits, and their products, and nuts, and low intake of fried foods were inversely associated with MetS by 0.707, 0.864, 0.769, and 0.918 times, respectively, after adjusting for covariates. More women and participants with more educated and lower income belonged to the high-hansik group, and participants with high self-rated health scores consumed more hansik. All participants on a high-hansik diet were associated with a 0.87 time lower risk of MetS. Specifically, the association between hansik intake and MetS risk was not significant among men following stratification by gender. Body composition, including the body mass index, waist circumference, and fat mass, was inversely associated with hansik intake, while the skeletal muscle mass index was positively associated with the hansik intake in each gender and all participants. In all the participants in the high-hansik group, no significant changes were seen in the serum glucose and HDL concentration. However, a high-hansik intake showed lower blood pressure and serum LDL and triglyceride concentrations only in men and a higher glomerular filtration rate in both genders.

Conclusions: Hansik intake might improve MetS risk, with its primary beneficial effects on body composition, dyslipidemia, and blood pressure gender-dependently.

KEYWORDS

traditional Korean diet, hansik, K_{diet} -index, metabolic syndrome, abdominal obesity, hyperglycemia, dyslipidemia, skeletal muscle mass

Introduction

Metabolic syndrome (MetS) is a cluster of several physiological and metabolic abnormalities that raise the risk of atherosclerotic cardiovascular disease, insulin resistance, and type 2 diabetes. A diagnosis of metabolic syndrome is made when a patient presents with three or more of the following: abdominal obesity, hyperglycemia, hypertriglyceridemia, reduced high-density lipoprotein cholesterol (HDL), and hypertension. The overall prevalence of MetS has risen worldwide, with different rates of prevalence in various countries (1). Gender-specific differences in the prevalence of MetS and its components have also been reported (2). In the United States (US), the MetS prevalence has increased in adults aged over 20 years from 32.5 to 36.9% over the period 2011–2012 to 2015–2016, according to the National Health and Nutrition Examination Survey (NHANES) (3). A significant increment in the 20–39 year age group, women, Asians, and Hispanics was seen (3). However, in Korea, the MetS prevalence in men increased from 24.5 to 28.1% between 2008 and 2017, while no significant increase was seen among women (20.5 and 20.7%), according to the Korea National Health and Nutrition Examination Survey (KNHANES) (4). Therefore, the differences in the MetS prevalence among Asian women in the US and Korea may be related to various lifestyle factors, especially dietary patterns (5). Scientists have made efforts to identify a dietary pattern that is easy to follow to prevent and alleviate MetS risk, such as the Mediterranean diet and Dietary Approaches to Stop Hypertension (DASH) (6). However, it is challenging to incorporate these diets into Asian dietary patterns. Hence, there is a need for a country-specific beneficial dietary pattern to alleviate the rising risk of MetS.

Koreans have traditionally consumed a Korean-style diet called “hansik (K-diet; 韓食).” The basic meal of hansik includes cooked multigrain rice (a mixture of cooked rice and other grains such as brown rice, beans, barley, oat, or other grains; japgogbab; 雜穀飯), soup made of fermented soybeans, braised or grilled fish, two servings of seasoned, cooked, or raw vegetable dishes with sesame or perilla oil (namul or saengchae, respectively), and fermented cabbage (kimchi) (7). Its basic meal pattern is called three cheopbansang (椀飯床), which includes three dishes in addition to multigrain rice, soup, and kimchi.

The soups contain various vegetables and small amounts of meat. A variety of vegetables are used for making namul. Grilled, baked, or stirred meats are also served instead of fish in the meal (7). Koreans ordinarily consume 3–5 cheopbansang each meal, and after each meal, fruits, nuts, and tea are served as desserts. Although the table setting is similar in every meal, the variety of vegetables used in namul and soups and the different meats and fish makes the meal nutritionally complete and healthy. Thus, the three cheopbansang in hansik can provide nutrients for meeting the dietary reference intake. The dietary pattern is easy to adhere to and provides Koreans with a balanced diet, including diverse foods, and could also be propagated to other Asian countries. Traditionally, Koreans do not consume milk and milk products that are not included in the hansik. However, their consumption as snacks has been recommended for over 50 years.

The diet pattern consumed by an individual is a crucial risk factor for the development of MetS. However, individuals find it difficult to adhere to particular foods and diets. Dietary patterns have been studied over the last decade to explore ways to reduce MetS risk while ensuring adherence. Korean-balanced diets have been shown to have an inverse association with MetS risk in randomized clinical trials (RCT) and observational studies (8–10). RCT studies are limited in the aspects of intervention periods and diet types to show the effects of traditional Korean diets. Korean food intake has been categorized into 3–4 clusters by the principal component analysis (PCA) of observational studies, which included a Korean-balanced diet. However, hansik, a traditional Korean diet, was somewhat different from the Korean-balanced diet in which multigrain, fermented soybeans, fruits, and nuts were excluded as the primary food items (11). However, the traditional Korean dietary pattern has not been studied for the association with MetS risk. In the present study, we defined the traditional Korean dietary pattern as the K_{diet} -index score to show its efficacy quantitatively. Therefore, we hypothesized that adherence to the hansik diet might be inversely related to the risk of MetS and its components. We also analyzed the gender-based differences of the hansik diet on MetS risk. The hypothesis was examined in a large city hospital-based cohort in Korea. Hansik intake of the participants was evaluated with 14 components to describe a traditional Korean diet, which was scored to

make the K_{diet} -index. The association of hansik with MetS and its components was examined using the K_{diet} -index in the present study.

Materials and methods

Participants

The urban hospital-based cohort, a multi-institution hospital-based registry, was part of the Korean Genome and Epidemiology Study (KoGES) conducted by the Korean National Research Institute of Health (NIH), Centers for Disease Control and Prevention and the Ministry of Health and Welfare in Korea. The purpose of this cohort was to attempt to resolve the public health issues about increasing metabolic diseases related to lifestyle changes (12). Volunteers who visited the general hospitals in the metropolitan area and cities were recruited, and the inclusion criteria were aged ≥ 40 years at baseline. All participants ($n = 58,701$; 20,293; men and 38,408 women) in the cohort were included for the present study (12). The study was approved by the Institutional Review Board (IRB) of the National Institute of Health, Korea (KBP-2015-055) and Hoseo University (HR-034-01). The participants signed a written informed consent form.

Anthropometric and biochemical measurements

Basic measurements such as height, weight, waist, and hip circumference were taken (13). The body mass index (BMI) was calculated by dividing the body weight (kg) by height squared (m^2) measured while wearing light clothes and bare feet. Body fat and skeletal muscle masses were determined using the Inbody 3.0 (Cheonan, Korea) based on the Bioelectrical impedance analysis in Ansan/Ansung cohort. However, they were estimated in the city hospital-based cohort using a machine learning prediction model generated from the Ansan/Ansung cohort (14). Skeletal muscle index (SMI) was defined as dividing appendicular skeletal muscle mass by height. A doctor measured blood pressure in the left arm in a sitting position with a sphygmomanometer. Blood was collected in vacuum blood collection tubes with and without ethylenediaminetetraacetic acid (EDTA) after participants fasted overnight, and plasma and serum samples were separated for biochemical analysis. In fasting serum or plasma, biochemical parameters [glucose, total cholesterol, HDL cholesterol, triglyceride, and creatinine concentrations and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities] were assayed using a Hitachi 7,600 automatic analyzer (Hitachi, Tokyo, Japan). Blood HbA1c contents were determined with an HbA1c Analyzer from EKF Diagnostics (Manchester, UK). Insulin resistance was

calculated using Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation by fasting serum glucose (mg/dL) and insulin (mU/L) concentrations divided by 405 (15). Serum LDL cholesterol concentrations were calculated with the Friedewald equation, excluding serum triglyceride concentrations ≥ 500 mg/dL. Serum high-sensitive C-reactive protein (hs-CRP) concentrations were determined with an enzyme-linked immunoassay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). Exercise levels were determined based on answers to a question on exercise intensity and duration: The participants in the high-exercise group did more than three sessions per week of moderate exercise (brisk walking, mowing, badminton, swimming, tennis, and others) for >30 min or intensive exercise (climbing, running, football, basketball, volleyball, and others) for >20 min per session. The participants who did not exercise belonged to the low-exercise group. Smoking status was categorized into current, past, or never based on their smoking more than 100 cigarettes over their lifetime and smoking during the last 6 months before participating in the study. Alcohol consumption was calculated based on the type, amount, and frequency of alcoholic beverages in the last 6 months. It was calculated by multiplying the drinking frequency by the average alcohol consumed each time.

MetS definition

The definition of MetS was based on the 2005 revised National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) criteria modified for Asians reducing the waist circumference (WC) and approved by the Korean Society for the Study of Obesity (KSSO). MetS was defined as the presence of three or more of the following: (1) abdominal obesity (waist circumference ≥ 90 cm for men and >85 cm for women), (2) low HDL-cholesterol level (<40 mg/dL for men and <50 mg/dL for women); (3) elevated serum triglyceride level (≥ 150 mg/dL) or current anti-dyslipidemic medication use; (4) elevated fasting blood glucose level (≥ 100 mg/dL) or current anti-diabetic medication use; (5) elevated blood pressure (average systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg) or current blood pressure medication use (16, 17).

Daily nutrient intake and dietary pattern analysis

The committee of KoGES designed and validated a semi-quantitative food frequency questionnaire (SQFFQ) that included 103 foods commonly consumed by Koreans (18). It estimated the usual Korean daily food intake during the last 6 months. SQFFQ requested information regarding the consumption frequency and amounts of 103 food items with assigned serving sizes. The results were converted into the

TABLE 1 The definition of hansik (traditional Korean diet) by the K_{diet} -index.

	Portion size	Criteria (times)	Score
Meals with multigrain rice		$\geq 2/\text{day}$	1
Eating frequencies of grains	210 g	$\geq 2/\text{day}$	1
Eating frequencies of kimchi	50 g	$\geq 2/\text{day}$	1
Eating frequencies of soup made with fermented soybeans	200 g	$\geq 1/\text{day}$	1
Eating frequencies of seaweeds	50 g	$\geq 2/\text{day}$	1
Cooked vegetables with garlic, onions, and ginger	70 g	$\geq 2/\text{day}$	1
Using frequencies of sesame or perilla oil, among other oils	1 teaspoon	$\geq 50\%$	1
Eating frequencies of meats including beef, pork, and chicken	60 g	$< 3/\text{week}$	1
Eating frequencies of fried foods	60 g	$< 3/\text{week}$	1
Eating frequencies of fish or clams	60 g	$\geq 1/\text{day}$	1
Eating frequencies of soybean products	60 g	$\geq 1/\text{day}$	1
Eating frequencies of fruits and fruit juicy	200 g	$\geq 2/\text{day}$	1
Eating frequencies of processed foods	60 g	$< 1/\text{day}$	1
Eating nuts	15 g	$\geq 1/\text{day}$	1

When meeting the criteria, the score of each K_{diet} -index component was 1. If not, the score was 0. The K_{diet} -index score was calculated with the sum of scores in each component.

quantity of intake of 23 nutrients using the computer-aided nutritional analysis program 3.0 developed by the Korean Nutrition Society (KNS) (18).

The 103 food items and alcohol were categorized into 29 pre-combined food groups and used as independent variables in a PCA to find the optimal number of diet factors. The number of factors was assigned according to satisfying eigenvalues of >1.5 in the PCA, which was met in 4 criteria. The orthogonal rotation procedure (Varimax) was applied to generate the clusters, indicating dietary patterns (19). Dietary factor-loading values of ≥ 0.40 were used to indicate significant contributions of food items by assigning names to the dietary patterns (19). The main food groups of the Korean balanced diet (KBD) included fish, crabs, red meats, vegetables, kimchi, pickles, seaweeds, and mushrooms as main food groups. Noodles, bread, fast foods, soups, and meats were high in the Western-style diet (WSD). A plant-based diet (PBD) included beans, potatoes, green vegetables, seaweed, milk, nuts, and eggs as predominant food groups. Rice-main diet (RMD) was rich in rice.

Hansik quantification by Korean dietary pattern index (K_{diet} -index)

Hansik was quantified based on commonly consumed Korean dishes or foods to determine its effect on MetS. Hansik was defined based on 14 components of traditional Korean meals shown in Table 1, and each component was given the portion size and criteria for a score. Each question about hansik provided portion size and criteria for individual food items to quantify the nutritional status of the participants for their eating

as per the hansik pattern. However, the component of “meals with multigrain rice” did not provide the portion size since it indicated the type of the meal, compared to having noodles or bread instead of multigrain rice. Each question was scored 1 if the hansik criteria were satisfied, else it was scored zero. The meal with multigrain rice (a mixture of cooked rice and other grains; jabgogbab) was 1 when the participants had ≥ 2 meals with multigrain instead of noodles or bread per day. The score of the 14 components was summed, and the summed score was assigned to the K_{diet} -index (11). The higher the total K_{diet} -index score, the higher the intake per the hansik pattern. The participants were divided into two groups by K_{diet} -index score, and they were called the low- (< 8) and high-hansik (≥ 8) groups.

Dietary inflammatory index

The DII was calculated by an equation reported in an earlier study, which was generated based on the dietary inflammatory potential of foods and nutrients having anti- or proinflammatory properties (energy, 32 nutrients, 4 food products, 4 spices, and caffeine). The inflammatory weights of the nutrients were adopted from the earlier report (20). However, some spices, including garlic, ginger, saffron, and turmeric, were omitted from the original equation since their intake was not reported as part of the SQFFQ. The DII calculation was conducted by multiplying the proinflammatory weights of the 38 dietary components by their daily intake and then dividing the sum for each item by 100. The association between DII, K_{diet} -index, and MetS risk was calculated with adjusted logistic regression.

Self-rated health and stress status

Self-rated health (SRH) was determined by the participant's perception of their wellbeing and health. The participants scored their health status from 1 (feeling very healthy) to 5 (feeling very unhealthy). Stress status was evaluated with 18 questions about physical and psychological stress at home and work, and each question was scored from 0 (lowest stress) to 3 (highest stress). The overall stress status scores were estimated by a summation of the scores of the 18 questions. Higher stress status scores indicated the presence of higher stress.

Statistical analyses

Statistical analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC, USA). When the sample size was determined using the G*Power program with effect size (0.05), power (0.99), and significant level (0.05), the sample size was 1,036. The sample size for each gender was sufficient to satisfy the sample size. The descriptive statistics for categorical variables (e.g., gender and lifestyle) were evaluated based on the frequency distributions of the hansik categories. Chi-squared tests were used to analyze the frequency distributions for categorical variables. Adjusted means and standard errors were calculated for continuous variables based on the hansik categories. The statistical differences between low and high hansik intake were determined using the analysis of covariance (ANCOVA) after adjusting for covariates, including age, residence area, BMI, education, income, energy intake, alcohol drinking, physical exercise, and smoking status. The adjusted odds ratio (ORs) and 95% confidence intervals (CI) for the high-intake of hansik as defined by the K_{diet} -index score were determined by multiple regression analysis after covariate adjustment. P -values < 0.05 were considered to be statistically significant.

Results

Characteristics of the participants according to their gender and hansik intake

The present study included 58,701 participants, 20,293 men, and 38,408 women, who were categorized into low-hansik and high-hansik intake groups based on the criteria of being under or over the K_{diet} -index score of 8, respectively. The low-hansik group (< 8 hansik scores) included 6,271 men (30.9%) and 7,728 women (69.1%), and the high-hansik group included 14,022 men (20.1%) and 30,680 women (79.9%). More women belonged to the high-hansik group than men (Table 2). The average age of the participants was higher in the high-hansik group than in the low-hansik group. Across both genders,

participants were more educated (\geq high school) and had lower incomes ($< \$4,000/\text{month}$) in the low-hansik group compared to the high-hansik group. A higher proportion of participants exercised regularly and were former smokers in the high-hansik group than in the low-hansik group. The stress scores were much lower in the high-hansik group than in the low-hansik group and lowered in men than women. These results indicated that people with higher hansik intake had lower stress levels. The SRH scores were lower in the high-hansik group than in the low-hansik group, indicating that the participants in the high-hansik group thought themselves healthier than those in the low-hansik group.

Food intake according to gender and hansik intake

The major food intake in low- and high-hansik was presented in Table 3. Multigrain rice intake was much higher in the high-hansik group than in the low-hansik group. However, white rice, noodles, and bread intakes were much lower in the high-hansik group in both genders (Table 3). Vegetables, fruits, kimchi, and seaweeds exhibited a much higher intake in the high-hansik group than in the low-hansik group in both genders. The adults in the high-hansik group had higher fish intake and lower meat intake than those in the low-hansik group (Table 3). Furthermore, beans, fermented beans, and nuts showed a higher intake in the high-hansik group than in the low-hansik group (Table 3). The results suggested that the 14 components of the hansik definition in Table 1 made the participants well-separated into low- and high-hansik groups.

Nutrient intake according to gender and hansik intake

The energy intake divided by estimated energy requirement was higher in the high-hansik participants and women (Table 4). An analysis of the percentage of macronutrient intake indicated that carbohydrates and protein intakes were higher, and fat intake was lower in the high-hansik group compared to the low-hansik group in both genders (Table 4). The intakes of fiber, calcium, vitamins C and D, and flavonoids were much higher in the high-hansik group than in the low-hansik group. However, only vitamin C intake met the recommended intake in the high-hansik group in both genders. Sodium intake was higher in the high-hansik group than the low-hansik group in both genders, and its average intake, except the high-hansik in men, was less than the recommended intake (2,300 mg/day) in the American Heart Association (21). Furthermore, potassium intake was also higher in the high-hansik group than in the low-hansik group in both genders. On the other hand, vitamin D and calcium intake

TABLE 2 Demographic and sociographic characteristics according to gender and hansik intake defined by the K_{diet} -index score.

	Men (<i>n</i> = 20,293)		Women (<i>n</i> = 38,408)	
	Low-hansik (<i>n</i> = 6,271)	High-hansik (<i>n</i> = 14,022)	Low-hansik (<i>n</i> = 7,728)	High-hansik (<i>n</i> = 30,680)
Age (years)	54.1 ± 0.10 ^b	57.5 ± 0.07 ^a	50.7 ± 0.09 ^d	52.8 ± 0.04 ^{c***++++##}
Education				
≤Middle school	433 (11.9)	1,320 (14.9)	1,173 (20.3)	5,565 (22.6)
High school	2,773 (76.2)	8,661 (75.4)	4,225 (73.0)	17,646 (71.7)
≥College	432 (11.9)	859 (9.72) ^{##}	388 (6.71)	1,394 (5.67) ^{##}
Income				
≤\$2,000	407 (6.92)	1,199 (8.92)	739 (10.4)	3,433 (11.8)
\$2,000–4,000	2,359 (40.1)	5,848 (43.5)	2,952 (41.7)	13,033 (44.9)
>\$4,000	3,112 (52.9)	6,388 (47.6) ^{##}	3,396 (47.9)	12,574 (43.3) ^{##}
Exercise (%)	3,359 (54.1)	8,593 (61.3) ^{##}	3,421 (44.8)	16,603 (54.2) ^{##}
Former smoking	2,424 (39.0)	6,371 (45.5)	112 (1.47)	348 (1.14)
Smoking (%)	2,011 (32.3)	3,653 (26.1) ^{##}	219 (2.87)	530 (1.73) ^{##}
Stress (scores)	14.4 ± 0.15 ^c	13.2 ± 0.10 ^d	16.1 ± 0.15 ^a	15.3 ± 0.07 ^{b***++++}
Self-rated health (scores)	2.58 ± 0.02 ^b	2.53 ± 0.01 ^c	2.75 ± 0.02 ^a	2.73 ± 0.01 ^{a***++++}

The score of each component meeting the criteria was 1. If not, the score was 0. The K_{diet} -index score was calculated by summing the scores of each component in the 14 questionnaires. High- and low-hansik intake indicated the K_{diet} -index scores ≥ 8 or < 8 , respectively. A Higher K_{diet} -index score indicated a higher hansik intake. Stress status was estimated with 18 questions about the physical and psychological at home and work, and each question was scored 0 (lowest stress) to 3 (highest stress). The scores of the stress status were estimated with a summation of the scores of 14 questions. The higher scores of the stress status indicated having higher stress. ***Significant differences by genders at $P < 0.001$. ++Significant differences by hansik intake at $P < 0.01$, +++ $P < 0.001$. *Significant interaction between genders and hansik intake at $P < 0.05$, *** $P < 0.001$. ##Significantly different from the low-hansik group in χ^2 test in each gender at $P < 0.001$. ^{a,b,c,d}Different superscripts on the values indicated significant differences among the groups in Tukey's test at $p < 0.05$.

did not reach their recommended intake levels. DII scores were lower in the high-hansik group than in the low-hansik group in both genders (Table 4).

When the diet was clustered into 4 dietary patterns, the consumption of a Korean balanced diet (KBD) or a plant-based diet (PBD) was higher, and a Western-style diet (WSD) or a rice-main diet (RMD) was lower in the high-hansik group compared to the low-hansik group. Multigrain rice intake was higher in the high-hansik group than the low-hansik group, and noodle and bread intakes were opposite to multigrain intake (Table 4). It indicated that the Korean meal pattern was changed from a meal with multigrain rice to a meal with flour-based foods such as noodles and bread due to westernization. Alcohol intake was lower in the high-hansik group than in the low-hansik group for both genders (Table 4).

Scores for individual components of the K_{diet} -index to represent hansik intake

The score for “meal with multigrain rice” was lower in the MetS group than in the non-MetS group but only for men, indicating that men in the MetS group ate less rice than those in the non-MetS group. However, the scores for the eating frequencies of grains, kimchi, seaweed, cooked vegetables, meats, and soybean products were significantly higher in women

than men. However, there was no significant difference in MetS incidence between the genders (Table 5). The results suggested that though women consumed higher levels of grains, kimchi, seaweed, cooked vegetables, and soybean products and less meat from the hansik components than men, this did not affect the incidence of MetS (Table 5). The score for the eating frequency of soup did not show significant variations by gender or MetS incidence. The eating frequency of fruits and fruit products, processed foods, and nuts were affected by gender and MetS: the scores were higher in women than men and the non-MetS group than in the MetS group (Table 5). The sum of the K_{diet} -index scores of each component, called a total K_{diet} -index score, was higher in women than men and the non-MetS group than the MetS group, suggesting that women without MetS had a higher hansik intake than the others (Table 5).

Association of the K_{diet} -index and DII scores with MetS risk

Among all the components of hansik evaluated by the K_{diet} -index, it was inversely associated with MetS risk by 0.903 times (95% CI = 0.821–0.993) in total participants and women by 0.817 times (95% CI = 0.707–0.943) but not in men, after adjusting for covariates including age, gender, residence area, education, income, energy intake,

TABLE 3 Food intake according to gender and hansik intake defined by the K_{diet} -index score.

	Men (<i>n</i> = 20,293)		Women (<i>n</i> = 38,408)	
	Low-hansik (<i>n</i> = 6,271)	High-hansik (<i>n</i> = 14,022)	Low-hansik (<i>n</i> = 7,728)	High-hansik (<i>n</i> = 30,680)
Multigrain rice (g/day)	424 ± 5.24 ^c	569 ± 3.50 ^a	396 ± 5.06 ^d	475 ± 2.36 ^{b***++++##}
White rice (g/day)	230 ± 4.58 ^a	86.7 ± 2.94 ^c	148 ± 4.44 ^b	50.8 ± 2.05 ^{d***++++##}
Noodles (g/day)	85.6 ± 1.49 ^a	57.6 ± 0.96 ^b	59.9 ± 1.45 ^b	36.8 ± 0.66 ^{c***++++#}
Bread (g/day)	33.3 ± 0.79 ^a	29.1 ± 0.51 ^b	31.9 ± 0.77 ^a	25.3 ± 0.35 ^{c***++++#}
Fruits (g/day)	144 ± 4.36 ^c	229 ± 2.81 ^b	154 ± 4.24 ^c	262 ± 1.92 ^{a***++++##}
Vegetables (g/day)	217 ± 3.50 ^c	309 ± 2.25 ^a	193 ± 3.40 ^d	266 ± 1.54 ^{***++++##}
Kimchi (g/day)	119 ± 2.24 ^c	169 ± 1.45 ^a	96.9 ± 2.18 ^d	131 ± 0.99 ^{b***++++##}
Seaweeds (g/day)	1.32 ± 0.04 ^d	2.01 ± 0.03 ^b	1.49 ± 0.04 ^c	2.28 ± 0.19 ^{a***++++##}
Fish (g/day)	35.3 ± 0.80 ^c	45.0 ± 0.51 ^a	33.7 ± 0.77 ^c	39.5 ± 0.35 ^{b***++++##}
Meats (g/day)	55.5 ± 0.86 ^a	46.4 ± 0.55 ^b	44.1 ± 0.84 ^b	30.2 ± 0.38 ^{c***++++##}
Beans (g/day)	20.8 ± 0.48 ^b	28.1 ± 0.31 ^a	20.7 ± 0.47 ^b	27.5 ± 0.21 ^{a+++}
Fermented soybeans (g/day)	2.97 ± 0.08 ^c	4.27 ± 0.05 ^a	2.84 ± 0.08 ^c	3.95 ± 0.04 ^{b***+++}
Nuts (g/day)	1.07 ± 0.09 ^d	1.94 ± 0.06 ^b	1.42 ± 0.09 ^c	2.46 ± 0.04 ^{a***+++}
KBD (%)	1,886 (30.1)	6,515 (44.3) ^{##}	1,562 (20.2)	9,906 (32.3) ^{###}
PBD (%)	736 (11.7)	3,462 (24.7) ^{##}	1,952 (25.3)	13,426 (43.8) ^{###}
WSD (%)	4,074 (65.0)	6,357 (45.3) ^{##}	3,891 (50.4)	9,230 (30.1) ^{###}
RMD (%)	2,885 (45.7)	3,600 (25.7) ^{##}	3,346 (43.3)	9,760 (31.8) ^{###}
Alcohol (g/day)	32.0 ± 1.22 ^a	30.3 ± 0.72 ^a	9.68 ± 1.23 ^b	8.99 ± 0.49 ^{b***+}

The score of each component meeting the criteria was 1. If not, the score was 0. The K_{diet} -index score was calculated by summing the scores of each component in the 14 questionnaires. High- and low-hansik intake indicated the K_{diet} -index scores ≥ 8 or < 8 , respectively. A Higher K_{diet} -index score indicated a higher hansik intake. KBD, Korean-balanced diet; PBD, plant-based diet; WSD, Western-style diet; RMD, rice-main diet. **Significant differences by genders at $P < 0.01$, *** $P < 0.001$. +++Significant differences by hansik intake at $P < 0.001$. #Significant interaction between genders and hansik intake at $P < 0.05$, ## at $P < 0.01$, ### $P < 0.001$. ###Significantly different from the low-hansik group in χ^2 test in each gender at $P < 0.001$. ^{a,b,c,d}Different superscripts on the values indicated significant differences among the groups in Tukey's test at $p < 0.05$.

TABLE 4 Nutrient intake according to gender and hansik intake defined by the K_{diet} -index score.

	Men (<i>n</i> = 20,293)		Women (<i>n</i> = 38,408)	
	Low-hansik (<i>n</i> = 6,271)	High-hansik (<i>n</i> = 14,022)	Low-hansik (<i>n</i> = 7,728)	High-hansik (<i>n</i> = 30,680)
Energy (EEE %)	86.1 ± 0.42 ^c	91.9 ± 0.28 ^b	92.8 ± 0.38 ^b	101 ± 0.19 ^{a***++++##}
Carbohydrate (En %)	70.9 ± 0.09 ^c	71.7 ± 0.06 ^b	71.0 ± 0.09 ^c	72.1 ± 0.04 ^{a***++++#}
Fat (En %)	14.6 ± 0.07 ^a	13.8 ± 0.05 ^b	14.5 ± 0.07 ^a	13.6 ± 0.03 ^{c***++++#}
Protein (En %)	12.8 ± 0.04 ^c	13.4 ± 0.02 ^b	13.2 ± 0.03 ^b	13.6 ± 0.02 ^{a***++++#}
Fiber (g/day)	14.3 ± 0.11 ^d	17.2 ± 0.07 ^b	12.1 ± 0.10 ^c	14.1 ± 0.05 ^{a***++++##}
Calcium (mg/day)	380 ± 2.64 ^b	475 ± 1.77 ^a	372 ± 2.39 ^b	457 ± 1.18 ^{a***++++#}
Potassium (mg/day)	2,034 ± 15.6 ^c	2,503 ± 10.4 ^a	1,882 ± 15.0 ^d	2,299 ± 7.02 ^{b***++++##}
Sodium (mg/day)	2,267 ± 25.0 ^b	2,863 ± 16.7 ^a	1,918 ± 24.2 ^c	2,338 ± 11.3 ^{b***++++##}
Vitamin C (mg/day)	79.6 ± 0.75 ^b	113 ± 0.51 ^a	80.8 ± 0.68 ^b	113 ± 0.34 ^{a+++}
Vitamin D (ug/day)	5.28 ± 0.07 ^d	6.49 ± 0.05 ^b	5.70 ± 0.06 ^c	6.79 ± 0.03 ^{***+++}
DII (scores)	−17.6 ± 1.28 ^{ab}	−26.6 ± 1.02 ^c	−14.6 ± 1.64 ^a	−20.8 ± 0.85 ^{b***+++}
Total polyphenol (mg/day)	2,308 ± 19.9 ^c	2,959 ± 13.3 ^a	2,127 ± 19.2 ^d	2,566 ± 8.94 ^{b***++++##}
Flavonoids (mg/day)	25.3 ± 0.39 ^d	38.9 ± 0.26 ^b	28.0 ± 0.36 ^c	43.3 ± 0.18 ^{a***++++##}

The score of each component meeting the criteria was 1. If not, the score was 0. The K_{diet} -index score was calculated by summing the scores of each component in the 14 questionnaires. High- and low-hansik intake indicated the K_{diet} -index scores ≥ 8 or < 8 , respectively. A Higher K_{diet} -index score indicated a higher hansik intake. EEE, estimated energy requirement; En%, energy percent; DII, dietary inflammation index.

Significant differences by genders at $P < 0.01$, * $P < 0.001$. +++Significant differences by hansik intake at $P < 0.001$. #Significant interaction between genders and hansik intake at $P < 0.05$, ## at $P < 0.01$, ### $P < 0.001$. ^{a,b,c,d}Different superscripts on the values indicated significant differences among the groups in Tukey's test at $p < 0.05$.

TABLE 5 The K_{diet} -index score of each component for hansik definition according to gender and metabolic syndrome (MetS).

	Men (<i>n</i> = 20,293)		Women (<i>n</i> = 38,408)	
	Non-MetS (<i>n</i> = 16,695)	MetS (<i>n</i> = 3,598)	Non-MetS (<i>n</i> = 33,706)	MetS (<i>n</i> = 4,702)
Meals containing multigrain rice	0.86 ± 0.004 ^b	0.81 ± 0.008 ^c	0.90 ± 0.003 ^a	0.88 ± 0.007 ^{ab***++##}
Eating frequencies of grains	0.80 ± 0.005 ^b	0.81 ± 0.009 ^b	0.89 ± 0.003 ^a	0.89 ± 0.008 ^{a***}
Eating frequencies of kimchi	0.65 ± 0.006 ^a	0.63 ± 0.011 ^a	0.54 ± 0.004 ^b	0.56 ± 0.010 ^{b***#}
Eating frequencies of soup made with fermented soybeans	0.20 ± 0.005	0.21 ± 0.01	0.19 ± 0.004	0.19 ± 0.009
Eating frequencies of seaweeds	0.19 ± 0.006 ^b	0.20 ± 0.01 ^b	0.25 ± 0.004 ^a	0.25 ± 0.009 ^{a***}
Cooked vegetables with garlic, onions, and ginger	0.20 ± 0.006 ^b	0.22 ± 0.01 ^b	0.31 ± 0.004 ^a	0.31 ± 0.010 ^{a***}
Using frequencies of sesame or perilla oil	0.05 ± 0.003 ^a	0.07 ± 0.005 ^a	0.03 ± 0.002 ^b	0.04 ± 0.005 ^{b***+}
Eating frequencies of meats including beef, pork, and chicken	0.40 ± 0.006 ^b	0.39 ± 0.012 ^b	0.58 ± 0.004 ^a	0.61 ± 0.011 ^{a***}
Eating frequencies of fried foods	0.6222 ± 0.007 ^b	0.612 ± 0.012 ^b	0.650 ± 0.005 ^a	0.621 ± 0.011 ^{b+}
Eating frequencies of fish or clams	0.15 ± 0.005 ^b	0.15 ± 0.009 ^b	0.20 ± 0.003 ^a	0.20 ± 0.008 ^{a***}
Eating frequencies of soybean products	0.19 ± 0.006 ^b	0.19 ± 0.010 ^b	0.26 ± 0.004 ^a	0.25 ± 0.009 ^{a***}
Eating frequencies of fruits and fruit juicy	0.33 ± 0.007 ^c	0.31 ± 0.012 ^c	0.53 ± 0.005 ^a	0.49 ± 0.011 ^{b***+++}
Eating frequencies of processed foods	0.82 ± 0.005 ^c	0.82 ± 0.008 ^c	0.90 ± 0.003 ^a	0.86 ± 0.008 ^{b***##}
Eating frequencies of nuts	0.16 ± 0.01 ^c	0.13 ± 0.01 ^d	0.29 ± 0.004 ^a	0.24 ± 0.01 ^{b***+++}
Total K_{diet} -index score	6.38 ± 0.03 ^c	6.34 ± 0.05 ^c	6.94 ± 0.02 ^a	6.78 ± 0.05 ^{b***++}

The score of each component meeting the criteria was 1. If not, the score was 0. The K_{diet} -index score was calculated by summing the scores of each component in the 14 questionnaires. High- and low-hansik intake indicated the K_{diet} -index scores ≥ 8 or < 8 , respectively. A Higher K_{diet} -index score indicated a higher hansik intake. Adjusted means and standard errors were calculated with adjusted for confounding factors such as age, energy intake, body mass index (BMI), residence area, education, income, alcohol intake, smoking status, and physical activity. ***Significant differences by genders at $P < 0.001$. + Significant differences by hansik intake at $P < 0.05$, at ++ $P < 0.01$, +++ $P < 0.001$. #Significant interaction between genders and hansik intake at $P < 0.05$, ## at $P < 0.01$. ^{a,b,c,d} Different superscripts on the values indicated significant differences among the groups in Tukey's test at $p < 0.05$.

exercise, alcohol intake, and smoking status (Figure 1A). K_{diet} -index and DII showed an inverse relationship in both genders ($P < 0.05$). DII was positively associated with MetS risk in total participants and women, not in men ($P < 0.05$; Figure 1A). These results suggested that hansik intake could be an anti-inflammatory diet to decrease MetS risk.

In the component of the K_{diet} -index, the high intake of a meal with multigrain rice, fruits, and their products, and nuts lowered the risk of MetS 0.707, 0.864, and 0.769 times, respectively, after adjusting for covariates. The low intake of fried foods resulted in a 0.918 time lower risk of MetS in all participants (Figure 1B). The differences in some components were shown in the MetS and non-MetS groups in both genders, but their differences were weaker in men than women. In men, the risk of MetS was 0.663 times lower with the intake of rice as part of the hansik. The scores for processed and fried food intake were higher in the MetS group than in the non-MetS group in women but not in men (Figure 1B), indicating that women with MetS were a lower intake of processed and fried foods. In contrast, in men, the meal with multigrain rice and eating frequencies of nuts were higher in the non-MetS group than in the MetS (Figure 1B). The scores of total K_{diet} -index was higher in non-MetS and MetS in both

gender. Therefore, people consuming a meal containing rice, fruits, and nuts as a dessert or snack and less processed and fried foods might be less susceptible to the risk of MetS.

Hansik intake scored by the K_{diet} -index and MetS and its components according to gender

The frequency of MetS incidence did not vary significantly between the low-hansik and high-hansik groups. However, the adjusted odds ratio (OR) for MetS was inversely related to high-hansik intake in all participants, especially women (Table 6). In the two-way ANCOVA adjusted for covariates, the body composition indices significantly differed with gender and hansik intake. The BMI, waist circumferences, and fat mass were inversely associated with the high-hansik group, and the skeletal muscle mass index (SMI) was positively associated with the high-hansik group in all participants regardless of gender (Table 6). It indicated that the high-hansik intake was inversely associated with obesity and abdominal obesity. However, glucose metabolism, including fasting serum glucose concentrations, glycosylated hemoglobin (HbA1c), and insulin resistance, did not significantly differ between the low-hansik and high-hansik

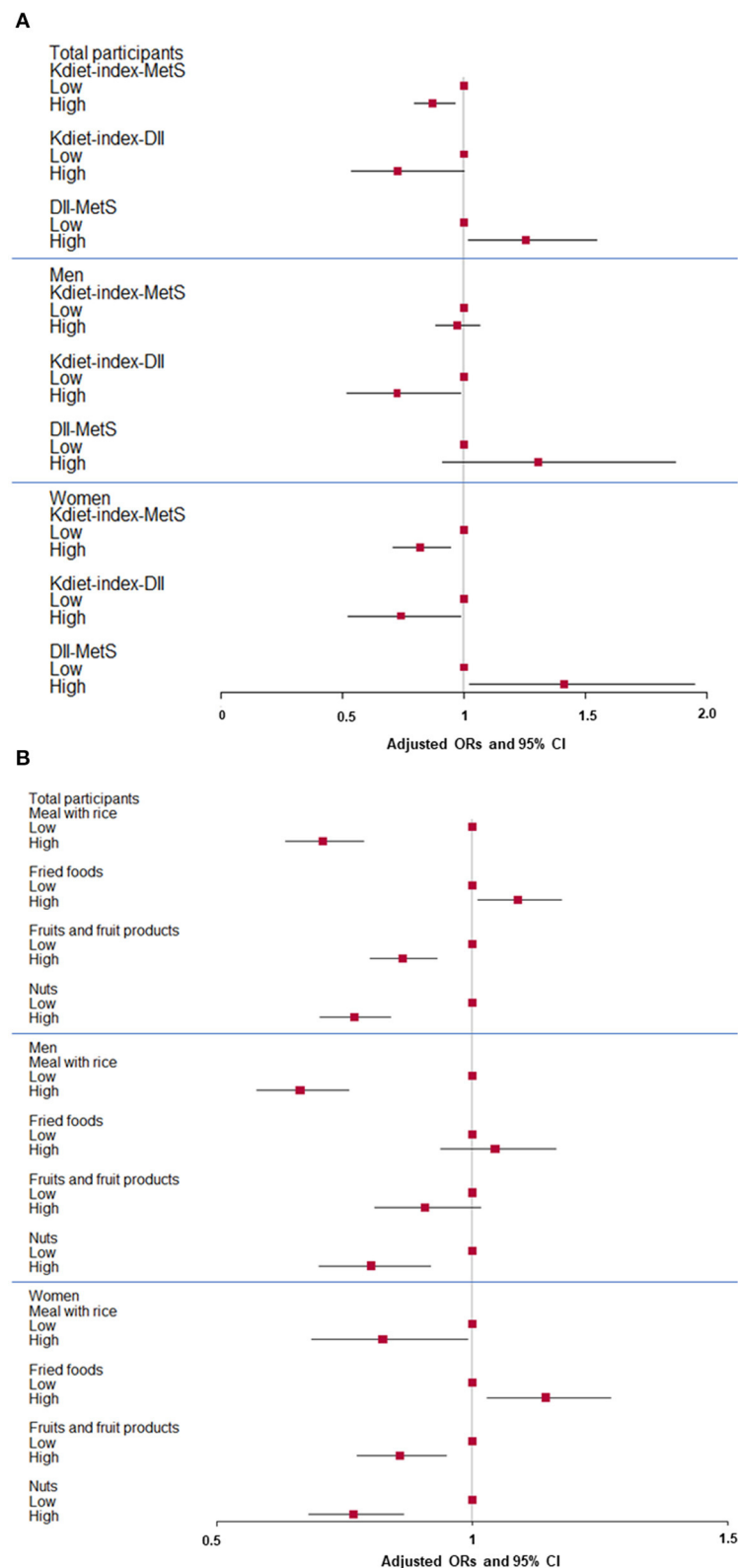


FIGURE 1

Adjusted odds ratio (ORs) and 95% confidence intervals (CI) of hansik intake evaluated with K_{diet} -index score and metabolic syndrome risk (MetS). **(A)** Association of K_{diet} -index score with MetS and dietary inflammatory index (DII) and association of MetS and DII in men and women. **(B)** Association of some components of K_{diet} -index with MetS in men and women. Hansik was defined with 14 components with portion sizes and scores. The cutoffs of a meal with multigrain rice, fried food intake, fruits, and fruit products, and nut intake were \geq twice per day, <3 times per week, ≥ 2 servings per day, and \geq once a day, respectively. Red squares and lines indicated adjusted ORs and 95% CI, respectively. They were shown in total participants, men, and women.

TABLE 6 Characteristics of metabolic parameters according to gender and hansik intake defined by the K_{diet} -index score and their associations.

	Men (<i>n</i> = 20,293)			Women (<i>n</i> = 38,408)			Total
	Low-hansik (<i>n</i> = 6,271)	High-hansik (<i>n</i> = 14,022)	Adjusted ORs and 95% CI	Low-hansik (<i>n</i> = 7,728)	High-hansik (<i>n</i> = 30,680)	Adjusted ORs and 95% CI	Adjusted ORs and 95% CI
MetS (%) ¹	1,084 (17.3)	2,514 (18.0)	0.923 (0.843–1.010)	888 (11.5)	3,814 (12.4)	0.876 (0.799–0.961)	0.874 (0.820–0.932)
BMI (mg/kg ²) ²	24.6 ± 0.04 ^a	24.4 ± 0.03 ^b	0.926 (0.863–0.992)	23.6 ± 0.04 ^c	23.6 ± 0.02 ^{c***++###}	0.886 (0.830–0.946)	0.888 (0.847–0.931)
Waist (cm) ³	86.0 ± 0.11 ^a	85.4 ± 0.07 ^b	0.911 (0.842–0.985)	78.0 ± 0.10 ^c	78.1 ± 0.05 ^{c***++###}	0.886 (0.830–0.946)	0.909 (0.861–0.961)
SMI ⁴	7.14 ± 0.01 ^a	7.18 ± 0.01 ^a	1.912 (1.731–2.113)	6.11 ± 0.01 ^b	6.12 ± 0.01 ^{b***}	1.673 (1.530–1.828)	1.759 (1.646–1.879)
Fat mass (%) ⁵	23.2 ± 0.07 ^b	23.0 ± 0.04 ^c	0.899 (0.809–0.999)	30.7 ± 0.07 ^a	30.6 ± 0.03 ^{c***++}	0.866 (0.786–0.955)	0.877 (0.816–0.942)
Serum glucose (mg/dL) ⁶	98.6 ± 0.42 ^a	98.2 ± 0.27 ^a	1.036 (0.918–1.169)	94.0 ± 0.41 ^b	93.6 ± 0.18 ^{b***}	0.897 (0.769–1.047)	0.968 (0.880–1.065)
HbA1c (%) ⁷	5.72 ± 0.02	5.72 ± 0.01	1.397 (1.121–1.740)	5.71 ± 0.02	5.71 ± 0.01	1.141 (0.885–1.472)	1.214 (0.948–1.500)
Insulin resistance ⁸	700 (11.2)	1,612 (11.5)	0.967 (0.831–1.125)	419 (5.42)	1,873 (6.10)	0.866 (0.711–1.056)	0.917 (0.813–1.034)
Serum total cholesterol ⁹	195 ± 0.77 ^b	189 ± 0.52 ^c	0.964 (0.855–1.086)	200 ± 0.74 ^a	201 ± 0.35 ^{a***++###}	0.963 (0.865–1.071)	0.937 (0.866–1.014)
Serum HDL ¹⁰	49.3 ± 0.28 ^b	49.3 ± 0.19 ^b	0.933 (0.826–1.053)	57.0 ± 0.27 ^a	57.3 ± 0.13 ^{a***++}	1.103 (1.001–1.215)	1.039 (0.963–1.120)
Serum LDL ¹¹	117 ± 0.71 ^b	113 ± 0.47 ^c	0.978 (0.850–1.125)	121 ± 0.68 ^a	120 ± 0.32 ^{a***++###}	0.943 (0.834–1.066)	0.935 (0.854–1.025)
Serum TG ¹²	141 ± 1.77 ^a	134 ± 1.18 ^b	0.990 (0.899–1.090)	114 ± 1.71 ^c	115 ± 0.80 ^{c***++}	0.970 (0.870–1.083)	0.955 (0.889–1.026)
SBP (mmHg) ¹³	127 ± 0.31 ^a	125 ± 0.20 ^b	0.905 (0.823–0.996)	121 ± 0.30 ^c	121 ± 0.14 ^{c***++}	1.031 (0.930–1.142)	0.947 (0.883–1.015)
DBP (mmHg) ¹⁴	79.2 ± 0.21 ^a	78.2 ± 0.14 ^b	0.859 (0.750–0.984)	74.1 ± 0.20 ^c	74.2 ± 0.09 ^{c***++###}	0.864 (0.729–1.023)	0.849 (0.765–0.944)
Serum hs-CRP ¹⁵	0.16 ± 0.01 ^a	0.14 ± 0.01 ^a	0.832 (0.592–1.169)	0.13 ± 0.01 ^{ab}	0.13 ± 0.004 ^{b*}	0.903 (0.585–1.393)	0.848 (0.650–1.106)
GFR ¹⁶	81.8 ± 0.33 ^c	84.0 ± 0.22 ^b	1.363 (1.204–1.543)	84.1 ± 0.32 ^b	87.3 ± 0.45 ^{a***++###}	1.784 (1.572–2.025)	1.535 (1.405–1.678)
Serum AST (U/L) ¹⁷	25.5 ± 0.25 ^a	24.9 ± 0.17 ^b	0.856 (0.698–1.050)	23.1 ± 0.24 ^c	22.9 ± 0.11 ^{c***+}	0.991 (0.763–1.289)	0.897 (0.765–1.053)
Serum ALT(U/L) ¹⁸	26.7 ± 0.37 ^a	25.7 ± 0.25 ^a	1.010 (0.891–1.144)	19.7 ± 0.36 ^b	19.8 ± 0.17 ^{b****}	0.956 (0.801–1.141)	0.969 (0.875–1.072)

The score of each component meeting the criteria was 1. If not, the score was 0. The K_{diet} -index score was calculated by summing the scores of each component in the hansik definition. High- and low-hansik intake indicated the K_{diet} -index scores ≥ 8 or < 8 , respectively. A Higher K_{diet} -index score indicated a higher hansik intake. ORs, Odds ratio; CI, confidence intervals. Covariates for adjustment included age, energy intake, body mass index (BMI), residence area, education, income, alcohol intake, smoking status, and physical activity. The cutoff points of hansik intake for logistic regression were as following: ¹Metabolic syndrome (MetS) criteria; ² < 25 kg/m² for body mass index (BMI); ³ < 90 cm for men and 85 cm for women waist circumferences; ⁴ < 29.0 % for men and 22.8 % for women in skeletal muscle index (SMI; defined as appendicular skeletal muscle mass/height); ⁵ < 25 % for men and 30% for women for fat mass; ⁶ < 126 mg/dL fasting serum glucose plus diabetic drug intake; ⁷ < 6.5 % HbA1c plus diabetic drug intake; ⁸ < 2.54 HOMA-IR; ⁹ < 230 mg/dL serum total cholesterol concentrations; ¹⁰ < 40 mg/dL for men and 50 mg/dL for women serum HDL cholesterol; ¹¹ < 160 mg/dL serum LDL cholesterol concentrations; ¹² < 150 mg/dL serum triglyceride concentrations; ¹³ < 140 mmHg systolic blood pressure (SBP); ¹⁴ diastolic blood pressure (DBP) < 90 mmHg plus hypertension medication; ¹⁵ < 0.5 mg/dL serum high sensitive-C-reactive protein (hs-CRP) concentrations; ¹⁶ Estimated glomerular filtration rate (GFR) < 60 ; ¹⁷ < 40 U/L Aspartate aminotransferase; ¹⁸ < 35 U/L Alanine aminotransferase. *Significant differences by genders at $P < 0.05$, *** $P < 0.001$. +Significant differences by hansik intake at $P < 0.05$, at ++ $P < 0.01$, +++ $P < 0.001$. #Significant interaction between genders and hansik intake at $P < 0.05$, ## at $P < 0.01$, ### $P < 0.001$. ^{a,b,c}Different superscripts on the values indicated significant differences among the groups in Tukey's test at $P < 0.05$.

groups (Table 6). There was no significant association between serum glucose concentration, HbA1c, and insulin resistance in the high-hansik group in both genders. After adjusting for covariates, serum total cholesterol, LDL cholesterol, and triglyceride concentrations were significantly affected by gender and hansik intake scored by the K_{diet} -index in all participants (Table 6). Serum concentrations of total cholesterol and LDL cholesterol but not triglyceride were higher in women than men, while they were lower in the high-hansik group than in the low-hansik group in men but not women. Serum HDL cholesterol concentrations were higher in women than men, but they were not significantly different between low- and high-hansik groups (Table 6). Men with a low-hansik intake had higher SBP and DBP, and high-hansik intake was associated with lower SBP

and DBP in men. Serum high-sensitivity C-reactive protein (hsCRP) concentrations were not significantly related to the high-hansik group for both genders. However, the estimated glomerular filtration rate (GFR) was much higher in the high-hansik group than in the low-hansik group for both genders, and it was positively associated with high-hansik intake in men, women, and all participants by 1.36, 1.78, and 1.54 times, respectively (Table 6). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were higher in men than women. However, only serum AST activities were higher in the low-hansik group than in the high-hansik group in men. However, there was no association between the high-hansik and low-hansik groups in serum ALT activities (Table 6).

Discussion

The prevalence of MetS increased in Korean men but not women from 2008 to 2017, according to the Korea National Health and Nutrition Examination Survey (KNHANES) (22). It may be related to the differences in diet among men and women. Korean women usually adhere to the hansik diet pattern with multigrain rice, soup, kimchi, and three side dishes. Hansik is known to be high in salt, and there have been diverse opinions about the health benefits of salt intake (23). However, the salts in hansik mainly come from jangs made with solar salts and fermented soybeans, which have been reported to be beneficial for metabolic diseases (24–26). The present study demonstrated that a high meal containing multigrain, fruits, and nuts and a low intake of fried foods based on a K_{diet} -index composed of 14 hansik-related components was inversely associated with MetS risk.

Further, the participants with high SRH scores had higher K_{diet} -index scores than those with low SRH. women on a high-hansik diet had 0.88 times lower risk of MetS. The high-hansik diet was inversely associated with the MetS components, namely BMI, waist circumference, and fat mass, and positively associated with SMI, an indicator of skeletal muscle mass in both genders. However, among the lipid parameters measured, high hansik intake was inversely associated only with hypo-HDL cholesterolemia. Therefore, hansik intake lowered MetS risk mainly by modulating body composition, particularly in women. Meals with rice might be a primary factor for Asians being leaner than Caucasians.

In Korea, from 2008 to 2017, the MetS prevalence in men increased by 3.6%, but that in women decreased marginally by 1.8%. It may be linked to several lifestyle factors other than diet alone. In the present study, a higher proportion of men belonged to the former and current-smoker group than women, while men and women drank about 31 and 9 g/day of alcohol, respectively. However, men exercised more than women and had lower stress scores. Moreover, men consumed a higher proportion of balanced KBD and WSD than women but less PBD and RMD. Energy (EER %), carbohydrates, and protein intakes were much higher in women than men, but fat intake was higher in men than women in the present study. It is difficult to explain the gender differences in the MetS risk based on lifestyles and nutrient intake. Gender-specific risk factors for MetS have been demonstrated in some studies (27, 28). In two studies in China, a more diversified diet was associated with a lower MetS risk among young women (27), while “animal and fried food” and “high-salt and energy” dietary patterns were observed to be related to a higher risk of MetS in men and women, respectively (28). In the KNHANES, high fruit and juice intakes were lower in the MetS group than in the non-MetS group in men, while breakfast intake was lower in the MetS group than in the health group in women (29). Therefore, the individual

components of the diet may influence MetS risk differently for each of the genders.

Previous studies have demonstrated that SRH is influenced by disease status, mental health, nutrient intake, and other risk factors such as smoking and alcohol drinking. A higher SRH is strongly linked to lower mortality risk (30, 31). However, the relationship between SRH and diet quality is inconsistent. In KHANES 2007–2014, SRH was strongly related to a higher intake of nutritious foods such as vegetables and milk in adults (32). However, SRH was not associated with the overall diet quality in adolescent children, although it was positively associated with a high vegetable intake and negatively linked to a high fat intake (33). Therefore, people in Korea consider hansik containing vegetables, fruits, and fish to be a healthy diet; however, they should be taught the right way to consume hansik to prevent metabolic diseases in the adult population.

Hansik is a traditional Korean balanced diet: it includes cooked multigrain rice (jabgogbab), soup (kuk), kimchi, fish, and 2 different vegetables (banchan). Traditional Korean cooking methods are boiling, blanching, seasoning, fermenting, and picking, but frying and baking are not commonly used (7). Kuk is made of jangs instead of salt, with vegetables and meat. Banchan is seasoned with jangs, and herbs, including red pepper, garlic, onion, sesame oil, and perilla oil. The hansik diet pattern was defined using 14 components, and the cutoff of each questionnaire was assigned to describe hansik according to the present study based on the previous studies (7). The Mediterranean diet described by the 14 items exhibits a strong inverse linear association with the adiposity index in adults at high cardiovascular risk. Among the 14 items of the K_{diet} -index, a high intake of nuts and a low intake of sweetened/carbonated beverages exhibited the highest inverse relationship with abdominal obesity (34). These results suggest that adherence to the Mediterranean diet prevents MetS risk. Like the Mediterranean diet, a higher intake of hansik (higher total hansik scores) was inversely associated with MetS, especially abdominal obesity-related components in MetS, only in women through low-fat mass and high skeletal muscle mass. Among the 14 components, the scores of meals with rice, eating frequency of fried foods, frequency of consumption of fruits and their products, and eating frequency of processed foods and nuts were significantly different between the non-MetS and the MetS groups only in women in the present study. The sum of 14 hansik component scores was higher in women than men and in the non-MetS group rather than the MetS group. They were highest in the women without MetS. These results indicate that women had better adherence to hansik intake than men and were inversely linked to MetS risk. However, hansik intake was not associated with hyperglycemia in both genders.

Meals with multigrain rice, fish, and vegetables, representing a balanced diet, have been reported to be inversely associated with abdominal obesity in Korea, China, and Japan (35–37),

as seen in the present study. Residents of Southern China have traditionally consumed multigrain rice with pork and vegetables and have a lower risk of abdominal obesity (35). Unlike Southern China, people in Northern China traditionally have flour made of wheat, which is positively associated with abdominal obesity (35). Whole grain intake is prospectively and inversely associated with abdominal obesity in adults in the Framingham Offspring cohort and HUNT study (38, 39). However, a white rice-main diet with a lower nutrient score similar to RMD is positively associated with obesity and MetS risk (8, 40). In Korea, a WSD high in noodles, bread, and meats, is positively associated with MetS risk, and KBD alleviated dyslipidemia in RCT (9, 10, 41). Therefore, a Korean-balanced diet with multigrain rice may ameliorate abdominal obesity and MetS risk compared to flour-based meals, including noodles and bread. In the present study, the participants who adhered to the hansik diet pattern had a higher SRH, indicating that people thought a hansik diet would be healthier than a WSD.

Consuming vegetables, seaweed, and fruits is known to reduce MetS risk. Vegetable and fruit intakes are inversely associated with MetS risk (OR: 0.86 and 95% CI: 0.80–0.92 for vegetables; OR: 0.86 and 95% CI = 0.77–0.96 for fruits) in meta-analysis with the studies in Asia (42). However, the meta-analysis with RCTs with MetS patients has shown that vegetables were inversely associated with diastolic blood pressure but no other metabolic syndrome components (43). Seaweed intake (4–6 g/day) is inversely associated with MetS risk in an RCT (44). They are known as healthy foods, but their cooking methods are crucially linked to health. In hansik, raw and cooked vegetables and seaweeds are seasoned with fermented soybeans called jang, perilla oil, and sesame oil in every meal with multigrain rice. Perilla oil and sesame oil include high linoleic acid and linolenic acid, respectively, which are known to prevent and alleviate dyslipidemia and blood pressure (45). Oils can make fat-soluble components in vegetables be absorbed better (46). Therefore, vegetable dishes in hansik are cooked to be healthier.

Furthermore, fruits are used as a dessert instead of sweets. A portion size of fruits is about 2 pieces of seasonal fruits such as apple, pear, persimmon, melon, and tangerine. Their intake of high-hansik (229 ± 2.81 g for men and 262 ± 262 g for women per day) met the recommended intake from the Korea Nutrition Society and Center for Disease Control and Prevention (2 servings/day; about 200–300 g/day) (47). Nuts have been consumed as a component of snacks and side dishes. Peanuts, walnuts, and dried seeds, including sunflower, sesame, and perilla seeds, have been traditionally used as the components of seasoning and snacks. Nut intake is beneficial for preventing MetS, overweight, and obesity in a meta-analysis of six prospective cohort studies and 62 RCTs (48). In addition, a 13-year population-based prospective study has demonstrated that nuts intake is inversely associated with MetS risk and severity (49). Therefore, the nut intake in adults with high-hansik may have a beneficial impact on reducing MetS risk.

Although hansik is considered a healthy diet in Korea, the impact of its high sodium content on health has been controversial. In the present study, sodium intake was higher in the high-hansik group than the low-hansik group. However, it was lower than the sodium recommended intake (2,500 mg/day) in women (2,338 mg/day) and slightly higher in men (2,863 mg/day). Potassium intake was also higher in the high-hansik than in the low-hansik. Furthermore, GFR was higher in the high-hansik than the low-hansik, suggesting that sodium excretion was better in the high-hansik group than the low-hansik group. Consistent with the GFR result, blood pressure was lower in the high-hansik group than in the low-hansik group in the present study. Consistent with the result, a previous study has shown that MetS is positively associated with urinary sodium and potassium ratio and GFR regardless of sodium intake (50). However, high salt intake is well-known for hypertension and MetS development, and less salt usage in hansik cooking has been promoted in Korean Health Department. Hansik containing less salt would be a better diet for preventing MetS risk.

In addition, hansik has not traditionally included milk and milk products which are critical nutritious factors, especially for bone health and metabolic diseases (29, 51). The intake of milk and milk products is low among Koreans, which is related to their exclusion from the components of hansik. However, participants with high-hansik intake had a higher calcium intake (M: 116 ± 2.77 ; F: 137 ± 1.29 mL) than those with low-hansik (M: 107 ± 2.87 ; F: 120 ± 1.92 mL) in the present study. It suggests that the participants with a high-hansik intake had a high SHR score and consumed more healthy foods, including milk and milk products, than those with a low-hansik intake. However, they still did not meet the recommended milk intake levels (52). Thus, efforts should be made to include milk and milk products in hansik meals.

The strength of the present study was that we were able to demonstrate that hansik intake scored by the K_{diet} -index was inversely associated with MetS as observed with the Mediterranean diet. However, there were some limitations. First, the results potentially included reverse causality bias due to the use of data from cross-sectional design studies. Second, the measurements for food intake were self-reported and could be over- or underreported: the participants were more likely to report beneficial food intake if they had diseases or were overweight/obese. Therefore, the results might have some potential bias.

Conclusions

Consuming hansik, consisting of multigrain rice with fruits and nuts, might improve MetS risk, primarily modulating body fat and increasing muscle mass, particularly in women, and blood pressure and dyslipidemia in men. As Koreans

believe that consuming a hansik diet improves health status, compliance with the hansik diet pattern might prevent and alleviate MetS risk. However, the hansik diet pattern did not meet the recommended calcium intake levels; hence, milk and milk products should be included as part of the side dishes in the hansik-based diet.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board (IRB) of the National Institute of Health, Korea (KBP-2015-055) and Hoseo University (HR-034-01). The patients/participants provided their written informed consent to participate in this study.

Author contributions

HY and SP conceptualized the study. M-SK acquired funding and supervised the research projects. MK, HH, and

SP analyzed the data. MK, M-SK, DJ, and SP prepared the manuscript with contributions. All authors approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Olive oil, Greek Mediterranean diet heritage and honoring the past to secure our future: Priorities for research and education

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Introduction

The Nobel prize winning Greek poet Odysseas Elytis wrote, “If Greece is completely destroyed, what will remain is an olive tree, a vine and a boat; this is enough to begin again” (1). Indeed, some plants, like the olive tree, and the grapevines, have evidently been in Greece forever. In the 1953 Rockefeller Report entitled “Crete: a case study of an underdeveloped area,” Allbaugh mentions: “olives, cereal grains, pulses, fruit, wild greens and herbs, together with limited quantities of goat meat and milk, game, and fish consist the basic Cretan foods... no meal was complete without bread... Olives and olive oil contributed heavily to the energy intake... food seemed literally to be “swimming” in oil” (2). In traditional Greek cuisine, olive oil is used in almost all culinary practices and applications. The cooking term *ladera*, originates from the word *ladi*, Greek for oil, and generally describes vegetables cooked in plenty of olive oil, onions, garlic, tomatoes and various herbs. These are the initial steps of making *ladera* dishes and the cooking practice of Greek cuisine called *tsigarisma* (sautéing)—basically to “sauté.” Onions and garlic would first be wilted or softened, for seconds, in a frying pan with a few tablespoons of hot olive oil, a practice that adds a certain depth of flavor. Subsequently, vegetables, grated tomatoes, various aromatic herbs, and a small amount of water is then added and the food is essentially left to cook on its own, traditionally over a low flame (3).

This combination of tomato, olive oil, garlic, onion and herbs in *ladera* increases the amount of polyphenols and carotenoids. Lightly frying vegetables with olive oil makes the vegetables healthier. Olive oil has the ability to act as a food excipient, which helps to release and absorb bioactive compounds from garlic, onion and tomato (4)¹.

1 On 3 May 2022, Dr Antonia Trichopoulou was invited to the Pontifical Academy of Sciences Conference on “The art of science of olive oil: nutrition, health and planetary health”, in Rome, and addressed the participants with the lecture entitled: “Olive Oil and the Heritage of the Greek Mediterranean diet: Priorities for Research and Education”. The address was based on the findings of the Greek National Health and Nutrition Survey HYDRIA.

Indeed, we know the importance of olive oil both culturally and for health—but do we still respect our heritage? What are Greek people eating today? Are they still eating the “healthy” traditional Mediterranean diet (5)? A diet with olive oil at its core and which has been a central part of Greek culture and daily life evidently forever (6).

Greek national health and nutrition survey -HYDRIA and dietary patterns

Answers to these questions are offered by the Greek National Health and Nutrition survey—HYDRIA, where food and macronutrient intake of the population in Greece were investigated along with an evaluation of adherence to the traditional Greek Mediterranean diet. In this survey, implemented in 2013–2014, adults over 18 years old ($n = 4,011$) were included (7). The HYDRIA survey design followed the recommendations of the European Health Examination Survey (EHES) (8) and the European Food Safety Authority (EFSA) (9) for the collection of health and dietary data at national level, allowing for inter-country comparisons (10).

The HYDRIA survey is a nationally representative survey. The selection of eligible individuals was based on the latest Greek general population census completed in 2011, of men and women aged 18 years and over, residing permanently in the country. The sampling frame covered all the 51 prefectures of the 13 regions of Greece and implemented a harmonized EU Member States protocol to collect data which allowed for direct comparison with other European countries (10). The HYDRIA survey provides a robust nationally representative sample, standardized data collection, and a comprehensive picture of today's Greek dietary pattern (7). Key elements of the findings provide important insights. Significantly, it was found that the contribution of total fat in adults was higher than the 20–35% of dietary reference intake ranges suggested by EFSA (11). In both men and women, 42–43% of total energy intake was from fat. Monounsaturated fatty acids (MUFA) represented 20.3% of men's and 20.6% of women's total energy intake. Saturated fatty acid (SFA) represented 13.1 and 13.5% of men's and women's total energy intake, respectively. Polyunsaturated fatty acid (PUFA) represented 5.7% of men's total energy intake with 5.9% for women. A higher MUFA percentage of total energy intake was noted for those individuals above 65 years of age at 21.9–21.3 vs. 20.1% for the younger adults surveyed. The high consumption of monounsaturated fats from olive oil was the main contributor of overall fat intake, with median distribution of the usual daily intake from fats and oils being 44 g for men and 32 g for women overall, with olive oil alone representing 34 g and 25 g for men and women, respectively (11).

When looking at the usual mean intakes in a diet of 2,000 kcal/day, it becomes clear that both men and women over the age of 65 eat less meat than their younger counterparts,

balanced by more fish, dairy, legumes, fruit and vegetables (excluding potatoes), and olive oil. Similarly, older men and women have a higher median intake of fats and oils at 46 and 32 g/day, respectively, mainly from olive oil, than younger age groups. Younger adults eat more meat, cereals and sugar products, along with consuming more non-alcoholic and alcoholic beverages (11).

Understanding dietary supplement use

The HYDRIA survey provides comprehensive data on dietary supplement use in Greece. Dietary supplements (DS) are used by 31% of the overall Greek population. DS use is higher by those living in urban areas and a considerably higher percentage of women, 40%, take DS in Greece, particularly those who eat a higher amount of fruit and those with a chronic medical condition. Men DS users represent only 22% of the population and tend to be those who have a good or very good self-reported health status (12).

Multivitamins with minerals (MVM) were reported most frequently at 5.4% of DS users, with men preferring this type of supplement. Following that, Iron at 4.6%, and Calcium DS are consumed by 4.7% of users and more often by women and survey participants with lower education levels. Plant- and oil-based supplements were used by <5% of the participants (12).

With studies finding that dietary supplements (DS), in many cases, are not being used to supplement nutrient deficiencies but on the understanding that they play a preventive role against disease (13), further exploration is warranted as to whether this “nutritional transition” (14) and its newly established habits actually benefit health. In addition, the efficiency of recommended food intake in comparison to substituting intake with DS is a pertinent question.

Addressing the move away from traditional dietary patterns

There is clear evidence that a significant portion of the population of Greece has moved away from the “healthy” traditional Mediterranean diet, with olive oil at its core. The HYDRIA survey population representative sample shows that, overall, only 28.3% of adults are now characterized as having a high adherence (score of 6–9 points) to the Mediterranean diet. About 39.7% of participants over 65 years old and 25.5% of participants under 65 years old were included in the high adherence category of the Mediterranean diet score (11).

Key is the observation that adults consume levels of red meat, fruit and vegetables that are not in line with international dietary recommendations. Based on the criteria, it is clear international dietary recommendations are not being met

by most adults when assessing their intake levels of several macronutrients and selected foods. With these insights into the dietary habits of adults in Greece it is possible to note that younger adults eat more meat, dairy and alcohol, which moves away from the lower to mid-level amounts that constitute the traditional Mediterranean diet consumption levels. They also eat lower amounts of fruit, legumes and vegetables, although older participants had comparably higher consumption in these categories (11). Even with contemporary moves toward acknowledging the benefits of the traditional Mediterranean diet translating into support for modifying dietary patterns toward incorporating key elements of the Mediterranean dietary pattern (15), younger generations in Greece are still moving away from beneficial food choices (11). The potential detrimental effects on mortality and morbidity in these observations are becoming clear (16).

Changes in diet could be attributed to the documented life-style changes impacted by the urbanization process. Urban living can have enormous and complex impacts on diets including increased participation in the workforce which has corresponded to moves toward convenience foods rather than quality food choices, increased income levels which enable access to a larger range of foods which are not necessarily as nutritious, the wider availability of inexpensive poor nutritional quality foods (frequently of animal origin), and more readily available unhealthy packaged foods (17).

The HYDRIA survey noted differences in adherence to the Mediterranean diet were observed among the four main geographical regions in Greece, in the highly urbanized area of Attica the Mediterranean dietary pattern was notably lacking with 35.6% of participants having a low level of adherence (11). Parallel with other studies the observed influence of urbanization and regional difference in diet quality emphasizes the need to consider tailored regional nutrition strategies (18).

The overall improvement in socioeconomic conditions in Europe and the globalization of the food supply has contributed to changes in amounts, types and costs of foods available across Europe and beyond. Just as policies should be distilled to provide regional effectiveness, attention should not be taken away from the effect of global economic policies such as those on trade, marketing and investment, and related global food and health policies have at all levels of stratified policy-making (19).

Tradition rarely honors unhealthy habits

Although there are some exceptions, tradition rarely honors unhealthy habits (20) with the United Nations Educational, Scientific and Cultural Organization (UNESCO) enshrining the intangible heritage of the Mediterranean diet and defining it as “... a set of skills, knowledge, rituals, symbols and traditions concerning crops, harvesting, fishing, animal

husbandry, conservation, processing, cooking, and particularly the sharing and consumption of food... and a way of life guided by respect for diversity” (20, 21).

In the past decades the pressures of modern life have changed the way people eat in Greece, resulting in spending less time sharing food and eating together. This loss of shared preparation and consumption of food generates a decline in the transfer of knowledge and skills related to food. In order to ensure the preservation of the traditional Mediterranean diet, before all that remains “*is an olive tree, a vine and a boat*” (1) priority could be the development of policies and actions related to the enhancement of knowledge and skills (e.g., “*tsigarisma*” -sautéing) related to food, at the very least in the populations that have a direct cultural heritage connection to it. Indeed, culinary habits could be a promoter of family and social cohesion at the local level. Education on the cultural elements inherent to the Mediterranean dietary pattern, extending healthy eating guidelines, through to training food producers, mass caterers, industry as well as faculty, students, and interested individuals, can work to preserve the traditional Mediterranean diet (22).

Considering the priorities for research and education on olive oil, as it is central to the Mediterranean diet, we assess each component of this dietary pattern, acknowledging that all are intertwined, since it is not easy to dissociate them. Within this context focus should be on the integrity and implementation of actions that will support and affect the development of compatible fiscal and pricing policies, school food and nutrition plans, food marketing, and nutrition labeling guiding principles (23).

One example is utilizing the current societal trend of “Healthy Eating” paralleled with an increased demand for traditional, local and seasonal foods, that can translate into business opportunities for the catering sector. The focus could be on traditional options emphasizing the use of olive oil and on the traditional dishes (24).

Traditional foods

The nutritional value of the Greek Traditional Mediterranean diet could be attributed to the combination of its constituents and not to a single component (25). Culinary practices seem to play a crucial role in the bioactivity of their ingredients and beneficial effects. Cooking methods, for example, that involve soaking herbs in a warm liquid, such as in the process of making soup, increase the antioxidant capacity of the herb extract, while extracts taken after grilling had a lower antioxidant capacity compared to the uncooked herb extract. Steaming and sautéing have been reported to increase the antioxidant capacity and phenolic content of extracts taken after cooking (26).

Tomatoes, onions, garlic and aromatic herbs are principal elements of the culinary practices of the Greek diet. Herbs

are rich in polyphenols, especially in their dried form, and generally contain higher amounts of polyphenols compared to other polyphenol-rich foods, such as dark chocolate, berries, and grapes. Polyphenols are well-known for their antioxidant properties and also for their anti-microbial, anti-diabetic Type II, and anti-asthma properties (27).

Accordingly, the systematic investigation of the nutritional value of traditional foods and recipes is needed, as well as the investigation of the historical and cultural identity of the simple and composite foods (recipes). In this way, scientific data would be gathered, which would substantiate the influence of the simple and composite foods on health.

Since in the preparation of traditional Mediterranean foods the use of olive oil is a central element, with its recognized health benefits, the study and rediscovery of these foods is crucial. Moreover, several traditional foods could represent healthy and ecologically friendly choices that also support local economies, because local products are generally used in their preparation. The cultivation of local products contributes to biodiversity and enhances the consumption of olive oil and to the employment of local people, thus promoting the balance between the territory and the people (28).

Sustainable nutrition—honoring our past to secure our future

The traditional Mediterranean diet embodies a sustainable dietary pattern in which culture, wellbeing and the environment are required to interact in harmony. As a plant-based diet, characterized by the low intake of meat and meat products, emphasizing traditional foods with their seasonal and regional produce, it is recognized as a sustainable dietary pattern (28).

Yet when imbalances occur there is a risk of losing essential elements, for example, the threat to the biodiversity of categorized medical and aromatic plants (MAPs) in Greece that climate change and unregulated harvesting may pose (3). The current food system should be reviewed within the context of ensuring the preservation and innovation of products considered to be exemplary in a sustainable food system. Any reform has to involve local communities and consider local needs alongside national ones, still within a global context.

In keeping with the need for a return to traditional foods access to the traditionally available produce is key. Farmers' markets may be instrumental in finding ways to increase production and encourage people to increase their consumption, in particular vegetables, at the population-level, which are consumed not only as salads but as main dishes, in both cases with considerable use of olive oil. The UNESCO Mediterranean diet definition adds "*Markets also play a key role as spaces for cultivating and transmitting the Mediterranean*

diet during the daily practice of exchange, agreement and mutual respect" (21). There are some indications that fruit and vegetable consumption was positively associated with the use of farmers' market shopping (29). Therefore, more information is needed regarding the use of farmers' markets, more specifically clarification of what are the barriers and facilitators to farmers' market shopping and the association between farmers' market access, use and socio-demographic characteristics of consumers.

The Mediterranean diet is more than just a "healthy diet," it is essential to build on the now robust documentation of its health benefits to support the evidence of its role as a sustainable dietary pattern. The Mediterranean diet warrants the convergence of knowledge with further multi-disciplinary studies encompassing the key elements of this dietary pattern, including economic, sustainability, cultural and environmental dimensions (30).

Mediterranean diet at the heart of a global approach to healthy dietary patterns

The respected contemporary report entitled 'Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems,' credits the Mediterranean diet as "*a diet that maximizes longevity, improves health-related quality of life and is ecologically sustainable and environmentally friendly.*" Significantly, the work details a universal healthy "reference diet" aimed at providing a way forward toward healthy dietary patterns as alternatives to standard current diets, many of which are high in unhealthy foods. This EAT "reference diet" encompasses many of the main elements of the traditional Mediterranean diet (15).

Practical approaches for dealing with any challenges in establishing the elements of the Mediterranean diet beyond the identified region of its heritage have already been tabled. Although issues still exist, examples such as the development of the Asian Diet Pyramid which respects the nutritional elements of the traditional Mediterranean Diet Pyramid and began over 30 years ago, following with the Latin American and African Heritage Diet Pyramids, represent efforts to transfer knowledge for the benefit of global populations (31). The concept of a "Planeterranean" diet is being addressed based on the premise that "*in every place of the world, it is possible to identify specific fruits, vegetables, legumes, wholegrain, and sources of unsaturated fats which present nutritional contents and characteristics similar to those provided by typical foods of Mediterranean diet, likely to have also similar health benefits for populations living far from the Mediterranean area*" (32). It is important, however, not to lose sight of the significance that the Mediterranean diet has at the global level, providing relevant characteristics of a sustainable diet beyond specific foods and nutrients (33).

Discussion: Priorities for research and education

Upon investigation it is possible to conclude that respect still exists for the heritage of the healthy, traditional Mediterranean diet, yet adherence is low as lifestyle choices, policies and global systems are removing essential access to key elements.

There is a need to move from the evidence to policies based in a long-term plan in which evaluation and actions should include the development of and implementation of global, regional and local food and nutrition policy that takes into account not only health issues but considers economy, culture and the environment, with a focus on olive oil.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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Religious fasting and its impacts on individual, public, and planetary health: Fasting as a “religious health asset” for a healthier, more equitable, and sustainable society

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Religious fasting is practiced by people of all faiths, including Christianity, Islam, Buddhism, Jainism, as well as Hinduism, Judaism, and Taoism. Individual/clinical, public, global, and planetary health has traditionally been studied as separate entities. Nevertheless, religious fasting, in conjunction with other religious health assets, can provide several opportunities, ranging from the individual to the population, environmental, and planetary levels, by facilitating and supporting societal transformations and changes, such as the adoption of healthier, more equitable, and sustainable lifestyles, therein preserving the Earth's systems and addressing major interconnected, cascading, and compound challenges. In this review, we will summarize the most recent evidence on the effects of religious fasting, particularly Orthodox and Ramadan Islamic fasting, on human and public health. Further, we will explore the potential effects of religious fasting on tackling current environmental issues, with a special focus on nutrition/food restriction and planetary health. Finally, specific recommendations,

particularly around dietary intake during the fasting rituals, will be provided to ensure a sustainable healthy planet.

KEYWORDS

faith-based fasting, sustainability, nutrition, lifestyle, global health, public health

Introduction

Human life expectancy is substantially higher today as opposed to 100 years ago, mainly attributed to advances in both preventive and clinical medicine and public health policies (1). However, increased longevity is associated with a growth in non-communicable and disabling diseases of old age such as neurodegenerative diseases, cardiovascular disease, and cancer. Furthermore, the western diet and lifestyle, characterized by unhealthy diet and sedentariness, has engendered many, so-called diseases of civilization, including obesity-associated metabolic syndrome, coronary heart disease, hypertension, stroke, type 2 diabetes, chronic liver disease, autoimmune disease, epithelial cell cancers, and osteoporosis, which are scarce or non-existent in hunter-gatherers and other non-westernized populations (2). As a consequence of civilization, life expectancy is expected, for the first time, to fall (3, 4). Moreover, the current western dietary pattern, high in animal-based and low in plant-based foods, is harmful; not only to personal health (5, 6), but also to public, global, and planetary health (7, 8). In this context, previous studies show meal consumption is linked to premature mortality and type 2 diabetes (9–11). Moreover, meat has been recognized as the food with the highest influence on greenhouse gas (GHG) emissions and land usage (12). In this context, producing 1,000 kcal of lamb or beef creates 14 and 10 kg of GHG emissions, respectively, compared to 1 and 3 kg for lentils or tofu (13). Additionally, meat production is deleterious for aquifers (14); one serving of beef or pork requires 1211 and 469 L of water, respectively, but one serving of dry beans, tofu, or tomatoes requires 220, 57, and 30 L, respectively (13). Several studies have found that reducing meat consumption can reduce GHG emissions, as well as land, water, and energy use, while concomitantly improving health outcomes (12). Therefore, an extension of a healthy life, while tackling transnational environmental challenges, is of utmost importance.

Religious fasting, or faith-based fasting, is predominantly practiced to satisfy prescribed religious requirements and is defined as a nutritional model characterized by a variance in the degree of caloric restriction and abstinence from specific foods (15). Interestingly, religious fasting could help to improve individual health as well as the community, and planet. Fasting rituals are followed by billions of individuals worldwide and their effects may differ from one religious community to

another (16). It should be acknowledged that 83% of the world's population self-identified as adhering to a religion in 2010, and this number is projected to rise to 87% by 2050 (17–19). In addition, Christians are the world's largest religious community, with Islam coming in second and being the fastest-growing faith (20). Skirbekk et al. (19) reported that countries with a greater proportion of religiously affiliated people are facing more environmental risks and are less prepared for those risks. Therefore, targeting the largest religious groups (i.e., Christians, Muslims) *via* specific recommendations may contribute to a “healthy planet, supporting healthy people.”

Using a comprehensive search, we will summarize in this review the latest evidence on the effects of religious fasting, particularly Orthodox and Ramadan Islamic fasting, on human health. Additionally, the potential effects of religious fasting on public and planetary health will be discussed and specific recommendations will be provided.

Methods

A two-step search process was conducted to identify (i) systematic reviews, with/or without meta-analyses, or scoping reviews and (ii) experimental studies evaluating the effects of any type of religious fasting on health outcomes in apparently healthy individuals (not physically active individuals and athletes) and patients.

In the first step, to identify systematic reviews, with/or without meta-analyses, or scoping reviews, a systematic literature search was conducted in five databases (PubMed, Web of Science, Cochrane, EBSCOhost, Scielo) on August 10th 2022. An additional search on Google Scholar was conducted on August 15th 2022. The following terms were used (see Table S1): “Religious fasting,” “faith-based fasting,” “human,” “health,” “scoping review,” “systematic review,” and “meta-analysis.” Appropriate Boolean operators were used to join the various keywords. Field tags, wild-card options (i.e., truncated words), and medical subject headings (MeSH) terms were also incorporated where appropriate. In the search strategy, no language or date limits were applied. The full search strategy for all databases is presented in Table S1. References of included papers were manually verified for a comprehensive search for relevant reviews. Personal files were also searched.

In the second step, experimental studies were searched on August 10th 2022 in Google Scholar using the following terms: “Religious fasting,” “faith-based fasting,” “human” and “health.” To identify experimental studies published after the date of the last search of the most recent comprehensive review, date limits were applied.

Results

For the first-step, the predefined search strategies yielded a preliminary pool of 443 possible papers. A total of 191 duplicates were removed. Then, 252 published papers were screened by titles and abstracts for eligibility, of which 36 published studies met the inclusion criteria. After a careful review of the 36 full texts, 27 reviews (systematic review with or without a meta-analysis) were included (Figure 1). Of the 27 included reviews, only one systematic review (21) and a scoping review (22) evaluated the effects of Orthodox fasting on health/nutritional outcomes. The remainder reviews ($n = 25$) focused on the effects of Ramadan fasting on anthropometric and metabolic markers (23–25), glucometabolic parameters (26–29), salivary flow rate, inflammatory and metabolic variables (30), immunity, inflammatory markers and infectious events (31–33), blood pressure and cardiovascular events (34–36), liver function (37), renal function and chronic kidney diseases (38, 39), body mass (i.e., body weight) and body composition (40–42), intestinal microbiome changes (43), hormones regulating appetite and satiety (44), psychiatric parameters (45), sleep quality (46) and pregnancy outcomes (47). The characteristics and the main outcomes of the included reviews are presented in Table S2.

For the second-step, 53 research articles evaluating the effect of Ramadan fasting on anthropometric parameters ($n = 10$), hydration status ($n = 11$), renal function ($n = 12$), metabolic health ($n = 9$), liver function ($n = 4$), markers of inflammation, immunity and oxidative stress ($n = 2$), the gut microbiome ($n = 3$), and sleep ($n = 2$) were included. Other empirical studies evaluating the effects of Orthodox fasting ($n = 13$), Daniel ($n = 3$) and Buddhist ($n = 2$) fasting were included.

Religious fasting, nutrition and individual health

In many religions, fasting is a fundamental practice with both spiritual and physical advantages (48). The health implications of religious fasting have been the topic of several scientific investigations, with the majority of studies conducted in the previous three decades. However, these implications have typically been studied at the individual level, with few studies exploring other domains across wellbeing from a transdisciplinary perspective. Besides the individual level of wellbeing (subjective hedonic and psychosocial wellbeing, “a

balanced mind and a healthy body,” and spiritual wellbeing), fasting impacts the community’s well-being (“social or collective wellbeing,” social capital, cohesion, connectedness, and “social identity” – a sense of belonging to a community), and the environmental and planetary wellbeing (“connection with nature,” nature-based mindfulness, “environmental and planetary wellbeing,” and nurturing environments). Over time, and throughout history, several religious actors and institutions, including trusted religious leaders, faith-based organizations, and faith communities, at all levels and of any religious creed/belief, have played a major role in the delivery of healthcare services and provisions and have contributed to shaping health emergency preparedness and response, as well as to mobilizing community-led action and catalyzing community partnership.

The effects of the religious Islamic fasting of Ramadan, the Greek Orthodox Christianity fasting, and the Biblical-based Daniel Fast, on subjects’ dietary intake and health-related outcomes, will be outlined. Other forms of religious fasting (i.e., Buddhism, Daniel fast, Judaism) are only briefly overviewed in this review given the paucity of health-related data available.

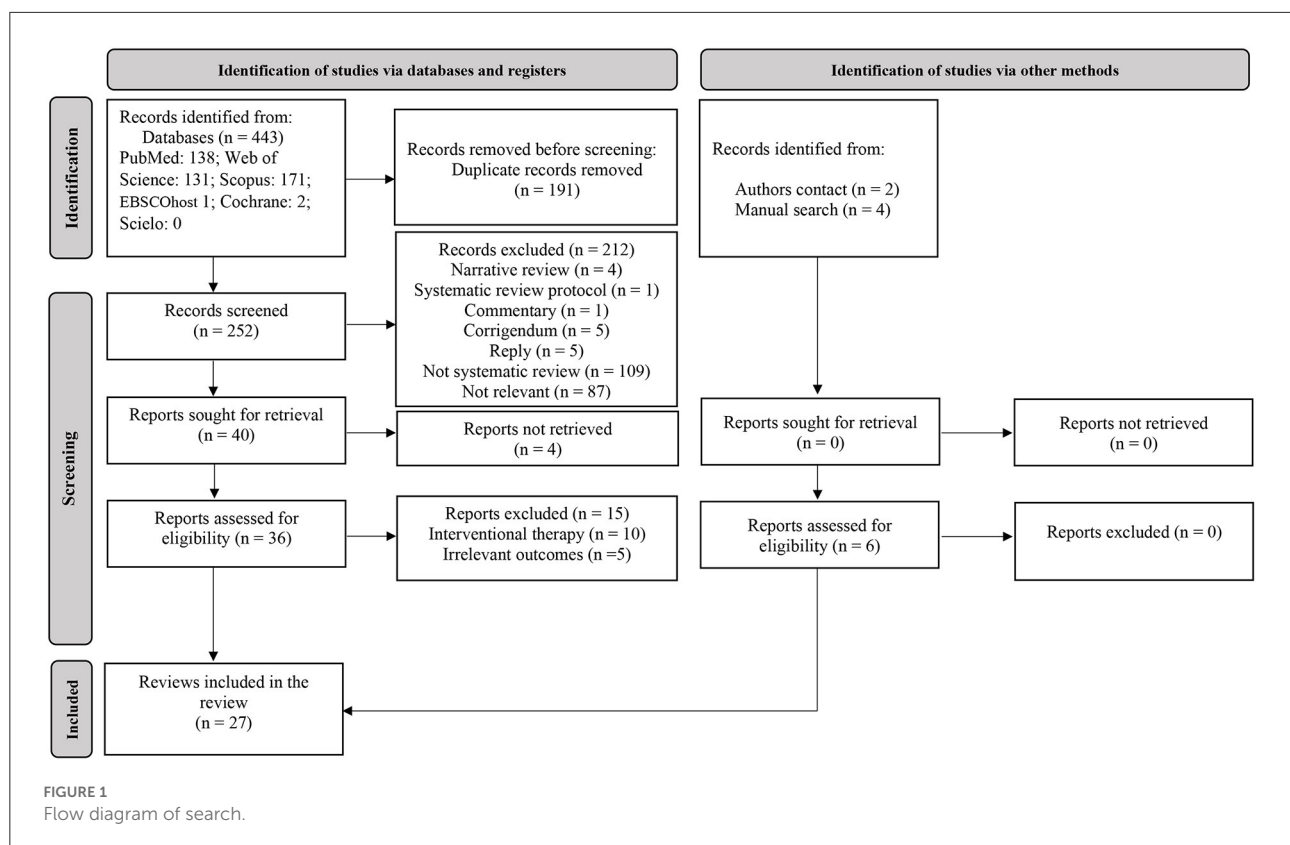
Islamic Ramadan fasting

During Ramadan fasting, one of the five pillars of the Islamic religion, more than 1.8 billion healthy pubescent Muslims worldwide must abstain from eating, drinking, and other specific behaviors (e.g., smoking, sexual intercourses, and all sensory pleasures) from dawn to sunset, during a period that lasts 29 or 30 days (lunar month) (15, 49). The daily fasting time length depends on the geographical location (latitude) and season. For example, in the north of the UK, daylight lasts <6 h in winter and about 19 h in midsummer (50).

During this month, water and food consumption are exclusively nocturnal and the typical dietary practice during Ramadan fasting consists of consuming one substantial meal after sunset and one lighter meal before dawn (51); however, an additional meal is sometimes consumed before sleeping (52). Interestingly, Ramadan fasting is similar to Alternate-Day Fasting in that the 12-h fast is followed by a 12-h feast. However, Ramadan fasting varies from Alternate-Day Fasting in that drinking water is not permitted throughout the 12-h fast (51, 53).

Food choices/preferences

Globally, there is large variability in dietary pattern between cultures in countries where individuals are observing Ramadan. Therefore, it is not surprising to observe different food preferences, specific to each culture and/or country. For example, Pirsahab et al. (54) found, in a study of 160 Iranian subjects, there was a higher consumption of vegetables and fruits



during Ramadan, whereas the consumption of dairy products, meat, and cereals decreased significantly. Contrarywise, in a study of 366 Ghanaian adolescents, it was found that the consumption of milk and vitamin A-rich fruits increased during Ramadan, while there was a lower consumption of dark leafy vegetables, legumes, and nuts (55). The authors also found that meat (beef, mutton, and chevon) consumption patterns remained relatively stable compared to before the month of Ramadan (~3 days/week), while poultry (chicken, guinea fowl, turkey) intake decreased marginally during Ramadan (55).

During a year-round study (56), including the month of Ramadan, Lebanese adults ($n = 62$), completed multiple (9–12, 14) 24-h dietary recalls. Dietary intake was estimated using food groups as well as energy, macro, and micronutrient consumption. The authors found that the intakes of cereals, cereal-based products, pasta, eggs, seeds and nuts, milk and dairy, and oils and fats were lower, while dried fruit, vegetables, cakes, pastries, and Arabic sweets, and sugar-sweetened-beverages intakes were higher during Ramadan as compared to the remainder of the year (56). In addition, the intake of poultry, meat, fish and seafood remained unchanged during vs. the remainder of the year (56). Nashvak et al. (57) evaluated the consumption of food groups before and during Ramadan in 160 healthy men from Iran. The authors found that, compared to before Ramadan, the consumption

of bread and cereals, dairy products, meat, and vegetables decreased, while the consumption of fruit, fat, and oil increased during Ramadan.

Anthropometric parameters

During the month of Ramadan, food and fluid intake becomes less frequent and, therefore, fluctuations in some anthropometric variables are to be expected.

The first published meta-analysis evaluating the effect of Ramadan observance on body mass was by Kul et al. (23). The authors found that Ramadan fasting decreases body mass (small effect). In addition, the subgroup analysis indicated that the decrease in body mass was sex-specific as it was observed in men but not in women.

The aforementioned meta-analysis (23) was updated by Sadeghirad et al. (40); in the updated version, the authors showed Ramadan fasting elicited a statistically significant decrease in body mass (-1.24 kg by the end of Ramadan). Again, the decrease in body mass was significant in both sexes (-1.51 kg in men and -0.92 kg in women). However, the body mass loss during Ramadan was continent-specific, as it was greater among Asian populations compared with Africans and Europeans. Furthermore, the body mass loss observed at follow-up (2–6 weeks after Ramadan) was less pronounced, albeit body

mass was still statistically significantly lower compared to before Ramadan (-0.72 kg).

The first systematic review with meta-analysis evaluating the effects of Ramadan fasting on body composition was conducted by Fernando et al. (41). The authors found that, from before to the end of Ramadan, there was a significant reduction in fat percentage in overweight or obese individuals, but not in those of normal weight. Similarly, Ramadan fasting yielded a significant loss of fat-free mass, but was about 30% less than the loss of absolute fat mass. After the end of Ramadan (2–5 weeks), there was a return toward, or to, pre-Ramadan measurements in body mass and body composition.

In another systematic review and meta-analysis, Jahrami et al. (42) concluded Ramadan fasting yielded a significant, but small reduction in body mass; this effect size translates into a difference in means of -1.022 kg. The regression analyses, used to explain sources of heterogeneity in the results, revealed that the decrease in body mass was directly associated with fasting time and correlated with country and season.

Regarding recent empirical studies, Agagündüz et al. (58) found, in 27 healthy Turkish participants (11 males, and 16 females) aged 27.6 ± 1.69 yrs, a significant decrease in body mass, body mass index (BMI), and fat-free mass. Similarly, Mengi Çelik et al. (59) reported a decrease in body mass and BMI during Ramadan in 32 (12 males 20 females) Turkish healthy adults aged 19 and 32 years. Similar findings were reported in 49 Indonesian healthy adults (15 males, 34 females) (60). Hasan et al. (61) found that Ramadan fasting decreased BMI and waist circumference in 55 overweight and obese participants (22 females and 33 males). Alsowaid et al. (62) enrolled only female individuals ($n = 20$) who were healthy students at the University of Bahrain. The authors found that Ramadan fasting decreased body mass, BMI, fat mass, and waist circumference (62). The decrease in body mass, BMI, and waist circumference could be attributed to the increased utilization of stored body fat as an energy substrate and/or the decreased energy intake as a result of fasting (63). Nevertheless, decreased total water intake during the month of Ramadan, possibly leading to a state of hypohydration, may also explain the decrease in body mass and BMI (63, 64).

Recently, Al-Maiman et al. (65) evaluated the effects of Ramadan fasting on anthropometric parameters of patients with type 1 ($n = 42$) and type 2 ($n = 62$) diabetes from Saudi Arabia, aged ≥ 20 years, and who fasted a minimum of 15 days during Ramadan. The authors concluded that Ramadan fasting decreased body mass and BMI in patients with type 2 diabetes. A significant decrease in body mass and BMI, with no significant effect of Ramadan fasting on body fat, visceral fat, and muscle mass was observed in Saudi patients ($n = 20$) with chronic kidney disease (CKD) (66). In contrast, Oueslati et al. (67) found, in 55 Tunisian type 2 diabetes (mean age = 54.5 ± 10.1 yrs) who fasted 29.3 ± 2.3 days during the month of Ramadan, a

significant decrease in body mass, BMI, waist circumference, fat body mass. The body mass loss was significantly correlated with the number of fasting days ($r = 0.348$, $p = 0.009$).

Badran et al. (68) found, in 98 patients with non-alcoholic fatty liver disease, that Ramadan fasting decreased significantly body mass and BMI, while waist circumference, hip circumference, waist/hip ratio, and triceps skin fold thickness remained unchanged.

It should be acknowledged that there are cases where body mass and body mass index may increase during the month of Ramadan, likely as a result of excess intake of fat, sugar, and energy, along with increased sedentariness (69).

Hydration status

Optimal hydration status is beneficial for physical and cognitive functioning, as well as health (70). Fluid restriction during daylight hours of the month of Ramadan may influence the hydration status of fasters. Indeed, previous reports have indicated that total water intake was less during Ramadan compared to before Ramadan (71–73), possibly leading to altered kidney function. Fortunately, fluid balance can be maintained by maximizing urinary concentration and decreasing obligatory urine output (74, 75). However, Leiper et al. (76) reported a prolonged state of hypohydration induces a degree of stress on kidney function, which may negatively affect the efficiency of the water-conserving mechanism. Hydration status can be assessed using urine (e.g., urine osmolality, urine-specific gravity) or blood (e.g., plasma/serum osmolality) markers, among others, such as the estimation of the total body water (64).

Ramadan et al. (77) evaluated the effect of Ramadan fasting on hydration status, assessed using plasma osmolality, in 13 healthy Kuwaiti adults who were either sedentary ($n = 7$) or physically active ($n = 6$). The authors found plasma osmolality increased significantly in sedentary individuals, suggesting a state of hypohydration (77). However, plasma osmolality remained unchanged in the physically active group, possibly explained by an adequate water intake after the break of the fast (77).

Ibrahim et al. (73) found, in 18 healthy men (mean age = 22.0 ± 2.4 yrs), a significant increase in urine specific-gravity measures, with mean values higher than 1.020, indicative of a state of hypohydration during Ramadan. Conversely, serum osmolality levels decreased significantly during Ramadan, but remained within normal ranges. It appears the homeostatic mechanisms may have kept serum osmolality at normal levels (73). The lack of change in urine osmolality during Ramadan was also observed in 16 male Sudanese students aged 20–22 years (78). Husain et al. (79) found, in 21 Malaysian healthy individuals (12 males and 9 females), that water intake and urine output volumes during the fasting and non-fasting

control periods were approximately the same. Again, the authors concluded fluid balance was maintained throughout the month of Ramadan (79).

Only three studies, with mixed findings, evaluated the effects of Ramadan fasting on the hydration status of patients. While Dikme and Dikme (80) reported the absence of the effect of Ramadan fasting on serum osmolality in 62 Turkish patients admitted to the emergency department, Azwany et al. (81) showed a significant increase in urine osmolality at the end of Ramadan vs. pre-Ramadan. In the latter study (81), urine osmolality remained within normal ranges. In patients ($n = 31$) with CKD (grades 2–4), Hassan et al. (82) found a reduction in the total body water as estimated using an impedancemeter, suggesting a state of hypohydration.

Collectively, information obtained to date does not suggest hydration status is adversely affected by Ramadan fasting. Moreover, changes in urine/plasma biomarkers are usually relatively small, with values remaining within the normal reference range. It appears fasting during Ramadan can be safely maintained for healthy individuals; however, future studies on patients with underlying chronic conditions are warranted.

Renal function

Optimal kidney function is of paramount importance for wellbeing and health (83). Creatinine clearance and the plasma/serum concentrations of creatinine, urea, and uric acid are all potential measures of renal function (84). Increases in serum urea and creatinine have been observed in sedentary men during Ramadan (77). However, possibly because of adaptations of renal function, the serum uric acid concentration is lower in physically active than in sedentary men (77). Other studies on healthy individuals have reported slight modifications in the levels of renal function markers, albeit with no clinical significance (85–87). To the best of our knowledge, no previous study has reported impairment of renal function in healthy individuals as a consequence of Ramadan fasting. Nevertheless, the impact of Ramadan fasting on renal function in patients with CKD is a subject of debate.

The first published systematic review evaluating the effects of Ramadan on renal function in patients with CKD was conducted by Bragazzi (38). After identifying and summarizing the results of 26 studies published between the years 1989 and 2013, the author concluded that recipients of kidney allograft can safely fast during the month of Ramadan (38). However, evidence regarding safety in patients with nephrolithiasis and CKD is equivocal. In addition, the authors pointed out the scarcity of information about Ramadan fasting falling in hot seasons (38). Finally, given the lack of evidence-based guidelines and protocols, which correctly address the issue of the impact of Ramadan fasting on CKD patients, the authors recommended the publication of randomized clinical trials.

In his systematic review and meta-analysis, Bragazzi (39) concluded patients with CKD can safely fast during Ramadan because glomerular filtration rates (GFR) do not significantly change. Additionally, sensitivity analyses revealed no impact of seasonality (39).

Other research articles have been published in an effort to better understand and evaluate the effects of Ramadan fasting in CKD patients. Mbarki et al. (88) evaluated the renal function in 60 CKD patients who fasted for the entire month of Ramadan. The authors found that 11.7% of CKD patients had an increase of serum creatinine by 442.1 $\mu\text{mol/L}$ on the initial serum creatinine level, and a 25% reduction of GFR, indicating the development of acute renal failure (ARF). The study by NasrAllah and Osman (89) showed compared to non-fasting CKD patients (control group), there were significant adverse effects in the fasted CKD patients [estimated GFR (eGFR) = 27.7 mL/min/1.73 m^2] and an increase of 60.4% in the serum creatinine after 1 week of Ramadan fasting. In addition, 3 months after the end of Ramadan, plasma creatinine remained high in 23% of the fasting CKD patients, with no significant difference vs. the non-fasting CKD patients' control group (89). The authors concluded the increase in creatinine levels was likely due to CKD progression, opposed to fasting (89). In a prospective study that enrolled 65 CKD (stage 3) patients, an increase of serum creatinine by $\geq 26.5 \mu\text{mol/L}$ in 33% of patients was reported (90). Chowdhury et al. (91) found in 68 CKD stage 3 diabetic patients who fasted ~ 19 h, a significant difference in proteinuria and ARF risk compared to 61 same-category patients who did not fast.

A significant improvement in eGFR (from 29.7 mL/min/1.73 m^2 to 32.7 mL/min/1.73 m^2 after fasting) in diabetic individuals was reported (92). Despite non-optimal hydration status and decreased serum basal B-type natriuretic peptide, Hassan et al. (82) found no significant differences in eGFR between fasting and non-fasting CKD patients (stages 2–4). In Turkish patients (45 fasters and 49 non-fasters CKD, stages 3–5), Kara et al. (93) found no significant changes in eGFR between non-fasters and fasters; however, individuals above the age of 72 appeared to be at a higher risk of renal function impairment than CKD patients under the age of 64. Recently, a retrospective study of 1,199 patients, who were not exempt from Ramadan fasting for 2 years (2016 and 2017), showed fasting significantly reduced the risk of developing ARF, particularly in patients with comorbidities (94). The authors concluded that Ramadan fasting conferred no negative effects on the majority of patients with comorbid disorders (94). Similarly, following Ramadan fasting, no significant impairment in renal function was found in autosomal dominant polycystic disease patients with early CKD (95). Baloglu et al. (96) found that hypertension and fasting days during Ramadan are strong predictors of ARF, where ARF developed in 27/117 patients with stage 2–3 CKD, with an average age of 60 years. The authors recommended adequate hydration and regular check-ups in order to lower the risk of developing ARF during Ramadan (96).

According to an Egyptian-based study (97), serum creatinine increased significantly after Ramadan fasting, although eGFR remained unchanged in individuals without CKD. Conversely, serum creatinine in CKD patients was lowered, and eGFR improved significantly during Ramadan, most likely as a result of better blood pressure control in hypertensive CKD patients (97).

In summary, Ramadan fasting appears to have no adverse effect on the renal function of healthy individuals. However, findings in CKD patients are mixed and it is difficult to determine the reasons for discrepancies in the identified studies. The differences between the studied populations in terms of CKD severity, hydration levels during non-fasting hours, fasting days, fasting length, observation period, and other lifestyle factors such as physical activity level may explain the contradictory findings. Fasting during the month of Ramadan tends to be more harmful in patients with increasing degrees of renal impairment; however, this is not universally true in all aforementioned papers. Recently, Malik et al. (98) recommended that stable CKD non-dialysis (up to Stage 3) patients may be able to fast with close monitoring, while hemodialysis and peritoneal dialysis patients are considered very high risk.

Metabolic health

Metabolic health is a broad term covering numerous elements of cellular, cardiovascular, and cardiorespiratory health and wellbeing (99). Anthropometrics, blood pressure, and blood-based indices, such as blood glucose and serum lipids can all be utilized in clinical settings to assess metabolic health (99). One well-established method is to use diagnostic criteria of metabolic syndrome (MetS) (100), a multidimensional disorder that predisposes people to serious health concerns such as atherosclerotic heart disease (101) and type 2 diabetes (102).

Kul et al. (23) conducted the first systematic review and meta-analysis evaluating the effects of Ramadan fasting on blood levels of lipids and fasting glucose, considering gender differences. The authors found that compared to pre-Ramadan levels, fasting during Ramadan significantly decreases LDL-cholesterol and fasting blood glucose levels in both sexes (23). In females, total cholesterol (TC) and triglyceride (TG) levels did not change, while HDL-cholesterol levels increased during vs. before Ramadan. In males, there was a significant decrease in total cholesterol, LDL-cholesterol and TGs.

The previous systematic review and meta-analysis was updated by Mirmiran et al. (26). The authors concluded that Ramadan fasting has no significant effect on circulating triglycerides, total cholesterol and LDL-cholesterol in apparently healthy individuals. Conversely, Ramadan fasting results in decreased levels of HDL-cholesterol and very low-density

lipoprotein cholesterol (VLDL-C), as well as increased LDL-cholesterol levels.

Faris et al. (28) evaluated the effect of Ramadan fasting on glucometabolic markers (i.e., fasting glucose, insulin, insulin resistance, leptin, adiponectin) in healthy non-athletic individuals. The authors concluded that fasting during Ramadan decreases significantly fasting glucose, with a minimal non-significant effect on insulin, insulin resistance, leptin, and adiponectin. The authors concluded Ramadan fasting has no adverse metabolic impacts, and could help improve glucometabolic markers, particularly fasting glucose levels, in healthy individuals.

Faris et al. (27) conducted a meta-analysis evaluating the effect of Ramadan fasting on MetS components among healthy Muslims. Results revealed that Ramadan fasting decreases significantly waist circumference, fasting blood glucose, serum triacylglycerol and systolic blood pressure, while it increases HDL-cholesterol (27).

Recently, Jahrami et al. (29) conducted a meta-analysis to evaluate the effect of Ramadan fasting on cardiometabolic risk factors in healthy adults. The authors found that Ramadan fasting significantly decreases total cholesterol, LDL-cholesterol, VLDL-C, TGs and diastolic blood pressure, while it increases HDL-cholesterol (29). Resting heart rate did not change during compared to before Ramadan (29). The authors concluded Ramadan fasting positively impacts cardiometabolic risk factors, which may provide healthy persons short-term protection against cardiovascular diseases (29). The beneficial effect of Ramadan fasting on systolic and diastolic blood pressure was originally proposed in the meta-analysis by Al-Jafar et al. (36).

The effect of Ramadan fasting on the main hormones regulating appetite and satiety (i.e., leptin, and adiponectin) was investigated in a systematic review and meta-analysis by Gaeini et al. (44). Accordingly, a significant decrease in leptin levels was observed after Ramadan fasting. Ramadan fasting had no significant effect on the levels of adiponectin. In addition, the sub-group analysis demonstrated a greater decrease in leptin levels among normal-weight individuals compared to those of overweight/obese subjects. The authors concluded Ramadan fasting may decrease leptin levels, particularly in normal-weight individuals.

The beneficial effects of Ramadan fasting on glycaemic parameters in patients with type 2 diabetes was also shown by Aydin et al. (24). In their systematic review and meta-analysis, the authors found Ramadan fasting significantly decreases fasting plasma glucose (24). Additionally, the subgroup analysis revealed the decrease in fasting blood glucose was observed in the monotherapy (single oral antidiabetics) group, the oral combination therapy (multi oral antidiabetics) group, and the multi-treatment (oral antidiabetics plus insulin or diet modification) group (24). However, postprandial plasma glucose, glycated hemoglobin, and fructosamine levels

remained unchanged during vs. before Ramadan (24). BMI decreased significantly in the oral combination therapy group alone (24). The authors concluded Ramadan fasting has no significant negative effects on postprandial plasma glucose and fructosamine levels. Interestingly, BMI and fasting plasma glucose were positively impacted by Ramadan fasting (24). Other recent empirical studies reported improvement in the metabolic profile in diabetics during Ramadan (103, 104).

In summary, Ramadan fasting improves the metabolic health of healthy individuals and type 2 diabetes patients. However, according to an epidemiological study of 13 countries with large Muslim populations in northern Africa, Asia, and the Middle East, hypoglycaemic episodes increased in people with diabetes (types 1 and 2) during Ramadan (105). Fortunately, the results of the systematic review and meta-analysis of Tahapary et al. (25) showed patients with diabetes who fasted during Ramadan had a low incidence of hypoglycemia and no studies reported fatal hypoglycemic events. Supporting this, Almulhem et al. (35) concluded there is insufficient evidence to link Ramadan fasting to an increased or decreased risk of cardiovascular events in diabetics. It is worth noting that Ramadan is challenging when it falls close to the summer (when daylight hours are longer) (106). Thus, contemporary recommendations for patients with diabetes seeking to participate in fasting during Ramadan should be followed (106, 107). Recent guidelines, authored by the International Diabetes Federation and the Diabetes and Ramadan International Alliance (IDF-DAR), pertaining to the management of patients with diabetes during the month of Ramadan have been developed (107). In brief, a health assessment, pre-Ramadan, should be conducted, taking place around 6–8 weeks prior to commencing Ramadan. This approach would permit health care professionals to obtain a detailed medical history, in addition to performing a risk assessment, thus facilitating the classification of diabetic patients into a low-risk (i.e., fasting is probably safe), moderate-risk (i.e., fasting safety is uncertain), or high-risk (i.e., fasting is probably unsafe) group (107). This classification system will underpin all subsequent recommendations, which will include guidance related to the safety of fasting, strategies/approaches for modifications or adjustments in dosage or treatment regimen, Ramadan-focused education, and dietary guidance (107). Following the above steps, individuals who elect to fast must adhere to diabetes management guidelines during Ramadan fasting, inclusive of changes to glycemic monitoring schedules and adjustments in medication dosing. Finally, a follow-up post-Ramadan is advised. This will assist healthcare professionals in identifying and understanding critical information about the individual's Ramadan successes and challenges, ensuring that subsequent participation in Ramadan fasting is more successful (107). Moreover, this process must be replicated each Ramadan because fasting safely at one-time point does not guarantee the same level of success the following year (107).

It should be acknowledged that utilizing analogs of basal insulin during Ramadan is recommended due to the relatively lower risk of hypoglycemia, as compared to regular human insulin (108, 109). For instance, for once-daily basal insulin analogs, the IDF-DAR guidelines advocate a reduced (15–30%) dose, administered at the break of fast (i.e., *iftar*) (107). Further, recent empirical evidence has highlighted the safety and effectiveness of insulin glargine 300 U/mL, a second-generation basal insulin analog, in patients with type 2 diabetes who fasted during Ramadan (110, 111). Further specific recommendations for type 1 and type 2 diabetics who observe Ramadan are also provided in the recent guidelines (107).

Structured diabetes education is important for diabetic patients who will observe Ramadan (112, 113). Previous studies concluded diabetic patients who benefited from a structured diabetes education (i) improved their blood glucose levels/glycemic control (112, 113), (ii) reduced the incidence of hypoglycemic events (112, 113) and hyperglycemic crises (113), (iii) decreased their body mass (112), and (iv) increased their acceptance and frequency of glycemia measurements (113). Interestingly, Bravis et al. (112) reported glycated hemoglobin reduction was sustained 12 months after attending the structured diabetes education.

The Diabetes and Ramadan International Alliance recommended practicing regular light-to-moderate exercise during Ramadan for patients with diabetes (114). In that regard, *Attarawih* prayer, which includes movements such as kneeling, bowing and rising, should be included in the daily exercise routine of diabetic patients (114).

Public health authorities should prioritize structured diabetes education for patients with diabetes who will fast during the month of Ramadan.

Liver function

The liver is a vital organ with multiple functions ranging from detoxification of drugs and toxic chemicals, regulation of red blood cells, and maintaining energy metabolism and bile acid homeostasis (115). The liver function tests typically include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), the international normalized ratio (INR) and albumin (116).

Faris et al. (37) conducted a systematic review and meta-analysis to evaluate the effects of Ramadan fasting on liver function tests in healthy people, and to examine the impact of different covariates using subgroup analysis and meta-regression. Faris et al. (37) found that Ramadan fasting induced a significant positive effect on markers of liver damage including AST, ALP, and bilirubin. The authors concluded Ramadan fasting could confer short-term protection against liver steatosis in healthy individuals (37), possibly attributed

to the improvement in the metabolic profile (e.g., increased HDL-cholesterol and decreased TC and LDL-cholesterol) and the decrease of body mass, waist circumference and body fat percentage (37). However, the small number of included studies, particularly those assessing GGT, warrant further research.

The effects of Ramadan fasting on salivary liver function tests were investigated by Besbes et al. (30) who concluded salivary ALP increased, whilst AMP decreased significantly during vs. before Ramadan.

Recently, the positive effect of Ramadan fasting on liver function in 70 healthy Iranian individuals was reported (117). Similar to healthy individuals, Ramadan fasting improved non-invasive measures for non-alcoholic steatohepatitis severity assessment (118, 119). Furthermore, it appears to have no negative effect on the liver function of obese males (120).

Collectively, Ramadan fasting appears to protect liver function in healthy individuals and improves non-invasive measures in patients with non-alcoholic steatohepatitis. However, more rigorous studies on patients are needed during Ramadan.

Markers of inflammation, immunity and oxidative stress

The imbalance between the generation of reactive oxygen species and the availability of antioxidants or radical scavengers results in oxidative stress (121). Excess reactive oxygen species can either oxidize biomolecules or structurally change proteins and genes, resulting in signaling cascades that can contribute to inflammatory-related diseases (121).

It is well-known that patients with chronic diseases are exempt from fasting during Ramadan. However, while some of these patients are eager to celebrate this time of year with their peers, there are no guidelines to assist physicians in dealing with the concerns of those with infectious diseases who choose to fast during Ramadan. In their systematic review, Bragazzi et al. (31) concluded that (1) patients with diabetes who are at risk of developing infectious complications should not fast, (2) Ramadan fasting has little effect on diarrheal patients (31), (3) HIV is a challenge, and patients should be advised to take *ad hoc* drug combinations, and to not eat fatty meals that could interfere with the treatment (31), (4) Ramadan has no effect on anti-helminthic therapy effectiveness (31), and (5) patients with active ulcers should not fast, because they are more likely to develop complications (31).

The second systematic review on the topic was conducted by Adawi et al. (32). The authors synthesized 45 studies and found that: (i) Ramadan fasting can mildly influence the immune system, with generally transient alterations, returning to baseline pre-Ramadan values immediately afterward; (ii) Ramadan

fasting during the second trimester of pregnancy is safe, not resulting in negative fetal outcomes or maternal oxidative status alterations; (iii) Ramadan fasting can enhance lipid profile and mitigate against oxidative stress in cardiovascular patients; (iv) Ramadan fasting is safe among asthmatic patients as well as in patients with HIV/AIDS and autoimmune disorders, and (v) Ramadan fasting can lead to increased immunological markers in psychiatric patients.

Faris et al. (33) performed a systematic review and meta-analysis to examine changes in inflammatory and oxidative stress markers in healthy individuals before and after Ramadan. The authors found that fasting during Ramadan resulted in very small reductions in interleukin (IL)-1, C-reactive protein (CRP)/high sensitivity-CRP, and malondialdehyde (MDA), and small reductions in tumor necrosis factor- α and IL-6. Faris et al. (33) concluded that fasting during Ramadan protects healthy people against elevated inflammatory and oxidative stress markers. This could be attributed to the reduction in caloric intake, and the associated decrease in body mass (predominately body fat).

In their systematic review, Besbes et al. (30) concluded that salivary immunoglobulin-A, playing an important role in mucosal immunity, decreased during the last week of Ramadan. It is worth noting that because there is no salivary IgA concentration threshold (122), a decrease in those levels does not necessarily imply that the participant is more susceptible to oral infection onset.

In obese and overweight subjects ($n = 114$), Makdour et al. (123) showed that Ramadan fasting ameliorates the genetic expression of antioxidant and anti-inflammatory and metabolic regulatory genes, which may confer protection against oxidative stress and its metabolic-related disorders in non-diabetic obese individuals. Compared to non-fasting obese males ($n = 14$), improvement in systemic inflammation biomarkers in obese males fasting during Ramadan ($n = 14$) has been reported (120).

The gut microbiome

The human gastrointestinal microbiome, which contains millions of organisms (e.g., bacteria, viruses, fungi, parasites), can be influenced by various environmental factors (e.g., diet). Conversely, various studies have shown that adverse changes in the intestinal microbiome can be associated with the development of various chronic diseases such as metabolic diseases (124), liver diseases (125, 126), neurodegenerative disorders (127), cancer (128), and others. Some findings have revealed that fasting diets can also cause changes in the microbiome (129, 130). In a recent systematic review, Mousavi et al. (43) reported that Ramadan fasting improves health parameters through positive effects on some bacterial strains such as *Akkermansia muciniphila* and *Bacteroides*.

The positive effects of Ramadan fasting on gut microbiota were recently demonstrated by Chen et al. (131). The authors

investigated the influence of fasting during Ramadan on the gut metabolic profiles in fecal samples obtained from healthy Chinese ($n = 16$) and Pakistani ($n = 18$) subjects, using liquid chromatography-mass spectrometry-based metabolomics analysis (131). Chen et al. (131) concluded fasting leads to changes in metabolite profiles specific to each ethnic group in a manner dependent on dietary components. Additionally, these changes are correlated with dynamic shifts in microbiota composition and diversity which, in conjunction with dietary changes during Ramadan fasting, lead to the enrichment or depletion of various functional metabolites (131).

Another study showed that Ramadan fasting resulted in significant beta diversity and enrichment in the *Bacteroidetes* phylum (132). The increase in the *Bacteroidetes* phylum after Ramadan fasting is important since a decrease in the *Bacteroidetes/Firmicutes* ratio has been found to play a key role in the development of obesity (133).

Su et al. (130) concluded that Ramadan fasting provokes substantial remodeling of the gut microbiome in healthy non-obese individuals and that this remodeling involves the upregulation of *Lachnospiraceae* species. It should be acknowledged that *Lachnospiraceae* and *Ruminococcaceae* are both bacteria that produce butyric acid, which helps to reduce oxidative stress, inflammation, and the risk of colon cancer (134–136).

In summary, Ramadan fasting appears to be beneficial for the gut microbiome of healthy individuals; however, future studies on patients are warranted.

Mental health

Good mental health is defined as a state of wellbeing that allows a person to cope with the normal stresses of life and function productively (137). Prevention of mental disorders (e.g., bipolar disorders) has emerged as an essential component of modern clinical psychiatry (137). Mental health can be assessed using psychometric interviews or specific questionnaires (137). One systematic review and meta-analysis evaluated the effects of Ramadan fasting on mental health in individuals without psychiatric disorders (45). The authors concluded that Ramadan observance was associated with lower stress, anxiety, and depression (45). However, given the small number of included studies ($n = 5$), the findings of Berthelot et al. (45) should be interpreted with caution. Additionally, the authors reported that no previous studies were carried out in psychiatric patients and recommended further studies should include these populations (45).

Current evidence suggests Ramadan fasting may not be considered a non-pharmacological therapy enhancing the mental health of healthy individuals, but promising results exist.

Sleep

Sleep plays a vital role in maintaining good overall health (138). Prolonged sleep loss is a risk factor for the development of non-communicable diseases (NCDs) such as diabetes, obesity, hypertension, heart disease and stroke and may contribute, in the long term, to premature death (139).

To date, the systematic review and meta-analysis of Faris et al. (46) is the only to estimate the effect size of Ramadan fasting on sleep duration and daytime sleepiness. The results revealed sleep duration decreased from 7.2 h per night before Ramadan to 6.4 h during Ramadan, while the Epworth sleepiness scale score, indicative of daytime sleepiness, increased slightly from 6.1 before Ramadan to 7.0 during Ramadan. Therefore, Faris et al. (46) concluded Ramadan fasting decreases sleep duration and increases daytime sleepiness levels. The increased night-time social activities (e.g., meetings in the coffee, prayer such as *Attarawih*, Quran reading group) and the large amount of food consumed after the break of fast, possibly delaying sleep, may explain these findings.

It should be acknowledged that almost all studies assessed sleep parameters using subjective tools. Therefore, the previous results should be interpreted with caution.

Recently, Mengi Çelik et al. (59) assessed sleep quality before and during the month of Ramadan, using the Pittsburgh Sleep Quality Index Questionnaire, in 32 (12 males 20 females) healthy Turkish adults. The authors found no significant effect of Ramadan fasting on sleep quality in healthy individuals. A similar result was observed in female healthy students from Bahrain (62). Alzhrani et al. (140) reported that sleep duration remained unchanged from before to during Ramadan in 115 adults (96 females and 19 males) from Saudi Arabia.

Collectively, Ramadan fasting appears to negatively affect sleep parameters. Future studies, with higher methodological rigor, evaluating strategies (e.g., sleep hygiene education, supplementation) aiming at improving sleep assessed using objective tools (e.g., polysomnography) are warranted.

Pregnancy outcomes

Despite being exempt, many pregnant Muslim women choose to fast during Ramadan. However, a sub-optimal diet during pregnancy could impact birth weight. Glazier et al. (47) investigated whether Ramadan fasting by pregnant women affects perinatal outcomes including perinatal mortality, preterm birth and small for gestational age infants, stillbirth, gestational diabetes, hypertensive disorders of pregnancy, congenital abnormalities, serious neonatal morbidity, birth weight, preterm birth, placental weight, neonatal death and maternal death. The authors concluded Ramadan fasting had no negative effects on birth weight, but there is

insufficient evidence on potential effects on other perinatal outcomes (47). As a result, future studies on this topic are needed.

Greek orthodox Christian fasting

The Christian Orthodox Church (COC) recommends that those who follow a long-term organized fasting diet must refrain from meat, dairy products, and eggs for 180–200 days every year while increasing their consumption of grains, fruits, legumes, vegetables, fish, seafood and olive oil. COC fasting consists of three main fasting periods: 40 days before Christmas (from 15 November to 24 December), 48 days before Easter (from Clean Monday to Holy Saturday), 14 days before Assumption (from 1 to 14 August), the fasting period before Holy Apostles (lasting 0–30 days depending on Easter feast), and three other daily feasts (5 January, 29 August, 14 September), in addition to every Wednesday and Friday (141).

Food choices

The COC fasting regime could be described as a periodical vegetarian diet, in which fish are occasionally and seafood always permitted (142), sharing numerous similarities with the typical Mediterranean diet (21, 143, 144). Therefore, it is not surprising previous studies reported increases in the consumption of fruits, vegetables and legumes in fasting individuals according to COC recommendations (142, 143, 145–147).

COC fasting and health-related indices

One systematic review (21) and one scoping review (22) evaluating the effects of COC fasting on health-related indices has been published. Interestingly, almost all included studies in those reviews were conducted in Greece.

Koufakis et al. (21) concluded that COC fasting has a beneficial effect on the lipid profile, with the decrease of TC and LDL-cholesterol levels being a consistent finding across trials (i.e., up to 17.8 and 31.4%, respectively). This was previously attributed to the decrease in the intake of energy and saturated fatty acids (148–150). However, the impact of COC fasting on HDL-cholesterol is controversial (21), with some studies reporting a significant decrease in HDL-cholesterol (151, 152), whilst others reported a lack of change (143, 153). It should be acknowledged that the decrease in HDL-cholesterol was previously observed after vegetarian diet intervention (154).

Conclusions on the influence of COC fasting on body mass, glucose homeostasis, blood pressure, antioxidant factors, iron status and relative hematological parameters cannot be drawn since relevant evidence is scarce and/or yields contradictory

findings (21). Koufakis et al. (21) noted that any potential negative effect of COC fasting, primarily due to the reduced intake of vitamin D and B12 and minerals (mostly calcium), warrants additional investigation. Additionally, the authors pointed out that incorporating this dietary pattern on a daily clinical basis as a health-promoting diet should be rigorously appraised and individually assessed, because current evidence is inconclusive, necessitating further investigations (21).

In their recent scoping review, Kokkinopoulou and Kafatos (22) suggested a beneficial effect of COC fasting on the lipid profile, marked by a decrease in total cholesterol and LDL-cholesterol. Additionally, the authors showed COC fasting reduced body mass, BMI and systolic blood pressure. Therefore, The COC fasting diet pattern may be recommended for the prevention of chronic diseases, as well as for persons who desire to adopt a plant-based diet for a healthier and/or more sustainable way of life (22). Although there is evidence in favor of health advantages, the COC fasting dietary recommendations, similar to any other dietary patterns, should always be followed under individualized supervision on effective meal planning (22). Nevertheless, long-term follow-up studies should be conducted to assess whether the favorable impacts of COC fasting on health markers are maintained over time (22).

A recent study by Kokkinopoulou et al. (155) investigated the relationship between COC fasting recommendations and cancer risk, specifically focusing on fiber, vegetables, fruit, and red and processed meat consumption. The authors found the diet of fasters to be healthy and follows the World Cancer Research Fund Cancer Recommendations (156), which is in favor of decreasing the chance of developing colorectal cancer compared to their non-fasting counterparts. Furthermore, eating more vegetables and fruits and eating less overall processed meat could reduce the risk of MetS.

In another study (157), plasma adiponectin, biochemical, and anthropometric data were collected from 55 COC fasters and 42 time-restricted eating (TRE) controls (all women, mean age = 47.8 years) at three different time points: baseline, the end of the dietary intervention (7 weeks), and 5 weeks after participants resumed their usual dietary habits (12 weeks from baseline). In the COC fasting group, adiponectin levels increased significantly at 12 weeks compared to baseline, and body fat mass decreased significantly between baseline and 12 weeks and between 7 and 12 weeks. Throughout the investigation, an inverse relationship between adiponectin and waist circumference values was detected in the same group. The authors concluded that COC fasting has beneficial metabolic effects linked to improved adiponectin levels.

Other recent studies showed the beneficial effect of COC fasting on metabolic health indices (148, 149, 158). For example, it has been shown that COC fasting has superior lipid-lowering effects compared to the TRE pattern in overweight adults (148). In addition, the favorable long-term effects of COC on irisin

levels (involved in the adipose tissue browning) in overweight, metabolically healthy, adults have been shown (158).

Interestingly, in the study of Papazoglou et al. (16), participants were 60 Greek Orthodox volunteers (30 with dyslipidemia and 30 without) who followed the COC fasting for 7 weeks, and 15 young (non-dyslipidemic) Muslim individuals who observed Ramadan fasting. The serum blood tests of study participants were measured pre- and post-fasting for biochemical (iron, ferritin, vitamin B12, calcium, LDL-cholesterol, HDL-cholesterol, TC, TG, and fasting glucose) and hematological (hemoglobin, hematocrit) (16). The results showed that COC fasting resulted in significant decreases in fasting glucose, HDL-cholesterol, LDL-cholesterol, and TC in both dyslipidemic and non-dyslipidemic Orthodox individuals. Hemoglobin, hematocrit, iron, and ferritin levels were significantly higher after COC fasting, although vitamin B12 and calcium levels were significantly lower (16). Further, a subanalysis of dyslipidemic and non-dyslipidemic Orthodox individuals found the former had a greater decrease in cholesterol levels (16). While TG, LDL-cholesterol, and TC levels were all higher in Muslim participants after Ramadan fasting, no significant effect was observed for the remaining assessed blood-based health indices (16). The authors concluded that prevention of calcium and vitamin B12 deficiency during COC fasting by supplement consumption is needed.

The sex-specific changes in lipid concentrations during COC fasting, as well as the potential role of vitamin D status in mediating these variations, have been investigated in a 12-week prospective intervention (159). In this study, biochemical data on serum lipids and vitamin D status were collected at baseline, 7 weeks after COC fasting, and 5 weeks after fasters resumed their standard dietary habits (12 weeks from baseline) (159). Based on 25-hydroxyvitamin D [25(OH)D] concentrations, participants (24 premenopausal females, 53%) were divided into two groups: those with concentrations above and below the median values (159). The study's findings revealed sex-specific differences in the pattern of lipid profile changes following COC implementation (159). Additionally, female participants with 25(OH)D concentrations below the median at each time point showed variability in TC and LDL-cholesterol responses; a 15% reduction was observed during the fasting period, followed by an appreciable increase slightly above baseline values 5 weeks after OF cessation (159). A similar pattern, albeit less pronounced, was observed for HDL-cholesterol concentrations, as well (159). For male participants, an inverse non-significant trend of TC, LDL-cholesterol, and HDL-cholesterol increase during the study period was observed; however, this was only seen in individuals with 25 (OH)D concentrations below the median (159).

Athonian COC fasting, practiced by Athonian monks, is a pescetarian variation of the typical COC fasting (160). Athonian monks must abstain from red meat consumption throughout the year, during both fasting and non-fasting periods (160). Karras et al. (160) showed lower BMI, body fat mass, and homeostatic

model assessment-insulin resistance in male Athonian monks ($n = 57$), compared to a general population practicing the typical COC fasting ($n = 43$). Additionally, the authors found that secondary hyperparathyroidism, resulting from profound hypovitaminosis D, was observed in the Athonian monks' group.

Nonetheless, larger sample size studies and/or long-term follow-ups are warranted before drawing firm conclusions on the effects of COC fasting on human health.

The effect of COC fasting on cognitive function and emotional wellbeing of healthy adults was evaluated by Spanki et al. (161). Two groups of fasting ($n = 105$) and non-fasting ($n = 107$) individuals were evaluated regarding their cognitive performance using the Mini Mental Examination Scale and the presence of anxiety and depression using the Hamilton Anxiety Scale, and the Geriatric Depression Scale, respectively. The authors concluded that COC fasting has a positive effect on cognition and mood in middle-aged and elderly individuals (161).

There is a growing body of evidence highlighting the beneficial effects of COC fasting on the health of Orthodox individuals. Unfortunately, studies evaluating the effects of COC fasting in patients are scarce. Future COC fasting-based studies in diverse ethnic populations, pregnant and breast-feeding women, and patients are also warranted.

Other types of religious fasting

Health-related indices

The Daniel fast

The Biblical-based Daniel fast, practiced by some Christians, prohibits the consumption of animal products, refined carbohydrates, preservatives, food additives, flavorings, sweeteners, alcohol, and caffeine (162). It is typically observed for 21 days, while fasts of 10 and 40 days have been reported (162).

Bloomer et al. (163) evaluated the effect of Daniel fast on human metabolic and cardiovascular health in 43 healthy individuals. The authors found that total energy, protein, fat, saturated lipids, trans-fat and dietary cholesterol intake decreased while carbohydrates, fibers, and vitamin C intake increased. They also found significant reductions in TC, LDL-cholesterol, HDL-cholesterol, systolic blood pressure, and diastolic blood pressure.

The effect of Daniel fast on glucose homeostasis has also been investigated (164). Following the Daniel fast, a significant decrease in glycemia, fasting blood insulin and HOMA-IR was observed (164). Interestingly, the Daniel fast appears to decrease blood oxidative stress (as measured by MDA), increase antioxidant capacity (as measured by TEAC), and increase nitrate/nitrite levels (165). The improved antioxidant

status was attributed to a combination of lower calorie and saturated fat intake and an increase in nutrient- and fiber-rich fruit, whole grain and vegetable consumption (165). In addition, the elimination of dietary additives, preservatives, and processing agents, as well as a reduction in protein consumption (methionine included) may explain the beneficial effect of the Daniel fast on the oxidative stress profile (165).

Unfortunately, given the scarcity of Daniel fast-related studies, no firm conclusions can be drawn on its health impacts.

The Jewish Yom Kippur fast

Yom Kippur occurs on the 10th day of the 7th month of the Hebrew calendar, after Rosh Hashanah (the Jewish New Year), and is distinguished by a prohibition on eating and drinking (166). The Jewish Yom Kippur fast differs from other fasting rituals in that it requires full abstinence from food and water for 25 h (167). The fast begins before sunset the evening before Yom Kippur and finishes after midnight the next day (166). It should be acknowledged that on Yom Kippur, ingestion of a shiur (~ a half mouthful of liquid) is allowed at intervals of between 4- and 9-min, and Jewish fasters can consume (if necessary) periodically 30 cc of food (MBE, 2009). This fast is short to generate major metabolic alterations leading to significant health implications (167). Unfortunately, available human studies regarding the effect of this fasting ritual on fasters' health are lacking, precluding a firm conclusion on its effects on health.

The Buddhist fasting

Buddhist fasting pattern, observed all year, is similar to a standard vegetarian diet that excludes meat and dairy products (occasionally milk). In addition, it is forbidden to consume five pungent vegetables (garlic, garlic chives, Welsh onion, leeks, and asant), processed foods, and alcohol (144). Food consumption varies by country and culture (168, 169). Few studies have evaluated the effects of Buddhist fasting on health-related indices. In a cross-sectional study, Ho-Pham et al. (170) evaluated the effects of a lifelong vegetarian diet on bone mineral density and body composition in a group of postmenopausal women. Participants were 105 Mahayana Buddhist nuns (vegans) and 105 omnivorous women (average age = 62, range = 50–85 yrs) (170). The results showed that although vegans consumed less calcium and protein than omnivores, veganism showed no negative effects on bone mineral density or body composition (170). Additionally, no significant adverse effects have been reported (170).

Lee and Krawinkel (168) compared body composition and nutrient intake data of 54 Buddhist vegetarian nuns and 31 omnivore Catholic nuns from South Korea. The authors found (i) nutrient intake of Korean Buddhist vegetarians was comparable to that of omnivores, and (ii) the intake of some

nutrients in Buddhist vegetarians was more favorable than in omnivores (168). Additionally, Buddhist vegetarians had higher fat-free mass, body fat, and BMI than omnivores, and body fat was inversely correlated with the duration of vegetarianism for vegetarians (168). Future studies evaluating the effect of Buddhist fasting in high-risk populations are warranted.

Religious fasting as a public health nutrition strategy

NCDs, often known as chronic diseases, are a growing concern for national governments and society throughout the world due to their high mortality and morbidity rates (171). NCDs are caused by a variety of factors, including behavioral, environmental, genetic, and physiological factors (171). They share four major behavioral risk factors: unhealthy diet, tobacco use, excess alcohol consumption, and sedentariness (172). According to current WHO projections, the total number of NCD deaths will rise to 55 million by 2030 (WHO). In 2012, all countries committed to lowering premature mortality from NCDs by 25% by 2025 (the 25 x 25 target) (173). Furthermore, the reduction in NCD premature mortality is included in the Sustainable Development Goals (SDGs), as SDG target 3.4 is to reduce NCD premature mortality one-third by 2030, compared to 2015 levels, and promote mental health and wellbeing (174). Public health practitioners and policymakers are responsible to ensure the health of all individuals by searching for and implementing effective public health strategies enabling the prevention and management of NCDs.

When examining the religious recommendations related to some types of fasting, some observations can be noticed. First, the consumption of alcohol is prohibited during Islamic, Daniel, and Buddhist fasting, and occasionally permitted during COC fasting. It is worth noting that alcohol consumption is a cause of over 200 diseases and injuries, accounting for 5.3% of premature deaths and 5.1% of the global disease and injury burden (174). Individuals strictly adhering to religious fasting will refrain from alcohol consumption, which will contribute to the improvement of their health status.

Second, during Ramadan fasting, smoking is not permitted during daylight. It should be acknowledged that tobacco smoking is the single leading cause of premature death worldwide (175), leading to the death of more than 8 million people each year (174). More than 7 million of these deaths are directly related to tobacco use (174). While adhering to Ramadan fasting will likely reduce smoking during this month (176, 177), the challenge is to maintain this habit following the break of the fast and after Ramadan (178). In this context, behavioral approaches may be effective to achieve this goal. For example, Ismail et al. (179) demonstrated a religious-based smoking cessation behavioral intervention delivered to Muslims from Malaysia during Ramadan resulted in sustained smoking

reduction after Ramadan. Unfortunately, to our knowledge, no data exists on the effects of other religious fasting regimens on smoking habits.

Third, the COC fasting regimen could be described as periodic vegetarian advocacy, with several similarities to the typical Mediterranean diet and incorporating almost all of its cardioprotective and health-promoting mechanisms, such as calorie restriction and a lower intake of dietary cholesterol and fatty acids (180). Consequently, observers of COC fasting will consume a healthy diet, in which macronutrients are consumed in appropriate proportions to support energetic and physiologic needs, without excess intake, while also providing adequate micronutrients and hydration to meet the body's physiologic needs (181). Similarly, the Buddhist diet appears healthy, as it is similar to a standard vegetarian diet (144).

Unfortunately, the diet of Muslim observers (consumed after the break of fast) during Ramadan could be characterized as “unhealthy.” In this context, Khaled and Belbraouet (182) reported that Ramadan observers consume more food rich in saturated fat, sugar, and processed carbohydrates, which may mask/reduce the beneficial effects of Ramadan fasting on health. Shifting toward a healthy diet (i.e., multiple plant-based diets) such as the Mediterranean diet or the Dietary Approaches to Stop Hypertension (DASH diet) will be beneficial for the health of Muslim observers. Current evidence suggests plant-based diets reduce the risk of NCDs such as obesity, cardiovascular diseases, type 2 diabetes, and some cancers (5). Interestingly, these dietary patterns might include animal products (e.g., dairy, moderate quantities of red meat), and have all been shown to be healthier and associated with less environmental impact than the average diet in the United States (12).

Given the lack of restriction on food choices during Ramadan fasting, and the possible lack of food literacy in many Muslim individuals (183), nutritional education for Muslims before the month of Ramadan is warranted. Additionally, stakeholders involved in public health should actively participate in consumer health programs focused on nutrition awareness.

Fourth, previous studies have revealed that level of physical activity is generally reduced during fasting rituals. For example, Farook et al. (184) observed decreases in the objectively assessed habitual physical activity of adults during the month of Ramadan. Similarly, Lessan et al. (185) reported that the total number of steps walked per day were significantly lower during Ramadan. Also, Buddhists are characterized by a sedentary lifestyle; they were only participating in some “religious” daily light activities (e.g., chanting, maintenance of the temple premises, meditation) (186). However, it is well-known that persistent low levels of physical activity are detrimental to individual health and wellbeing (187, 188) and that adhering to regular a physical activity program is effective in reducing the burden of NCDs (189), as well as premature morbidity and mortality (190). Unfortunately, the results of a recent study (191) revealed religiosity did not play a mediating role in physical

activity participation. Therefore, the promotion of physical activity during and outside fasting periods is needed. It should be noted that undertaking physical activity in a fasting state improves body composition (i.e., body fat) and some metabolic indices (e.g., HDL-cholesterol) (192, 193).

Finally, the frequency of emergency department admissions appears not to change (194–198) or even decrease (199) during Ramadan, suggesting no adverse effects regarding the safety of fasting during the month of Ramadan. Nevertheless, data related to the effect of other types of religious fasting on the frequency of emergency department admissions are lacking. Given the healthy diet and the possible intake of water characterizing other religious fasting regimens, it is hypothesized a decrease in the frequency of emergency department admissions will be observed.

Taken together, religious fasting regimens appear safe and beneficial for public health. Individuals involved in the public health sector such as physicians and nutritionists should encourage religious believers to adhere to fasting. Public health stakeholders should encourage public mass media to promote elements found in religious-based fasts as a nutritional public health strategy. Additionally, to optimize health gains from fasting, sessions of nutritional education (e.g., workshops, and seminars) should be organized during and outside the fasting rituals.

Religious fasting and planetary health

The prestigious medical journal, *The Lancet*, published at the beginning of 2014 a manifesto for planetary health, which was signed by 7,390 scientists from all over the world, primarily from the fields of medicine, public health, health care, environmental science, and ecology (200). *The Lancet* declared planetary health a new research area in its own right in 2017, requiring multidisciplinary, interdisciplinary, and transdisciplinary efforts to address unprecedented challenges. This relatively new integrating concept focuses on preserving “the health of human civilization and the state of the natural systems on which it depends” (201) and is gaining support from reputable funding and charitable organizations (e.g., the Rockefeller Foundation). Good human health cannot be preserved when the ecological systems that sustain life on Earth are in a poor or unsatisfactory condition (202). Planetary health is also considered a social movement that aims to transform current practices of living and conducting business at all levels (i.e., individual, societal, national, regional, global), in response to threats to human wellbeing, the sustainability of human civilization, and the health of the planet we inhabit and share with other species (200). Current evidence supports the assertion that global warming is human-induced (203). Religious fasting may help in tackling some environmental

issues. For example, during some religious fasting patterns (i.e., Muslim, Buddhism, Daniel), alcoholic drinks are not permitted, and in some Muslim countries (e.g., Tunisia), and are even regulated by a law to not be on sale during the month of Ramadan. It should be acknowledged that some alcoholic beverages (e.g., Whiskey, Vodka) are classified by the NOVA system (204) as ultra-processed foods (UPFs). UPFs include multiple industrial processing (e.g., extrusion, molding, and pre-frying) and manufacturing stages, usage of a large variety of components/additives which have potential dual detrimental impacts on the environment and health (205), and the usage of extensive synthetic packaging, a major source of environmental waste production, mostly containing compounds with carcinogenic and endocrine disruptor properties, UPFs seems to have the most harmful impact, not only on health, but also on the environment (206). It appears that decreasing the consumption of alcoholic drinks during religious fasting is beneficial for environmental health.

It is well-known that livestock is responsible for a significant portion of GHG emissions (14). Marinova and Raphaely (207) found that replacing meat (i.e., beef) with a plant-based option (i.e., wheat) generates 113 times less GHG emissions per nutrient. In addition, red meat is detrimental to planetary health through its direct impacts on land use and freshwater withdrawals (202). Meat consumption is also a significant contributor to biodiversity loss (208), and phosphorus depletion, which endangers future plant-based food production (209). Additionally, based on 800 studies, red meat was classified as carcinogenic to humans (Category 1 for processed meat and Category 2a for cooked meat) by the World Health Organization in 2015 (WHO, 2015). Clearly, red meat consumption is an issue that requires immediate attention in this social movement for restoring and preserving planetary health.

Red meat consumption is not permitted in some religious fasting, including COC fasting, Daniel fast, and Buddhist fasting, which may contribute to the planetary health preservation. However, observers of Ramadan are permitted consume red meat. It is, therefore, necessary to find a strategy that will enable the reduction of its consumption by the Muslim community. In this context, UNEP (210) reported religion continues to play a significant role in many societies including education, health, and politics, and could help to address environmental challenges. Indeed, elements from religion can influence public opinion on sustainability by changing adherents' daily practices, and providing ethical orientation and visions for addressing climate, environmental, and ecological crises. For example, Barclay (211) reported a Muslim fishing community from Tanzania implemented sustainable fishing methods resultant of scientific information provided by Islamic leaders on the subject. Fortunately, "Muslim eco-theologians believe that they have a personal and spiritual responsibility to prevent the spread of environmental

degradation because Islam encompasses not only humanity but also nature" (212). In addition, the duty to care for and protect nature is rooted in the Roman Catholic Church's Encyclical "Laudato Si" (213), and Pope Francis has publicly spoken of ecological sins when referring to the climate crisis (214). Likewise, the Interfaith Rainforest Initiative, which fights rainforest deforestation and protects indigenous peoples, uses religious texts from member religions (e.g., Buddhism, Catholicism, Hinduism, Islam, Judaism, Protestant) to advocate for protection (215).

In this context, Weder et al. (216) reported that communication based on religious texts is an effective tool for raising public awareness and shaping public opinion on sustainable development and the environment. Making the public aware of the environmental crisis during prayers is recommended. However, this can be achieved only when religious leaderships (e.g., Imam in the Islamic religion, Pope in the Christian religion) are appropriately educated.

Religious leaders should orient their dialogue toward the obligation to foster efforts of religious believers to decrease pollution, which is responsible for approximately 9 million deaths per year (217). For example, encouraging religious believers to limit (when possible) the use of fuel-powered means of transport will contribute to the decrease of ambient-air pollution, as well as tackling sedentariness. It is worth noting that, in the Muslim religion, avoiding means of transport to join a mosque is encouraged.

Religious leaders should pay attention to food security, which exists only when all individuals have access to physically, socially, and economically sufficient, nutritious, and safe food that meets their dietary needs (218). In this context, Islam does not encourage overeating during and outside the month of Ramadan. Therefore, religious leaders should focus their discussions on avoiding overeating, which could help to ensure food security.

Collectively, religious fasting can help tackle current environmental issues due to the restriction/decrease of the intake of some foods harmful to planetary health. Religious-based communication/dialogue must assert landscape values of respect, love, and care for current and future generations, the planet, and other species. Religiosity and, more broadly speaking, spirituality, are topics, generally, narrowly articulated and framed and have rarely been investigated at the intersection of other topics, such as health, from the individual, public, global, and planetary perspectives. Rethinking religiosity/spirituality means dissecting its profound impacts on human life and daily activities, as well as it means reflecting on public, global, and planetary health from "unusual" points of view. Both religion and health should be conceived and understood, not as abstract entities, but rather as interconnected parts of everyday life, like all those "activities that make us human and keep us in

motion: worship, travel, work, migration, the quest for health, and encounters with disease and death” (219). Integrating and incorporating elements of religiosity/spirituality and spiritual, religious, and faith-based practices in public, global, and planetary policies, fully respecting cultural traditions, cultivating and fostering religious and interfaith dialogue, and embracing diversity and pluralism as well as shifting toward more sustainable lifestyles, including dietary intake, may contribute to a more just and equitable society. In conclusion, fasting represents a fundamental “religious health asset,” that is to say, “an asset located in or held by a religious entity that can be leveraged for the purposes of development of public health” (220). Religion-based fasting can be, indeed, mobilized to improve individual health, as well as the health of communities, populations, and the planet.

Conclusions

Religious fasting is universally widespread—from Buddhism, Jainism, and Hinduism to Christianity, Islam, Judaism, and Taoism—and not the prerogative of a single religious belief. Individual/clinical, public, global, and planetary health have usually been investigated separately and siloed from each other. Religious fasting, alongside other religious health assets, can provide several opportunities across multiple levels of scale, ranging from the individual to the community, population, environmental, and planetary levels, by facilitating and supporting societal transformations and changes, including the adoption of healthier, more equitable, and sustainable lifestyles, preserving the Earth’s systems, and addressing major interrelated, cascading and compound challenges, with tremendous impacts on the “whole health”—from inequities and inequalities to NCDs and the climate crisis (221).

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Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

KT, SG, and NB conceived and designed the manuscript. AA, MB, LP, ES, OB, SK, CC, JG, OA, HJ, and HC critically revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.1036496/full#supplementary-material>

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Dietary patterns, nutritional status, and mortality risks among the elderly

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Introduction: While most epidemiological studies have focused on the effects of individual dietary patterns and nutritional status on health, the relationships between the combinations of these factors and patient prognosis requires further investigation.

Objective: This study explored mortality risk in individuals with different combinations of dietary patterns or nutritional status.

Methods: Unsupervised K-means clustering was used to classify populations. The analyses included Cox proportional risk and competing risk models.

Results: After considering a complex sampling design, the results showed that among 12,724 participants aged >60 years, 6.99% died from cancer and 10.47% from cardiovascular and cerebrovascular disease (CCVD). After correcting for participant baseline information and chronic conditions, the geriatric nutritional risk index and healthy eating index (HEI) were negatively associated with the risk of all-cause and cause-specific mortality. The opposite was true for the dietary inflammatory index (DII). After sorting the population three clusters based on study scores showed higher risks of all-cause mortality and cancer-related death in Cluster 2 and 3.

Discussion: These results suggest that different nutritional status and dietary patterns are associated with the risk of all-cause mortality and death from cancer and CCVD in people aged >60 years in the United States. Dietary patterns with high HEI and low DII were beneficial to health, whereas nutritional status needs to be maintained at a level that is not too low.

KEYWORDS

diet, k-means clustering, mortality, nutritional status, dietary patterns

Introduction

Typical dietary patterns are practical nutritional tools that reflect regular dietary habits. A nutritional gap in the diet refers to specific nutrient deficits that may lead to deficiencies and poor health. Dietary and nutritional factors may contribute to or mitigate disease development and significantly influence its outcome (1). The indicators commonly used to measure dietary patterns and nutritional status include the dietary

inflammatory index (DII), Healthy Eating Index (HEI), and Geriatric Nutritional Risk Index (GNRI).

Assessing the nutritional status of individuals is complex. The GNRI is widely used to measure nutritional status in older people, as it is derived from serum albumin levels and body mass indexes (2, 3). A scoring algorithm was developed for the DII to estimate an individual's dietary inflammatory potential (4). The National Cancer Institute developed the HEI to evaluate dietary quality in the US (5). While epidemiological studies have explored the relationship between the GNRI (nutritional indicator) and DII and HEI (dietary indicators) and all-cause, cardiovascular and cerebrovascular disease (CCVD), and cancer mortality (6–11), these conclusions remain inadequate. In addition, most studies assess one dimension of nutrition or dietary habits, with few studies focusing on the health effects of the multiple dimensions of food and nutritional habits.

The impact of different dietary patterns and nutritional status combinations on health warrants exploration. Classifying individuals according to different combinations can provide information on their dietary habits, nutritional status, and health in a broader dimension. This study examined this effect using information from the National Health and Nutrition Examination Survey (NHANES) database from 1999 to 2018. We explored the relationship of dietary patterns and nutritional status with all-cause and cause-specific mortality by classifying populations using k-means clustering methods.

Materials and methods

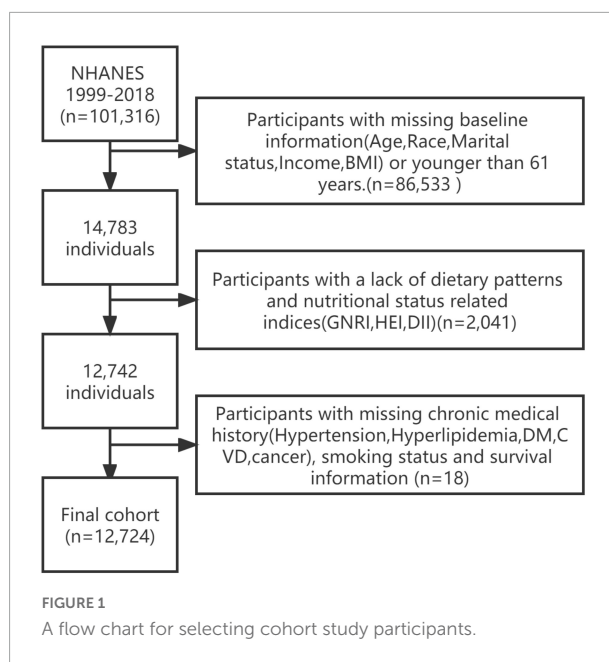
Study design

This prospective study included adults aged >60 years from 1999 to 2018. The NHANES is a cross-sectional, ongoing study of the US non-institutionalized population and includes data from household interviews, examinations, and post-examinations. The samples were selected using a complex multistage process. The participants' publicly available mortality data were obtained on December 31, 2019 (12). According to the International Classification of Diseases, the primary outcomes were all-cause mortality, as well as cancer (C00-C97), cardiac (I00-I09, I11, I13, I20-I51), cerebral (I60-I69), and vascular disease (CCVD). The participant selection and exclusion flow chart was shown in Figure 1. The NCHS research ethics review board approved the NHANES protocol.

Nutritional status and dietary patterns

The GNRI is calculated using the following formula:

$$\text{GNRI} = [1.489 \times \text{Alb (g/L)}] + \{41.7 \times [\text{weight (kg)}/\text{IBW (kg)}]\}.$$



Where Alb is serum albumin and the ideal body weight (IBW) indicates a body mass index (BMI) of 22 kg/m² (2).

The DII is an indicator of dietary inflammation that is used to assess the anti-and pro-inflammatory properties of the diet of different individuals. Specific descriptions are available elsewhere (4). Based on a literature review, the DII was used to report the effects of 45 different dietary or nutritional intakes on inflammatory markers [interleukin (IL)-1 β , IL-4, IL-6, IL-10, C-reactive protein, and tumor necrosis factor- α (TNF- α)]. The intake of each food component was subtracted from its standard evaluated intake and divided by its standard deviation. The resulting value was converted to an intermediate percentage and multiplied by the overall inflammatory effect score for the food component. The DII score is the sum of the food scores. A positive DII value indicates a pro-inflammatory diet, whereas the opposite is true for an anti-inflammatory diet. In our study, the food components included: carbohydrates, proteins, total fat, alcohol, fiber, cholesterol, saturated fat, monounsaturated fatty acids, polyunsaturated fatty acids, n-3 fatty acids, n-6 fatty acids, niacin, vitamins (A, B1, B2, B6, B12, C, and E), iron, magnesium, zinc, selenium, caffeine, and energy.

Diet quality was assessed using the 2015 version of the HEI (5), which included 13 ingredients (whole protein foods, total vegetables, whole fruits, whole grains, greens and beans, dairy, seafood, plant proteins, fatty acids, sodium, added sugars, refined grains, and saturated fats). The HEI is calculated based on density (amount of food components/1000 kcal). The higher the score, the higher the diet quality.

Covariates

The covariates in this study population included age, sex, race, household income, BMI, marital status, and smoking status. The baseline information of these participants was obtained from the responses to the NHANES' demographics or questionnaires. Hyperlipidemia was defined as (1) triglyceride level ≥ 150 mg/dl; (2) total cholesterol level ≥ 200 mg/dl, low-density lipoprotein level ≥ 130 mg/dl, high-density lipoprotein level < 40 mg/dl (male) and < 50 mg/dl (female), or (3) treatment with lipid-lowering drugs. Hypertension was defined as (1) self-reported use of hypertensive medication, (2) previously reported hypertension (from a questionnaire), or (3) current blood pressure measurement (mean systolic blood pressure ≥ 140 mmHg or mean diastolic blood pressure ≥ 90 mmHg). Diabetes mellitus (DM) was

defined as (1) self-reported or told by a physician to have diabetes; (2) glycated hemoglobin $\geq 6.5\%$; (3) fasting glucose ≥ 7.1 mmol/L, DM; ≥ 6.11 and < 7.0 , impaired fasting glycemia; (4) random glucose ≥ 11.1 mmol/L; (5) 2 h oral glucose tolerance test ≥ 11.1 mmol/L, DM; ≥ 7.7 and < 11.1 mmol/L, impaired glucose tolerance; (6) self-reported use of diabetes medication. Smoking was defined as (1) having smoked less than 100 cigarettes, no smoke; (2) having smoked more than 100 cigarettes and not currently smoking, former smoke; and (3) having smoked more than 100 cigarettes and currently smoking, now smoke. NHANES provides more details on the above covariates (websites in the [Supplementary material](#)). Publicly available participant mortality data were used to determine the causes of death of the patients who died.

TABLE 1 Baseline demographic characteristics of all participants in this study.

Characteristics	Total	Female	Male	P-value*
Age (years)	70.00 (65.00, 76.00)	70.00 (65.00, 77.00)	69.00 (65.00, 76.00)	< 0.001
BMI (kg/m ²)	28.07 (24.83, 32.09)	28.01 (24.38, 32.50)	28.10 (25.35, 31.70)	0.23
GNRI	115.23 (108.40, 122.94)	114.98 (107.96, 123.41)	115.47 (108.90, 122.36)	0.76
DII	1.30 (-0.32, 2.56)	1.66 (0.13, 2.78)	0.77 (-0.72, 2.17)	< 0.001
HEI	56.31 (47.17, 66.17)	57.42 (48.40, 66.99)	54.78 (45.84, 64.94)	< 0.001
Triglyceride (mg/dl)	129.00 (91.00, 189.00)	130.00 (92.00, 186.00)	128.00 (89.00, 192.00)	0.66
Total cholesterol (mg/dl)	195.00 (167.00, 224.00)	206.00 (180.00, 233.00)	182.00 (155.00, 210.00)	< 0.0001
LDL (mg/dl)	111.00 (87.00, 137.00)	115.00 (92.00, 142.00)	106.00 (81.00, 130.00)	< 0.0001
HDL (mg/dl)	53.00 (43.00, 65.00)	58.00 (48.00, 71.00)	46.00 (39.00, 56.00)	< 0.0001
Race/ethnicity	—	—	—	0.01
Non-Hispanic white	81.83 (75.76, 87.90)	54.83 (53.84, 55.81)	45.17 (44.19, 46.16)	—
Non-Hispanic black	7.71 (6.91, 8.52)	59.33 (57.62, 61.05)	40.67 (38.95, 42.38)	—
Mexican American	3.38 (2.75, 4.01)	55.20 (52.92, 57.48)	44.80 (42.52, 47.08)	—
Other Race	7.07 (6.25, 7.90)	57.21 (53.87, 60.56)	42.79 (39.44, 46.13)	—
Marital status	—	—	—	< 0.001
Cohabited	1.83 (1.39, 2.26)	37.39 (28.97, 45.81)	62.61 (54.19, 71.03)	—
Divorced	11.43 (10.46, 12.39)	64.40 (61.22, 67.58)	35.60 (32.42, 38.78)	—
Married	62.06 (57.78, 66.33)	45.53 (44.59, 46.46)	54.47 (53.54, 55.41)	—
Separated	1.25 (1.06, 1.45)	57.09 (48.40, 65.77)	42.91 (34.23, 51.60)	—
Single	3.06 (2.68, 3.43)	54.07 (47.34, 60.81)	45.93 (39.19, 52.66)	—
Widowed	20.38 (18.94, 21.83)	81.91 (80.41, 83.40)	18.09 (16.60, 19.59)	—
Income	—	—	—	< 0.001
\$0–19,999	19.76 (17.94, 21.58)	66.54 (64.75, 68.33)	33.46 (31.67, 35.25)	—
\$20,000–\$44,999	36.04 (33.48, 38.61)	57.59 (56.24, 58.94)	42.41 (41.06, 43.76)	—
\$45,000–74,999	20.55 (18.68, 22.42)	49.57 (47.65, 51.49)	50.43 (48.51, 52.35)	—
\geq \$75,000	23.65 (21.39, 25.90)	47.63 (45.80, 49.45)	52.37 (50.55, 54.20)	—
Smoke	—	—	—	< 0.001
Never	48.17 (45.39, 50.95)	68.21 (66.85, 69.57)	31.79 (30.43, 33.15)	—
Former	41.22 (38.38, 44.05)	42.25 (40.41, 44.09)	57.75 (55.91, 59.59)	—
Now	10.56 (9.63, 11.49)	47.81 (44.25, 51.38)	52.19 (48.62, 55.75)	—

LDL, low density lipoprotein; HDL, high-density lipoprotein, GNRI, geriatric nutritional risk index; DII, dietary inflammatory index; HEI, healthy eating index; BMI, body mass index.

*P-values were calculated by Rao-Scott chi-square test and Wilcoxon rank-sum test.

TABLE 2 Baseline disease characteristics of all participants in this study.

Characteristics	Total	Female	Male	P-value*
Hyperlipidemia	—	—	—	<0.001
No	16.09 (14.81, 17.37)	47.89 (45.52, 50.26)	52.11 (49.74, 54.48)	—
Yes	83.91 (79.08, 88.74)	56.79 (55.81, 57.76)	43.21 (42.24, 44.19)	—
Hypertension	—	—	—	<0.001
No	29.79 (27.52, 32.07)	51.30 (49.47, 53.13)	48.70 (46.87, 50.53)	—
Yes	70.21 (66.23, 74.18)	57.08 (55.99, 58.16)	42.92 (41.84, 44.01)	—
DM	—	—	—	<0.001
No	63.35 (59.48, 67.21)	58.00 (56.83, 59.18)	42.00 (40.82, 43.17)	—
DM	26.27 (24.51, 28.04)	50.11 (47.79, 52.43)	49.89 (47.57, 52.21)	—
IFG	6.56 (5.72, 7.40)	48.80 (43.52, 54.09)	51.20 (45.91, 56.48)	—
IGT	3.82 (3.20, 4.44)	58.74 (54.20, 63.27)	41.26 (36.73, 45.80)	—
Cause of death	—	—	—	<0.001
Cancer	6.99 (6.30, 7.68)	42.56 (38.50, 46.62)	57.44 (53.38, 61.50)	—
CCVD	10.47 (9.51, 11.43)	50.44 (47.61, 53.27)	49.56 (46.73, 52.39)	—
NO	68.06 (63.73, 72.39)	57.22 (56.16, 58.27)	42.78 (41.73, 43.84)	—
Other	14.48 (13.31, 15.64)	56.34 (53.84, 58.85)	43.66 (41.15, 46.16)	—

DM, diabetes mellitus; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; CCVD, cardiovascular and cerebrovascular diseases.

*P-values were calculated by Rao-Scott chi-square test and Wilcoxon rank-sum test.

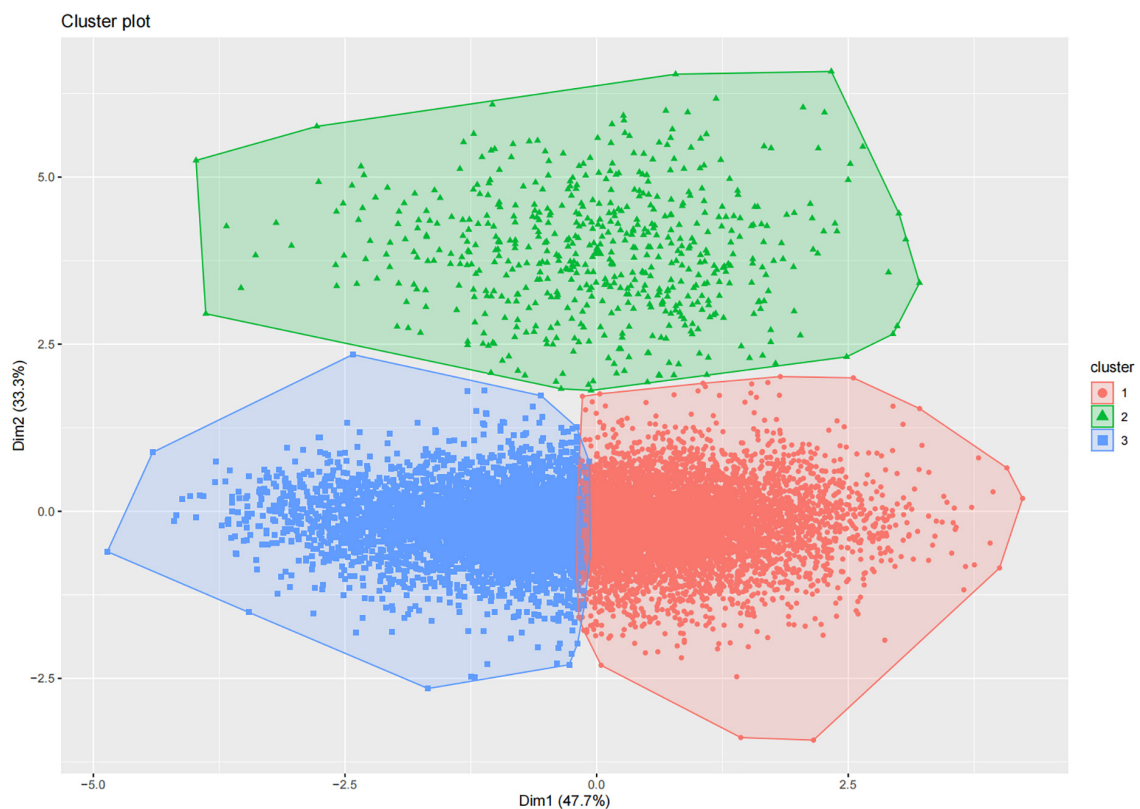


FIGURE 2

K-means clustering of participants based on three nutrition- and diet-related scores, and visualization. Red dots indicate cluster 1 [higher healthy eating index (HEI), lower dietary inflammatory index (DII)]; green dots indicate cluster 2 [lower geriatric nutritional risk index (GNRI)]; and blue dots indicate cluster 3 (lower HEI, higher DII).

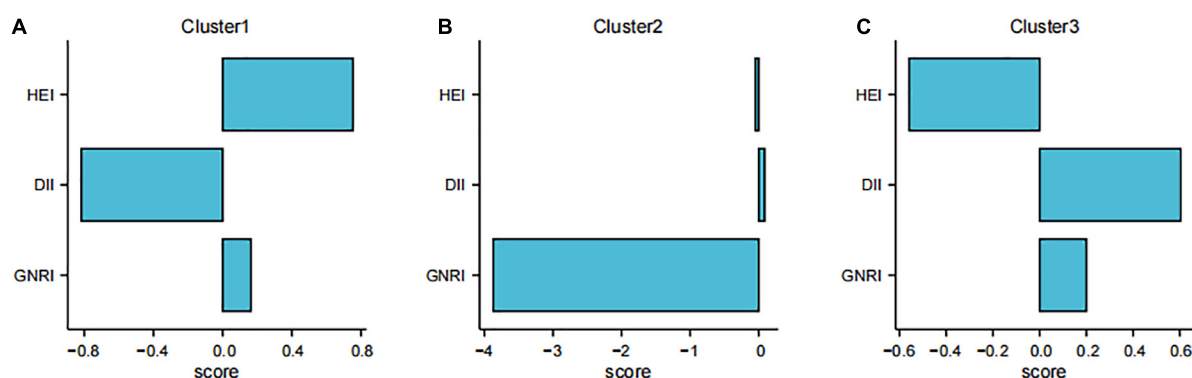


FIGURE 3

Distribution of cluster centers for the three scores in different clusters. (A) Cluster 1 [higher healthy eating index (HEI), lower dietary inflammatory index (DII)]; (B) Cluster 2 [lower geriatric nutritional risk index (GNRI)]; (C) Cluster 3 (lower HEI, higher DII).

Statistical analysis

Following the guidelines for using the NHANES database, sampling weights were considered in the analysis of the relationship between the variables and all-cause mortality. Weighted analyses were conducted to better reflect the overall picture. Categorical and continuous variables are expressed as percentages (%) and co-medians (interquartile range), respectively. Nutritional and dietary pattern scores were log-transformed and standardized for analysis. Differences in categorical and continuous variables were tested using Rao–Scott chi-square and Wilcoxon rank-sum tests. We used k-means clustering to identify groups of individuals with similar characteristics. K-means, a popular unsupervised algorithm, can be used to distinguish between groups. It is widely used in the medical field (13, 14). K-means groups patients by identifying the centroids of different groups. It was first proposed in 1967 (15). The algorithm divides the participants into clusters where the number of collections k is chosen autonomously. We used the “factoextra” package (16), clustering effects, and clinical significance to find the optimal number of clusters. The final result was three cluster centers, with the population belonging to each group labeled. Rao–Scott chi-square tests were used to compare the baseline characteristics of the participants in different clusters. Bar charts were used to describe the dietary structure or nutritional attributes of the different populations.

We used the Cox proportional risk model to determine the associations of all-cause mortality with variables and clusters and calculated the hazard ratios (HRs) and corresponding 95% confidence intervals (95% CIs). We performed competing risk analyses to compare different variables and clusters of cancer-specific or CCVD deaths and obtained HRs (17). The three models in the study were as follows: model one, which included only the study variables and did not adjust for other factors; model two, which adjusted for age, sex, race, marital status, and household income; and model three, which additionally

adjusted for smoking status, hypertension, hyperlipidemia, and diabetes. Ten participants were not included in model three owing to a lack of data on smoking status. All analyses were performed using R version 4.1.0. All statistical analyses were two-sided. Statistical significance was defined as $p < 0.05$.

Results

Baseline characteristics of participants

After excluding participants with missing study variables and covariates, this study included 12,724 participants aged >60 years. After weighting, 6.99% died of cancer and 10.47% died of cardiovascular disease over a median follow-up time of 7.1 years. The weighted baseline characteristics of the participants are shown in **Tables 1, 2**. The unweighted baseline characteristics are shown in **Supplementary Table 1**.

We classified the 12,724 participants into three clusters using k-means clustering. The graph of the classification results is shown in **Figure 2**.

The results of the analysis of the nutrition and diet-related index centers are shown in **Figure 3** and **Supplementary Table 2**.

We categorized participants with lower DII and higher HEI as the first cluster, those with lower GNRI as the second cluster, and those with lower HEI and higher DII as the third cluster. **Supplementary Table 3** shows the individual standardized scores. **Table 3** shows the baseline characteristics of the study participants in the three weighted collections.

Compared to the other two clusters, the second cluster had a larger proportion of participants aged 71–80 years (48%), a smaller proportion of caucasians (70.72%), a smaller proportion of people with hyperlipidemia (45.7%), and a higher proportion of people with hypertension (76.1%). Caucasians comprised the majority of participants.

TABLE 3 Baseline characteristics of participants in the national health and nutrition examination survey (NHANES) stratified by the three clusters of nutrition- and diet-related scores.

Characteristics	Total	Cluster 1	Cluster 2	Cluster 3	P-value*
Age (years)	—	—	—	—	0.02
60–70	53.88 (50.49, 57.27)	54.73 (52.73, 56.73)	45.08 (38.90, 51.26)	53.83 (52.19, 55.46)	—
71–80	41.35 (38.87, 43.83)	40.58 (38.71, 42.46)	48.05 (42.27, 53.83)	41.49 (39.87, 43.10)	—
> 80	4.77 (4.03, 5.52)	4.69 (3.78, 5.59)	6.87 (4.43, 9.32)	4.69 (4.02, 5.36)	—
BMI (kg/m ²)	—	—	—	—	<0.001
<18.5	1.11 (0.91, 1.32)	1.15 (0.82, 1.48)	2.13 (0.93, 3.34)	1.01 (0.74, 1.28)	—
18.5–23.9	18.30 (16.84, 19.76)	20.15 (18.52, 21.79)	20.98 (16.47, 25.49)	16.60 (15.23, 17.97)	—
24–28	29.83 (27.80, 31.86)	32.40 (30.72, 34.08)	31.19 (25.93, 36.46)	27.63 (25.89, 29.37)	—
≥28	50.76 (47.48, 54.03)	46.30 (44.58, 48.02)	45.70 (40.48, 50.91)	54.76 (52.92, 56.60)	—
Race/ethnicity	—	—	—	—	<0.001
Non-Hispanic white	81.83 (75.76, 87.90)	83.67 (82.00, 85.35)	70.72 (66.06, 75.39)	81.13 (79.12, 83.14)	—
Non-Hispanic black	7.71 (6.91, 8.52)	5.51 (4.74, 6.28)	16.77 (13.48, 20.05)	8.86 (7.63, 10.08)	—
Mexican American	3.38 (2.75, 4.01)	3.26 (2.62, 3.90)	3.05 (2.01, 4.08)	3.50 (2.67, 4.33)	—
Other Race	7.07 (6.25, 7.90)	7.55 (6.43, 8.68)	9.46 (6.23, 12.69)	6.51 (5.53, 7.49)	—
Marital status	—	—	—	—	<0.001
Cohabited	1.83 (1.39, 2.26)	1.48 (0.98, 1.99)	1.67 (0.34, 3.01)	2.12 (1.55, 2.68)	—
Divorced	11.43 (10.46, 12.39)	10.08 (8.85, 11.30)	14.36 (9.89, 18.82)	12.32 (11.15, 13.49)	—
Married	62.06 (57.78, 66.33)	65.83 (63.60, 68.05)	53.14 (47.39, 58.89)	59.62 (57.95, 61.30)	—
Separated	1.25 (1.06, 1.45)	1.11 (0.78, 1.44)	2.73 (1.11, 4.36)	1.27 (1.04, 1.49)	—
Single	3.06 (2.68, 3.43)	2.91 (2.32, 3.50)	2.14 (1.22, 3.06)	3.24 (2.71, 3.77)	—
Widowed	20.38 (18.94, 21.83)	18.59 (16.99, 20.20)	25.96 (21.25, 30.67)	21.44 (20.11, 22.77)	—
Income	—	—	—	—	<0.001
\$0–19,999	19.76 (17.94, 21.58)	15.18 (13.59, 16.76)	26.57 (21.84, 31.30)	23.01 (21.33, 24.69)	—
\$20,000–\$44,999	36.04 (33.48, 38.61)	33.21 (31.08, 35.33)	36.80 (31.12, 42.48)	38.30 (36.36, 40.24)	—
\$45,000–74,999	20.55 (18.68, 22.42)	20.99 (19.05, 22.93)	20.25 (14.99, 25.50)	20.21 (18.74, 21.69)	—
≥\$75,000	23.65 (21.39, 25.90)	30.62 (28.02, 33.22)	16.39 (12.30, 20.47)	18.48 (16.65, 20.31)	—
Hyperlipidemia	—	—	—	—	<0.001
No	16.09 (14.81, 17.37)	16.38 (14.96, 17.80)	54.26 (48.87, 59.64)	13.09 (12.11, 14.08)	—
Yes	83.91 (79.08, 88.74)	83.62 (82.20, 85.04)	45.74 (40.36, 51.13)	86.91 (85.92, 87.89)	—
Hypertension	—	—	—	—	<0.001
No	29.79 (27.52, 32.07)	32.33 (30.36, 34.30)	23.83 (18.15, 29.51)	28.16 (26.61, 29.70)	—
Yes	70.21 (66.23, 74.18)	67.67 (65.70, 69.64)	76.17 (70.49, 81.85)	71.84 (70.30, 73.39)	—
DM	—	—	—	—	<0.001
No	63.35 (59.48, 67.21)	65.15 (63.23, 67.07)	72.86 (67.89, 77.84)	61.18 (59.62, 62.75)	—
DM	26.27 (24.51, 28.04)	23.96 (22.44, 25.49)	26.47 (21.61, 31.33)	28.14 (26.75, 29.54)	—
IFG	6.56 (5.72, 7.40)	6.50 (5.55, 7.46)	0.08 (–0.05, 0.21)	7.08 (6.06, 8.09)	—
IGT	3.82 (3.20, 4.44)	4.38 (3.45, 5.31)	0.59 (0.01, 1.17)	3.60 (2.91, 4.28)	—
Cause of death	—	—	—	—	<0.001
Cancer	6.99 (6.30, 7.68)	5.95 (5.12, 6.78)	15.57 (11.86, 19.28)	7.22 (6.39, 8.05)	—
CCVD	10.47 (9.51, 11.43)	9.33 (8.31, 10.36)	10.41 (7.58, 13.25)	11.40 (10.36, 12.09.44)	—
NO	68.06 (63.73, 72.39)	72.84 (70.93, 74.76)	59.17 (53.64, 64.70)	64.80 (62.80, 66.81)	—
Other	14.48 (13.31, 15.64)	11.87 (10.72, 13.02)	14.85 (11.05, 18.65)	16.57 (15.39, 17.76)	—

GNRI, geriatric nutritional risk index; DII, dietary inflammatory index; HEI, healthy eating index; BMI, body mass index; DM, diabetes mellitus; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; CCVD, cardiovascular and cerebrovascular diseases.

*P-values were calculated by Rao-Scott chi-square test.

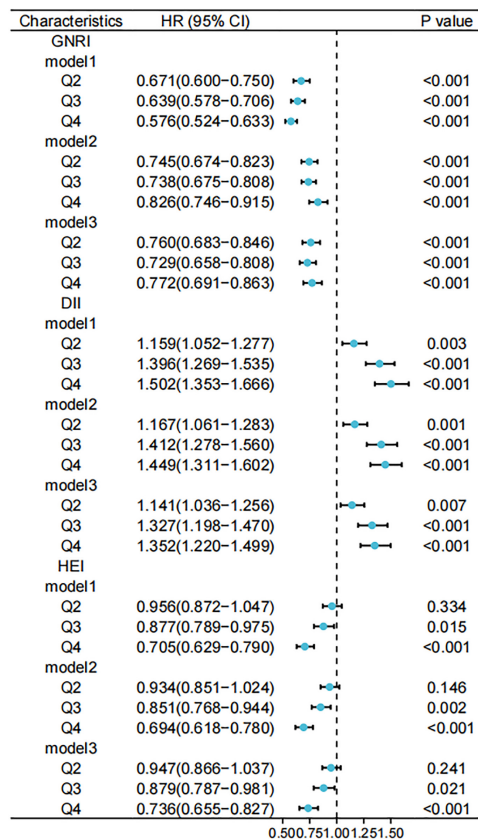


FIGURE 4

Relationship between different scores and risk of all-cause mortality. Standardized scores were divided into four groups (Q1, Q2, Q3, and Q4) according to the 25th, 50th, and 75th quartiles, with the Q1 group used as a reference. Model 1 was unadjusted for variables and Model 2 was adjusted for age, sex, race, marital status, and household income at baseline; Model 3 was further adjusted for history of hypertension, hyperlipidemia, and diabetes. GNRI, geriatric nutritional risk index; DII, dietary inflammatory index; HEI, healthy eating index.

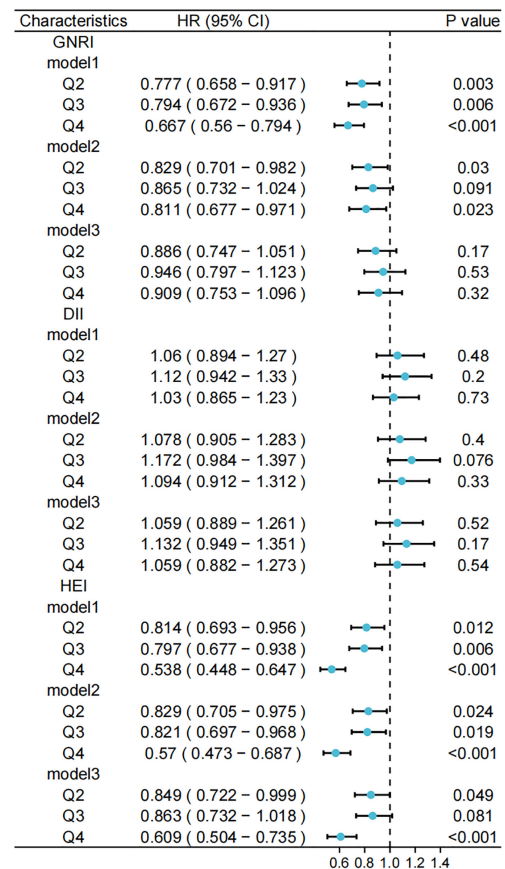


FIGURE 5

Relationship between different scores and risk of cancer mortality. Standardized scores were divided into four groups (Q1, Q2, Q3, and Q4) according to the 25th, 50th, and 75th quartiles, with the Q1 group used as a reference. Model 1 was unadjusted for variables and Model 2 was adjusted for age, sex, race, marital status, and household income at baseline; Model 3 was further adjusted for history of hypertension, hyperlipidemia, and diabetes. GNRI, geriatric nutritional risk index; DII, dietary inflammatory index; HEI, healthy eating index.

Nutrition and diet indices related to mortality risk

Figure 4 shows forest plots of the three scores against all-cause mortality, considering the complex survey design.

Furthermore, we used weighted to Cox regression calculate the HRs and 95% CIs. Figures 5, 6 show forest plots of the three scores (quartiles) against the risks of cancer-related and CCVD-related mortality.

Our unweighted competing risks model calculated the relationship between them.

We adjusted for participant baseline characteristics (sex and age) and patient-relevant medical history (hypertension and diabetes) to obtain HRs and 95% CIs for the different variable-adjusted indices versus all-cause mortality (Figure 4). We observed lower risks of death for participants with GNRI values in the second, third, and fourth quartiles compared to that

in the lowest quartile in the model. This trend was observed in the entire model. For the DII, the risk of death was higher in the other quartiles than in the lowest quartile. For HEI, the risks of death were lower in the third (HR: 0.879, 95% CI: 0.787–0.981) and fourth quartiles (HR: 0.736, 95% CI: 0.655–0.827) compared to that in the lowest quartile.

Using competing risk models, we obtained forest plots of the different indices for cancer-related mortality risk (Figure 5). A higher GNRI was associated with a lower risk of cancer-related death only in the unadjusted model (HR: 0.667, 95% CI: 0.56–0.794), a trend that was not significant in the adjusted model. No models showed a significant association between the DII and the risk of cancer-related deaths. The risk of cancer death was lower in the highest quartile of HEI compared to that in the lowest quartile (HR: 0.609, 95% CI: 0.504–0.735). In contrast, the tendency for a reduced risk of cancer death in the second and third quartiles of HEI was more significant in models 1 and 2.

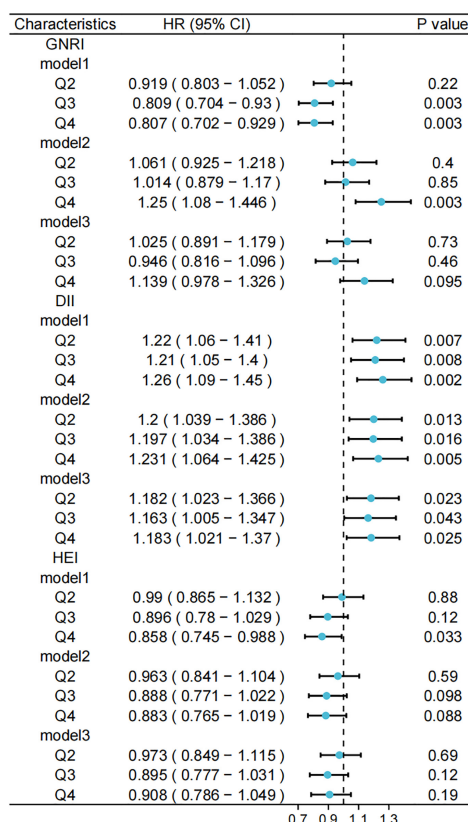


FIGURE 6

Relationship between different scores and risk of cardiovascular and cerebrovascular diseases (CCVD) mortality. Standardized scores were divided into four groups (Q1, Q2, Q3, and Q4) according to the 25th, 50th, and 75th quartiles, with the Q1 group used as a reference. Model 1 was unadjusted for variables and Model 2 was adjusted for age, sex, race, marital status, and household income at baseline; Model 3 was further adjusted for history of hypertension, hyperlipidemia, and diabetes. GNRI, geriatric nutritional risk index; DII, dietary inflammatory index; HEI, healthy eating index.

Figure 6 shows a forest plot of the risks of death associated with cardiovascular disease obtained from the unweighted competing risk model. After controlling for participant baseline information and chronic medical history, participants in the other three quartiles of the DII had an increased risk of death from cardiovascular disease compared to those in the lowest quartile (model 3, Q4; HR: 1.183, 95% CI: 1.021–1.37). For the GNRI and HEI, no significant trends were observed in the risk of death from cardiovascular disease in the adjusted models.

After adjusting for multiple variables, we determined the relationships between different clusters and the risk of all-cause mortality using a weighted Cox proportional risk model. An unweighted competing risk model was used to assess the relationship between different sets and the risk of death from cancer and cardiovascular disease (Figure 7).

Compared to Cluster 1, the risk of all-cause mortality and cancer-related mortality was higher in Clusters 2 and 3 in

the full model, with a more pronounced trend observed in Cluster 2 (model 3, HR: 2.627, 95% CI: 2.054–3.36). The risk of CCVD death was higher in Cluster 3 (model 3, HR: 1.13, 95% CI: 1.018–1.255) compared to Cluster 1, a trend that was not observed in Cluster 2.

Discussion

This study assessed the relationship of nutritional status and dietary patterns with all-cause and specific causes of death in individuals aged >60 years. We considered the effects of combinations of dietary habits and nutritional status with different distributions in the population on the risk of death. Compared to those in the lowest quartile, the GNRI and HEI had a better prognosis in the higher levels. A higher DII score was associated with a higher risk of mortality. After unsupervised clustering of the population based on the three indicators (GNRI, DII, and HEI), the population was divided into three clusters. We found that Cluster 2, with a lower GNRI, was associated with higher all-cause and cancer risks of death compared to Cluster 1. This tendency was more pronounced than that in Cluster 3 (lower HEI, higher DII, and moderate GNRI).

The result of our study revealed a negative correlation between the GNRI and all-cause mortality. The GNRI was calculated based on the serum albumin concentration, height, and weight. The simplicity of this calculation has led to the widespread use of this nutritional indicator. Some studies have reported lower activity levels in older patients in the low GNRI group with heart failure (18). A low preoperative GNRI was associated with increased postoperative complications and poor prognosis (6, 19), which is consistent with our findings. It was also an independent prognostic factor for some cancers (20, 21). These are probably related to the anti-inflammatory and antioxidant physiological properties of serum albumin (22).

Dietary inflammatory index was positively associated with all-cause mortality in participants aged >60 years in the present study. A previous meta-analysis demonstrated that a pro-inflammatory diet was associated with increased risks of cardiovascular disease and mortality (23). Deng et al. also reported this correlation in the NHANES III database (24). This is consistent with our findings. We validated this finding using the latest mortality data from the NHANES database (follow-up to 2018). Pro-inflammatory diets were scored based on inflammatory factors, and their close relationship may explain the correlation between DII and mortality. For example, TNF- α and IL-1 β play roles in vascular inflammation (25).

High-quality diets with higher HEI reduce all-cause mortality and the risk of cardiovascular disease and cancer death (26). A decreased HEI score indicates increased sugar or fat supply (27), leading to an increased risk of disease (28).

Owing to the individual variations in nutritional intakes and dietary patterns, the development of uniform standards provides

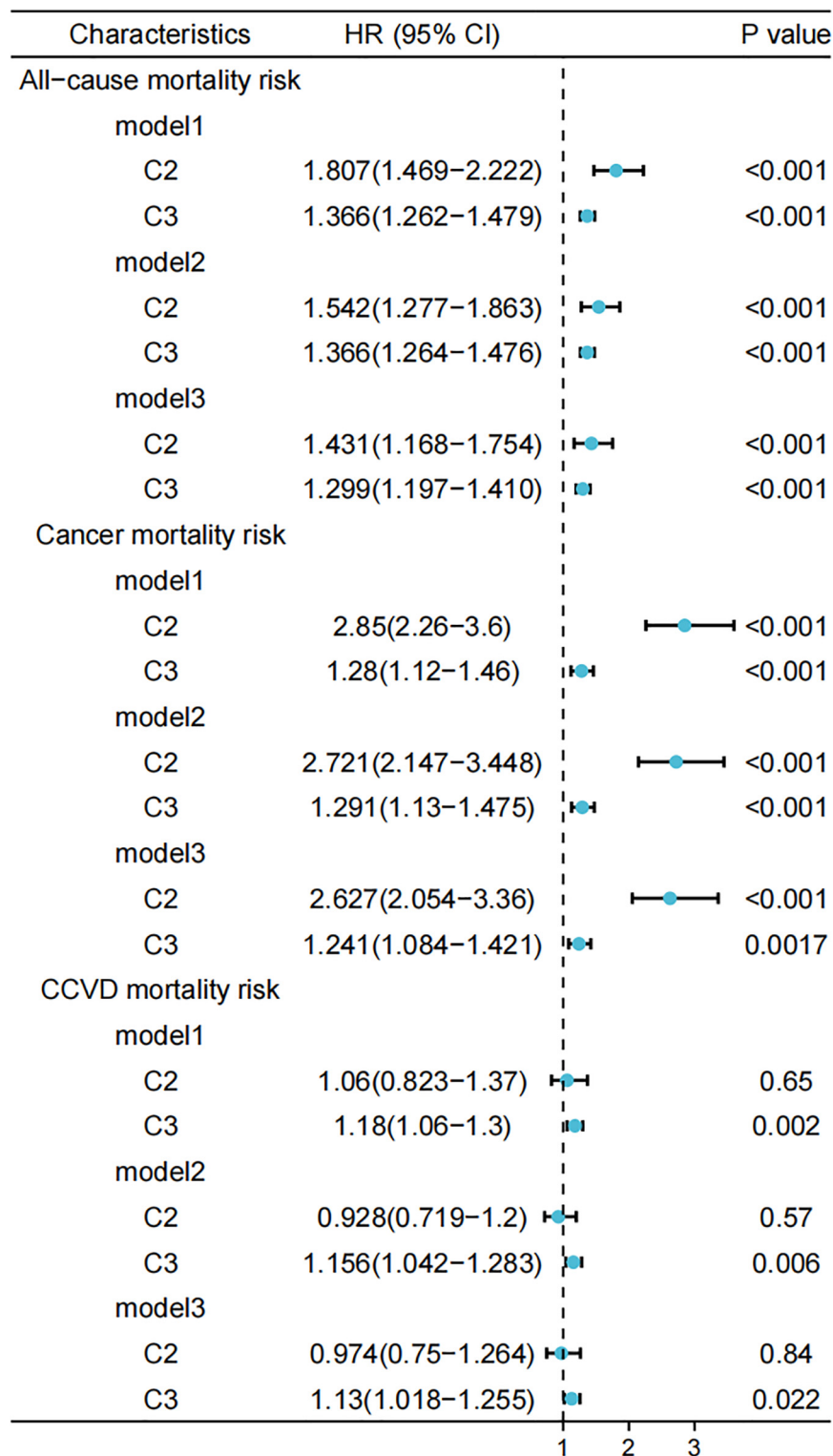


FIGURE 7

Relationship between different scores and risk of all-cause/cancer/cardiovascular and cerebrovascular diseases (CCVD) mortality. Standardized scores were divided into four groups (Q1, Q2, Q3, and Q4) according to the 25th, 50th, and 75th quartiles, with the Q1 group used as a reference. Model 1 was unadjusted for variables and Model 2 was adjusted for age, sex, race, marital status, and household income at baseline; Model 3 was further adjusted for history of hypertension, hyperlipidemia, and diabetes. GNRI, geriatric nutritional risk index; DII, dietary inflammatory index; HEI, healthy eating index.

a basis for studying the effects of diet on health (e.g., GNRI, DII, and HEI). However, single measures may affect the validity of the evaluation because they are developed for different populations or do not cover the full range of components. We clustered three different combinations of diet and nutrition scores on this study to evaluate the relationship between diet, nutrition, and health in older adults in a larger dimension. First, participants with a higher HEI and lower DII in Cluster 1 (moderate GNRI levels) had the best prognosis. Participants in Cluster 3 (higher DII, lower HEI, and average GNRI levels) had higher risks of all-cause mortality, cancer death, and CCVD death compared to that in Cluster 1.

In contrast, Cluster 2, with low GNRI (moderate DII and HEI), had a two-fold increased risk of cancer compared to Cluster 1. The high risk of CCVD death in cluster 3 may be associated with a high pro-inflammatory diet of inflammatory factors and increased energy supply from sugar or fat. These findings suggest that imbalances in nutritional status can have a significant impact on health.

This study had several strengths. First, the data were obtained from the newly updated NHANES database of survival data. Second, we classified the populations using unsupervised machine learning to identify distinctive characteristics. Additionally, we adjusted for the effects of covariates on the studied variables. However, this study has some additional limitations. First, the serum albumin measurements in the GNRI and diet collection in the diet model were not representative of the continuous state of the participants. Second, the k-means clustering method is sensitive to outliers; however, we normalized the data to reduce this possibility.

Conclusion

This study explored the relationship between nutritional status and dietary patterns and the risk of all-cause and cause-specific mortality in people aged >60 years in the US. Healthy diet with high HEI and low DII are beneficial to health, whereas nutritional status requires modest maintenance. Our findings provide a new perspective to exploring the relationship between nutritional status, dietary patterns, and health.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: <https://www.cdc.gov/nchs/nhanes/>.

Ethics statement

The studies involving human participants were reviewed and approved by National Center for Health Statistics Research Ethics Review Board. National Health and Nutrition Examination Survey (NHANES) website (<https://www.cdc.gov/>

[nchs/nhanes/](https://www.cdc.gov/nchs/nhanes/)). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZL and DX: conceptualization. ZL: methodology, resources, and visualization. W-XX: software. Y-JF, D-DW, and W-XX: validation. FD: investigation and supervision. DX: data curation. ZL and Y-JF: writing—original draft preparation. D-DW and FD: writing—review and editing. J-HT: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.963060/full#supplementary-material>

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Associations between dietary intake, physical activity, and obesity among public school teachers in Jeddah, Saudi Arabia

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Objective: We aimed to assess the dietary intake of certain food groups in a representative sample of public-school teachers living in Jeddah city. We also, examined the association of dietary intake with physical activity and obesity among schoolteachers.

Methods: The study was a cross-sectional online survey, conducted among 640 (177 male, 463 female) schoolteachers aged between 20 and 62 years old and working in public primary, intermediate, and high schools in Jeddah. Measurements included gender, weight, height, body mass index (BMI), health problems, and lifestyle behaviours, including physical activity levels, smoking status, and dietary intake.

Results: Based on gender, number of non-smoking women (94%) was higher than number of non-smoking men (57.1%) ($P < 0.001$). However, men were more active than women ($P = 0.03$). Regarding BMI, there were more overweight men than women, while obese women numbered more than men ($P = 0.003$). There was no significant difference in dietary intake between men and women except that men consumed more soft drinks than women ($P = 0.002$). Lower physically active schoolteachers were less likely to consume salad (OR = 0.6, 95% CI 0.4–0.9; $P = 0.02$), vegetables (OR = 0.6, 95% CI 0.3–0.9; $P = 0.01$), beans and legumes (OR = 0.4, 95% CI 0.2–0.7; $P = 0.005$), wholegrain bread (OR = 0.6, 95% CI 0.4–0.9; $P = 0.03$), dairy products (OR = 0.6, 95% CI 0.4–0.9; $P = 0.01$), snacks (OR = 0.5, 95% CI 0.2–0.8; $P = 0.01$), and fish (OR = 0.4, 95% CI 0.2–0.9; $P = 0.04$) compared to those with high levels of physical activity. Only fruit intake was considered statistically significant (OR = 0.4, 95% CI 0.3–0.7; $P = 0.003$). The study found a relationship between the BMI of schoolteachers and food intake. Obese schoolteachers had lower consumption of fruits (OR = 0.3, 95% CI 0.2–0.7; $P = 0.007$) and white meat (OR = 0.5, 95% CI 0.3–0.9; $P = 0.03$) than schoolteachers in the normal weight group.

Conclusion: The high prevalence of physical activity, dietary intake and body weight among Saudi teachers is a major public health concern. The present study identified several lifestyle factors associated with body weight that may represent valid targets for the prevention and management of obesity among Saudi school teachers. Promoting active lifestyles and healthy diets would be primary targets for obesity prevention.

KEYWORDS

obesity, dietary behaviour, diet, physical activity, schoolteachers

1. Introduction

Obesity and overweight are major public health problems that are considered a global pandemic (1) due to disease and biological risk factors linked to non-communicable diseases. The World Health Organization (WHO) defines obesity and overweight as abnormal or excessive accumulation of fat and classifies it according to a body mass index (BMI) of higher than 25 and 30 kg/m², for overweight and obesity, respectively (2). The prevalence of adult obesity has increased threefold globally in the last four decades (3), according to WHO. There is a growth in obesity rates around the world (>1 billion). In Europe, two thirds of the adult's population is obese (3), whereas, in Saudi Arabia, more than half the adults are considered obese, which is also the case in Arabian gulf countries (4). Obesity and overweight causes are complicated by environmental, genetic, behavioural, and socioeconomic factors. It is well-known that dietary intake and physical activity are risk factors linked with obesity and overweight (5). In Saudi Arabia, obesity and overweight increased due to shifting lifestyles as a result of the growth of the economy, technology, food availability, variety, and changes in the population's living standards. This led to changes in food choice, energy intake and energy expenditure. A high snack frequency (unhealthy crisps, sweets, and carbonated beverages), skipping daily breakfast, and emotional eating are harmful dietary habits significantly associated with a higher BMI that were found in the Saudi population (6). In addition, some Saudis are physically inactive because of the weather, culture, and lack of time and equipment (7). Sedentary lifestyles in the Saudi population as a result of sedentary jobs, long car trips, and watching screens (computer, mobile, television and games) for more than 8 hours per day contribute to the physical inactivity of Saudi adults (7).

Schoolteachers have specific environmental and psychological challenges that require ideal health conditions to meet their job requirements (8). Studies have reported that schoolteachers might not have a healthy dietary intake due to skipping breakfast, eating inadequate breakfast, and consuming foods high in fat and sugar or calorically dense snacks, which is not supported by dietary recommendations (9–11). This dietary behaviour among schoolteachers is more likely to lead to concentration problems and increase the risk of obesity (9). The change in socio-cultural and lifestyle for the Saudi population increase adults' consumption of unhealthy fast foods. The majority of schoolteachers in Saudi Arabia skip breakfast at home, especially females (74%); further, over 76% and 85% of males and females eat breakfast and/or snacks at school (5). A US study (12) found that the most frequently consumed foods by schoolteachers was candy (70%) and 33% of them provided it as a reward for students weekly. However, the consumption of cookies, doughnuts, sweetened drinks, and pizza was at 25%.

Additionally, most schoolteachers are moderately physically active (13–15), irrespective of their role in the classroom, and schoolteachers during teaching (14, 16). Among individuals, including schoolteachers, the obesity risk increases with the degree of physical inactivity, sedentary life, and dietary behaviour (17). The prevalence of obesity among schoolteachers was 30% in a Brazilian study (18) and 40% in a Saudi study in Jeddah (19). Obesity is associated with lifestyle and comorbidities among public school teachers (20). The higher obesity prevalence among schoolteachers may be related to high levels of sedentary behaviour during

leisure (20) and the association of prolonged screen time, sedentary behaviour, and unhealthy eating habits (15). In addition, sitting time and watching screen devices are associated with consuming products of high energy density and low nutritional content. Globally, one in four adults is physically inactive (21), compared to one in two in Saudi Arabia (22) and one-third in South Africa (23). A Brazilian study (24) found that more than 50% schoolteachers are physically inactive, and this is as result of low levels of physical activity, high levels of TV viewing and low energy expenditure. Generally, schoolteachers sit in an orthostatic position at school 95% of the time. Furthermore, previous scientific evidence indicated health impact of increased percentages of obesity among different populations including increase rates of associated chronic diseases such as cardiovascular disease, diabetes, hypertension (25), and cancer (26). Also, studies showed other undesirable health impact for obesity including the presence of psychological disorders (27, 28), body dissatisfaction (29). Moreover, the health of schoolteachers affects teaching quality. This may indirectly affect student achievement and students' subsequent success in society due to the role of a teacher in students' lives, especially at this critical age (30). Previous literature illustrated that schoolteachers have been affected by chronic diseases globally. For example, the rates of hypertension were reported by 25%, 29% and 84% among Saudi, Indian, and Nigerian schoolteachers, respectively (31–33).

As schoolteachers are considered role models for students, the dietary intake and physical activity of schoolteachers may influence their students' behaviours. Also, teachers spend ~7 h a day, 5 days per week at schools, working with students, which allows students to copy teachers' food choices during breakfast and snack times. Therefore, schoolteachers should be positive role models for students by setting an example of healthy dietary intake and healthy lifestyle habits (34, 35). Dietary intake, physical activity levels, and obesity are important indicators of health status among schoolteachers, which may affect their work performance. Hence, the current study hypothesises that public schoolteachers who are physically active and have a normal BMI have a healthy dietary intake. To the best of our knowledge, the association between physical activity, obesity status, and dietary intake has not been examined among schoolteachers in Saudi Arabia. Additionally, previous studies regarding school nutrition and health interventions focused considerably on students, not teachers. Therefore, this study aimed to fill this gap and assess the dietary intake of certain food groups in a representative sample of public-school teachers living in Jeddah city. We further examined the association of dietary intake with physical activity and obesity among schoolteachers. The finding of this study will enhance future health interventions in schools and lead policymakers in Saudi Arabia and beyond.

2. Methods

2.1. Study design and participants

This cross-sectional study was conducted between October 2021 and March 2022 on teachers working in public schools in Jeddah, Saudi Arabia. This study was first approved by the Biomedical Ethics Research Committee of King Abdulaziz University (Reference No. 159-21). In order to collect data from schoolteachers in Jeddah

city, approvals were also sought from the Education Department of Jeddah.

The inclusion criteria were male and female teachers, aged between 18 and 65 years old, working in public primary, intermediate, and high schools in Jeddah city. This study excluded teachers working in private schools. There are six administrative educational offices for public schools in Jeddah: North, East, Centre, Naseem, South, and Safa. According to the Ministry of Education statistics, the total number of male and female teachers working at public schools in Jeddah was as follows: in primary, intermediate, and high schools there were 6,046, 3,777, and 2,189 male teachers and 7,304, 4,323, and 3,219 female teachers, respectively (36). Hence, according to the online sample size calculator (37), the required sample size for the current study was 638 schoolteachers working in public schools in Jeddah.

2.2. Measures

An online self-reported survey was distributed electronically *via* par code and WhatsApp, e-mail to several educational offices and, in return, to all public schools in their area during the school year. A closed-ended multiple-choice questionnaire was used to collect and assess data in four categories: sociodemographic characteristics, health characteristics, health behaviours, and dietary intake. The online self-reported survey was used because it allowed the collection of data from a large and representative sample in a short time. At the beginning of the online survey, schoolteachers were informed about the research objectives and procedures, and their anonymity and voluntary participation in this research were guaranteed. Since the survey was distributed online, consent was obtained by adding a consent statement, respondents provided consent through this to participate in the research. Thus, only those who agreed to participate were able to complete the survey.

2.2.1. Sociodemographic characteristics

Sociodemographic characteristics of schoolteachers included age (18–65), sex (male; female), marital status (single, married, divorced, widowed), education level (diploma; bachelor; postgraduate), school stage (primary; secondary; high), and years of teaching experience (1–5; 6–10; 11–15; 16–20; and above 20 years).

2.2.2. Health characteristics

Self-reported health characteristics, including the presence of chronic disorders (e.g., diabetes, hypertension, coronary heart disease, high cholesterol levels) and self-reported height and weight, were collected to determine body mass index (BMI). BMI in kg/m² was categorised as normal weight (BMI between 18.5 and 24.99), overweight (BMI ≥ 25.0), or obese (BMI ≥ 30.0). BMI was classified according to the cut-off points of the World Health Organization (WHO) (38).

2.2.3. Health behaviours

Questions were related to participants' health behaviours, including physical activity, cigarette smoking, and dietary intake. For physical activity, questions were adapted from previous study

conducted in Saudi Arabia (39). Participants were asked to self-report their physical activity, such as walking, running, or swimming, with the responses being classified as never, rarely, 1–2 per week, 3–4 per week, and more than 5 per week. In the analysis, we categorised them into two groups: low (participants who exercised two times per week or less) and high (participants who exercised more than three times per week) physical activity (39). Smoking was assessed by asking the participants about their cigarette smoking habits during the previous year. The responses were categorised as smoker, ex-smoker, and non-smoker.

2.2.4. Dietary intake

A short, non-quantitative, validated Food Frequency Questionnaire (FFQ) was used to assess the weekly frequency (days/week) of dietary intake for 14 food items, including fruits, fruit juices, salads, vegetables, dairy products, beans and legumes, cereals, wholegrain bread, snacks, sweets, soft drinks, white and red meat, and fish. The validated questionnaire was adopted from Cleghorn et al. (40). Each FFQ item had seven frequency options including (never, once a week, 2 to 4 times per week, 5 to 6 times per week, 1 to 2 times per day, 3 to 4 times per day, or 5 or more times per day). The participants were asked on average how often they consumed these 14 food items during the past 12 months. Based on participants responses, consumption was categorised as “low intake” or “high intake”. A consumption frequency of at least 5 days/week was defined as high intake, whereas low intake was defined as <5 days/week. These categories were based on a study done by Delfino et al. (15).

2.3. Statistical analysis

All of the data presented in the current study were categorical variables. Descriptive data of the study participants are shown in tables as frequencies (*n*) and percentages (%). Correlations between categorical variables (demographic characteristics, gender, and dietary intake) were examined using the chi-square test. Logistic regression analyses were performed to obtain odds ratios (ORs) and 95% CIs of dietary intake by physical activity levels and BMI status. All models were adjusted for a selected set of covariates, including sex, age, marital status, education level, years of experience, health status, and smoking status. In the analysis model, physical activity and BMI status were considered the explanatory variables, while the dietary intake was considered the outcome variables. For all analyses, the *P* < 0.05 was considered borderline significant. However, due to multiple testing of 14 food groups, we applied Bonferroni correction, and the statistical significance level was set at *P* = 0.003. Statistical analyses were performed using SPSS software version 28.0.

3. Results

The demographic characteristics of public-school teachers from Jeddah, Saudi Arabia, based on gender are shown in Table 1. The sample consisted of 640 schoolteachers; 72.3% were female, and 27.7% were male. Among the study participants, 74.1% were married, and 68.2% were aged between 35 and 54 years old. The majority of the study participants (83.6%) held a bachelor's degree. There were significant differences between male and female teacher in marital

TABLE 1 Baseline characterisation of schoolteachers from public school stratified by gender.

Characterisation	Total (<i>n</i> = 640)	Male (<i>n</i> = 177)	Female (<i>n</i> = 463)	<i>P</i> -value
Age				
18–24	58 (9.1)	7 (4.0)	51 (11.0)	0.07
25–34	71 (11.1)	18 (10.2)	53 (11.4)	
35–44	194 (30.3)	59 (33.3)	135 (29.2)	
45–54	243 (38.0)	70 (39.5)	173 (37.4)	
55–65	74 (11.6)	23 (13.0)	51 (11.0)	
Marital status				
Single	106 (16.6)	24 (13.6)	82 (17.7)	0.03
Married	474 (74.1)	142 (80.2)	332 (71.7)	
Divorced	44 (6.9)	11 (6.2)	33 (7.1)	
Widow	16 (2.5)	0 (0)	16 (3.5)	
Education level				
Diploma	66 (10.3)	11 (6.2)	55 (11.9)	<0.001
Bachelor	535 (83.6)	144 (81.4)	391 (84.4)	
Postgraduate	39 (6.1)	22 (12.4)	17 (3.7)	
School stage				
Primary	221 (34.5)	65 (36.7)	156 (33.7)	0.61
Secondary	157 (24.5)	45 (25.4)	112 (24.2)	
High	262 (40.9)	67 (37.9)	195 (42.1)	
Experience years				
1–5	101 (15.8)	29 (16.4)	72 (15.6)	0.12
6–10	116 (18.1)	21 (11.9)	95 (20.5)	
11–15	99 (15.5)	33 (18.6)	66 (14.3)	
16–20	90 (14.1)	27 (15.3)	63 (13.6)	
Above 20	234 (36.6)	67 (37.9)	167 (36.1)	
Health condition				
Healthy	417 (65.1)	109 (61.6)	308 (66.5)	0.32
Having diabetes	86 (13.4)	26 (14.7)	60 (13.0)	
Having heart disease	12 (1.9)	6 (3.4)	6 (1.3)	
Having hypertension	65 (10.2)	21 (11.9)	44 (9.5)	
High cholesterol	60 (9.4)	15 (8.5)	45 (9.7)	
Smoking cigarette				
Smoker	65 (10.2)	47 (26.6)	18 (3.9)	<0.001
Quit smoking	39 (16.4)	29 (16.4)	10 (2.2)	
Non-smoker	536 (83.8)	101 (57.1)	435 (94.0)	
Physical activity				
Low active	297 (46.4)	70 (39.5)	227 (49.0)	0.03
High active	343 (53.6)	107 (60.5)	236 (51.0)	

(Continued)

TABLE 1 (Continued)

Characterisation	Total (<i>n</i> = 640)	Male (<i>n</i> = 177)	Female (<i>n</i> = 463)	<i>P</i> -value
BMI status				
Normal weight	220 (34.3)	53 (29.9)	167 (36.6)	0.003
Overweight	258 (40.3)	91 (51.4)	167 (36.6)	
Obese	155 (24.2)	33 (18.6)	122 (26.8)	

Data presented as frequency (%).

P-value was calculated based on chi-square.

status and education level. In comparison to men, the percentage of widowed, single, and divorced women teachers was higher than that of men ($P = 0.03$). A higher percentage of women teachers held diplomas, while a higher percentage of men held postgraduate degree ($P < 0.001$). Most of the participants (40.9%) taught in high schools, and 36.6% had more than 20 years of teaching experience. Approximately 36.6% of the schoolteachers included in this study were healthy, 13.4% were diagnosed with diabetes, and 10.2% were diagnosed with hypertension. The mean BMI was 27.2 kg/m² (SD = 4.9), and 34.4% of the participants were in the normal weight range, whereas 40.8% and 24.5% of the participants were overweight and obese, respectively. There were more overweight men teachers than women teachers, while there were more obese women teachers than men teachers ($P = 0.003$). In general, the percentage of non-smokers was 83.8%. There were more non-smoker women teachers (94%) than non-smoker men teachers (57.1%) ($P < 0.001$). A total of 53.6% of the participants were classified as highly physically active. Men teachers were more active than women teachers ($P = 0.03$). **Table 2** presented dietary intake according to gender. There was no significant difference in dietary intake between men teachers and women teachers except that men teachers consume more soft drinks than women teachers ($P = 0.002$).

The association between physical activity levels and dietary intake is shown in **Table 3**. Schoolteachers with low levels of physical activity exhibited a decreased consumption of salad (OR = 0.6, 95% CI 0.4–0.9; $P = 0.02$), vegetables (OR = 0.6, 95% CI 0.3–0.9; $P = 0.01$), beans and legumes (OR = 0.4, 95% CI 0.2–0.7; $P = 0.005$), wholegrain bread (OR = 0.6, 95% CI 0.4–0.9; $P = 0.03$), dairy products (OR = 0.6, 95% CI 0.4–0.9; $P = 0.01$), snacks (OR = 0.5, 95% CI 0.2–0.8; $P = 0.01$), and fish (OR = 0.4, 95% CI 0.2–0.9; $P = 0.04$) compared to those with high levels of physical activity. However, the difference was borderline significant. Only fruit intake was considered statistically significant (OR = 0.4, 95% CI 0.3–0.7; $P = 0.003$). The associations between BMI and dietary intake are shown in **Table 4**. Obese schoolteachers had a decreased consumption of fruits (OR = 0.3, 95% CI 0.2–0.7; $P = 0.007$) and white meat (OR = 0.5, 95% CI 0.3–0.9; $P = 0.03$) than schoolteachers in the normal weight group. Among the enrolled schoolteachers, the risk of being obese was associated with a decreased intake of vegetables (OR = 0.5, 95% CI 0.3–0.9; $P = 0.05$) compared to the group with normal body weight, but this did not reach statistical significance. However, the difference was borderline significant, and none of these associations were statistically significant.

TABLE 2 Dietary intake based on gender of public schoolteachers.

	Total	Male	Female	<i>P</i> -value
Fruit				
Low intake	525 (82)	142 (80.2%)	383 (82.7%)	0.49
High intake	115 (18)	35 (19.8%)	80 (17.3%)	
Fruit juice				
Low intake	559 (87.3)	154 (87.0%)	405 (87.5%)	0.89
High intake	81 (12.7)	23 (13.0%)	58 (12.5%)	
Salad				
Low intake	460 (71.9)	127 (71.8%)	333 (71.9%)	1.00
High intake	180 (28.1)	50 (28.2%)	130 (28.1%)	
Vegetables				
Low intake	500 (78.1)	142 (80.2%)	358 (77.3%)	0.45
High intake	140 (21.9)	35 (19.8%)	105 (22.7%)	
Bean and legumes				
Low intake	564 (88.1)	150 (84.7%)	414 (89.4%)	0.10
High intake	76 (11.9)	27 (15.3%)	49 (10.6%)	
Cereal				
Low intake	567 (88.6)	161 (91.0%)	406 (87.7%)	0.26
High intake	73 (11.4)	16 (9.0%)	57 (12.3%)	
Whole grain bread				
Low intake	462 (72.2)	125 (70.6%)	337 (72.8%)	0.62
High intake	178 (27.8)	52 (29.4%)	126 (27.2%)	
Dairy				
Low intake	416 (65)	107 (60.5%)	309 (66.7%)	0.13
High intake	224 (35)	70 (39.5%)	154 (33.3%)	
Snacks				
Low intake	560 (87.5)	154 (87.0%)	406 (87.7%)	0.79
High intake	80 (12.5)	23 (13.0%)	57 (12.3%)	
Sweet				
Low intake	500 (78.1)	144 (81.4%)	356 (76.9%)	0.24
High intake	140 (21.9)	33 (18.6%)	107 (23.1%)	
Soft drinks				
Low intake	560 (87.5)	143 (80.8%)	417 (90.1%)	0.002
High intake	80 (12.5)	34 (19.2%)	46 (9.9%)	
Red meat				
Low intake	559 (87.3)	150 (84.7%)	409 (88.3%)	0.23
High intake	81 (12.7)	27 (15.3%)	54 (11.7%)	
White meat				
Low intake	450 (70.3)	117 (66.1%)	333 (71.9%)	0.17
High intake	190 (29.7)	60 (33.9%)	130 (28.1%)	
Fish				
Low intake	599 (93.6)	158 (89.3%)	400 (86.4%)	0.35
High intake	41 (6.4)	19 (10.7%)	63 (13.6%)	

Data presented as frequency (%).
P-value was calculated based on chi-square.

TABLE 3 Odds ratios and 95% CIs of food group intake according to physical activity levels of public schoolteachers.

	Odds	95% CI	P-value
Fruit			
Low PA	0.4	0.3–0.7	0.003
High PA	1	Reference	
Fruit juice			
Low PA	0.8	0.4–1.4	0.50
High PA	1	Reference	
Salad			
Low PA	0.6	0.4–0.9	0.02
High PA	1	Reference	
Vegetables			
Low PA	0.6	0.3–0.9	0.01
High PA	1	Reference	
Bean and legume			
Low PA	0.4	0.2- 0.7	0.005
High PA	1	Reference	
Cereal			
Low PA	0.6	0.3–1.1	0.16
High PA	1	Reference	
Whole grain bread			
Low PA	0.6	0.4–0.9	0.03
High PA	1	Reference	
Dairy			
Low PA	0.6	0.4–0.9	0.01
High PA	1	Reference	
Snacks			
Low PA	0.5	0.2–0.8	0.01
High PA	1	Reference	
Sweet			
Low PA	0.8	0.5–1.2	0.39
High PA	1	Reference	
Soft drinks			
Low PA	1.1	0.7–2.1	0.51
High PA	1	Reference	
Red meat			
Low PA	0.5	0.3–1.0	0.05
High PA	1	Reference	
White meat			
Low PA	0.7	0.5–1.1	0.09
High PA	1	Reference	
Fish			
Low PA	0.4	0.2–0.9	0.04
High PA	1	Reference	

P-value was calculated using logistic regression. The model adjusted for gender, age, marital status, education level, experience years, body mass index, health status, and smoking.
PA, physical activity.
Physical activity was explanatory variables and dietary intake was outcomes variables.

TABLE 4 Odds ratios and 95% CIs of food group intake according to body mass index categories of public schoolteachers.

	Odds	95% CI	P-value
Fruit			
Normal weight	1	Reference	
Overweight	0.6	0.4–1.1	0.13
Obese	0.3	0.2–0.7	0.007
Fruit juice			
Normal weight	1	Reference	
Overweight	0.7	0.4–1.4	0.41
Obese	0.6	0.2–1.3	0.21
Salad			
Normal weight	1	Reference	
Overweight	0.8	0.5–1.2	0.43
Obese	0.8	0.4–1.4	0.48
Vegetables			
Normal weight	1	Reference	
Overweight	0.7	0.4–1.2	0.28
Obese	0.5	0.3–0.9	0.05
Bean and legume			
Normal weight	1	Reference	
Overweight	0.6	0.3–1.2	0.22
Obese	1.5	0.7–3.2	0.21
Cereal			
Normal weight	1	Reference	
Overweight	0.6	0.3–1.3	0.26
Obese	1.1	0.5–2.2	0.78
Whole grain bread			
Normal weight	1	Reference	
Overweight	0.7	0.4–1.1	0.21
Obese	1.1	0.6–1.9	0.62
Dairy			
Normal weight	1	Reference	
Overweight	0.7	0.4–1.1	0.09
Obese	0.6	0.4–1.1	0.11
Snacks			
Normal weight	1	Reference	
Overweight	0.5	0.3–1.1	0.02
Obese	0.9	0.4–1.9	0.96
Sweet			
Normal weight	1	Reference	
Overweight	0.7	0.4–1.2	0.29
Obese	1.1	0.6–1.9	0.69

(Continued)

TABLE 4 (Continued)

	Odds	95% CI	P-value
Soft drinks			
Normal weight	1	Reference	
Overweight	1.3	0.7–2.4	0.38
Obese	1.3	0.6–2.8	0.39
Red meat			
Normal weight	1	Reference	
Overweight	1.2	0.6–2.1	0.50
Obese	0.6	0.3–1.4	0.32
White meat			
Normal weight	1	Reference	
Overweight	0.8	0.5–1.2	0.40
Obese	0.5	0.3–0.9	0.03
Fish			
Normal weight	1	Reference	
Overweight	0.8	0.3–1.9	0.68
Obese	1.5	0.6–3.7	0.37

P-value was calculated using logistic regression. The model adjusted for gender, age, marital status, education level, experience years, physical activity, health status, and smoking. BMI status was explanatory variables and dietary intake was outcomes variables.

4. Discussion

To prevent and control the rise of obesity prevalence and its complications, it is fundamental to establish healthy eating habits in the early development of a child (41). Consequently, the most effective way to promote health in the education system is through schoolteachers who can encourage healthy eating (41). Studies show that being proactive can improve health outcomes and promote healthy lifestyles (42, 43). In addition, the essential aspects of implementing health promotion in schools have been achieved with the aid of teachers' interest and engagement (43, 44). Thus, this study aimed assess the dietary intake of certain food groups in a representative sample of public-school teachers living in Jeddah city. Also, it aimed to examine the association of dietary intake with physical activity and obesity among schoolteachers. The main study findings regarding health behaviours of Saudi public-school teachers showed that the prevalence of overweight and obesity was 65%, the percentage of non-smokers was 84%, and 54% of the study participants met the definition of high physical activity. Our data show that physically active teachers consume more fruits, vegetables, beans, legumes, and dairy products than teachers who are less physically active. Less red meat and fewer snacks were consumed by teachers who were less physically active than by those who were more active. Compared to teachers with normal weight, those with a high BMI consumed fewer fruits, vegetables, snacks, and white meat. Hence, the main hypothesis was confirmed. To the best of our knowledge, no previous studies have examined the association of dietary intake with physical activity and obesity among schoolteachers in Saudi Arabia.

In this study, the obesity prevalence of schoolteachers was twice as high as the global obesity prevalence of adults (24.5 vs. 13.0%), and further analysis of the determinants is needed to investigate this alarming finding. This increased obesity frequency is consistent with earlier findings by Lizana et al. (45) reporting an obesity frequency of 25.7% among 70 rural Chilean teachers. Other studies among different populations, including 305 Tanzanian health workers, teachers, and bankers reported an even higher obesity prevalence (37.8%) with the prevalence of overweight and obesity among teachers reported by 62.6% in Tanzanian (46), which was similar to the results of the current study (65% when considering both overweight and obesity). It should be emphasised that the age of the current study population may have contributed to the increased obesity prevalence, as obesity increases progressively with age, reaching its peak in age groups from 40 to 60 years (47); these age groups were predominant in our study population. The risk of developing a several non-communicable diseases, including obesity, cardiovascular diseases, diabetes and hypertension increases with age (48). The results of the current study also revealed that the number of overweight men teachers was higher than that of women teachers, while that of obese women teachers was higher than that of men teachers ($P = 0.003$). However, the reason for such a difference may be the hormonal differences between men and women, genetic factors, and variations in clinical severity are also thought to play a role in the interaction influence of sex in the correlations with obesity (48). Previous studies conducted in Saudi Arabia reported similar results as men had higher levels of overweight and women had higher levels of obesity (49).

The present study demonstrated that the level of physical activity in Saudi Arabia has increased, although it is still relatively low. According to the Saudi STEPwise survey, the prevalence of moderate and high levels of physical activity among Saudis aged 15–64 years was 32.3% (50). The level of physical activity reported in this study was 54%. Despite not being comparable to our study in methodology or national representativeness, other studies among Saudi adults aged 15 and older have reported increases in physical activity, ranging from 50 to 70% (7, 51). According to the WHO, around 30% of the world's population and 30–70% of those living in countries in the eastern Mediterranean region do not meet the recommended minimum level of physical activity (52). In the present study, women teachers were found to be less physically active than men teachers. A possible explanation for this might be that men in Saudi Arabia generally have more opportunities than women to engage in outdoor physical activity especially considering the hot weather in the kingdom, which may affect women who wear hijab to exercise (49). However, there are plenty of health clubs for both sexes; nevertheless, the high cost for health clubs might be an issue that impact practising physical activity (49). It is notable that women in the Middle East have generally been reported to be less physically active than men (53, 54), and lower than that in many developed countries (53).

The current study findings indicated that the consumption of food from various categories was considered poor, as only a small percentage of schoolteachers' food intake met the dietary recommendations. These results were consistent with the findings of a nationally representative survey on the Saudi adult population that revealed that the consumption of fruits, vegetables, beans, legumes, dairy products, and fish did not meet the dietary recommendations (55). Unhealthy eating habits are the leading risk factor for poor

health, with an estimated 11.3 million attributed deaths and 241.4 million attributed disability-adjusted life years per annum globally (56). In line with our findings, the Global Burden of Diseases, Injuries, and Risk Factors study reported that in Saudi Arabia, the average intake of fruits, vegetables, nuts, whole grains, and seafood (polyunsaturated and omega 3) fatty acids was considerably below optimal levels, whereas the consumption of red meat, processed meats, saturated fatty acids, sugar-sweetened beverages, and salt was significantly above optimal levels (57). Interventions to enhance teacher eating habits are necessary because they may positively impact both teachers' and students' health-related behaviours. This is because healthier diets are strongly associated with a lower risk of developing chronic diseases (58). This study also reported that the teachers' dietary intake did not differ by gender except that men teachers consume more soft drinks than women teachers ($P = 0.002$). Previous studies showed similar results revealed similar findings (59, 60). A study that examined the consumption pattern of sugar-sweetened beverages among college students in Jordan and its impact on their body weight, reported that men consumed greater amounts of sugar-sweetened beverages than women ($P = 0.016$) (59). This might be because women are more aware of the risk factors of soft drinks and are more concerned with their body image than men (61, 62). Another reason for such a difference reported by Xi et al. is that men drink less water than women, which leads to increased sugar-sweetened drink consumption (63). Men are also likely to consume more soft drinks overall since their overall food consumption is larger than that of women (62).

The present study findings showed that schoolteachers with high physical activity consumed more fruits, vegetables, beans, legumes, and dairy products than those with low physical activity. A sufficient degree of physical activity leads to improve health, including decreased overweight and obesity levels. Conversely, a lack of physical exercise is strongly associated with an elevated risk of cardiometabolic and vascular diseases (64). This is probably because most study participants had university degrees (bachelor's and postgraduate), which indicates that people who are well-educated are more aware about nutritional aspects and have a favourable attitude towards leading a healthy lifestyle (54). Previous studies have shown a close relationship between exercise and healthy food choices (65–67), which appears to be associated with improved health knowledge and awareness, as well as perceiving a healthy diet and physical activity requirements as beneficial and practical (68). Similarly, a study concluded that low levels of physical activity were associated with inadequate fruit and vegetable intake among young adults in Brazil (low consumption of fruits and vegetables by 79 and 90% respectively) (69). Additionally, in previous study among college students in Saudi Arabia physically active students tend to consume higher amounts of fruits, vegetables, and dairy products than inactive students (70). Other studies found that adults with an active lifestyle eat healthier diets including more fibre and fruits and vegetables, less saturated fat, and fewer servings of fried food and sweets than sedentary adults (65, 66, 68). Furthermore, in the current study, schoolteachers with low physical activity consumed fewer snacks and less red meat than those with high physical activity. These findings are consistent with a previous study conducted among Polish adults (71). The Polish study reported that adults who considered themselves physically active consumed less red meat and meat products (71). Although, few studies investigated the relationship between physical

activity and the consumption of red meat, a previous study in adult women aged 20–50 years old reported that lower physical activity is associated with low consumption of red meat, which is consistent with our findings (72). However, in a study conducted among female undergraduate students, the high consumption of unhealthy snacks was significantly associated with increased levels of physical activity (73), which is the opposite of our findings as the present study showed that those who were physically active had higher intake of healthy food and less intake of unhealthy snacks.

The present study results showed that schoolteachers with high BMI have a decreased consumption of fruits compared to those with normal weight, which is aligned with previous studies (74). These findings are supported by the fact that a higher intake of fruits and vegetables lowers the risk of developing obesity (75). Increasing fruit and vegetable intake may also contribute to weight management because fruits and vegetables are low in energy but high in fibre and water, thereby producing a satiating effect (76). The satiating properties of fruits and vegetables lead to a reduction in energy-dense, nutrient-poor food consumption, thereby reducing overall caloric intake (76, 77). The intake of snacks, depending on the quantity and frequency of consumption, is unlikely to increase body weight. However, our findings showed that schoolteachers with high BMI consumed fewer snacks than schoolteachers with normal weight. Several studies have found that snacking frequency and daily calorie consumption from snacks have a negligible or even inverse relationship with the risk of becoming overweight or obese (78, 79). However, these associations may be confounded due to the underreporting of snacking behaviours among obese people compared to normal-weight respondents (78, 80). The consumption patterns of main meals and the choice of foods and beverages consumed as snacks appear to influence the associations between snacking, diet quality, and body weight (81). In particular, frequent snacks as substitutes for main meals may have a negligible impact on body weight and daily energy balance. Moreover, our findings indicated that less intake of vegetables is associated with increased risk of obesity. Data from the Atlantic Partnership for Tomorrow's Health Study demonstrated that increased fruit and vegetable consumption is inversely associated with BMI risk (82). Previous study showed that low vegetable intake was strongly associated with obesity risk among Japanese patients with type 2 diabetes in both sexes (83). These findings are supported by the results of a systematic review confirming that increased vegetable intake is associated with weight loss of 0.09–0.1 kg over 4 years, decreased risk of weight gain, and lower risk of overweight or obesity (84). Vegetables may contribute to the beneficial effects of weight changes, as they abundantly contain vitamins, minerals, fibres, and proteins (16, 85). In the present study findings, white meat consumption had a negative association with increased BMI. Red meat consumption has been linked to general and abdominal obesity in several epidemiological studies, but the results have been inconsistent (86, 87). Several studies have indicated that red meat consumption is positively associated with obesity (88, 89), whereas others have not found a significant correlation (90, 91). Because red meat and its products are a rich source of protein, cholesterol, and saturated fatty acids, they are considered high-energy-density foods (88). Moreover, the association between white meat consumption and obesity has received less attention in previous studies than red meat consumption, and the results in this regard have been

more controversial (92, 93). According to Vergnaud et al., who assessed the association between the consumption of total meat, red meat, poultry, and processed meat in a large European population, higher poultry consumption was found to be associated with a lower risk of obesity (93), whereas Maskarinec et al. found a significant positive relationship between white meat consumption and obesity in Hawaii (94). Furthermore, a longitudinal study of ~90,000 adults whose dietary habits and anthropometric data were measured for 6.5 years showed a statistically significant relationship between the consumption of animal proteins and long-term weight gain (95).

4.1. Strengths and limitations

The main strength of this research is that it is the first study to examine the associations of dietary intake with physical activity and obesity using a representative sample of public school teachers living in Jeddah. In our analysis, we adjusted for socioeconomic factors that may significantly influence dietary intake. However, the current study has certain limitations. First, information about dietary intake, physical activity level, health status and body height and weight were collected using a self-reported questionnaire, which may raise concerns about recall bias, as some participants responded in socially desirable ways for some variables, including dietary intake. Second, the dietary intake was not measured using the semi-quantitative FFQ; only the intake frequency, i.e., the number of days per week, was recorded. Future studies should consider FFQ which captures more food items than the one used in this study. Likewise, future studies should also consider using the semi-quantitative FFQ with both frequency and portion sizes. Another point is that body weight can affect the quantity and quality of food consumed, and vice versa (96); thus, the association between dietary intake and obesity can be ambiguous. Third, the cross-sectional design prevents drawing conclusions about causal relationships. Finally, the study findings cannot be generalised to all schoolteachers in Saudi Arabia, as this study examined only schoolteachers in Jeddah city; thus, extending the findings to other populations is not possible. Future studies should collect data from all regions in Saudi Arabia in order to generalise the findings for all the Kingdom.

5. Conclusions

The current study is one of the first to examine the associations of dietary intake with physical activity and obesity among schoolteachers in Saudi Arabia. Schoolteachers are a vital group that is neglected in scientific research, and they play an important role in promoting health in education systems by encouraging healthy lifestyles and healthy eating habits among students. The findings of this study revealed that more than half of the schoolteachers reported high physical activity levels; however, a high prevalence of overweight and obesity was also reported by the study participants. These high rates of overweight and obesity might increase the current or future risk of non-communicable diseases among schoolteachers. Our results also showed that high physical activity was associated with higher consumption of healthy food, including fruits, vegetables, beans, legumes, and dairy products. Conversely, higher BMI was associated with lower consumption

of fruits, vegetables, snacks, and white meat. Adjustments to the lifestyle of teachers are required, aiming to increase their healthy diet and reduce several risk factors leading to cardiovascular disease, dyslipidaemia and diabetes. Schoolteachers are expected to be role models for students and future generations. Healthy schoolteachers influence the health and educational performance of students. However, health promotion interventions at schools focus on students, not teachers. Strategies to reduce the obesity risk among schoolteachers should focus on increasing the intake of a healthy diet, as well as increasing physical activity. Nutrition education programs are vital to encourage schoolteachers in Saudi Arabia to consume healthy food and adopt a healthy lifestyle, thereby enhancing public health.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Education Department of Jeddah City and the Biomedical Ethics Research Committee of King Abdulaziz University

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Author contributions

Conceptualisation: NAlm, IS, and NAlj. Methodology: NAlj and NAlm. Formal analysis: IS. Data collection and writing—original draft preparation: NAlm, IS, MA, MH, HW, and NAlj. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Changes in French purchases of pulses during an FAO awareness campaign

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Background: Pulses can play a key role in a well-balanced diet and are now recognized for their health and sustainability benefits. However, consumption remains quite low, motivating promotion efforts such as the "International Year of Pulses" declared by the Food and Agriculture Organization (FAO) in 2016. The present study aims to evaluate the changes in the purchase of pulses before and after the FAO's awareness campaign promoting the consumption of pulses in France and investigate the potential differences across sub-populations.

Methods: Purchase data come from Kantar Worldpanel 2014–2017. First, in order to understand demand for pulses, the influence of sociodemographic variables on the purchase of pulses in different forms (raw, processed, ultra-processed) is analyzed using a Box-Cox heteroskedastic double-hurdle model. Then, changes in purchasing before and after the FAO campaign were estimated using a two-way fixed-effects model, controlling for price and sociodemographic variables.

Results: On that period, the purchasing of pulses increased by 8.4% overall. The increase was greater for younger participants (+11.8%), people living in urban areas with over 200,000 inhabitants (+8.4%), and lower-income households (+7.1%). The 8.4% increase observed indicated that there were gradual preference change in favor of pulses and the impact of the awareness campaign was to boost expenditure on pulses by a further 2%.

Conclusion: The FAO campaign coincided with an increase in the purchasing of pulses and may have had an enhancing effect. However, consumption still remains below the level advised by dietary guidelines. There is a need for more public information and communication on the health and sustainability benefits of pulses, the consumption of which can be promoted through supply and education interventions.

JEL codes: D12; Q18; I18.

KEYWORDS

awareness campaign, pulses purchases, Box-Cox double-hurdle model, pseudo-panel, nutritional guidelines, sustainability

Introduction

Health and sustainability benefits of pulse consumption

Pulses have been a focus of attention for several years in the developed economies, as a food group favorable to health and sustainability. According to the Food and Agriculture Organization (FAO), pulses have significant health benefits and are conducive to a well-balanced diet. Legumes/pulses¹ are an interesting source of protein. They have a higher micronutrient density (high folate, iron, magnesium, potassium, and zinc content) than cereals, almost double the protein content, and a high lysine content (the main limiting amino acid in cereals), making them a perfect complement to cereals in diets with low amounts of animal products. In some countries, legumes/pulses are classified as protein foods, along with the traditional animal-protein group (meat, eggs, and fish) (1, 2). Beyond proteins, legumes are a dietary source of fiber and various micronutrients of interest (3, 4). Epidemiological studies have reported health benefits associated with consuming legumes.² In addition, from an ecological point of view, the environmental cost of plant proteins is reduced compared to animal proteins, and pulses can be considered a sustainable alternative (9). Moreover, the benefits of meat consumption remain a topic of debate (9, 10). Dietary guidelines recommend the consuming pulses^{3,4} and limiting meat intake.⁵

In this context, pulse-based foods display several advantages at the consumption level. First, they are protein-rich and can be used as substitutes for meat in a healthy diet. Second, they have a lower cost, making these foods a cheap source of alternative proteins. Finally, they are easily storable, as they are mainly consumed in a non-perishable form (dry or canned). Despite these advantages, pulse consumption remains low in the Western diet (13). In France, the recommendation is “intake at least twice a week,” representing 200 g/week,⁶ far from

what French dietary surveys register at the national level. The third National Food Consumption survey, INCA3,⁷ realized in 2014–2015, found an average consumption of 7.7 g/day in an adult sample, with only 14.7% of respondents reporting being consumers of pulses.⁸ In 2019, French consumers still considered pulses a traditional and old-fashioned food, and these were sometimes perceived as being part of low-budget diets (15), less fancy, food for vegetarians, requiring long preparation, not very well liked, or used as a side and not a main dish (16). Other studies have uncovered similar perceptions. In addition, consumption can be hindered by the presence of antinutrients causing digestive discomfort (17).

Few studies have investigated the demand for pulses in developed countries using household surveys.⁹ In the estimation of household demand systems, pulses and derived products are frequently included in large aggregated categories, such as fruits and vegetables or starchy foods (19). The substitution potential between animal products and more eco-friendly or plant-based alternatives remains the main issue. Studies investigate the potential reductions in meat consumption by assessing consumers' consumption of animal products associated with lower greenhouse gas emissions and, more rarely, of plant-based alternatives (20, 21). Few consumers replace meat by pulses to such a degree that a decrease in meat consumption is observed. Most consumers consume comparable amounts of meat but vary with respect to their acceptance of substitutions, and studies vary with respect to the options assessed: health information campaign, redesign of meal composition in convenient products, and meat substitutes. Consumers may also consider a change in meat portions (inducing the consumption of larger portions of legumes/pulses) or may lower their meat-eating frequency (with a higher intake frequency of legumes/pulses) (22). Different consumer groups favor different substitution options, indicating a role of sociodemographic characteristics in the demand for pulse-based products (23). According to a meta-analysis of meat-eating behavior, the most influential factors are gender, age, and socioeconomic status (SES) (24). Research has shown that young people are more open to flexitarianism, and that health concerns are associated with older age (in particular people 45–60 years of age), while younger consumers are more receptive to

1 Legumes are soybeans and beans, lentils, chickpeas, and peas. Pulses are defined as dry-harvested leguminous crops, excluding soybeans.

2 For example, inverse relationships between the consumption of legumes and the risk of cardiovascular disease (5), type 2 diabetes (6), and cardiometabolic risk factors such as overweight/obesity (7) and dyslipidemia (8).

3 https://www.dietaryguidelines.gov/sites/default/files/2019-05/2015-2020_Dietary_Guidelines.pdf

4 <https://www.mangerbouger.fr/Les-recommandations/Augmenter/Les-legumes-secs-lentilles-haricots-pois-chiches-etc>

5 Based on epidemiological studies, consumption recommendations indicate a maximum of <500 g/week of red meat and <50 g/day of processed meat (11). French guidelines advise a maximum of 500 g/week and 150 g/week for red meat and processed meat, respectively (12).

6 On the basis of an average portion of 100 g.

7 “Étude Individuelle Nationale des Consommations Alimentaires”, based on food recall over three 24-h periods (14). Note that this survey reports information on less-processed pulses (raw, preserved, or frozen products) only and does not include ultra-processed products such as preparations based on pulses.

8 These, however, consume an average of 52.4 g/day, meeting the 200 g/week recommendation.

9 Some works rely on balance-sheet data, which only refer to apparent consumption (18).

minimizing environmental impacts, as are people with a higher level of education (25, 26).

The potential of information policies

The “mismatch” between the many benefits of pulses and the low levels of consumption has recently motivated promotion efforts. A lack of knowledge regarding the environmental issues associated with meat consumption (27–29) and the possibilities for the substitution of meat by plant protein sources (30) are cited as reasons for the mismatch. However, public awareness was raised through the dissemination of scientific information regarding meat consumption,¹⁰ and official promotion was undertaken through an international FAO campaign declaring 2016 as the International Year of Pulses (IYP) (32).¹¹ In France, 24 national and regional events took place in 2016, promoted on the Ministry of Agriculture’s website and relayed by the media. The campaign raised awareness among different food-system actors, including policymakers, pulse producers, processors, and traders, restaurant and catering operators, health and nutrition practitioners, and end-users such as schoolchildren. The general public was the focus of an information and educational campaign based on mass media and face-to-face events (cooking demonstrations, exhibitions, and museum displays—in particular, at the SIAL¹² (Salon International de l’Alimentation) international food exhibition held in Paris. Recently, a study found that the message regarding the environmental benefits of pulses and crop diversification tended to have a greater impact on intentions to purchase lentils than information about their nutritional benefits (34). Firms understood the new opportunities: they began displaying environmental claims on pulse packaging (35) and invested in innovations based on pulses. New and exotic products emerged on the market, such as prepared snacks (hummus and other chickpea-based foods) or dishes (lentil- or pea-based), pasta made with pulse flour, soy preparations, and many combinations of pulses and other ingredients (36). A two-tier market thus appeared, with both a traditional segment of pulses (unprocessed raw products, canned, or less processed, such as preserved or frozen) and an innovative one including ultra-processed products, and in particular, meatless substitutes (soy

steaks, vegan sausages, etc.). Firms anticipated this surge—by 2016, large agri-food companies had started acquiring successful meat-substitute firms around the world (37).

Several methods have been developed to evaluate the impact of information policies. Contrary to the abundant literature on trials enrolling control groups to evaluate the impact of a specific intervention, the FAO campaign focused on here involved global exposure, without the possibility of a control/non-exposed group. Moreover, we cannot rely on an estimation of variation in the degree of exposure of the population to the campaign (38). Some previous works have also faced these issues. A first approach, used by Capacci and Mazzocchi (39) in a study on the UK’s 5-a-day promotion of fruit and vegetables, involves estimating the counterfactual, i.e., the outcome expected in the absence of an information campaign. The authors mentioned measured the average treatment effect on the exposed subjects, corresponding to the entire population. Another approach successfully used by Shankar et al. (40) in a study of a campaign aiming to limit salt consumption involved (i) a fixed effects model to analyze the trend in salt intake over the period considered and (ii) a two-way fixed-effects model to estimate group-specific responses to the campaign, where each cross-sectional unit was considered as its own control group. In both works, the impact of the health information campaigns was found to be positive. One study also evaluated the impact of a campaign across income quartiles (39). The campaign was found to be more effective for those in the 3rd income quartile and therefore somewhat regressive in terms of reducing nutritional inequalities. Information campaigns are one of the tools that are frequently used to implement public food policy (for instance, by the French Nutritional and Health Program). However, two issues can affect their efficiency. First, public budgets for safe and nutritious foods appear limited compared to the large budgets earmarked by the agroindustry to advertise “junk food.” Second, information is disproportionately well received by more educated consumers, inducing nutritional and health inequalities (41, 42). Nevertheless, as this policy tool can be used at the meta-level of countries when international organizations such as FAO get involved, it can have a significant impact.

In this context, our study aims to analyze the changes in pulse purchasing before and after the FAO campaign and discuss the possible effects of this awareness policy on pulse consumption in France, with particular reference to different sub-populations. To this aim, we consider a fixed-effects model to estimate the changes in purchasing concomitant with the FAO campaign. Our data consist in purchases for food-at-home. In a first step, as the demand for pulses has not been the focus of many works and remains poorly understood, we analyze the influence of sociodemographic factors on the purchase of pulses using a Box-Cox double-hurdle model. In a second step, we analyze the changes in purchasing associated with the 2016 FAO campaign, at the population level and across sociodemographic groups, to test whether it might have had a regressive effect by

10 Particularly influential was a report on red meat consumption and colon cancer (IARC/WHO) published in 2007 (31).

11 The key slogans to raise awareness were “Pulses are highly nutritious”; “Pulses are economically accessible and contribute to food security at all levels”; “Pulses have important health benefits”; “Pulses foster sustainable agriculture and contribute to climate change mitigation and adaptation”; “Pulses promote biodiversity”. The International Year of Pulses generated 225 events in 63 countries (33).

12 [http://www.fao.org/pulses-2016/events/fr/?page=30&ipp=5&tx_dynalist_pi1%5bpar%5d=YToxOntzOjE6IkwiaO3M6MToiOC17fQ\\$==\\$](http://www.fao.org/pulses-2016/events/fr/?page=30&ipp=5&tx_dynalist_pi1%5bpar%5d=YToxOntzOjE6IkwiaO3M6MToiOC17fQ$==$)

affecting population subgroups differently, which could deepen nutritional inequalities.

The remainder of the paper is organized as follows. Section Methods describes the data and methodology. Section Results presents the results. Section Discussion discusses the results and policy implications. Section Conclusions offers some concluding remarks.

Methods

Data

The data employed here are from Kantar Worldpanel surveys conducted in 2014, 2015, 2016, and 2017. These surveys were administered to over 20,000 households, which reported their weekly food acquisition. All participating households registered grocery purchases through the use of barcode scanning. Then, a group of households (half of the participating households) was asked to also specifically report the purchases of fresh products (meat, fish, fruit, and vegetables). These purchasing data provide an inventory (in quantity and expenditure) of the French population's consumption of food-at-home. However, these data do not provide information on food-away-from-home or self-produced food consumption (for example, from a home garden), thus they represent around 80% of total consumption.¹³

For our study, we selected the group of households with complete information on purchases, i.e., who reported both their grocery purchases and their fresh produce acquisition (over 12,000 households). It is worth noting that the Kantar data distinguish 13 periods of 4 weeks each throughout the year. Therefore, we retained households that reported their food acquisition for at least 1 week. We excluded households that did not communicate information about sociodemographic and economic variables (such as income or education level). Consequently, our sample includes 10,914 households in 2014, 11,074 households in 2015, 11,031 households in 2016, and 10,764 households in 2017. Each database is considered as a cross-section.¹⁴ We selected a set of products including

pulses in different forms, including raw and subject to various preparations: dried (lentils, beans, flageolet beans, split peas, and chickpeas), canned (flageolet beans, white and red beans, lentils, and chickpeas), frozen (flageolet beans), preparations/recipes (falafel, tofu, sausages with lentils, soy steaks, and other meat substitutes), desserts made of soy, soy beverages, and soy ingredients.

The dependent variable used in our analysis was the annual purchase of pulse-based products at the individual level. We obtained this by cumulating the period quantities registered in the Kantar database at the household level for each year of study. We then divided these quantities by the number of persons in the household. Therefore, the purchase of pulse-based products in kg/year/pers is expressed as follows:

$$q_{\text{Year}}^{\text{Kg}}/\text{pers} = \frac{\text{quantity}_{\text{kg}} * \frac{52}{N_w}}{N},$$

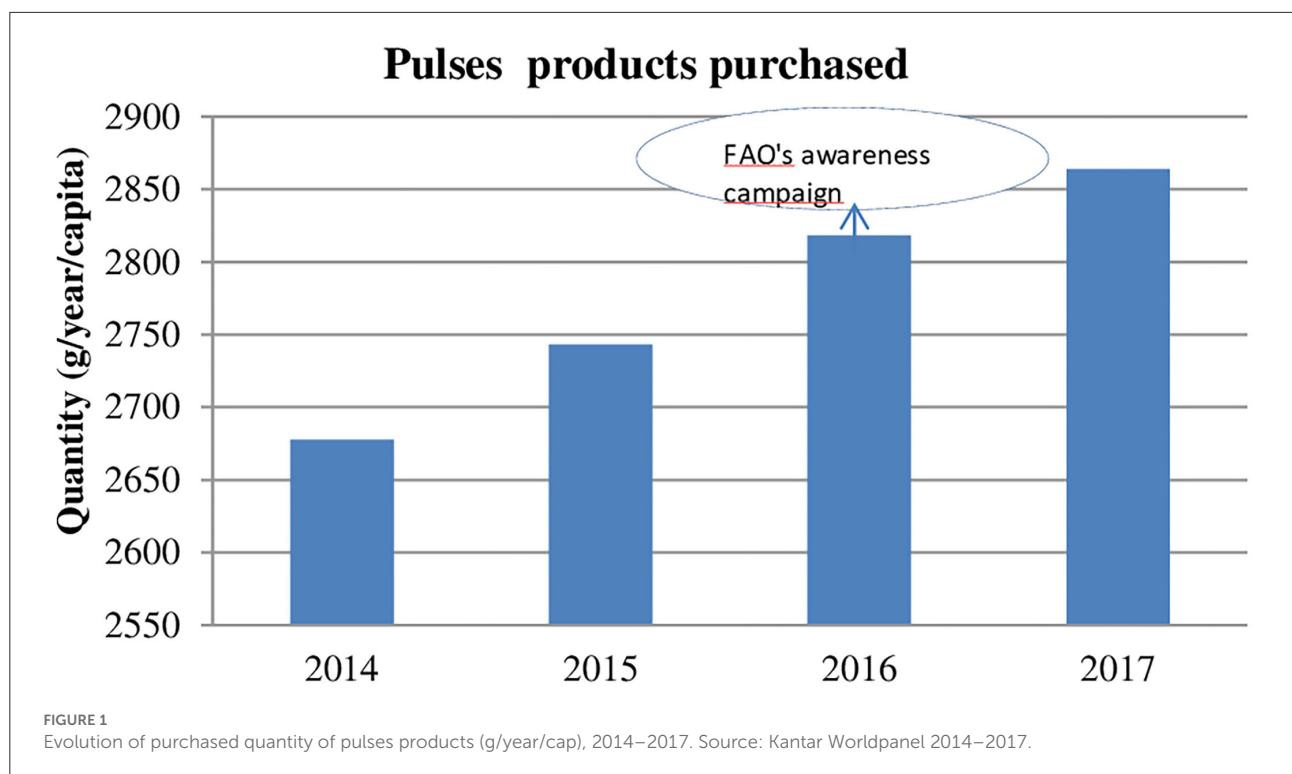
where N_w is the number of weeks of food purchases in a given year and N is the number of persons in the household.

The purchasing of pulse products increased by 6.9% between 2014 and 2017 (Figure 1; 2.68 kg/year per capita in 2014 and 2.86 kg/year in 2017, Appendix A). A large majority of consumers (84.9–88.4%) bought pulse-based products (Figure 2; Appendix A). Taking into account only pulse consumers, quantities per capita represented 3.24 kg in 2017. It must be noted that, in contrast to the purchases by the total sample, purchases attributed only to pulse consumers show stagnation between 2016 and 2017, at the time of the FAO awareness campaign. Relative to quantity, expenditure per capita showed a greater increase of 12.2% (Appendix A), meaning an increasing average unadjusted unit value,¹⁵ from 2.77 to 2.94 €/kg. The market broadened throughout the period under analysis. This can first be captured in terms of the increase in the diversity of products, observable in the 16.3% increase in the number of different barcodes in the Kantar categories “pulses” and “preparations from pulses” (2,049 items in 2015 and 2,383 in 2017). To capture the polarization of the market, we separated the pulse-based products into two segments: less-processed products (unprocessed and preserved, i.e., traditional consumption) and ultra-processed products (preparations from pulses, including meat substitutes). The proportion of purchasers of both categories increased across the period under investigation (73.2–77.2% for less-processed products, or LPs, and 60.0–63.6% for ultra-processed products, ULPs), with a stable repartition of quantities and close proportions between these two segments: LPs represented 59.3% of the market in 2014 and 58.6% in 2017. However, average prices (unadjusted unit values) were quite different. Not only was the price difference between LPs and ULPs in a range of one to two, but the price increase was also higher, in a range of one

¹³ Food-away-from-home was estimated to constitute 20% of all food consumed in 2015 (43) and over 25% of household food expenditure in 2018 (44).

¹⁴ Kantar data make it possible to employ panel data and to follow the same households over multiple years. In our sample, fewer than 60% of households present in 2014 recorded their purchases in 2017; therefore, we did not work with panel data. We considered our databases as cross-sectional and constructed a pseudo-panel in section Estimation of changes in pulse purchasing over the awareness campaign period using a two-way fixed-effects model, which has the advantage of avoiding selection bias associated with attrition effects (which increase with the number of periods) and bias associated with learning effects.

¹⁵ Computed as the ratio of expenditure to quantity.



to five, with an increase of 2.7% for LPs and 14.0% for ULPs (Appendix A). The increase in consumption over this period may be attributable to various drivers, such as greater awareness (due in part to the FAO campaign), greater diversity in supply, mainly driven by consumer demand and firm restructuring (36, 37), and the flexitarian behavior of consumers with a growing acceptance of alternatives to meat (20) and concern for animal welfare. Heterogeneity in purchasing is observed according to sociodemographic characteristics. In particular, higher quantities are purchased by people with an education level lower than the baccalaureate diploma, those 65 years and over, and in the southern region (Appendix B).

Econometric methodology

Estimation of the relationship between sociodemographic characteristics and pulse purchases using a Box-Cox double-hurdle model

Our aim is to identify the sociodemographic factors associated with the purchase of pulse products. Linear regression models are generally inappropriate for analyzing such data since some households may not purchase pulses in a given year, i.e., the presence of zeros. The Tobit model (45) is often used when a dependent variable is zero for a part of the population but positive (and with different outcomes) for the rest of the

population. Here, we specify the Tobit model as follows:

$$y_h^* = x_h \beta + \varepsilon_h \quad \forall h = 1, \dots, N$$

$$y_h = \begin{cases} y_h^* & \text{if } y_h^* > 0 \\ 0 & \text{if } y_h^* \leq 0, \end{cases} \quad (1)$$

where y_h^* is the latent variable and y_h is the quantity of pulse products purchased. $x_h = (x_h^1 \dots x_h^K)$ is a vector of explanatory variables, $\beta = (\beta_1 \dots \beta_K)'$ is a vector of unknown coefficients, and ε_h 's are independent, identical, and normally distributed random variables with a mean of zero and variance σ^2 . The estimation procedure used was the maximum likelihood method.

The Tobit model assumes that households simultaneously decide to purchase pulse products and what quantity to purchase. In other words, this model assumes that the sign of a given determinant's effect will be the same for both the probability of purchasing pulse products and the quantity purchased.

The double-hurdle model by Cragg (46)¹⁶ is an alternative method that supposes sequential behavior. In a first step,

¹⁶ Similar to Cragg's double-hurdle model, the Heckman model is an alternative method that supposes sequential behavior. In the first step, households decide to participate or not in the market. However, Heckman's model is less flexible. In this model, once consumers pass the participation hurdle they are assumed to have positive consumption, whereas Cragg's model assumes that a potential buyer could have zero

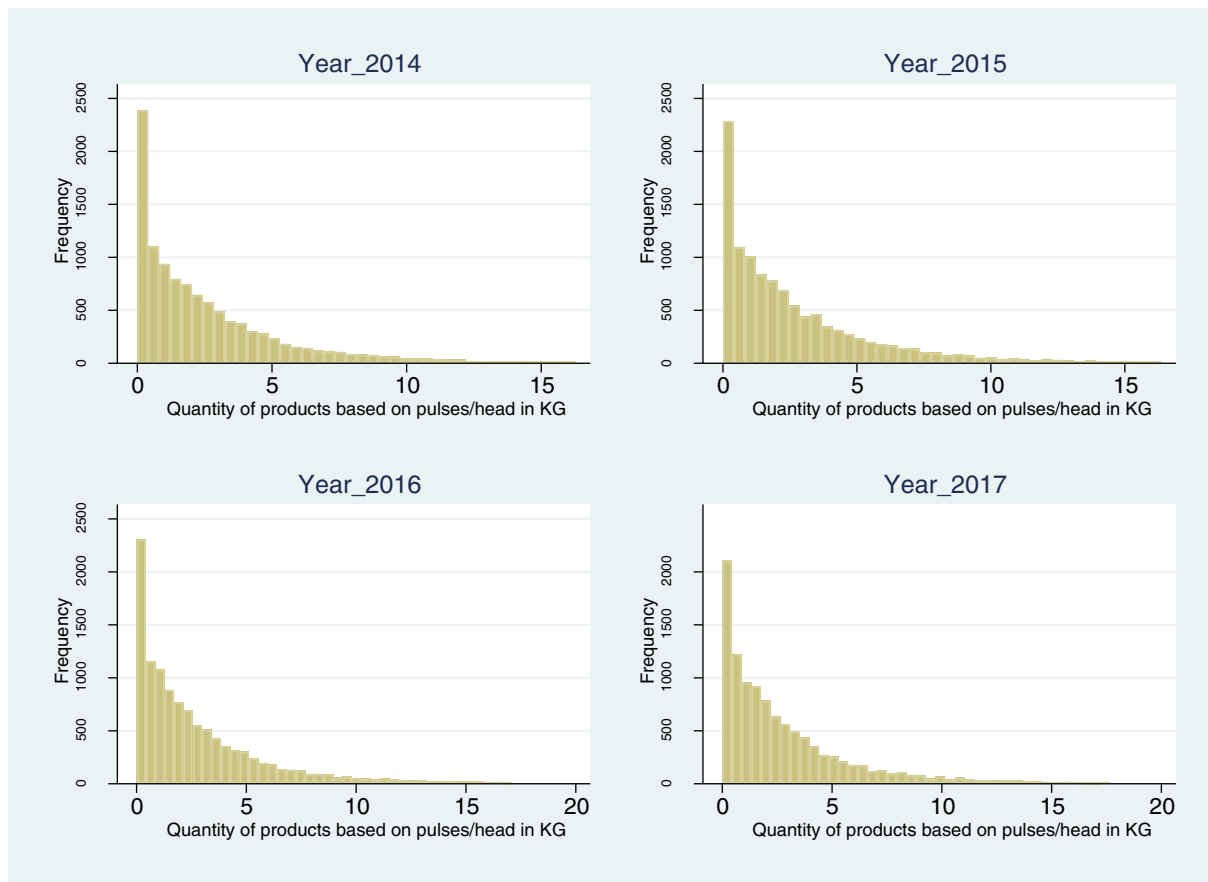


FIGURE 2

Proportion of zero purchases in the sample 2014–2015. Source: Kantar Worldpanel 2014–2017.

households decide whether to purchase pulse products. The second step models the quantity they will buy, conditional on the first decision. Therefore, in Cragg's model, the decision to participate in the market (i.e., purchase pulse products) and the level of participation (i.e., the quantity of pulse products purchased) are determined by two separate mechanisms (47). The participation (P) equation is as follows:

$$P_h = 1 \text{ if } P_h^* > 0 \text{ and } 0 \text{ if } P_h^* \leq 0; \\ P_h^* = \alpha z_h + u_h \quad u_h \sim N(0, 1), \quad (2)$$

consumption. Indeed, Cragg's model postulates that, in addition to an individual being a potential buyer, favorable circumstances have to arise for positive consumption to be observed (i.e., a person is a potential buyer and has sufficient funds to purchase pulses). In other words, in Cragg's model the consumer must pass two hurdles: (1) be a potential buyer of pulses (participation hurdle) and (2) actually buy pulses (consumption hurdle). The first hurdle allows for the possibility that zero consumption is due to non-economic considerations (preferences), while the second hurdle allows for the possibility that zero consumption could be a corner solution.

where P^* is a latent participation variable that takes a value of 1 if the household purchased pulse products and 0 otherwise; z is a vector of household characteristics; and α is a vector of parameters. The level of participation (Y) equation is follows:

$$\begin{cases} y_h = y_h^* \text{ if } y_h^* > 0 \text{ and } P_h^* > 0 \\ y_h = 0 \text{ otherwise} \\ y_h^* = x_h \beta + \varepsilon_h \quad \varepsilon_h \sim N(0, \sigma^2), \end{cases} \quad (3)$$

where y_h is the quantity of pulse products purchased, x is a vector of household characteristics, β is a vector of parameters, and u_h and ε_h are independently distributed. The estimation procedure used was the maximum likelihood method. To test which model—the Tobit or Cragg model—best identifies the determinants of pulse products purchases, a likelihood ratio (LR) test was conducted (Appendix C) (48).

Specification issues

According to Arabmazar and Schmidt (49), the standard double-hurdle model built on the assumption of normality of the error may be inconsistent when this normality assumption does

not hold. Thus, we tested normality by conducting the Doornik–Hansen (50) tests. The test indicated that we could reject that the residuals were normally distributed (Appendix C). One way to manage the non-normality of the error is to apply a Box-Cox transformation to the dependent variable (51–53), such as

$$y^T = \frac{y^\lambda - 1}{\lambda}, \quad (4)$$

where y^T corresponds to the transformed variable and λ is the transformation parameter to be estimated, which is between 0 and 1. To take into account the presence of heteroskedasticity,¹⁷ or the variation in the variance values across the observations, we specified the variance of the error terms as a function of a set of variables. The standard deviation can be written as follows

$$\sigma_h = \exp(\gamma M'_h), \quad (5)$$

where M_h is a vector of the variables¹⁸ and γ is a vector of the coefficients (54, 55).

Estimation of changes in pulse purchasing over the awareness campaign period using a two-way fixed-effects model

Estimation strategy

To more accurately evaluate the changes in purchase behavior during the awareness campaign period, the standard procedure would be to define a treated and a control group and to apply an estimation method such as difference-in-difference. In our case, the entire population was exposed to the awareness campaign considered as the treatment, and there is no possibility of distinguishing a control group from the treated one. Therefore, we follow the strategy proposed by Lee and Jones (56) and used also by Shankar et al. (40). This method consists in using panel data to estimate

- 1- A one-way fixed-effects model including a variable that identifies the change in household quantities of pulse products purchased over time, after controlling for observable factors that may affect the outcome. This is described as follows:

$$Y_{ht} = \alpha + \beta X_{ht} + \rho D_t + \vartheta_h + \varepsilon_{ht} \quad (6)$$

where Y_{ht} is the logarithm of quantity of pulse products purchased per/head for household h at time t .

X_{ht} stands for a vector of sociodemographic and economic variables. D_t is a dummy variable taking a value of 0 and 1 for the pre-campaign and post-campaign periods, respectively. ϑ_h represents individual effects, and ε_{ht} is the residual.

- 2- A two-way fixed-effects model used to separately estimate the individual fixed-effects before and after the awareness campaign. This is described as follows:

$$\begin{aligned} Y_{ht}^0 &= \alpha^0 + \beta^0 X_{ht}^0 + \tau^0 \delta_t^0 + \vartheta_h^0 + \varepsilon_{ht}^0, \\ Y_{ht}^1 &= \alpha^1 + \beta^1 X_{ht}^1 + \tau^1 \delta_t^1 + \vartheta_h^1 + \varepsilon_{ht}^1. \end{aligned} \quad (7)$$

The superscripts pertain, respectively, to pre- (noted 0) and post-campaign (noted 1), and δ_t represents time dummies. With this method, each value of the individual effect is predicted and then used to compute the *full* individual fixed effect, which is equal to the mean of time fixed effects plus individual fixed effects. The differences between the “pre-campaign” full individual fixed effects and the “post-campaign” ones indicate the individual response in this period of the awareness campaign. In other words, holding other observable variables constant, they tell us how the pulse-product purchasing of each household changed in the post-campaign period.

Since our data are cross-sectional, we used them to establish a pseudo-panel. The idea is to identify households belonging to the same cohort¹⁹ and to monitor the mean behavior of the cohorts established (57). In total, two types of problem tend to be generated by estimations from pseudo-panels. The first concerns measurement errors for the different variables, which can lead to estimation biases. The model variables are not directly observed but, rather, the mean values are calculated using survey data. Nevertheless, these are close to their true values when there are a large number of individuals in the cohort. Verbeek and Nijman (58, 59) showed that measurement errors and estimation biases are negligible if the size of cohorts reaches 100. However, establishing large cohorts involves reducing the number of observations used (here, the number of cohorts) across a given sample, leading to less precise estimations. Reducing the number of cohorts can also increase the heterogeneity of individuals in a single unit and, therefore, increase the variance of estimators, making them less effective. A compromise needs to be found between the cohorts large enough to limit measurement errors, sufficiently homogeneous cohorts, and sufficient observations

17 The Breusch–Pagan test of heteroskedasticity that was carried out led to a rejection of the homoskedasticity assumption (Appendix C).

18 We included variables that were more likely to cause the heteroskedasticity according to the Breusch–Pagan test. The Breusch–Pagan test consists of regressing the squared residuals of the model on the independent variables. Thus, we were able to identify the variables that were sources of heteroskedasticity.

19 Here, we deal with statistical cohorts, in contrast to cohorts identified from health surveys. In this paper, a cohort is a group of households with the same characteristics (e.g., “year of birth” of the reference person in the household, or “county size”).

to obtain adequately precise estimators. We have four cross-sectional databases spanning 2 years (2014 and 2015) before the awareness campaign and 2 years (2016 and 2017) after. Each “pre-campaign” and “post-campaign” period includes 8 quarters. Each dataset contains more than 10,000 households for 13 periods of 4 weeks (52 weeks of the calendar year). We used the sum of those 13 purchasing periods in order to have quarterly purchasing information for each household.²⁰ We defined our cohorts using two variables: the “year of birth” of the reference person in the household and “county size,” composed of three levels (rural area with fewer than 2,000 inhabitants, urban area with 2,000 to 199,999 inhabitants, urban area of 200,000 inhabitants or more). Thereby, we formed 165 annual cohorts observed at minimum over 8 quarters and at maximum over 16 quarters (4 years*4 quarters). The first three cohorts include the heads of household born in 1936 and living, respectively, in the three types of counties mentioned above. The last three cohorts include the heads of household born in 1990 and living, respectively, in the three types of counties mentioned. Our pseudo-panel includes 2,592 observations (study sample). Not all cohorts are observed in each survey, and the mean size of the observed cohorts is 64 individuals.

Robustness check

Although our pseudo-panel provides a large number of cohorts, which is important to obtain precise estimations, the number of observations per cohort is a little less than recommended. For this reason, we challenge the estimation from this pseudo-panel with a robustness check consisting of using the same sample and constructing another pseudo-panel—pseudo-panel 2—with cohorts of, on average, 100 individuals or more (59). Pseudo-panel 2 was constructed similarly, based on “year of birth” and “county size” variables (Appendix H). This pseudo-panel is composed of 84 biannual cohorts observed at minimum over 8 quarters and at maximum over 16 quarters. It includes 1,320 observations, and each cohort includes 127 individuals, on average. Considering the pseudo-panel nature of the data, equations take the following form:

$$Y_{ct} = \alpha + \beta X_{ct} + \rho D_t + \vartheta_c + \varepsilon_{ct}, \quad (8)$$

$$Y_{ct}^0 = \alpha^0 + \beta^0 X_{ct}^0 + \tau^0 \delta_t^0 + \vartheta_c^0 + \varepsilon_{ct}^0, \quad (9)$$

$$Y_{ct}^1 = \alpha^1 + \beta^1 X_{ct}^1 + \tau^1 \delta_t^1 + \vartheta_c^1 + \varepsilon_{ct}^1,$$

where the household index h has been replaced by the cohort index c . As in equations (6) and (7), X_{ct} stands for a vector of sociodemographic economic variables: age, access to an orchard,²¹ access to a vegetable

garden, education level, region of residence, body mass index, income class, and the logarithm of the price of pulse-based products.

Independent variables

Price is a key factor; however, the database does not provide a price variable. We computed the unit value of pulse-based products as the ratio between expenditure and the quantity purchased and adjusted the unit value for quality by following the procedure of Crawford et al. (60), who attributed variation in actual price to spatial and time differences only.

Other independent variables included were the socioeconomic and demographic characteristics of the household. These include the characteristics of the participant in the survey, namely, age, level of education, and socioprofessional category, which are considered major individual drivers of dietary patterns in protein intake (24). Our age variable has 3 levels, distinguishing 2 potentially active phases (18–44 and 45–64 years) from the retirement period (from 65 years). Our educational variable has 4 levels, distinguishing survey participants with less than post-secondary qualifications (around one third of the sample), post-secondary qualifications, and 2 categories above that level: those who completed up to 3 years of university and those who completed more than 3 years of university education (this latter group represents around 20% of the sample, depending on the year). The socioprofessional category of survey participants is expressed in 5 categories to differentiate labor status and consumption patterns: farmer, self-employed, employee/manual worker, associated professional, senior executive, and student/unemployed. Body mass index (BMI) was introduced as a health-related variable with 5 levels.²² Inverse associations were found between a higher consumption of pulses and a lower risk of overweight/obesity (7). At the level of the household, variables include household income, which was corrected by consumption units (CU) according to the OECD-modified scale. By taking into account demographic variation during the life cycle, the measure of income per consumption unit allows for a comparison of the incomes of households of different sizes and composition. Our income/CU variable has 4 levels: lower than poverty line; from poverty line to median income; from median income to 7th decile; 7th decile and over. We also introduced variables describing the household-specific access to fruit, vegetables, and pulses from self-production

variables and became proportional when constructing the pseudo-panel. For example, the region of residence variable comprises 2 levels (North and South), and in the pseudo-panel it indicates the proportion of households in cohort c living in the North and in the South.

²² BMI levels are *thin* ≤ 18.5 kg/M²; *normal weight* < 25 kg/M²; *overweight* < 30 kg/M²; *moderate obesity* < 35 kg/M²; anything above this level is considered *severe obesity*.

²⁰ Each quarter is composed of three Kantar four-week periods, with the last quarter including the remaining four periods.

²¹ Access to an orchard, access to a vegetable garden, education level, region of residence, and body mass index are all originally dummy

in orchards or vegetable gardens. Finally, we added location variables such as the region, distinguishing South from North based on the different pulse production capacities and consumption patterns. We also introduced county size, distinguishing rural areas from less and more urbanized areas (respectively, 2,000–199,999 inhabitants and 200,000 inhabitants and over). Descriptive statistics are presented in [Table 1](#).

In the estimation of the Box-Cox double-hurdle model for pulse product purchases, the sociodemographics introduced could differ for each decision, separating the different determinants of the probability of purchasing from those of the decision regarding the amount purchased. Therefore, the decision to buy a specific product, a behavior related to taste, is separated from the decision about how much to buy, which could be related to health or sustainability behaviors. In the participation equation (decision to purchase), sociodemographic characteristics (age, BMI of the participant), SES (household income, socioprofessional status/education of the participant), self-production (orchard, vegetable garden), and location (region) were introduced. In the equation related to the decision regarding the yearly amount purchased, sociodemographic characteristics (age, BMI of the participant), SES (socioprofessional status/education of the participant), and location (region, county size) were used. For each equation, we estimated two specifications depending on the SES variables included. When simultaneously using SES variables within the same equation, problems of collinearity can occur. Specification 1 included income and socioprofessional status (excluding education). Specification 2 included income and education (excluding the socioprofessional status). In the fixed-effects model explaining the change in purchases concomitant with the FAO campaign, we introduced the price of pulses (corrected unit value) and all household characteristics described above. Concerning SES status, we chose Specification 2, which includes education, as knowledge (for which education level is considered a proxy) is assumed to be an important driver of receptiveness to health information ([41](#)).

Results

According to the specification tests ([Appendix C](#)), the Box-Cox model was the best model, and we discuss these results in the following.

What is the profile of consumers of pulse products?

The Box-Cox heteroskedastic double-hurdle model was estimated for 2015 and 2017, which correspond to the pre- and

post-campaign years, respectively (Specification 1 is presented in [Appendix D](#), Specification 2 in [Table 2](#)). Comparable estimations were performed for LPs ([Appendix E](#)) and ULPs ([Appendix F](#)).

2015 purchases

The *probability of purchasing pulse products* (Specification 2) was associated with age, with a lower probability of purchase observed for younger panelists (at the global sample level) compared to individuals in the 45- to 64-year age group, and a higher probability of purchase for senior participants (65 years and over) when it comes to ULPs. Regarding education, we observed a lower probability of purchase for those holding a bachelor degree and over (with a level over 3 years of university) compared to those with post-secondary education, a difference significant at the 10% level. As for BMI, both an overweight and a severe/morbid obesity status were statistically associated with a lower probability of purchasing pulses (compared to those with a normal weight). Lower-income categories (under the poverty line and under the median income) had a higher probability of purchase compared to those with a higher-than-median income). Regarding socioprofessional status, farmers had a lower probability of purchase at the global level and for LPs (compared to employees/workers, in Specification 1; [Appendix D](#)). We observed a positive association between access to an orchard or a vegetable garden and the purchase of pulse products. Depending on the degree of processing of pulse products, we found similar associations but also some different effects, such as a negative association in the northern region (compared to the South) with LPs purchases.

The *quantity of pulse-based products purchased* showed a negative association with younger panelists and a positive one with older ones (reference: median category of 45–64 years of age). A severe obesity status was associated with greater quantities of pulse products purchased, as were the categories of overweight and moderate and severe obesity for LPs specifically. For ULPs, only the thin category showed a relationship to purchasing quantity. Income was negatively associated with the low-to-median category in both specifications and also to the highest category (reference: median income to the 7th decile). This latter effect was not found in the case of LPs. In Specification 1, categories of socioprofessional status such as associated professionals and senior executive showed a negative effect (reference: employees/workers). This latter effect was not observed for ULPs, however. Concerning education (Specification 2), a positive association was observed with the lowest level and a negative one with the level of 1–3 years of university (reference: post-secondary qualification), while only the highest level was significantly negative for ULPs. We also found a negative association of the northern region with quantities purchased, at the global level and for LPs. For ULPs,

TABLE 1 Descriptive characteristics of the study population.

Variables	2014		2015		2016		2017	
	N = 10914	%	N = 11074	%	N = 11301	%	N = 10764	%
Age Group								
18–44 yrs	5,638	51.66	5,752	51.94	5,517	48.82	5,123	47.60
45–64 yrs	3,511	32.17	3,550	32.06	3,910	34.60	3,788	35.19
65 yrs and +	1,765	16.17	1,772	16.00	1,873	16.58	1,852	17.21
Education level								
<Post-secondary qualifications	3,112	28.51	3,073	27.75	3,131	27.71	2,954	27.44
Post-secondary qualifications	2,879	26.38	2,967	26.79	3,089	27.33	2,952	27.42
1st, 2nd, 3rd year university	2,531	23.19	2,557	23.09	2,612	23.11	2,485	23.09
Bachelor's degree and +	2,392	21.92	2,477	22.37	2,469	21.85	2,373	22.05
Monthly income €/CU								
<poverty line	1,839	16.85	1,895	17.11	1,885	16.68	1,728	16.05
Poverty line to median income	4,145	37.98	4,181	37.76	4,303	38.08	4,788	44.48
Median income to 7th decile	2,341	21.45	2,363	21.34	2,419	21.41	1,616	15.01
> 7th decile	2,589	23.72	2,635	23.79	2,694	23.84	2,632	24.45
Body Mass Index								
Thinness: BMI < 18.5 kg/M2	400	3.67	401	3.62	400	3.54	368	3.42
Normal weight: 18.5 ≤ BMI < 25 kg/M2	5,807	53.21	5,820	52.56	5,859	51.84	5,512	51.21
Overweight: 25 ≤ BMI < 30 kg/M2	2,884	26.42	2,985	26.96	3,102	27.45	2,985	27.73
Moderate obesity: 30 ≤ BMI < 35 kg/M2	1,128	10.34	1,127	10.18	1,192	10.55	1,208	11.22
Severe and morbid obesity: BMI > 35 kg/M2	497	4.55	520	4.70	532	4.71	499	4.64
No answer	198	1.81	221	2.00	216	1.91	192	1.78
County Size								
Urban area- 2,000–199,999 inhabitants	3,890	35.64	3,961	35.77	4,056	35.89	3,893	36.17
Urban area 200,000 inhabitants+ and Paris	3,790	34.73	3,722	33.61	3,686	32.62	3,415	31.73
Rural area	3,234	29.63	3,391	30.62	3,559	31.49	3,456	32.11
Region of residence								
North	7,068	64.76	7,138	64.46	7,231	63.99	6,879	63.91
South	3,846	35.24	3,936	35.54	4,070	36.01	3,885	36.09
Orchard owner								
No	6,024	55.20	6,053	54.66	6,040	53.45	5,722	53.16
Yes	4,890	44.80	5,021	45.34	5,261	46.55	5,042	46.84
Vegetables garden								
No	6,509	59.64	6,493	58.63	6,572	58.15	6,149	57.13
Yes	4,405	40.36	4,581	41.37	4,729	41.85	4,615	42.87
Socio-professional category								
Farmer	55	0.50	55	0.50	60	0.53	51	0.47
Senior executive	729	6.68	741	6.69	718	6.35	726	6.74
Student/Unemployed person	927	8.49	923	8.33	837	7.41	773	7.18
Employee/Manual worker	5,339	48.92	5,412	48.87	5,525	48.89	5,264	48.90
Associated professionals	3,480	31.89	3,534	31.91	3,711	32.84	3,533	32.82
Self-employed	384	3.52	409	3.69	450	3.98	417	3.87

we observed a negative association with urban areas with over 200,000 inhabitants (reference 2,000–199,999 inhabitants).

It is interesting to note that for several characteristics, opposite effects were observed for the probability of purchase

and the quantities purchased. Concerning income, the two categories of poorer households (under the median income) had a higher probability of purchasing LPs, but they purchased a lower amount. For ULPs, this effect was found only for the

TABLE 2 Associations of sociodemographic and economic variables with purchases of products based on pulses 2015 and 2017 Box-Cox heteroscedastic double-hurdle model.

Variables	Year 2015		Year 2017	
	Specification 2		Specification 2	
	Participation	Y^T	Participation	Y^T
Age				
18–44 years	−0.33*** (0.037)	−0.26*** (0.029)	−0.22*** (0.038)	−0.30*** (0.028)
45–64 years	Ref	Ref	Ref	Ref
65 years +	−0.05 (0.050)	0.12*** (0.035)	−0.06 (0.050)	0.09** (0.035)
County size				
rural		−0.00 (0.029)		0.04 (0.029)
Urban area - from 2,000 to 199,999 inhabitants		Ref		Ref
Urban area of 200,000 inhabitants+ and Paris		−0.05 (0.029)		0.03 (0.030)
Region of residence				
North	0.02 (0.032)	−0.07*** (0.025)	0.00 (0.033)	−0.05* (0.025)
South	Ref	Ref	Ref	Ref
Monthly income €/CU				
<Poverty line	0.11** (0.049)	−0.14*** (0.040)	0.05 (0.058)	−0.03 (0.045)
Poverty line to median income	0.18*** (0.041)	−0.16*** (0.033)	0.06 (0.048)	−0.02 (0.037)
Median income to 7th decile	Ref	Ref	Ref	Ref
>7th decile	0.01 (0.044)	−0.08** (0.036)	0.02 (0.052)	−0.01 (0.041)
Body mass index				
Thinness : BMI < 18.5 kg/M2	−0.04 (0.080)	0.09 (0.065)	0.06 (0.091)	−0.02 (0.068)
Normal weight: 18.5 ≤ BMI < 25 kg/M2	Ref	Ref	Ref	Ref
Overweight : 25 ≤ BMI < 30 kg/M2	−0.13*** (0.036)	0.03 (0.028)	−0.06* (0.038)	−0.00 (0.029)
Moderate obesity: 30 ≤ BMI < 35 kg/M2	−0.00 (0.053)	0.05 (0.040)	−0.10* (0.052)	0.03 (0.040)
Severe and morbid obesity: > 35 kg/M2	−0.15** (0.070)	0.13** (0.058)	−0.10 (0.075)	0.05 (0.058)
No answer	−0.05 (0.109)	0.03 (0.085)	0.14 (0.131)	−0.12 (0.089)
Orchard owner				
Yes	0.14*** (0.036)		0.15*** (0.037)	
No	Ref		Ref	

(Continued)

TABLE 2 (Continued)

Variables	Year 2015		Year 2017	
	Specification 2		Specification 2	
	Participation	γ^T	Participation	γ^T
Vegetables production at home				
Yes	0.13*** (0.036)		0.11*** (0.038)	
No	Ref		Ref	
Education level				
< Post-secondary qualifications	−0.02 (0.044)	0.10*** (0.033)	0.09** (0.046)	0.11*** (0.033)
Post-secondary qualifications	Ref	Ref	Ref	Ref
1st, 2nd, 3rd year university	−0.05 (0.043)	−0.10*** (0.034)	0.02 (0.045)	−0.10*** (0.034)
Bachelor's degree and +	−0.08* (0.045)	−0.15*** (0.036)	0.02 (0.047)	−0.10*** (0.037)
Constant	1.16*** (0.056)	1.14*** (0.045)	1.16*** (0.063)	1.01*** (0.048)
Box-Cox Parameter (λ)		0.19*** (0.009)		0.18*** (0.009)
Log likelihood		−24,606.52		−24,194.85
Observations	11,074	11,074	10,764	10,764

Standard errors in parentheses: ***p < 0.01, **p < 0.05, *p < 0.10. Specification 2 includes all variables except socioprofessional status variable. Specification 1 is in [Appendix D](#).

poverty-to-median income level. Concerning BMI, participants with severe and morbid obesity had a lower probability of purchasing pulses, but they purchased greater quantities. This was also the case for overweight participants purchasing LPs. We also found a similar pattern for the oldest age category and ULPs, with a lower probability but greater quantities purchased.

2017 purchases

Some interesting changes were observed in 2017 compared to 2015. Concerning the *probability of purchasing pulse products*, income was no longer significant in 2017, for either LPs or ULPs. As for BMI, moderate obesity was negatively associated with the probability of purchase in 2017 (at the 10% level), at the global level and for LPs. The socioprofessional status of farmer was no longer significant.

Concerning the *quantities* purchased, no income effect was observed at the global level in 2017. However, we still observed—as in 2015—a negative effect of the lower income category (under the poverty line) for LPs. In contrast, this effect was positive for ULPs in 2017. For LPs, county sizes under and above the reference category (2,000–199,999 inhabitants) showed a positive relationship to quantities purchased in 2017.

The purchase of pulse products during the FAO campaign

The coefficient associated with price (adjusted unit value) was significantly negative, indicating that a 1% rise in the price was accompanied by a 0.70% decrease in the quantity purchased ([Table 3](#)). Controlling for sociodemographic variables, the change in purchasing ρ was significantly positive. Indeed, the purchasing of pulses increased by 8.4%. The [Figure 1](#) shows a steady increase in pulses purchasing since 2014, indicating a gradual preferences change in favor of pulses. For this reason, we estimated a specification that includes a time trend variable. The coefficient associated with this variable is statistically significant and the change in purchasing after the campaign is estimated by 2% ([Table 3](#)). Hence, the 8.4% increase observed indicated that there was a gradual preference change in favor of pulses and the impact of the awareness campaign was to boost expenditure on pulses by a further 2%. Our results from the separate two-way fixed-effects model also showed heterogeneous effects observed across various sociodemographic groups (age of the reference person, county size, and household income; cf. [Figure 3](#) and [Table 3](#)).

We found that the awareness campaign coincided with an increase in purchases for all sociodemographic groups. The change in quantities purchased over the pre- and post-campaign

TABLE 3 Changes in purchases of pulses after the FAO awareness campaign.

	Method 1	Method 1 (with time trend)	Method 2	
	Whole sample	Whole sample	Sample before information campaign	Sample after information campaign
log(price)	−0.698*** (0.00118)	−0.695*** (0.0007963)	−0.699*** (0.000683)	−0.699*** (0.000692)
Information campaign (ρ)	0.0815*** (0.00109)	0.02027*** (0.001348)		
Age				
18–44	−0.0237*** (0.00471)	−0.0119*** (0.003177)	−0.00554* (0.00288)	0.00262 (0.00260)
45–64	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>
65 years +	−0.00137 (0.00473)	−0.0160*** (0.00319)	−0.00805*** (0.00282)	−0.0111*** (0.00260)
Monthly income €/CU				
[1,000–1,500]	−0.0229*** (0.00487)	−0.01041** (0.00328)	0.00264 (0.00217)	−0.0178*** (0.00269)
[1,500–2,000]	−0.00388 (0.00257)	0.00030 (0.0017)	−0.000720 (0.00153)	−0.00257*** (0.00110)
[2000]	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>
Fruit tree owner				
Yes	0.513*** (0.0174)	0.53011*** (0.0117)	0.494*** (0.00993)	0.503*** (0.00760)
No	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>
Vegetables production at home				
Yes	−0.198*** (0.0160)	−0.2499*** (0.0107)	−0.310*** (0.0102)	−0.270*** (0.00787)
No	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>
Education level				
<Post-secondary qualifications	0.0239 (0.0219)	−0.0059 (0.0147)	−0.0811*** (0.0127)	−0.0117 (0.0110)
Post-secondary qualifications	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>
1st, 2nd, 3rd year university	−0.0141 (0.0258)	0.0156 (0.0173)	−0.0381** (0.0154)	−0.0592*** (0.0136)
Bachelor degree and +	0.0411 (0.0258)	0.0325* (0.0173)	0.0653*** (0.0165)	−0.0174 (0.0119)
Region of residence				
North	0.0956*** (0.0170)	0.1068*** (0.0114)	0.158*** (0.0101)	0.119*** (0.00931)
South	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>
Body mass index				
Thinness	−0.0609 (0.0430)	−0.071** (0.0289)	−0.0924*** (0.0230)	−0.105*** (0.0182)
Normal weight	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>	<i>Ref</i>

(Continued)

TABLE 3 (Continued)

	Method 1	Method 1 (with time trend)	Method 2	
	Whole sample	Whole sample	Sample before information campaign	Sample after information campaign
overweight	0.181*** (0.0177)	0.097*** (0.0119)	−0.0281*** (0.0101)	0.0491*** (0.00902)
Moderate obesity	0.290*** (0.0243)	0.1926*** (0.0164)	−0.0276** (0.0130)	0.107*** (0.0127)
Severe and morbid obesity	0.177*** (0.0320)	0.1396*** (0.0215)	−0.0131 (0.0160)	0.0939*** (0.0177)
No answer	0.103** (0.0492)	0.131*** (0.0331)	−0.0429 (0.0266)	0.144*** (0.0209)
Trend		0.0081*** (0.00015)		
Time effects				
2014T1			<i>Ref</i>	
2014T2			−0.0000723 (0.000745)	
2014T3			−0.000164 (0.000782)	
2014T4			0.000134 (0.000767)	
2015T1			0.0445*** (0.000750)	
2015T2			0.0444*** (0.000764)	
2015T3			0.0443*** (0.000823)	
2015T4			0.0447*** (0.000795)	
2016 T1				<i>Ref</i>
2016T2				−0.0000533 (0.000683)
2016T3				−0.000163 (0.000768)
2016T4				0.000119 (0.000725)
2017T1				0.0429*** (0.000695)
2017T2				0.0428*** (0.000708)

(Continued)

TABLE 3 (Continued)

	Method 1	Method 1 (with time trend)	Method 2	
	Whole sample	Whole sample	Sample before information campaign	Sample after information campaign
2017T3				0.0427*** (0.000811)
2017T4				0.0429*** (0.000720)
Constant	−0.213*** (0.0205)	−0.2081*** (0.01378)	−0.0500*** (0.0132)	−0.0694*** (0.0109)
N		2592	1296	1272

Standard errors in parentheses; * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Models include the sociodemographic variables (age, BMI of the participant), SES (household income, education of the participant), self-consumption availability (orchard, vegetable garden) and localization (region, county size).

ρ : change in purchases before and after the campaign period.

As the dependent variable is the logarithm of the quantity purchased, before interpreting coefficients associated to the information campaign, sociodemographic variables (age, BMI of the participant), SES (household income, education of the participant), self-consumption availability (orchard, vegetable garden) and localization (region, county size), it is important to apply this transformation: $100 \times [\exp(\alpha_a) - 1]$ where α_a is the coefficient associated with variables. For example, the change in purchases before and after the campaign period is $100 \times [\exp(0.0815) - 1] = 8.4\%$.

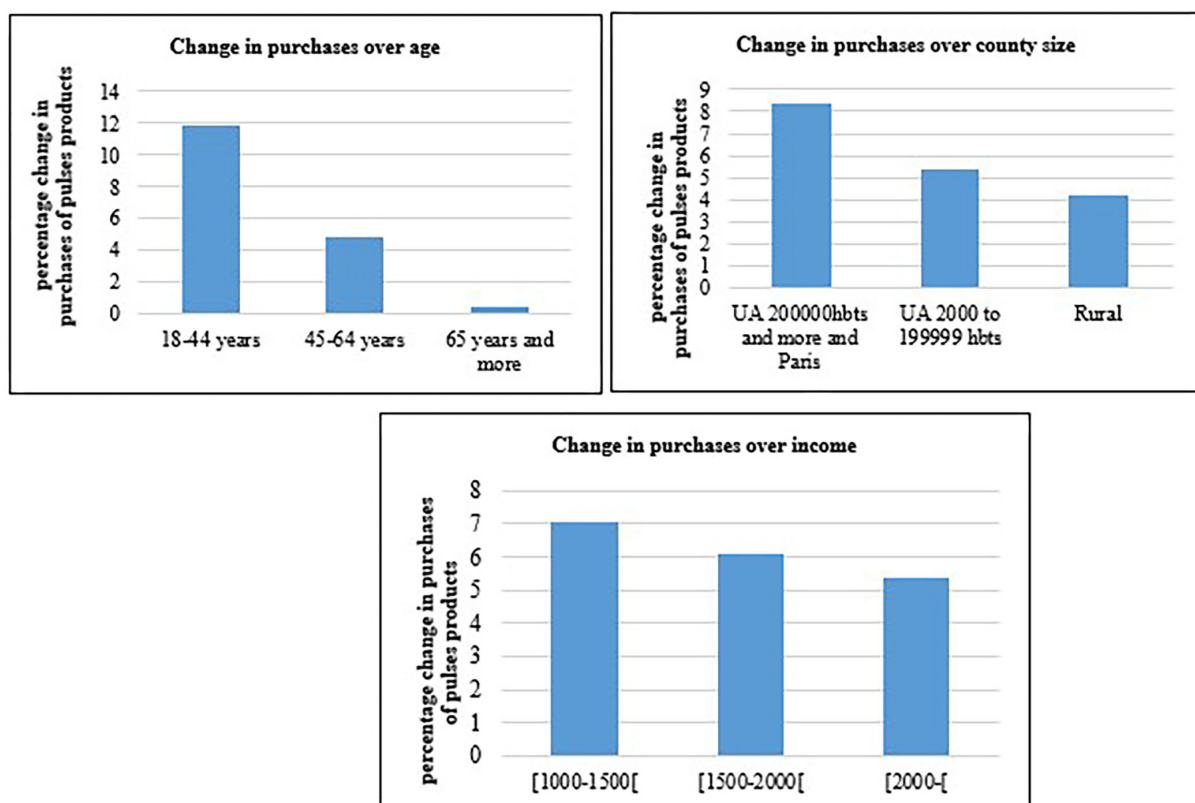


FIGURE 3
Change (%) in quantities purchased of pulses products over age groups, county size and income classes.

time period was greater among young people and people living in large urban areas with over 200,000 inhabitants (Figure 3).

For instance, the campaign coincided with an 11.8% increase in the purchase of pulse products for participants aged 18–44 years

of age, whereas stability was observed for senior participants (65 years and more), with a very small increase estimated at 0.51%. For urban households (200,000 inhabitants and over), an 8.4% increase was observed, vs. 4.2% for rural areas. Regarding income (Figure 3), we found two types of positive effects. The first was overall, and the second involved a stronger effect at the lower income level: 7.1% for the lower-income group and 5.4% in the upper-income group. Regarding the robustness check, the estimations run on the second pseudo-panel sample (59) showed a similar positive effect of ρ , along with a negative association with price (Appendix H).

Discussion

Did the profile of pulse consumers change?

Our results show that pulse purchasing was associated with sociodemographic and economic characteristics of households in the pre-campaign and the post-campaign periods. Some effects were comparable between 2015 and 2017, such as the negative effect of younger panelists and the positive effect of older ones on the quantity purchased, in line with the characteristics of an old-fashioned/traditional consumption (61). In 2017, as in 2015, older participants had a lower probability of purchasing ULPs but not LPs. Clearly, older participants did not change their habits and were less attracted to innovative products than younger participants (reference: 45–64 years). In line with the idea of pulses being a traditional food, purchasing is still positively associated with the lowest level of education and negatively with the highest levels of education and higher socioprofessional status.

Interesting differences arose in 2017, indicating a lower importance of sociodemographic variables. In particular, in the “post-campaign” period, pulse product purchasing was not related to BMI or income. Looking at the degree of processing of pulse products, we indeed found that most associations with BMI status seen in 2015 (except for severe/morbid obesity) were not observed in 2017 for LPs. Being thin was also no longer associated with the purchase of ULPs after the campaign. This suggests that before the campaign, LPs were purchased by heavier consumers while ULPs were used by specific consumers, probably those with more plant-based diet patterns, such as vegetarians. In the “post-campaign” period, these patterns were no longer observed. This may be linked to information on the benefits of LPs and ULPs becoming more mainstream as supply developed (36). Accordingly, a larger share of the population purchased these types of products in 2017 (Appendix A).

Our study highlights a complex effect of income. Pulses are traditionally viewed as a low-status food. We observed a

positive relationship between the probability of purchase and a lower income in 2015, at the global level and for LPs (this effect disappears in 2017), but a negative association between that income level and the quantity purchased was found in 2015 (and remained in 2017). This means that poorer households buy in total fewer quantities, but they shop more often. Note that Kantar data do not register food-away-from-home. Yet, a French study found that, in the context of choice between dishes meat-based or pulses-based, consumers chose pulses dishes when they were in contexts of « restaurant » or “self-service » rather than in at-home preparation (16)²³. As higher income households are known to eat more frequently out-of-home than other households, their lower frequency of pulses purchase for at-home-consumption may be more than compensated by their choice of pulses-based dishes when out-of-home ²⁴.

Meanwhile, note that for the quantity of ULPs purchased, the association with a lower income level, which was negative in 2015, turned positive in 2017. Therefore, despite increasing prices (Appendix A), the market for processed pulse products appears to have developed even for lower-income households. Since the quantities purchased by all income levels increased (Appendix B), this does not appear to be a pure substitution effect at the expense of raw products but, rather, a real expansion of the market at the household level. Finally, is there a geographical issue? The negative effect of the northern region at the global level and for LPs suggests an influence on consumption of living in areas of production. There could be a self-production effect here, as being a farmer had a negative effect on purchasing in 2015.

Therefore, some differences were observed in 2017 compared to 2015, indicating that changes in behavior occurred between these years. The pure effects of the FAO’s information campaign cannot be separated from other factors, however. Besides the change in preferences from the consumer side, some changes regarding supply can also be noted, as illustrated by the different effects observed between LPs and ULPs. Between both years, the variety of products offered in the market increased, and in particular on the consumption innovative ultra-processed preparations (36).

²³ One motivation could be saving time of preparation since pulses are perceived as requiring a long preparation (16).

²⁴ French consumption surveys, INCA3 (14) and CAP Protéines/CREDOC (62) registered pulse consumption, but not income information. Literature on the effect of economic crisis (such as in 2008) which induced an income shock on food consumption, does not bring clear results. Pulses consumption was found to decrease in Greece (63) and in Italy (64), while it increased in France (65), in Spain (66) and in Portugal (67). Besides, it is not clear whether it results from the crisis impact or from the existing trend.

An increase in purchasing concomitant with the FAO campaign

Our method, which uncovered an increase in pulse purchasing during the FAO campaign, provides an estimated average effect, as in Shankar et al. (40), but this does not imply a causal analysis. Our results may support a positive effect of the FAO campaign, which coincided with an 8.4% increase in the quantity of pulses purchased. Compared with other informational campaigns with positive effects, the FAO campaign's resonance in France appears lower than that of the salt campaign in the UK, which was estimated to have reduced the consumption of salt by 11% (40). The 5-a-day fruit and vegetable campaign in the UK was also found to have a positive effect, since the estimated intake would have been 0.3 portions lower without the campaign (39). Therefore, in France as in other countries, large institutional campaigns do come with and probably enhance positive changes in purchasing behavior.

Recent private initiatives have also been launched to attempt to change people's eating habits, including the "Meatless Monday" campaign, which advocates avoiding meat or fish on Mondays.²⁵ However, as for these latter types of information campaign, the positive effect observed after the FAO campaign differed across sociodemographic groups. It was greater for households with younger participants and those living in urban areas, which corresponded to households with lower levels of baseline purchases in 2014 and 2015, before the campaign, thus shortening the relative differences within those population groups (Appendix B). Indeed, younger consumers had been found by Stoll-Kleeman and Schmidt (24) to be more open to flexitarianism, which might help explain this demographic effect.

Concerning income, the greatest increase in pulse purchasing was in lower-income households (1,000–1,500€ monthly). Overall, the increase in purchasing concomitant with the FAO campaign matches changing consumer behavior with the development of the market (36). The anticipation of food manufacturers and their back-and-forth interactions with public policy are well known (68). However, there are still gaps between perceptions of supply and demand (69). Our results suggest a two-tier market, as LP and ULP purchases are clearly differentiated in terms of sociodemographic groups, and in particular with regard to income and BMI. This could mean that both institutional and private strategies—the FAO campaign and market forces—favor a gap between well-informed consumers ready to pay the price for innovative foods, and consumers less focused on pulses, identified as traditional LPs, for reasons of lack of information and price. The launching of these on the market exploits the demand for healthy and sustainable

foods and benefits from institutional communication regarding healthy dietary habits. In this context, an encouraging finding is the greater increase in purchasing by young participants and lower-income households, which partly corrects consumption discrepancies between sub-populations and, in particular, income classes. The largest increase in purchasing is registered for the lowest income class (7.1 vs. 5.4% for the highest income class), despite the moderating influence of the rise in prices throughout the period. Such an encouraging effect was not found by Capacci et al. (39) for the fruit and vegetable campaign in the UK, where the 3rd income quartile benefited the most from the campaign effects, indicating a regressive effect in terms of nutritional inequalities. This might be explained by the different profiles of consumers of fruit and vegetables compared to pulses: higher SES in the first case, lower SES in the second case.

Overall, our results suggest that a large institutional campaign may be a useful tool at the global level and also in the perspective of minimizing nutritional inequalities through the reduction of consumption disparities.

Strengths and limitations

One of the strengths of our study is that it evaluates the change in pulse purchasing before and after a large FAO campaign. Although our method does not allow the identification of a causal effect, the results reveal an increase in purchasing, which has some interesting policy implications (discussed below). In addition, we evaluated the change in pulse purchasing for different subgroups based on income, age, and region and found differential purchasing patterns, in particular with reference to income.

Our study also has some limitations, however. First, the size of the cohorts in our pseudo-panel is restricted (64 households, on average), although we conducted a robustness check by calculating estimates on a sample with larger cohorts (127 households, on average). Second, the length of the period is short (2014–2017), a common drawback shared by other studies (39, 40). Third, Kantar purchasing data are incomplete, as they do not include food-away-from-home and do not represent whole-household consumption. Thus, our evaluation of the increase in pulse purchases during the FAO campaign can only be considered a benchmark.

Conclusions

Our paper aimed to analyze the change in pulse purchasing before and after an FAO information campaign and discuss the possible effects of this push for awareness. In a first step, we analyzed the influence of sociodemographic characteristics on

²⁵ <https://www.lundi-vert.fr/index.php/2020/09/22/les-bienfaits-des-legumineuses/>

the purchasing of pulse products. In a second step, we provided an estimation of the change in purchasing patterns.

Then, two main results can be highlighted. First, our results suggest a positive effect of the FAO campaign. There was a gradual preference change in favor of pulses and the impact of the awareness campaign was to boost expenditure on pulses by a further 2%. Our method provides an estimated average effect and not a causal effect, however, similar to the work of Shankar et al. (40). Second, we observed a larger increase in purchasing for low-income households compared to high-income households. We tested the robustness of our results by conducting a similar estimation on a second pseudo-panel sample with larger cohorts and found consistent results.

Our study has certain policy implications. Our results show that there is still a long way to go to reach the level of consumption advised by France's nutritional recommendations ("Programme National Nutrition Santé"). The timing and the extent of the consumer response observed here results both from the FAO design of the campaign and its implementation by local institutions, as well as from market forces that led to greater variety in supply and an increase in prices. Pulse-based products are still considered an old-fashioned food, sometimes perceived as being consumed by those on tight budgets, in spite of efforts to give them a better image in terms of their health benefits. The FAO campaign might have been an initial signal to everyday consumers, as it launched various national initiatives, and this type of awareness raising should be pursued at the level of both public institutions and private stakeholders.

Additional work is needed to build a comprehensive understanding of changes in food purchasing following the campaign. Future work should investigate the potential substitution of animal-protein products by plant-protein ones across a longer timeframe. The results of such work can help support future food policies aimed at improving health and sustainability, as well as the development of the market. Improving communication about the benefits of pulses may help promote this food category, and several channels can be used simultaneously to this end—in particular between the food industry and consumers (69)—as new food market segments develop. Nudging in shopping environments could also favor the purchase of pulses and promote more sustainable consumer behavior.

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Data availability statement

The datasets presented in this article are not readily available because our data come from Kantar Worldpanel, which is a private company, and data users are not at liberty to share the raw data nor any transformed version thereof. Consequently, we do not provide a data appendix. Requests to access the datasets should be directed to france.caillavet@inrae.fr.

Author contributions

IB, FC, and MA designed the study. IB performed data management and data analysis. FC and IB wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.971868/full#supplementary-material>

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Correlation between dietary patterns and cognitive function in older Chinese adults: A representative cross-sectional study

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Objective: The objective of this study was to investigate the relationship between dietary patterns and cognitive function in older adults (≥ 60 years old).

Methods: Food intake was quantitatively assessed by the Food Frequency Questionnaire (FFQ), and cognitive function was assessed by the Chinese version of the Simple Mental State Examination Scale (MMSE). Four major dietary patterns were identified by the factor analysis (FA) method. The relationship between dietary patterns and cognitive function was evaluated by logistic regression.

Results: A total of 884 participants were included in the study. Four dietary patterns (vegetable and mushroom, oil and salt, seafood and alcohol, and oil tea dietary patterns) were extracted. In the total population, Model III results showed that the fourth quartile of dietary pattern factor scores for the vegetable and mushroom pattern was 0.399 and 7.056. The vegetable and mushroom dietary pattern may be a protective factor for cognitive function, with p -value = 0.033, OR (95% CI): 0.578 (0.348, 0.951) in Model III (adjusted for covariates: sex, ethnic, marital, agricultural activities, smoking, drinking, hypertension, diabetes, dyslipidemia, BMI, and dietary fiber). In the ethnic stratification analysis, the scores of dietary pattern factors of the vegetable and mushroom among the Yao participants were 0.333 and 5.064. The Vegetable and mushroom diet pattern may be a protective factor for cognitive function, p -value = 0.012, OR (95% CI): 0.415 (0.206, 0.815).

Conclusion: The fourth quartile of the vegetable and mushroom dietary pattern scores showed dose-dependent and a strong correlation with cognitive function. Currently, increasing vegetable and mushroom intake may be one of the effective ways to prevent and mitigate cognitive decline. It is recommended to increase the dietary intake of vegetables and mushroom foods.

KEYWORDS

cognitive function, dietary patterns, older adults, vegetables, mushrooms

1. Introduction

As global lifespan increases, there is a concern about diseases that significantly impact older adults, including those that cause chronic ailments, impact physical functioning, and lead to cognitive decline. Cognitive decline can have multiple causes, with Alzheimer's disease being the most common, with more than 46 million people worldwide suffering from dementia, which is impacted strongly (1, 2). It is expected that the number of Alzheimer's disease cases will increase to 131.5 million by 2050, and the aging population in China is constantly increasing. In 2020, the prevalence of cognitive decline and dementia in elderly individuals aged 60 years and above in China was 15.54% and 6.04%, respectively (3–5). Areas of cognitive dysfunction, such as progressive memory loss and language decline, are progressively impairing the ability of older adults to live independently.

The implementation of healthy dietary patterns is a potential preventive strategy for declining cognitive function and can mitigate the cognitive decline that occurs with age (6). The effects of single nutrients or foods on cognitive function have been well documented. For example, high intakes of fish, omega-3 polyunsaturated fatty acids, and high linoleic acid may reduce cognitive decline (7–9). Nutrients such as monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and antioxidants (e.g., beta-carotene and vitamin C) can protect the nervous system and mitigate cognitive decline (10–12).

However, it has been found that one's entire diet should be considered and that there are synergistic effects between the nutrients consumed (stronger than their single components), meaning that dietary patterns may have a more significant impact on cognitive function (13, 14). In recent years, the focus has shifted from investigating specific nutrients or foods to generalizing dietary patterns, and some dietary patterns have become representative of the theoretical basis that the whole diet reduces cognitive decline, such as the Mediterranean, Dietary Approaches to Stop Hypertension (DASH), Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND), low-fat and plant-based, traditional food, and Westernized and seafood-rich dietary patterns (15–19). The findings on dietary patterns and cognitive function vary, with plant-based dietary patterns and Mediterranean dietary patterns alleviating the decline in cognitive impairment (17, 20). These results offer hope for dietary patterns to prevent cognitive impairment. However, the greatest limitation of theoretically guided dietary patterns is that they do not provide complete access to the full information of the dietary matrix and do not reflect the true picture of dietary intake, especially for specific populations in particular regions that cannot strictly adhere to typical dietary patterns. For example, the Mediterranean dietary model does not make use of the accumulated knowledge on the relationship between dietary intake of nutrients and neurodegeneration and only considers nine food groups (13).

The most commonly used method to explore dietary patterns is factor analysis (FA) using a data-driven approach. This method is not limited by theory but reflects dietary intake in a specific population. For example, adherence to a “grain” pattern may be a risk factor for cognitive function and a “plant” pattern may be a protective factor for cognitive function (21). Gongcheng

County is the “hometown of longevity” in China, and oil tea is a daily beverage for people in southwest Guangxi, among which Gongcheng oil tea (22) is the most famous. According to the analysis of China's National Food hygiene standard method, the main components of oil tea are green tea, ginger, and oil (mainly peanut oil), and its main nutritional components include tea polyphenols, gingerol, total fat, and dietary fiber. As a specialty drink of the Yao people, Chinese medicine considers oil tea to be a compound preparation of traditional Chinese medicine, which has the functions of strengthening the spleen and eliminating food, treating the common cold and loosening the bowels, relieving symptoms of diarrhea, and stopping diarrhea (22). Ginger (23) and tea polyphenols (24) have been shown to have neuroprotective effects. We found that the dietary structure of the local elderly is relatively stable and less affected by external influences. However, there are few studies on the relationship between the dietary patterns of oil tea drinkers and cognitive function. Therefore, we further investigated the relationship between dietary patterns and cognitive function in Gongcheng County to provide an epidemiological basis for the prevention of cognitive impairment in local older adults.

2. Participants and methods

2.1. Participants

The study site was Gongcheng Yao Autonomous County, Guilin, Guangxi, China. According to the cross-sectional sampling survey, the sample size calculation formula is as follows:

$$N = (Z_{\alpha}^2 \times pq) / d^2,$$

where N is the sample size, Z is the statistic, $\alpha = 0.05$, $Z = 1.96$, p is the expected prevalence, $q = 1 - p$, and d is the allowable error ($d = 0.1p$). The prevalence of cognitive dysfunction in rural Chinese older adults was 42.9% (25). The estimated sample size for this study was 589, with an additional 10–15% of the sample. The number of people surveyed in this cross section was $N = 1,246$. Each participant completed the MMSE and FFQ questionnaire and underwent a physical examination and laboratory tests.

Inclusion criteria were as follows: older adults who (a) resided in the study area; (b) were over 60 years of age; (c) had blood sample, height, weight, and questionnaire information; and (d) had a reasonable energy intake of 500–6000 kcal (26). Finally, the participants were propensity score matched (PSM) (1:3) by age and years of education. A total of 884 adults aged 60 years and older were included in this study (2018–2019). The study included men ($n = 370$) and women ($n = 514$).

This research was conducted in accordance with the guidelines set forth in the Declaration of Helsinki, and all procedures involving human participants were approved by the Ethics Committee of Guilin Medical College. Written informed consent was obtained from each participant (No. 20180702-3).

2.2. Assessment of cognitive function

The MMSE questionnaire has high reliability and validity for the assessment of cognitive function. The questionnaire has the advantage of being easily applied and implemented in a short period. To assess the relationship between dietary patterns and the risk of cognitive dysfunction, MMSE scores have been classified according to the level of literacy: illiterate group ≤ 17 , primary group ≤ 20 , secondary and higher group ≤ 24 as the cutoff point, regarded as the cognitive impairment group (CI), compared to the non-CI group, and the rest are classified as the non-cognitive impairment (non-CI) group (27, 28).

2.3. FA and identification of dietary patterns

The main food intake during the previous year was determined by reference to the food frequency questionnaire (FFQ), which included the frequency and quantity of food consumed (29). The intake of each group (g/day) was then calculated by adding the intake of the member's food. Daily nutrient intake was calculated by multiplying the frequency of consumption of each component of each food by the nutrient content of that particular component. In this study, foods were combined into different food groups according to their nutritional content and use. To extract dietary patterns, we divided 109 foods into 14 food groups with reference to similar nutrients: cereals and potatoes, vegetables, fruits, soybeans and nuts, poultry, gut, aquaculture, egg, milk, mushroom, alcohol, oil, salt, and oil tea. Dietary patterns were generated by utilizing the factor analysis method (FA) for 14 predefined food groups. Factor analysis is a nutritional epidemiological method. By distinguishing one or more dietary factors, we found that certain types of food tend (or not) to be ingested in the same sitting.

Feature values of >1 and scree plots were used to determine the number of dietary patterns. Furthermore, by using orthogonal transformations of rotation factors, the results were easier to interpret. Food groups with absolute factor loadings of >0.3 were significant in the dietary pattern because the food items in these food groups appeared to be strongly associated with the identified factors. Factor scores were calculated by weighting each participant's intake of the 14 food groups by factor loadings. We named dietary patterns based on food groups with factor loadings of >0.6 .

2.4. Definition of covariates

Diabetes was defined as fasting blood glucose (FBG) of ≥ 7.0 mmol/L or a regimen of glucose-lowering medications (30). Hypertension was defined as systolic blood pressure (SBP) of ≥ 140 mmHg and/or diastolic blood pressure (DBP) of ≥ 90 mmHg or a regimen of antihypertensive medication (31) (for this study, hypertension includes having had hypertension and not knowing that you have hypertension but having

hypertension on your test.). Dyslipidemia was defined as total cholesterol (TC) of ≥ 6.22 mmol/L, triacylglycerol (TG) of ≥ 2.26 mmol/L, low-density lipoprotein cholesterol (LDL-C) of ≥ 4.14 mmol/L as high LDL-hemorrhage, and high-density lipoprotein cholesterol (HDL-C) of <1.04 mmol/L as hypo-HDL-C (32). The following demographic information was collected through questionnaires: age (60–69, 70–79, and ≥ 80 years), sex (male or female), ethnicity (Han, Yao, or other), marital status (unmarried, married, or other), education (illiterate [uneducated], primary education [educated for 0–6 years], and higher education [educated for more than 6 years]), agricultural activities [involved in agriculture (plowing, planting, and weeding)], and BMI (body mass index). Participants who were retired, homemakers, unemployed for more than 1 year, or physically disabled were considered nonworking individuals, unlike those involved in physical activities in agriculture. Smoking was defined as currently smoking at least one cigarette per day. Alcohol consumption was defined as drinking at least 50 g of alcohol or more once a month (33).

2.5. Statistical methods

The chi-square test and Student's *t*-test were applied for continuous and categorical variables, respectively, and the Wilcoxon rank-sum test was used for comparisons between groups of nonnormally distributed information. The energy and nutrient contents of food were obtained from the Chinese Food Composition Table (2009 version) (34). Nutrients were corrected using the residual method. Variance inflation factors (VIF <10) (35) were employed to avoid multicollinearity between nutrients. The ranges of the included nutrients are as follows: SFAs, 229.324; MUFAs, 1159.459; PUFAs, 220.699; energy, 336.380; protein, 13.121; fat, 3052.548; carbohydrates, 81.488; and dietary fiber, 2.182. Case matching and controls by age and educational years were performed using the propensity score matching method. Four dietary patterns were extracted using the factor analysis (FA) method with a factor loading matrix criterion of 0.3. Food groups that exceeded the criterion of 0.6 showed extreme correlation with their corresponding dietary patterns and were then named according to those patterns. Unconditional regression of binary logistic regression was used to fit a continuous intake of 14 food groups to the cognitive function model, and linear trend analysis was implemented. Simultaneous restricted cubic spline (RCS) plots were used to represent the relationship between continuous dietary pattern scores and cognitive function. The lowest quartile (first quartile) was defined as the reference group in each model. The results were expressed as weighted OR (95% CI). After adjusting for potential confounders, unconditional binary logistic regression was applied to analyze the relationship between dietary patterns and cognitive function and to perform between-group trend analysis stratified by ethnicity. All analyses were performed in R (4.1.3), and two-tailed *p*-values of <0.05 were considered statistically significant. The authors can be contacted for the source code should there be any questions about the analysis.

3. Result

3.1. Participant characteristics

The propensity score matching (PSM) results were as follows: non-CI (non-cognitive impairment) ($N = 663$) and CI (cognitive impairment group, compared to the non-CI group.) ($N = 221$). The jitter scatter plot and the histogram of the propensity value distribution show good results (Supplementary Figure 1).

Statistically significant differences occurred between the sex (P -values = 0.001), hypertension (P -values = 0.049), MMSE scores, orientation, memory, attention calculation, language skills, and visuospatial skills (P -values < 0.001) of the non-CI and CI groups, as shown in Table 1. Different ethnic groups had statistically significant variations in sociodemographic characteristics, including marital status (P -values = 0.009) and agricultural activities (P -values = 0.009), as shown in Table 2.

Significant differences were observed in the dietary pattern scores of SFAs (P -values = 0.040), protein (P -values = 0.010), carbohydrate (P -values = 0.027), dietary fiber (P -values = 0.025), fruit (P -values = 0.008), soybeans and nuts (P -values = 0.037), poultry (P -values = 0.030), aquaculture (P -values = 0.005), eggs (P -values < 0.001), mushrooms (P -values < 0.001), and vegetables and mushrooms (P -values = 0.001) of the non-CI and CI groups, as shown in Table 3. For the non-CI and CI groups, dietary pattern scores for PUFAs (P -values = 0.008), soybeans and nuts (P -values = 0.002), oil tea (P -values = 0.020), oil and salt (P -values = 0.042), and seafood and alcohol (P -values = 0.049) significantly varied among different ethnic groups, as shown in Table 4.

3.2. Dietary patterns

Kaiser–Meyer–Olkin (KMO) factor adequacy is equal to 0.81, Bartlett's test of sphericity is less than 0.001, and the cumulative variance explained is 44.64%, all of which meet the conditions for the use of factor analysis. The left side of the Scree plots shows the characteristic roots, and the slope for 4–5 factors becomes flatter, with the first four factors already covering most of the information, as shown in Supplementary Figure 2. Thus, four can be chosen as the number of factors. The dietary patterns were named according to the factor loadings of the 14 food groups in the dietary patterns (vegetables and mushrooms, oil, and salt, seafood and alcohol, and oil tea), as shown in Table 5. The first factor, which was defined by a high intake of vegetables, mushrooms, fruits, cereal potatoes, soybean nuts, gut, egg, and milk, was labeled “vegetables and mushroom dietary pattern.” The second factor, which was defined by a high intake of oil, salt, poultry, and soybean nuts, was labeled the “oil and salt dietary pattern.” The third factor, which was defined by a high intake of aquaculture, alcohol, poultry, and fruits, was labeled “seafood and alcohol dietary pattern.” The fourth factor, which was defined by a high intake of oil tea, milk, and gut, was labeled the “oil tea dietary pattern.”

3.3. Linear trend of 14 food groups and cognitive function scores

There was a nonlinear relationship between consumption and cognitive function values in the cereal and potato, fruit, and poultry food groups, with P -values of $p = 0.009$, $p = 0.01$, and $p = 0.024$, respectively (Supplementary Figure 3-1). There was also a non-linear relationship between dietary intake and cognitive function in the aquaculture, egg, milk, and mushroom food groups, with P -values of $p = 0.001$, $p < 0.001$, $p = 0.037$, and $p = 0.045$, respectively (Supplementary Figure 3-2).

3.4. Linear trend of factor scores of dietary patterns and cognitive function

We used restricted cubic spline (RCS) to flexibly model and visualize the relationship between dietary pattern scores and cognitive function. The seafood and alcohol dietary pattern showed a non-linear relationship with cognitive function, with a trend consisting of an initial decline, a brief increase, and a final flattening off, P -values = 0.003 (Supplementary Figure 4).

3.5. Association of dietary patterns with the risk of cognitive function

In Model I, after adjusting for sex and ethnicity, the score of the vegetable and mushroom dietary pattern factor was in the fourth quartile (0.399, 7.056); the vegetable and mushroom dietary pattern may be a protective factor for cognitive function, with an OR (95% CI) of 0.484 (0.302, 0.769). Other dietary patterns were not associated with cognitive function. In Model II, after adjusting for sex, ethnicity, marital status, agricultural activities, smoking, alcohol consumption, hypertension, diabetes, dyslipidemia, and BMI, when the score of the vegetable and mushroom dietary pattern factor was in the fourth quartile (0.399 and 7.056), it was found that the vegetable and mushroom dietary pattern may be a protective factor of cognitive function, with an OR (95% CI) of 0.517 (0.320, 0.830). Other dietary patterns were not associated with cognitive function. In Model III, after adjusting for sex, ethnicity, marital status, agricultural activities, smoking, alcohol consumption, hypertension, diabetes, dyslipidemia, BMI, and dietary fiber, when the score of the vegetable and mushroom dietary pattern factor was in the fourth quartile (0.399, 7.056), it was found that the vegetable and mushroom dietary pattern may be a protective factor of cognitive function, with an OR (95% CI) of 0.578 (0.348, 0.951). Other dietary patterns were not associated with cognitive function. A linear trend was noted between the quartiles of the dietary scores of the vegetable and mushroom (P -values = 0.017) and oil tea (P -values = 0.042) dietary patterns, as shown in Table 6.

TABLE 1 Sociodemographic characteristics of participants.

Characteristics	All participants (<i>n</i> = 884)	Non-CI (<i>n</i> = 663)	CI (<i>n</i> = 221)	<i>P</i> -value
Age, (years)^a				
60–69	662 (74.89)	500 (75.41)	162 (73.30)	0.547
70–79	185 (20.93)	138 (20.81)	47 (21.27)	
≥ 80	37 (4.19)	25 (3.77)	12 (5.43)	
Sex				
Male	370 (41.86)	299 (45.10)	71 (32.13)	0.001
Female	514 (58.14)	364 (54.90)	150 (67.87)	
Ethnic				
Han	321 (36.31)	249 (37.56)	72 (32.58)	0.254
Yao	537 (60.75)	397 (59.88)	140 (63.35)	
Other	26 (2.94)	17 (2.56)	9 (4.07)	
Marital				
Unmarried	23 (2.60)	18 (2.71)	5 (2.26)	0.098
married	652 (73.76)	500 (75.41)	152 (68.78)	
Other	209 (23.64)	145 (21.87)	64 (28.96)	
Education				
Illiteracy	530 (59.95)	402 (60.63)	128 (57.92)	0.743
Primary	130 (14.71)	97 (14.63)	33 (14.93)	
Above education	224 (25.34)	164 (24.74)	60 (27.15)	
Agricultural activities				
No	50 (5.66)	42 (6.33)	8 (3.62)	0.179
Yes	834 (94.34)	621 (93.67)	213 (96.38)	
Smoking				
Yes	181 (20.48)	142 (21.42)	39 (17.65)	0.268
No	703 (79.52)	521 (78.58)	182 (82.35)	
Alcohol drinking				
Yes	307 (34.73)	234 (35.29)	73 (33.03)	0.596
No	577 (65.27)	429 (64.71)	148 (66.97)	
Hypertension				
Yes	557 (63.01)	405 (61.09)	152 (68.78)	0.049
No	327 (36.99)	258 (38.91)	69 (31.22)	
Diabetes				
Yes	80 (9.05)	58 (8.75)	22 (9.95)	0.685
No	804 (90.95)	605 (91.25)	199 (90.05)	
Age, (years) ^b	67.59 (5.53)	67.48 (5.43)	67.92 (5.84)	0.312
MMSE SCORE	22.97 (5.29)	25.11 (3.41)	16.54 (4.67)	<0.001
BMI	22.52 (7.38)	22.63 (8.34)	22.17 (2.95)	0.422
Orientational ^c	9.00 [7.00, 10.00]	9.00 [8.00, 10.00]	6.00 [4.00, 8.00]	<0.001
Memory	5.00 [3.00, 6.00]	5.00 [4.00, 6.00]	3.00 [2.00, 4.00]	<0.001
Attention calculation	3.00 [1.00, 5.00]	4.00 [1.00, 5.00]	1.00 [0.00, 1.00]	<0.001
Language	8.00 [7.00, 8.00]	8.00 [7.00, 8.00]	7.00 [6.00, 8.00]	<0.001
Visual Space	0.00 [0.00, 1.00]	1.00 [0.00, 1.00]	0.00 [0.00, 0.00]	<0.001

^a*n* (%), chi-square.^b[mean (SD)], Students' *t*-test.^cMean [25, 75], Wilcoxon rank-sum test.

TABLE 2 Sociodemographic characteristics of participants.

Characteristics	Non-CI			CI		
	Han (<i>n</i> = 249)	Yao (<i>n</i> = 397)	<i>P</i> -value	Han (<i>n</i> = 72)	Yao (<i>n</i> = 140)	<i>P</i> -value
Age, (years)						
60–69	183 (73.49)	301 (75.82)	0.210	49 (68.06)	105 (75.00)	0.606
70–79	59 (23.69)	78 (19.65)		19 (26.39)	27 (19.29)	
≥80	7 (2.81)	18 (4.53)		4 (5.56)	8 (5.71)	
Sex						
Male	102 (40.96)	191 (48.11)	0.147	20 (27.78)	48 (34.29)	0.628
Female	147 (59.04)	206 (51.89)		52 (72.22)	92 (65.71)	
Marital						
Unmarried	12 (4.82)	6 (1.51)	0.071	0 (0.00)	4 (2.86)	0.009
married	177 (71.08)	309 (77.83)		45 (62.50)	104 (74.29)	
Other	60 (24.10)	82 (20.65)		27 (37.50)	32 (22.86)	
Education						
Illiteracy	166 (66.67)	226 (56.93)	0.170	44 (61.11)	77 (55.00)	0.069
Primary	30 (12.05)	65 (16.37)		5 (6.94)	28 (20.00)	
Above education	53 (21.29)	106 (26.70)		23 (31.94)	35 (25.00)	
Agricultural activities						
No	12 (4.82)	27 (6.80)	0.092	6 (8.33)	1 (0.71)	0.009
Yes	237 (95.18)	370 (93.20)		66 (91.67)	139 (99.29)	
Smoking						
Yes	46 (18.47)	91 (22.92)	0.292	9 (12.50)	29 (20.71)	0.289
No	203 (81.53)	306 (77.08)		63 (87.50)	111 (79.29)	
Alcohol drinking						
Yes	85 (34.14)	143 (36.02)	0.888	20 (27.78)	50 (35.71)	0.508
No	164 (65.86)	254 (63.98)		52 (72.22)	90 (64.29)	
Hypertension						
Yes	163 (65.46)	235 (59.19)	0.066	51 (70.83)	94 (67.14)	0.721
No	86 (34.54)	162 (40.81)		21 (29.17)	46 (32.86)	
Diabetes						
Yes	20 (8.03)	37 (9.32)	0.780	8 (11.11)	12 (8.57)	0.383
No	229 (91.97)	360 (90.68)		64 (88.89)	128 (91.43)	
Age	68.09 (5.11)	67.23 (5.64)	0.013	68.94 (5.95)	67.52 (5.79)	0.138
MMSE SCORE	24.77 (3.48)	25.30 (3.35)	0.114	16.96 (4.49)	16.61 (4.41)	0.010
BMI	22.18 (3.12)	22.89 (10.45)	0.550	22.43 (2.74)	22.03 (3.06)	0.647
Orientational	9.00 [8.00, 10.00]	9.00 [8.00, 10.00]	0.633	6.00 [3.75, 8.00]	6.00 [4.00, 7.00]	0.212
Memory	5.00 [4.00, 6.00]	5.00 [4.00, 6.00]	0.084	3.00 [1.75, 4.00]	3.00 [2.00, 4.00]	0.088
Attention calculation	4.00 [1.00, 5.00]	4.00 [2.00, 5.00]	0.400	1.00 [0.00, 2.00]	1.00 [0.00, 1.00]	0.959
Language	8.00 [7.00, 8.00]	8.00 [8.00, 8.00]	0.869	7.00 [6.00, 8.00]	7.00 [7.00, 8.00]	0.303
Visual Space	0.00 [0.00, 1.00]	1.00 [0.00, 1.00]	0.245	0.00 [0.00, 0.00]	0.00 [0.00, 0.25]	0.964

TABLE 3 Nutrients and factor scores of participants with cognitive impairment and normal group.

Characteristics	All participants (N = 884)	Non-CI (N = 663)	CI (N = 221)	P-value
SFAs, (g)	15.93 [9.70, 24.30]	16.25 [10.18, 24.80]	14.61 [9.02, 22.90]	0.040
MUFA, (g)	23.56 [15.33, 35.56]	23.89 [15.47, 35.80]	22.05 [15.20, 34.88]	0.242
PUFA, (g)	12.93 [7.56, 21.03]	12.79 [7.64, 20.35]	13.24 [7.46, 22.16]	0.417
Energy, (kcal)	1382.30 [1014.15, 1848.75]	1401.30 [1029.37, 1871.07]	1278.63 [948.68, 1721.94]	0.041
Protein, (g)	33.66 [22.38, 48.63]	34.47 [23.29, 50.36]	32.01 [19.84, 43.07]	0.010
Fat, (g)	57.64 [39.54, 88.76]	58.57 [39.66, 88.76]	54.62 [38.91, 88.28]	0.299
Carbohydrates, (g)	171.02 [123.26, 225.95]	173.81 [126.23, 230.37]	164.88 [115.47, 215.53]	0.027
Dietary fiber, (g)	12.75 [9.68, 16.60]	12.98 [9.94, 16.91]	11.63 [9.05, 15.86]	0.025
Cereals and potatoes, (g/day)	519.38 [362.80, 763.26]	519.55 [365.58, 757.31]	518.29 [355.05, 776.62]	0.460
Vegetables, (g/day)	212.66 [114.88, 393.45]	217.01 [117.80, 394.58]	210.16 [105.27, 376.70]	0.254
Fruits, (g/day)	123.90 [54.73, 275.35]	126.53 [58.47, 287.20]	114.59 [34.32, 211.63]	0.008
Soybeans and nuts, (g/day)	16.06 [5.84, 35.12]	16.91 [6.23, 35.67]	11.73 [4.86, 30.84]	0.037
Poultry, (g/day)	46.27 [18.55, 93.15]	50.00 [19.94, 96.38]	36.70 [16.06, 81.18]	0.030
Gut, (g/day)	0.00 [0.00, 1.68]	0.00 [0.00, 1.68]	0.00 [0.00, 1.68]	0.360
Aquaculture, (g/day)	6.70 [2.57, 16.75]	6.70 [3.35, 16.94]	5.03 [0.00, 13.40]	0.005
Egg, (g/day)	15.84 [4.42, 50.00]	20.11 [7.24, 50.00]	11.56 [3.62, 37.00]	<0.001
Milk, (g/day)	0.00 [0.00, 21.53]	0.00 [0.00, 23.45]	0.00 [0.00, 16.75]	0.284
Mushrooms, (g/day)	1.34 [0.00, 3.36]	1.68 [0.00, 3.36]	0.16 [0.00, 2.35]	<0.001
Alcohol, (g/day)	0.00 [0.00, 25.12]	0.00 [0.00, 25.00]	0.00 [0.00, 26.68]	0.499
oil, (g/day)	25.80 [16.00, 41.55]	25.80 [15.90, 42.00]	25.80 [16.50, 40.50]	0.733
Salt, (g/day)	6.90 [4.50, 10.80]	6.90 [4.50, 10.50]	6.90 [4.80, 12.00]	0.383
Oil tea, (g/day)	360.00 [180.00, 640.00]	360.00 [180.00, 640.00]	360.00 [180.00, 640.00]	0.447
Vegetables and mushrooms dietary patterns score	−0.30 [−0.69, 0.40]	−0.25 [−0.66, 0.46]	−0.42 [−0.74, 0.15]	0.001
Oil and salt dietary patterns score	−0.16 [−0.56, 0.34]	−0.16 [−0.58, 0.32]	−0.16 [−0.55, 0.43]	0.446
Seafood and alcohol dietary patterns score	−0.24 [−0.46, 0.19]	−0.23 [−0.45, 0.21]	−0.26 [−0.48, 0.13]	0.147
Oil tea dietary patterns score	−0.09 [−0.52, 0.46]	−0.09 [−0.49, 0.49]	−0.09 [−0.55, 0.35]	0.253

Mean [25, 75], Wilcoxon rank-sum test.

4. Discussion

This research was a case-control study based on a cross-sectional survey ($N = 884$). In a study of elderly people in Gongcheng County, female participants had a higher prevalence of cognitive impairment than other rural Chinese populations. There is substantial variation when considering the beneficial effects of diet in studies of the association of diet and cognitive impairment with age, and one potential reason for this variation in dietary pattern-cognitive function association may be sex (36, 37). Female participants should give greater attention to dietary intake. Our study points to a higher rate of cognitive impairment in hypertensive patients than in participants without cognitive impairment. Previous studies have suggested that hypertension is a risk factor for cognitive decline (38), and this association is mediated through microvascular brain damage (39). Appropriate blood pressure management may help alleviate cognitive decline in participants.

Four dietary patterns (vegetables and mushroom, oil and salt, seafood and alcohol, and oil tea) were extracted. The vegetable and mushroom dietary pattern (a) showed significant outcomes when the dietary pattern scores were at the fourth quartile after different levels of adjustment for potential confounders and (b) may be a protective factor for cognitive function. Among the Yao respondents, the vegetable and mushroom dietary pattern showed strong correlations with cognitive health when the dietary pattern scores were at the fourth quartile and after adjusting for different degrees of potential confounding factors. It is suggested that vegetable and mushroom dietary pattern may be a protective factor for cognitive function. After reviewing the previous literature, we made even more surprising findings. The Mediterranean dietary pattern includes various vegetables and mushrooms. Strict adherence to the Mediterranean diet (MD) may result in better cognitive status (40, 41). In a cross-sectional and longitudinal study of older adults in Taiwan, a dietary pattern high in phytonutrient-rich plant foods (fruits, whole grains, nuts/seeds, and vegetables)

TABLE 4 Nutrients in the cognitively impaired and normal group, and factor scores by ethnicity.

Characteristics	Non-CI			CI		P-value
	Han (n = 249)	Yao (n = 397)	P-value	Han (n = 72)	Yao (n = 140)	
SFAs, (g)	16.52 [10.09, 26.25]	15.85 [10.42, 23.60]	0.190	12.96 [8.83, 20.79]	16.51 [9.19, 24.12]	0.361
MUFA, (g)	24.16 [15.11, 36.37]	23.45 [15.79, 35.32]	0.082	20.41 [14.87, 30.35]	24.95 [15.92, 39.02]	0.173
PUFA, (g)	12.17 [7.01, 19.49]	12.87 [7.67, 20.32]	0.008	11.05 [6.78, 17.20]	14.48 [7.77, 24.41]	0.093
Energy, (kcal)	1339.24 [976.07, 1894.25]	1412.47 [1067.05, 1850.93]	0.155	1246.82 [925.87, 1629.49]	1312.30 [1026.62, 1922.56]	0.644
Protein, (g)	33.77 [22.61, 49.62]	34.83 [23.57, 50.40]	0.251	32.37 [21.14, 42.84]	31.78 [18.87, 45.39]	0.551
Fat, (g)	57.20 [38.45, 85.71]	58.87 [40.65, 88.59]	0.061	51.16 [36.08, 74.20]	61.09 [40.42, 98.76]	0.169
Carbohydrates, (g)	166.96 [128.86, 219.32]	181.25 [123.95, 234.53]	0.378	154.33 [121.20, 215.59]	165.60 [116.52, 214.76]	0.882
Dietary fiber, (g)	12.90 [9.90, 16.23]	13.01 [9.96, 17.08]	0.128	11.13 [8.52, 14.53]	12.25 [9.90, 16.28]	0.093
Cereals and potatoes, (g/day)	519.50 [377.97, 710.70]	519.55 [362.90, 768.70]	0.976	582.00 [344.97, 880.20]	490.84 [359.11, 722.39]	0.522
Vegetables, (g/day)	203.09 [113.72, 378.10]	217.35 [124.71, 398.77]	0.391	206.78 [118.39, 325.82]	210.51 [104.50, 407.10]	0.942
Fruits, (g/day)	120.86 [58.10, 269.76]	128.83 [59.90, 286.69]	0.567	120.87 [32.96, 204.61]	112.67 [44.89, 236.90]	0.315
Soybeans and nuts, (g/day)	12.37 [4.16, 32.30]	18.75 [8.24, 36.88]	0.002	13.36 [5.29, 29.57]	10.78 [4.85, 32.96]	0.402
Poultry, (g/day)	49.53 [19.04, 100.99]	49.50 [20.94, 93.64]	0.13	36.08 [12.61, 68.02]	37.44 [16.80, 91.26]	0.541
Gut, (g/day)	0.00 [0.00, 1.34]	0.00 [0.00, 1.68]	0.5	0.00 [0.00, 0.00]	0.00 [0.00, 2.05]	0.301
Aquaculture, (g/day)	6.70 [3.35, 16.75]	6.70 [3.35, 16.75]	0.766	5.03 [0.00, 10.21]	5.35 [0.00, 15.32]	0.288
Egg, (g/day)	22.26 [6.42, 51.88]	18.53 [7.64, 50.00]	0.798	11.56 [3.62, 40.62]	11.56 [3.35, 33.31]	0.169
Milk, (g/day)	0.00 [0.00, 23.45]	0.00 [0.00, 21.44]	0.897	0.00 [0.00, 16.75]	0.00 [0.00, 16.75]	0.937
Mushrooms, (g/day)	0.84 [0.00, 3.35]	1.68 [0.00, 4.02]	0.067	0.00 [0.00, 1.79]	0.60 [0.00, 3.35]	0.526
Alcohol, (g/day)	0.00 [0.00, 12.40]	0.00 [0.00, 44.22]	0.462	0.00 [0.00, 10.91]	0.00 [0.00, 37.11]	0.469
oil, (g/day)	25.80 [16.80, 42.00]	25.20 [15.00, 40.80]	0.369	24.90 [16.42, 39.90]	26.85 [16.42, 45.00]	0.504
Salt, (g/day)	6.60 [4.20, 9.60]	7.50 [4.80, 10.80]	0.085	6.00 [4.72, 9.90]	7.35 [5.10, 12.30]	0.100
Oil tea, (g/day)	400.00 [160.00, 640.00]	360.00 [180.00, 640.00]	0.343	440.00 [175.00, 740.00]	360.00 [180.00, 640.00]	0.020
Vegetables and mushrooms dietary patterns score	−0.44 [−0.75, 0.06]	−0.75 [−0.85, −0.31]	0.184	−0.44 [−0.75, 0.06]	−0.75 [−0.85, −0.31]	0.184
Oil and salt dietary patterns score	−0.11 [−0.51, 0.59]	−0.20 [−0.49, 0.47]	0.042	−0.11 [−0.51, 0.59]	−0.20 [−0.49, 0.47]	0.042
Seafood and alcohol dietary patterns score	−0.23 [−0.43, 0.18]	−0.13 [−0.39, −0.06]	0.049	−0.23 [−0.43, 0.18]	−0.13 [−0.39, −0.06]	0.049
Oil tea dietary patterns score	−0.07 [−0.53, 0.22]	−0.25 [−0.90, −0.13]	0.216	−0.07 [−0.53, 0.22]	−0.25 [−0.90, −0.13]	0.216

was associated with better cognitive function in older adults (42). A large epidemiological study in rural Shanxi, China, reported that two dietary patterns, the MVF (mushroom, vegetable, and fruit) and the MS (meat and soybean products) patterns, were predominant in the area, with greater adherence to the former being a possible protective factor for cognitive function (43). In the Chinese Longitudinal Healthy Longevity Survey (CLHLS), which assesses cognitive function using the Chinese version of the MMSE, a lower intake of fresh fruits and vegetables was significantly associated with a higher risk of cognitive impairment (44). Dietary intake patterns dominated by vegetables and mushrooms appear to

have a protective effect on cognitive function, an outcome that is consistent with our findings. The likely reason for this protective effect is that antioxidants in the brain protect brain tissue from damage caused by free radicals, and eating more antioxidant-rich vegetables and fruits is strongly linked to the mitigation of damage in Alzheimer's patients (45). More prospective studies are needed to explore the correlation between diet and cognitive function and to determine the underlying molecular mechanisms. However, other studies indicate that cognitive function is not associated with the vegetable and mushroom dietary pattern. As discussed, the Mediterranean dietary pattern is known to include

TABLE 5 Factor loadings of four principal components of 14 food groups extracted from principal component analysis (PCA) of eating frequency data of 884 adults aged 60 years or older.

Food groups	Vegetables and mushrooms dietary patterns	Oil and salt dietary patterns	Seafood and alcohol dietary patterns	Oil tea dietary patterns
Cereals potatoes	0.462	−0.101	0.083	0.23
Vegetables	0.604	0.193	0.186	0.006
Fruits	0.581	−0.004	0.362	−0.071
Soybeans nuts	0.564	0.408	0.034	0.08
Poultry	0.249	0.389	0.378	0.272
Gut	0.334	0.0616	0.082	0.518
Aquaculture	0.112	0.082	0.734	0.017
Egg	0.553	0.073	−0.057	−0.048
Milk	0.479	−0.172	−0.094	−0.489
Mushrooms	0.602	0.006	−0.008	0.225
Alcohol	−0.015	−0.102	0.697	−0.043
oil	0.059	0.696	−0.042	−0.104
Salt	−0.001	0.703	0.007	−0.008
Oil tea	0.028	−0.163	−0.112	0.644
Factor variance	2.323	1.426	1.374	1.127
Variance contribution	16.59%	10.19%	9.82%	8.05%
Cumulative variance contribution	16.59%	26.78%	36.59%	44.64%

Food groups with factor loadings: food groups ≥ 0.600 are marked in bold.

an abundance of vegetables and mushrooms. A New Zealand cohort study of older adults reported no significant associations between the Mediterranean dietary pattern and cognitive function or components of cognitive function (16). A study in Taiwan noted that a) in adults aged 65–74 years, a Western diet was detrimental to the maintenance of cognitive function, and b) in adults aged ≥ 75 years, the dietary pattern may not be correlated with cognitive function (46). The reason for those outcomes may be as follows: first, the Taiwan study adjusted for lifestyle factors and ADL, but no adjustment was made for nutrients, BMI, common diseases (including hypertension, diabetes, and dyslipidemia), and the covariate adjustment effect on the relationship between dietary pattern and cognitive function. Second, the healthy diet pattern involved a small sample size, thereby weakening the research strength. Finally, obvious differences in eating habits occur between rural and urban populations.

Oil and salt were not correlated with cognitive function. This finding is inconsistent with previous studies, which suggest that the oil and salt dietary pattern may be a risk factor for cognitive function (47). Dietary salt promotes neurovascular and cognitive dysfunction through gut-initiated TH17 participants (48), and high salt intake is associated with an increased risk of hypertension (49). Hypertension is an important risk factor for cognitive impairment (38), resulting in a small sample size that may lead to reduced statistical power for oil and salt dietary patterns to correlate with cognitive function.

The seafood and alcohol dietary pattern may not be associated with cognitive function. Previous reports suggest that the dietary

patterns of older adult French people may be beneficial for cognitive function (fish for males and fruits and vegetables for females) (50). However, alcohol was not a protective factor for cognitive function (51). The complexity of this pattern could explain, to some extent, this inconsistent finding. Furthermore, the reason for the lack of association between the seafood and alcohol dietary pattern and cognitive function may be due to reverse causality. People with cognitive impairment may be advised to change their dietary habits and food choices. In conclusion, these possibilities cannot be ruled out in our analysis.

Oil tea dietary patterns were not associated with decreased cognitive function. Previous studies have reported that high doses of oil tea intake may be associated with a low risk of abnormal HDL cholesterol (29). This study explored the relationship between oil tea intake and blood lipids by grouping oil tea intake doses into more detailed quartiles. Recent studies have suggested that the weekly dietary intake of oil tea may be associated with diabetes under different dietary patterns (22). It may be implied that more detailed investigations should be conducted to explore the relationship between oil tea dietary patterns and cognitive function.

4.1. Limitations

This study also has some limitations that must be carefully considered. First, this study is a cross-sectional study, consisting

TABLE 6 Different potential confounders entered the logistic regression model and participants adhered to the relationship between different dietary patterns and cognitive functioning.

Subgroups		Model I ^a		Model II ^b		Model III ^c		P for trend
		P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	
Vegetable and mushroom dietary pattern								
[−2.458, −0.686]	(N = 221)	ref	ref	ref	ref	ref	ref	0.017
(−0.686, −0.304]	(N = 221)	0.216	0.763 (0.496, 1.170)	0.244	0.772 (0.499, 1.191)	0.256	0.777 (0.503, 1.199)	
(−0.304, 0.399]	(N = 221)	0.086	0.683 (0.441,1.053)	0.158	0.670 (0.425, 1.050)	0.236	0.762 (0.484,1.194)	
(0.399, 7.056]	(N = 221)	0.002	0.484 (0.302, 0.769)	0.007	0.517 (0.320, 0.830)	0.033	0.578 (0.348, 0.951)	
Oil and salt dietary pattern								
[−2.3456, −0.565]	(N = 221)	ref	ref	ref	ref	ref	ref	0.713
(−0.565, −0.163]	(N = 221)	0.755	1.074 (0.687, 1.681)	0.781	1.066 (0.678, 1.680)	0.837	1.049 (0.666, 1.654)	
(−0.163, 0.344]	(N = 221)	0.885	0.967 (0.611, 1.530)	0.911	0.974 (0.611, 1.552)	0.801	0.942 (0.589, 1.505)	
(0.344, 11.417]	(N = 221)	0.457	1.186 (0.757, 1.862)	0.433	1.199 (0.762, 1.893)	0.55	1.150 (0.727, 1.824)	
Seafood and alcohol dietary pattern								
[−1.866, −0.456]	(N = 222)	ref	ref	ref	ref	ref	ref	0.444
(−0.456, −0.237]	(N = 219)	0.464	0.843 (0.534, 1.330)	0.481	0.848 (0.534, 1.342)	0.541	0.866 (0.545, 1.373)	
(−0.237, 0.192]	(N = 222)	0.863	1.039 (0.671, 1.610)	0.797	1.060 (0.679, 1.655)	0.737	1.079 (0.691, 1.687)	
(0.192, 14.337]	(N = 221)	0.814	0.944 (0.585, 1.521)	0.675	0.898 (0.542, 1.482)	0.714	0.910 (0.550, 1.503)	
Oil tea dietary pattern								
[−4.010, −0.515]	(N = 222)	ref	ref	ref	ref	ref	ref	0.042
(−0.515, −0.086]	(N = 220)	0.055	0.640 (0.405, 1.006)	0.052	0.634 (0.399, 1.002)	0.057	0.639 (0.401, 1.010)	
(−0.086, 0.46]	(N = 221)	0.929	1.020 (0.664, 1.565)	0.947	1.015 (0.658, 1.566)	0.977	1.006 (0.652, 1.553)	
(0.46, 9.162]	(N = 221)	0.176	0.729 (0.459, 1.150)	0.13	0.700 (0.439, 1.108)	0.101	0.677 (0.424, 1.076)	

^aThis logistic regression is adjusted by the following potential confounding factors: sex and ethnic.

^bThis logistic regression is adjusted by the following potential confounding factors: sex, ethnic, marital, agricultural activities, smoking, drinking, hypertension, diabetes, dyslipidemia, and BMI.

^cThis logistic regression is adjusted by the following potential confounding factors: sex, ethnic, marital, agricultural activities, smoking, drinking, hypertension, diabetes, dyslipidemia, BMI, and dietary fiber.

mainly of older Chinese adults, and therefore, it is not possible to establish a clear chronological order or to exclude the possibility of reverse causality and the existence of Neyman bias. Second, the dietary intake assessment was not performed before the cognitive function measures, and we cannot infer the existence of the resulting causality. Third, the dependence of the FFQ method on memory or cognitive performance in this study did not allow for a precise estimation of nutrient intake. Fourth, we controlled for the effects of nutrient dietary intake in the model using the residual method and VIF. Fifth, we were unable to adjust for genetically related lipoprotein E status, but we controlled for a wide range of health-related factors as potential confounders. Sixth, because we conducted a large cross-sectional epidemiological survey, the MMSE questionnaire is not clinically adequate to accurately classify cognitive impairment, and our data do not adequately support studies with a detailed classification of patients with cognitive impairment. The MMSE questionnaire can only be used to initially assess the status of cognitive function. Therefore, to obtain more precise findings, longer follow-up and larger prospective studies are necessary to explore the relationship between dietary patterns and cognitive function.

5. Conclusion

In this population-based study, the vegetable and mushroom dietary pattern showed a strong correlation with cognitive function, and this association was dose-dependent. Therefore, the vegetable and mushroom dietary pattern is the safest choice at this time. Finally, the vegetable and mushroom dietary pattern may be a protective factor for cognitive function, suggesting that more vegetables and mushroom foods should be consumed in the diet to prevent cognitive impairment.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Guilin Medical College. The

patients/participants provided their written informed consent to participate in this study.

Author contributions

RG and ZZ had full access to all of the data in the study, took responsibility for the integrity of the data, and the accuracy of the data analysis. RG drafted the manuscript and performed the statistical analysis. WP, JC, TL, KH, SX, and XT critically revised the manuscript for important intellectual content. YL obtained funding. YL and JQ supervised the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2023.1093456/full#supplementary-material>

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Nutrition, lifestyle, and cognitive performance in esports athletes

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Introduction: Electronic sports, termed esports, is a growing athletic activity in which high levels of attention and cognitive performance are required. With its increasing popularity and competitiveness, interest in strategies to improve performance have emerged. Improving esports athlete performance, namely cognitive endurance, and resilience, may lie in nutritional or lifestyle factors. The Nutrition, Vision, and Cognition in Sport Studies (IONSport) investigated nutritional and behavioral factors that can influence cognition via 3-dimensional multiple objects tracking test (3DMOT) via Neurotracker X (NTx) software. The purpose of this study was to characterize the lifestyle of high level esports athletes with detailed nutrition, sleep, and physical activity assessments, and their association to gaming related cognitive performance.

Methods: 103 male and 16 elite female esports athletes aged 16 to 35 years old completed surveys, food records, and cognitive testing sessions over 10 days. Participants were instructed to maintain their normal dietary and lifestyle habits.

Results: There were positive significant associations between average NTx scores and the following nutrients: magnesium, phosphorous, potassium, sodium, zinc, selenium, thiamin, niacin, vitamins B6 and B12, folate, cholesterol, saturated, polyunsaturated, and monounsaturated fats, omega-6 and omega-3 fatty acids, and choline. Majority of participants did not meet recommended dietary allowances (RDAs) for these micronutrients nor the recommended intakes for dairy, fruit, and vegetables. There was a significant ($p = 0.003$) positive ($r = 0.272$) association between total vegetable intake and average NTx score. There was a significant negative association ($p = 0.015$) with our final sustain session, which measured cognitive resilience, and the Stanford Sleepiness Scale score. Repeated measures analysis was done with these groups over the 18 core NTx sessions. There were significant ($p = 0.018$) differences between the two groups with those who consumed the recommended amount of protein or more performing significantly better on NTx over the 18 sessions than those that did not consume enough protein. Those who consumed the recommended intakes for riboflavin, phosphorous, vitamin B12, and selenium performed significantly better over the 18 core NTx sessions than those that did not meet the recommended amounts.

Discussion: The need for a nutrition intervention that is rich in protein, vitamins, and minerals is warranted in this population.

KEYWORDS

esports, cognitive performance, protein, micronutrients, athletes, cognition, dairy

1. Introduction

Electronic sports, or esports, are athletic activities consisting of various avenues of digital gameplay including video and personal computer (PC) modalities (1). The esports industry has been growing exponentially with an estimated global revenue of 66.6 million in 2021 (2) and an expected worldwide audience of 577.2 million by 2024 (3). Esports athletes undergo intense training leading up to competitions (4) with the potential to win prize purses of \$121 million in 2017 and \$100 million in the 2018 *Fortnite* competition season (5).

In many of the games, elevated levels of attention and cognitive performance are needed to remain competitive in the esports world. The available research on esports and cognitive performance has primarily been on action-based video games, showing that these esports athletes have a higher processing speed than the controls (6). However, a recent review showed that players of non-action-based video games did not have a significantly greater cognitive ability (7). Cognitive testing, for esports athletes, traditionally utilizes a battery of tests from the validated National Institutes of Health (NIH) Toolbox for Neurological and Behavioral Function. That is, these tests singly evaluate executive function, episodic memory, language, processing speed, and attention (8). Uniquely, task-switching tests, such as 3-Dimensional (3D) Multiple Object Tracking (MOT) integrate these skills (9) and may represent a more realistic, yet controlled testing environment (10).

With the growing esports industry and the popularity of avid gaming, keys to improving performance are crucial. As with traditional athletes, one aspect of improving performance is optimizing nutrition with a primary emphasis on neurological function rather than just skeletal muscle or cardiovascular systems. Esports athletes share many attributes with traditional athletes (11) and thus utilize similar training paradigms to those who compete in physical sports (12). Research on nutritional factors and esports athletes has been limited to individual micronutrients like creatine, or non-nutritive substances such as caffeine (13–16). Specifically, a recent study investigating ingestion of a caffeine metabolite, paraxanthine, was executed by Yoo et al. to determine its efficacy in improving cognitive performance. After supplement ingestion, cognitive performance increased in some but not all cognitive tests. Regardless, caffeine and its metabolites have been vastly studied for their performance benefits and have been found to promote an overall increase in cognitive outcomes with the consequences of diminishing returns, caffeine addiction and continued elevation of doses to achieve benefits (17).

A descriptive study that analyzed esports athletes of different ranks in Germany found that most of the athletes assessed engaged in positive health behaviors as defined by a health score, adequate physical activity, and body mass index (BMI). However, none of the groups, as defined by their ranking, consumed the recommended five servings of fruits and vegetables assessed by a single question of intake, although the professional esports athletes consumed more fruits and vegetables than any other group (18).

Nutrition is just one aspect of overall lifestyle that could benefit esports performance. Other components of lifestyle to achieve optimal performance include physical activity and sleep; however, they have not been studied extensively in high level esports athletes. A global study investigating health behaviors and esports athletes found that the majority of those sampled did not meet the World Health

Organization (WHO) guidelines (19) of 150–300 min moderate physical activity per week or 75–150 min of intense/vigorous activity per week for physical activity despite maintaining a normal BMI. They also found that most esports athletes did not smoke and consumed less alcohol than the global average. Interestingly, players in the top 10% of their gaming rank reported greater amounts of physical activity than 90% of the total esports athletes sampled (20). Esports athletes are generalized as having poor sleep due to their increased time spent gaming. This colloquialism has some truth, as a study investigating three professional esports teams demonstrated that these athletes were sleeping less than 7 h per day and had significantly increased awake time after initial sleep onset with subsequent daytime sleepiness. It is hypothesized that these professional athletes have training times that could impact their sleep opportunity (21). Everyday esports athletes could also experience the deleterious effects of lack of sleep as evidenced by Ketelhut et al. (22); however, as Peracchia and Curcio explain, there is conflicting evidence to support the impact of sleep based on results from the literature (23).

While these previous findings in esports athletes are beneficial, the importance of nutrition, physical activity, and sleep on performance in high level esports athletes is lacking. Therefore, this study aimed to observe the role of nutrition, physical activity, and sleep on cognitive training/testing. It was hypothesized that esports athletes would not be consuming a well-balanced diet, have adequate sleep habits, or meet the recommended levels of physical activity, and these factors would be associated with impairment of cognitive performance.

2. Methods and materials

2.1. Participants

Healthy males ($n=103$) and females ($n=16$) volunteered to participate in this fully remote study. All participants were classified as professional, elite, or avid esports athletes. Participants were included in this study if they were between the ages of 16 to 35 years old with acceptable vision (best-corrected vision of 20/40 or better in each eye). Participants were excluded if they had a pacemaker, had an untreated psychiatric disorder, were color blind, had a diagnosis or condition of vertigo, macular degeneration, diabetic retinopathy, glaucoma, retinitis pigmentosa, optic neuropathy, retinal vascular occlusions, strabismus, or other autoimmune disorders related to visual health. Participants completed an informed consent form before the preliminary questionnaires ensuring their eligibility for the study. All methods and study procedures were approved by the Texas A&M University Institutional Review Board for Human Subjects in research. A total of 384 participants consented; however, 186 withdrew. Additionally, 77 participants started the study but did not finish. One hundred twenty-one participants completed the study; however, two were not included in data analyses due to incomplete data (Figure 1).

2.2. Recruitment

Participants were recruited using social media platforms including Instagram, Twitter, Facebook, and Discord, and through emailing esports teams. Additionally, flyers were distributed in College Station, and Austin, Texas. Participants were prompted to fill out a Google

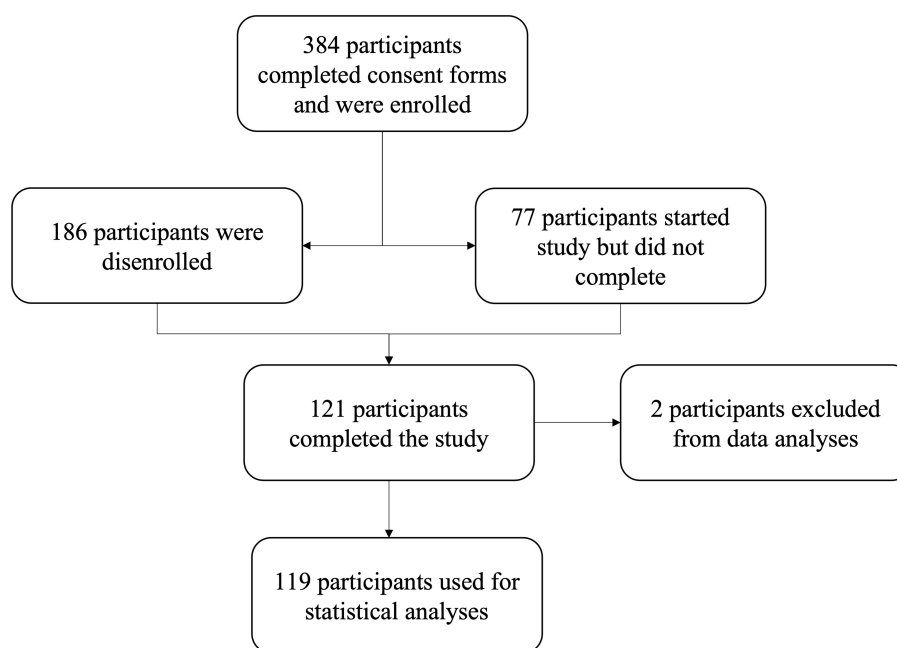


FIGURE 1
Subject enrollment data.

form, which provided emails for further communication. Consent, and assent forms, if needed, were signed. Participants were then assigned a participant ID followed by a brief informational Zoom meeting to answer questions and provide precise instructions on conducting the study.

2.3. Surveys

The following forms were completed via REDCap (an online survey and study management platform): a Gaming History Questionnaire, Demographics, Medical History, Vision Screening, Pittsburgh Sleep Quality Index (PSQI), Modifiable Activity Questionnaire and a daily pretesting survey. The Gaming Questionnaire, a survey developed by our lab team, was designed to assess participant skill levels and gaming habits. Questions included tournament rankings, gamer rank, and games played. The Demographics, Medical History, and Vision Screening surveys were used to obtain baseline health data and study eligibility. The PSQI, a questionnaire that generates a score based on individual and bed partner feedback, characterized sleep behavior and clarified sleep patterns and quality. A higher PQSI score reflected worse sleep quality (24). The Modifiable Activity Questionnaire was used to assess the frequency and duration of several types of physical activity for the past year and lifetime (25).

2.4. Nutrition tracking and monitoring

Participants were asked to continue their normal eating behaviors and physical activity throughout the study. They were instructed to complete 10 days of food records via the Automated Self-Administered

24-Hour Dietary Recall (ASA24) software, a standardized and validated tool from the National Cancer Institute (26), which allows for accurate estimation of energy and nutrient intake. In ASA24, participants were guided through a series of questions as they completed their food records using multiple passes and probes to enhance memory and recall of foods consumed and provided visual aids to determine portion sizes. At study completion, nutrition reports were created and sent to participants after being reviewed by a Registered Dietitian, and if necessary, feedback was provided.

2.5. Physical activity and sleep monitoring

The LETSCOM Smart Band was used as a wearable device that monitored steps, heart rate (HR), and sleep (LETSCOM. (2017) Smart Band ID107Plus HR). Participants were instructed to consistently wear the Smart Band on their nondominant wrist, only removing it for necessary events (e.g., showering). It was worn for at least 8 continuous days after an initial full charge. Data from the tracker was obtained from the VeryFit Pro app, which is the associated app for the tracker. Participants entered this data into their pretesting survey, which they completed prior to every cognitive training session.

2.6. Visual cognitive performance testing

During each of the 8 days of cognitive training, participants entered the following information on a pretesting survey: recent physical activity, fluid intake, most recent urine color via a validated urine color scale (27), HR, readiness to perform, body composition, Stanford Sleepiness Scale (28), and hours of sleep the previous night.

Participants completed 20 cognitive training sessions, remotely, using the Neurotracker X (NTx) 3D software program over 8 training days. Days one and eight included the baseline testing consisting of four training sessions (three core training sessions and one sustain training session) while days two through seven consisted of two core training sessions. Participants were instructed to set up their screen according to the NTx guidelines and confirm that the distance between the eyes and the display was the same as the width of the display. For example, participants using a 17-inch laptop should have their eyes approximately 17-inches away from the screen. In addition, the center of the screen remained at eye level during training. Participants conducted the testing in a dimly lit room with no distractions while wearing 3D glasses. Participants, who relied on glasses for corrected vision wore them under the 3D glasses when training.

NTx trained four aspects of perceptual-cognitive function: division of attention while tracking multiple objects, large visual field, performance at one's maximal speed threshold, and 3D visual cues. Each core training session required tracking the spatial location of four pre-identified target spheres initially highlighted from the other four. Once identified, these spheres became identical in color to the four other spheres. All eight identical spheres moved among each other at a given speed within a 3D virtual space: passing in front of or behind each other, colliding with each other or the edges of the screen, or changing directions. After six-seconds (6s) of movement, the spheres stopped, and the participant identified the four pre-identified spheres. If the subject selected all four of the correct spheres, the speed of sphere movement increased for the next 6s-trial. If one or more spheres were missed, the speed of sphere movement decreased for the next trial in a staircase pattern. Subjects performed 20 trials within a single training session obtaining a "speed threshold," which was the level at which the participant correctly tracked and selected the correct objects 50% of the time. The final speed threshold for each training session and the progression over 20 sessions were the primary outcomes of cognitive performance.

The sustain training sessions, used to measure cognitive resilience, included one 60s trial at 8s duration. Four targets would highlight and then return to the starting color. The first trial was self-paced with all targets continuously moving. At the conclusion of the session, the participant received a speed threshold score. Sustain sessions trained and assessed the participants' ability to maintain fluid attention over time and were used as a stamina assessment, which is potentially related to the ability to stay focused during game play.

2.7. Statistical analyses

Descriptive statistics were performed on all data. Initial student's t-test was performed to assess baseline differences between males and females. Correlations were performed using Spearman's rho to guide further investigation. Analyses with significant correlation ($p < 0.05$) were considered key variables and used for repeated measures ANOVA. For repeated measures, variables were recoded into new variables to generate groups for the analyses. The significance level was $p < 0.05$ for all analyses. All analyses were performed using IBM SPSS Statistics 29.

3. Results

3.1. Subjects

Three hundred eighty-four participants enrolled in the study; however, there are complete data sets for analyses on 119 participants (103 males and 16 females). A student's t-test was performed, and there were no significant differences ($p < 0.05$) between male and female baseline characteristics (Table 1).

As shown in Table 2, participants gamed an average of 6.33 days with 4.82h spent gaming in one sitting (Table 2). The most popular game type, with 96.6% of total participants playing, included those in the category of action (platformers, shooters, racing, and fighting) and action-adventure, with 71.4% of total participants playing (action games with strong storylines) (Figure 2). An example of the action game type is *Fortnite* and an example of the action-adventure game type is *Halo*.

3.2. Nutrition

Participants were instructed to maintain their normal eating habits for the study. They recorded all food and beverage intake for 10 days via ASA24. However, 4 participants included in analyses did not fully adhere to the protocol and only recorded their food and beverage intake for 1 to 9 days ($n = 4$). The average macronutrient intake of participants is shown in Table 3. The average intake over the 10 days was 1,852 kilocalories (kcal) with 79.9 grams (g.) protein, 76.2 g. fat, and 210.8 g. carbohydrates (Table 3). Moisture represented water intake and was measured in milliliters (ml.). Participants consumed 2,041.8 ml. of total water (including moisture from foods, beverages, and separate water intake).

Spearman's correlations were performed using average NTx score (speed threshold) and all micronutrient data to determine variables of interest for further investigation. Micronutrients of interest, as shown in Table 4, were determined from significant ($p < 0.05$) correlations between average NTx score and the nutrient and *a priori* analysis based on our prior work. There were significant positive associations between average NTx scores and the following nutrients: magnesium, phosphorous, potassium, sodium, zinc, selenium, thiamin, niacin, vitamins B6 and B12, folate, cholesterol, saturated, polyunsaturated, and monounsaturated fat, omega-6 and omega-3 fatty acids, and choline (Table 4). Average intakes over the 10-day period and recommended intakes were analyzed with most participants not meeting USDA guidelines for magnesium, zinc, folate, omega-6 and omega-3 fatty acids, vitamin D, and choline. Most participants exceeded cholesterol, sodium, and saturated fat recommendations (Table 4).

Spearman's correlations between these dietary factors and average NTx scores were performed and used to guide further analysis (Table 5). There was a significant ($p = 0.003$) positive ($r = 0.272$) association between total vegetable intake and average NTx score. In comparison to the Dietary Guidelines for Americans 2020–2025 participants did not meet recommendations for total vegetables, fruits, whole grains, or dairy intakes (Figure 3).

TABLE 1 Participant characteristics.

	Male				Female				p value
	N	Mean	Std. Dev	Min–Max	N	Mean	Std. Dev	Min–Max	
Age (yrs)	103	23.1	5.0	16–36	16	24.4	5.0	18–33	0.319
Height (in)	103	69.2	4.0	60.0–81.0	16	68.8	3.1	63.0–74.0	0.724
Weight (lbs)	103	187.6	59.9	97.0–420.0	16	176.5	52.6	120–300.0	0.477
BMI	103	27.5	8.4	16.0–63.9	16	26.0	6.7	18.6–39.50	0.241

* $p < 0.05$. Student's test performed assessing significant differences between male and female baseline characteristics. No significant differences.

TABLE 2 Gaming frequency of participants.

	N	Mean	Std. Dev	Min–Max
Days spent gaming per week	119	6.3	1.1	2–7
Hours spent gaming in one sitting	119	4.8	2.0	3–14

3.3. Physical activity and sleep

Participants wore an activity tracker (LETSCOM Smart Band) for each day they participated in cognitive testing resulting in 8 consecutive days of wearing the device. Average daily steps for participants were 3,941 steps with 2.64 total miles traveled per day walking (Table 6). The average resting HR was 68.6 beats per minute (bpm) with an average minimum HR of 57.5 bpm and an average maximum HR of 95.7 bpm (Table 6). Participants slept for an average of 445.2 min or 7.42 h. The average time awake after sleep onset was 9.85 min (Table 7). Sleep health was also measured by two subjective sleep questionnaires, the PSQI and Stanford Sleepiness Scale, and our pretesting survey. The average PSQI global score was 6.13. A PSQI global score greater than 5 is associated with severe or moderate sleep disturbances (24). The Stanford Sleepiness Scale measured participant alertness via a self-assessed seven-point scale with 1 being very alert and 7 being excessively sleepy. The participant was also able to select X_1 which indicated they were barely conscious due to sleepiness. For our data analysis, X was calculated as an 8 (28). The average Stanford Sleepiness Scale score was 2.77.

3.4. Visual cognitive performance testing

For 8 consecutive days, participants completed visual cognitive performance testing via NTx. On the first (day 1–baseline) and last (day 8–elevated baseline) day of the testing, participants completed three core sessions and one sustain session. During the middle days of the study (days 2 through 7), participants completed two core sessions. All sessions were completed in one sitting. Speed threshold, the level at which the participant correctly tracked and selected the correct objects 50% of the time, was determined for each session. The average speed threshold was 1.52. Additionally, a sustain score was given for each sustain trial completed. On average, the initial sustain score was 1.04 while the average final sustain score was 1.53 (Table 8). The sustain score was the speed threshold score derived from the sustain trial. Additionally, NTx reference data, which highlights the difference in performance between non-athletes, elite athletes, and

professional athletes was considered in the evaluation and showed that our participants performed with the elite group but could not reach the same level of performance as professional athletes (29).

3.5. Sleep and visual cognitive resilience

There were no significant associations between NTx speed threshold scores and thus, visual cognitive performance, and sleep health. However, there was a significant negative association ($r = -0.230$, $p = 0.015$) between the final sustain session and the Stanford Sleepiness Scale score. The sustain sessions measure cognitive resilience, and it was found that participants with an increased Stanford Sleepiness Scale score performed worse on the sustain session. Specifically, the significant negative association was in the second sustain session, although there was a downward trend in the first sustain session ($r = -0.069$). Associations of average sleep hours, as measured by a wearable device, and cognitive performance were not significant but also showed a negative association.

3.6. Physical activity and cognitive performance and resilience

There were no significant associations between physical activity metrics and cognitive performance or cognitive resilience.

3.7. Nutrient impact on cognitive performance

Protein was normalized for body weight for each participant in grams protein per kilogram of bodyweight (g/kg). Dichotomous groups were created with one group representing individuals who consumed less than 0.8 g/kg and the other group being those who consumed 0.8 g/kg or more. This protein value was used based on the USDA Dietary Guidelines for Americans 2020–2025 protein recommendations (30). Repeated measures analysis was done with these groups over the 18 core NTx sessions. There were significant ($p = 0.018$, Observed Power $\beta = 0.812$) differences between the two groups with those who consumed the recommended amount of protein performing significantly better on NTx over the 18 sessions than those that did not consume enough protein (Figure Error! Reference source not found.).

Additionally, similar methods in transforming variables into above and below the recommended intakes were used for vitamin D, riboflavin, calcium, phosphorous, vitamins B6 and B12, selenium,

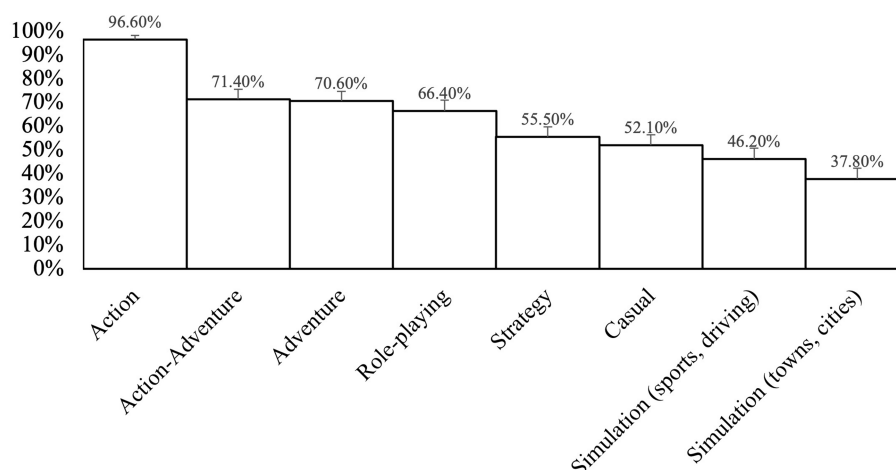


FIGURE 2
Game types played by participants. Error bars represent SEM for column percent.

TABLE 3 Macronutrient intake over 10 days.

	N	Mean	Std. Dev	Min–Max
Kilocalories	119	1852	698	521–4,136
Protein (g)	119	79.9	32.9	21.6–203.6
Total Fat (g)	119	76.2	30.8	22.1–193.8
Carbohydrates (g)	119	210.8	91.1	36.0–592.4
Moisture (mL)	119	2042	907	179–4,862

zinc, niacin, and magnesium. Those who consumed the recommended intakes for riboflavin, phosphorous, vitamin B12, and selenium all performed significantly better over the 18 core NTx sessions than those that did not meet the recommended amounts. However, this result reflects analysis of uneven groups. Nonetheless, our data shows that most participants were not consuming these nutrients adequately and this was associated to reduced performance on NTx (Table 4).

4. Discussion

In this study, we examined the role of nutrition, physical activity, and sleep on cognition related gaming performance in esports athletes. We tested the assumption that most esports athletes do not consume a nutritious, well-balanced diet. We hypothesized that cognition related gaming performance, as shown through NTx cognitive testing scores, would be enhanced if esports athletes consumed proper nutrition, were physically active, and had quality sleep habits.

The results demonstrated that the average food intake of esports athletes did not meet the standards for a nutritious, well-balanced diet based on the USDA Dietary Guidelines for Americans 2020–2025 (30). The recommendation for total caloric intake is 2,400 kcals and 2000 kcals, for males and females, respectively, for this age group (30). However, the average intake of the 10 days of food and beverage logs was 1852kcals, which was below the recommendation for both males and females. The average macronutrient intake for participants

included 79.9g. protein, 76.2g. fat, and 210.8g. carbohydrates, which exceeds the RDA of 46g. protein for females, 56g. protein for males, and 130g. carbohydrates for both males and females based on their age. However, on average, protein and carbohydrate intake were within acceptable macronutrient distribution ranges (AMDR) at 17.3 and 45.5%, while fat was slightly above the recommendation at 37% of total intake (30).

The majority of micronutrient intake did not meet the USDA Dietary Guidelines for Americans 2020–2025 (30). As shown in Table 4, the average intake of magnesium, zinc, folate, omega-6 and omega-3 fatty acids, vitamin D, and choline across the 10 days did not meet recommendations. However, there were positive significant ($p < 0.05$) associations between average NTx score and the following nutrients: magnesium, phosphorus, potassium, sodium, zinc, selenium, thiamin, niacin, vitamins B6 and B12, folate, cholesterol, saturated, polyunsaturated, and monounsaturated fats, omega-6 and omega-3 fatty acids, and choline. Micronutrients including thiamine, niacin, vitamins B6 and B12, folate, magnesium, and zinc were most closely associated with cognitive functioning and have been shown to influence cognitive performance through neurotransmitter synthesis, neuronal membrane and receptor modification, and energy metabolism (31). While key nutrients for improving performance were decreased, nutrients that may have an impact on health were increased and exceeded recommendations including average cholesterol, saturated fat, and sodium. A study analyzing cognitive performance in children found that saturated fat intake was related to longer reaction times during tasks that require more cognitive flexibility. In addition, high saturated fat and cholesterol intake were associated with higher switch costs demonstrating a decrease in working memory and reaction time (32). These negative effects on cognitive performance due to saturated fat and cholesterol could have impacted the esports athletes NTx scores, as NTx core sessions test an individual's division of attention, reaction time, and memory.

The overall lack of micronutrient intake can be attributed to esports athletes not satisfying the Dietary Guidelines for Americans 2020–2025 for consumption of dairy, fruit, vegetables, and whole grains (30). The average recommended intake of dairy, fruit, vegetables, and whole grains, according to the Dietary Guidelines for

TABLE 4 Micronutrient intake over 10days.

	N	Mean	Std. Dev	Min–Max	% Participants not meeting recommendation	Spearman’s rho	p value
Magnesium	119	239.0	96.2	59.1–626.5	95.7%	0.166	0.070
Phosphorous	119	1250.2	463.2	331.7–2937.9	11.2%	0.195	0.033
Potassium	119	1992.0	773.8	676.0–4882.9	NA	0.211	0.021
Sodium	119	3352.8	1193.8	1185.4–8341.2	16.5%	0.218	0.017
Zinc	119	10.2	4.4	2.4–26.7	69.0%	0.232	0.011
Selenium	119	113.3	46.8	33.1–278.1	4.3%	0.254	0.005
Thiamin	119	1.5	0.6	0.3–3.6	36.4%	0.203	0.026
Niacin	119	26.9	13.6	4.7–76.8	21.6%	0.224	0.014
Vit. B6	119	2.3	1.7	0.4–9.3	27.3%	0.204	0.026
Folate	119	329.3	145.0	87.2–866.5	76.9%	0.191	0.037
Vit. B12	119	5.2	3.5	0.7–20.3	13.8%	0.181	0.048
Cholesterol	119	291.5	168.6	57.4–1003.0	63.6%	0.213	0.020
Saturated fat	119	25.6	11.5	6.8–65.4	*66.1%	0.173	0.059
Monounsaturated fat	119	26.3	10.8	7.8–62.7	NA	0.252	0.006
Polyunsaturated fat	119	17.3	7.1	4.4–46.2	NA	0.277	0.002
Omega-6	119	15.3	6.4	3.8–40.9	57.0%	0.275	0.002
Omega-3	119	1.6	0.7	0.4–4.5	52.1%	0.227	0.013
Vit. D	119	3.9	4.0	0.4–25.2	97.4%	0.157	0.088
Choline	119	285.6	136.3	54.5–843.9	94.2%	0.245	0.007

*The recommendation for saturated fat is less than 10% of total kcal intake. Percent of participants not meeting recommendations is based on average participant caloric intake. *NA indicates there is no current USDA recommendation for this nutrient. Recommendations are based on USDA Dietary Guidelines for Americans 2020–2025. Spearman’s rho is association to average Neurotracker scores.

TABLE 5 Food groups, fiber, caffeine, and added sugars.

	N	Mean	Std. Dev	Min–Max	Recommendation	Spearman’s Rho	p value
Total Dairy (cup eq.)	119	1.4	0.8	0.1–4.7	3	0.065	0.483
Total Fruit (cup eq.)	119	0.4	0.6	0.0–3.4	2	0.065	0.478
Total Vegetable (cup eq.)	119	1.1	0.6	0.2–3.5	3	0.272	0.003
Whole grains (oz. eq.)	119	0.7	0.9	0.0–3.7	8	−0.048	0.603
Fiber (g)	119	13.1	5.6	3.6–37.1	14 g/ 1,000 kcal	0.177	0.054
Caffeine (mg)	119	74.6	90.7	0.0–520.3	NA	−0.001	0.990
Added Sugar (g)	119	14.1	10.8	0.3–54.0	NA	0.166	0.070

Americans is 3.0 cups eq, 2.0 cups equivalents (eq.), 3.0 cups eq., and 8.0 oz. eq. respectively (30). As shown in Table 5, average total dairy, fruit, vegetables, and whole grains intake were, respectively, 0.4 cups eq., 1.1 cups eq., 0.7 oz. eq., and 1.4 cups eq., which are all much lower than the daily recommendations. This finding is consistent with a prior study that investigated esports athletes where they also did not meet the recommended servings of fruits or vegetables (18). However, as seen in Table 4, there is a significant ($p = 0.003$), positive ($r = 0.272$) association between total vegetable intake and average NTx score indicating that more nutritious foods improve cognitive performance. Prior research has found caffeine doses between 32 to 300 mg enhance various facets of cognitive performance including attention, vigilance, and reaction times (33–35). However, our results, as shown in Table 5, did not find a significant association between caffeine intake and

cognitive performance. This could be due to the average caffeine intake being on the lower end at only 74.6mg, which is about the equivalent of one cup of coffee. The esports athletes who habitually consume this lower dose of caffeine may not achieve performance benefits. The decrease in caffeine-induced benefits at the ergogenic level has been seen in athletes who are habitual caffeine users and, increasing caffeine doses has been required to maintain ergogenic effects (36), which may translate to maintaining cognitive performance enhancements as well.

Sedentary behavior is often linked to increased screen-time activities. Esports athletes have been assumed to have more of a sedentary lifestyle due to their increased hours spent on gaming. As seen in Table 2, the average days spent gaming per week was 6.3 days with 4.8 h spent gaming per day. When examining physical activity,

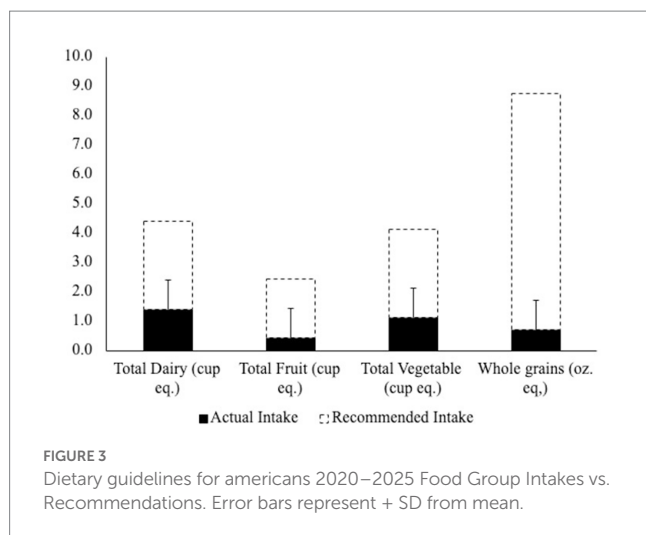


TABLE 6 Physical Activity as measured by a wearable device.

	N	Mean	Std. Dev	Min-Max
Avg distance (miles)	114	2.6	4.5	0.1–35.4
Avg steps	114	3,941	2,817	336–21,173
Avg calories	113	4740.1	9223.4	15.2–9223.4
Avg heart rate (bpm)	114	68.6	13.9	44–146
Max heart rate (bpm)	114	95.7	36.8	53–193
Min heart rate (bpm)	114	57.5	9.6	3–100

TABLE 7 Sleep health as measured by a wearable device.

	N	Mean	Std. Dev	Min-Max
PSQI global score	119	6.1	2.7	1.0–14.0
Stanford sleepiness scale	114	2.8	1.0	1.0–6.0
Avg sleep minutes	112	445.2	76.0	225.0–682.0
Avg awake minutes	112	9.9	3.2	1.0–13.0

which was measured through the participant's wearable device, the average amount of steps the esports athletes took per day was 3,941 steps, and an average distance of 2.64 miles per day. This is much lower than the Centers for Disease Control's (CDC) recommendation of 10,000 steps per day, the equivalent of about 5 miles (37). In addition, Table 6 shows that the average maximum HR was only 95.7 bpm, indicating that most esports athletes were not participating in moderate to vigorous intensity physical activity per day as the target HR for moderate-intensity activity is between 64–76% of max HR (37). This would have been 126–150 bpm for the male esports athletes and 125–149 bpm for the female esports athletes based on their average age. These data suggest that the esports athletes were physically inactive, which may have negatively affected their NTx scores. Chang et al. found a dose–response relationship between resistance exercise and cognitive performance where high-intensity exercise improves the speed of processing and moderate intensity exercise improves executive function (38). Another study evaluating the effects of exercise on gaming performance of *League of Legends*, a popular video game, found that 15 min of high-intensity interval

TABLE 8 Neurotracker X Scores.

	N	Mean	Std. Dev	Min-Max
Average NTx Score	119	1.52	0.43	0.34–2.75
Low NTx Score	119	0.83	0.39	0.01–2.20
Top NTx Score	119	2.18	0.58	0.73–4.71
Sustain 1 Score	118	1.04	0.44	0.12–2.45
Sustain 2 Score	115	1.53	0.53	0.19–3.41

training exercise prior to playing the video game enhanced performance as the video gamers improved their accuracy and capacity to eliminate targets (39).

Similarly, the increased hours esports athletes spend on gaming have been assumed to negatively impact their sleep quality. Esports athletes have been associated with having non-traditional sleep characteristics and, in fact, have been found to have shorter overall sleep durations of about 7 h, including a later sleep onset and sleep offset than traditional athletes (40). This is consistent with the current study as the participants slept for an average of 7.42 h (Table 7). In addition, the average global PSQI score, which was used to examine overall sleep quality, was 6.1, indicating poor sleep, as a PSQI global score > 5 is associated with severe or moderate sleep disturbances (24). Furthermore, as shown in Figure 5, there was a negative association ($p=0.015$) with the final sustain session and the Stanford Sleepiness scale (28). As the score increased on the Stanford Sleepiness Scale, the participants' sustained session score decreased. Sleep restriction was shown to impair various aspects of cognitive performance including working memory and attention-based tasks such as sustained attention, visuo-spatial attention, serial attention, reaction time, and subtraction tasks (41). These executive functions are crucial to being a successful esports athlete as they need to have quick reaction times and sustained attention, so improving sleep health will be instrumental in enhancing esports performance.

It was expected that esports athletes would have higher NTx scores than the average individual due to the cognitive skills associated with being a successful esports athlete. However, as displayed in Table 8, the NTx score, which was the speed threshold, was 1.52. The speed threshold refers to the participant correctly tracking and selecting the object 50% of the time. When compared to NTx standards, as shown in Figure 6, the esports athletes' average score was not vastly different from elite-amateurs and was lower than professionals. This finding coincides with a study investigating the cognitive profile of experienced video game players where it was found that they had higher processing speeds, task switching abilities, and responded faster on a Stroop test, displaying a quick reaction time, but made significantly more errors compared to individuals with little or no video game experience. This could be due to esports athletes employing the use of pre-firing, a strategy used in action games, where they react to a stimulus prior to its occurrence, which may lead to an increased error rate (6).

Specific macro and micronutrients and their relationship to cognition related gaming performance were also analyzed. The results demonstrated, as seen in Figure 4, that participants who consumed 0.8 g/kg protein or more performed better during the 18 NTx core sessions than those who consumed less protein. This could in part be due to protein intake enhancing working memory and episodic memory, especially when task demands are increased (42). In

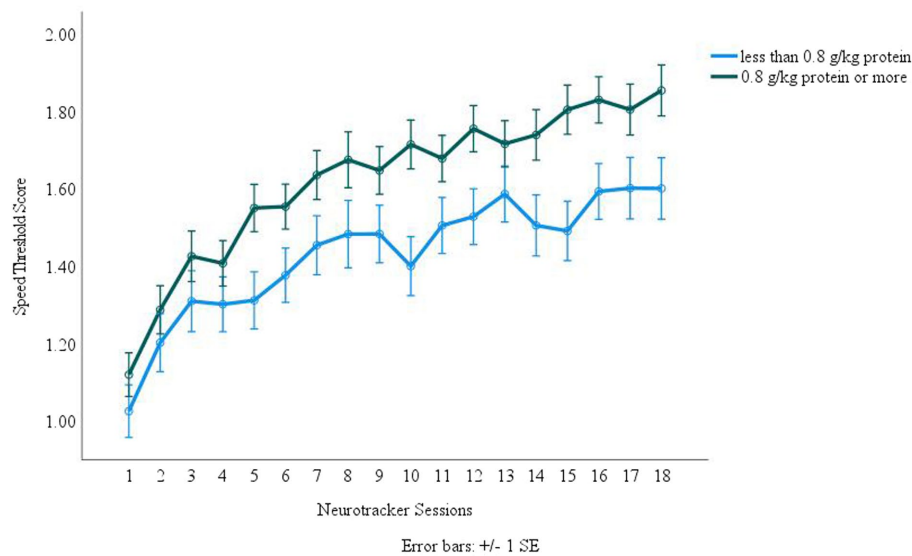


FIGURE 4
Protein Intake and Neurotracker Performance. Error bars represent ± 1 SEM.

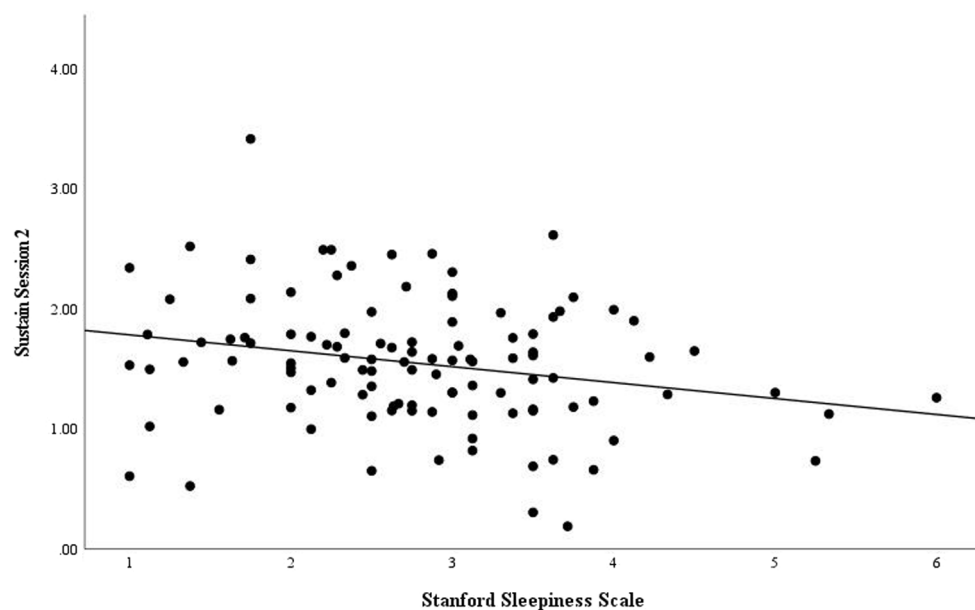
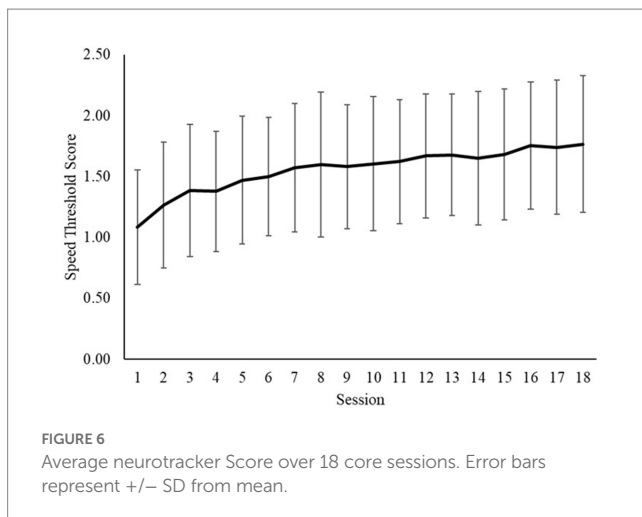


FIGURE 5
Association of cognitive resilience and Stanford Sleepiness Scale.

addition, daily tyrosine intake has been positively associated with fluid intelligence and working memory (43). A study investigating macronutrient composition and risk of mild cognitive impairment found that individuals who had a high percent protein intake were at reduced risk for mild cognitive impairment (44). Similarly, a review on protein intake and cognitive performance by van de Rest, van der Zwaluw, & de Groot demonstrated that there is an established link between protein intake and cognition (45). Furthermore, improvements to cognitive performance and function come from the amino acids found in proteins which support the genesis and turnover of neurotransmitters (46).

It was also observed that participants who consumed the recommended intakes of riboflavin, phosphorous, vitamin B12, and selenium all performed better than those who did not meet the recommended intakes. Prior studies have also found similar positive effects between riboflavin, vitamin B12, and selenium and cognitive function; however, this research has mainly been done on an elderly or diseased population. For example, riboflavin has been shown to improve multi-dimensional cognitive functioning in middle-aged and elderly people (47) and vitamin B12 has been found to positively affect memory functioning scores that were tested using the Wechsler Memory Scale-Revised in mild cognitive impairment patients (48). Previous research has



also linked vitamin B12 deficiency to possible cognitive decline and cognitive function although higher quality evidence is needed (49). Additionally, in a study investigating older Chinese adults, high selenium intake was linked to having a high global cognition score and better memory, possibly due to selenium's antioxidative properties (50). On the other hand, high phosphorus intake has typically been associated with impaired cognitive functioning. One study using middle-aged participants found that an increased phosphorus intake was associated with a lower composite cognitive score (51). Similarly, another study investigating United States (US) veterans found that increased dietary phosphorus intake led to increased serum phosphorus levels, which was associated with a greater risk of incident dementia (52).

Our study was limited in the use of a 100% remote platform, so we could not assess true compliance to study protocols. In addition, participants may not have been entirely truthful or accurate when completing the surveys/questionnaires. However, this study provided us the opportunity to capture sleep, physical, dietary habits, and cognitive performance in a group of elite esports athletes from across the US that would have otherwise been inaccessible. Another limitation was the use of food records as it is known that this method results in underreporting of intake. Despite this, ASA24 is a clinically validated tool developed from the NIH that uses images for portion sizes and a second pass technique for interviewing thus mitigating underreporting. NTx core session scores were increased with higher protein intake as seen in Figure 5. NTx scores were expected to increase from consumption of adequate nutrients, but as seen in Table 4, most participants did not meet recommended intakes, which could have played a role in the scores being lower than expected. Additionally, sleep quality as measured by the Stanford Sleepiness Scale, had an impact on cognitive resilience with those with worsened quality being associated with worsened performance. Furthermore, esports athletes could improve their performance through lifestyle modifications.

Our results indicate that sufficient protein and certain micronutrients are associated with improved cognitive performance in esports athletes. In addition, physical activity levels and sleep habits are not optimized in this population which if corrected may help to further optimize cognitive performance. It remains to be determined how a nutrition intervention that is high in protein and rich in vitamins and minerals could attenuate cognitive fatigue rather than solely improve performance. For example, dairy has a promising

potential for improving cognitive performance in esports athletes due to its balanced and varied food matrix (53). In particular, bovine milk is rich in vitamins A, D, B, and C and minerals including calcium, magnesium, and potassium (54) which could make it a promising nutritional intervention for esports athletes (55–57). Total dairy, protein, vegetable, fruit, and whole grain intake, all of which are components of a well-balanced diet were not sufficiently consumed in this population, to correct macro and micronutrients deficiencies that essential for optimizing cognitive functioning. However, much work is needed to identify specific nutrients, foods and food matrices that can be used to optimize high level cognitive performance applicable to esports athletes as well as many other high-level performers.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Texas A&M University Institutional Review Board for Human Subjects in research. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SR is responsible for study design and conception, data analysis, and revision of the manuscript. KB and SR supervised all data collection, analysis, and manuscript editing. JG executed study experiments, supervised study activities, analysed data, and wrote the draft of the manuscript. LA is responsible for study recruitment and the draft of the discussion along with final edits. JG, LA, SS, MC, and JC collected data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Novel approach to investigate the association between type 2 diabetes risk and dietary fats in a dietary pattern context: a scoping review

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The effect of dietary fat on type 2 diabetes (T2D) risk is unclear. A *posteriori* dietary pattern methods have been increasingly used to investigate how dietary fats impact T2D risk. However, the diverse nutrients, foods and dietary patterns reported in these studies requires examination to better understand the role of dietary fats. This scoping review aimed to systematically search and synthesize the literature regarding the association between dietary patterns characterized by dietary fats and T2D risk using reduced rank regression. Medline and Embase were searched for cross-sectional, cohort or case-control studies published in English. Of the included studies ($n = 8$), five high-fat dietary patterns, mostly high in SFA, were associated with higher T2D risk or fasting glucose, insulin and Homeostasis Model Assessment (HOMA) levels. These were mostly low-fiber ($n = 5$) and high energy-density ($n = 3$) dietary patterns characterized by low fruit and vegetables intake, reduced fat dairy products and higher processed meats and butter intake. Findings from this review suggest that a *posteriori* dietary patterns high in SFA that increase T2D risk are often accompanied by lower fruits, vegetables and other fiber-rich foods intake. Therefore, healthy dietary fats consumption for T2D prevention should be encouraged as part of a healthful dietary pattern.

KEYWORDS

dietary patterns, dietary fats, type 2 diabetes, reduced rank regression (RRR), review

Introduction

Type 2 diabetes (T2D) is a chronic condition characterized by elevated glucose levels, or hyperglycaemia (1). If left untreated, chronic hyperglycaemia can lead to an increased risk of cardiometabolic complications and early death (1). In the past 30 years, the prevalence of T2D has increased from approximately 108 million to 422 million adults worldwide (2). The projections estimate that by 2045, 700 million individuals will develop diabetes (3). Therefore, understanding how T2D progresses is critical for developing preventative methods to lower its incidence worldwide.

An unhealthy diet is considered one of the key risk factors for the onset of T2D (4). Whilst the benefits of managing some dietary risk factors, such as carbohydrate intake, have been well researched (5) the role of dietary fats is less clear and this is likely due to the varying sources of fat in our diet (6). Higher polyunsaturated fatty acids (PUFA) intake has been linked to lower fasting blood glucose levels (7) and reduced T2D incidence (8, 9),

whilst higher saturated fatty acid (SFA) intake has been linked to the development of insulin resistance (10). However, SFA intake may also differentially affect the risk of developing T2D, depending on its food source; SFA from meat may increase risk (11, 12) whereas SFA from dairy may decrease risk of T2D (13). Moreover, some studies also report no association between dietary fat type and risk of T2D (6, 14). The majority of studies to date have focused on a single nutrient approach (10, 14–16). However, as foods and nutrients are not eaten in isolation, understanding of the role of dietary fat within the context a dietary pattern is warranted.

There are three main delineations of dietary pattern methodologies: *a priori*, empirical and *a posteriori* (16, 17). The *a priori*, or hypothesis-oriented approach, derives dietary patterns using a pre-defined criterion (16) whilst empirical methods use a data-driven approach to create dietary patterns according to the combinations of foods consumed in a specific population (16). Extensive research has been conducted to investigate the association between *a priori* and empirical dietary patterns and T2D (18, 19). However, the use of *a posteriori* dietary pattern methods to understand T2D is only beginning to be explored.

A *a posteriori* methods, such as reduced rank regression, combine both hypothesis-oriented and data driven approaches to create patterns of food intake according to pre-defined response variables (16, 20, 21). The response variables selected for use in reduced rank regression are known to be on the causal pathway between diet and the health outcome of interest, which can include biomarkers or nutrient intake. While increasing research has used reduced rank regression to investigate the association between diet and T2D using biomarkers as response variables (22–25) few studies have used nutrient intakes as response variables (16, 26–28). An understanding of how dietary fats, within the context of overall dietary patterns, impact on T2D risk is important for advancing T2D research beyond a single nutrient-focus. Therefore, the aim of this scoping review was to systematically search and synthesize the literature regarding the association between dietary fats and risk of T2D while using reduced rank regression.

Methods

Eligibility criteria

This scoping review included publications from human observational studies (cross-sectional and cohort studies) from inception to November 2021. To be eligible for inclusion, studies were required to include information on: (i) *a posteriori* dietary patterns that used dietary fat [saturated fat (SFA), monounsaturated fat (MUFA), polyunsaturated fat (PUFA), total fat, unsaturated fat, omega-3, omega-6, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), arachidonic acid (AA)] as at least one of the response variables regardless of the other response variables (could be nutrient or biomarker); and (ii) T2D risk, insulin resistance, homeostasis model assessment of insulin resistance (HOMA-IR), fasting insulin, fasting glucose, glycated hemoglobin (HbA1c), oral glucose tolerance test (OGTT) or gestational diabetes as an outcome. As a preliminary search identified few studies that investigated T2D as an outcome, the authors have included related outcomes to provide a more

comprehensive overview of the research question. All population groups were included. Non-English publications were excluded. This scoping review was undertaken in accordance with Preferred Reporting Items for Systematic Reviews and Meta-analysis—Extension for Scoping Reviews (PRISMA-ScR) (29).

Search strategy

Information sources

Two electronic databases (Medline and Embase) were searched in November 2021. An updated search was run in July 2022.

Literature search

The search strategy was developed and piloted in consultation with a librarian. It involved combining two search themes using the Boolean operator “and”, while only searching titles and abstract. The first theme was (“reduced rank regression” or “rrr”) and the second theme was (“type 2 diabetes” or “T2D” or “T2DM” or “type 2 diabetes mellitus” or “T1DM” or “insulin resistance” or “insulin resistant” or “IR” or “impaired glucose tolerance” or “pre-diabetes” or “glucose intolerance” or “impaired fasting glucose” or “gestational diabetes” or “GDM”). To identify all possible studies that used reduced rank regression, “fat” and “dietary fats” was not used as a search term. Instead, these terms were included during the screening of titles and abstracts by two independent reviewers.

Selection of sources of evidence

The search results were exported to Covidence. Initial screening of articles titles and abstracts was performed by two independent reviewers (BB and LM) according to the inclusion and exclusion criteria. If both reviewers agreed on the suitability of the article, it was moved to full text screening and again reviewed independently by two reviewers. If there was any disagreement, a third reviewer (KML) was consulted. Duplicates were removed via the in-built function in Covidence.

Data extraction

Data extraction was performed by using a pre-piloted Excel template. The following information was extracted: (i) study design, sample size; (ii) dietary assessment method; (iii) dietary patterns: food groups (predictors) and nutrient intakes (response variables); (iv) outcomes; (v) adjustments made in the analysis; (vi) main results. In studies investigating multiple outcomes, only the outcomes listed in the eligibility criteria were summarized. Where response variables included a combination of nutrient intakes and other measures, these were also extracted.

Synthesis of results

A narrative approach was used to summarize the main findings of included studies. Results were presented by grouping included studies based on their outcome to better describe the level of evidence for T2D and related outcomes and highlight any gaps in knowledge.

Results

The initial search identified a total of 270 records. After removal of duplicates, titles and abstracts of 207 records were screened and 188 were excluded for not meeting our pre-defined inclusion criteria. Full texts for 19 reports were screened. Of these, 11 articles were excluded: $n = 5$ exposure didn't match inclusion criteria (reduced rank regression response variable didn't include dietary fat), $n = 4$ did not have a full-text available, $n = 1$ was a duplicate of another study and $n = 1$ had an ineligible study design (meta-analysis). In total, eight studies were deemed eligible and were included in the present review (Figure 1).

Study characteristics

The description of the studies included in this scoping review is presented in Table 1. Six studies had a prospective design (16, 26, 28, 30, 32) and two were cross-sectional (27, 33). All studies

included both male and female participants, except for one study, which investigated the odds of developing GDM in pregnant women (33). Sample sizes ranged from 249 (33) to 120,343 (31). Three studies were conducted in the United Kingdom (26, 28, 31) and others were conducted in Germany (16) Sweden (32) Australia (30) United States (33) and Malaysia (27). Most of the studies ($n = 6$) were conducted in adults (aged 18 to 69 years), except for two in adolescents (27, 30). One study was conducted in adults with T2D (16) and one in severely obese adults (32). All other studies ($n = 6$) were conducted in healthy populations (26–28, 30, 31, 33).

Dietary intake assessments

Two studies used food frequency questionnaires (FFQ) to collect dietary data (16, 27) two used semi-quantitative FFQs (30, 32), two used a 24-hour dietary assessment tool (Oxford WebQ) (28, 31) one used 24-hour dietary recalls (28, 31, 33) and one used food diaries (Table 1) (26).

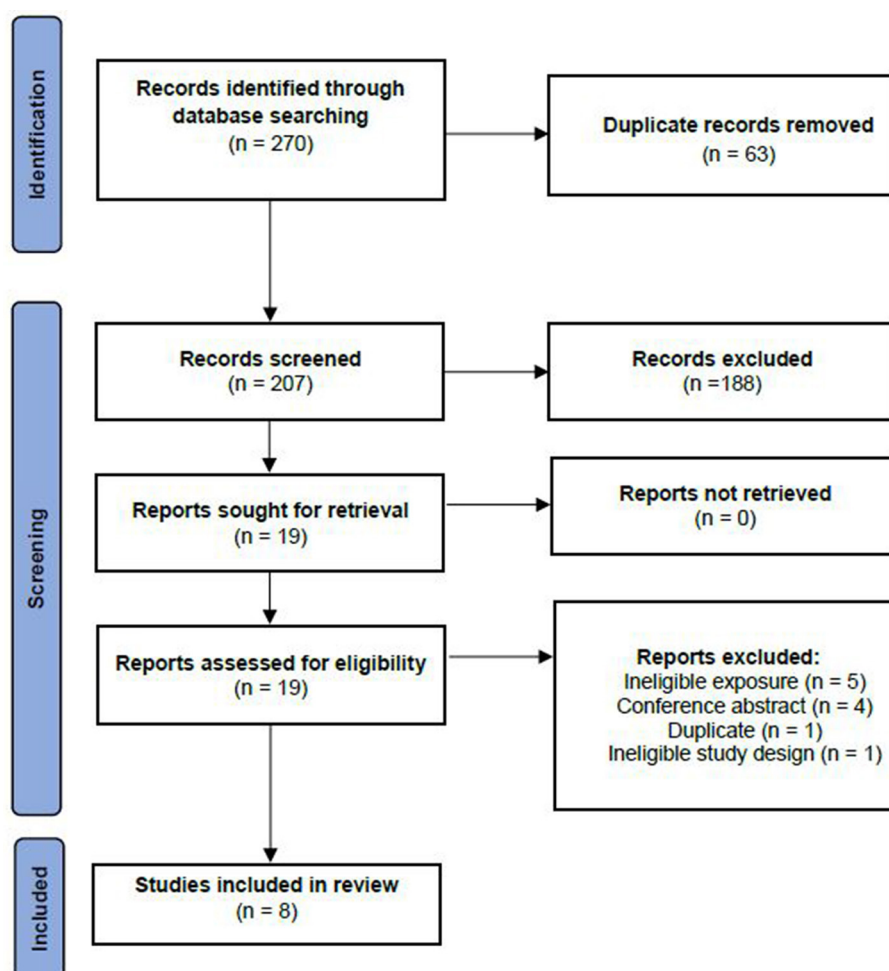


FIGURE 1

Study selection for inclusion in the systematic review based on the preferred reporting items for systematic reviews and meta-analyses —extension for scoping reviews (PRISMA-ScR).

TABLE 1 Summary of studies investigating the association between *a posteriori* dietary patterns (reduced rank regression) and T2D and related outcomes [insulin resistance, HOMA-IR, fasting insulin, fasting glucose, oral glucose tolerance test (OGTT) and gestational diabetes].

References, Country	Study design, population	Dietary assessment method	Dietary patterns		T2D outcomes	Adjustments	Results
			Predictors	Response variables			
Pastorino et al. (26) United Kingdom	Prospective cohort, n at baseline = 1180, 55.5% female Age: 16-35y at baseline Follow-up: up to 11 years	Five-day food diary	45 food groups (g/day)	Dietary fiber density (g/1000kcal), Glycemic Index (units) Total dietary fat density (g/1000kcal)	Doctor diagnosed self-report T2D (yes/no; binary)	Demographic characteristics: Socioeconomic position, education Dietary intake: energy intake, energy misreporting Lifestyle risk factors: smoking, physical activity, medications for hypertension and dyslipidemia, BMI and waist circumference	An increasing trend in OR for T2D with increasing quintile of DP z-scores in women. Highest z-scores quintiles had: OR: 5.45, 95% CI: 2.01, 14.79
Hoffman et al. (16) Germany	Prospective cohort, n at baseline = 193 (cases of T2D) and 385 (controls) Each case matched by age and sex to 2 controls Follow-up: up to 3 years Age: Men 40 – 65y and women 35 – 64y at baseline	FFQ	49 food groups (g/day)	PUFA:SFA, dietary fiber (g/day), magnesium intake (g/day) alcohol (g/day)	Self-reported T2D development (yes/no; binary)	Demographic characteristics: age, sex, education Dietary intake: total energy intake Lifestyle factors: BMI, waist-hip ratio, sports activity, smoking status	DP was associated with lower risk of T2D. Relative risk from highest quintile: 0.49(95% CI: 0.25, 0.94)
Appannah et al. (30) Australia	Prospective cohort study, n at baseline = 1,605, 49% female Age at baseline: 14y Follow-up: up to 3 years	2 Semi-quantitative FFQ	47 food groups (g/day)	Dietary energy density (kJ/g), fiber density (g/MJ), percentage of energy from total fat (%)	Serum Glucose (mmol/L; continuous), Insulin (%; continuous) and HOMA (%; continuous)	Demographic characteristics: age Dietary intake: energy misreporting Lifestyle factors: smoking status, physical fitness, BMI z-scores	A 1 SD unit increase in DP z-score was associated with higher fasting glucose in boys and higher insulin and HOMA in both boys and girls. Glucose: 0.04 (95% CI: 0.01, 0.08) Insulin: 3% (95% CI: 1%, 7%) HOMA: 4% (95% CI: 1%, 7%)
Brayner et al. (28) United Kingdom	Prospective cohort study, n at baseline = 16,523, 53.2% female Age: 40-69y at baseline Follow-up: mean 6.3 years	≥2 dietary assessment (Oxford WebQ)	48 food groups (g/day)	MUFA (%E), PUFA (%E), SFA (%E)	Doctor diagnosed self-reported T2D (yes/no; binary)	Demographic characteristics: age, sex, Townsend deprivation index Dietary intake: energy misreporting Lifestyle factors: physical activity, smoking status, BMI, blood pressure medication use and family history	The DPs were NS associated with T2D.

(Continued)

TABLE 1 (Continued)

References, Country	Study design, Population	Dietary assessment method	Dietary patterns		T2D outcomes	Adjustments	Results
			Predictors	Response variables			
Gao et al. (31) United Kingdom	Prospective cohort study, n at baseline = 120,343, 56.5% female Age: 40–69y at baseline Follow-up: mean 8.4 years	≥2 dietary assessment (Oxford WebQ)	50 food groups (g/day)	Dietary energy density (kJ/g) SFA (%E) free sugars (%E) fiber density (g/MJ)	Diabetes incidence – from hospital admission registries (yes/no; binary)	Demographic characteristics: ethnicity, Townsend deprivation index, education Dietary intake: energy intake Lifestyle factors: smoking status, physical activity, family history (yes/no: diabetes, hypertension, cardiovascular disease, high cholesterol), menopause, BMI	A DP characterized by high intakes of butter, low-fiber bread, sugars and preserves, chocolate and confectionary, was associated with T2D. HR: 1.09, 95% CI: 1.06, 1.12. The DP characterized by high intake of sugar-sweetened beverages, fruit juice, table sugars and preserves and low intake of high-fat cheese and butter was NS associated with T2D.
Emi et al. (27) Malaysia	Cross-sectional study, n = 335, 68% female Age: 13y	FFQ	13 food groups (g/day)	Dietary energy density (kJ/g), fiber density (g/MJ), percentage of energy from total fat (%E) and from free sugars (%E)	Serum High HOMA (4.0 unit; binary), high serum insulin (25.0uIU/mL; binary), high blood glucose (5.6mmol/L; binary)	Demographic characteristics: sex, school location, mother's educational level Dietary intake: energy misreporting Lifestyle factors: physical activity, and BMI z-score	The identified DPs were NS associated with high HOMA, high serum insulin or high glucose.
Johns et al. (32) Sweden	Prospective cohort study (in severely obese individuals), n at baseline = 6,897 (for dietary patterns creation), females n = 4,264 and n = 2,307 (for longitudinal analysis), females n = 1,447 Follow-up: mean 10 years	Semi quantitative FFQ	39 food groups (g/day)	Dietary energy density (kcal/g), fiber density (g/kcal), percentage of energy from SFA (%E)	Serum Blood glucose (mmol/L; continuous), Serum insulin (mmol/L; continuous)	Demographic characteristics: sex, age, education level Lifestyle factors: physical activity, smoking status, medications for blood pressure, lipid reducing or diabetes, baseline concentrations of the outcome (i.e. blood glucose or serum insulin)	The DP z-scores were NS with blood glucose, but were associated with higher serum insulin Insulin: beta coefficient: 1.22, $p < 0.001$
Shin et al. (33) United States	Cross-sectional study (in pregnant women), n = 249 Age: <25y: 6%, 25–29y: 43.6%, 30–34y: 35.2%, ≥35y: 15.2% Age: 16–41y	24-hour recall	28 food groups (g/day)	Pregnancy BMI (kg/m ²), dietary fiber (g/day), PUFA + MUFA: SFA	Gestational diabetes mellitus (yes/no; binary) defined by fasting plasma glucose level 5.1 mmol/L before 24 weeks of gestation	Demographic characteristics: age, race/ethnicity, family poverty income ratio, education, marital status Dietary intake: energy intake Lifestyle factors: physical activity, pregnancy BMI and gestational weight gain	Highest tertile of “high in refined grains, fats, oils and fruit juice” pattern had higher odds of GDM (OR: 4.9; 95% CI: 1.4, 17.0). Highest tertile of “high nuts, seeds, fat and soybean, low milk and cheese” pattern had higher odds of GDM (OR: 7.5; 95% CI: 1.8, 32.3). Highest tertile of “high added sugar and organ meats, low fruits, vegetables and sea foods” pattern had higher odds of GDM (OR: 21.1; 95% CI: 4.0, 109.8)

DP, dietary patterns; GDM, gestational diabetes mellitus; MUFA, monounsaturated fat; NS, not significant; PUFA, polyunsaturated fat; SFA, saturated fat; %E, percentage from total daily energy intake.

Dietary patterns

In terms of dietary pattern predictor variables, food groups were presented in grams per day in all studies, with the number of food groups ranging from 13 (27) to 50 (Table 1) (31). All studies included a rationale for food groups creation. Detailed information on the included food groups is presented in Table 2. Regarding dietary pattern response variables, three studies included a variable for total fat intake: two as percentage from total energy intake (%E) intake (27, 30) and one as fat density (g/1000kcal) (26). Two studies included %E from SFA (29, 33) and one used %E from SFA, PUFA and MUFA (25). Two studies used a ratio of individual dietary fats: one used PUFA:SFA (16) and one used MUFA+PUFA:SFA (33). Seven of the eight included studies used other dietary components in addition to dietary fat as response variables. This included dietary fiber density (g/MJ) (26, 27, 30–32), fiber intake (g/day) (16, 33) glycemic index (%) (26) magnesium intake (g/day) (16) alcohol intake (g/day) (16) %E from free sugars (27, 31) with one study including an anthropometric component [Body Mass Index (BMI kg/m²)] (33). One study included only dietary fats as response variables (28). As summarized in Table 2, each study reported results for multiple dietary patterns. One study reported results for all dietary patterns generated (33). Four studies included dietary patterns that explained as much of the response variable as possible, using subjective cut offs in explained variation ranging from 10 to 20% (26, 28, 30, 31). Three studies only included one of the dietary patterns generated (16, 27, 32).

Type 2 diabetes and related outcomes

Four studies investigated T2D incidence (16, 26, 28, 31), two studies investigated associations with HOMA, fasting insulin and glucose levels (27, 30), one study investigated associations with fasting insulin and glucose levels (32) and one study investigated associations with GDM (Table 1) (33).

Adjustments

All included studies adjusted for demographic characteristics, dietary intake and/or lifestyle characteristics (Table 1). All studies adjusted for sex, age, socioeconomic position, education or ethnicity (16, 26–28, 30–33). Seven studies adjusted for either total energy intake or energy intake misreporting (16, 26–28, 30, 31, 33). A combination of some lifestyle factors such as physical activity, BMI, smoking, family history of T2D and/or hypertension and waist to hip ratio were adjusted for in all studies (16, 26–28, 30–33).

Dietary patterns and type 2 diabetes

Four of the eight studies were prospective studies examining T2D incidence (Table 1). Of these, two studies identified a high-saturated fat dietary pattern associated with higher risk of T2D (26). These studies ($n = 2$) reported lower intake of foods such as fruit and vegetables (Table 2). Pastorino et al. (26) identified a

high-fat, high-GI, low-fiber dietary pattern, characterized by high intake of butter, animal fat and processed meat and low intake of fruits and vegetables, that was associated with higher odds of developing T2D in women (OR: 5.45; 95% CI: 2.01 to 14.79), but not in men. Hoffman et al. (16) generated a low PUFA:SFA ratio and magnesium and high alcohol and fiber pattern, which was significantly associated with lower risk of T2D (relative risk of highest vs lowest quintile: 0.49, 95% CI: 0.25, 0.94). Brayner et al. (28) aimed to investigate the prospective associations between fat-derived dietary patterns and obesity, abdominal obesity and T2D incidence. Of the two dietary patterns investigated, a high SFA and low PUFA and MUFA dietary pattern was associated with higher obesity and abdominal obesity (OR: 1.24, 95% CI: 1.02, 1.45; and OR: 1.19, 95% CI: 1.02, 1.38, respectively), but not with T2D. Gao et al. (31) investigated two dietary patterns. Of these a dietary pattern associated with high intake of chocolate, confectionary, butter, low-fiber bread and low intake of fruits and vegetables (high energy density, SFA, and free sugars and low fiber density pattern) was associated with higher risk of T2D (HR: 1.09, 95% CI: 1.06, 1.12).

Dietary patterns and type 2 diabetes-related outcomes

Three of the eight studies investigated either prospective ($n = 2$) or cross-sectional ($n = 1$) associations with either HOMA, fasting insulin and/or glucose (Table 1). Of these, two studies identified a high-saturated fat dietary pattern associated with higher levels of T2D biomarkers such as fasting insulin, glucose and HOMA (30, 32). These studies reported lower intake of fiber-rich foods such as fruit and vegetables (Table 2). Appannah et al. (30) identified that an energy dense, high-fat, low fiber dietary pattern was associated with 0.04 mmol/L (95% CI: 0.01, 0.08) higher fasting glucose in boys, 3% (95% CI: 1%, 7%) higher insulin and 4% (95% CI: 1%, 7%) higher HOMA in both boys and girls. In contrast, Emi et al. (27) determined that a dietary pattern high in sugar, fiber, high dietary energy density and low fat, and characterized by high intake of sugar sweetened beverages, sweets and low intake of cereal and meat, was not associated with any T2D markers. Johns et al. (32) identified that an energy-dense, low-fiber and higher saturated fat dietary pattern characterized by higher intake of foods such as chocolate, low-fiber bread, cheese and fast food was not associated with blood glucose, but was associated with higher insulin levels (beta coefficient: 1.22 ± 0.17 , $p < 0.001$). Lastly, Shin et al. (33) identified three dietary patterns, “high refined grains, fats, oils and fruit juice”, “high nuts, seeds, fat and soybean; low milk and cheese”, and “high added sugar and organ meats; low fruits, vegetables and seafood”, which were associated with higher odds of GDM (OR: 4.9, 95% CI: 1.4, 17.3; OR: 8.2, 95% CI: 1.8, 37.4; OR: 21.1, 95% CI: 4.0, 109.8, respectively).

Discussion

This is the first scoping review to synthesize information on the associations between dietary fats and risk of T2D in an *a posteriori* dietary patterns context. The main findings are that

TABLE 2 Summary of food groups and dietary patterns of included studies.

References	Food groups	Dietary patterns
Pastorino et al. (26)	Food groups were created based on their response variables (glycaemic index, fat and fiber) content. Food groups: Pizza, pasta, rice, cereals and other, high-fiber cereals, low-fiber cereals, white bread, whole meal bread, crisp and other bread, biscuit, pastry, cakes, whole milk, skimmed milk, low-fat dairy desserts, full-fat yogurt, low-fat yogurt, full fat dairy dessert, cream, butter and animal fat, cheese, eggs, oils, plant fat solid, plant fat solid low fat, fish, red meat, offal, white meat, processed meat, vegetables, pulses, fruit, potatoes, fried potatoes, nuts and seeds, soups, dressing and sauces, jam and chutney, table sugar, honey and syrup, confectionery, savory snacks, alcoholic drinks, squashes and juices, pure fruit juice, soft drinks, coffee and tea	Three dietary patterns were generated, but only DP1 was further investigated as it explained >15% of the variation in the response variables. The dietary pattern was characterized by low intake of fruit, vegetables, low-fat yogurt, wholemeal bread, high-fiber cereals and high intakes of white bread, processed meat, fried potatoes, butter and animal fat and added sugar.
Hoffman et al. (16)	Food groups were created based on their culinary usage and nutrient content. Food groups: Cooked vegetables, cabbage family, legumes, cooked potatoes, mushrooms, sauce, poultry, meat except fish and poultry, animal fat except butter, dessert, cake, cookies, confectionary, ice cream, jam, honey, chocolate spread, canned fruit, fruit juice, tea, muesli, cornflakes, pasta, rice, pizza, vegetarian dishes, garlic, wholemeal bread, other bread, olive oil, fresh fruit, raw vegetables, other vegetable oils and fats, water, fish, nuts, chips, salt sticks, fried potatoes, beer, spirits, wine, other alcoholic beverages, eggs, coffee, soup, processed meat, low-fat dairy products, high-fat dairy products, low-fat cheese, high-fat cheese, butter, margarine	Although four dietary patterns were generated, food group correlations were only provided to dietary pattern 4, the only dietary pattern associated with type 2 diabetes. The dietary pattern was characterized by high intake of whole grain bread, fresh fruit, spirits and wine and low intake of low-fat and high-fat dairy, coffee, fruit juice, processed meats and margarine.
Appannah et al. (30)	Food groups creation was based on nutrient profiles or culinary usage, and their hypothesized contribution to diet-disease relationships Food groups: High-fat milk and cream, low-fat milk, yogurts, cheese, butter and animal fat, margarine and vegetable oils, eggs and egg dishes, low-fiber bread, high-fiber bread, other bread products, high-fiber breakfast cereals, other breakfast cereals, rice, pasta, and other grains, cereal-based mixed meals, pizza, biscuits and cakes, puddings, ice creams, chocolate and confectionery, sugar-free confectionery, spreads, meat and poultry, meat mixed dishes, processed meat, coated or breaded meat and fish, meat substitutes, fish, fried or roast potatoes, boiled or baked potatoes, vegetables (raw or boiled), fried vegetables, legumes, vegetable mixed dishes, fresh fruit, other fruit, nuts and seeds, crisps and savory snacks, soups, sauces (low energy dense), sauces (high energy dense), condiments, sugar-sweetened beverages, low-energy beverages, fruit juice, hot and powdered drinks, water, alcoholic drinks	Although this study generated three dietary patterns, only dietary pattern 1 was further investigated as it explained >15% of the variation in the response variables. The dietary pattern generated was characterized by high intakes of processed meat, chocolate and confectionery, low-fiber bread, crisps and savory snacks, fried and roasted potatoes and low intakes of fresh fruits, vegetables, legumes, high-fiber bread and yogurts
Brayner et al. (28)	Food groups were created based on the food groupings used in the UK National Diet and Nutrition Survey and were adapted according to differences in the response variables (SFA, PUFA, and MUFA) content of food items Food groups: Pasta, rice and cereals, whole meal pasta, rice and cereals, white bread, whole meal bread, high fiber breakfast cereals, other breakfast cereals, whole milk, skimmed milk, other milk, cheese, low fat cheese, yogurt low fat, yogurt full fat, ice cream, cream and dairy desserts, butter, margarine, olive oil, high-fat sauces, low-fat sauces, bacon and ham, beef and veal, non fried chicken, turkey pork and dishes, fried poultry, other meats, white fish, battered and fish products, oily fish, other seafood, eggs and eggs dishes, meat alternatives, vegetables raw and boiled, vegetables (mixed dishes), legumes, fruits, boiled and baked potato, soups, nuts and seeds, crisps, chips and savory snacks, buns, cakes, pastries and fruit pies, puddings, biscuits, sugar, preserves and confectionery, fruit juice, high sugar beverages, soft drinks, diet, tea and coffee, water, spirits and liqueurs, wine, beer and cider	Three dietary patterns identified, but only dietary patterns 1 and 2 were further investigated as they explained >10% of the variation in the response variables. Two dietary patterns were identified: one was characterized by higher intake of nuts, seeds, and butter and lower intake of fruit and low-fat yogurt and the other was characterized by higher intake of butter and high-fat cheese and lower intake of nuts and seeds.
Gao et al. (31)	Food groups were created aligned to the U.K. National Diet and Nutrition Survey and according to the similarity of their nutritional composition and culinary use. Food groups: Chocolate and confectionary, butter and other animal fat spreads, low-fiber bread, table sugars and preserves, grain-based desserts, sugar-sweetened beverages and other sugary drinks, high-fat cheese, crisps and savory snacks, alcoholic drinks (wine, beer, spirits), milk-based desserts, processed meat, red meat, high-fat milk and cream pizza, fried or roast potatoes, other bread products, coated or breaded meat and fish, fruit juice, egg and egg dishes, animal fat spread lower fat, plant-based spread normal, sauces and condiments, low/non sugar ssbs, nut-based spreads, milk-based and powdered drinks, sauces and condiments (low-fat), vegetable side dishes and dips, plant-based spread lower fat, pasta and rice, nuts and seeds, poultry, olive oil, other breakfast cereals, coffee and tea, other fish, low fat cheese, high-fiber bread, low-fat milk, oily fish, non-dairy milk, meat substitutes, legumes and pulses, boiled or baked potatoes, soups, wholemeal pasta and rice, dried and stewed fruit, high-fiber breakfast cereals, vegetables, fresh fruit.	Four dietary patterns were generated, but only 2 were further investigated as they explained >20% of the variation in the response variables. Two dietary patterns were identified: one was characterized by high intakes of chocolate and confectionery, butter, low-fiber bread, and sugars and preserves, and low intakes of fruits and vegetables and the other was characterized by high intakes of sugar-sweetened beverages, fruit juice, table sugars and preserves, and low intakes of high-fat cheese and butter.

(Continued)

TABLE 2 (Continued)

References	Food groups	Dietary patterns
Emi et al. (27)	Food groups were created based on their nutritional characteristics Food groups: Cereal and cereal based dishes, meat and poultry, seafood and shellfish, milk and dairy products, egg and egg dishes, nuts, vegetables, fruits, local desserts, sweet sweetened beverages, sweets, processed food, fast foods and snacks	Four dietary patterns were generated, but only dietary pattern 1 was further investigated as it explained the most variation in response variables (35%). The dietary pattern identified was characterized by high intakes of sugar-sweetened beverages, fruits, sweets and low intakes of meat and cereal
Johns et al. (32)	Food groups were created based on according to usual culinary usage and within the constraints of the food groupings in the dietary questionnaire Food groups: Chocolate, low fiber bread (swedish), full fat spread, cheese, fast food, cake, white bread, candy, fatty meat, full fat milk, pizza, crisps, soft drink, cookie, semi skimmed milk, nuts, oil, hot drink, dessert, spirits, beer, jam, potatoes, lean meat, egg, low fat spread, wine, skimmed milk, juice, full fat yogurt, light meals, fish, crisp bread, meat alternative, wholemeal bread, cereal, low fat yogurt, vegetables, fruit.	Three dietary patterns were generated, but only dietary pattern 1 was further investigated as it explained the majority of the variation in the response variables (54%). The dietary pattern identified was characterized by higher intake of chocolate, low-fiber bread, cheese, fast food, and cake and low intake of fruit and vegetables
Shin et al. (33)	Food groups were created based on grouping schemes reported in the Food Patterns Equivalents Database (FPED) 2011–2012 Food groups: Citrus, melons, and berries, other fruits, fruit juice, dark green vegetables, tomatoes, other red and orange vegetables (excludes, tomatoes), potatoes (white potatoes), other starchy vegetables (excludes white potatoes), other vegetables, beans and peas computed as vegetables, whole grains, refined grains, meat (beef, veal, pork, lamb, game), cured meat (frankfurters, sausage, corned beef, cured ham and luncheon meat made from beef, pork, poultry), organ meat (from beef, veal, pork, lamb, game, poultry), poultry (chicken, turkey, other fowl), seafood high in n-3 fatty acids, seafood low in n-3 fatty acids, eggs, soybean products (excludes calcium fortified soy milk and mature soybeans), nuts and seeds, milk (includes calcium fortified soy milk), yogurt, cheese, oils, solid fats, added sugars, alcoholic drinks	Three dietary patterns were identified: the first was characterized by high intake of refined grains, solid fats, oils, and fruit juice; the second was characterized by high intake of nuts and seeds, solid fats, soybean products and low loadings of milk and cheese; the third one was represented by high intake of added sugars and organ meats and low loadings of fruits and vegetables and seafood

of the eight articles included, five high-fat dietary patterns were identified that were positively associated with either T2D incidence or elevated fasting glucose, insulin and/or HOMA levels. All five dietary patterns were characterized by other nutrients in addition to dietary fats, such as low dietary fiber and high dietary energy density, characterized by low intake of fruit and vegetables, reduced fat dairy products and higher intake of processed meats and butter. The only dietary pattern that used exclusively dietary fat as the response variables (namely PUFA, MUFA and SFA) did not find any associations with T2D risk. This suggests that intake of unhealthy fats is often accompanied by lower intake of fruits, vegetables and other fiber-rich foods, which may need to be considered when deriving *a posteriori* dietary patterns for assessing T2D risk. Therefore, consumption of healthy dietary fats for the prevention of T2D should be encouraged as part of a healthful overall dietary pattern.

Although all studies in this review used dietary fat as one of the response variables, the way these were used varied between studies. Regardless of whether total fat, or individual dietary fats were used, most dietary patterns generated included high factor loadings for meat and dairy (16, 26, 28, 30, 33). This is consistent with literature on the main food sources of saturated fat, where high processed meats intake has been consistently associated with higher risk of T2D and cardiovascular diseases (34, 35) whereas findings for dairy are mixed, and often depend on whether full fat or low fat dairy products are considered separately (36). For the two studies that included saturated and unsaturated fats as response variables, high factor loadings for nuts and seeds were also observed. However, findings from these studies were conflicting; Shin et al. (33) reported that a dietary pattern high in nuts, seeds, fat and soybean was associated with higher odds of developing GDM,

whilst Brayner et al. (28) reported that a dietary pattern high in nuts, seeds and butter was not associated with T2D incidence. This difference may be partially attributable to the sample populations, since Shin et al. (33) examined GDM in pregnant women and Brayner et al. (28) examined T2D incidence in healthy adults. Taken together, these findings suggest that the underlying foods that contribute to a high-fat dietary pattern should be taken into consideration to ensure these foods are lower in SFA.

Outcomes from this review align with literature on the effect of dietary fats on fasting serum glucose and insulin levels. The study by Appannah et al. (30) reported an association between a dietary pattern characterized by high intakes of high-SFA foods, such as processed meat, chocolate, savory snacks and fried foods, and higher fasting serum glucose and insulin, which could indicate insulin resistance. This supports evidence from randomized controlled trials (RCT), which have shown that high-SFA diets can decrease insulin sensitivity (37, 38). For example, a 12-week RCT of 486 European adults investigated two high-fat (either high in SFA or high in MUFA) and two low-fat (with or without 1.2 g/day n-3 PUFA supplementation) isocaloric diets. In healthy individuals, the high MUFA diet increased participants' insulin response to glucose, whilst the high SFA diet reduced insulin response (37). Similarly, a meta-analysis of RCTs reported that replacing 5% of energy from SFA with PUFA lead to significant decreases in glucose, HbA1c and HOMA levels. Although the mechanisms behind high-SFA diets and insulin resistance are still unclear, there is evidence to suggest that it might be linked to ceramide production (39). High-SFA diets increase ceramide production, which in turn can impair insulin signaling (39). High-SFA diets have also been suggested to be more inflammatory and chronic low-grade inflammation can inhibit insulin action (40, 41). Although RCTs can provide insight

into the causal relationship between dietary fats and glucose or insulin, prospective cohort studies can better understand these associations in a dietary pattern context. Moreover, as fasting glucose and insulin are important biomarkers in T2D development, more prospective studies investigating their long-term associations are needed to better elucidate the associations between dietary fat and T2D incidence in an *a posteriori* dietary pattern context.

Consistent with this review, evidence suggests that intake of specific nutrients, in addition to dietary fats, may play an important role in the development of T2D (42). Higher dietary fiber, found in fruits and vegetables, for instance, has been shown to modulate blood glucose concentration following a meal (43) and has thus been linked to a lower risk of T2D (5). Conversely, higher intake of carbohydrate, especially refined carbohydrate has a strong positive association with T2D risk (5). High dietary sodium is also linked to insulin resistance (44). Dietary patterns such as Dietary Approaches to Stop Hypertension (DASH) and the Mediterranean diet have dietary fat as a key focus, but are also a rich source of other key nutrients, such as fiber and antioxidants (19). A systematic review and meta-analysis of 48 studies has shown that a higher adherence to either DASH (RR: 0.81; 95% CI: 0.72, 0.92) or Mediterranean diet (RR: 0.87; 95% CI: 0.82, 0.93) was associated with substantial reduction in T2D incidence. Both dietary patterns encourage a high consumption of fiber-rich foods such as fruits and vegetables, along with lower intakes of SFA rich foods such as red meat and processed foods (45, 46). Therefore, the combination of dietary fats with other dietary components may be a stronger predictor for T2D, than any nutrient in isolation. However, as only one study was identified that derived dietary patterns based on dietary fat alone, further prospective evidence is needed to confirm this.

The present review provides supporting evidence for the role of energy-dense dietary patterns as a risk factor for T2D development (47). Half of the studies included in this review included energy density as a response variable (27, 30–32) which has an established positive association with obesity risk (48) whereas associations with T2D are less clear (49). Energy dense foods are also often high in SFA and added sugars, low in fiber and have a high glycaemic load, all of which have been linked to a higher risk of T2D (50). Interestingly, the study by Brayner et al. (28) in which only dietary fats were used to derive dietary patterns, there was no association with T2D. However, there was an association between higher SFA-rich foods and lower PUFA-rich foods with increased odds of developing overall and abdominal obesity. Therefore, although there are no clear associations between dietary fats and T2D, it may be that obesity is on the causal pathway as a key risk factor for T2D development (51). As many as 85% of individuals with T2D have either overweight or obesity (52). Further, obesity can contribute to low-grade chronic inflammation (53) whereas PUFA intake can counteract pro-inflammatory pathways (54).

This review has identified some discrepancies in the design and reporting of reduced rank regression methods that limit the interpretability of results. As the number of dietary patterns generated depended on the number of response variables, studies included in this review ranged from three to four response variables (16). This created challenges when the number of patterns reported differed, as the rationale for which dietary patterns to report also varied. Often, the dietary patterns that explained the most variation

in the response variables were reported, regardless of how much was explained by the other dietary patterns. However, this cut off ranged from >10% to 20% (26, 28, 30, 31) of explained variation, with some studies not providing a numerical cut off (16, 27, 32, 33). The number of food groups also varied, ranging from 13 to 50. Evidence from principal component analysis, another data-driven dietary pattern method, has shown that changes in the number of food groups can influence the associations with health outcomes (16). Thus, more consistent definition of this reporting of minimum cut off points and the rationale for classifying food groups would improve the reporting of this dietary pattern method in the literature.

This review acknowledges some strengths and limitations. Firstly, as a scoping review, this study followed systematic searching methods based on PRIMSA-ScR guidelines. Secondly, it identified reporting discrepancies in the use of reduced rank regression that should be addressed. In line with scoping review methodology, no critical appraisal of the evidence has been conducted, and therefore the quality of studies has not been considered. However, most studies were large prospective studies that adjusted for demographic characteristics, dietary intake and lifestyle risk factors.

In conclusion, this scoping review has identified eight studies using reduced rank regression to derive dietary patterns based on dietary fat. Overall, findings suggest that high-fat dietary patterns, especially high-SFA containing foods, were positively associated with T2D incidence and glucose, insulin and HOMA levels. While dietary fat may be an important predictor of T2D risk, evidence from a dietary pattern perspective suggests that foods such as processed meats, low-fiber cereals and low intakes of fruit and vegetables are likely to contribute to an increased T2D risk. Therefore, consumption of healthy dietary fats for the prevention of T2D should be encouraged as part of a healthful overall dietary pattern.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

BB and LM performed the screening of articles. BB drafted the manuscript. KL reviewed first draft of the manuscript. All authors contributed to the design of the study, the interpretation and critical evaluation of the review, provided edits, and approved final submission of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2023.1071855/full#supplementary-material>

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Be aware of the sodium intake outside student canteens: development and validation of a sodium food frequency questionnaire in Chinese undergraduates

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Background: Chinese college students used to eat in student canteens, making dietary consumption outside the cafeterias the main reason for the difference in sodium intake. This study aims to develop and validate a food frequency questionnaire (Sodium-FFQ) targeting dietary sodium intake outside the canteens among undergraduates in China.

Methods: This cross-sectional study included 124 and 81 college students from comprehensive universities in the development and validation stage. A 24h dietary recall and a food frequency questionnaire were used to develop the Sodium-FFQ. Food items were selected according to the foods that contributed more to the total sodium intake. Test-retest correlation coefficients with an interval of 14days were employed to evaluate reproducibility. Validity was assessed against a single 24h urine collection and a 3-day dietary record using correlation coefficients, Bland-Altman analyses, and cross-classification analysis of Kappa coefficients.

Results: The Sodium-FFQ consists of 12 groups of foods with 48 items. The Spearman correlation coefficient of test-retest on sodium intake was 0.654 ($p < 0.05$), and that between the Sodium-FFQ, 3×24h dietary record, and 24-h urinary sodium were 0.393 ($p < 0.05$) and 0.342 ($p < 0.05$), respectively. The Sodium-FFQ was correlated to 24h urinary sodium-to-potassium ratio, with a Spearman coefficient of 0.370 ($p < 0.05$). The classification agreement of the Sodium-FFQ and 24h urinary sodium was 68.4%, and the Kappa coefficient was 0.371 ($p < 0.001$).

Conclusion: The Sodium-FFQ developed in this study presented an acceptable reproducibility, validity, and classification agreement. It indicates that the Sodium-FFQ could be a potential tool for promoting sodium restriction in college students.

KEYWORDS

food frequency questionnaire, sodium, validity, college students, salt reduction

1. Introduction

Excess dietary sodium intake is associated with high blood pressure (1–3), high risk of cardiovascular disease, all-cause mortality, and other chronic diseases (1, 2). Salt reduction programs, regarded as one of the most cost-effective strategies (4), have been implemented worldwide with a great diversity of approaches (4–7). However, the problem remains: most people have no idea how much sodium they have taken in (8). Therefore, an assessment tool that monitors sodium intake and identifies the source of sodium is strikingly needed.

Biomarker detection and dietary survey are the main ways of assessing sodium intake (9). Compared with detecting sodium concentration in the urine or blood, the latter is more straightforward, convenient, and widely applied to collect dietary information in epidemiological studies (10). The food frequency questionnaire (FFQ) is a common dietary survey method (10, 11). A well-designed FFQ could better reflect the dietary intake level of certain nutrients and the risk of malnutrition and even be transformed into a practical tool for self-assessment (10, 12, 13). Several studies have evaluated residents' dietary sodium (or salt) intake by designing a sodium-related FFQ (7, 14–17). In contrast, such a study is scarce (18, 19) in China due to the patterns and typical communal meals.

College students, who are in the emerging period (usually defined as 18–25 years old), are unique and vulnerable (20, 21). Most undergraduates eat in the student canteens or restaurants around the school (including eating take-out foods). Thus, deciding and estimating how much salt and high-sodium seasonings to add and eat is hard. Consequently, the foods outside the canteens, like processed foods, have become the leading cause of dietary sodium differences among this population. College years are vital for transforming and establishing dietary behaviors and habits (20, 21). Forming and keeping a low-sodium diet will help college students' life-long health. A practical tool would present the daily sodium intake of the undergraduates, act as an alert, and guide them to make better food choices.

Therefore, this study aimed to develop a sodium food frequency questionnaire focused on foods outside student canteens for college students and validate it via testing against 24-h urinary sodium excretion and 3-day dietary records.

2. Materials and methods

2.1. Ethical approval

The study was approved by the Ethics Review Committee of the Xiangya School of Public Health, Central South University (XYGW-2020-087).

2.2. Study design and participants

This is an observational study with a cross-sectional design. The study was conducted from November 2020 to October 2021 in Changsha City, Hunan Province, China. College students studying in the comprehensive universities were recruited according to the following criteria: (1) without severe liver and kidney insufficiency, cardiovascular and cerebrovascular diseases, benign tumors, or

mental diseases, (2) with a regular diet in the past month, and (3) ability to read and write. The following exclusion criteria were added for the students required to collect urine: (1) being menstruating, (2) taking diuretics in the last 2 weeks, and (3) being unable to collect 24-h urine. All participants signed the informed consent before the study. A total of 124 and 81 students participated in the development and validation stages, respectively.

2.3. Development of The sodium-FFQ

2.3.1. Stage 1

A convenience sample of 151 students from comprehensive universities who met the abovementioned criteria was recruited, and 124 provided complete dietary information. A one-day dietary recall and food frequency questionnaire targeting the past 12 months was employed to identify the college students' high-sodium foods frequently consumed in winter, spring, and autumn. Food items were first selected from one-day dietary recall data and supplemented by data from an FFQ derived from China Health and Nutrition Survey (CHNS) (22). The contribution rate of each food to the total sodium intake was calculated, and the foods with a contribution rate reaching 90% were selected for the food list. Besides, the foods with a content ≥ 200 mg Na/100 g in the Chinese Food Composition List and the commercially processed foods ($\text{Na} \geq 500$ mg Na/100 g) were also added (10, 12, 23). In this stage, a total of 12 categories with 55 items were included in the draft version of the FFQ.

Responses to each item were designed in a fill-in-blank format. The consumption frequency, with the units of 'time(s)/month,' 'time(s)/week,' and 'time(s)/day,' was converted as the daily frequency divided by 28 days a month. Standard portion sizes were determined referring to the most frequent answers from the one-day dietary recall data and varied by different eating ways, such as 'a spoon/portion from aunts in the canteen' or 'a bowl from homemade dishes.'

- The total sodium intake was estimated using the formula below:

$$Total\ Na = \sum_{i=0}^n F_i \times I_i \times C_{Na,i}$$

Total sodium intake from FFQ (*Total Na*, mg/d), food items (*i*), frequency (*F*, times/d), intake of each time (*I*, g, or mL), and sodium content of each food (*C*, mg/g or mL/L), and *n* is a natural number.

- Adjusted sodium intake was the total Na adjusted by the items regarding "re-adding salt to the cooked meals" of the subjects, and the model was as follows:

$$Adjusted\ Sodium\ Intake\ (mg/d) = Total\ Na + AS \times F_{AS} \times m_{AS} \times 400$$

Total sodium intake from FFQ (*Total Na*, mg/d), add salt again or not (*AS*, Yes = 1, no = 0), frequency of adding salt (*F_{AS}*, occasionally = 1/7 = 0.1429, often = 3/7 = 0.3571), grams of salt added each time (*m_{AS}*, one spoonful = 2 g, unclear = 3 g, or the actual amounts added each time), and the conversion factor (1 g salt \approx 400 mg sodium).

2.3.2. Stage 2

Two experts in nutrition evaluated the representativeness, response format, and standard portion sizes of each item for college students. Then, thirteen foods rich in sodium (4) or potassium (9) were added to the food list; the high-potassium foods were served as the adjustment factor and a reminder of a healthy diet. Here developed the original version of a 68-item Sodium-FFQ.

2.3.3. Stage 3

The original Sodium-FFQ was pre-tested among nineteen volunteer students (five boys and fourteen girls). The comprehensibility of the FFQ was assessed via face-to-face interviews with trained research assistants. Moreover, a single 3-day dietary record and twice food frequency questionnaire were conducted to validate the FFQ preliminarily and confirm the data collection process. After the pre-tested stage, twenty-five items were eliminated, three added, and two modified; the result was the forty-eight-item version of the FFQ, named the 1st version of Sodium-FFQ (Sodium-FFQ 1.0, [Supplementary Material](#)).

2.4. Validation of the sodium-FFQ

According to the previous research (24, 25), the sample size was calculated at 100 in this stage (24, 25). Through campus advertisement and online webpage recruitment, one hundred and twenty-four students registered for participation, of which 102 were recruited. In this stage, the online questionnaires were delivered twice, with a 3-day diet record and a single 24 h urine collected then. [Figure 1](#) shows the timeline of this stage.

2.4.1. Test–retest of sodium-FFQ 1.0

The test–retest procedure evaluated the reproducibility over a 14-day interval. Uniformly trained research assistants guided the college students to complete the Sodium-FFQ 1.0 on the 4th and 18th days. The Sodium-FFQ 1.0 was delivered online via The Questionnaire Star, a tool for developing electronic questionnaires. Each questionnaire had a unique linkage. All food items and each type of portion size were displayed with pictures for reference.

2.4.2. Three-day dietary record

Research assistants delivered the standardized dietary record toolkits to the participants and guided them to fill in the record book the day before the start. The toolkit consists of a record book of 3-day diets, an estimated food weight list, a piece of graph paper to examine the amount the participants estimated, and a pen. The participants

were asked to choose one of the 3 days to take photos of all the foods on graph paper before and after eating and then send photos to research assistants for examination (26).

2.4.3. Twenty-four-hour urine collection

Since approximately 85 to 90% of sodium is ingested over 24 h of excretion in the urine, the 24 h U_{Na} is considered the gold standard for measuring sodium intake (2, 3). The 24 h urinary toolkits were sent on the second day to the participants. The toolkits included a polypropylene bucket (4 liters, with a lid), a urine collection instruction, and two label stickers for writing names, dates, and ID. The instruction was to help the subjects collect urine correctly, including (1) starting the collection after the first urination in the morning, (2) collecting all the urine within 24 h, especially the first one of the next day, (3) keeping the container in the shade and avoiding contamination by blood or stools, and (4) keeping usual eating and drinking habits during collection.

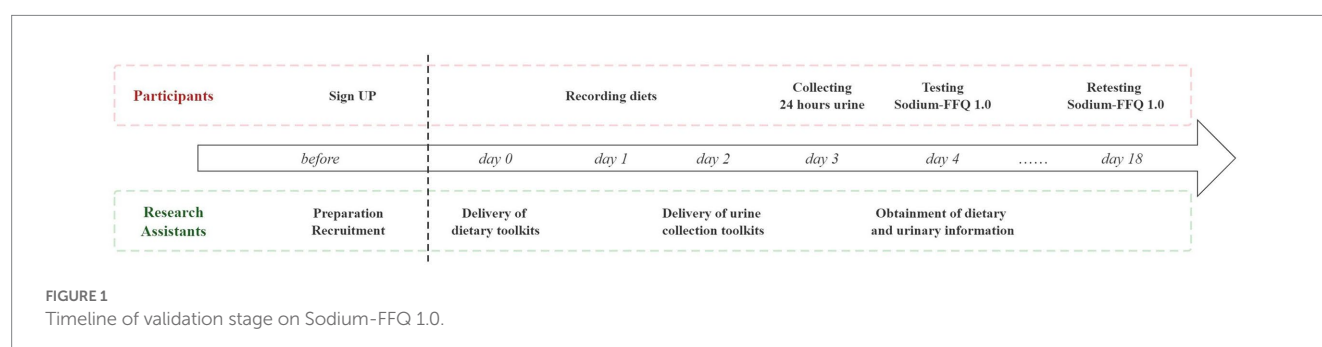
Participants were required to submit their urine and dietary records on the fourth day. Research assistants checked urines by asking whether the subjects adhered to the collection steps and then observed the total volumes via naked eyes. The urinary buckets were transported to the laboratory for processing. The total volume of the sample was uniformly measured by research assistants using a measuring cup of 5 liters; the samples of more than 500 ml were considered valid. All the samples were then sub-packaged and sent to the clinical laboratory department within 2 h. Twenty-four-hour urinary sodium and potassium concentration was detected by ion-selective electrode method (Beijing LEADMAN Biochemical Co., Ltd., China) and converted into milligrams (one mmol/24 h U_{Na} = 23 mg/24 h sodium, one mmol/24 h U_K = 39 mg/24 h potassium).

2.5. Covariates

Information on the characteristics and eating habits of the subjects was collected along with the dietary surveys through online questionnaires. Characteristics data includes students' age, sex, ethnicity, grade, major, and monthly pocket money. Eating habits, main ways of eating, and the frequency (amount) of re-adding salt to cooked meals were collected by self-designed questions.

2.6. Statistical analyses

EpiData 3.1 software (The Epi Data Association, Odense, Denmark), the Nutrition Star Expert System software (Zhending



Health Technology Co., LTD., Shanghai, China), and Microsoft Excel software were employed to create the dietary database. IBM SPSS 26.0 (IBM, Washington, United States.) was used for data analyses. The figures were presented by R and MedCalc (Version 20.027, MedCalc Software, Ostend, Belgium). The Shapiro–Wilk test was used to identify the data distribution, and the non-parametric methods were employed for further analysis. Values are described as mean with standard deviation or median with quartiles. The *Spearman* correlation and intra-class coefficients (ICC) were calculated for the reproducibility between the test and retest results. The correlation coefficients <0.5 represent poor reliability, between 0.5 and 0.75 as moderate reliability, 0.75 and 0.9 as good reliability, and >0.9 as excellent reliability (27). For validity, both 24 h U_{Na} and 3-day food records were tested against the results from Sodium-FFQ 1.0 via the following analyses (24, 28): (1) *Spearman* correlation coefficient to evaluate the correlation between Sodium-FFQ and other methods, (2) de-attenuated correlation coefficient to correct the observed correlation for the attenuating effect of random within-person error, (3) receiver operating characteristic analyses to identify the cut-off values, (4) *Bland–Altman* method and cross-classification proportion to assess the agreement of bias. According to Lombard (28), correlation coefficients >0.5 were considered good outcomes, between 0.2 and 0.49 as acceptable outcomes, and <0.2 as poor outcomes. For the *Bland–Altman* analyses (12), the presence of bias, direction, and extent was described; the absence of significant correlations between the differences and the means of the tools was considered a good outcome. For the cross-classification, the agreement of $\geq 50\%$ in the same category and $\leq 10\%$ in the opposite is acceptable (12). Significant levels were set at $p < 0.05$.

3. Results

3.1. Characteristics of participants in the validation stage

A total of 102 college students were recruited into the study; 10 did not return their diet records and urinary sample, and 11 had abnormal urines. Eighty-one college students finished the FFQ survey and 24-h urine collection. The average age of the participants was 20.86 ± 6.51 years old (Table 1), including 21 males (25.9%) and 60 females (74.1%). Among them, 88.9% of college students are of Han nationality, and 55.6% have lived in Hunan Province for less than 3 years. There were 28 students (34.6%) from senior grades or above, and most (71.6%) were from medical majors. More than half of college students eat in student canteens (59.3%), followed by take-out (24.7%). Fifteen college students (18.5%) re-added salt to their cooked meals. There was no difference between the sex in most characteristics.

According to 24 h urinary sodium excretion, dietary sodium intake was estimated to be 2998.72 ± 1338.31 mg/d, with a median of 2664.55 (2011.93, 3901.95) mg/d. The dietary sodium intake of 81 college students from 3-day diet records was 1200.47 ± 575.84 mg/d, with a median of 1097.27 (784.87, 1425.70) mg/d. Since no college students cooked alone, the subjects could not record salt and most high-sodium seasonings in the 3×24 h dietary records. There was a difference between the 3×24 h dietary record and the sodium intake estimated by 24 h urinary sodium. The average urinary potassium was 992.43 ± 388.23 mg/d, and the median was 991.18 (671.78, 1265.26)

mg/d. The median of 24 h urinary sodium to potassium ratios in the 81 participants was 5.52 (3.59, 7.38).

3.2. Reproducibility

The sodium intake, regardless of adjusting by re-adding salt, was significantly higher in the first FFQ survey than in the second (Table 2, Wilcoxon $U = -2.856$, $p = 0.004$). The intra-class correlation (ICC) between the test and retest results of Sodium-FFQ and the adjusted one was 0.571 ($p < 0.001$) and 0.575 ($p < 0.001$), respectively. The ICC of the food categories ranged from snacks of 0.619 ($p < 0.001$) to seasonings and others of 0.820 ($p < 0.001$), while the associations of staples, beans and products, meat and aquatic, eggs, and beverages were not observed.

The *Spearman* correlation coefficients between the test–retest results on Sodium-FFQ were 0.654 ($p < 0.001$) and 0.672 ($p < 0.001$) on adjusted results. The *Spearman* coefficients ranged from 0.273 ($p < 0.05$) for staple foods to 0.678 ($p < 0.05$) for fast food. The food categories, except meat & aquatic, and eggs, presented moderate reproducibility in the test–retest investigation.

3.3. Validity

The *Spearman* coefficients of 3-day dietary records with Sodium-FFQ and the adjusted one were 0.393 ($p < 0.05$) and 0.409 ($p < 0.01$), respectively (Table 3); the *Spearman* correlation coefficients between them with 24 h U_{Na} were 0.342 ($p < 0.01$) and 0.329 ($p = 0.004$), respectively. Compared with the unadjusted FFQ, the adjusted one performed poorly, correlating with 24 h U_{Na} . After adjustment using 24 h U_{Cr} and U_K , the *Spearman* partial correlation coefficients between the Sodium-FFQ, adjusted one and 24 h U_{Na} were both 0.352 ($p = 0.002$); that of the Sodium-FFQ, adjusted one with 24 h sodium-to-potassium ratio were 0.370 ($p = 0.001$) and 0.358 ($p = 0.001$), respectively.

De-attenuated *Spearman* correlation coefficients presented the corrected correlation between Sodium-FFQ with the calibrated indices. The correlation was all expanded after correcting, with about 1.234 folds between Sodium-FFQ with 3-day dietary records and 1.190 folds with urinary indices.

In order to assess the agreement, the presence, and the direction of bias at a group level, *Bland–Altman* analyses were used (Figure 2). For the FFQ, the mean bias with the reference measures was 0.04 g (Figure 2A, 95% CI: $-0.043, 0.121$, $p = 0.351$) for the 3 day food record and 12.2 g (Figure 2C, 95% CI: $9.706, 14.687$, $p < 0.001$) for the 24 h U_{Na} . The FFQ-adj presented a mean bias of 0.02 g with the 3 day food record (Figures 2B) and 11.6 g with the 24 h U_{Na} (Figures 2D).

3.4. Agreement of the sodium-FFQ and 24 h U_{Na}

3.4.1. Roc analyses for the Cut-Off value

According to the sodium intake estimated by 24 h urine, participants were divided into “normal intake” and “high intake” groups, referred to the threshold of 2300.0 mg/d (2). ROC curves were drawn to identify the cut-off value of the Sodium-FFQ (Table 4; Figure 3). The area under the curve (AUC) of Sodium-FFQ was 0.728

TABLE 1 Characteristics of participants in validation study ($n=81$, % or mean \pm SD).

Variables	Total 81 (100.0)	Sex	
		Boys 21 (25.9)	Girls 60 (74.1)
Age	20.86 \pm 6.51	23.10 \pm 12.61	20.08 \pm 1.14
<i>Ethnicity</i>			
Han	72 (88.9)	19 (90.5)	53 (88.3)
Others	9 (11.1)	2 (9.5)	7 (11.7)
<i>Self-reported BMI (kg/m²)</i>			
< 18.5	13 (15.9)	2 (9.5)	11 (18.0)
18.5 ~ 23.9	55 (67.1)	14 (66.7)	41 (67.2)
24.0 ~ 27.9	11 (13.4)	4 (19.0)	7 (11.5)
\geq 28.0	3 (3.7)	1 (4.8)	2 (3.3)
<i>Grade</i>			
Freshman	12 (14.8)	4 (19.0)	8 (13.3)
Sophomore	17 (21.0)	3 (14.3)	14 (23.3)
Junior	24 (29.6)	7 (33.3)	17 (28.3)
Senior or older	28 (34.6)	7 (33.3)	21 (35.0)
<i>Major</i>			
Humanities & Social Sciences	7 (8.6)	2 (9.5)	5 (8.3)
Literature/Sciences and Engineering/ Agriculture	16 (19.8)	7 (33.3)	9 (15.0)
Medicine	58 (71.6)	12 (57.1)	47 (76.7)
<i>Pocket Money (RMB, yuan / monthly)</i>			
< 1,000	11 (13.6)	2 (9.5)	9 (15.0)
1,001 ~ 1,500	25 (30.9)	7 (33.3)	18 (30.0)
1,501 ~ 2000	31 (38.3)	12 (57.1)	19 (31.7)
> 2000	14 (17.3)	0 (0)	14 (23.3)
<i>Main ways of eating</i>			
Student canteen	48 (59.3)	11 (52.4)	37 (61.7)
Take-out eating	20 (24.7)	5 (23.8)	15 (25.0)
Restaurant	11 (13.6)	5 (23.8)	6 (10.0)
Home	2 (2.5)	0 (0)	2 (3.3)
<i>Re-adding salt to cooked meals</i>			
Yes	15 (18.5)	5 (23.8)	10 (16.7)
No	66 (81.5)	16 (76.2)	51 (83.3)

BMI, Body mass index; RMB, renminbi; Chinese official coupons, 1 RMB \approx 0.15 USD.

($p=0.001$), with the threshold for classification of 1078.89 mg/d. The threshold of the adjusted one was 1150.92 mg/d (AUC=0.719, $p=0.001$). The two curves had no statistical difference in AUC ($p=0.292$).

3.4.2. Kappa coefficients of the two methods

A cross-classification analysis was applied to evaluate the agreement at the individual level by cut-off values of 1078.89 mg/d and 1150.92 mg/d separately. The proportion of agreement was 68.4%, and that in the opposite group was 10.5%. The *Kappa* coefficients were both 0.371 ($p=0.001$) of the Sodium-FFQ and the adjusted one.

4. Discussion

This study aimed to develop an assessment tool on dietary sodium intake (Sodium-FFQ) and present the measurement properties among college students. The Sodium-FFQ included the high-sodium foods consumed by undergraduates outside the campus cafeterias. Results showed acceptable reproducibility, validity, and agreement on the classification of the Sodium-FFQ. This Sodium-FFQ might be a helpful tool to assess dietary sodium intake and identify the primary sources for undergraduates. Further, it could also act as an alert to excessive sodium, contributing to sodium reduction in China.

TABLE 2 The sodium intake from FFQ in the test–retest investigation, mg/day ($n=81$, Median, $P_{25}\sim P_{75}$).

Variables	Test	Retest	ICC (p-value)
FFQ	1509.58 (699.67, 2406.56)	1040.44 (502.17, 1929.88)	0.571 (< 0.001)
FFQ-adj	1566.18 (699.67, 2506.56)	1078.89 (566.91, 1964.92)	0.575 (< 0.001)
Staple food	41.06 (0.00, 100.35)	34.06 (0.00, 79.02)	0.230 (0.122)
Beans and products	0.34 (0.00, 20.27)	0.00 (0.00, 4.29)	0.254 (0.097)
Vegetable & Fungi	38.94 (18.66, 81.01)	35.41 (11.46, 73.09)	0.639 (< 0.001)
Fruits	0.10 (0.00, 0.27)	0.00 (0.00, 0.14)	0.750 (< 0.001)
Dairy and products	61.08 (29.89, 157.61)	39.86 (6.64, 93.00)	0.626 (< 0.001)
Meat & Aquatic	152.36 (79.89, 245.02)	86.78 (33.95, 171.59)	−0.104 (0.671)
Eggs	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.217 (0.138)
Snacks	220.30 (113.10, 358.15)	185.06 (68.76, 365.23)	0.619 (< 0.001)
Fast food	380.44 (116.63, 1163.84)	230.25 (20.22, 1100.66)	0.773 (< 0.001)
Beverages	0.00 (0.00, 1.79)	0.00 (0.00, 1.79)	0.276 (0.076)
Seasonings & Others	52.88 (0.00, 165.26)	26.44 (0.00, 86.32)	0.820 (< 0.001)

FFQ, the Sodium-FFQ; FFQ-adj, adjusted by salt re-added in cooked meals.

TABLE 3 Spearman Correlation coefficients between the Sodium-FFQ, food records, and 24h U_{Na} ($n=81$).

Variables	FFQ		FFQ-adj	
	Crude	De-attenuated	Crude	De-attenuated
3-day dietary record	0.393 (< 0.001)	0.485	0.409 (< 0.001)	0.459
24h U_{Na}	0.342 (0.003)	0.407	0.329 (0.004)	0.351
24h U_{Na} (Adjust: 24h U_{Cr})	0.319 (0.005)	0.379	0.318 (0.005)	0.339
24h U_{Na} (Adjust: 24h $U_{Cr} + U_K$)	0.352 (0.002)	0.419	0.352 (0.002)	0.376
Na/K ratio	0.370 (0.001)	0.440	0.358 (0.001)	0.382

FFQ-adj, adjusted by salt re-added in cooked meals. Crude: crude Spearman correlation coefficients with p -value. Deattenuated: de-attenuated correlation coefficients: correcting coefficients referring to Liu. and Beaton., et al. (10). Deviation of 3 day dietary record, within-person, $s^2_{w_1} = 3668.568^2$. Between person, $s^2_{b_1} = 6493.668^2$, $\lambda_a = 0.319$. Deviation of test and retest results, within-person, $s^2_{w_2} = 6879.920^2$. Between person, $s^2_{b_2} = 12959.571^2$, $\lambda_b = 0.282$. Deviation of adjusted test–retest results, within-person, $s^2_{w_3} = 6879.920^2$. Between person, $s^2_{b_3} = 13044.052^2$, $\lambda_c = 0.278$.

In this study, the Sodium-FFQ is little different from sodium-related FFQ published yet (12, 15, 29). College students, who hardly cook themselves, are the target population of the Sodium-FFQ. Neither can they accurately estimate the amount of salt and high-sodium seasonings added by cooks nor restrict the intake of these seasonings. Hence, natural, processed, and fast foods, rather than salt and most high-sodium seasonings, are the body of the Sodium-FFQ. Additionally, Sodium-FFQ contained the foods in different seasons since food intake varies with time (30). Some foods rich in potassium ($K > 150\text{ mg}/100\text{ g}$), such as fresh vegetables and fruits, were also added to the food list due to the relationship between dietary potassium intake and sodium excretion (31). Despite being different from the previous FFQ, it is still available to assess sodium intake for college students.

The results showed significant differences among 24h U_{Na} , 3-day dietary records, and the Sodium-FFQ. The possible explanation might be that the 3-day dietary records and FFQ survey did not ask about the students' salt and high-sodium seasonings intake, while 24h urine contained total sodium intake daily. This could also be reflected by the results of Bland–Altman analyses, that the Sodium-FFQ had a good

consistency with the dietary recording method but poorly with the 24h U_{Na} method. Moreover, the difference between 24h U_{Na} and dietary records were consistently observed in previous studies (12, 15, 29). The extensive distribution of sodium in natural foods, variation in the proportion of salt retained in food, the widespread utility of sodium compounds in food process and medication, as well as uncommon foods unlisted in food composition tables, could contribute to such a difference, too (25, 32, 33).

Our study found that sodium intake measured in the retest survey was lower than in the test survey, regardless of whether the Sodium-FFQ was adjusted. This is similar to most FFQ reproducibility research (15, 17, 24, 34). The training effect could explain it partially (30). Repeated measurement would make subjects sensitive to dietary intake and cautious about answering FFQ in the retest survey (30). Besides, the list of food weights in the toolkit was gifted; it might help some participants learn to estimate portion sizes between the two surveys. Other possible reasons are the Hawthorne effect (35) and volunteer bias (36).

Reproducibility presented the repeatability of the FFQ evaluated at two different time points (10). The current study demonstrated an

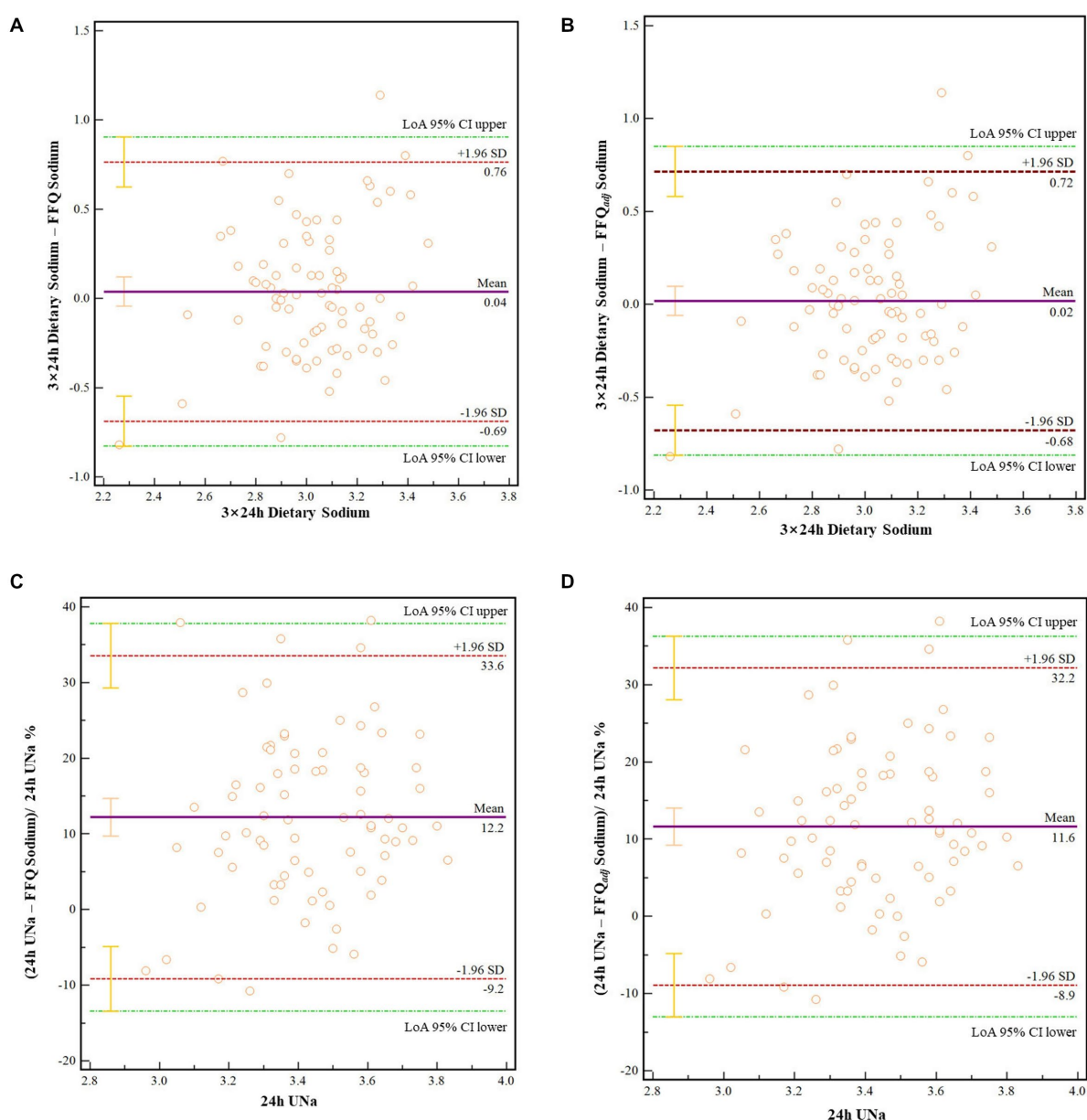


FIGURE 2

(A) Bland–Altman for the Sodium-FFQ and 3-day dietary record. (B) Bland–Altman for the adjusted FFQ and 3-day dietary record. (C) Bland–Altman for the Sodium-FFQ and 24h U_{Na} . (D) Bland–Altman for the adjusted FFQ and 24h U_{Na} . FFQ-adj, adjusted by salt re-added in cooked meals. LoA 95% CI upper/lower: Upper or lower of the 95% Confidence interval of the limits of agreements.

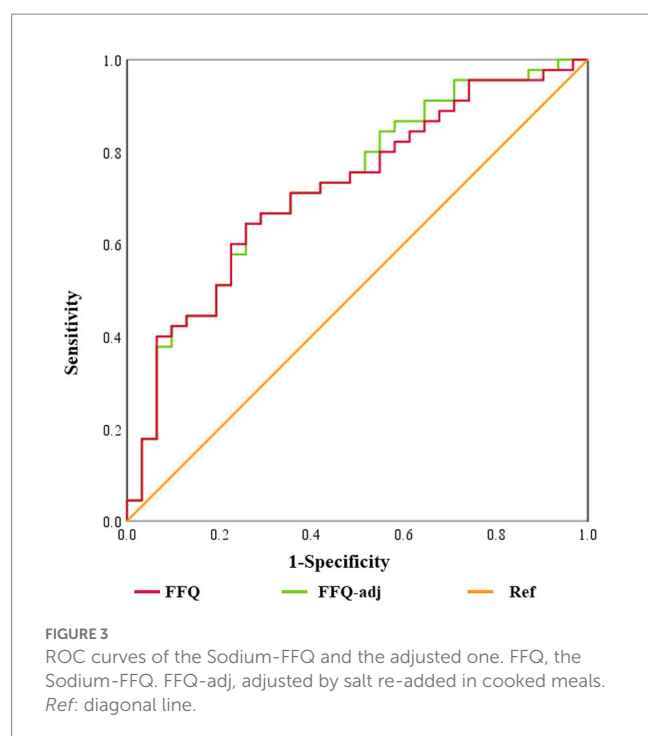
TABLE 4 ROC analyses of the Sodium-FFQ and adjusted one for cut-off values.

Variables	AUC	<i>p</i>	Youden-index	sensitivity	specificity	cut-off value
FFQ	0.728	< 0.001	0.386	64.44%	74.19%	> 1078.89
FFQ-adj	0.719	< 0.001	0.386	64.44%	74.19%	> 1150.92

acceptable reproducibility of the Sodium-FFQ, consistent with those of other studies (12, 34). The intra-group correlation coefficient (ICC) also revealed a moderate association between the test and retest of 0.571, within the range of previous studies from 0.43 to 0.97 (33, 34, 37). The ICC varies significantly among studies might be because of the test–retest interval (25), sample size (10, 24), dietary disparities

(38), and sex and age differences of subjects (13, 33). In our study, taking the day-to-day variation of sodium intake and excretion (39, 40), as well as the possibility of high compliance (10), 2 weeks were chosen (12, 30, 34) to represent the short-term stability of it.

Consistency with the previous works (14, 15, 17, 24, 25, 33, 34, 37, 41), the correlation coefficient between the Sodium-FFQ and 3-day



dietary records were 0.393 (*Spearman*) and 0.485 (de-attenuated), respectively. The correlation coefficients between Sodium-FFQ and 24h U_{Na} were lower than Sodium-FFQ with dietary records; this was in line with the existing studies (ranging from 0 to 0.37) (15, 24, 25). However, after adjusting for 24h U_{Cr} and 24h U_{K} , the partial correlation coefficient increased to 0.352 (*Spearman*) and 0.419 (de-attenuated), respectively. Such a weak correlation between 24h U_{Na} and the Sodium-FFQ could be explained by the temperature effect on sodium excretion (42), single 24-h urine collection (24, 25), and relatively small sample sizes (10, 25). Nonetheless, these results presented an acceptable performance (both reproducibility and validity) of the Sodium-FFQ.

One notable result of our study is the association between Sodium-FFQ and 24h urinary sodium-to-potassium ratio. Previous studies revealed a positive association between 24h urinary Na/K ratio and blood pressure (40, 43). It has recently been reported to be a better predictive index for cardiovascular risk than urinary sodium or potassium excretion alone (40, 43, 44). Though the correlation performed poorly, it reveals the possibility of using Sodium-FFQ for college students' diet guidance.

According to the criteria, the Sodium-FFQ presented a moderate reproducibility with 0.571 of ICC, and acceptable validity, with a series of *Spearman* correlation coefficients within 0.2~0.49, 68.4% being classified in the same category and 10.5% to opposite ones, and non-significant *p*-value in Bland-Altman test. Though the Sodium-FFQ did not meet the criteria of tools being applied to nutritional epidemiological studies (12), the results confirmed that foods outside canteens have become dominant among college students. These also hinted that timely approaches to establishing a low-sodium diet are necessary. Furthermore, the ultimate purpose of our study was to develop a screening tool that makes it possible to classify individuals according to intake levels, raise public awareness of the low-sodium diet, and promote healthier eating habits in college students. Therefore, we hope this study will help not only the practitioners of salt reduction but also college students and their parents.

This is the first study focused on developing and validating the food frequency questionnaire targeting college students' sodium intake in China, which provides information and helps to salt reduction initiatives. The primary strength of the present study is that the tool was developed for a specific-vulnerable population (college students), who could contribute a lot to themselves and surrounding people. Another advantage is that two reference methods were used to test the validation of this Sodium-FFQ. Rigorous quality control procedures were carried out throughout the study. All the urine samples were examined, measured, and sub-packaged by uniformly trained assistants, guaranteeing the reliability of biochemical information. Additionally, each food item of the Sodium-FFQ was accompanied by pictures with portion size; this could help the respondents fill it more accurately.

Our study also had several limitations. First, a single 24-h urine collection could not fully represent an individual's dietary sodium intake since it is associated with intra-individual variability under a controlled environment, furtherly contributing to the limitations. Furthermore, we collected the anthropometric information via a questionnaire instead of measurement by ourselves. College students, who are sensitive to body shape and dietary intake, might underreport their daily dietary consumption, BMI, and other actual situations; these could result in a lower intake from dietary records than actual ones. Moreover, the performance of the Sodium-FFQ was susceptible to relatively small sample sizes due to twenty-one participants' drop-out. In addition, the students were convenience sampled from four comprehensive universities in the capital city of Hunan Province in central China. Hence, the food items in Sodium-FFQ could not be extrapolated to students all over China due to dietary disparities and the non-probability sampling methods.

5. Conclusion

A food frequency questionnaire targeting dietary sodium intake among college students has been developed and validated. The questionnaire includes forty-eight items from 11 food groups, demonstrating acceptable reproducibility and validity against the gold standard indicator of 24h U_{Na} , and a moderate classification agreement with the cut-off value of 1078.89 mg/d. Sodium-FFQ would be a potential tool to address exceedingly sodium intake from foods outside student canteens among college students. Further study should focus on modifying and re-validating the Sodium-FFQ for extrapolation. Simultaneously, it is necessary to develop Sodium-FFQ for people in different life stages and combine it with various platforms or electronic devices to contribute to sodium reduction.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Review Committee of the Xiangya School of Public Health, Central South University. The participants provided their written informed consent to participate in this study.

Author contributions

YX, YL, and QL made concepts, designed the study, acquired funding, and managed data preparation. YX, LY, JC, JD, and YQ recruited participants. YX, CX, CY, JL, JH, HZ, MW, YP, and QX conducted the investigation, analyzed, and interpreted data. YX drafted the original manuscript. QL and YL reviewed and edited the manuscript. All authors interpreted the results, made a substantial contribution to the improvement of the manuscript, read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2023.1062845/full#supplementary-material>

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