Plant-based diets for a sustainable future

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Plant-based diets for a sustainable future

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Editorial: Plant-based diets for a sustainable future

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Editorial on the Research Topic
Plant-based diets for a sustainable future

In an era when our choices profoundly impact the planet, the way we eat takes on new significance. Plant-based diets have emerged as a sustainable solution for both our health and the environment. This issue delves into the world of plant-based diets, exploring their health benefits and their role in creating a sustainable future.

The human diet continually evolves, initially relying solely on vegetable foods collected from nature and transitioning to plant-based diets with the introduction of agriculture. Throughout civilizations, preferences for plant-based foods are shaped by religion, culture, health, personal choices, and economic factors (1). Plant-based diets are associated to vegetarianism or other diets that naturally promote and priorize the consumption of foods from vegetable sources and can be more or less flexible in the inclusion of animal foods. These include flexitarian, semi-vegetarian, pesco-vegetarian, lacto-ovo-vegetarian and vegan diets (2). Additionally, they may align with traditional and intricate dietary styles like the Mediterranean Diet, Nordic Diet, or DASH (2). Some of the reasons for not eating animal-based foods may be related to personal, cultural and religious beliefs mostly focused on animal wellbeing, but can also emanate from the loss of confidence on animal-based products, which have affected the food choices of the modern consumers (1). Following the tendency for plant-based diets, the meat substitute products are a promising option and an alternative source for protein intake. Still, Garaus and Garaus found that US consumers' predominantly negative perceptions of meat substitutes, identifying gender-based differences and consumer profiles, offering insights to help market and promote plant-based alternatives more effectively.

In practice, we can analyse the nutritional challenges and the possible health gains of plant-based diets. First, comparing plant-based with meat-based diets, the nutritional intake of the first was found to be lower for vitamin B12, vitamin D and iodine, but also for calcium, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The average intake of iron and zinc were also considered inadequate since their requirements are higher due to the lower bioavailability presented from the vegetable sources. Nevertheless, the meat-based was higher for fiber, polyunsaturated fatty acids (PUFA), α-linolenic acid (ALA), vitamin E, folate, and magnesium (3). But if this is true for adults, in contrast to children, adolescents, pregnant or lactating women, and older adults, authors tend to be more cautious (2). Another focus for concern is the adequacy of dietary protein and aminoacid intake from plant-based diets. Recent studies in adults following classic vegetarian diets indicate they can supply protein and aminoacids above the body requirements (4). A recent study investigating the ideal percentage of protein in plant diets and its correlation with nutrition and sustainability found no singular optimal value, proposing a broad range of 25–70%. The study revealed that a well-balanced plant-based diet, rich in high-quality proteins and diverse plant foods, could potentially safeguard against bone loss. Conversely, an unhealthy plant-based diet might adversely affect bone mineral density (BMD). Despite potential limitations such as reliance on self-reported dietary data, the research suggests that meticulously planned vegetarian diets merit further exploration for their potential bone health benefits across diverse populations (Fouillet et al.).

Precisely, we can consider that the nutritional adequacy may be compromised for the people following plant-based diets but also for the general population mainly if they follow unhealthy food habits, with a lack of food variety but rich in processed foods. It can also be critical specifically for the more vulnerable population groups and the ones with specific and higher requirements, such as the older adults or athletes, and the populations from poor countries where the minimum requirements are compromised (1–3). Nutrition education strategies, along with food fortification may be an important means to fight against nutrition deficiencies and malnutrition.

Nutritional adequacy can also be affected by the food composition, which is variable in terms of the different nutrients depending on many factors from soil and agriculture to the intrinsic genotypic variations of plants, or food innovation and processing technologies. The study highlights that certain Indian onion cultivars, especially the yellow bulb "Arka Pitamber," have a high potassium-to-sodium ratio, which could be beneficial in dietary interventions to manage hypertension and reduce cardiovascular disease risks in the Indian population (Singh et al.). Modern techniques for food production can work as an ally on the creation of foods with more interesting nutrient profiles for certain conditions or pathologies. Shi et al. 's study aims to transform spent barley grains into a nutritious ingredient for making starch less noodles with minimal impact on blood sugar, presenting a sustainable food option particularly suitable for individuals with diabetes and those concerned about blood sugar and weight management.

Following the advances of Epidemiology but also the achievements of clinical and basic science, since the beginning of the twenty-first century a shift has occurred and the benefits for health from a plant-based diet has become stronger based on evidence, with particular attention to the management of chronic diseases risk, the lower mortality rates and the increased longevity (1–3, 5). Noteworthy, we still don't have consistent conclusions as the health benefits associated to plant-based diets are still a matter for debate (2, 5). Although some studies did not find any association or benefits on vegetarian diet for weight management (3), studies focusing on physiological changes found that vegan diets reduced body weight (6) and a clinical trial found these diets were more effective for weight loss (7). More recently, Magkos et al. concluded there was still limited evidence that the severe restriction of meat-based foods will impact on the reduction of overweight and obesity (2). Ghadiri et al. found that among Iranian postmenopausal women with osteopenia/osteoporosis, adherence to a healthy plant-based diet is associated with a lower likelihood of bone mineral density abnormalities, whereas an unhealthy plant-based diet correlates with a significantly increased risk of such abnormalities. Analyzing the benefits from more balanced diets, such as DASH, Mediterranean and Nordic diets, the associations to longevity and health are well-known, namely in terms of lower mortality and lower risk for non-communicable diseases (2). Several studies also indicated that plant-based diets were associated with lower risk for cancer, for type 2 diabetes and cardiovascular diseases (2, 8), However, a stronger evidence to support these arguments is still necessary (2). More frequently, people choose plant-based or popular diets for health reasons, and usually those individuals are more interested in nutritional rationale, but the study from Jontez et al.'s study compares the nutrient intake and serum metabolic biomarkers of individuals on self-selected diets like LCHF, vegan, vegetarian, and omnivorous, finding that while LCHF adherents consume comparable micronutrients, their higher intake of saturated fats and cholesterol, and lower intake of fibers, may necessitate healthier fat sources and more plant-based foods to optimize nutrient levels.

More recently, plant-based diets have been associated with a need to save the planet and promote the environmental causes. A sustainable diet should ensure a good nutritional status and health of individuals. It should also have a low environmental impact and contribute to the health and wellbeing of future generations. This concept comprises a range of very different dimensions, including nutritional, social, ecological, and economical (2).

Mannucci et al. examines the complexity of sustainable nutrition with a focus on vegetable oils, emphasizing the need for interdisciplinary research and holistic approaches to address the health, environmental, and socio-economic factors in food production and consumption for future global food security.

The planet is exceeding its limits and "Planetary health" is at risk, with serious adverse effects, such as climate change, decrease in freshwater reserves, the loss of biodiversity and changes at the biogeochemical flows (8). The unprecedented growth of the world population over the past 70 years, soaring from 2.5 to 8 billion, has intensified the challenge of ensuring a sustainable and nutritious food supply for the global population. One of the reasons for having more sustainable diets is that they can contribute to the reduction of the greenhouse gases (GHG) emissions. But if the traditional food production and food patterns have an impact at the planetary health, the opposite is also true, with recent problems related to the impact of natural disasters or adverse climatic conditions at food production. In general, the main findings reveal that plan-based diets are more sustainable. It is also important to analyse the impact of the different foods and not only the type of diet since there is a high variability regarding environmental impact for each food, even if they are part of the same diet (8). Another issue to discuss is the cost of foods that have a smaller environmental footprint, since the higher prices are a limitation for the populations with lower economic resources (1). Rochefort et al. found that among French Canadian adults, diets with lower animal-based and higher plant-based protein intakes are associated with better diet quality and are more cost-effective, suggesting the benefits of such diets for health and sustainability. Following a sustainable and healthy diet was also associated to a better knowledge about sustainable nutrition and environmental responsible choices (Yassibaş and Bölükbaşi), which points the challenge of improving the populations literacy concerning this Research Topics. Nevertheless, Macit-Çelebi et al. revealing a low compliance with EAT-Lancet recommendations that is associated with higher obesity rates, highlighting the need for education to improve diet sustainability. The studies mentioned above collectively reinforce the idea that our dietary choices are not just about personal health but also about the health of our planet. As we move toward a more sustainable future, plant-based diets may hold the key to achieving both personal and environmental wellness. It's worth mentioning that more research and continued awareness are necessary to fully embrace plant-based diets and make them an integral part of our sustainable future. As individuals, we can take steps toward a more plant-centric diet, and as a society, we can support policies and practices that promote the sustainability of our food choices. By doing so, we contribute to a healthier and more sustainable future for ourselves and generations to come.

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Conflict of interest

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Healthy plant-based diet index as a determinant of bone mineral density in osteoporotic postmenopausal women: A case-control study

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Introduction: The association between plant-based diet indices and bone mineral density (BMD) of women with osteoporosis have not been studied in Iranian women. This study aimed to evaluate the association between plant-based diet indices and BMD in postmenopausal women with osteopenia/osteoporosis.

Materials and methods: The present research was a case-control study conducted on 131 postmenopausal women with osteoporosis/osteopenia and 131 healthy women. The BMD of the femoral neck and lumbar vertebrae was measured by the Dual-energy X-ray absorptiometry (DXEA) method. Participants were asked to complete a validated semi-quantitative food frequency questionnaire (FFQ). We used three versions of plant-based diet indices, including plant-based diet index (PDI), healthy plant-based diet index (hPDI), and unhealthy plant-based diet index (uPDI). Two different multivariable logistic regression was used for the crude and adjusted model to assess the relationship between PDI, hPDI, and uPDI with odds of femoral and lumbar BMD.

Results: There was a reverse association between last tertile of hPDI with femoral BMD abnormality in the both adjusted model [Model 1: odds ratio (OR): 0.33; 95% confidence interval (CI): 0.19–0.63 and Model 2: OR: 0.30; 95% CI: 0.15–0.58, respectively]. Furthermore, we found a reverse relationship between hPDI with lumbar BMD abnormality in the first adjusted model (OR: 0.36; 95% CI: 0.19–0.67). On the other hand, a negative association was observed in the second and last tertile of hPDI with lumbar BMD abnormality

(OR: 0.47; 95% CI: 0.24–0.90 and OR: 0.34; 95% CI: 0.17–0.64, respectively). According to the results, the association of femoral BMD abnormality in the last tertile of uPDI compared to the first tertile in the both adjusted models (Model 1: OR: 2.85; 95% CI: 1.52–5.36 and Model 2: OR: 2.63; 95% CI: 1.37–5.06) were significant. Also, we observed a positive relationship between the last tertile of uPDI with lumbar BMD abnormality compared to the lowest tertile in the both adjusted models (Model 1; OR: 4.16; 95% CI: 2.20–7.85, Model 2; OR: 4.23; 95% CI: 2.19–8.19).

Conclusion: Overall, the findings indicated that in postmenopausal women with osteoporosis, a healthy plant-based diet could prevent bone loss, and an unhealthy plant-based diet might have detrimental effects on BMD.

KEYWORDS

plant-based diet index, healthy plant-based diet index, unhealthy plant-based diet index, bone mass density, osteopenia

Introduction

Osteoporosis (OP) is characterized by a decrease in bone mineral density (BMD) (1), which in turn reduces bone strength and makes it susceptible to fracture (2). OP usually develops gradually and does not show symptoms until a fracture occurs (3). Although OP can affect all bones, the bones of the pelvis, ribs, lumbar vertebrae, and wrists are most commonly affected (4). The worldwide prevalence of OP is reported to be 18.3% (5). Also, the prevalence of this disease in Iranian elderly is estimated at 41.5% (6).

It has been observed that the average and maximum amount of bone mass in women is lower than in men (7). One of the reasons can be hormonal changes that cause a 40–50% decrease in maximum bone mass in women (8). However, OP affects both genders of all ages. A reduction in BMD is more common in women with the onset of menopause, which occurs around age 50 (7).

Osteoporosis (OP) is a multifactorial disease (9). Lifestyle is one of the most critical factors affecting bone density. Among the lifestyle factors, nutrition plays an essential role in bone health (10). Intake of fruits, vegetables, calcium, potassium, magnesium, vitamins D and K can help bone health (10).

Plant-based diets have become popular due to the belief that healthier diets prevent chronic diseases (11). Some studies have investigated the relationship between plant-based diets and BMD (12, 13). Plant-based diets are rich in potassium, magnesium, vitamins C and K (10, 11), and other essential nutrients in bone matrix synthesis. Although the source of plant-based dietary proteins (such as legumes, grains, nuts, seeds, vegetables, etc.) provides low biological value, a recent systematic review found no significant difference in the consumption of plant and animal protein on bone health (14). On the other hand, a plant-based diet is generally lower in saturated fat and cholesterol, and increased dietary fiber and many phytochemicals promote bone health (15).

In addition, a meta-analysis study found that participants who followed a vegetarian diet had a 27% lower risk of OP (8). Contrary to the mentioned study, one study showed that the prevalence of OP was higher in Chinese postmenopausal women who frequently consumed vegetables (16). Also, in some studies, no association was observed between the consumption of a plant-based diet and the risk of developing OP (17, 18).

So, further studies are needed to demonstrate the association between the plant-based diet and OP. To the best of our knowledge, there is little information on the relationship between plant-based diet indices and BMD in Iranian postmenopausal women with OP/osteopenia. Therefore, this study investigated the relationship between these two variables in Iranian postmenopausal women with OP/osteopenia.

Materials and methods

Study population

The present research was a case-control study conducted on 131 postmenopausal women with OP/osteopenia and 131 healthy postmenopausal women aged 45–65 years who were admitted to the Bone Densitometry Center in Isfahan, Iran (May 2021 to Dec 2021). The sample size was calculated based on the previous study considering OR = 2.30 (19). The exclusion criteria were premenopausal, use of glucocorticoids, alcohol, diabetes, renal disease, rheumatoid, cancer, and history of chemotherapy (**Figure 1**). Menopause was the absence of a menstrual cycle in the last 12 months.

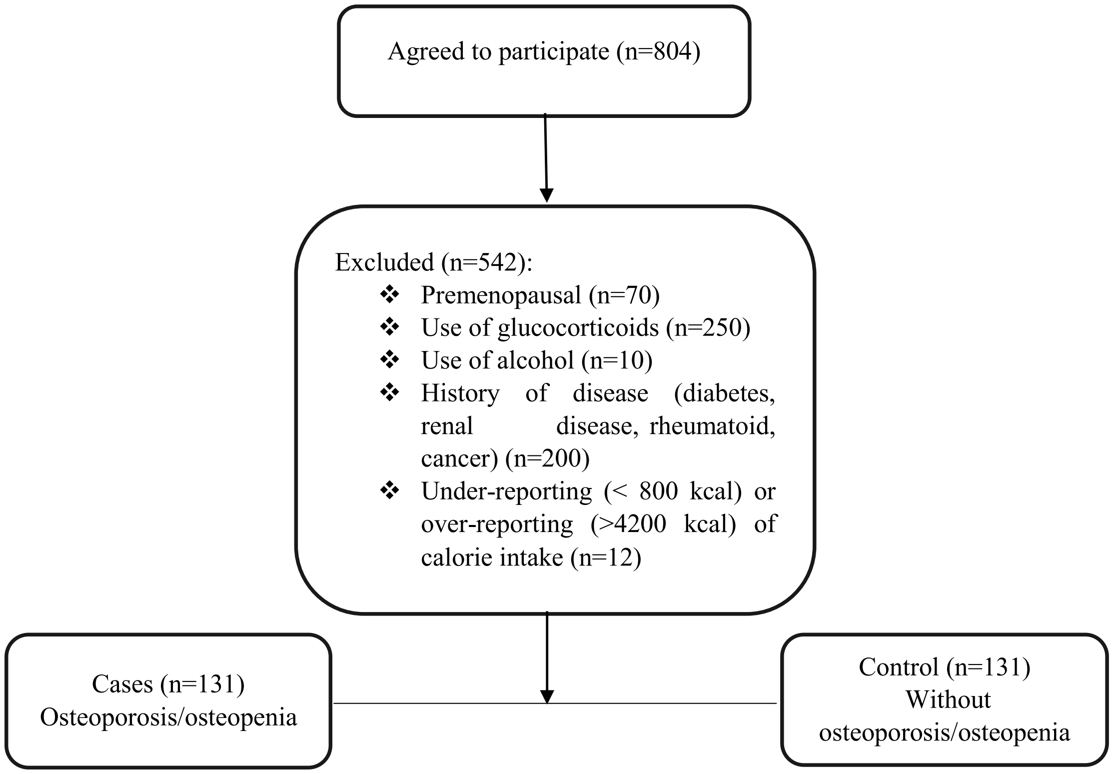

FIGURE 1
Flow chart of the study.

The height was measured without shoes using a stadiometer and the stretch stature method with an accuracy of 0.5 cm. Also, body weight was measured without shoes and with the least clothes by a digital scale and recorded with an accuracy of 100 g. In addition, body mass index (BMI) was calculated as weight divided by height (square meters). A general information questionnaire collected information on socio-demographic, confounding, and contextual variables such as socioeconomic status and taking of drugs and supplements that affect BMD.

Bone mineral density measurement

Bone mineral density (BMD) of the femoral neck and lumbar vertebrae in grams per square centimeter was measured by a technician using the Dual-energy X-ray absorptiometry (DXEA) method, and bone mass status was determined by a physician [device model: Horizon Wi (S/N 200451)]. Bone mass status was determined based on World Health Organization (WHO) criteria, according to which T-score values greater than −1 indicate normal bone mass, between −1 and −2.5 indicate osteopenia, and less than −2.5 indicate osteoporosis (20). Case samples were selected from individuals diagnosed with osteopenia and osteoporosis by a physician. But, control participants were selected from healthy postmenopausal volunteers with normal bone mass and other inclusion criteria who referred to the Bone Densitometry Center in Isfahan at the same time.

Information about the level of physical activity was collected using the International Physical Activity Questionnaire (IPAQ) (21). Physical activity of the participants based on MET (metabolic equivalent of task)-minutes and using the standard protocol were divided into three physical activity classes including low activity (below 600 MET-minutes/week), moderate (between 600 and 3,000 MET-minutes/week), and intense activity (above 3,000 MET-minutes/week).

Dietary assessment and food grouping

Participants were asked to complete a validated semi-quantitative food frequency questionnaire (FFQ) (22) that included questions on their habitual daily consumption of food items during the past year. We used previous method to create three versions of plant-based diets, including plant-based diet index (PDI), healthy plant-based diet index (hPDI), and unhealthy plant-based diet index (uPDI) (23–25). All foods reported through the FFQ were classified into 18 food groups with three main classes: healthy plant foods (i.e., whole grains, fruits, vegetables, nuts, legumes, vegetable oils, and tea/coffee), unhealthy plant foods (i.e., fruit juices, sugar-sweetened beverages, refined grains, potatoes, and sweets/desserts), and animal foods (i.e., animal fat, dairy, egg, fish/seafood, meat, and miscellaneous animal-based foods). In PDI and hPDI, the highest consumption of plant foods and healthy plant foods got 10 scores, and the lowest consumption got 1 score. For unhealthy, a plant food score of 1 was considered for the highest consumption and 10 scores for the lowest consumption of unhealthy plant food. Scores for each of the PDI, hPDI, and uPDI were summed to obtain a score ranging from 18 to 180. A higher total score for each index indicated higher adherence to that dietary pattern.

Ethics statement

The Ethics Committee of Tabriz University of Medical Sciences approved the protocols and procedures (Ethical Approval code: IR.TBZMED.REC.1400.114), and informed written consent was obtained from all participants after being informed about the purpose of this research.

Statistical analysis

Participants were classified based on tertiles of PDI, hPDI, and uPDI. First, we used the residual method to adjust all the consumed food item (26). The Kolmogorov-Smirnov test was used to examine the normal distribution of the data. The baseline characteristics of the participants were described by means and standard deviations (SD) for the continuous variables and frequencies (percentages) for the categorical variables. Independent samples *T*-test and chi-square test were used to compare the continuous and categorical variables, respectively. Also, the ANOVA test was used for nutrient and food group analysis. Two different multivariable logistic regression were used for the crude and adjusted models to assess the relationship between PDI, hPDI, and uPDI with the odds of femoral and lumbar abnormality. We controlled the effects of BMI and age in the first model. Income, education, physical activity, and taking calcium supplements were added to the second model. Statistical analyses were carried out using SPSS (version 20.0, Inc., Chicago, IL, USA). *P*-value < 0.05 was considered statistically significant.

Results

Mean age, BMI, femoral and lumbar BMD, taking vitamin D supplements, and demographic data of the case and control groups are shown in **Table 1**. According to the results, age ($P = 0.03$), femoral and lumbar BMD ($P < 0.001$ for both), physical activity level ($P = 0.01$), education level ($P < 0.001$), and vitamin D use ($P = 0.01$) was different between the case and control groups.

Nutrient intake between tertiles of PDI, hPDI, and uPDI are reported in **Table 2**. Intakes of energy ($P < 0.001$), carbohydrate ($P < 0.001$), fat ($P < 0.001$), fiber ($P = 0.001$), MUFA ($P < 0.001$), PUFA ($P < 0.001$), vitamin E ($P < 0.001$), folate ($P < 0.001$), sodium ($P < 0.001$), magnesium ($P < 0.001$), iron ($P < 0.001$), selenium ($P < 0.001$), copper ($P < 0.001$), phosphorus ($P = 0.036$), and zinc ($P = 0.02$) was higher in the last tertile of PDI compared to the first tertile.

In the last tertile of hPDI, the consumption of fiber ($P < 0.001$), vitamin C ($P < 0.001$), vitamin K ($P = 0.001$), vitamin A ($P = 0.003$), phosphorus ($P = 0.003$), and magnesium ($P = 0.009$) was higher compared to the first tertile, but intakes of energy ($P = 0.006$), carbohydrate ($P = 0.02$), fat ($P = 0.001$), sodium ($P < 0.001$), saturated fatty acid (22) ($P < 0.006$), and vitamin B_{12} ($P = 0.02$) was lower in the last tertile (**Table 2**). In the uPDI group, the consumption of all nutrients [vitamin E ($P = 0.009$), sodium ($P = 0.01$), and other nutrients ($P < 0.001$)] were lower in the last tertile than in the first tertile (**Table 2**).

The intake of food groups among tertiles of PDI, hPDI, and uPDI were presented in **Table 3**. According to **Table 3**, the consumption of whole ($P = 0.001$) and refined grains ($P = 0.003$), legumes ($P = 0.03$), vegetable oils ($P < 0.001$), tea/coffee ($P < 0.001$), fruit juice ($P = 0.003$), potato ($P < 0.001$), sugar-sweetened beverages ($P < 0.001$), sweets and desserts ($P < 0.001$) of participants in the last tertile of PDI was significantly higher, but intakes of dairy ($P = 0.03$), fish and seafood ($P = 0.01$) were lower in the last tertile compared to the first tertile.

Individuals in the last tertile of hPDI had higher intakes of whole grains ($P < 0.001$), fruits ($P < 0.001$), vegetables ($P < 0.001$), and legumes ($P = 0.004$) compared to the first tertile. In contrast, consumption of refined grains ($P < 0.001$), potato ($P < 0.001$), sugar-sweetened beverages ($P < 0.001$), sweets and desserts ($P < 0.001$), animal fat ($P < 0.001$), egg ($P < 0.001$), meats ($P = 0.001$), and animal-based foods ($P < 0.001$) was lower than the first tertile (**Table 3**).

The lower intakes of whole grains ($P = 0.002$), fruits ($P < 0.001$), vegetables ($P < 0.001$), nuts ($P < 0.001$), legumes ($P < 0.001$), vegetable oils ($P = 0.03$), dairy ($P < 0.001$), egg ($P = 0.01$), fish and seafood ($P < 0.001$), meat ($P < 0.001$), and higher intakes of refined grains ($P < 0.001$), sweets, and desserts ($P = 0.01$) was seen in the participants of the last tertile compared to the first tertile of uPDI group (**Table 3**).

According to **Table 4**, there was no relationship between PDI and femoral and lumbar BMD abnormalities in the crude and both adjusted models. As shown in **Table 4**, there was a reverse association between the second and last tertile of hPDI with femoral BMD abnormality in the crude model [odds ratio (OR): 0.43; 95% confidence interval (CI): 0.23–0.80 and OR: 0.35; 95% CI: 0.19–0.66, respectively] and both adjusted model (Model 1: OR: 0.38; 95% CI: 0.20–0.72 and OR: 0.33; 95% CI: 0.19–0.63,

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TABLE 1 Baseline characteristics of the study participants.

Variables	Control (n = 131)	Case (n = 131)	P-value
Age (year)	56.47 ± 5.91	57.95 ± 5.42	**0.03**
BMI (kg/m^2)	29.13 ± 3.31	29.78 ± 3.99	0.15
BMD femoral (g/cm^2)	0.78 ± 0.07	0.64 ± 0.09	**<0.001**
BMD lumbar (g/cm^2)	1.00 ± 0.08	0.81 ± 0.09	**<0.001**
Income, average (%)	53 (40.5)	65 (49.6)	0.08
Physical activity, moderate (%)	22 (16.8)	9 (6.9)	**0.01**
Education level (%)			**<0.001**
Under diploma	65 (49.6)	98 (74.8)	
Diploma	52 (39.7)	25 (19.1)	
Higher diploma	14 (910.7)	8 (6.1)	
Calcium supplement (%)			0.55
Yes	32 (24.4)	32 (24.4)	
No	99 (75.6)	99 (75.6)	
Vitamin D supplement (%)			**0.01**
Yes	76 (58.0)	58 (44.3)	
No	55 (42.0)	73 (55.7)	

BMI, body mass index; BMD, bone mineral density.
Values are shown as mean for continuous and percentage for categorical variables.
Using independent samples T-test for continuous and chi-square test for categorical variables. Significant p-values are indicated in bold.

respectively and Model 2: OR: 0.31; 95% CI: 0.15–0.61, and OR: 0.30; 95% CI: 0.15–0.58, respectively).

Furthermore, we found a reverse relationship between hPDI with lumbar BMD abnormality in the crude model (OR: 0.36; 95% CI: 0.20–0.68) and the first adjusted model (OR: 0.36; 95% CI: 0.19–0.67). On the other hand, a negative association was observed in the second and last tertile of hPDI with lumbar BMD abnormality (OR: 0.47; 95% CI: 0.24–0.90 and OR: 0.34; 95% CI: 0.17–0.64, respectively).

According to the results, the association of femoral BMD abnormality in the last tertile of uPDI compared to the first tertile in the crude (OR: 2.64; 95% CI: 1.43–4.88) and both adjusted models (Model 1: OR: 2.85; 95% CI: 1.52–5.36 and Model 2: OR: 2.63; 95% CI: 1.37–5.06) were significant. Also, we observed a positive relationship between the last tertile of uPDI with lumbar BMD abnormality compared to the lowest tertile in the crude (OR: 3.97; 95% CI: 2.12–7.42) and both adjusted models (Model 1; OR: 4.16; 95% CI: 2.20–7.85, Model 2; OR: 4.23; 95% CI: 2.19–8.19).

Discussion

In the present case-control study, it was shown that higher scores of PDI indicating a higher intake of whole grains, legumes, vegetable oil, tea and coffee, fruit juices, refined grains, potatoes, sugar-sweetened beverages, sweets, and desserts were not significantly related to BMD abnormality of the femoral neck and lumbar vertebrae. Previous studies have shown conflicting findings regarding the relationship between vegetarian diet and bone density (27). In a study conducted by Shahinfar et al., it was shown that there is no significant relationship between PDI and osteocalcin as a biomarker of bone formation among older adults (28). It has also been shown that vegetarians are not subjected to a higher risk of developing OP than non-vegetarians (29). In contrast, a review study demonstrated that a plant-based diet reduces the density of the femoral neck, lumbar spine, and whole body compared to an omnivorous diet (11).

Bone is a living tissue sensitive to body conditions. Subtle changes in acid-base balance and nutrient intake can alter bone metabolism and long-term bone density (30, 31). Decreased BMD is an important risk factor for fractures throughout life (32). A plant-based diet has positive effects on bones, including the consumption of fruits and vegetables, which help to maintain calcium in the body due to the presence of potassium (33). This diet has more antioxidants, phytochemicals, and vitamins and imposes less acid load on the body (34). Also, the negative effects of this diet include lower amounts of protein, higher phytic acid and as a result less absorption of zinc from the diet (35), and lower amounts of calcium and vitamin D, all of which are considered important for improving bone health (34, 36). The interaction between the positive and negative effects of a plant-based diet neutralizes each other, which may be why a plant-based diet does not significantly affect BMD. However, plant-based diets affect multiple inflammatory and

TABLE 2 Nutrients intakes between tertiles of plant-based diet index (PDI), healthy plant-based diet index (hPDI), and unhealthy plant-based diet index (uPDI).

Variables	PDI				hPDI				uPDI			
	T1 (*n* = 88)	T2 (*n* = 85)	T3 (*n* = 88)	*P*	T1 (*n* = 90)	T2 (*n* = 87)	T3 (*n* = 84)	*P*	T1 (*n* = 88)	T2 (*n* = 83)	T3 (*n* = 90)	*P*
Energy (kcal/d)	1961.95 ± 247.88	2161.47 ± 337.90	2257.15 ± 409.28	**<0.001**	2219.61 ± 376.18	2104.17 ± 362.39	2050.18 ± 316.83	**0.006**	2324.99 ± 400.36	2100.44 ± 276.48	1959.64 ± 287.00	**<0.001**
Carbohydrate (g/day)	286.81 ± 36.84	319.59 ± 49.66	336.79 ± 56.70	**<0.001**	325.19 ± 58.96	313.30 ± 52.00	303.92 ± 43.44	**0.02**	399.07 ± 57.96	310.57 ± 42.46	294.10 ± 45.90	**<0.001**
Protein (g/day)	65.55 ± 11.93	68.17 ± 11.81	67.90 ± 14.95	0.34	68.29 ± 11.77	66.00 ± 13.53	67.26 ± 13.74	0.50	77.35 ± 12.64	67.98 ± 9.15	56.67 ± 6.79	**<0.001**
Fat (g/day)	68.41 ± 8.80	75.34 ± 14.35	78.69 ± 16.92	**<0.001**	78.45 ± 14.65	73.03 ± 15.07	70.65 ± 12.23	**0.001**	81.75 ± 15.96	72.85 ± 11.52	67.99 ± 11.72	**<0.001**
Fiber (g/day)	29.41 ± 6.38	30.98 ± 4.95	32.83 ± 6.32	**0.001**	28.48 ± 4.96	30.90 ± 5.25	34.09 ± 6.65	**<0.001**	36.35 ± 5.79	30.39 ± 4.13	26.61 ± 3.35	**<0.001**
SFA (g/day)	18.20 ± 3.92	18.80 ± 4.44	19.15 ± 6.09	0.43	20.02 ± 4.59	18.26 ± 5.19	17.78 ± 4.70	**0.006**	21.63 ± 5.20	18.80 ± 4.09	15.82 ± 3.44	**<0.001**
MUFA (g/day)	25.11 ± 3.07	27.03 ± 4.56	28.08 ± 5.86	**<0.001**	27.33 ± 4.40	26.64 ± 5.31	26.21 ± 4.61	0.29	29.55 ± 5.85	26.46 ± 3.69	24.29 ± 2.67	**<0.001**
PUFA (g/day)	17.52 ± 2.98	18.89 ± 3.71	20.47 ± 3.67	**<0.001**	19.33 ± 3.84	19.11 ± 3.52	26.21 ± 4.61	0.23	20.74 ± 4.16	18.58 ± 3.38	17.61 ± 2.59	**<0.001**
Vitamin A (RAE/day)	464.05 ± 265.35	481.53 ± 255.65	508.93 ± 293.57	0.54	432.59 ± 207.29	461.34 ± 178.98	566.18 ± 377.31	**0.003**	677.67 ± 337.66	452.03 ± 156.62	328.66 ± 140.59	**<0.001**
Vitamin E (mg/day)	20.87 ± 4.09	21.77 ± 4.33	23.63 ± 4.73	**<0.001**	21.75 ± 4.77	22.62 ± 4.00	21.94 ± 4.78	0.40	23.31 ± 5.09	21.50 ± 4.10	21.47 ± 4.13	**0.009**
Vitamin K (μg/day)	123.60 ± 74.79	123.50 ± 70.15	142.60 ± 80.40	0.15	113.53 ± 57.30	123.45 ± 54.06	154.70 ± 102.02	**0.001**	169.72 ± 89.67	126.14 ± 63.24	95.18 ± 48.59	**<0.001**
Vitamin B_6 (mg/day)	1.64 ± 0.36	1.70 ± 0.31	1.73 ± 0.39	0.19	1.64 ± 0.30	1.67 ± 0.33	1.76 ± 0.42	0.05	1.98 ± 0.37	1.66 ± 0.23	1.43 ± 0.17	**<0.001**
Folate (μg/day)	427.99 ± 71.79	465.38 ± 69.25	498.01 ± 83.92	**<0.001**	474.71 ± 79.41	457.38 ± 79.04	458.95 ± 82.66	0.28	500.72 ± 88.87	456.18 ± 61.03	435.35 ± 74.33	**<0.001**
Vitamin B_{12} (μg/day)	2.88 ± 1.08	2.98 ± 1.43	2.79 ± 1.69	0.68	3.14 ± 1.37	2.95 ± 1.59	2.55 ± 1.24	**0.02**	3.61 ± 1.62	3.05 ± 1.15	2.04 ± 0.95	**<0.001**
Vitamin C (mg/day)	137.52 ± 66.41	137.98 ± 68.13	153.21 ± 75.41	0.24	118.37 ± 62.76	142.88 ± 57.01	169.80 ± 80.55	**<0.001**	196.23 ± 76.98	135.91 ± 47.24	97.99 ± 41.05	**<0.001**
Vitamin D (μg/day)	1.09 ± 0.80	0.78 ± 0.74	0.60 ± 0.59	**<0.001**	0.93 ± 0.79	0.78 ± 0.76	0.72 ± 0.63	**0.203**	1.08 ± 0.79	1.00 ± 0.80	0.41 ± 0.36	**<0.001**
Sodium (mg/day)	3499.82 ± 412.96	3664.86 ± 525.91	3888.30 ± 579.82	**<0.001**	3863.37 ± 564.62	3599.56 ± 509.74	3581.28 ± 477.37	**<0.001**	3815.11 ± 595.41	3598.98 ± 441.12	3638.57 ± 530.06	**0.01**
Calcium (mg/day)	511.09 ± 275.79	490.20 ± 251.98	466.16 ± 360.78	0.61	475.28 ± 253.04	458.98 ± 299.20	535.12 ± 342.90	0.21	705.79 ± 301.86	501.55 ± 243.30	268.05 ± 153.57	**<0.001**
Magnesium (mg/day)	390.43 ± 71.38	418.62 ± 61.09	439.97 ± 80.52	**<0.001**	403.94 ± 73.10	410.13 ± 70.96	436.41 ± 75.37	**0.009**	473.77 ± 67.50	418.10 ± 60.50	359.38 ± 41.41	**<0.001**
Phosphorus (mg/day)	2953.33 ± 742.80	3200.72 ± 759.01	3297 ± 924.99	**0.036**	3008.40 ± 721.25	3042.03 ± 751.09	3417.48 ± 931.17	**0.003**	3809 ± 798.74	3181.24 ± 645.23	2523.86 ± 398.38	**<0.001**
Iron (mg/day)	13.99 ± 1.89	15.31 ± 1.70	16.18 ± 2.229	**<0.001**	15.34 ± 2.21	14.94 ± 2.19	15.21 ± 2.12	0.46	16.36 ± 2.48	15.07 ± 1.69	14.10 ± 1.59	**<0.001**
Selenium (μ/day)	116.65 ± 16.60	123.02 ± 16.38	129.83 ± 18.30	**<0.001**	125.38 ± 17.69	121.42 ± 18.63	122.69 ± 17.39	0.32	129.65 ± 20.68	124.03 ± 16.63	116.20 ± 13.25	**<0.001**
Zinc (mg/day)	10.46 ± 2.05	11.22 ± 2.19	11.35 ± 2.58	**0.02**	11.27 ± 2.48	10.77 ± 2.17	10.99 ± 2.26	0.34	12.48 ± 2.14	11.26 ± 2.07	9.36 ± 1.50	**<0.001**
Copper (mg/day)	1.45 ± 0.24	**1.60** ± 0.25	1.68 ± 0.30	**<0.001**	1.57 ± 0.29	1.57 ± 0.30	1.59 ± 0.25	0.91	1.77 ± 0.27	1.58 ± 0.24	1.38 ± 0.18	**<0.001**

PDI, plant-based diet index; hPDI, healthy plant-based diet index; uPDI, unhealthy plant-based diet index; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; RAE, retinol activity equivalents.
Values are presented as mean ± SD.
Using one-way ANOVA. Significant *p*-values are indicated in bold.

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TABLE 3 The intake of food groups between tertiles of plant-based diet index (PDI), healthy plant-based diet index (hPDI), and unhealthy plant-based diet index (uPDI).

Variables	PDI				hPDI				uPDI			
	T1 (*n* = 88)	T2 (*n* = 85)	T3 (*n* = 88)	*P*	T1 (*n* = 90)	T2 (*n* = 87)	T3 (*n* = 84)	*P*	T1 (*n* = 88)	T2 (*n* = 83)	T3 (*n* = 90)	*P*
Whole grains (g/day)	200.15 ± 45.20	211.35 ± 43.33	226.98 ± 49.45	**0.001**	194.84 ± 39.93	213.30 ± 46.73	232.04 ± 47.98	**<0.001**	226.53 ± 50.09	210.94 ± 49.34	201.49 ± 38.93	**0.002**
Fruits (g/day)	443.75 ± 203.74	443.41 ± 197.47	469.34 ± 210.85	0.62	369.47 ± 178.37	452.78 ± 165.30	541.65 ± 228.41	**<0.001**	604.35 ± 207.80	439.53 ± 144.01	317.01 ± 136.12	**<0.001**
Vegetables (g/day)	235.82 ± 118.26	232.32 ± 110.12	241.03 ± 120.84	0.88	198.83 ± 85.97	231.10 ± 98.20	282.76 ± 143.86	**<0.001**	322.35 ± 129.27	216.97 ± 81.23	171.15 ± 71.50	**<0.001**
Nuts (g/day)	8.11 ± 1.36	12.42 ± 1.37	9.92 ± 1.25	0.07	11.60 ± 1.85	9.43 ± 0.88	9.22 ± 0.96	0.37	13.18 ± 1.05	11.94 ± 1.55	5.50 ± 1.25	**<0.001**
Legumes (g/day)	23.77 ± 16.64	26.73 ± 12.03	29.48 ± 14.35	**0.03**	24.91 ± 13.10	24.35 ± 11.91	30.98 ± 17.65	**0.004**	32.85 ± 16.62	26.78 ± 13.09	20.58 ± 11.05	**<0.001**
Vegetable oils (g/day)	26.82 ± 5.10	29.24 ± 6.04	32.02 ± 5.56	**<0.001**	29.27 ± 6.17	30.38 ± 6.03	28.44 ± 5.52	0.10	30.56 ± 7.65	28.22 ± 5.30	29.27 ± 4.23	**0.03**
Tea and coffee (g/day)	584.82 ± 326.66	770.48 ± 374.91	948.74 ± 444.43	**<0.001**	757.74 ± 396.28	758.08 ± 417.55	791.47 ± 427.11	0.82	735.38 ± 360.80	729.64 ± 353.02	836.46 ± 496.05	0.15
Fruit juices (g/day)	1.36 ± 0.41	3.43 ± 0.85	7.26 ± 1.87	**0.003**	4.40 ± 0.86	4.49 ± 1.12	3.10 ± 1.69	0.68	3.37 ± 0.83	5.76 ± 1.87	3.05 ± 0.86	0.25
Refined grains	207.84 ± 82.71	244.01 ± 96.70	256.79 ± 108.47	**0.003**	290.56 ± 97.85	235.42 ± 75.78	178.14 ± 86.57	**<0.001**	199.65 ± 91.16	236.47 ± 76.85	271.30 ± 110.19	**<0.001**
Potatoes (g/day)	10.30 ± 1.05	15.59 ± 1.14	19.68 ± 1.32	**<0.001**	21.46 ± 1.25	15.29 ± 1.03	8.33 ± 1.01	**<0.001**	13.45 ± 1.48	14.77 ± 1.12	17.29 ± 1.08	0.08
Sugar-sweetened beverages (g/day)	8.91 ± 2.15	15.81 ± 2.47	31.85 ± 6.05	**<0.001**	30.07 ± 4.20	23.05 ± 5.21	2.64 ± 1.02	**<0.001**	13.12 ± 4.65	19.83 ± 3.51	23.76 ± 4.06	0.17
Sweets and desserts (g/day)	23.95 ± 15.00	41.29 ± 37.91	41.18 ± 21.73	**<0.001**	49.58 ± 38.68	33.76 ± 21.64	21.83 ± 13.86	**<0.001**	31.42 ± 31.24	32.20 ± 19.14	42.25 ± 29.41	**0.01**
Animal fat (g/day)	2.36 ± 0.42	2.91 ± 0.39	1.86 ± 0.36	0.17	3.90 ± 0.50	2.33 ± 0.34	0.76 ± 0.18	**<0.001**	2.51 ± 0.43	2.68 ± 0.41	1.95 ± 0.34	0.39
Dairy (g/day)	279.22 ± 141.56	246 ± 142.58	215.57 ± 194.68	**0.03**	250.79 ± 140.82	239.57 ± 172.14	250.58 ± 177.85	0.87	340.01 ± 173.88	266.04 ± 146.49	139.68 ± 90.44	**<0.001**
Egg (g/day)	14.21 ± 6.81	11.95 ± 6.56	12.85 ± 7.42	0.10	15.39 ± 6.67	11.91 ± 6.53	11.58 ± 7.16	**<0.001**	14.39 ± 7.77	13.43 ± 6.25	11.32 ± 6.52	**0.01**
Fish and seafood (g/day)	4.79 ± 0.64	3.70 ± 0.60	2.54 ± 0.26	**0.01**	3.17 ± 0.32	3.43 ± 0.38	4.47 ± 0.79	0.19	6.54 ± 0.77	2.97 ± 0.28	1.54 ± 0.16	**<0.001**
Meat (g/day)	37.03 ± 12.59	39.66 ± 11.80	36.22 ± 15.81	0.21	40.85 ± 13.76	38.55 ± 14.09	33.12 ± 11.62	**0.001**	42.71 ± 14.74	38.00 ± 10.55	32.32 ± 12.98	**<0.001**
Animal-based foods (g/day)	4.77 ± 0.58	5.56 ± 0.49	5.90 ± 0.56	0.33	8.40 ± 0.60	4.98 ± 0.47	2.62 ± 0.31	**<0.001**	5.74 ± 0.56	5.85 ± 0.53	4.69 ± 0.55	0.25

PDI, plant-based diet index; hPDI, healthy plant-based diet index; uPDI, unhealthy plant-based diet index.
Values are presented as mean ± SD.
Using one-way ANOVA. Significant *p*-values are indicated in bold.

TABLE 4 Crude and multivariable-adjusted odds ratios and 95% CIs across tertile of plant-based diet index (PDI), healthy plant-based diet index (hPDI), and unhealthy plant-based diet index (uPDI).

Variables	Femoral BMD abnormality			Lumbar BMD abnormality		
	Crude	Adjusted model 1	Adjusted model 2	Crude	Adjusted model 1	Adjusted model 2
PDI						
T1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
T2	0.62 (0.34–1.14)	0.68 (0.36–1.26)	0.71 (0.37–1.36)	0.81 (0.44–1.47)	0.86 (0.47–1.58)	0.90 (0.48–1.69)
T3	0.75 (0.41–1.36)	0.80 (0.43–1.48)	0.74 (0.39–1.40)	1.11 (0.61, 2.02)	1.20 (0.65–2.20)	1.10 (0.57–2.06)
P_{trend}	0.35	0.48	0.37	0.71	0.55	0.72
hPDI						
T1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
T2	0.43 (0.23–0.80)	0.38 (0.20–0.72)	0.31 (0.15–0.61)	0.58 (0.31–1.06)	0.55 (0.30–1.01)	0.47 (0.24–0.90)
T3	0.35 (0.19–0.66)	0.33 (0.19–0.63)	0.30 (0.15–0.58)	0.36 (0.20, 0.68)	0.36 (0.19–0.67)	0.34 (0.17–0.64)
P_{trend}	**0.001**	**0.001**	< **0.001**	**0.001**	**0.001**	**0.001**
uPDI						
T1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
T2	1.09 (0.59–1.99)	1.03 (0.55–1.90)	1.08 (0.57–2.04)	1.40 (0.76–2.58)	1.36 (0.74–2.52)	1.37 (0.73–2.59)
T3	2.64 (1.43–4.88)	2.85 (1.52–5.36)	2.63 (1.37–5.06)	3.97 (2.12–7.42)	4.16 (2.20–7.85)	4.23 (2.19–8.19)
P_{trend}	**0.002**	**0.001**	**0.004**	< **0.001**	< **0.001**	< **0.001**

BMD, bone mass density; PDI, plant-based diet index; hPDI, healthy plant-based diet index; uPDI, unhealthy plant based diet index.
Model 1: adjusted for BMI and age.
Model 2: additionally, adjusted for income, education, physical activity, and calcium supplement.
These values are shown as odds ratio (95% CIs). Obtained from logistic regression. Significant *p*-values are indicated in bold.

systemic responses, such as microbiota metabolites (37, 38). There are findings in animal models that microbiota act as crucial mediators in bone remodeling (12). It is also shown that diet can alter the diversity of an individual's microbiota in human models, suggesting a mechanism by which plant-based diets can affect bone health (12).

As shown in the current study, the higher hPDI scores are correlated with greater protective effects against the decrease in the femoral neck and lumbar vertebrae BMD. A study implemented by Shahinfar et al. it was indicated that there was no significant relationship between hPDI and osteocalcin (28). The present case-control study indicated that higher hPDI scores are associated with a higher intake of fruits and vegetables, which was statistically significant. It has been demonstrated that more fruits and vegetables are related to more BMD and OP risk reduction in middle-aged and older adults (39). This effect of fruits and vegetables may be because they are good potassium, calcium, and magnesium sources, which can neutralize the effects of calcium excretion in urine caused by dietary acid (40).

It was observed that uPDI significantly increases the chance of abnormality in BMD in both the femoral neck and lumbar vertebrae regions. As shown in the present study, uPDI is associated with a decrease in the intake of whole grains, fruits, vegetables, nuts, legumes, and dairy products, all of which contribute to increased bone density (15, 41, 42). The results of a study investigating the relationship between uPDI and osteocalcin showed that there is an inverse relationship between uPDI and osteocalcin (28). Osteocalcin plays an important role in regulating bone mineralization, osteoblast, and osteoclast activity (43). Therefore, an unhealthy plant-based diet can reduce the amounts of osteocalcin, which may reduce bone formation and BMD.

If we want to point out the limitations of the present study, we can mention examining dietary intake through the FFQ questionnaire, which depends on long-term memory. Also, this questionnaire can overestimate the intake of nutrients. However, FFQ is the most common dietary assessment method in epidemiological studies and is an easy and effective tool for collecting dietar y information.

Overall, the findings indicated a healthy plant-based diet could exert a protective effect in preventing bone loss. In contrast, an unhealthy plant-based diet can negatively affect BMD in postmenopausal OP women. Consuming proteins with high biological value, vegetables, fruits, legumes, whole grains, soy products, and nuts can help bone health. Therefore, properly planning a vegetarian diet is not only harmful to bone health but can also hold protective effects in preventing bone loss. Further studies are necessary to investigate the generalizability of these findings to other populations with different demographic or biological characteristics.

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 Research Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (IR.TBZMED.REC.1400.114). The patients/participants provided their written informed consent to participate in this study.

Author contributions

EC, MG, ZS, and SG contributed to the data collection and writing the first draft. MN and SG contributed to the statistical analysis and interpretation of data. BG contributed in funding and project management. All authors 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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Genotypic variation in Na, K and their ratio in 45 commercial cultivars of Indian tropical onion: A pressing need to reduce hypertension among the population

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The intake of diets with higher sodium (Na) and lower potassium (K) has been considered a leading factor for the development of hypertension (HTN). Majority of junk, processed and packaged food have higher Na contents. To counter the effects of diet on HTN, the identification of high K/Na ratio plant-based food is needed. Among fruits and vegetables, onion could be the ideal option since it contains high K content. Keeping this in mind, 45 commercially well adapted short day Indian onion cultivars were evaluated for K and Na content and their ratio to isolate suitable cultivars to prevent HTN in the Indian population. The data suggested wide variation among the genotypes for K, Na, and K/Na ratio ranging from 490.2 ± 17.0 to 9160.0 ± 96.7 mg/kg on dry matter basis, 52.7 ± 3.0 to 458.2 ± 61.7 mg/kg on dry matter basis and 3.1 ± 0.7 to 109.5 ± 17.3, respectively. The K content was recorded as significantly highest in the yellow-coloured bulb variety "Arka Pitamber" (9160.1 ± 96.7) followed by Pusa Sona (7933.2 ± 292.8). On the other hand, minimal K was assessed in the white-coloured bulb variety "Agrifound White" (490.3 ± 17.0) followed by Udaipur Local (732.9 ± 93.4). Twelve cultivars exhibited > 7000 mg K content, while nine cultivars recorded < 1500 mg. On the contrary, Na was recorded as significantly highest in the dark-red-coloured bulbs and the lowest in white bulbs. Furthermore, it was determined that there was a more than 35-fold difference observed between the highest (109.5) and lowest (3.1) K/Na ratio in the bulbs of tested cultivars. Cluster analysis revealed three major groups comprising of 23, 13 and 9 genotypes. This information could form the base for public health, food and onion researchers to design suitable cultivars to prevent HTN as a population-wide approach. The next century is going to be food-based for the amelioration of human diseases in a sustainable way without any after-effects on the human body.

KEYWORDS

***Allium cepa* L., cardiovascular diseases, hypertension, onion bulbs, potassium, potassium/sodium ratio, sodium**

1. Introduction

Globally, non-communicable diseases (NCDs) are becoming the foremost cause of death. Approximately 80% of deaths due to NCDs occur in countries with low to middle incomes (1). Principally, NCDs consisting of cardiovascular diseases (CVDs), diabetes, various types of cancer and chronic lung dysfunction are responsible for the majority of deaths. According to one of the estimates in 2010, about 1.39 billion individuals (31.1%) in the adult population were suffering from hypertension (HTN); this number has been constantly increasing since then. Therefore, HTN has now become a major health issue worldwide (2). Among the various factors causing NCDs and metabolic disorders, unhealthy diets are a prominent cause of HTN. According to WHO observations, people from low to middle income countries consume table salt much more than is recommended (1). According to a previous study, global mean sodium intake was quite high (3.95 gm per day) in 2010 compared to the recommended intake (< 2.3 gm per day) in major published guidelines.

Onion (*Allium cepa* L., $2n = 2\times = 16$), a bulbous vegetable and condiment crop, belonging to the *Amaryllidaceae* family, is one of the most important crop which has been domesticated and cultivated worldwide for more than 5000 years due to their peculiar properties as food, their therapeutic value and ethnopharmacological properties. This crop is grown in all climates worldwide (3–6) and is the third most important horticultural crop after potato and tomato (7). Its bulbs are an enriched source of various health promoting phytochemicals and nutrients and this crop has an utmost valorisation globally due to its multifarious uses in every community and society across the globe. The Queen of French cuisine, Julia Child, stated: "It is hard to imagine a civilisation without onions." Every community, region and country have various traditional and folk remedies but there is a great need to document them in a systematic way to form the foundation of more scientific and modern research on that particular aspect.

The World Health Organization (WHO) also endorses the use of fresh onion extracts for treating colds, coughs, bronchitis, asthma, and appetite loss, as well as relieving hoarseness and preventing atherosclerosis (8). Because of its naturally possession of higher amounts of flavonoids and widely popular across the world, the onion crop became an interesting and fascinating vegetable (9, 10). Onions are recommended to lighten blood and lymph stagnation and to improve sexual debility or weakness. Regularly taken on an empty stomach, a mixture of white onion and honey was considered as an exceptional aphrodisiac tonic (11). Being the leading country in onion production, Indian farmers harvested 26.7 million tons from 1.4-million-hectares (12).

The 21st century is going to work on the principle of "Food as Medicine" and onion will surely play a large role in this. Since antiquity, the bulbous onion has played an important role in human health as it is being used in every kitchen in India. Most of the breeding experiments focused only on enhancing yield and yield-attributing components. However, little focus has been given to improving various quality characteristics. In Indian onions, not much scientific data on nutritional properties are available (13). Onion bulbs are enriched with potassium, vitamin C, folic acid and dietary fibre, also possessing good amounts of iron and calcium; however, they are lower in sodium and fat (8, 14–16). In USA onion cultivars, Metrani et al. (17) quantified 13,550.1 mg/kg potassium in red onion bulbs.

The comprehensive information of the genotypic difference in the potassium and sodium concentration in onion bulbs could be an epitomized contribution for people who are suffering from HTN and prone to CVD. The ratio of potassium and sodium across the genotypes varies with bulb colour and geographical location, which may support the development of future cultivars which are nutrition- and disease-specific.

HTN and CVD may be the result of metabolic syndrome; this has received the global attention of nutrition and health researchers (18). Potassium and sodium are the most important elements, being essential for normal and proper cellular functioning in the body. As it enhances the risks of high blood pressure, HTN (19, 20), CVD (21, 22), and obesity (23, 24) higher sodium and lower potassium dietary intake has become a serious global health challenge. Global research reports revealed that adverse ratio of both electrolytes is strongly linked to blood pressure (20, 21, 25). It is well documented that the dietary Na:K ratio is an independent risk factor for metabolic syndrome. Furthermore, it was suggested to modify ratios, including lower Na intakes and higher K intakes, to prevent metabolic disorders (18).

Keeping this in mind, the present study was conducted with the aim (a) to evaluate the potential cultivars representing diverse bulb colours and geographical locations with higher available potassium levels for utilisation in future onion breeding programs, (b) to identify the genotypes of Indian onions possessing the lowest sodium content in their bulbs, and (c) to select cultivars exhibiting higher potassium and sodium ratios for the regulation of blood pressure in hypertensive people.

2. Materials and methods

2.1. Location and climate

This experiment was carried out in the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, which is situated at 28.63°N latitude and 77.15°E longitudes and a mean height of 228 m above mean sea level. This geographical location falls in the *Trans*-Gangetic agro-climatic zone of India.

2.2. Plant material

A total of 45 different commercially grown varieties (**Table 1**) comprising different bulb colours from white to dark red were collected from different states of the country (representing more than 10 onion-producing states) and evaluated. Seeds of all varieties were maintained and produced during 2019–2020 at the Vegetable Research Farm, Division of Vegetable Science, IARI, New Delhi. After proper cleaning, harvested seeds were stored under ambient conditions. In October 2020, fungicide-treated seeds were sown for nursery production. After 6–7 weeks, the seedlings of all genotypes were transplanted in January 2021. All of the agronomical packages and practices recommended by the IARI for raising successful bulb crops were followed. This experiment was laid out in a Randomised Block Design, with three replications. Each replication included about 200 plants per plot of each variety.

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TABLE 1 List of open pollinated short day commercial Indian onion varieties used for assessment of bioactive compounds.

S. No	Variety	Code	Bulb colour	Institute/ University	Releasing state	Country region
1	Akola Safed	AKLS	White	IARI	Maharashtra	W
2	Early Grano	EG	Yellow	IARI	New Delhi	N
3	Bhima Shubra	BSBR	White	DOGR	Maharashtra	W
4	JWO-1	JWO1	White	JAU	Gujarat	W
5	Bhima Shweta	BSWT	White	DOGR	Maharashtra	W
6	Pusa Riddhi	PRDI	Red	IARI	New Delhi	N
7	Pusa White Round	PWR	White	IARI	New Delhi	N
8	NHRDF Fursungi	NFRS	Red	NHRDF	Maharashtra	W
9	PKV White	PKVW	White	NHRDF	Maharashtra	W
10	Bhima Shakti	BSKT	Red	DOGR	Maharashtra	W
11	Pusa White Flat	PWF	White	IARI	New Delhi	N
12	VL Pyaz	VLPZ	Red	VPKAS	Uttarakhand	N
13	RO-252	R252	Red	RAU	Rajasthan	N
14	Udaipur Local	ULCL	Red	RAU	Rajasthan	N
15	GJWO-3	GJW3	White	JAU	Gujarat	W
16	GJWO-11	GJ11	White	JAU	Gujarat	W
17	JNDWO-085	JNW8	White	JAU	Gujarat	W
18	Arka Pitamber	APTB	Yellow	IIHR	Karnataka	S
19	Bhima Kiran	BKRN	Red	DOGR	Maharashtra	W
20	Phursungi Local	PHLC	Pink	NHRDF	Maharashtra	W
21	Pusa Shobha	PSOB	Brown	IARI	New Delhi	N
22	Agrifound White	AFW	White	NHRDF	Maharashtra	W
23	Pusa Sona	PSON	Yellow	IARI	New Delhi	N
24	Talaja Red	TZRD	Red	JAU	Gujarat	W
25	JRO-11	JR11	Red	JAU	Gujarat	W
26	Bhima Raj	BRAJ	Red	DOGR	Maharashtra	W
27	HOS-4	HOS4	Red	CCSHAU	Haryana	N
28	Bhima Light Red	BLRD	Red	DOGR	Maharashtra	W
29	Pusa Madhavi	PMDV	Red	IARI	New Delhi	N
30	Arka Bheem	ARBM	Red	IIHR	Karnataka	S
31	NHRDF Red-4	NRD4	Red	NHRDF	Maharashtra	W
32	L-819	L819	Red	NHRDF	Haryana	N
33	Punjab Naroya	PBNR	Red	PAU	Punjab	N
34	Bhima Super	BSPR	Red	DOGR	Maharashtra	W
35	Hisar-2	HSR2	Red	CCSHAU	Haryana	N
36	B-780	B780	Red	MPKV	Maharashtra	W
37	Bhima Safed	BMSF	White	DOGR	Maharashtra	W
38	Pusa Red	PRED	Red	IARI	New Delhi	N
39	PRO-6	PRO6	Red	PAU	Punjab	N
40	Kalyanpur Round Red	KRR	Red	CSAUAT	Uttar Pradesh	N
41	Sukhsagar	SSR	Red	LOCAL	West Bengal	W
42	Bhima Dark Red	BDR	Red	DOGR	Maharashtra	W
43	RO-59	RO59	Red	RAU	Rajasthan	N
44	NHRDF-Red L-28	NL28	Red	NHRDF	Haryana	N
45	XP Red	XPR	Red	Local	New Delhi	N

2.3. Estimation of potassium and sodium content

Replication-wise, fully dried samples of the edible portion of the bulb were homogenised using a pestle and mortar. Half a gram of powdered sample (three replications) was taken for digestion in 20 ml of an acid solution of nitric acid (HNO_3) and 4-perchloric acid in the ratio of 9:4 and placed in a 500 ml conical flask. The corresponding mixture was kept overnight and was placed on a hot plate the next morning for digestion until white fumes had appeared for about 2 h. After digestion, the clear solution was diluted with double-distilled autoclaved water up to 100 ml. After dilution, the mixture was filtered with Whatman Filter Paper Number-1. An Atomic Absorption Spectrophotometer (Model AA-6880, Shimadzu, Japan) was used to measure absorbance and calculate sodium and potassium contents (Table 2). Air acetylene gas was used for this study. Each sample was measured twice (n = 6 for each variety, 3 replications and two replicates) to avoid any handling mistakes.

2.4. Statistics

Analysis of variance (ANOVA), box plot analysis, DMRT and cluster analysis was calculated by the use of SAS software version 9.3 (SAS Institute, Cary, NC, USA). For cluster analysis, hierarchical clustering technique was used and calculating the distance between the two clusters, a complete linkage algorithm was used which works on the principle of distant neighbours or dissimilarities.

3. Results

3.1. Potassium (K) content (mg/kg of DWB)

A wide genotypic variation in K concentrations was recorded in the onion bulbs (Table 3). The average potassium content in onion bulbs was recorded to be 4679.3 mg/kg on DWB (dry weight basis), whereas it ranged from 490.3 to 9160.1. It was determined that there was a more than 18-fold difference between the highest and lowest potassium contents in the bulbs of tested onion cultivars. It was also observed that bulb colour impacted K concentration.

The K content was recorded to be significantly highest in the yellow-coloured bulb variety from Southern India “Arka Pitamber” (9160.1 ± 96.7 mg/kg of DWB) followed by the Pusa Sona (7933.2 ± 292.8 mg/kg of DWB), GJWO-11 (7875.9 ± 572.8 mg/kg of DWB), and PRO-6 (7690.0 ± 188.2 mg/kg of DWB) varieties. However, minimum K content was assessed in the white-coloured bulb variety “Agrifound White” (490.3 ± 17.0 mg/kg of DWB) followed by Udaipur Local (732.9 ± 93.4 mg/kg of DWB), GJWO-3

TABLE 2 Details of autosampler atomic absorption spectrophotometer parameters.

Element	Symbol	Burner height (mm)	Wavelength for OD value (nm)	*R*-value
Potassium	K	7	766.4	0.95
Sodium	Na	7	588.0	0.94

TABLE 3 Estimation of potassium (K) and sodium (Na) of fresh bulbs on dry weight basis in Indian onion varieties.

S. No	Variety name	Potassium (mg/kg)	Sodium (mg/kg)
1	Arka Pitamber	9160.1 ± 96.7^{a}	120.9 ± 13.8^{mnopq}
2	Pusa Sona	7933.2 ± 292.8^{b}	73.8 ± 13.9^{opq}
3	GJWO-11	7875.9 ± 572.8^{bc}	341.0 ± 31.6^{de}
4	PRO-6	7690.0 ± 188.2^{bcd}	244.2 ± 13.9^{fghi}
5	VL Pyaz	7660.5 ± 68.7^{bcd}	347.8 ± 39.8^{de}
6	Bhima Safed	7514.5 ± 181.8^{bcde}	292.8 ± 28.8^{ef}
7	Pusa Red	7467.1 ± 252.7^{bcde}	379.7 ± 11.9^{bcd}
8	Punjab Naroya	7428.6 ± 332.9^{bcde}	205.6 ± 38.3^{hijk}
9	Bhima Dark Red	7258.5 ± 155.2^{bcde}	181.9 ± 17.9^{ijklm}
10	Pusa Riddhi	7245.7 ± 236.5^{bcde}	103.4 ± 11.9^{nopq}
11	Kalyanpur Round Red	7214.3 ± 130.2^{bcde}	279.0 ± 46.7^{efg}
12	Bhima Super	7186.6 ± 106.4^{bcde}	303.8 ± 39.5^{ef}
13	Pusa White Round	6942.4 ± 220.8^{bcde}	202.9 ± 29.4^{hijkl}
14	Pusa Madhavi	6796.3 ± 433.5^{bcde}	298.0 ± 39.3^{ef}
15	Sukhsagar	6621.9 ± 71.3^{bcde}	280.6 ± 41.3^{efg}
16	XP Red	6539.3 ± 183.4^{bcde}	446.8 ± 17.6^{ab}
17	Bhima Kiran	6485.3 ± 145.3^{cde}	147.0 ± 12.9^{jklmno}
18	Akola Safed	6340.8 ± 135.1^{de}	68.8 ± 4.2^{pq}
19	NHRDF-Red L-28	6333.3 ± 411.3^{de}	458.2 ± 61.7^{a}
20	HOS-4	6322.5 ± 284.8^{de}	202.2 ± 12.4^{hijkl}
21	RO-252	6321.8 ± 125.6^{de}	421.9 ± 16.9^{abc}
22	Pusa White Flat	6142.1 ± 168.3^{e}	395.2 ± 43.2^{abcd}
23	Bhima Light Red	4831.7 ± 69.7^{f}	129.2 ± 15.0^{lmnop}
24	Pusa Shobha	4681.3 ± 109.9^{fg}	236.5 ± 21.5^{fghi}
25	Arka Bheem	4017.2 ± 148.6^{fgh}	291.6 ± 21.3^{ef}
26	B-780	3647.7 ± 88.0^{fghi}	352.6 ± 28.9^{cde}
27	JNDWO-085	3429.2 ± 251.3^{ghi}	382.4 ± 11.2^{bcd}
28	NHRDF Fursungi	3368.3 ± 159.9^{hi}	183.3 ± 11.2^{ijklm}
29	JRO-11	3279.0 ± 10.3^{hi}	70.2 ± 8.7^{pq}
30	Hisar-2	3207.1 ± 119.9^{hi}	92.7 ± 11.9^{nopq}
31	L-819	3035.5 ± 161.3^{hi}	119.3 ± 12.7^{mnopq}
32	Bhima Shakti	2529.2 ± 142.8^{ij}	306.3 ± 32.9^{ef}
33	Phursungi Local	1740.7 ± 203.2^{jk}	133.2 ± 6.7^{klmnop}
34	Early Grano	1699.9 ± 178.7^{jk}	209.4 ± 11.7^{ghij}
35	NHRDF Red-4	1662.2 ± 129.2^{jk}	263.3 ± 15.7^{fgh}
36	PKV White	1551.9 ± 143.2^{jk}	202.4 ± 12.0^{hijkl}
37	RO-59	1496.3 ± 27.2^{jk}	291.8 ± 10.4^{ef}
38	JWO-1	1415.8 ± 123.4^{jk}	121.9 ± 8.5^{mnopq}
39	Bhima Shubhra	1230.6 ± 13.5^{jk}	94.2 ± 6.3^{nopq}
40	Talaja Red	1165.1 ± 121.1^{k}	292.8 ± 40.5^{ef}
41	Bhima Raj	928.3 ± 70.8^{k}	83.7 ± 10.9^{nopq}
42	Bhima Shweta	892.7 ± 76.7^{k}	155.2 ± 13.8^{jklmn}
43	GJWO-3	752.7 ± 112.9^{k}	112.1 ± 15.2^{mnopq}
44	Udaipur Local	732.9 ± 93.4^{k}	239.0 ± 23.8^{fghi}
45	Agrifound White	490.3 ± 17.0^{k}	52.7 ± 3.0^{q}

The values are presented as replicated mean ± standard deviation. $^{a-q}$Means followed by the same letters within a column do not differ significantly.

(752.7 ± 112.9 mg/kg of DWB), Bhima Shweta (892.7 ± 76.7 mg/kg of DWB), and Bhima Raj (928.3 ± 70.8 mg/kg of DWB).

It was concluded that yellow-coloured bulb varieties (Arka Pitamber and Pusa Sona) exhibited significantly higher K contents than red and white varieties. Twenty-three varieties showed higher potassium contents than the overall mean (4679.3), while 22 exhibited lower values.

Twelve cultivars, including Arka Pitamber, Pusa Sona, GJWO-11, PRO-6, VL Pyaz, Bhima Safed, Pusa Red, Punjab Naroya, Bhima Dark Red, Pusa Riddhi, Kalyanpur Round Red, and Bhima Super, exhibited K content of more than 7000 mg/kg on DWB, whereas nine cultivars, including RO-59, JWO-1, Bhima Shubhra, Talaja Red, Bhima Raj, Bhima Shweta, GJWO-3, Udaipur Local and Agrifound White, had a DWB content of less than 1500 mg/kg in their bulbs when evaluated under the *trans*-gangetic plain zone of New Delhi conditions.

3.2. Sodium (Na) content (mg/kg of DWB)

Like K, Na also exhibited broad variation in its concentrations among the tested genotypes (Table 3). The overall average sodium content in onion bulbs was 229.0 mg/kg of DWB, ranging from 52.7 to 458.2. It was determined that there was a more than eightfold difference observed between the highest and lowest Na content in the bulbs of tested cultivars.

In the reverse trend, like K, the Na content was found to be significantly higher in the dark-red-coloured bulb variety "NHRDF-Red L-28" (458.2 ± 61.7 mg/kg of DWB) followed by XP Red (446.8 ± 17.6), RO-252 (421.9 ± 16.9), and Pusa White Flat (395.2 ± 43.2). However, the lowest content was recorded in the white-coloured bulb variety Agrifound White (52.7 ± 3.0 mg/kg of DWB) followed by Akola Safed (68.8 ± 4.15), JRO-11 (70.2 ± 8.7), Pusa Sona (73.8 ± 13.9), and Bhima Raj (83.7 ± 10.9). Among white varieties, the highest Na content was recorded in Pusa White Flat (395.2 ± 43.2), whereas the lowest was found in Agrifound White (52.7 ± 3.0). Twenty-two varieties possessed higher Na than the overall mean (229.0), while 23 recoded values less than this. On the whole, it was further observed that red-coloured varieties elicited higher sodium contents compared to yellow, brown, and white bulb-coloured varieties on a dry weight basis.

Eleven cultivars, including NHRDF-Red L-28, XP Red, RO-252, Pusa White Flat, JNDWO-085, Pusa Red, B-780, VL Pyaz, GJWO-11, Bhima Shakti and Bhima Super, exhibited a DWB Na content of more than 300 mg/kg, whereas ten cultivars including L-819, GJWO-3, Pusa Riddhi, Bhima Shubhra, Hisar-2, Bhima Raj, Pusa Sona, JRO-11, Akola Safed, and Agrifound White elicited a level of less than 120 mg/kg of DWB.

3.3. Potassium and sodium (K/Na) ratio

The overall average K/Na ratio in onion bulbs was shown to be 25.5, ranging from 3.1 to 109.6. A greater than 35-fold difference was observed between the highest and lowest ratio in the bulbs of tested onion cultivars (Figure 1).

This ratio was significantly higher in the yellow-coloured Northern Indian variety "Pusa Sona" (109.6 ± 17.3) followed by Akola Safed (92.5 ± 7.5), Arka Pitamber (76.5 ± 9.3), Pusa Riddhi (70.6 ± 6.3), and JRO-11 (47.3 ± 6.3). On the other hand, the minimum ratio was estimated in the red-coloured bulb variety from Rajasthan "Udaipur Local" (3.1 ± 0.7) followed by Talaja Red (4.1 ± 0.9), RO-59 (5.1 ± 0.3), Bhima Shweta (5.8 ± 0.9), and NHRDF Red-4 (6.3 ± 0.5). Among the white-coloured bulbs, the maximum ratio was record in Akola Safed (92.6 ± 7.5), while the minimum ratio was reported in GJWO-3 (6.8 ± 1.1). Sixteen varieties showed a higher ratio than the overall mean,

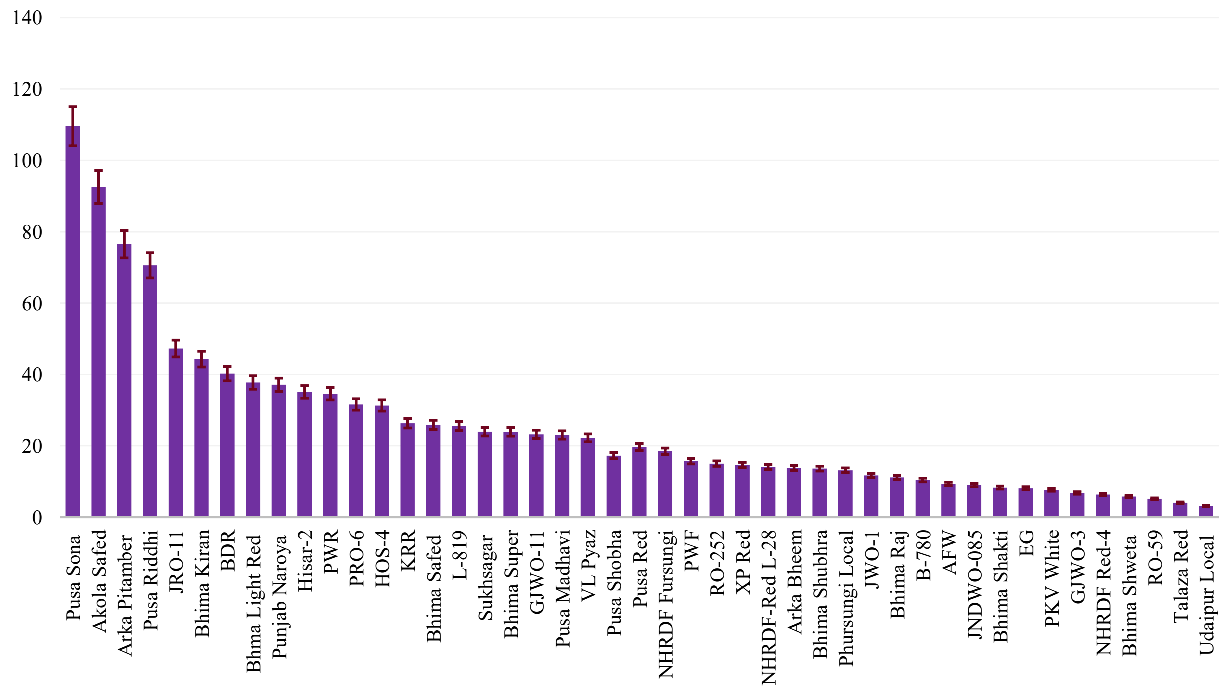

FIGURE 1
Potassium to sodium ratio in the 45 Indian short day onion cultivars.

while 29 were lower than this value. On the whole, it was further observed that yellow-coloured varieties elicited higher potassium and sodium ratios compared to red, brown, and white bulb-coloured varieties.

Thirteen cultivars, including Pusa Sona, Akola Safed, Arka Pitamber, Pusa Riddhi, JRO-11, Bhima Kiran, Bhima Dark Red, Bhima Light Red, Punjab Naroya, Hisar-2, Pusa White Round, PRO-6 and HOS-4, exhibited a K/Na ratio of more than 30, whereas 11

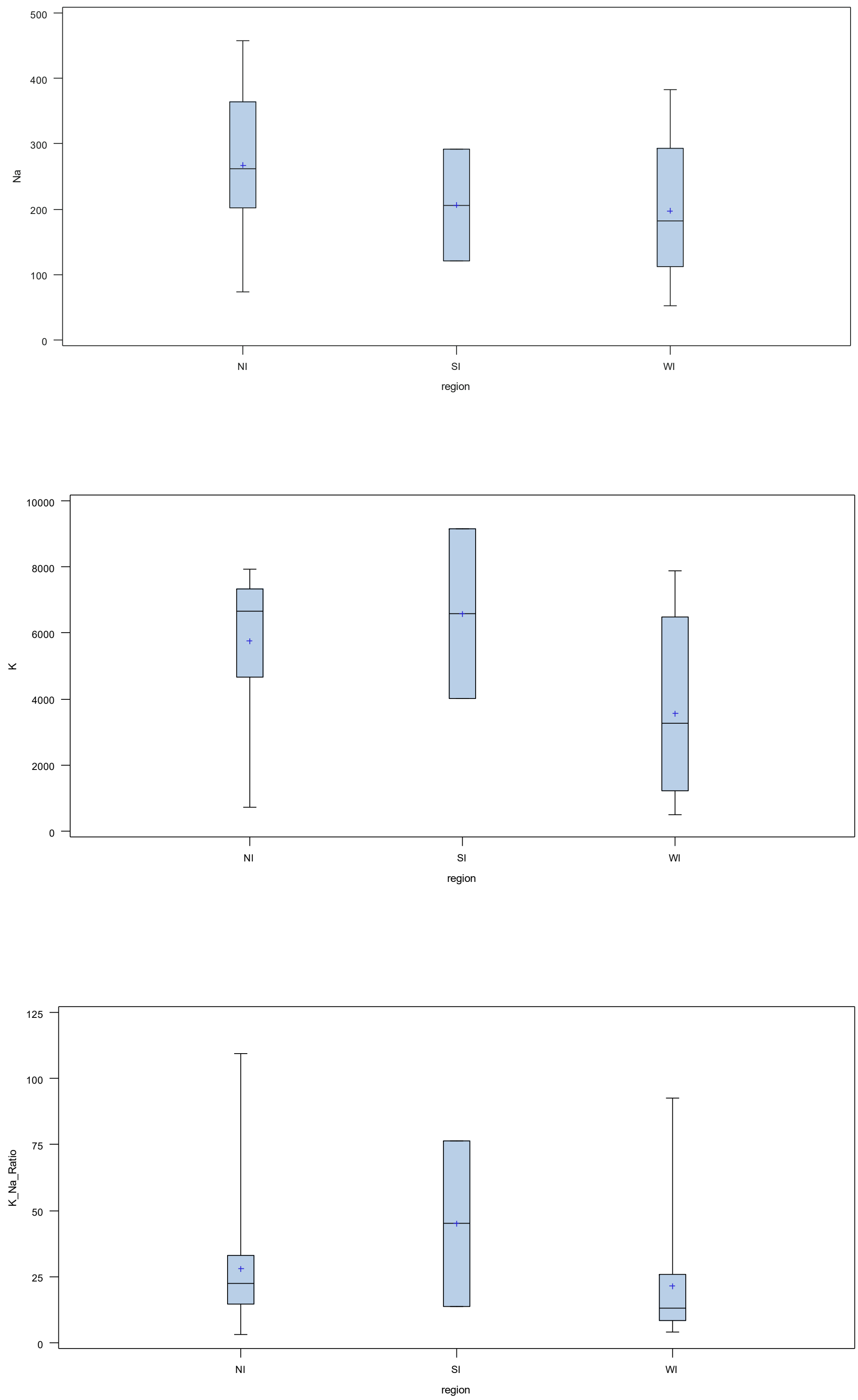

FIGURE 2
Box plot analysis of K, Na, and K/Na ratio on the basis of region.

cultivars, including Agrifound White, JNDWO-085, Bhima Shakti, Early Grano, PKV White, GJWO-3, NHRDF Red-4, Bhima Shweta, RO-59, Talaja Red and Udaipur Local, showed a ratio of less than 10 when evaluated under the *trans*-gangetic plain zone of New Delhi conditions. Varieties (RO-59 and Udaipur Local) selected from Rajasthan, showed a significant yet very low ratio. While two varieties

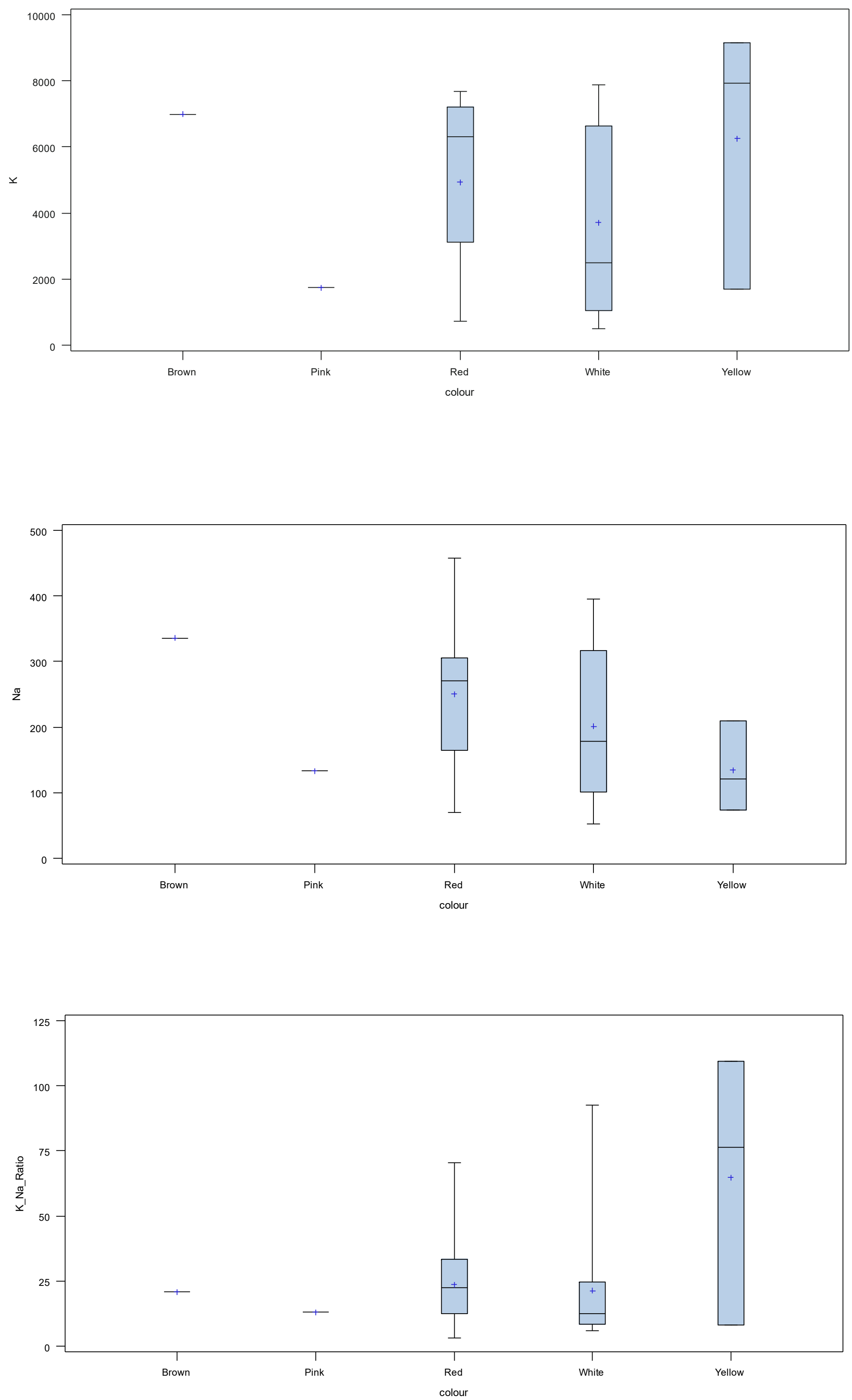

FIGURE 3
Box plot analysis of K, Na, and K/Na ratio on the basis of bulb colour.

released by IARI, New Delhi *viz.*, Pusa White Flat (15.7 ± 2.1) and Pusa White Round (34.6 ± 3.8), showed highly significant differences, almost twofold differences were obtained, these varied depending on the shape of the bulbs. This confirmed that the shape of bulbs is also associated with the K/Na ratio.

3.4. Impact of bulb colour and region

The box plot analysis, based on region, showed that the mean Na and K were highest in the varieties from the North Indian region (NI) followed by South India (SI) and Western India (WI). The highest mean Na/K ratio was observed in onions from SI followed by NI and WI grown onions (**Figure 2**). In terms of colour, the highest Na was found in brown onions, followed by red-, white-, pink-, and yellow-coloured onions. The highest K was observed in yellow-coloured onions, followed by brown, red, white, and pink onions. The highest mean Na/K ratio was observed in yellow onion, followed by red, brown, pink, and white onion bulbs (**Figure 3**).

3.5. Cluster analysis

The Hierarchical Clustering of all the genotypes was done based on distant neighbours or dissimilarities. The dendrogram is presented in **Figure 4**. The genotypes grouped in clusters and sub-clusters are presented in **Table 4**. The dendrogram exhibited three major clusters including 23, 13, and 9 genotypes in cluster C1, C2, and C3, respectively. The cluster I was divided into two groups; group C1A and C1B. The group C1A contained one genotype, i.e., Arka Pitamber which is a yellow-coloured bulb variety. The cluster C1B again subdivided into two categories. The cluster C2 grouped 13 genotypes and divided into two categories included 5 and 8 genotypes. The third cluster C3 contained nine genotypes and mainly consisted of red coloured bulb varieties except JNDWO-085. Except few, most of the northern Indian cultivars grouped into cluster C1.

4. Discussion

Across the globe, people are facing major health issues in the form of metabolic disorders and chronic NCDs. Coronary artery and cerebrovascular (heart stroke) are the most prevalent among cardiovascular diseases (26) and the frequency of deaths due to CVDs increased significantly (27). HTN was found to be the major risk factor (28–30) for CVDS. The intake of higher amounts of sodium and lower amounts of potassium is one of the main reasons for HTN. There is strong evidence that increasing dietary potassium intake reduces both systolic and diastolic blood pressure. Intake of potassium-enriched diets not only reduces blood pressure, but also the risk factors for various CVD, even in elderly and obese subjects with HTN (31–37). A renowned Canadian physician stated that the "*prevalence of arterial hypertension on this continent is in large part due to potash poor diet and an excessive use of salt*" (38). Current diets contain lower potassium (70–80 mmol/day) and higher sodium (150–200 mmol/day), whereas ancestral diets contained much higher potassium (230–300 mmol/day) and negligible (1–10 mmol/day) sodium (39, 40). With the advancements in diet and lifestyle, a major sustainable change was observed after using artificial salt in cooking led to reduction in dietary intake of potassium (31, 41, 42).

In a recent study, Bibbins-Domingo et al. (43) estimated that a reduction in dietary sodium intake of only 1,200 mg per day would reduce the number of stroke cases in the USA from 32,000 to 66,000. Now, it has been scientifically established that a reduction in dietary sodium intake can reduce the risk factors for various CVDs (44). Furthermore, according to the WHO, K is important for blood pressure regulation in hypertensive persons and recommends at least 3510 mg of potassium per day to maintain blood pressure and reduce the risk of CVD. A meta-analysis of 33 randomised controlled trials concluded that potassium supplementation led to a significant reduction in mean systolic and diastolic blood pressure of 3.1 and 2 mmHg, respectively (45).

As an enriched source of dietary K, along with other beneficial bioactive compounds, onion bulbs could be a potential source for

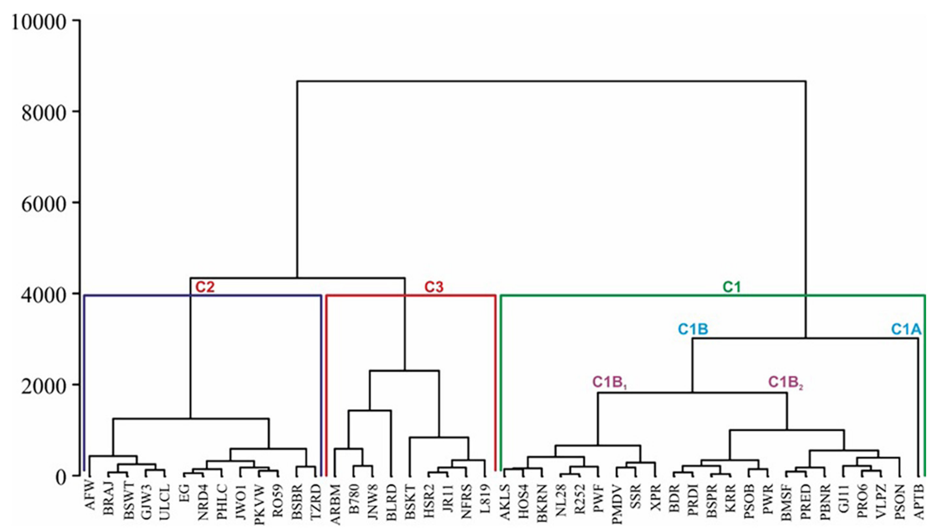

FIGURE 4
Hierarchical Clustering of 45 genotypes for K, Na, and P/Na ratio based on the distant neighbour and dissimilarities.

TABLE 4 Cluster analysis of 45 short day Indian onion genotypes for sodium, potassium contents, and potassium/sodium ratio.

Cluster 1 (23) *			Cluster 2 (13)		Cluster 3 (9)	
1A (1)	1B (22)		2A (5)	2B (8)	3A (4)	3B (5)
	$1B_1$ (9)	$1B_2$ (13)				
Arka Pitamber	Akola Safed	Bhima Dark Red	AFW	EG	Arka Bheem	Bhima Shakti
	HOS-4	Pusa Riddhi	Bhima Raj	NHRDF Red-4	B-780	Hisar-2
	Bhima Kiran	Bhima Super	Bhima Shweta	Phursungi Local	JNDWO-085	JRO-11
	NHRDF Red L-28	Kalyanpur Red	GJWO-3	JWO-1	BLR	NHRDF Fursungi
	RO-252	Pusa Shobha	Udaipur Local	PKV White		L-819
	Pusa White Flat	Bhima Safed		RO-59		
	Pusa Madhavi	Pusa Red		Bhima Shubhra		
	Sukhsagar	PB Naroya		Talaja Red		
	XP Red	GJWO-11				
		PRO-6				
		VL Pyaz				
		Pusa Sona				
		Pusa White Round				

*Number of genotypes grouped.

hypertensive people to reduce their elevated blood pressure. In low- and middle-income countries, rapid demographic growth and the lower availability of resources have become essential to create cheaper plant-based functional foods that could replace high-cost allopathic medicines, avoid their side effects and ensure nutritional security. Being a versatile crop, onion bulbs are the best option for Indian people since this crop is used in almost all Indian kitchens. Since antiquity, various physicians have prescribed this crop to prevent various ailments and diseases, well documented in the historical literature. India is also a global leader in onion production. Wide genotypic variation in potassium and sodium concentrations and their ratios was recorded in the Indian short day onion bulbs. Our results were supported by the findings of Metrani et al. (17) in the USA. They estimated 12720.7 and 13550.1 mg/kg of DWB K concentrations in red onion long day varieties. However, they found significant differences in the concentration of Na between the two genotypes: 314.1 and 1001.3 mg/kg of DWB. This shows that concentrations of Na and K are highly dependent on the genotype and growing environmental conditions. In onion, growing environmental conditions, agronomic management and genetic makeup of the cultivars determined the variation minerals composition in bulbs.

On the basis of bulb colour, yellow varieties recorded the highest K contents. Varieties like Arka Pitamber and Pusa Sona could be recommended to the Indian population after clinical assessment and bioavailability studies. Twenty-three varieties showed higher potassium contents than the overall mean (4679.3), which clearly showed that the Indian onion population has a higher K content. The major reason of this variability is likely to be due to the genotypic makeup of the cultivar (46). However, this aspect was not even considered for the exploration of a potential source of dietary K. Our results suggest that onion bulbs may offer a sufficient amount of potassium to meet the recommended dietary allowance (RDA) for humans. However, there is no clear-cut RDA for potassium (47).

The human body requires traces of sodium for some metabolic functions, but the consumption of too much sodium results in elevated blood pressure (1), eventually leading to CVD or heart failure. In the current scenario, the identification of plant-based food with low sodium contents is important for decreasing or minimising the risk of CVD and heart attacks. In the present study, an overall average of 229.0 mg/kg of DWB, ranging from 52.7 to 458.2 was observed. Much higher differences were determined between the highest and lowest values. On the contrary, Na content was significantly highest in the dark-red-coloured variety, while K was highest in yellow-coloured onions. Here, our interest is to identify the genotype with low sodium and high potassium contents for further breeding programs. Eleven cultivars exhibited Na content of more than 300 mg/kg of DWB, whereas ten cultivars recorded values of less than 120. Being versatile, with the peculiar flavour of Indian onions, no commercial hybrid is there at national level from the public sector. Cultivators mostly grow open pollinated varieties, so this study might be useful for breeders aiming to develop hybrids with higher contents of potassium using identified genotypes as parents (8).

Various scientific studies have proven that unhealthy diets and HTN play a chief role in the development of various heart diseases. The intake of higher dietary salt and the lower intake of vegetables and fruits are directly associated with a higher risk of CVDs (41, 48) because of elevated blood pressure. The higher intake of potassium and lower intake of sodium is quietly helpful for regulating blood pressure and decreasing the risk of CVDs, especially in hypertensive adults (44, 49). Therefore, the WHO recommended reducing the intake of sodium to less than 2000 mg per day (50) and significantly enhancing the intake of potassium in the diet to a minimum of 3510 mg per day to reduce blood pressure (51). Sodium is usually considered responsible for enhancing blood pressure while potassium antagonistically acts to keep blood pressure within

TABLE 5 Potassium/sodium ratio (K/Na ratio) in short-day Indian OP commercial varieties grown under the *trans*-gangetic plains of India.

S. No	Variety name	K/Na ratio ± SD
1	Pusa Sona	109.6 ± 17.3^{a}
2	Akola Safed	92.5 ± 7.5^{b}
3	Arka Pitamber	76.5 ± 9.3^{c}
4	Pusa Riddhi	70.6 ± 6.3^{c}
5	JRO-11	47.3 ± 6.3^{d}
6	Bhima Kiran	44.3 ± 3.1^{de}
7	Bhima Dark Red	40.2 ± 4.6^{def}
8	Bhima Light Red	37.7 ± 4.7^{efg}
9	Punjab Naroya	37.1 ± 8.0^{efg}
10	Hisar-2	35.1 ± 5.6^{fg}
11	Pusa White Round	34.6 ± 3.8^{fg}
12	PRO-6	31.6 ± 2.4^{gh}
13	HOS-4	31.3 ± 1.4^{gh}
14	Kalyanpur Round Red	26.3 ± 4.0^{hi}
15	Bhima Safed	25.9 ± 2.9^{hi}
16	L-819	25.5 ± 1.4^{hi}
17	Sukhsagar	23.9 ± 3.5^{hij}
18	Bhima Super	23.9 ± 2.9^{hij}
19	GJWO-11	23.2 ± 2.5^{ijk}
20	Pusa Madhavi	23.0 ± 2.8^{ijk}
21	VL Pyaz	22.2 ± 2.5^{ijkl}
22	Pusa Red	19.7 ± 1.1^{ijklm}
23	NHRDF Fursungi	18.4 ± 2.0^{ijklmn}
24	Pusa Shobha	17.2 ± 1.3^{jklmno}
25	Pusa White Flat	15.7 ± 2.1^{klmnop}
26	RO-252	15.0 ± 0.9^{lmnopq}
27	XP Red	14.6 ± 0.3^{lmnopqr}
28	NHRDF-Red L-28	14.1 ± 2.6^{mnopqrs}
29	Arka Bheem	13.8 ± 0.6^{mnopqrs}
30	Bhima Shubhra	13.6 ± 0.9^{mnopq}
31	Phursungi Local	13.1 ± 2.1^{mnopqrst}
32	JWO-1	11.7 ± 1.8^{mnopqrstu}
33	Bhima Raj	11.2 ± 0.9^{nopqrstuv}
34	B-780	10.4 ± 1.0^{nopqrstuv}
35	Agrifound White	9.3 ± 0.7^{opqrstuv}
36	JNDWO-085	9.0 ± 0.4^{pqrstuv}
37	Bhima Shakti	8.3 ± 0.6^{pqrstuv}
38	Early Grano	8.1 ± 0.5^{pqrstuv}
39	PKV White	7.7 ± 0.5^{pqrstuv}
40	GJWO-3	6.8 ± 1.1^{qrstuv}
41	NHRDF Red-4	6.3 ± 0.5^{rstuv}
42	Bhima Shweta	5.8 ± 0.9^{stuv}
43	RO-59	5.1 ± 0.3^{tuv}
44	Talaja Red	4.1 ± 0.9^{uv}
45	Udaipur Local	3.1 ± 0.7^{v}

The values are presented as replicated mean ± standard deviation. $^{a-v}$Means followed by the same letters within a column do not differ significantly.

the desired range. Instead of looking at these two elements distinctly, the ratio of the two in the diet has greater significance than the amount of either one alone. Interestingly, the recorded data pertaining to this ratio on the dry weight basis exhibited significant differences and it was further determined that there was a more than 35-fold difference observed between the highest and lowest values.

The K/Na ratio was highest in the yellow-coloured bulb varieties than in red and white onions (Table 5). Sixteen varieties showed higher ratios than the overall mean, while 29 were lower than this. Despite that, the excessive intake of any mineral may prevent other mineral elements from being properly absorbed and utilised in the body. Therefore, the K/Na ratio in onion bulbs is more important to avoid any imbalance. Results of the current research work provide beneficial preliminary information for nutritionists and dieticians involved in developing diet plans and potassium-restricted meals for hypertensive individuals.

One of the possible contraindications to onion consumption is the FODMAPs- content. FODMAPs are a category of carbs and fibres that many people cannot tolerate. They may cause unpleasant digestive symptoms, such as bloating, gas, cramping, and diarrhoea. Individuals suffering from irritable bowel syndrome are often intolerant to FODMAPs and may need to avoid onions. (52).

Conclusion

From the findings of the current study, it could be determined that the Indian onion may be considered a potential source of potassium, as well as having low sodium contents. Yellow bulb onions recorded higher potassium levels than red- and white-coloured onions, which may be useful for hypertensive individuals to prevent various CVDs. Further, it should be explored using comprehensive investigations as a potential source of dietary potassium. That information could be used to develop diet plans and further breeding programs to develop specific cultivars for populations with improved potency. Although the comprehensive clinical and physiological implications remain to be established, our findings and information generated on Indian onion further cater to emphasise the need for future studies focused on the development of functional foods as a public health approach. Still, imperative questions with respect to bioavailability and physiological and molecular pathways still need much more attention from plant physiologists. Additionally, molecular level studies for the identification of genotypes with significantly higher potassium-to-sodium ratios will be beneficial for breeders and geneticists aiming to develop new climate smart resilient cultivars with desired quality and nutrition factors. Ultimately, all such novel approaches may help to alleviate HTN effects in the global population, which is an alarming worldwide challenge faced by public health and plant scientists. In the 21st century, dietary interventions to reduce the occurrence of HTN should have immense potential to considerably decrease CVD morbidity and mortality. Furthermore, well-designed clinical trials are required to test the probable effects of various Indian varieties with high potassium-to-sodium ratios on blood pressures and various cardiovascular and cerebrovascular events.

Data availability statement

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

Author contributions

HS and AnK: conceptualization, formal analysis, and methodology. HS: investigation and writing – original draft. HS, AnK, and AmK: software. AnK: supervision. HS: writing – original draft. AG, AmK, AnK, HS, and ML: interpretation of data. AnK, ML, and AG: writing – review and editing. All authors 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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Corrigendum: Genotypic variation in Na, K and their ratio in 45 commercial cultivars of Indian tropical onion: A pressing need to reduce hypertension among the population

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US consumers' mental associations with meat substitute products

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Negative impacts of meat consumption on both consumers' health and the environment call for alternative sources for protein intake. In the last decades, the development of meat substitute products has made enormous progress. Given the beneficial aspects of reduced meat consumption, meat substitutes might be a promising approach for a more plant-based diet. However, despite the continuous improvement of meat substitute products and their increasing market potential, meat consumption in the US is still at a high level. Extant literature acknowledges that meat substitute products prompt several negative thoughts and feelings in various European countries, while US consumers' perceptions of meat substitute products have not been investigated so far. However, understanding consumers' thoughts and feelings toward meat substitute products provides valuable insights which can help policymakers and marketers to efficiently promote meat substitute products. Against this background, the current research investigates US consumers' mental associations (i.e., connections of information and prior experiences with the product category stored in memory) with meat substitute products and explores if there are any differences between women and men. A sample of 175 US citizens acquired through an online panel provider completed a free word association technique resulting in 824 mental associations that qualified for the subsequent analysis. In a deductive-inductive content analysis, we assigned the mental associations to 20 categories (e.g., taste, health, environment) and determined their valence (i.e., positive, neutral, or negative). Frequencies and relationships among the categories were analyzed by employing frequency analyses, Chi-square difference tests, and multidimensional correspondence analysis. The findings reveal that meat substitute products elicit more negative mental associations than positive ones. Results validate categories identified in existing literature, but also reveal new categories of mental associations. Furthermore, the findings demonstrate that mental associations differ between women and men, with women tending to perceive meat substitutes more negatively than men. The multiple correspondence analysis resulted in four different consumer profiles (skeptics, innovators, health-oriented consumers, and avoiders) which can guide policymakers and brand managers on the effective promotion of meat substitute products.

KEYWORDS

meat analogs, plant-based diet, taste, health, mental associations, meat alternatives, perceptions

1. Introduction

Despite its negative impacts on both health and the environment, global meat consumption is projected to increase by 14% by 2030 compared to the average of the baseline period 2018–2020 (1). Over the last 50 years, meat production has more than tripled resulting in more than 340 million tons of meat each year (2). This increase is well-reflected in the enormous meat market revenue. The meat market's revenue was 1,206 bn US dollars in 2022, with an expected annual growth of 7.73%. The most portion of meat is consumed in the US, accounting for a revenue of 159.2 bn US dollars in 2022 (3). In terms of meat consumption, this relates to 224.6 pounds of red meat and poultry (4). In a global comparison, Americans are on top of the *per capita* meat consumption. On average, an American consumer eats more than three times more than the worldwide average (5). This immersive meat consumption is problematic from many different perspectives. Meat consumption is associated with several issues, such as food security concerns, animal welfare concerns, environmental concerns which relate to the depletion of natural resources, pollution, and emission of greenhouse gases, and public health concerns due to zoonotic and cardiovascular diseases (6).

In more detail, intensive meat consumption has been identified as a severe health risk. Studies confirm that meat consumption represents a risk factor for heart attack, stroke, and type 2 diabetes (7). Especially in high-income Western countries, the consumption of red and processed meat increases mortality rates at a modest level, often caused by colorectal and other forms of cancer (8, 9). Furthermore, potential explanations for these diseases refer to chemicals that are naturally contained in meat and/or released during processing and cooking (9).

Furthermore, meat products have adverse effects on the environment. Meat production signifies one of the major polluters in the food supply chain since meat requires enormous waste and causes wastewater generation and discharge (10). The meat supply chain starts with agriculture (i.e. e.g., feed storage, farm management, and primary packaging production), and ends at the final consumption stage. Between these steps, slaughterhouse activities as well as meat processing and packaging activities require additional energy resources (11). Food waste is one of the major issues in the context of meat production. Following the suggestion of Amicarelli et al. (12), food waste is considered as "food (including inedible parts) discharged, lost, degraded, consumed by pets or utilized in non-food or energy fields." Research reveals that in Italy, from 2,678,878 t animals bred, only 1,154,393 t (i.e., 43%) are brought to the slaughterhouse. At the slaughterhouse stage, material use efficiency is estimated to be 82% (unconscious food waste), demonstrating the large potential for improvement. Additionally, only a small percentage of the energy required for beef production comes from renewable resources (a maximum of 5%). The entire Italian beef production requires approximately 11,500 t of packaging, with an energy-hidden flow (i.e., water, energy) of 1,500 TJ and more than 780,000 liters of water (11). In contrast to direct flows, which account for the actual mass of materials and hence, do not require additional material in the production chain, hidden flows (also indirect flow or embodied materials) define all materials required in the production stage for manufacturing a product (13).

On a global level, meat production is recognized as the most relevant source of methane which considerably contributes to global warming (8). Today, meat production accounts for more than half (54%) of the total emissions from agriculture (1). A recent systematic meta-analysis reviewing 369 studies report that beef and lamb meat produced the highest greenhouse gases. In comparison, field-grown vegetables account for 0.37 kg CO2-eq/kg, while beef generates 26.61 kg CO2-eq/kg and lamb meat produces 25.58 kg CO2-eq/kg (14). Among other natural sources, the production of meat requires extensive grassland which is frequently obtained by cutting down trees, causing an additional release of carbon dioxide (15).

Plant-based meat substitutes (meat alternatives, meat analogs) that describe vegetable-based food products which often include proteins from pulses, algae, cereal protein, and fungi (16), are much more sustainable than traditional meat. These plant-based products are manufactured with the overall objective to mimic aspects of meat (6, 17). As compared to conventionally produced meat, plant-based meat substitutes require significantly fewer natural resources. For instance, a beef burger causes 9.3 times more greenhouse gas emissions than a plant-based burger. Even more remarkable is the comparison of land use between plant-based and beef burgers: the latter requires 9.5 times more land use, and 546 times more water (18). In addition to these ecological benefits, the plant-based meat market has enormous growth potential. 2022, the global market value of plant-based meat reached 10.11 billion US dollars in 2022, with a predicted steady increase over the next 5 years reaching roughly 34 billion US dollars in 2027 (19).

Against the serious health and environmental consequences of meat consumption, an increase in the consumption of meat substitutes while reducing at the same time meat intake is desirable from many perspectives. A reduction in meat consumption would have a considerable positive impact on greenhouse gas emissions and health. It is predicted that a shift to a flexitarian lifestyle would reduce greenhouse gases by 583 MtCO2e per year (20) Meat substitute products are a good source of protein while at the same time, they reduce the intake of saturated fat and cholesterol as compared to meat (6). A study reports that a plant-based diet is effective in treating obesity (21) and eventually the nutritionally beneficial effects could reduce up to 52,700 premature deaths per year (20).

Despite these acknowledged positive influences, not all consumers are willing to adopt meat substitutes. Extant studies recognize the need for future research to better understand the drivers and barriers of meat substitute consumption among different consumer groups (16, 22) and the factors that encourage consumers to eat less meat (23) as well as consumers' knowledge about the market (24). A common way to explore barriers and consumers' expectations of plant-based meat is the assessment of consumers' mental associations. Some prior studies have already revealed that in Germany (22), the UK and the Netherlands (13), and Portugal (25) consumers perceive meat substitute products mainly negatively. Nevertheless, knowledge of consumers' mental associations with meat substitute products is limited, especially in the US. Given the vast amount of meat consumed in the US, knowledge of consumers' associations with the product category meat substitute products would help policymakers and marketers to understand the drivers and barriers in the adoption process of meat alternatives. Knowledge of product categories as represented in mental associations serves as an important retrieval cue in the decision process (26), and literature recognizes the importance of food cues in determining food preferences (27). The product category itself represents an important mental association with the product (28) and as such, likely informs brand evaluations. In this context, studies verify that a strong link between the brand and the product category positively impacts memory-based brand choice (29). Against this background, the current study has the overall objective to investigate how US consumers perceive meat substitutes, and how these perceptions differ among women and men.

2. Literature review

This section is organized around two major themes. The first theme reviews prior studies exploring consumers' perceptions and mental associations with meat substitute products which form the basis for the category system used in the method section. The second theme discusses the relevance of product category associations and provides theoretical arguments on the importance of exploring them in a food context.

2.1. Meat substitute products

Limited empirical evidence exists reporting US consumers' mental associations with meat substitute products. A mental association is defined as a link between representations of aspects of reality or in other words, the internal cognitive structures that mirror the real world in the mind (30, 31). Individuals form mental representations about a new aspect of reality, such as a new dish, food product, or category, based on the information they gain about it and the experience they make (32). The mental representation of the new food is stored in memory along with the sensory and contextual associations. When individuals encounter the new food again, the prior mental associations such as previous judgments about the taste, texture, and smell of the product or dish are retrieved from memory as well (33). In other words, the new food serves as a stimulus that can influence the future perception of the new dish, food product, or category as well as the individual behavior (32, 33).

Studies concentrating on US samples have employed experimental designs to demonstrate that the majority (72%) of US citizens prefer a food product consisting of farm-raised beef as compared to alternative meat products (34). Other research reports that meat alternatives seem to not substitute ground meat: utilizing household scanner data, Neuhofer and Lusk (35) report that 86% of consumers who regularly consume meat substitute products consume ground meat as well. This finding was supported by Talyor et al. (36). Only 6% of the respondents acquired in a longitudinal survey (February 2020 to January 2022) indicated eating plant-based protein, while 4% indicated eating both plant-based and beef proteins on the same day. The same study collected US citizens' perceptions of meat substitute products vs. grounded meat using closed-ended questions. Results revealed that meat substitute products are perceived to be environmentally friendly and healthy, however, they scored low on taste, price, appearance, nutrition, and naturalness (36). Other studies found that US consumers had a significantly lower likelihood of purchasing meat substitute products as compared to Chinese or Indian citizens (37). Overall, these studies agree that US citizens prefer meat over meat substitute products, however, consumers' mental associations with meat substitutes have not been investigated that far. In support of this notion, a recent literature review on consumers' mental associations with food consumption did not identify any study conducted in the US (38).

Although to the best of our knowledge, no study exists which explores US consumers' mental associations with meat substitute products, some studies provide valuable insights into consumers' mental associations with meat substitute products in Europe. First exploratory studies found that two major drivers prompt consumers to consume meat products, namely ecological welfare and political values (16, 39). However, on a general level, it seems that meat outperforms meat substitutes in terms of positive mental associations. A study with a sample from the UK and the Netherlands reveals that meat is associated with good health and mood, convenience, sensory attractiveness, and luxury. On the contrary, only a few positive mental associations were identified for meat substitutes, namely ethical aspects and weight control (16). Similar results were obtained by a study conducted in Germany. The most frequent mental associations with meat substitute products were "tofu", "vegan", and "disgust", while meat products prompted the mental associations "delicious," "food," and "taste" most frequently (22). Supporting these findings, a study conducted in Scotland reports that various alternatives of meat substitutes (tofu, seitan, legumes, insects, and lab-grown meat) prompt feelings of disgust (25). Whereas UK respondents indicated a considerably lower utility level for meat substitutes than any other type of meat, there are certain attributes with high utility levels. Country of origin, low-fat content, and low carbon footprint are positively associated with meat substitute products and hence might be fruitful as a promotion strategy (40). In support of these findings, organic and local represent important attributes predicting the choice for meat substitute products based on micro-algae (41).

Other research employing closed-ended questions report that ethical concerns are a strong driver for willingness to eat cultured meat in Germany, while perceptions of unnaturalness and potential damage to farmers represent negative drivers (42). Unnaturalness has also been identified as a barrier to meat substitute adoption in the US, in addition to the limited taste and appeal (43). On the one hand, health and sustainability aspects have been identified as the main drivers for consumers' intention to consume meat substitute products in China (39). On the other hand, other research revealed a lack of awareness of the mental association between meat consumption and climate change (44). In depth interviews with 20 Dutch consumers revealed mainly positive or neutral mental associations with meat substitutes, such as "traditional meat replacement," "nutrition substitution" or "specific meat substitute products" (45). Interestingly, there was no consensus on the requirements for meat substitute products, some respondents indicated that meat substitute products should be similar to real meat while others held the opposite opinion. It needs to be mentioned that all of the 20 respondents were familiar with meat substitute products and some of them were vegetarian (45). However, other studies acknowledged the challenge to attract not only vegetarians but people who regularly eat meat (16). Given the low proportion of 1–2% of vegetarians of US citizens (6), it is important to collect mental associations of the broader population.

Related to this, some demographic variables have been identified to impact consumers' mental associations with meat substitute products. For instance, a positive impact of education level on meat substitute consumption was observed. Individuals with higher education consumed significantly more meat substitute products in the UK and the Netherlands (16). The mental associations with meat alternatives in the UK and Netherlands did not differ between gender (22). Nevertheless, another study reported differences in motives for eating meat substitute products between men and women. Women tend to base their decision to eat meat alternatives on sustainability reasons and health concerns, while additionally, other personality characteristics, such as low disgust sensitivity, higher education, and lower age are additional drivers of meat substitute consumption (46). Interestingly, men in the US seem to be more willing to switch to another meat alternative—*in-vitro* meat—on a regular basis as compared to women (43), which is why a higher intention to change could be assumed for men. On the contrary, a comprehensive literature review reveals that men in general consume more meat and have a lower willingness to eat plant-based meals (47). Similarly, educational status predicted the willingness to follow a plant-based diet (47). Hence, educational status as well as gender seem to influence consumers' mental associations with meat substitute products. In order to gain a better understanding of the importance of product category associations, and their potential to transfer any thoughts and feeling to a brand belonging to this product category, the next section elaborates on the formation of category-based brand associations.

2.2. Product category associations

Meat substitutes represent a new product category, including several brands, such as Impossible Foods, Beyond Meat, Gardein, and Amy's Kitchen. Rather than exploring the brand associations on a brand level, the current research concentrates on mental associations with the product category (i.e., a particular group of related products) (48). Mental associations with product categories are an important predictor of brand associations, and brand associations play an important role in consumers' product evaluations and choices (49). Brand associations describe any type of information that is linked to the brand node in memory, such as the product category itself, the usage context, or any other evaluative thoughts (e.g., taste, texture) (50, 51). The allocation of a brand to a specific brand category is the first step in building strong brands (52).

Extant research acknowledges the relevance of categorization in the context of meat substitute products (53). In 2011, consumers merely associated meat substitute products as different as compared to processed meat substitute products, while some product categories (e.g., sausages, burger patties) included both, meat and meat substitutes (53). The authors call for research that explores the underlying attributes which determine the product category classification. Product classification is important for a brand to make it into the awareness and evoked set during the purchase process (54) and for the identification and differentiation of brands (55). There is consensus in extant literature that each product category has its own specific mental associations (56).

Schema theory represents a theoretical explanation for the relevance of product categories and their corresponding mental associations. In the branding context, schemas are cognitive structures that define the expectations of specific product categories in the form of values on attributes, the weight of these attributes, and the variability across different brands (57). If consumers encounter a new product or brand, they will first process the product category knowledge to which this product or brand belongs. Research confirms that category schemas influence consumers' responses to local and global brands (58). Other studies show that products need to match the category color norms in order to prompt favorable attitudes and purchase intention (59). Individuals compare a stimulus (brand) to the exemplar (product category) and if the stimulus fits the exemplar, an affective transfer occurs (60). In other words, in such a category-based judgment, the thoughts and emotions are transferred from the product category to the brand. In the context of meat substitutes, research reveals that not taste itself, but the symbolic meaning associated with the product category of meat substitutes determines taste evaluations. The authors conclude that heavy meat eaters should be addressed with values they endorse when promoting meat alternatives (60). However, if a product does not fit a product category by threatening existing beliefs, consumers most likely react negatively (61). Hence, it is important to know which favorable mental associations exist for meat substitutes. At the same time, knowledge of potential negative mental associations would provide new knowledge on barriers to meat substitute consumption and might guide manufacturers on strategies aiming at eliminating these negative mental associations.

3. Materials and methods

3.1. Sample selection

To answer our research questions, we drew on an online sample acquired through the online panel provider "Clickworker" in September and October 2022. An invitation to participate in our study was posted on Clickworker's web platform and respondents were offered a small compensation for their participation. Only US citizens were allowed to participate in our study. As is typical with online panel providers, potential participants decided for themselves whether they wanted to participate in our study. Because the resulting self-selection bias is an issue in online panels, our sampling strategy was non-probabilistic (62). While the non-probabilistic samples are unlikely to be perfectly representative of the target population, they are particularly useful for qualitative methods (63) such as the one employed in our study. For a detailed discussion of the advantages and disadvantages of non-probabilistic samples for qualitative studies see Patton (63).

3.2. Data collection

To collect the data, we applied the free word association technique, which is particularly suited to studying the structure of mental representations (32). The idea underlying the free word association technique is that the responses that are triggered by an unstructured and ambiguous stimulus, elicit the participants' deep feelings, beliefs, and attitudes (37). Following the steps in the procedure of the free word association technique, we first familiarized the participants with the task and assured them that there are no right or wrong answers. We also instructed them to enter only single words or expressions. Then, we asked them to list those words that spontaneously come to their mind when we presented them with the stimulus: "Please let us know what you think about meat substitute products (e.g., meat-free minced meat, meat-free sausages)." Afterward, participants could enter their verbal responses into open text boxes in a questionnaire that we created using the web application "SoSci Survey." We asked the respondents to write down as many words as came to their minds. We deliberately did not limit the number of words and provided them with unlimited time to not restrict the thought process (32).

Once participants had shared all of their thoughts and feelings about meat substitutes in the free word association technique (one open question), we collected several demographic and sociodemographic data. In specific, we asked respondents to indicate their answers for the variables age (one item), gender (one item), educational attainment (one item), ethnicity (two items), employment status (one item), and personal income (one item).

It is important to mention that the study was conducted in accordance with the revised version of the Declaration of Helsinki and was approved by the first author's institutional review board. Informed consent was obtained right on the first page of the questionnaire. More specifically, the following statement was included "Your participation is voluntary and you are free to withdraw at any time, without giving a reason and without cost. By clicking the "Next" button, you voluntarily agree to take part in this study." Respondents who failed attention checks (please tick the middle of the scale) were automatically excluded from the survey.

3.3. Data analysis

The data were analyzed using a deductive-inductive content analysis. Deductive-inductive approaches combine the strengths of deductive and inductive content analysis (64). In the first step, deductive categories are developed based on existing literature. Each category is precisely defined, clear coding rules are established (i.e., when a text passage is assigned to that category), and reference examples are given (65). Once the resulting category system is finalized, the actual coding takes place, in which passages are assigned to the categories. In the second step, inductive categories are developed from the text material that contains new aspects that have not yet been adequately described in the existing literature and thus, could not be assigned to an existing category. To code these new aspects, new categories are formulated based on the key information contained in the text (64). In the final step, these categories are revised and integrated into the category scheme.

We followed this deductive-inductive procedure by first deductively developing categories based on extant literature exploring mental associations with meat-substitute products. Starting with this preliminary category scheme, we coded the data. All mental associations were assigned to these semantic categories by the two researchers. Inconsistencies were discussed until a consensus regarding a specific category was reached. In the process of coding the data, not all mental associations could be categorized, requiring the inductive creation of new categories.

Once our category scheme was complete, we performed frequency analyses of the categories. Furthermore, we investigated differences between gender using Chi-square tests. For a deeper analysis, we then conducted a Multiple Correspondence Analysis (MCA). MCA is an exploratory multivariate technique of data analysis (66). It is used to study relationships between categorical (nominal) variables and graphically represent them in the form of a biplot, which makes it a useful tool for analyzing free word associations (67). When used to analyze data on free word associations, each point represents a category of mental associations and the position of the points in the biplot reflects the relationship with other categories. MCA thus enables the identification of patterns and clusters within categorical data. For this reason, we analyzed our study participants' positive and negative mental associations with meat substitute products using MCA. The multiple correspondence analysis represents the final step in our data analysis. For a better overview of all methodological steps in our study, we have summarized the methods of sampling, data collection, and data analysis in Figure 1.

4. Results

4.1. Sample characteristics

175 participants completed the survey. In the first step, the data were cleaned from non-meaningful terms, leading to an exclusion of three respondents. As a result, a total of 824 mental associations elicited by 172 participants qualified for the data analysis. Participants were between 18 and 66 years old, with an average age of 36.4 years. Among the participants, 59.9% were female and 34.3% were male, 1.7% were transgender, and 4.1% did not answer this question. As regards the highest educational attainment, 46.5% had an advanced or bachelor's degree, 29.7% held a college or associate degree, 18.6% finish high school, and 1.2% did not complete high school. The majority of participants were employed, with 40.1% working full time, 5.8% with a part-time employment, and 16.3% being self-employed. 5.2% were students, 8.1% indicated "homemakers" as employment status, and 3.5% preferred not to stated their employment status. Participants' income was assessed in eight categories, whereas 3.5% indicated to have no income at all, 25% earned between $1 and $9,999, 13.4% had an income between $10,000 and $24,999, 23.8% confirmed

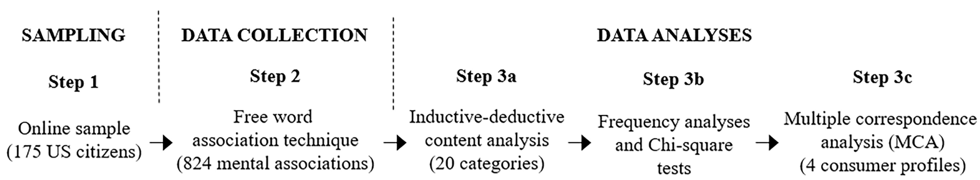

FIGURE 1
Overview of the methods applied for sampling, data collection, and data analyses.

to have an income between $25,000 and $49,999, 8.7% indicated to earn between $50,000 and $74,999, 7.6% received an income between $75,000 and $99,999, 6.4% earned between $100,000 and $149,999, and finally, 2.9% reported to earn $150,000 and greater (8.7% did not report their income). Hence, about two-thirds of the sample earned less than the median income. Most respondents were White, not Hispanics (59.9%) and White Hispanics (11.6%) followed by African American (7.6%), Asian (5.8%), Native American (4.1%), and Others (2.9%).

The distribution of ethnicities in our sample closely mirrors the US population. In terms of employment, those who work full-time are also comparable to the US population. Our sample is also broad and diverse for the other sample characteristics. However, as common for online samples, our participants are more often self- or unemployed, tend to be younger, and are better educated (68). Women and transgender persons are overrepresented in our sample, and their income is lower. Approximately two-thirds of the sample earns less than the median income.

4.2. Categorization of mental associations

Three new categories emerged during the coding process: "innovation," "nutrients," and "additives." The cycling between the data and the coding scheme also resulted in the deletion of the preliminary categories "mood," "ethics," "social influence," "risk," "Should not be similar to meat," "Should be similar to meat," "Not all are good," and "satiety," because they were not mentioned. In addition, the sensory appeal category was divided into more specific categories, namely "general appeal," "taste," "texture," and "smell." Furthermore, the preliminary categories "alternative protein foods" and "specific meat substitutes" were renamed into "protein sources" and "specific brands," respectively to better reflect the customers' knowledge about specific brands or protein sources as represented in meat substitute products. Multiple mental associations from one person that could be classified into the same category were counted as one, resulting in 437 distinct case-category codes. For further analysis, the mental associations were further split up into mental associations related to diet (Table 1) and mental associations related to motives for meat substitute consumption (Table 2). For the motives for meat substitute products, the mental associations were further classified into positive or negative mentions to allow a distinction between positive and negative mentions. This step resulted in 136 neutral mentions and 301 with positive or negative valence. The analysis proceeded with an investigation of the most frequent mental associations, the portion of positive and negative mental associations as well as differences between women and men.

TABLE 1 Mental associations related to diet.

Mental associations related to diet	Exemplary mental associations
No meat	Meat-free, plant-based, sourced from plants
Meat products	Beef, pork, chicken
Protein sources	Pea protein, soya protein, lupin protein
Vegetables	Potato, vegetable, fruit
Meat substitute brands	Beyond Meat, Impossible Burger, MorningStar Farms
Meat substitute products	Meat-free minced, meat-free sausages, veggie burgers
Meat replacement	Substitute, switch
Vegetarian/vegan diet	Vegetarian-friendly, vegan-friendly, vegan eating

4.3. Frequencies of mental associations with meat substitutes

The three most frequent positive mental associations are "healthy", "tasty", and "innovative". Three most frequent negative mental associations are "not tasty", "disgusting", and "fake". Some categories feature only positive but no negative mental associations. For instance, the new category "innovation" prompted only positive mental associations. Likewise, there was general broad agreement that meat substitute products are good for the environment, while no participants verbalized thoughts that meat substitutes could harm the environment. On the contrary, several consumers perceived meat substitute products to be unnatural, while only one respondent associated meat substitutes with "natural." Meat substitute products are often perceived as pricy. Eleven price associations were "expensive", while only two were "cheap". Several positive and negative mental associations were classified into the health category. In this category, an interesting pattern was observed: the positive mental associations in the health category were quite unspecific (e.g., "healthy," "nutritious," "longer life"), while negative mental associations are specific and seem to focus on short-term consequences (e.g., "stomach aches," "heartburn," "diarrhea") as well as medium-term consequences (e.g., "anemia"), and long-term consequences (e.g., "cancer-causing"). Mental associations which expressed respondents' general attitude toward meat substitute products, either positively (e.g., "great," "decent," "useful"), neutrally (e.g., "fact," "different," "recipes") or negatively (e.g., "bad," "waste," "depressing") were classified into the category "other." The frequencies of the positive, neutral, and negative mental associations in the category "other" were about the same.

4.4. Valence and gender differences of mental associations with meat substitutes

Overall, more negative (56%) than positive (44%) mental associations were observed. There was a remarkable difference in positive and negative mental associations between women and men though. Men had much more positive mental associations (63%) than negative ones (37%), while the opposite was true for women, who had 33% positive and 67% negative mental associations. Additional analyses (Chi-square tests) provided more detailed insights into the specific mental associations causing this significant difference. Women associated meat substitutes significantly more often with "vegan" or "vegetarian" (17%), while this mental association was not elicited by men [only 3%; $X^2(1, 162) = 6.88$, $p = 0.01$]. On the contrary, men associated meat substitute products more often with tastiness (20%), while only 5% of women mentioned this positive mental association [$X^2(1, 162) = 9.58$, $p = 0.00$]. Furthermore, women considered meat substitute products more often as fake (15%) than men [3%; X^2(1,

TABLE 2 Mental associations reflecting motives and barriers for meat substitute consumption.

Mental associations related to motives	Exemplary positive mental associations	Exemplary negative mental associations	Frequency of mental associations	
			Pos.	Neg.
Taste	Tasty, yummy, delicious	Tasteless, bland, bad flavor	19	28
Health	Healthy, weight control, longer life	Unhealthy, stomach aches, cancer-causing	32	11
Disgust	n.a.	Gross, nasty, Eww, Yuck	0	24
Fake	It's real	Fake, misleading, deceptive	1	18
General appeal	Appetizing, mouth-watering	Unattractive, unappealing, unappetizing	2	16
Innovation	Innovative, new, unique	n.a.	16	0
Skepticism	Reliable, trusting	Weird, strange, questionable	1	15
Environment	Eco-friendly, planet-friendly, combats climate change	n.a.	14	0
Texture	Toothsome, satisfied with the consistency	Gritty, rubbery, chewy	2	12
Price	Cheap, cheaper	Expensive, pricey, overpriced	2	11
Natural content	Natural	Unnatural, against nature, lab-made	1	10
Curiosity	Curious, interesting	Uninterested, I do not care	8	3
Familiarity	Know	Unknown, Idk, not formed opinion	1	7
Animal welfare	Cruelty-free, less animal cruelty	n.a.	7	0
Additives	Clean, free	Additive, preservatives, meat glue	4	3
Variation	Versatile	Average	3	2
Convenience	Easy, quick, convenient	n.a.	3	0
Smell	n.a.	Stinky, odd smell	0	2
Nutrition	n.a.	High sodium	0	3
Other	Good, improved, feasible	Bad, waste, depressing	12	11

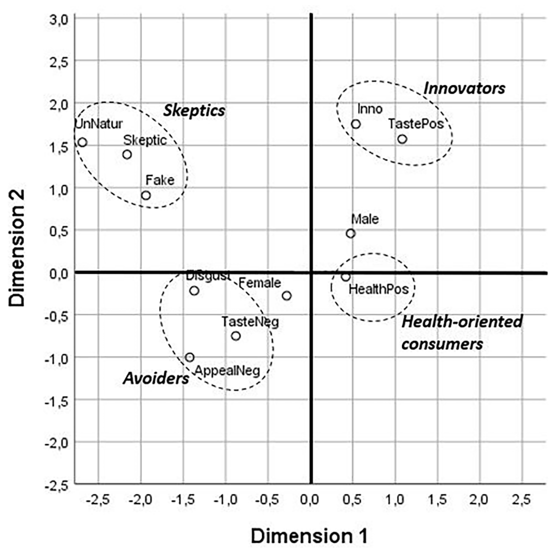

FIGURE 2
Biplot of the relationships between mental associations with meat substitutes.

162) = 4.99, $p = 0.03$]. Also, in the "others" category, women had more negative mental associations (10%) than men [0%; Chi-square test $X^2(1, 162) = 6.11$, $p = 0.01$]. These gender differences in mental associations with meat substitute products were also reflected in the MCA as we will elaborate on below.

We conducted the MCA to identify profiles of individuals based on their mental associations related to motives for meat substitute consumption (Table 2). Following the suggestion of Sester et al. (46) we utilized only categories mentioned by more than 5% of mental associations (minimum threshold = 15) for the MCA. The MCA revealed two dimensions: the first dimension with an inertia of 23.2%, and the second dimension with an inertia of 13.8%. The biplot, which depicts a topological representation is illustrated in Figure 2. The two diagonals divided people in terms of (1) taste perceptions and (2) healthy/unnatural perceptions. The first diagonal classifies consumers into individuals who appreciate the taste of meat substitutes vs. individuals who do not like the taste of meat substitutes and do not experience them as appealing. The second diagonal classifies individuals that perceive meat substitute products to be healthy vs. those that consider them unnatural. The latter individuals had mental associations such as "overprocessed," "artificial," and "lab-made."

Four different consumer profiles were identified. The first profile, which we call "skeptics" consists of individuals that are skeptical and consider meat substitutes as unnatural or even fake. The second profile, which we call "innovators" includes individuals that perceive meat substitutes as innovative and tasty. The third profile, which we call "health-oriented consumers" includes individuals that consider meat substitutes as healthy. Finally, the fourth profile, which we call "avoiders," reflects individuals that perceive meat substitutes as distasteful and unappealing, and even feel disgusted.

With regard to gender, we again observed differences. From Figure 2, it can be seen that the barycenters of both genders are on the tasty/untasty diagonal. Women are located near profile 4 ("avoiders") close to the barycenter of the negative mental associations with "taste," while the barycenter of men is between profile 2 ("innovators") and profile 3 ("health-oriented consumers). We turn to the interpretation of these results in the next section.

5. Discussion

Despite alarming obesity figures, the detrimental effect of immoderate meat consumption on health as well as the negative consequences of excessive meat production on the environment, only a minority of US citizens follows a plant-based diet. While there are three different ways to reduce meat consumption— (1) eat less but higher quality meat, (2) replace animal proteins with plant-based foods that have no similarities to meat (e.g., beans), (3) consume meat substitute products that mimic meat—our study focused on the last of these ways. Since meat substitute products can often substitute meat one-to-one in conventional dishes, consuming them is a particularly easy way to promote healthier and more sustainable eating habits. Nevertheless, little is known about the mental associations with meat substitute products among US citizens. The current study set out to fill this gap. It employed a free word association task and surveyed 175 US citizens on their mental associations with meat substitutes. Product categorization is important in the context of meat substitute products (52) and enables marketers and researchers to understand how consumers perceive these new products. Brand managers need to be aware of consumers' mental associations with the product category to effectively promote brands belonging to this product category (54, 55). The data analysis is based on 824 mental associations. Frequency analyses and a multiple correspondence analysis reveal new and interesting insights into US consumers' mental associations with meat substitute products.

Overall, the findings indicate that US citizens have more negative mental associations with meat substitute products than positive mental associations. This finding expands prior research reporting that consumers have more positive mental associations with meat as compared to meat substitute products (13), while our study concentrates only on meat substitutes. In contrast to prior studies, our findings further demonstrate that gender represents an important variable in explaining consumers' positive and negative perceptions of meat substitute products. In doing so, we advance existing research reporting inconclusive findings on differences in men's and women's mental associations with meat substitute products.

Mental associations with meat substitutes differ between men and women. It seems that women consider meat substitute products part of a vegan or vegetarian diet, and not as an alternative source of protein that could substitute meat occasionally. Women seem to have rather utilitarian associations, by concentrating on the nutritional aspect rather than on hedonic associations. This pattern has been observed in prior research as well (46). Indeed, women associated meat substitute products with a bad taste and disgust, mental associations that have been reported by prior studies employing non-US samples as well (25).

In this context, the unhealthy-tasty intuition might explain the prevailing negative mental associations of women with meat substitute products (69). In essence, the unhealthy-tasty intuition postulates that individuals associated unhealthy products with good taste, while the opposite is true for healthy products (69). The unhealthy-tasty intuition has been confirmed in various contexts. For instance, one study validates the unhealthy-tasty intuition in the context of recipes by reporting that a health claim negatively affects taste expectations (70). Hence, if meat substitute products prompt merely nutritional associations together with health inferences (as revealed in the MCA), women might draw the conclusion that meat substitute products are healthy but at the same time, also less tasty. Nutrition information indeed prompts more health inferences than taste inferences. A recent study demonstrates that presenting nutrition information before tasting plant-based products causes consumers to pay more attention to health inferences rather than taste inferences (71). Furthermore, women tend to perceive meat substitutes as fake products and are skeptical of these products. One possible explanation for these mental associations is that women are in general responsible for the nutrition of the family and hence, more cautious (72). Furthermore, these mental associations might be caused by little awareness of the benefits of meat substitute products. Prior research acknowledges that a lack of knowledge on how a plant-based can positively impact health and the environment represents a major barrier to plant-based diets (73).

On the contrary, men experience meat substitute products as innovative, which at the same time also represents one of the new categories that emerged in this study. Indeed, prior research has not identified "innovation" as an important mental association with meat substitute products. However, given the trend of variety seeking (74), new and innovative products seem to prompt favorable mental associations, while this mental association is predominantly elicited by men. Men associated meat substitute products with good taste and had fewer negative mental associations as compared to women. These findings contribute to the debate on men's willingness to consume plant-based meals (47) and potentially also the related research streams on alternative proteins such as *in-vitro* meat (43).

Overall, the study reveals four consumer profiles. The first profile represents the "skeptics," who mistrust information about meat substitute products and who experience them as fake and unnatural. The second profile is the "innovators," who have a generally positive attitude towards meat substitute products and associate them with good taste. The third profile is the "avoiders," who experience meat substitute products as unappealing, disgusting, and unappealing. Finally, the fourth profile represents the "health-oriented consumers," who consider meat substitutes as a good alternative for a healthier (vegan or vegetarian) diet. While the profile of health-oriented consumers has also been found in European studies and also the "avoiders" profile was similarly described by Possidonio et al. (25), US citizens still seem to differ in their mental associations. Ethical considerations play a subordinate role, while the novel character of meat substitutes was clearly more important as the profile of the "innovator" demonstrates. Also, the profile of "skeptics" is interesting, as it has not been observed in this pronounced way by prior studies.

When these profiles are combined with the genders, interesting patterns can be observed. Women seem to be best represented by the profile "avoiders," while men's mental association justified a classification into the profile "innovators." These new insights have several important practical implications: Policymakers and brand managers of meat substitute products need to have different targeting strategies based on gender. Men's consumption of meat substitute products could be stimulated by highlighting the innovative character and the good taste of the products. For women, a focus on the elimination of negative mental associations by providing more information on the processing of meat substitutes might be a good strategy. In general, reducing skepticism toward meat substitute products through governmental campaigns might be a fruitful policy, which should mainly target women. Additionally, highlighting good taste seems to be a good strategy for all consumer profiles, since taste represents one of the most important predictors of food consumption (75).

6. Conclusion

In conclusion, plant-based diets are beneficial for individuals and society. The current research contributes to the ongoing discussion on consumers' mental associations and perceptions of meat-substitute products in an effort to provide new insights which help both policymakers and marketers to better promote meat-substitute products. In addition to the identification of gender differences in terms of mental associations with meat substitute products, the current study identifies four different consumer profiles, which can be used for targeting purposes. Further studies might test specific promotional strategies (i.e., taste vs. health claims) for the identified consumer profiles.

One of the strengths of our study is that we discovered gender differences in mental associations with meat substitutes. Future studies could collect other person-specific data, such as actual meat consumption, and relate it to mental associations with meat substitutes.

Although our sample was large and diverse, it is an online sample and thus limited in its representativeness (e.g., concerning age or educational attainment). Thus, further work could use other sampling strategies that allow for examining our findings in larger and more representative samples to validate the results for the U.S. population. Another promising avenue is to replicate our study in other countries to compare the mental associations with meat substitutes across cultural contexts (37).

Our research could present the starting point for future research. On the one hand, additional qualitative work could be conducted using projective techniques (e.g., construction, completion, order of choice, or expressive) to make systematic comparisons between the different methods of analysis. On the other hand, quantitative work could develop scales to gain additional information about consumers' perceptions of meat.

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 Institutional Reviewer Board of Modul University Vienna. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MG and CG: conceptualization, data curation, and writing – review and editing. CG: methodology and formal analysis. MG: writing – original draft preparation and project administration. 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.

Publisher's note

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Evaluation of adherence to the Mediterranean diet with sustainable nutrition knowledge and environmentally responsible food choices

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Background: Dietary patterns and their possible effects on health and the environment are becoming increasingly important. It is thought that nutritionally balanced diets can also be compatible with environmental targets and, therefore, the Mediterranean diet (MD), which is regarded as a sustainable diet model, comes to the fore. This study was carried out to evaluate adherence to the MD with sustainable nutrition knowledge and environmentally responsible food choices and to determine the factors affecting adherence.

Methods: A questionnaire prepared by the researchers was sent to individuals online and 1732 adults living in Turkey participated in this cross-sectional study. Adherence to the MD was evaluated with the Mediterranean Diet Adherence Screener (MEDAS). In addition, questions were asked about nutritional knowledge and environmentally responsible food choices to evaluate the sustainable nutritional behaviors of individuals.

Results: Half of the participants (51.1% of men / 53% of women) adhere to the MD at a moderate level. Even the individuals with the highest adherence to the MD had low compliance with the recommendations for fruit (43.4%) and fish (37.3%) consumption. A one-unit increase in age, sustainable nutrition knowledge score, and environmentally responsible food choices score increases the MD adherence score by 0.08, 0.125, and 0.148 points, respectively ($p<0.005$). Individuals with high adherence to the MD avoid consuming genetically modified organism food more ($p<0.001$), prefer to consume environmentally labeled foods ($p<0.001$), and buy food more from local businesses ($p<0.001$), while they prefer to buy imported food less ($p=0.034$).

Conclusion: The results of this study showed that some strategies should be developed to increase the adaptation of individuals to the MD and sustainable nutritional behaviors. Nutritionally adequate, sustainable, and eco-friendly nutritional behaviors should be encouraged to increase the possible health benefits of nutrition and minimize environmental effects. To promote sustainable nutrition, firstly it is important to determine the knowledge level of individuals concerning sustainable nutrition and, for this purpose, it is thought that an international valid sustainable nutrition knowledge assessment tool is needed.

KEYWORDS

sustainable nutrition, Mediterranean diet, environmentally responsible food, nutrition knowledge, healthy nutrition

1. Introduction

As a result of the increase in the world's population, natural resources are faced with the danger of depletion, and thus issues such as social inequality, environmental degradation, and climate change have begun to be addressed within the framework of 'sustainability' (1). In this context, it is aimed to meet the needs of current generations without compromising the ability of the next generations to meet their own needs (2).

Nutrition plays a key role in promoting healthy and sustainable diets and ensuring food security globally in achieving the "zero hunger" goal, which is among the 17 sustainable development goals (3). Increasing global population, developing technology, urbanization, and changes in consumption patterns cause individuals' nutritional habits to change. Traditional diets are being replaced by diets in which more refined sugars, fats, and meats are consumed. These dietary changes greatly increase the incidence of type 2 diabetes, coronary heart disease, and other chronic diseases that reduce global life expectancy, while also increasing greenhouse gas emissions and use of land, livestock, water, and agrochemical (4, 5). Recent studies focus on nutritional habits and dietary patterns and their effects on health, the environment, and food system (5–7). With the concept of a sustainable diet, initiatives are planned to eradicate poverty, food and nutrition insecurity, and poor health outcomes (8).

A sustainable diet contributes to food and nutrition security and healthy life for current and future generations; has a low environmental impact; is culturally acceptable, accessible, affordable, nutritionally sufficient, safe, and healthy; makes the best use of natural and human resources; and respects ecosystems (9). There is evidence that nutritionally balanced diets may also be compatible with environmental goals (10). The Mediterranean diet (MD) has four main sustainability dimensions: health and nutrition benefits, low environmental impact and rich biodiversity, cultural heritage with high sociocultural food values, and positive local economic returns (11, 12). The MD is a dietary pattern characterized by high consumption of olive oil, vegetables, fruits, legumes, whole grains, and nuts; moderate whole milk and dairy products, wine, eggs, chicken, fish, and seafood (depending on the proximity of the population to the seashore); and low consumption of red meat, saturated fat and sweets, and its main feature is nutritional diversity (13).

The MD reduces environmental impact by focusing on increased consumption of plant-based foods compared to animal-derived and processed foods. It has a positive effect on environmental sustainability as it has a lower carbon and water footprint, lower greenhouse gas emissions, and less land and energy use compared to existing Western-type dietary patterns (5, 6). It provides a reduction in water and fuel consumption by reducing the consumption of processed food, and it also supports the consumption of local products, especially by including fish and seafood in the diet (5). It is predicted that transition to the MD will reduce environmental impact (−72%), land use (−58%), energy use (−52%), and water consumption (−33%) (14).

The MD is a healthy eating pattern involving interactions with cultures, people, and the environment, as well as sustainability. Educating the younger generations about the benefits of healthy and sustainable diets and raising consumer awareness of local and sustainable foods will be the first and easiest steps towards achieving global goals (15). It is extremely important to consume healthy and eco-friendly foods, to prevent non-communicable diseases (which are one of the main causes of death in the world) for the protection of human health, and to prevent excessive use of natural resources, climate change, and pollution in order to protect the health of the planet (16).

In the literature, studies on the MD as a sustainable dietary pattern and its possible effects on the environment are increasing (6, 15). However, to the best of our knowledge, there has been no study examining the relationship between individuals' knowledge of sustainable nutrition and their adherence to the MD. At this point, it is important to investigate the role of individuals' adherence level to the MD and their level of knowledge about sustainable nutrition in this adherence. It is thought that our study would be important in terms of filling this gap in the literature. Furthermore, practices such as eco-labeling are considered an important indicator of the increase in individuals' awareness of environmentally responsible foods and their interest in these products (17). Therefore, it is regarded as essential to evaluate the preferences of individuals for traditional/local foods, products with environmental labels, and especially ultra-processed foods in food preferences within the framework of sustainable nutrition.

Accordingly, in the present study it was aimed to evaluate adherence to the MD among adults as a sustainable dietary pattern and to determine the factors affecting adherence to this diet, especially sustainable nutrition knowledge and environmentally responsible food choices.

2. Materials and methods

2.1. Participants

Individuals selected by snowball sampling living in different cities in Turkey participated in this cross-sectional study. A pilot study was conducted with 15 individuals to determine the sample size. With the data obtained from the pilot study, the sample size was determined as at least 100 individuals using the program Power Analysis and Sample Size (PASS), provided that alpha (α) = 0.05, power (1–β) = 0.95, and the correlation coefficient was 0.35.

2.2. Ethical considerations

In order to carry out the research, approval was obtained from Gazi University Ethics Committee (Research Code No: 2021–04). Volunteers who agreed to participate in the study were informed about the study and their consent was obtained. This study was conducted by the principles of the Declaration of Helsinki.

2.3. Data collection

The data were collected through an online questionnaire sent to the participants. In the first part of the questionnaire, there were questions about sociodemographic characteristics (age, sex, educational status, etc.). The Mediterranean Diet Adherence Screener (MEDAS) was used to evaluate adherence to the MD, which is accepted as a sustainable nutritional model. In addition, questions were asked about nutritional knowledge and environmentally responsible food choices to evaluate the sustainable nutritional behaviors of individuals. The body weight and height of the individuals were recorded based on the declaration. Body mass index (BMI) was calculated and evaluated according to the classification of the World Health Organization.

2.4. Data collection tools

2.4.1. Mediterranean diet adherence screener

Mediterranean Diet Adherence Screener, which was developed by Martínez-González et al. (18) in 2012, was adapted into Turkish by Pehlivanoğlu et al. (19). It is a valid and reliable tool for assessing adherence to the MD. The habits of consuming foods that are characteristic of the MD are established with two questions on the scale and food consumption frequency with 12 questions. Each question is scored as 0 or 1 point. The total score ranges from 0 to 14, with 0 indicating the lowest adherence to the MD and 14 the highest adherence. An MD adherence score of ≤5 indicates low adherence, 6–9 moderate adherence, and ≥9 high adherence (19).

2.4.2. Sustainable nutrition knowledge

To determine sustainable nutrition knowledge, 15 items (in Supplementary material) prepared by Gülsöz (20) for her master's thesis, using international literature, were used. The calculation was made by giving 1 point to the correct answer for each item. The highest score that can be obtained in total is 15, and a higher score indicates greater sustainable nutrition knowledge (20).

2.4.3. Environmentally responsible food choices

To evaluate the environmentally responsible food choices of individuals, the 7-item "Environmentally Responsible Food Choices" instrument developed by Başar was used (17). The items are as follows: "I can pay more for organically grown food," "I avoid consuming food with genetically modified organisms (GMOs)," "I prefer to consume eco-label food," and "I am careful not to consume too much meat," "I prefer to buy dairy products from local producers," "I avoid consuming imported food such as a variety of exotic fruits," and "I avoid consuming canned "ready-made" food." Item responses are 5-point Likert type and each item is scored between 1 and 5. The total score is at most 35 and a higher score indicates more environmentally responsible food choices.

2.4.4. Statistical analysis

Statistical analyses of the data obtained were conducted using IBM SPSS Statistics 22 (IBM SPSS, Turkey). In the descriptive analyses, categorical data were used as numbers and percentages, mean, and standard deviation values were used according to the normality of the numerical data. Compliance with the normal distribution was examined by Kolmogorov–Smirnov/Shapiro–Wilk tests and histogram plot. The Chi-square test was used to compare the categorical data. For the numerical data, the t-test was used to compare two groups. A multiple linear regression model was used to identify independent predictors of the MD. The differences in compliance rates for each food and food group according to the MEDAS items were calculated and the difference between the current status (percentage of participants currently adhering to each dietary recommendation) and the ideal situation (100% compliance) is shown in radar charts. The statistical significance level was set at 0.05.

3. Results

The study was conducted with 1732 adults and their general characteristics are shown in Table 1. The mean age of the participants was 27.7 ± 10.00 years, and the majority (72.1%) were between 18 and 29 years old. Most of the participants (78.1%) were university students/graduates. The mean BMI was 22.8 ± 4.21 kg/m^2 in women and 25.3 ± 4.07 kg/m^2 in men and 59.2% of all participants had a normal BMI (Table 1).

The participants' MEDAS scores and levels, sustainable nutrition knowledge scores, and environmentally responsible food choices scores are given in Table 2. It was determined that 51.1% of men and 53% of women showed moderate adherence to the MD. While there was no significant difference between the MEDAS scores according to sex ($M = 6.76 \pm 2.08$; $W = 7.01 \pm 2.06$ $p > 0.05$), sustainable nutrition knowledge ($M = 8.43 \pm 2.72$; $W = 9.25 \pm 2.51$, $p < 0.05$) and environmental responsible food choices scores ($M = 22.36 \pm 5.40$; $W = 22.67 \pm 4.81$, $p < 0.05$) were higher in the women than in the men. According to the level of adherence to the MD, the answers given by the participants regarding sustainable nutrition knowledge and environmentally responsible food choices are given in Supplementary Table 1 and Table 2.

TABLE 1 Sociodemographic characteristics of the participants.

Sociodemographic characteristics	Men (*n* =615)		Women (*n* =1.117)		Total (*n* =1732)	
	n	%	*n*	%	*n*	%
Age (years)						
18–29	387	62.9	862	77.2	1.249	72.1
30–39	76	12.4	153	13.7	229	13.2
40–49	78	12.7	63	5.6	141	8.1
≥50	74	12.0	39	3.5	113	6.5
Age (years) (mean ± SD)	30.7 ± 12.05		26.1 ± 8.24		27.7 ± 10.00	
Educational level						
Primary school	7	1.1	23	2.1	30	1.7
Secondary school	11	1.8	24	2.1	35	2.0
High school	97	15.8	110	9.8	207	12.0
University	451	73.3	901	80.7	1.352	78.1
Postgraduate	49	8.0	59	5.3	108	6.2
Length of education (years; mean ± SD)	15.3 ± 2.20		15.3 ± 2.28		15.3 ± 2.25	
Body Mass Index (BMI) (kg/m^2)						
<18,5 (underweight)	20	3.3	127	11.4	147	8.5
18,5–24,9 (normal)	289	47.0	736	65.9	1.025	59.2
25,0–29,9 (overweight)	224	36.4	183	16.4	407	23.5
>30 (obese)	82	13.3	71	6.4	153	8.8
Body Mass Index (BMI) (kg/m^2) (mean ± SD)	25.3 ± 4.07		22.8 ± 4.21		23.7 ± 4.33	

TABLE 2 Adherence to the Mediterranean diet, sustainable nutrition knowledge and environmentally responsible food preference scores of the participants.

	Men (*n* =615)		Women (*n* =1.117)		Total (*n* =1732)	
	n	%	*n*	%	*n*	%
Level of adherence to the Mediterranean diet						
Low adherence	170	27.6	257	23.0	427	24.7
Moderate adherence	314	51.1	592	53.0	906	52.3
High adherence	131	21.3	268	24.0	399	23.0
	$p = 0.083$*					
Adherence to the Mediterranean diet scores (mean ± SD)	6.76 ± 2.08		7.01 ± 2.06		6.92 ± 2.07	
	$p = 0.233$**					
Sustainable nutrition knowledge scores (mean ± SD)	8.43 ± 2.72		9.25 ± 2.51		8.96 ± 2.62	
	$p = 0.015$**					
Environmentally responsible food preference scores (mean ± SD)	22.36 ± 5.40		22.67 ± 4.81		22.56 ± 5.03	
	$p = 0.00$**					

*Chi-square test.
**Student *t* test.
The bold values indicate statistically significant values ($p < 0.05$).

The compliance of the men and women with MEDAS items according to their adherence to the MD is shown in Figure 1. The use of olive oil as the main oil type was high only in the group with high adherence to the MD among the women. However, olive oil use was high in all men, regardless of MD adherence. Even the men and women who showed the highest adherence to the MD had low compliance (below 50%) with the recommendations for fruit (43.4%) and fish consumption (37.3%; Figure 1; Supplementary Table 3). Moreover, 58% of the men and 64.9% of the women who adhered closely to the MD did not comply with the recommendation regarding fish consumption. Individuals were in greater compliance with the recommendation of low consumption of butter/margarine, sweet or carbonated beverages, and bakery products (Supplementary Table 3). In addition, compliance with the recommendations for red wine consumption was low in both sexes (Figure 1).

Regression models were created to identify potential factors affecting individuals' adherence to the MD (Supplementary Table 4). In the first stage, sex, age, and BMI and, afterward, length of education, sustainable nutrition knowledge score, and environmentally responsible food choices score were added to the models. In the last stage, model 5 was created with the significant parameters. According to model 5, a one-unit increase in age, sustainable nutrition knowledge score, and environmentally responsible food choices score increases the MD adherence score by 0.08, 0.125, and 0.148 points, respectively ($p < 0.005$). In the multilinear model created, sex, age, sustainable nutrition knowledge score, and environmentally responsible food choices score with a 5% change in MD adherence score are explained (Table 3).

4. Discussion

The MD is considered a sustainable diet model when regarded together with its socio-cultural, economic, and environmental benefits (21). In the present study, most of the individuals (52.3%) living in Turkey, a country with a coast on the Mediterranean, had a moderate level of adherence to the MD. Similarly, in studies conducted in Italy (22), Portugal (23) and different European countries (24) the most of individuals (59.4, 62.7 and 68.3%, respectively) adhered to the MD at a moderate level. On the other hand in a study conducted in Lithuania and Serbia, which are not Mediterranean countries, most of the participants (47.7%) had moderate levels of adherence but the rate of low levels of adherence was also high (39%) (25). These data emphasize that geography is important for individuals' nutritional habits, food preferences, and diet quality. Although the availability of and accessibility to olive oil, legumes, whole grains, and fresh products (fruits, vegetables, and fish) are easier in Mediterranean countries, the low rate of individuals with high adherence to the MD in these studies is confusing. In a study comparing the trends of adherence to the MD at the global level over time, there was a significant decrease in the level of adherence to the MD between 1961 and 1965 and between 2000 and 2003 in many countries, including Turkey, but this decrease was smaller between 2004 and 2011 (26). This situation may have resulted from Westernization together with the changes in cultural, social, and political factors greatly impacting the changes in nutritional habits.

While the sociocultural level is one of the variables consistently associated with better adherence to the MD in the literature, it is controversial whether a higher education level is generally associated with adopting healthier diets and consuming healthier foods (27). Especially with Westernization, university students take an active role in the modern age. In a review, it is stated that the majority of university students show moderate adherence to the MD, and this is evident even among university students living in Mediterranean countries, especially those living far from their families (28). Same results were also found in studies evaluating the adherence of university students to the MD in Turkey (29, 30). Similarly, in the present study, in which most of the participants (78.1%) were university students/graduates, the adherence of individuals to the MD was moderate. It is thought that the changes in the food preferences of university students, their access to fresh food, the inadequacy of food preparation conditions due to their staying in dormitories and student houses, and negative financial conditions cause this situation.

The MD, which is described as sustainable, is associated with 4 criteria: 1. high consumption of vegetables and fruits, 2. low consumption of cheese, meat, and meat products, 3. low intake of sugar, sodium, total fat, saturated fat, and cholesterol, and 4. high consumption of fish and olive oil (13). A traditional MD includes the consumption of seasonal vegetables and fruits every day. In the

FIGURE 1
Compliance to Mediterranean diet adherence screener (MEDAS) items according to sex **(A)** men, **(B)** women, and level of adherence to the Mediterranean diet.

TABLE 3 Regression models for potential factors affecting adherence to the Mediterranean diet.

	Model 1			Model 2			Model 3			Model 4			Model 5		
	β	*se*	*p*	*β*	*se*	*p*	*β*	*se*	*p*	*β*	*se*	*p*	*β*	*se*	*p*
Sex	0.077	0.108	0.002	0.081	0.109	0.001	0.063	0.109	0.012	0.053	0.108	0.033	0.053	0.105	0.030
Age	0.123	0.006	<0.001	0.136	0.006	<0.001	0.130	0.006	<0.001	0.090	0.006	0.001	0.080	0.005	0.001
BMI	−0.029	0.013	0.281	−0.022	0.013	0.421	−0.011	0.013	0.685	−0.009	0.013	0.736			
Length of education				0.055	0.023	0.030	0.024	0.024	0.352	0.025	0.023	0.320			
Sustainable nutrition knowledge scores							0.134	0.020	<0.001	0.119	0.019	<0.001	0.125	0.019	<0.001
Environmentally responsible food preference scores										0.148	0.010	<0.001	0.148	0.010	<0.001
Adjusted R^2	0.014			0.016			0.032			0.052			0.052		

Multiple linear regression.

present study, it is noteworthy that individuals of both sexes have levels lower than the recommendation (2 servings of vegetables/day, 3 servings of fruit/day) in terms of fruit and vegetable consumption. A study conducted by Marendić et al. (31) in Italy with a similar age group also determined that individuals' adherence to vegetable and fruit consumption recommendations was very low, similar to our study. In another Italian study conducted with adults, it was also stated that adherence to the recommendations of fruit and vegetable consumption was low (32). These plant-derived foods, which have less environmental impact and high nutritional value, were consumed less than recommended may be an important problem in terms of sustainable nutrition.

The most characteristic feature of the MD is olive oil. In the present study, although most individuals with low, moderate, and high adherence to the MD indicated olive oil as the main culinary fat, compliance with the recommendation for olive oil amount was found low in both sexes. In a study conducted with Lebanese university students, the type of oil consumed by individuals, in general, was olive oil, but, similar to our study, the amount of olive oil consumed daily by the participants was below the recommendations (33). This may be due to the fact that olive oil is more expensive than other types of vegetable oil.

In the present study, legumes and nuts were the foods for which individuals' adhered to the MD recommendations most. In particular, legume consumption among the men with high adherence to the MD is higher than among both the men and women with lower adherence. Nut consumption by individuals is compatible with the MD adherence level and is similar between the sexes. In a study conducted in Gulf countries (Saudi Arabia, Oman, and Kuwait), the highest consumption of legumes was seen in individuals with moderate adherence to the MD, and the highest consumption of nuts was determined in individuals with high adherence. However, in line with the recommendations, the rates of individuals consuming legumes and nuts were quite low (34). The fact that Turkey is located in geography rich in cereals and legumes may have caused a high level of adherence to these recommendations.

As an important food source, fish has various health benefits due to its antioxidant, anti-inflammatory, cardioprotective, and neuroprotective properties (35). However, the effect of seafood on a sustainable diet is not very clear in the literature. The health benefits of seafood consumption and the lower impact of fish consumption on greenhouse gas emissions compared to other animal-derived proteins are good points to be highlighted; however, the ecological impacts as a result of overfishing create a dilemma (36). Therefore, it is important to strengthen recommendations for consumption from accepted sustainable sources and species that are not overfished. Considering its effects on both health and the environment, the MD, which supports local and seasonal fish consumption, is seen as one of the healthiest nutritional patterns with low environmental impact (37). In the present study, although fish consumption by individuals with high adherence to the MD in both sexes is higher than in those with moderate and low adherence, it is noteworthy that only 37.3% of individuals with high adherence to the MD complied with the recommendations.

Age is associated with food choices, and it is stated that older individuals show higher adherence to the MD (27, 38). In our study, similar to the literature, a significant positive correlation was found between age and MEDAS score. Accordingly, it was determined that a one-unit increase in age increased the MD adherence score by 0.08 points. It is thought that this situation may be since elderly individuals behave more traditionally in their food preferences and that chronic diseases requiring nutrition therapy are seen more frequently with increasing age. On the other hand, because of changing living conditions and fast changes in their food preferences, younger people play a more active role in Westernization. In the present study, the majority of participants (72.1%) were aged between 18 and 29. Therefore, future studies with larger samples in the older age group would be a better guide for evaluating this relationship.

As a sustainable dietary pattern, the MD gains importance not only with its nutritional recommendations but also with the protection of biodiversity, local production, and the culture of each community. It has been shown that there is a relationship between the health-promoting and eco-friendly behaviors of individuals and their adherence to the MD (39). Further, it is also known that there is a relationship between the level of nutrition knowledge, which is influential in improving health, and the adherence of individuals to the MD (40). In the present study, individuals with a higher level of knowledge about sustainable nutrition adopted a more sustainable nutrition model. In fact, an increase of one unit in the sustainable nutrition knowledge score increases the MD adherence score by 0.125 points. According to the MD model, plant-derived foods, which are recommended to be consumed more frequently in order to provide a healthy diet, also have less impact on the environment during production and consumption (39).

Eco-friendly approaches such as organic agriculture and the MD are seen as promising models for sustainable diets (41). In the present study was found that environmentally responsible food choices were a factor affecting adherence to the MD and this result is in agreement with the literature. Individuals with high adherence to the MD stated that they preferred to consume environmentally labeled foods more than those with moderate or low adherence. As a result of the study conducted by Yardimci and Demirer (42) to examine the relationship between the level of adherence to the Mediterranean diet and the awareness of the Ecological Footprint of Turkish adults, it was determined that as individuals' adherence to the Mediterranean diet increases, their awareness of ecological footprints will also increase. In the literature, it is stated that the use of eco-labels for sustainable food shopping may be beneficial for consumers with a preference for sustainable nutrition but without sufficient knowledge. The use of eco-labels in food shopping affects positively the organic food choices of individuals and becomes a source of motivation to choose these foods (43).

One of the factors evaluated within the scope of environmentally responsible food choices was GMOs in our study. It was determined that individuals with high adherence to the MD avoided consuming food with GMOs more than those with moderate or low adherence ($p < 0.001$). Although there is concern about the possible harmful effects of foods with GMOs on the environment, when evaluated in terms of sustainability it is stated that the correct use of GMOs technology may be important in achieving the goal of preventing global hunger. More studies are needed to determine the economic, environmental, and health-related indirect effects of foods with GMOs (44).

In addition, the role of the MD in strengthening sustainable food systems through regional development strategies and traditional local products is emphasized (45). Regarding its environmental impact, the MD supports the consumption of local and seasonal products (11). It is recommended to consume more seasonal and locally produced foods in order to reduce the energy inputs and greenhouse gas emissions caused by household food consumption (46). In our study, individuals with high adherence to the MD preferred to buy milk and dairy products from local markets and they preferred imported foods less. In a study with Italian families, households with high adherence to the MD were more likely to purchase both organic and local products, while an increase in household size decreased the likelihood of purchasing local products (41).

The adherence of individuals to the MD was evaluated with MEDAS, which is an internationally valid and reliable scale, and the evaluation of adherence to the MD from both a nutritional and sustainable perspective constitutes the strength of the present study. Since there is no valid and reliable tool developed to evaluate the sustainable nutrition knowledge level in the international literature, the use of statements prepared in line with the existing literature for this purpose is one of the limitations of our study. Another limitation is that although the sample size was high, most of the participants consisted of young adults and individuals with a high level of education. It is thought that this may have been since participation in online studies is easier for this age group. For the sample to represent the universe, it is recommended to ensure a homogeneous distribution of different age groups for future research. Evaluation of weight and height according to the declaration is also among the limitations of this study.

As a result, investigating individuals' diet quality and their perceptions of food preferences is a valuable approach to appropriately shifting dietary behavior in the desired direction and identifying effective strategies. In the present study, the factors that may affect adherence to the MD were examined by asking questions about the sustainable nutritional knowledge and environmentally responsible food choices of individuals. It was found that some changes are needed in the dietary habits of Turkish adults to meet the nutritional recommendations and environmental guidelines for sustainability. Since nutritious foods such as fresh vegetables and fruits, which are an important component of the MD, are consumed less than recommended, multidimensional approaches should be developed both to increase individuals' awareness of the importance of fruit and vegetable consumption and to provide access to these foods. Insufficient fish consumption is an important problem in terms of both healthy nutrition and the environmental effects of nutrition. In order to increase fish consumption among individuals, not only ensuring adequate access to fish but also increasing awareness of cultural adoption and the possible benefits of fish consumption is important. The development of policies that will increase adherence to the MD is extremely important in terms of its contribution to the health of the public as well as to the protection of the world. For this purpose, it is necessary to encourage nutritionally adequate, sustainable, and eco-friendly nutritional behaviors in terms of nutrition. To promote sustainable nutrition, determining the knowledge level of individuals concerning sustainable nutrition is very important, so there is a need for an internationally valid sustainable nutrition knowledge assessment tool.

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 Gazi University Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EY contributed to the conception, design of the study, and wrote the first draft of the manuscript. HB organized the database and performed the statistical analysis. EY and HB wrote sections of 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1158155/full#supplementary-material

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Habitual low carbohydrate high fat diet compared with omnivorous, vegan, and vegetarian diets

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Background: Dietary patterns which exclude whole food groups, such as vegetarian, vegan and low carbohydrate high fat diet (LCHF), are increasingly popular in general public. When carefully planned, all these diets have some known benefits for health, but concerns are also raised in particular for LCHF. The quality of LCHF diet which individuals follow in real life without supervision is not known.

Methods: One hundred thirty healthy individuals with stable body mass following LCHF, vegan, vegetarian and omnivorous diet for at least six months, were compared in a cross-sectional study. Diet was analyzed through 3-day food records and FFQ, anthropometric measurements were performed and serum metabolic biomarkers determined from fasting blood.

Results: Participants on LCHF diet had the intakes of micronutrients comparable to other groups, while the intakes of macronutrients differed in line with the definition of each diet. The intakes of saturated fats, cholesterol and animal proteins were significantly higher and the intakes of sugars and dietary fibers were lower compared to other groups. Healthy eating index 2015 in this group was the lowest. There were no differences in the levels of glucose, triacylglycerols and CRP among groups. Total and LDL cholesterol levels were significantly higher in LCHF group, in particular in participants with higher ketogenic ratio. Fatty acids intakes and intakes of cholesterol, dietary fibers and animal proteins explained 40% of variance in total cholesterol level, with saturated fatty acids being the strongest positive predictor and monounsaturated fatty acids a negative predictor.

Conclusion: None of the self-advised diets provided all the necessary nutrients in optimal levels. Due to the detected increased levels of serum cholesterols, selection of healthy fat sources, higher intake of dietary fibers and partial replacing of animal sources with plant sources of foods should be recommended to the individuals selecting LCFH dietary pattern.

Clinical Trial Registration: ClinicalTrials.gov, identifier NCT04347213.

KEYWORDS

low carbohydrate high fat diet, vegan diet, vegetarian diet, omnivorous diet, serum cholesterol, diet quality, saturated fatty acid, monounsaturated fatty acid

1. Introduction

Traditionally the majority of people in Western countries and also in Slovenia were omnivores. National guidelines for healthy nutrition are thus based on omnivorous diet (1). In the last decades the popularity of different dietary patterns such as vegetarian, vegan and low carbohydrate high fat (LCHF) is rising, according to lay publications, social media and cross sectional surveys (2, 3). The motivators for choosing such a dietary pattern may be ethical and environmental issues, weight loss or improving fitness, but the main motivation is improving health (4, 5). All mentioned dietary patterns are in fact advertised in public as health beneficial and safe for long term practicing, but for LCHF, most scientific evidence originates from intervention studies, mostly with ketogenic diet and rarely longer than 15 weeks. On the other hand, little is known of the long-term effects in healthy adults, especially when the dietary choices in diets that omit whole food groups are made without proper counseling. Although nation-representative scans, performed under the umbrella of EFSA, investigate the connections between habitual dietary pattern and health (6), LCHF, vegetarian and vegan dietary patterns are rare, so the data of individuals following those dietary patterns cannot be extracted from such studies and no data is available about them.

In order for a diet to be considered low carbohydrate, carbohydrate intake is limited to 130 g daily or <26% of daily energy intake (EI) for a 2,000 kcal/day diet (7), and thus this pattern excludes or strongly limits the intake of starchy foods, legumes, sugars and fruits (8). To compensate for the described omissions, subjects following such a dietary pattern consume higher amounts of concentrated fats, meat, poultry, fish, eggs and cheese as well as red and processed meat (9). The majority of energy therefore derives from fat, making their fat intake higher than in dietary patterns that do not restrict carbohydrates. Some LCHF diets restrict carbohydrate intake to the extent to promote ketogenesis, making them ketogenic LCHF, while the others allow enough carbohydrate that the ketogenesis does not take place, making them nonketogenic LCHF (10). For the purpose of this article, we will call LCHF a dietary pattern that limits carbohydrates to 26% EI or less and has fat intakes more than 50% EI which is 167% of the recommended fat intake of 30% EI (11). LCHF is also the term with which such a dietary pattern is presented in lay literature in Slovenia and under which the persons that follow it identify themselves. Vegetarian and vegan dietary patterns also exclude whole food groups, as vegetarians eliminate meat, poultry and often fish, and vegans in addition reject all animal products such as dairy and eggs, and other products from animal origin, such as honey (12). The predominance of different food groups in the diet reflects in an altered intake of macronutrients which have differential effects on metabolism and health. LCHF is defined by high fat intake, and there is typically low intake of dietary fibers, while vegetarians and especially vegans usually have higher intakes of carbohydrate, omega-6 fatty acids and dietary fibers, but lower intake of protein, saturated fatty acids (SFA) and long chain omega-3 fatty acids (13). All restrictive dietary patterns also carry the risk of micronutrient deficiencies. Thus, restricting carbohydrate rich foods in LCHF may lead to low intake of thiamin, folate, niacin, riboflavin, vitamins A, C, and E, pyridoxine, calcium, magnesium, iron, potassium, selenium, and zinc (14, 15). Without supplementation, deficiencies in vitamin K, linolenic acid and water-soluble vitamins, excluding vitamin B_{12}, are common (9). Vegetarians and more often vegans may be vitamin B_{12} deficient, because vitamin B_{12} is mainly found in animal source foods (2). More severe vitamin D deficiencies are also found among vegans, compared with other dietary patterns (16).

As with all major lifestyle changes, health benefits were found to be among the main motivators also for people who have switched to LCHF (4). Indeed, metabolic disturbances that may be improved with LCHF diet [such as glucose metabolism, triacylglicerols levels and blood pressure (17)] are often the cause of various diseases, especially chronic noncommunicable diseases. Diet quality and intake of bioactive compounds are independent risk factors for non-communicable diseases and all-cause mortality (18, 19). Certain promotors of vegan, vegetarian or LCHF dietary patterns often consider only their dietary pattern as healthy and all the other dietary patterns wrong, creating myths in the public. Whether any of these patterns can be considered healthy when it is self-advised and used over a long term, may depend on the choice and quality of the food chosen from allowed food groups by the individual and observed/measured parameter, and remains controversial, especially for LCHF, for which epidemiological research is scarce. A high fat intake, high in SFA, is a well-established risk factor for high serum triacylglycerols and cholesterol levels. However, in majority of population high fat and particularly high SFA intake is associated with high sugar intake as part of a Western diet. Metabolic millieu of individuals on LCHF dietary pattern is different to the ones on Western diet due to low carbohydrate intake (20, 21). Short-term studies have been inconclusive about the effects of LCHF dietary pattern on serum cholesterol and triacylglycerols levels (9, 20, 22, 23). LCHF dietary pattern in overweight and obese subjects with weight loss has been associated with improved fat and glucose metabolism on short-term (20, 24), but long-term effects without energy restriction are unknown. On the upside, adherence to LCHF dietary pattern for a short period was associated with increased insulin sensitivity, better glucose regulation and lower risk for metabolic syndrome (9). Contrary to the LCHF diet, vegetarians and vegans consume less dietary fats, in particular SFA, and also exhibit lower total serum and LDL cholesterol levels than omnivores (2). In fact, vegetarian dietary pattern was associated with cardiovascular disease prevention (25). Energy intake is an important parameter as well, as it is important to maintain body mass. Lower body mass index (BMI) was determined among vegetarians and vegans, which was associated with a lower risks of developing obesity, metabolic syndrome and type 2 diabetes mellitus (25).

It has been established that vegetarians and vegans have lower mortality rates from chronic noncommunicable diseases (13), but this result may be partly due to an overall healthier lifestyle. For instance, they often avoid smoking and drinking alcohol, have lower BMI and higher levels of physical activity than omnivores (2). However, diets low in carbohydrate are also associated with improved health parameters (20). Ketone bodies, which are synthesized when the usage of fatty acids is elevated, have been linked to the alleviation of several age-related diseases and to longevity [reviewed in (26)]. It is known that with careful planning, one can achieve the recommended levels of all nutrients regardless of the omission of separate food groups (15, 27, 28). The intake of SFA, dietary fibers and bioactive compounds is largely dependent on food choices within a given food group. However, only a small proportion of people seek the advice from an expert dietitian about proper replacement of omitted foods when transitioning to a new dietary pattern. The aim of this study was therefore to compare diet quality and serum biomarkers in healthy normal weight adults with constant body mass, who followed either LCHF, or vegan, vegetarian or omnivorous diets for at least six months, without supervision of a dietitian.

2. Materials and methods

2.1. Study design and subjects

The present study is a cross-sectional study that took place at the Faculty of Health Sciences, University of Primorska in Izola from December 2019 to October 2021. The study protocol was approved by the Slovenian National Medical Ethics Committee (No. 0120–557/2017/4) and was registered at ClinicalTrials.gov (NCT04347213).

Volunteers, highly interested in healthy nutrition, were recruited through a web survey posted on social media in groups dedicated to nutrition or specific dietary pattern. The survey inquired on dietary pattern, motives for its selection, duration of adherence to present dietary pattern, and self-reported body height and body mass, stability of body mass in the last three months, age, presence of chronic diseases, presence of medication, pregnancy or lactation and an invitation to give a contact for a potential invitation to participate in the study. The including criteria were adherence to dietary pattern (LCHF, vegan, vegetarian or omnivorous) for a minimum of six months, BMI between 18.5 and 30 kg/m^2 and age between 20 and 60 years. Any chronic disease, taking medications (except contraception), being pregnant or lactating and a change in body mass (more than 3 kg) three months prior to the measurement served as the exclusion criteria. Using G*Power 3.1.9.7 (Heinrich-Heine-Univesität Düsseldorf, Germany) it was *a priori* calculated, that we need sample size N = 76 for parameter comparison between four equal groups and sample size *N* = 86 for Pearson correlation, for statistical power 0.8 at 5% 1. type error and 20% 2. type error. After reaching 90 participants, new recruits were accepted based on their sex and BMI to obtain homogenous groups (Figure 1).

Interested volunteers with no exclusion criteria received all the instructions regarding the measurements and questionnaires through an online session. Measurements included anthropometric and blood pressure measurements, blood withdrawal for biochemical analysis, 3-day Food Diary, Food Frequency Questionnaire (FFQ), Lifestyle questionnaire, International Physical Activity Questionnaire (IPAQ) and Socio-Economic Questionnaire.

2.2. Dietary assessment

Subjects completed 3-day Food Diary during the week before the visit at the University. They were instructed to record food intake for three days, two weekdays and one weekend day, to weigh and record all foods and beverages immediately before eating and to weigh any leftovers. They were asked to include food labels and recipes for mixed dishes and in addition, to report taking any food supplements, including the dose, and to describe if they pursue any type of fasting.

Participants also completed FFQ validated for Slovene population (29). FFQ includes nine food groups: milk and dairy products, vegetables, fruits, starchy foods, legumes, meat and meat products, fat and fatty foods, sugar and beverages. It consists of eight frequency measures: never, once per month, 2 to 3 times per month, 1 to 2 times per week, 3 to 4 times per week, 5 to 6 times per week, 1 to 2 times per day and 3 or more times per day; and 3 portion sizes: small, medium and large. Participants received visualization tools for more accurate portion assessment.

All 3-day Food Diaries and FFQ were checked by dietitian and any ambiguities and inaccuracies were addressed on the day of the measurements, to ensure the reliability of the data. Dietary data from Food Diaries and FFQ were analyzed with the Open Platform for Clinical Nutrition (OPEN).[1] Data of macronutrient intake, micronutrient intake, energy intake, and energy density were obtained from these analyses.

2.2.1. Healthy eating index

Healthy Eating Index 2015 [HEI (30)] was calculated from food diary data to evaluate diet quality according to the developers' protocol (31). HEI evaluates intake of 13 food groups: total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant protein foods, fatty acids ratio (sum of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA)/SFA), refined grains, sodium, added sugars and SFA, all estimated per energy intake unit (1,000 kcal). The first nine categories are scored positively and the last four are scored negatively. The sum of all categories is HEI (0–100), with the higher score representing higher diet quality.

2.2.2. Dietary inflammatory index

Dietary Inflammatory Index (DII) was calculated according to author's description (32), from 3-day Food Diaries and included 36 food parameters out of 45 (alcohol, vitamin B6, β-carotene, caffeine, dietary fibers, folic acid, garlic, ginger, magnesium, MUFA, niacin, omega-3 and omega-6 fatty acids, onion, PUFA, riboflavin, selenium, thiamin, turmeric, vitamin A, vitamin C, vitamin D, vitamin E, zinc, green/black tea, pepper, thyme/oregano, and rosemary and pro-inflammatory parameters were vitamin B12, carbohydrate, dietary cholesterol, energy, total fat, iron, protein, and SFA). Intake of every food parameter was used to calculate *z*-score based on world mean consumption, obtained from 11 datasets (32). *Z*-score was converted in percentile and centered on zero by multiplying with 2 and subtracting 1. The result was then multiplied with the overall inflammatory effect score, reported by the authors based on review of 1943 articles (32). Overall inflammatory effect scores smaller than zero were considered anti-inflammatory and scores greater than zero pro-inflammatory. Subjects' DII score is the sum of food parameter specific DII scores. Higher DII scores represent more pro-inflammatory diets.

2.2.3. Processed foods index

To assess the overall use of processed and highly processed foods in different dietary patterns, we established a new Processed Foods Index (PFI), based on the NOVA classification of processed foods (33), which serves as an indication of the degree of processed food intake:

$$PFI = \sum_{n=1}^{m} \frac{E_{food_n}}{EI} \, food\ group$$

where E_{food_n} is energy value of food item n, EI is daily energy intake, $food\ group$ is the number of processed food group the food item belongs to and m is the number of food items consumed.

1 http://opkp.si/

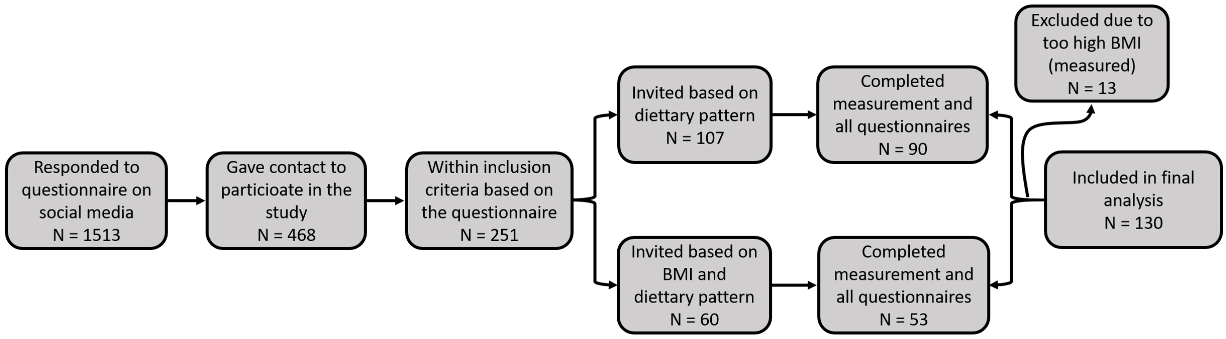

FIGURE 1
Flow diagram of the recruitment process.

NOVA classification of processed foods classifies foods into four groups based on the processing degree: unprocessed or minimally processed foods, processed culinary ingredients, processed foods and ultra-processed foods. Group 1 includes unprocessed foods such as fresh fruits, vegetable, seeds, fungi and algae and animal foods such as muscle, offal, eggs and milk. Minimally processed foods include foods that undergo processes including drying, crushing, grinding, powdering, fractioning, filtering, roasting, boiling, non-alcoholic fermentation, pasteurization, chilling, freezing, placing in containers and vacuum packaging. Group 2 includes processed culinary ingredients such as oils, butter, lard, sugar and salt. Allowed processes in this group are pressing, refining, grinding, milling and drying. Group 3 includes canned and bottled vegetables or legumes preserved in brine, whole fruit preserved in syrup, tinned fish preserved in oil; some types of processed animal foods such as ham, bacon, pastrami, and smoked fish, freshly baked breads, and simple cheeses to which salt is added. Processes for group 3 consist of adding salt, oil, sugar or other substances from group 2 to group 1 foods and also cooking, baking and non-alcoholic fermentation. Group 4 foods are created by series of industrial techniques and processes, including carbonated soft drinks, sweets, fatty or salty packaged snacks, candies, mass produced packaged breads, buns, cookies, pastries, cakes, margarine and other spreads, sweetened breakfast cereals, fruit yoghurt, energy drinks, pre-prepared meat, cheese, pasta and pizza dishes, poultry and fish nuggets, sausages, burgers, hot dogs, powdered and packaged soups, noodles and desserts (33).

2.2.4. Ketogenic ratio determination

To determine a possible effect of dietary pattern on metabolic milieu, we calculated ketogenic ratio (KR) (34, 35) with the following equation:

$$KR = \frac{0.9\,F + 0.46\,P}{C + 0.58\,P + 0.1\,F}$$

where F is fat intake [g], P is protein intake [g] and C is carbohydrate intake [g]. KR = 1.5 was considered threshold of ketogenesis (35, 36).

2.3. Anthropometric measurements

Anthropometric measurements were performed after an at least 12 h overnight fast in standardized conditions with light clothing, without shoes and by the same examiner. Body height, waist and hip circumference were measured. Body mass was measured using bioelectric impedance analyzer Tanita BC 418MA (Tanita Corporation, Arlington Heights, IL, USA). Body fat percentage, fat mass, lean mass, muscle mass, total body water and phase angle were measured using bioelectrical impedance analyzer Bodystat Quadscan 4000 (Bodystat Ltd., Isle of Man, British Isles). Blood pressure was measured with an automatic blood pressure monitor Model SEM-1 (Omron Healthcare Company, Singapore).

2.4. Serum biomarkers

Blood samples were collected in morning hours after an overnight fast (at least 12 h). Samples were set to clot at room temperature for 30–60 min and then centrifuged at 2000 rpm for 10 min at room temperature. Serum was aliquoted and stored at −80°C until further analysis. Cobas c111 analyser (Roche, Basel, Switzerland) with a specific Cobas c111 reagent for each parameter (Roche, Basel, Switzerland) was used to determine serum glucose, triacylglycerols, total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC) and C-reactive protein (CRP). Non-HDL cholesterol was calculated as c(TC) – c(HDLC).

2.5. Gene expression analysis

To control for endogenous cholesterol synthesis, we determined the expression of HMG-CoA synthase and HMG-CoA reductase in leukocytes of participants. For the isolation of RNA from peripheral lymphocytes, blood was collected into EDTA-vacutainers (BD, Franklin Lakes, NJ, USA). Mononuclear cells were isolated from 3 ml of full blood using Histopaque-1077 (Sigma-Aldrich, St.Louis, MO, USA). Buffy coat was washed in PBS and dissolved in TriZol reagent (ThermoFisher Scientific, Waltham, MA, USA); total RNA was isolated following the manufacturers' protocol. One µg of RNA was reverse transcribed with High-capacity cDNA Archive kit (Applied biosystems, Foster city, CA, USA). RT-PCR reaction was performed with Quant studio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) and SYBR-green reagent (Qiagen, Hilden, Germany) under the following reaction conditions: 2 min at 95°C and 40 cycles of 5 s at 95°C and 10 s at 60°C. The primer sequences 196049379c2 for 3-hydroxy-3-methylglutaryl–CoA reductase (HMG-CoA-R) and 148298676c2 for 3-hydroxy-3-methylglutaryl–CoA synthase 1 (HMG-CoA-S) were selected from Primerbank (Spandidos, 2010) and 18S rRNA was used as internal control (F-GTAACCCGTTGAACCCCATT and R-CCATCCAATCGGT AGTAGCG). Primer specificity was confirmed by melting curve inspection and relative gene expressions were calculated using Δct method.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 26.0 (IBM, NY, USA). Means and standard deviations, minimum and maximum were calculated. Shapiro–Wilk test was used to evaluate the normality of data distribution. ANOVA was used to compare groups for normally distributed data and Kruskall-Wallis' test was used for non-normally distributed data. As age has an impact on observed biochemical parameters, ANCOVA with age as a covariate was performed to compare biochemical data among the groups. Hierarchical regression analysis was performed to examine the effects of age, nutritional parameters known to affect serum cholesterol levels and dietary indices on TC. p-values < 0.05 were considered statistically significant.

3. Results

3.1. Subjects characteristics

Two hundred thirty seven individuals, identifying themselves as practicing LCHF diet, replied to our online survey, 98 of whom gave their contact for a potential participation in the study. After screening for inclusion criteria, we were able to recruit 24 adults with 3-months stable body mass, practicing LCHF diet for at least six months (58% from 1–3 years and 25% more than 3 years). They mostly reported choosing their diet for health (Figure 2). For comparison, we recruited 32 vegans, 37 vegetarians and 37 omnivores, the sample thus included a total of 130 subjects (97 females and 33 males) (Table 1). The subjects were healthy adults without any chronic disease, and with comparable BMI and other anthropometric parameters. A total of 76% of subjects were in relationship or married and 29% had high school diploma, 58% bachelor's degree and 13% master's degree or PhD. More than half (62%) of the subjects never smoked, 21% were former smokers and 17% were current smokers (regular or occasional).

3.2. Nutritional intake

Energy intake did not differ among the groups ($p = 0.607$; Figure 3B). However, there were significant differences in the intake of food groups (Figure 3A). LCHF had significantly higher intake of fats and fatty foods, and lower intakes of starchy foods, fruits, sugars and sweets than all other groups. Additionally, it differed from vegan group in the intake of milk and dairy products and from both vegan and vegetarian group in the intake of legumes and meat and substitutes. LCHF group had significantly different macronutrient distribution than other groups ($p < 0.001$; Figure 3C): it had the lowest intakes of carbohydrates and the highest intakes of fats and proteins. All participants from LCHF group met the reference values of at least 0.8 g protein per kg body mass per day (11) (Table 2). The minimal recommended protein intake was not reached in 31% vegans, 35% vegetarians and 8% omnivores. For the intake of carbohydrates, the recommended 50% EI (11) was achieved in 84% vegans, 54% vegetarians and 22% omnivores. Fat intake below the recommended maximum of 30% EI (11) was determined in 53% vegan, 22% vegetarian and 19% omnivorous participants. LCHF group had significantly higher SFA and MUFA intake than other groups (Figure 3D).

LCHF group had the lowest number of meals per day and the lowest eating time frame (Table 3). LCHF group had the highest intake of animal proteins while vegans had the highest intake of plant proteins. LCHF group had the lowest sugar and free sugar intake (Table 3). For this parameter, the highest intake was determined in omnivorous group, but also here it did not exceed reference values (RV) in 81% of the group. LCHF group had the lowest dietary fibers intake ($p < 0.001$; Table 3), but a marked proportion of individuals in other three groups also did not meet the RV (Table 2).

In LCHF group, analysis of micronutrient intakes revealed significantly lower intake of copper and significantly higher intake of vitamin A, phosphorus and manganese than in vegan and vegetarian group, significantly higher intake of vitamin C and pyridoxine than in omnivorous group, and higher intake of vitamin E, riboflavin,

	LCHF	Vegan	Vegetarian	Omnivorous
Social environment	17	15	12	67
Health	96	58	68	64
Being fit	50	23	41	39
Weight loss	50	4	21	3
Social media	4	0	0	0
Ethics	4	92	56	0

0-20 %
20-40 %
40-60 %
60-80 %
80-100 %

FIGURE 2
Motives for choosing the current dietary pattern. More than one motive could have been chosen by each participant. Results are shown as percentage of each group.

TABLE 1 Characteristics of study subject by dietary pattern.

Dietary pattern	LCHF	Vegan	Vegetarian	Omnivorous	All
N (M/F)	24 (5/19)	32 (9/23)	37 (7/30)	37 (12/25)	130 (33/97)
Age (years)*	41.2 ± 5.7[a,b,c]	34.0 ± 10.1	37.4 ± 10.7	36.2 ± 11.5	36.9 ± 10.2
Height (cm)	170 ± 9	170 ± 9	169 ± 8	171 ± 9	170 ± 9
Body mass (kg)	67.9 ± 13.0	62.9 ± 8.0	64.6 ± 10.0	66.2 ± 13.3	65.2 ± 11.2
BMI (kg/m^2)	23.4 ± 3.1	21.8 ± 2.1	22.4 ± 2.6	22.6 ± 3.0	22.5 ± 2.8
Fat mass (%)	23.9 ± 6.0	20.8 ± 8.4	24.3 ± 7.1	21.9 ± 7.1	22.7 ± 7.3
Fat free mass (kg)	51.3 ± 11.4	49.8 ± 8.9	48.9 ± 9.3	51.9 ± 12.8	50.4 ± 10.6
TBW (%)	56.2 ± 4.8	57.8 ± 7.2	55.2 ± 5.8	57.0 ± 5.5	56.6 ± 6.0
SBP (mmHg)	121 ± 9	123 ± 16	121 ± 16	119 ± 12	121 ± 14
WHR	0.79 ± 0.05	0.79 ± 0.06	0.80 ± 0.05	0.79 ± 0.06	0.79 ± 0.06
WHtR	0.45 ± 0.04	0.44 ± 0.05	0.45 ± 0.04	0.45 ± 0.06	0.45 ± 0.05
PA (MET)	7.7 ± 6.1	11.4 ± 11.5	10.2 ± 10.3	13.4 ± 10.0	10.9 ± 10.0
Smoking (N (%))	6 (25)	4 (13)	6 (16)	6 (16)	22 (17)

LCHF, low carbohydrate high fat diet; BMI, Body Mass Index; WHT, Waist to Hip Ratio; WHtR, Waist to Height Ratio; TBW, Total body water; SBP, Systolic blood pressure; PA, Physical activity. *Kruskal-Wallis H test, $p < 0.05$; Student's or Mann–Whitney test was performed for all the parameters expressed as mean ± standard deviation between all pairs of groups. Statistically significant differences ($p < 0.05$) are reported with superscript indices close to the first value of the pair: [a]LCHF and vegan group; [b]LCHF and vegetarian group; [c]LCHF and omnivorous group.

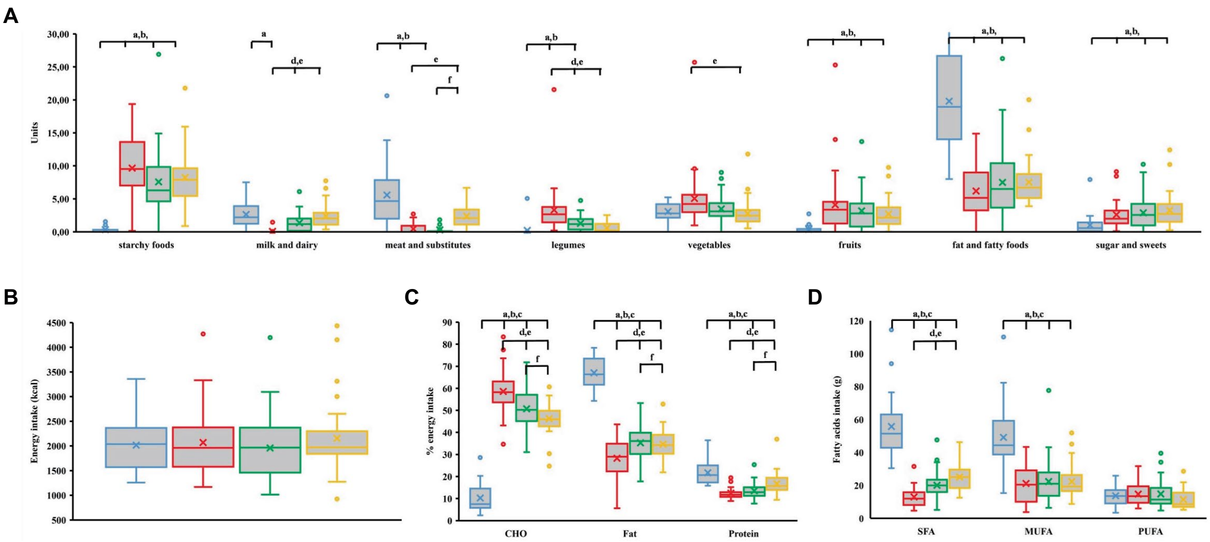

FIGURE 3
(A) Food group unit intake (meat and substitutes include vegan meat substitutes). **(B)** Daily energy intake. **(C)** Contribution of macronutrients to daily energy intake. **(D)** Fatty acids intake. LCHF, low carbohydrate high fat; CHO, carbohydrate; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Blue—LCHF, red—Vegan, green—Vegetarian, yellow—Omnivorous. [index]$p<0.05$, Student's or Mann–Whitney test between: [a]LCHF and vegan group; [b]LCHF and vegetarian group; [c]LCHF and omnivorous group; [d]vegan and vegetarian group; [e]vegan and omnivorous group; [f]vegetarian and omnivorous group.

pantothenic acid, biotin, zinc and selenium than in all other groups (Figure 4). For iodine, only LCHF group reached RV (11). Vegans had the lowest calcium intake, below RV. Omnivores had vitamin B_{12} intakes around RV, whereas the other groups exceeded RV by multiples of tens. The majority of participants on LCHF and vegan diet have taken at least one dietary supplement per day (75 and 84%, respectively), while this percentage was lower among vegetarians (46%) and omnivores (43%). The three most common dietary supplements used were vitamin B_{12}, vitamin D and vitamin C. All the supplements were included in the analysis of micronutrient intakes (Figure 4).

3.2.1. Dietary indices

In addition to the analysis of macro- and micronutrient intakes, three dietary parameters, HEI, DII and PFI, were calculated to evaluate the quality of the diet (Table 4). LCHF had the lowest diet quality as determined by HEI, followed by omnivores, vegetarians and vegans. Average HEI for all dietary groups together was 65.6 ± 13.5. DII calculated from the total intakes of foods and dietary supplements was significantly different between groups. The lowest DII, which indicates the lowest intake of potentially inflammatory foods, was observed in vegan group. PFI did not significantly differ between groups. HEI negatively correlated with both DII and PFI, while DII and PFI positively correlated.

TABLE 2 Percentage of participants meeting reference value.

Nutrient	RV	LCHF % meet RV	Vegan % meet RV	Vegetarian % meet RV	Omnivorous % meet RV
Carbohydrate	>50% EI[1]	0	84.4	54.1	21.6
Fat	<30% EI[1]	0	53.1	21.6	18.9
Protein	>0.8 g/kg BM[1]	100	68.8	64.9	91.9
Free sugar 1	<10% EI[2]	95.8	93.8	86.5	81.1
Free sugar 2	<5% EI[2]	95.8	68.8	40.5	35.1
Dietary fibers	>30 g[1]	8.3	71.9	43.2	35.1
ω-3/ω-6	>0.20[1]	45.8	40.6	43.2	54.1

BM, body mass; EI, energy intake; LCHF, low carbohydrate high fat; RV, reference value. [1]Slovenian RV (11), [2]WHO RV (37).

TABLE 3 Energy and macronutrient intake.

Variable (Unit)	LCHF	Vegan	Vegetarian	Omnivorous
Meals (N/day)*	2.8 ± 0.5 [a,b,c]	3.8 ± 0.7	3.7 ± 0.9 [f]	4.1 ± 0.8
Eating time frame (h/day)*	8.7 ± 1.9 [a,b,c]	11.3 ± 1.4 [e]	11.0 ± 1.8 [f]	12.0 ± 1.6
Energy density (kcal/g)	1.22 ± 0.44	1.12 ± 0.29	1.15 ± 0.36	1.07 ± 0.33
Ketogenic ratio*	1.50 ± 0.36 [a,b,c]	0.27 ± 0.10	0.35 ± 0.10	0.38 ± 0.10
Carbohydrate (g)*	51 ± 35 [a,b,c]	307 ± 123 [d,e]	252 ± 106	254 ± 107
Sugar (g)*	28.3 ± 22.6 [a,b,c]	90.4 ± 58.7	85.9 ± 42.4	90.7 ± 42.6
Free sugar (g)*	9.2 ± 14.4 [a,b,c]	22.0 ± 19.2 [d,e]	30.9 ± 21.8	36.6 ± 24.8
Free sugar (%)*	1.7 ± 2.3 [a,b,c]	4.3 ± 3.2 [e]	6.1 ± 3.5	6.7 ± 3.9
Fat (g)*	150 ± 46 [a,b,c]	64 ± 27	76 ± 27	81 ± 20
SFA (% EI)*	25.0 ± 6.0 [a,b,c]	5.8 ± 2.9 [d,e]	9.6 ± 3.7	10.8 ± 2.7
MUFA (% EI)*	21.9 ± 6.1 [a,b,c]	9.4 ± 4.7	10.3 ± 4.6	9.6 ± 3.4
PUFA (% EI)*	6.1 ± 2.2	6.5 ± 2.5 [e]	6.8 ± 2.9 [f]	4.8 ± 2.2
ω-6 FA (% EI)*	5.0 ± 1.9 [a,b,c]	3.1 ± 2.2	3.6 ± 2.4	2.9 ± 1.9
ω-3 FA (% EI)*	0.9 ± 0.5 [a,b,c]	0.7 ± 0.6	0.7 ± 0.8	0.6 ± 0.5
ω-3/ω-6 FA ratio	0.24 ± 0.15	0.35 ± 0.60	0.23 ± 0.20	0.27 ± 0.21
Cholesterol (mg)*	1,078 ± 500 [a,b,c]	12 ± 16 [d,e]	162 ± 140 [f]	314 ± 166
Protein (g)*	108 ± 35 [a,b,c]	65 ± 31[e]	65 ± 23 [f]	91 ± 42
Protein (g/kg BM)*[1]	1.60 ± 0.45 [a,b,c]	1.02 ± 0.36 [e]	1.02 ± 0.41 [f]	1.40 ± 0.61
Animal protein (%)*[2]	86.0 ± 12.8 [a,b,c]	1.7 ± 4.8 [d,e]	29.8 ± 20.2 [f]	59.9 ± 15.1
Dietary fibers (g)*	17.9 ± 21.1 [a,b,c]	50.4 ± 42.7 [d,e]	31.7 ± 14.9	27.1 ± 15.3
Alcohol (g)*	4.9 ± 9.1	2.7 ± 7.5 [e]	2.1 ± 4.9 [f]	6.6 ± 10.6

All values are expressed as mean ± standard deviation. LCHF, low carbohydrate high fat; FA, fatty acids; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; EI, energy intake; BM, body mass. *$p < 0.05$, ANOVA or Kruskal-Wallis H test; Student's or Mann–Whitney test was performed for all the parameters between all pairs of groups. Statistically significant differences ($p < 0.05$) are reported with superscript indices close to the first value of the pair: [a]LCHF and vegan group; [b]LCHF and vegetarian group; [c]LCHF and omnivorous group; [d]vegan and vegetarian group; [e]vegan and omnivorous group; [f]vegetarian and omnivorous group. [1]Recommended values for protein intake is 0.8 g/kg BM (11). [2]Percent of protein intake (animal protein + plant protein = 100%).

3.3. Serum biomarkers

To analyze whether the different nutrient intakes reflect in serum biochemical parameters, lipid profile, glucose and CRP were measured. Among the participants in the LCHF group, 71% had TC above the reference value and 67% had increased LDLC level (Table 5). As the age of participants in the LCHF group was higher compared to other groups and due to the known association of TC with age, ANCOVA model with the age as a covariate was additionally performed. With this covariate considered, the levels of TC [F (3, 125) = 11.23; $p < 0.001$], LDLC [F (3, 125) = 10.41; $p < 0.001$] and non-HDLC [F (3, 125) = 7.89; $p < 0.001$], but also HDLC [F (3, 125) = 8.43; $p < 0.001$] were still significantly different among groups. TC and LDLC levels were the lowest in vegan group, where the levels were significantly lower also when compared to the omnivorous group not only to LCHF. The same was observed for HDLC level. In fact, 19% of vegan participants had LDLC levels below the reference value. For HDLC, the percentage of participants with too low levels was similar for vegan, vegetarian and omnivorous (22, 19, 19%, respectively), whereas in the LCHF group it was only 4%. In the levels

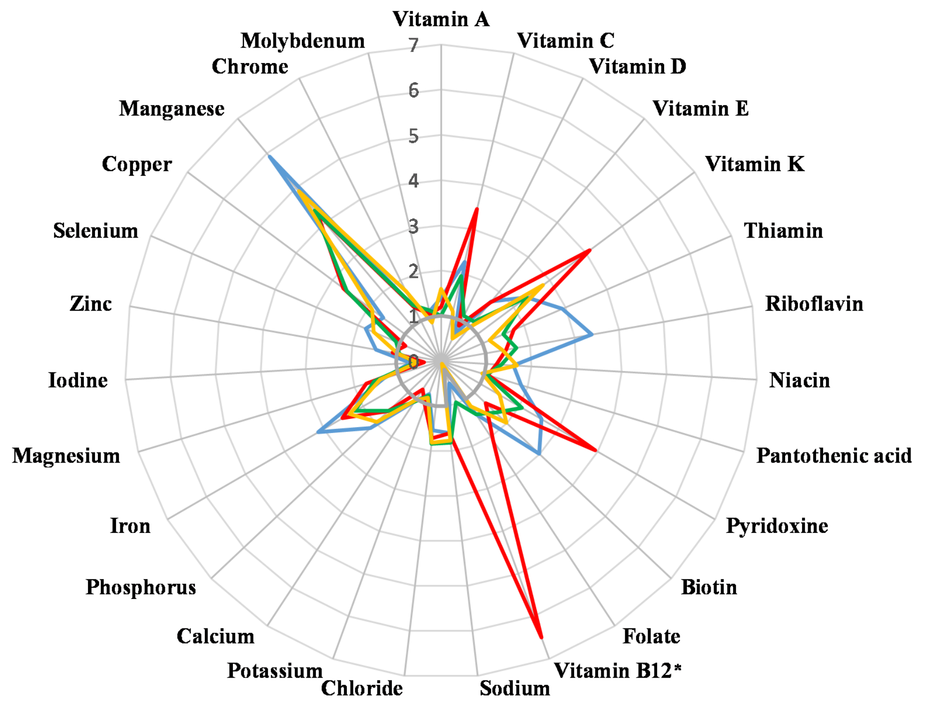

FIGURE 4
Micronutrient intake in multiples of Slovene Reference Values [SRV, (11)]. *Vitamin B_{12} values were divided by 20 to fit in the chart. Blue—Low carbohydrate high fat, red—Vegan, green—Vegetarian, yellow—Omnivorous.

of glucose, triacylglycerols and CRP there were no important differences between groups, also, all the levels were within reference values.

Further, hierarchical regression analysis was performed to examine the effects of age, nutritional parameters known to affect serum cholesterol levels and dietary indices on the total serum cholesterol levels (Table 6). In the first step, age was entered, followed by specific nutrients intake (SFA, MUFA, PUFA, dietary cholesterol, animal protein, and dietary fibers) in the second step. In the third step, dietary indices were entered (HEI, DII and PFI). Stage one (age) explained 9.7% of the variation of TC [F (1, 128) = 13.709; $p < 0.001$]. Stage two (SFA, MUFA, PUFA, cholesterol, dietary fibers and animal protein intakes) explained additional 40.0% of the variation of TC [F (7, 122) = 17.211, $p < 0.001$]. And at stage three, the proposed regression model explained 51.4% of total variance in cholesterol levels [F (10, 119) = 12.608; $p < 0.001$]. Of the chosen independent variables, age and daily intakes of SFA and MUFA proved to significantly impact TC level (Table 6).

For some insight whether the observed differences in the cholesterol levels might be due to different endogenous synthesis, we further analyzed the expression of HMG-CoA synthase and HMG-CoA reductase in peripheral lymphocytes. The analysis was performed on a subsample (N = 88), where 22 participants were randomly selected from each group. No statistically significant difference was found among groups (respectively $p = 0.742$ and $p = 0.945$), controlling for age.

The calculated ketogenic ratio was in LCHF group from 0.74 to 2.10. The participants were therefore on both sides of the threshold of ketogenesis, which is set at KR = 1.5 (35, 36). When we divided LCHF group into two subgroups, those below the threshold of ketogenesis (N = 12) and those above (N = 12), subjects above the threshold had significantly higher levels of TC, LDL and non-HDL ($p = 0.008$, $p = 0.009$ and $p = 0.014$, respectively; Figure 5).

4. Discussion

LCHF is advertised as long-term safe either for improving health or for improving sport performance, but in scientific literature some concerns are raised. To assess the quality and health effects of long-term self-planned LCHF diet in healthy lean adults we compared it to the most commonly followed omnivorous diet and to two patterns with whole food group omission – vegan and vegetarian. We have focused only on the participants who had stable body mass for at least three months; as LCHF is often used as a weight loss program, potential nutrient deficiencies may be due to the restriction of energy intake and not only arising from omitting whole food groups, but on the other hand, energy restriction may also mask some negative effects of high fat intakes. Although studies report lower BMI in vegetarians compared to non-vegetarians and even lower in vegans (16, 41), in order to objectively compare the four dietary patterns, we recruited groups of participants with comparable BMI, as BMI is an independent risk factor for low grade inflammation, metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases (42).

4.1. Macronutrient intakes

By definition, LCHF diet differs from other diets in the intake of carbohydrates and fats. Consistent with LCHF definition (7, 10), the

TABLE 4 Dietary indices.

Dietary index	LCHF	Vegan	Vegetarian	Omnivorous
HEI*	51.1 ± 8.1 [a,b,c]	73.4 ± 11.9 [d,e]	68.2 ± 10.9	65.5 ± 13.1
Total fruits*	0.83 ± 0.96 [a,b,c]	3.84 ± 1.44	3.30 ± 1.85	3.30 ± 1.33
Whole fruits*	1.29 ± 1.63 [a,b,c]	4.12 ± 1.52	3.68 ± 2.02	4.03 ± 1.48
Total vegetables*	3.63 ± 1.35 [c]	4.06 ± 1.01 [e]	3.68 ± 1.23 [f]	2.95 ± 1.13
Greens and beans*	1.33 ± 1.76 [a,b,c]	4.31 ± 1.20 [d,e]	3.14 ± 1.72	2.68 ± 1.75
Whole grains*	0.37 ± 1.28 [a,b,c]	6.47 ± 3.95	5.73 ± 3.91	5.62 ± 3.93
Dairy*	6.62 ± 2.96 [a,b]	0.38 ± 1.13 [d,e]	4.30 ± 3.53 [f]	6.14 ± 2.72
Total protein food*	4.92 ± 0.41 [a,b,c]	3.53 ± 1.52	3.27 ± 1.48 [f]	4.00 ± 1.08
Seafood and plant proteins*	3.21 ± 2.13 [a]	4.50 ± 1.34 [e]	4.22 ± 1.40	3.78 ± 1.69
Fatty acids*	1.54 ± 1.96 [a,b]	8.19 ± 2.92 [d,e]	5.16 ± 3.94 [f]	2.32 ± 3.14
Refined grains*	10.00 ± 0.00 [a,b,c]	7.94 ± 3.38	8.49 ± 2.78	8.11 ± 2.68
Sodium	7.37 ± 3.26	7.03 ± 3.96	7.19 ± 3.49	7.35 ± 3.20
Added sugar*	9.92 ± 0.41 [b,c]	9.66 ± 0.97 [d,e]	9.30 ± 1.13	9.00 ± 1.45
Saturated fats*	0.08 ± 0.41 [a,b,c]	9.41 ± 1.90 [d,e]	6.81 ± 3.17	6.27 ± 2.91
DII*	1.85 ± 1.45 [a]	1.02 ± 1.75 [d,e]	1.89 ± 1.79	2.43 ± 2.39
PFI	2.23 ± 0.29	2.26 ± 0.42	2.42 ± 0.44	2.42 ± 0.39
Ketogenic ratio	1.50 ± 0.36 [a,b,c]	0.27 ± 0.10 [d,e]	0.35 ± 0.11	0.38 ± 0.10

All values are expressed as mean ± standard deviation. LCHF, Low carbohydrate high fat; HEI, Healthy Eating Index; DII, Dietary Inflammatory Index; PFI, Processed Food Index. *ANOVA or Kruskal-Wallis H test, $p < 0.05$; Student's or Mann–Whitney test was performed for all the parameters between all pairs of groups. Statistically significant differences ($p < 0.05$) are reported with superscript indices close to the first value of the pair: [a]LCHF and vegan group; [b]LCHF and vegetarian group; [c]LCHF and omnivorous group; [d]vegan and vegetarian group; [e]vegan and omnivorous group; [f]vegetarian and omnivorous group.

intake of carbohydrates in our participants was up to 26% EI and was significantly lower than in other groups. A complete omission of starchy foods, legumes and fruits was observed. Some of the LCHF participants followed a ketogenic diet, while the rest lowered their carbohydrate intake without an interest in being ketotic, which was manifested in a wide range of KR. The observed variety of low-carbohydrate dietary patterns is likely a consequence of diverse lay guidelines and publications, available online and advertised by various nutritional coaches and was therefore expected in self-advised planning. Increased fat intake in LCHF group was mostly achieved with milk and dairy, meat and substitutes and fat and fatty food intake, which reflected in higher intakes of SFA, MUFA and cholesterol, while PUFA intake in LCHF did not differ from other groups. LCHF group had also the highest protein intake, which was again achieved through the intake of animal protein. In contrast, this group had the lowest intake of plant proteins among the groups. Of the three control groups, vegan had the highest intake of carbohydrates and the lowest intakes of fats and proteins.

We have analyzed to what extent the other dietary patterns were in line with the national recommendations (11). In vegan group, the highest proportion of participants met the RV for the intake of carbohydrate and fat. Similar to what was reported on a national level (6), our participants exhibited a tendency towards lower carbohydrate intake and higher fat intake, not just those following LCHF but also others. The majority of participants reached the RV for proteins [0.8 g/kg BM day (11)], however the percentage was somewhat lower in vegan and vegetarian group (69 and 65%, respectively, compared to 100% in LCHF and 92% in omnivorous). Among vegans, 31% did not reach the RV, which is additionally concerning because plant-derived proteins have lower absorbability and may have lower content of essential amino acids; therefore higher intakes of plant proteins are recommended (43). All groups had low intake of free sugars with the majority of participants meeting the WHO recommendations (37). LCHF group had low dietary fibers intake; only 8% met the RV and the average intake was 17.9 g per day. In other groups the intakes were better but still low; two thirds of omnivores, more than half of the vegetarians and one third of vegan did not meet the RV. However, when we consider the average intake of dietary fibers in each group, the participants in the three control groups had higher dietary fibers intakes than previously reported for Slovenian adults. There, nearly 90% of adults did not meet the RV (44).

4.2. Micronutrients intakes

Despite the fears of micronutrient insufficiencies (14, 15) due to omissions of starchy foods, legumes and fruits, mean values of micronutrient intake in LCHF group met or even exceeded recommended intakes for the majority of micronutrients, but mostly remained under the upper tolerable limits, where established. The result can be partially explained by the fact that 75% of the participants from this group regularly took at least one dietary supplement. In addition, in comparison to some previous reports (15), where low micronutrient intakes were reported, participants in the present study had no EI restriction. Nevertheless, we observed too low intakes of potassium, iodine and vitamin D, while mean value of calcium intake coincided with RV, indicating a presence of insufficient intakes in a part of participants. Inadequate micronutrient

TABLE 5 Serum biomarkers.

Biomarker (Unit)	LCHF		Vegan		Vegetarian		Omnivorous		RV
Glucose (mmol/L)	4.64 ± 0.60		4.71 ± 0.44		4.67 ± 0.54		4.79 ± 0.46		3.6–6.1
	<0%	>0%	<0%	>0%	<0%	>0%	<0%	>0%	
Total cholesterol (mmol/L)*	7.48 ± 4.27[a,b,c]		4.01 ± 0.86[d,e]		4.46 ± 0.90		4.84 ± 1.84		4.0–5.2
	<8%	>71%	<50%	>6%	<41%	>19%	<22%	>22%	
HDLC (mmol/L)*	2.10 ± 0.47 [a,b]		1.58 ± 0.41[e]		1.72 ± 0.42		1.87 ± 0.47		>1.4
	<4%		<22%		<19%		<19%		
LDLC (mmol/L)*	5.85 ± 4.47 [a,b,c]		2.66 ± 0.76[e]		3.00 ± 0.89		3.14 ± 1.30		2.0–3.3
	<0%	>67%	<19%	>16%	<8%	>35%	<11%	>30%	
Non-HDLC (mmol/L)*	5.38 ± 4.26 [a,b,c]		2.42 ± 0.91 [e]		2.74 ± 0.93		2.98 ± 1.90		<3.4[1]
		>59%		>9%		>19%		>22%	
TAG (mmol/L)	0.91 ± 0.66		0.88 ± 0.39		0.91 ± 0.39		1.05 ± 1.02		0.6–1.7
	<25%	>8%	<13%	>3%	<16%	>5%	<24%	>11%	
TAG/HDL	0,466 ± 0,357		0,672 ± 0,628		0,584 ± 0,362		0,663 ± 0,856		♂ < 2.967[2]
		>0%		>3%		>0%		>5%	♀ < 2.237[2]
CRP (mg/L)	0.61 ± 0.50		0.80 ± 1.08		0.75 ± 1.01		1.20 ± 1.77[#]		<2.0[3]
		>4%		>6%		>3%		>19%	

All values are expressed as mean ± standard deviation. LCHF, Low carbohydrate high fat; CRP, C-reactive protein; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; non-HDLC = TC - HDLC; TAG, triacylglycerols; RV, reference values (unless a reference is indicated, Slovene reference values are reported); [<]% of participants with levels below recommended values; [>]% of participants with levels above recommended values; [#]value for 36 participants, one was excluded due to very high CRP values of 15.61 mg/L, indicating an acute infection (CRP values indicating chronic low-grade inflammation are between 2 and 10 mg/L (38); *ANOVA or Kruskal-Wallis H test controlled for age, $p < 0.05$. Student's or Mann–Whitney test was performed for all the parameters between all pairs of groups. Statistically significant differences ($p < 0.05$) are reported with superscript indices close to the first value of the pair: [a]LCHF and vegan group; [b]LCHF and vegetarian group; [c]LCHF and omnivorous group; [d]vegan and vegetarian group; [e]vegan and omnivorous group; [f]vegetarian and omnivorous group. [1]Reference (39), [2]Reference (40), [3]Reference (38).

intakes were noted also in other groups. Intakes of vitamin D were insufficient in all groups which is in line with a previous report on a national level (45); despite the endogenous biosynthesis of vitamin D, the majority of Slovene population has insufficient serum 25(OH) D levels, especially in winter. Iodine intakes were also too low in all groups. The main source of iodine in Slovenia is iodized salt and iodine sufficiency in Slovenia is reportedly achieved due to highly excessive salt intake (46). Our participants did not exceed salt intake recommendations to such a high degree as reported for general population in Slovenia (47). Further, non-iodized salt is believed to be more natural and healthy in some laic nutritional information sources preferred by people on special dietary patterns, especially vegetarian and vegan, and is available on market (48), which is of concern. Similar to LCHF group, calcium intake was problematic in part of vegetarians and omnivores, and majority of vegans. Other insufficient intakes were group specific: vegans did not meet the RV for selenium; vegetarians did not meet RV for potassium and zinc; while omnivores had too low intakes of vitamin E, pantothenic acid, zinc and molybdenum. The use of supplements was common also in these groups, as 84% of vegan, 46% of vegetarians and 43% of omnivorous participants took at least one dietary supplement per day. The results suggest that the participants following restrictive dietary patterns are familiar with potential insufficiencies and try to correct them with supplements. However, none of the self-advised diets was fully sufficient in providing all nutrients, thus further education of the public is necessary.

4.3. Dietary quality assessed through dietary indices

Assessing a diet on the level of singular nutrients does not give the exact picture of the possible impact of the ingested food mixtures on health. An overall assessment of diet quality gives further information (49, 50) and different indices have been developed for this purpose. We have chosen three: HEI, which assesses overall diet quality and was associated with better cardiovascular markers, overall health and lower mortality rates (49), DII that measures inflammatory potential of the diet and has been associated with cardiovascular diseases incidence and related mortality (51) and PFI, a novel index which was based on NOVA classification of processed foods (33), since the consumption of ultra-processed food was also associated with adverse health effects (52). LCHF group had significantly lower HEI than other groups. Their score was low for whole grains, which was expected in line with LCHF definition; fatty acids ratio and SFA intake, which could be improved with a better choice of fatty foods; and was the best of all the groups for refined grains and free sugars intake, again in line with the definition. LCHF had a better score in total vegetables than omnivorous and the best score of all the groups for total protein food. Omnivorous had very similarly low score for fatty acids ratio, but higher for SFA than LCHF group. The highest HEI was observed in vegans, followed by comparable results in vegetarians and omnivorous group. We point out that the lowest HEI observed in LCHF group was still relatively high as it was comparable

TABLE 6 Hierarchical regression model for total serum cholesterol.

Predictor	Dependent variable: total serum cholesterol			
	ΔR^2	β	F	p
Step 1	0.097		13.709	0.000
Age (years)		0.311		0.000
Step 2	0.400		17.211	0.000
Age (years)		0.204		0.003
SFA intake (g)		0.644		0.000
MUFA intake (g)		−0.273		0.016
PUFA intake (g)		−0.081		0.353
Cholesterol intake (mg)		0.234		0.065
Dietary fibers (g)		−0.020		0.792
Animal protein (g)		−0.066		0.603
Step 3	0.018		12.608	0.000
Age (years)		0.176		0.012
SFA intake (g)		0.713		0.000
MUFA intake (g)		−0.292		0.010
PUFA intake (g)		−0.106		0.259
Cholesterol intake (mg)		0.165		0.218
Dietary fibers (g)		−0.071		0.369
Animal protein (g)		−0.071		0.575
HEI		0.032		0.727
DII		0.001		0.994
PFI		−0.145		0.070
Model	0.514		12.608	

SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; HEI, Healthy Eating Index; DII, Dietary Inflammatory Index; PFI, Processed Food Index.

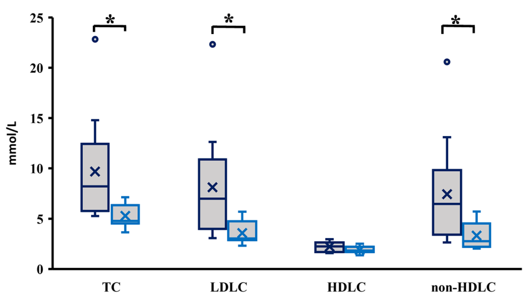

FIGURE 5
Cholesterol levels in low carbohydrate high fat group divided based on ketogenic ratio (KR). Dark blue—KR > 1.5; light blue—KR < 1.5. *$p < 0.05$; TC, Total Cholesterol; LDLC, Low-density lipoprotein cholesterol; HDLC, High-density lipoprotein cholesterol; non-HDLC = TC − HDLC.

to HEI observed for overall population in several European countries (50). DII of LCHF group was comparable to that of vegetarians and omnivores, while vegans had significantly lower DII, which points to the consumption of less pro-inflammatory food constituents (32) in this group. As DII is a population-based index, it is difficult to compare our results to the results reported in literature. There was no difference in PFI among our groups. In all groups it was between 2 and 3, which suggests a low presence of ultra-processed foods in their diets.

4.4. Serum biomarkers

Serum biomarkers were assessed to observe the health footprint of the observed diets. Relatively high diet quality and a comparable low consumption of pro-inflammatory foods in all groups reflected in low mean CRP levels of all groups [much lower than 2 mg/L which is a well-accepted cut-off level indicating chronic low-grade inflammation (38)], without statistical difference among the groups. The biggest part of participants exceeding the cut-off of chronic low-grade inflammation was in omnivorous group. All the participants had glucose levels within the recommended values and there were no differences among the groups. Mean triacylglycerols levels were within the recommended values in all groups and there were no differences among the groups. LCHF group had significantly higher cholesterols levels. Seventy-one percent of participants in LCHF group had TC levels above the upper limit, many had also too high LDLC (67%) and non-HDLC (59%) – the latter is recommended as a cardiovascular disease prediction factor by European Society of Cardiology (53). The literature suggests low carbohydrate diets might have an impact on LDL particle size, increasing LDL peak, but not mean, particle size and decreasing the numbers of small dense LDL and total LDL particles, which may reflect a decreased atherogenicity of the LDL particles, but the clinical significance of LDL particles is still unknown (54). TAG/HDLC ratio (40) and non-HDLC [in individuals without hypertriacylglycerolaemia (55)] have been suggested as potential biomarkers of small dense LDL particles with high specificity and sensitivity determined by ROC curves. Our LCHF participants had significantly higher non-LDLC, but did not differ from other groups in TAG/HDLC ratio. For a final conclusion LDL subfraction analysis would have to be performed. Previously, we and others have not observed an increase in cholesterols levels in shorter weight-loss ketogenic diet interventions in participants with obesity (22, 24, 56). Ketosis induces a different metabolic milieu than diets where the presence of carbohydrate and/or protein is sufficient to elicit an insulin response and KR was suggested as suitable to determine the metabolic effect of a diet (35). We therefore divided the participants of the LCHF group according to their KR to those with higher probability of being in ketosis (KR > 1.5) and those with lower. The participants with KR > 1.5 had a worse cholesterol profile. Compared to the aforementioned ketogenic interventions with positive results on cholesterol profile that were hypocaloric and where the participants had regular meetings with dietitians (22, 24, 56), participants in this study had stable body mass, an eucaloric diet and did not consult a dietitian. Research suggests that lowering carbohydrate intake and subsequent lowering of insulin levels may inhibit hepatic cholesterol synthesis which might result in a lower TC and LDLC as long as one does not increase SFA and dietary cholesterol intakes while lowering carbohydrate intake (20). Significant correlations between SFA intake and TC, LDLC, HDLC and non-HDLC have been observed (39, 57). To control for endogenous cholesterol synthesis, we determined the expression of HMG-CoA synthase and HMG-CoA reductase in leucocytes of participants and observed no differences among groups. Even though liver expressions should be analyses for a final answer, this points to exogenous factors to be the explanation for the observed cholesterol profile.

Cholesterol has a fundamental role in cellular membrane in all cell types, in steroid hormones and bile acid production, which raises a concern that very low cholesterol levels observed in some participants, mostly vegan and vegetarian, might also have a negative impact on health. Very low LDLC and HDLC levels were associated with increased risk for stroke, cataract and all-cause mortality (58, 59). Some of our participants had LDLC (19% vegans, 8% vegetarians and 11% omnivorous) and HDLC (22% vegans and 19% vegetarians and omnivorous) levels below RV. Of note, the reported level of LDLC that increased the risk for all-cause mortality [<1.55 mmol/L (59)] is lower than Slovene recommended minimum.

4.5. Nutritional choices that might explain the observed cholesterol profile in LCHF participants

Our present LCHF participants had higher absolute SFA intake than those reported in the aforementioned ketogenic interventions (22, 24, 56). Although our participants were motivated for LCHF diet for health-related reasons, it seems that their only focus was lowering the carbohydrate intake without paying much attention to the quality of other macronutrients and diet quality in general. Mediterranean ketogenic diet low in SFA intake and high in MUFA and PUFA intake was reported to maintain normal cholesterol levels (20, 60). In agreement, our linear regression model identified SFA intake as a positive predictor for total serum cholesterol and MUFA intake as a negative predictor. A higher emphasis on selection of healthy fat sources from vegetables, high in MUFA and PUFA, or fish, high in omega-3 PUFA, when substituting carbohydrate as source of energy with fat in LCHF diet should be made in lay literature.

Apart from SFA intake, high dietary fibers intake is demonstrated to have a positive effect on cholesterol profile (61). High viscosity dietary fibers trap and eliminate bile, consequently lowering TC and LDLC without affecting HDLC (62). Although the present regression model did not reveal dietary fibers intake as a significant predictor of TC, LCHF group had low intakes of all dietary-fibers-rich foods such as whole grains, fruits and vegetables. Dietary fibers intake below recommendations was observed also in omnivores, who had low intakes of vegetable and whole grains and in fact, they had significantly higher TC levels than vegetarian and vegan group, although those were within reference values. In public, carbohydrates are often blamed for obesity, high serum glucose and development of chronic noncommunicable diseases (63), but omitting complex carbohydrates has a negative effect on dietary fibers intake, which was previously associated with increased risks for noncommunicable diseases (64). Since carbohydrate intake is limited in LCHF diet, RV for dietary fibers intake is hard to reach without inclusion of processed foods in this group. We should point out, that some processed foods are healthy and have the potential to lower serum cholesterol levels. This is true for processed extra virgin olive oil and canola oil, processed foods with added plant sterols or stanols, and foods with added soluble dietary fibers and some fermented foods (65); all of these food groups produced at least a moderate (i.e., 0.20–0.40 mmol/L) reduction in LDL cholesterol levels and are suitable for a LCHF diet. Functional foods enriched with dietary fibers content such as inulin, galactooligosaccharides, fructooligosaccharides and lactulose are good examples of foods for increasing the dietary fibers content without increasing digestible carbohydrate intake (66) and are again suitable for LCHF diet. Functional foods with added inulin were associated with improved serum lipid and glucose profile (67). Different dietary fibers supplements are appearing on the market, also the ones containing high viscous dietary fiber such as β-glucan, psyllium, and raw guar gum, and have been shown to lower LDLC (62).

Another possible explanation for different effects of LCHF diets on cholesterol profile lies in the fact that controlled studies of ketogenic diet with favorable lipid profile results had a controlled protein intake (22, 24, 56). High protein intake in the present LCHF group could influence metabolic regulation and cause a shift in macronutrient consumption for energy source. Further, animal protein sources contributed mainly to this high protein intake. Although our regression model did not reveal animal protein intake as significant predictor of TC, increased animal protein intake was previously associated with decreased longevity (68), contrary to the intake of plant proteins which was associated with decreased risk for cardiovascular and all-cause mortality (69). Similar conclusion was drawn from cohort studies investigating low-carbohydrate diet. There, low-carbohydrate diet with predominant animal food sources was associated with higher all-cause, cardiovascular and cancer mortality, while low-carbohydrate diet with predominant plant food sources was associated with lower all-cause and cardiovascular mortality (70, 71). In this context it is important that plant protein intake is accompanied with higher intake of phytochemicals and dietary fibers (72), while animal protein sources contain a lot of SFA. The public, interested in LCHF diet, should therefore be encouraged to substitute some of their animal protein sources with plant protein sources.

4.6. Limitations

The relatively small sample size in the present study may be considered a limitation, as we were able to recruit 24 participants practicing LCHF diet with stable body mass and suitable BMI. The prevalence of people who follow LCHF diet in Slovenia is not known, but national dietary study Si.Menu 2017/18, performed using EFSA methodology on a representative sample of 364 Slovenian adults (18–64 years old), did not detect people on LCHF diet (6). The fact that we were only recruiting participants following the same pattern for a minimum of six months while keeping stable body mass, strongly limited our sample size. The target sample size for vegan and vegetarian participants was easier to reach, as these patterns are more frequent in Slovenian population; according to the national survey approximately 1.6% of population does not consume meat (6). Although participants in all groups fell within the same age-range, participants in LCHF group were statistically older. This might have an impact on cholesterol levels. Indeed, age explained 10% of TC variability in our model, but an additional 40% of TC variability was explained by SFA and MUFA intakes.

5. Conclusion

Most of our vegan and vegetarian participants have chosen their diet for health reasons and to be fit, but many did so also for ethical reasons. In contrast, LCHF pattern was chosen exclusively to improve or maintain health. Furthermore, all of our participant were individuals with an above average interest in nutrition. This reflected in a higher HEI than reported for European adults (50). In spite of that fact, self-planned LCHF diet showed poor nutritional choices – in particular by simply substituting carbohydrate-derived energy with fat without selecting the fat source. Literature suggests a restriction or elimination of consumption of processed and unprocessed red meat, starchy vegetables and refined grains and an emphasis on dietary fibers derived from whole grains, dietary-fibers-rich fruit, low-carbohydrate vegetables (cruciferous and green leafy vegetables and legumes), avocado, olive and vegetable oils, soy, fish and chicken to constitute a healthy LCHF diet (23). Low dietary fiber intakes could be mitigated also with functional foods with added dietary fibers or with dietary fiber supplements, especially the highly viscous ones. Further effort on educating the public about healthy low-carbohydrate choices but also correct replacements of omitted foods in vegan and vegetarian patterns should be made to achieve healthier diets in "real life" while respecting individuals' decision on which dietary pattern they wish to follow.

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 Republic of Slovenia Ministry of Health National Medical Ethics Committee (No. 0120-557/2017/4). The patients/participants provided their written informed consent to participate in this study.

Author contributions

NM and ZJ: conceptualization, supervision, and project administration. NB, KS, SK, AP, ZJ, and NM: methodology and investigation. NM and NB: formal analysis and data curation. ZJ: resources. NB: writing—original draft preparation. NM, ZJ, SK, and AP: writing—review and editing. NM: visualization. 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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Transitioning to sustainable dietary patterns: learnings from animal-based and plant-based dietary patterns in French Canadian adults

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Introduction: Many dietary guidelines promote the substitution of animal proteins with plant-based proteins for health benefits but also to help transitioning toward more sustainable dietary patterns. The aim of this study was to examine the food and nutrient characteristics as well as the overall quality and costs of dietary patterns consistent with lower intakes of animal-based protein foods and with higher intakes of plant-based protein foods among French Canadian adults.

Methods: Dietary intake data, evaluated with 24 h recalls, from 1,147 French-speaking adults of the PRÉDicteurs Individuels, Sociaux et Environnementaux (PREDISE) study conducted between 2015 and 2017 in Québec were used. Usual dietary intakes and diet costs were estimated with the National Cancer Institute's multivariate method. Consumption of animal- and plant-based protein foods was classified into quarters (Q) and differences in food and nutrient intakes, Healthy Eating Food Index (HEFI)-2019 scores and diet costs across quarters were assessed using linear regression models adjusted for age and sex.

Results: Participants with lower intakes of animal-based protein foods (Q1 vs. Q4) had a higher HEFI-2019 total score (+4.0 pts, 95% CI, 0.9 to 7.1) and lower daily diet costs (-1.9 $CAD, 95% CI, −2.6 to -1.2). Participants with higher intakes of plant-based protein foods (Q4 vs. Q1) had a higher HEFI-2019 total score (+14.6 pts, 95% CI, 12.4 to 16.9) but no difference in daily diet costs (0.0$CAD, 95% CI, -0.7 to 0.7).

Discussion: In a perspective of diet sustainability, results from this study among French-speaking Canadian adults suggest that a shift toward a dietary pattern focused primarily on lower amounts of animal-based protein foods may be associated with a better diet quality at lower costs. On the other hand, transitioning to a dietary pattern focused primarily on higher amounts of plant-based protein foods may further improve the diet quality at no additional cost.

KEYWORDS

animal-based protein, plant-based protein, dietary pattern, diet quality, diet cost, healthy eating food index (HEFI)-2019, sustainability, sustainable diet

1. Introduction

Current global food production has a major impact on the environment by contributing to 19–29% of total greenhouse gas emissions, land degradation and biodiversity loss (1, 2). A shift to more sustainable food production and consumption practices is therefore necessary to achieve the United Nations 2030 Sustainable Development Goals (3). As production of plant-based foods is less resource-intensive than production of animal-based foods (4), replacing the latter with the former has become one of the cornerstones of the sustainable diet paradigm (5–7). In this regard, the healthy and sustainable diet proposed in 2019 by the EAT-Lancet Commission advocates for a reduction in the global consumption of animal-based foods and an increase in the consumption of plant-based foods, including plant-based protein foods, so that efforts to feed the world's population remain within the planetary boundaries (8). The Canada's Food Guide (CFG)-2019 (9) also recognizes the impact of food choices on the environment, something that the Dietary Guidelines for Americans (DGA) 2020–2025 have yet to do (10).

In addition to pressuring the environment, intake of animal-based protein foods, especially red and processed meats, has been associated with unfavorable health outcomes in many studies. For example, consumption of red and processed meats has been associated with greater risks of type 2 diabetes, colorectal cancer, cardiovascular mortality and all-cause mortality (11–13). By contrast, intake of plant-based protein foods such as legumes, soy and nuts has been associated with lower total cholesterol, LDL cholesterol and triglycerides (14) as well as with lower risks of mortality (12, 15). Consequently, the health benefits of consuming plant-based protein foods are recognized in both the CFG-2019 (16) and the DGA 2020–2025 (10).

Animal-based protein foods represent a large share of the plate of typical dietary patterns in North America. For example, it has been estimated that two-thirds of the protein intake among Canadian adults came from animal-based foods in 2015 (17). This implies that transitioning the population's intake from animal- to plant-based protein foods represents a sizeable task. Moreover, a decrease in the intake of animal-based protein foods may not be automatically compensated by an increase in the consumption of plant-based protein foods, and vice versa. In that context, a better understanding of the dietary patterns associated with lower intakes of animal-based protein foods and with higher intakes of plant-based protein foods is key to identifying the most feasible and acceptable dietary patterns consistent with health and diet sustainability in a given population. Thus, the aim of this study was to examine the food and nutrient characteristics as well as the overall quality and costs of dietary patterns consistent with lower intakes of animal-based protein foods and with higher intakes of plant-based protein foods among French Canadian adults. We hypothesized that a dietary pattern comprising more plant-based protein foods is associated more strongly with overall diet quality than a dietary pattern comprising less animal-based protein foods.

2. Materials and methods

2.1. Study design and population

Data from the web-based multicenter cross-sectional PRÉDicteurs Individuels, Sociaux et Environnementaux (PREDISE) study, which aimed to document associations between individual, social and environmental factors, and the adherence to dietary guidelines, were used for these analyses. Recruitment and complete procedures of the PREDISE study have been previously described (18). In short, between August 2015 and April 2017, participants aged 18–65 years from five different administrative regions of the Province of Québec (i.e., Capitale-Nationale/Chaudière-Appalaches, Estrie, Mauricie, Montreal, and Saguenay-Lac-St-Jean) were recruited through the services of a survey firm. Stratified sampling was used to obtain an age- and sex-representative sample of French-speaking adults from each of these five administrative regions. To be eligible, participants needed to have Internet access to complete the questionnaires. Exclusion criteria were pregnancy and lactation. Participants had a 3 weeks period to complete online questionnaires on sociodemographic characteristics and web-based 24 h dietary recalls (R24W). Afterward, participants were invited to an in-person visit at a research center where anthropometric measurements (i.e., height and weight) were taken. A total of 1849 participants met the inclusion criteria and gave their written consent and, among those, 1,147 completed at least one 24 h recall and were included in the study sample. The project was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committees of Université Laval (ethics number: 2014-271), Centre hospitalier universitaire de Sherbrooke (ethics number: MP-31-2015-997), Montreal Clinical Research Institute (ethics number: 2015-02), and Université du Québec à Trois-Rivières (ethics number: 15-2009-07.13).

2.2. Dietary intake assessment

Dietary intakes were evaluated using three unannounced R24W (19, 20) in which participants were asked to report all foods they consumed the day before in their prepared and cooked forms, where applicable, thus accounting for food or nutrient losses and moisture change due to preparation (18). Nutrient intake values from the R24W are derived from the Canadian Nutrient File 2015. Foods reported were classified into seven food categories corresponding to the broad categories of foods consumed and of public health interest: (1)- animal-based protein foods (including yogurts, cheeses and unsweetened milk), (2)- plant-based protein foods (including plant-based yogurts, fortified plant-based cheeses that contain sufficient proteins (i.e., not less than 25 g per 100 g or 15 g per 100 g for products intended to resemble fresh cheese) and unsweetened plant-based beverages that contained at least 2.5 g of proteins per 100 ml), (3)- vegetables and fruits, (4)- refined grains, (5)- whole grains, and finally foods not recommended in the CFG-2019, which were further divided into (6)- processed meats and (7)- other foods group (see **Supplementary Table 1** for further description of the food group classification). Consumption of foods in each food group was expressed in reference amount (RA) per

2,500 kcal. One reference amount corresponds to the portion size of one typical serving of each food in Canada (21). Nutrients consumption was expressed either in percentage of energy intake or in mg per 2,500 kcal.

2.3. Healthy eating food index-2019

Data from R24W were used to calculate the Healthy Eating Food Index (HEFI)-2019, which assesses the alignment of dietary patterns with recommendations on healthy food choices in the CFG-2019 (22, 23). The HEFI-2019 consists of 10 components: five that are based mostly on foods (Vegetables and fruits, Whole-grain foods, Grain foods ratio, Protein foods, Plant-based protein foods), one that is based on beverages (Beverages) and four that are based on nutrients (Fatty acids ratio, Saturated fats, Free sugars, Sodium). Scores for each component range from 5 to 20 points, and the HEFI-2019 total score has a maximum of 80 points (see **Supplementary Table 2** for the HEFI-2019 components, points and scoring system). Higher HEFI-2019 scores reflect a greater adherence to recommendations on healthy food choices in the CFG-2019 and consequently, a better diet quality.

2.4. Daily diet costs

The daily diet cost was calculated for each participant and each 24 h recall by matching dietary recall data to a food price database created by our research team in collaboration with the Institut national de santé publique du Québec (INSPQ). The detailed methods for the creation of this food price database and the matching procedures have been described elsewhere (24). Briefly, in the R24W, each food reported is linked to a Bureau of Nutritional Science food group. A 2015–2016 Nielsen food price database was used to compute a standard price for each Bureau of Nutritional Science food group (n = 180) of the 2015 Canadian Nutrient File used in the R24W. This standard price was adjusted for material loss and for food preparation to account for moisture, fat loss and cooking gains. Then, the amount of each food or beverage reported in the R24W expressed in kilogram was multiplied by the corresponding Bureau of Nutritional Science food group price per kilogram for each 24 h recall and summed to obtain a daily diet cost. For the present study, the daily diet cost was adjusted to 2,500 kcal/day to estimate a cost for isocaloric dietary patterns and energy-adjusted daily diet costs were used in the statistical analyses.

2.5. Statistical analyses

2.5.1. Estimating usual dietary intakes and diet costs

To account for within-individual random errors that affect dietary intakes measured with 24 h recalls, the National Cancer Institute (NCI)'s multivariate Markov Chain Monte Carlo method was used (25). This method allows the estimation of the distribution of usual (i.e., long term) food and nutrients intakes as well as of daily diet costs using regression calibration of data from repeated 24 h recalls. To better reflect variations in dietary intakes within individuals, the model was stratified by sex. The model included the following covariables: age and indicators for the sequence of 24 h recalls (i.e., first, second or third recall) and the day of the week (i.e., weekdays vs. weekend days including Friday). Certain foods were considered to be consumed episodically in the model if 10% or more of the population did not report consumption on the first dietary recall. Based on this criterion, the consumption of whole-grain foods, plant-based protein foods, processed meats and some beverages (i.e., sugary drinks, artificially sweetened beverages, vegetable and fruit juices, sweetened milk and plant-based beverages, alcohol, unsweetened milk and unsweetened plant-based beverages that are not a source of proteins) was considered episodic. All remaining foods and nutrients were considered to be consumed daily. The diet cost was also considered as a "daily" variable in the model. Estimated usual dietary intakes and costs among pseudo-individuals generated in the Monte Carlo simulation step of the multivariate method were pooled within each stratum. The HEFI-2019 total score and component scores were calculated from estimated usual intakes among pseudo-individuals.

2.5.2. Descriptive statistics

SURVEY procedures were used when appropriate to account for the stratified design of the PREDISE study. To ensure sex- and age- representativeness in each administrative region, balancing weights were used since the final sample size of the PREDISE study was larger than originally planned. Consumption of animal- and plant-based protein foods, expressed in RA per 2,500 kcal, was first categorized into quarters based on raw intakes in the overall population. The distribution of sociodemographic variables across quarters of animal- and of plant-based protein foods consumption was estimated using the SURVEYFREQ procedure and differences were assessed with a chi-square test. Sociodemographic variables considered were sex (men and women), age (18 to $<$35, 35 to $<$49, 50–65 years), body mass index (BMI; normal $<$ 25.0, overweight 25.0–29.9, obese $\geq$ 30), smoking status (never, former, occasionally, or daily), education (none/high school/trade, CEGEP, university) and household income ($<$30 000 $CAD, 30 000 to $<$60 000 $CAD, 60 000 to $<$90 000 $CAD, $\geq$90 000 $CAD).

2.5.3. Association with food and nutrient intakes, diet quality, and diet costs

Linear regression models were used to examine the association between usual intakes of animal- or plant-based protein foods (independent variables, categorized as quarters) and usual intakes of other food groups and nutrients, HEFI-2019 scores and daily diet costs (dependent variables). For animal-based protein foods, quarter 4, which represents the group of participants with the highest consumption, was used as the reference. For plant-based protein foods, quarter 1, which represents the group of participants with the lowest consumption, was used as the reference. Models were adjusted for sex and age. Standard errors and 95% CI were estimated using 200 bootstrap resamples and normal approximation. All analyses were performed in SAS Studio (version 3.81 SAS Institute, Cary, NC, United States) and figures were generated in R Studio (version 2022.02.0; R Foundation for Statistical Computing, Boston, MA, United States).

TABLE 1 Participants' sociodemographic characteristics according to quarters of animal-based protein food intake[1].

Characteristic (n = 1147)	Q4[2] (>5.6–13.5) (%)	Q3 (>4.5–5.6) (%)	Q2 (>3.3–4.5) (%)	Q1 (0.0–3.3) (%)
Sex				
Female	26.8	26.9	24.1	22.1
Male	23.3	23.7	25.5	27.5
p	*0.11*			
Age group				
18–34 years	25.6	21.0	25.8	27.7
35–49 years	26.7	25.9	25.2	22.3
50–65 years	23.2	29.3	23.5	24.0
P	*0.16*			
Body mass index group[3]				
Normal (<25.0)	26.6	23.4	24.4	25.7
Overweight (25.0–29.9)	21.1	29.0	25.9	24.0
Obese (≥30.0)	26.5	24.8	24.1	24.6
P	*0.48*			
Smoking				
Never	26.3	23.9	25.6	24.2
Former	24.7	27.8	22.6	24.9
Occasional or daily	21.5	24.8	27.0	26.7
P	*0.67*			
Education[3]				
High school or less	24.7	21.9	27.1	26.3
CEGEP	23.8	25.1	28.8	22.4
University	25.5	27.5	21.4	25.6
P	*0.22*			
Income[3]				
<30 000 $CAD	22.1	22.7	25.6	29.6
30 000 to <60 000 $CAD	23.9	27.7	22.8	25.6
60 000 to <90 000 $CAD	28.7	23.2	24.6	23.5
≥90 000 $CAD	23.5	26.9	26.4	23.2
P	*0.68*			

[1] Values are percentages and will sum across columns. CAD, Canadian dollars; CEGEP, Collège d'Enseignement Général et Professionnel; Q, Quarter.
[2] Because these are only descriptive data, quarters of animal-based protein food intake were not identified based on usual dietary intakes obtained by the National Cancer Institute's multivariate method. Range of animal-based protein food intake for each quarter is expressed as RA per 2,500 kcal.
[3] Body mass index group, n = 1,022 (125 missing values); Education, n = 1,087 (60 missing values); Income, n = 988 (159 missing values).
The italic values correspond to the p-value of the chi-square test.

3. Result

3.1. Characteristics of participants

As presented in Table 1, there was no major difference in the participants' sociodemographic characteristics across quarters of animal-based protein food intake. In contrast, participants with the highest self-reported intake of plant-based protein foods were older, tended to have a lower BMI, were less likely to be occasional or daily smokers and had a higher education level than participants with lower intakes of plant-based protein foods (Table 2).

If the high plant-based protein food dietary pattern was the mirror of the low animal-based protein food dietary pattern and vice versa, then all (100%) participants would have been categorized into corresponding quarters of the two patterns. However, less than 30% of the entire cohort was categorized into corresponding quarters of usual plant-based and animal-based protein food consumption (Table 3), indicating a relatively strong mismatch between the two patterns.

3.2. Food and nutrient intakes

Table 4 presents the food and nutrient intakes across quarters of usual animal-based protein food intake. In this population, high

TABLE 2 Participants' sociodemographic characteristics according to quarters of plant-based protein food intake[1].

Characteristic (n = 1147)	Q1[2] (0.0–0.0) (%)	Q2 (>0.0–0.6) (%)	Q3 (>0.6–1.5) (%)	Q4 (>1.5–9.1) (%)
Sex				
Female	24.9	22.5	25.0	27.7
Male	29.4	23.1	25.4	22.1
P	*0.12*			
Age group				
18–34 years	31.6	24.2	21.9	22.3
35–49 years	26.0	24.2	24.9	24.9
50–65 years	23.6	20.1	28.8	27.5
P	*0.05*			
Body mass index group[3]				
Normal (<25.0)	21.9	22.7	25.4	30.1
Overweight (25.0–29.9)	27.3	21.6	27.9	23.2
Obese (≤30.0)	29.6	24.8	23.6	22.1
P	*0.08*			
Smoking				
Never	24.7	23.7	24.8	26.8
Former	25.9	21.3	26.7	26.1
Occasional or daily	38.9	22.8	23.2	15.0
P	*0.01*			
Education[3]				
High school or less	34.0	22.2	23.5	20.3
CEGEP	25.5	23.0	25.0	26.4
University	23.1	23.4	27.4	26.1
P	*0.06*			
Income[3]				
<30 000 $CAD	30.6	24.4	26.0	19.0
30 000 to <60 000 $CAD	29.6	19.2	26.3	24.9
60 000 to <90 000 $CAD	24.4	22.3	25.8	27.6
≥90 000 $CAD	20.6	26.3	27.5	25.6
p	*0.14*			

[1]Values are percentages and will sum across columns. CAD, Canadian dollars; CEGEP, Collège d'Enseignement Général et Professionnel; Q, Quarter.
[2]Because these are only descriptive data, quarters of plant-based protein food intake were not identified based on usual dietary intakes obtained by the National Cancer Institute's multivariate method. Range of plant-based protein food intake for each quarter is expressed as RA per 2,500 kcal.
[3]Body mass index group, n = 1,022 (125 missing values); Education, n = 1,087 (60 missing values); Income, n = 988 (159 missing values).
The italic values correspond to the p-value of the chi-square test.

TABLE 3 Proportion of all participants in each quarter of animal- and plant-based protein food intake[1,2].

		Quarters of animal-based protein food intake			
		Q4	Q3	Q2	Q1
Quarters of plant-based protein food intake	Q1	8.4%	6.7%	5.5%	4.4%
	Q2	6.7%	6.5%	6.2%	5.6%
	Q3	5.6%	6.3%	6.6%	6.6%
	Q4	4.3%	5.5%	6.7%	8.4%

[1]Quarters of usual intake of animal- and plant-based protein foods are based on the National Cancer Institute's multivariate method. Gray cells indicate group overlap.
[2]Q, Quarter.

TABLE 4 Usual food and nutrient intakes across quarters of animal-based protein food intake in French-speaking adults from Québec, Canada[1,4].

	Q4 (Reference)	Q3	Q2	Q1
Animal-based protein foods, RA/2,500 kcal				
Mean (SE)	6.5 (0.3)	4.8 (0.1)	3.9 (0.1)	2.8 (0.1)
Range	(>5.4–16.9)	(>4.3–5.4)	(>3.5–4.3)	(0.5–3.5)
	Mean (SE)[3]	Difference vs. Q4 (95% CI)[2,3]		
Vegetables and fruits, RA/2,500 kcal	4.5 (0.2)	−0.0 (−0.3, 0.2)	0.0 (−0.4, 0.4)	0.0 (−0.6, 0.6)
Refined grains, RA/2,500 kcal	2.0 (0.1)	0.0 (−0.1, 0.1)	0.0 (−0.2, 0.2)	0.1 (−0.3, 0.4)
Whole grains, RA/2,500 kcal	1.2 (0.1)	0.1 (0.0, 0.2)	0.2 (0.0, 0.3)	0.3 (0.1, 0.6)
Plant-based protein foods, RA/2,500 kcal	0.8 (0.1)	0.1 (0.0, 0.2)	0.3 (0.1, 0.4)	0.5 (0.1, 0.8)
Processed meats, RA/2,500 kcal	0.6 (0.0)	−0.0 (−0.1, 0.0)	−0.1 (−0.2, 0.0)	−0.1 (−0.2, 0.0)
Other foods, RA/2,500 kcal	4.6 (0.2)	0.3 (0.1, 0.6)	0.5 (0.1, 1.0)	0.8 (0.0, 1.7)
MUFA, % energy intake	13.0 (0.2)	−0.1 (−0.4, 0.1)	−0.2 (−0.6, 0.2)	−0.3 (−1.0, 0.3)
PUFA, % energy intake	6.6 (0.1)	0.4 (0.3, 0.6)	0.7 (0.4, 0.9)	1.1 (0.6, 1.5)
SFA, % energy intake	12.9 (0.2)	−0.8 (−1.1, −0.4)	−1.3 (−1.8, −0.8)	−2.1 (−2.8, −1.4)
Free sugars, % energy intake	10.8 (0.4)	1.0 (0.5, 1.4)	1.6 (0.8, 2.4)	2.5 (1.2, 3.8)
Sodium, mg/2,500 kcal	3,671 (65.6)	−120 (−203, −36.2)	−206 (−337, −74.5)	−332 (−528, −135)

[1]Usual food and nutrient intakes are based on the National Cancer Institute's multivariate method. All values were estimated using linear regression models adjusted for age and sex.
[2]Difference vs. the reference quarter (Q4) corresponds to the regression coefficient in the linear regression models (see section "2. Materials and methods").
[3]SE and 95% CI are calculated using 200 bootstrap resamples.
[4]MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Q, quarter; RA, reference amount; SFA, saturated fatty acids.

intakes (Quarter 4) of animal-based protein foods corresponded to a mean of 6.5 RA/2,500 kcal (SE, 0.1) while low intakes (Quarter 1) corresponded to a mean of 2.8 RA/2,500 kcal (SE, 0.3). Participants with low compared to those with high intakes of animal-based protein foods (Quarter 1 vs. Quarter 4) had higher intakes of whole grains (+0.3 RA/2,500 kcal, 95% CI, 0.1 to 0.6), plant-based protein foods (+0.5 RA/2,500 kcal, 95% CI, 0.1 to 0.8), other foods not recommended in the CFG-2019 (+0.8 RA/2,500 kcal, 95% CI, 0.0 to 1.7), PUFA (+1.1%E, 95% CI, 0.6 to 1.5) and free sugars (+2.5%E, 95% CI, 1.2–3.8) as well as lower intakes of SFA (-2.1%E, 95% CI, -2.8 to -1.4) and sodium (-332 mg/2,500 kcal, 95% CI, -528 to -135).

The food and nutrient intakes across quarters of usual plant-based protein food consumption is presented in Table 5. Low intakes (Quarter 1) of plant-based protein foods corresponded to a mean of 0.2 RA/2500kcal (SE, 0.0) while high intakes (Quarter 4) corresponded to a mean of 2.2 RA/2,500 kcal (SE, 0.1) in this population. Participants with high compared to low intakes of plant-based protein foods (Quarter 4 vs. Quarter 1) had higher intakes of vegetables and fruits (+1.8 RA/2,500 kcal, 95% CI, 1.2 to 2.4) and whole grains (+0.9 RA/2,500 kcal, 95% CI, 0.7 to 1.1), as well as lower intakes of refined grains (-0.5 RA/2,500 kcal, 95% CI, -0.8 to -0.2), animal-based protein foods (-0.8 RA/2,500 kcal, 95% CI, -1.4 to -0.2), processed meats (-0.4 RA/2,500 kcal, 95% CI, -0.5 to -0.2) and other foods not recommended in the CFG-2019 (-1.5 RA/2,500 kcal, 95% CI, -2.2 to -0.8). Participants with higher intakes of plant-based protein foods also had higher intakes of MUFA (+1.0%E, 95% CI, 0.5 to 1.6) and PUFA (+1.4%E, 95% CI, 0.9 to 1.8), and lower intakes of SFA (-1.5%E, 95% CI, -2.2 to -0.7), free sugars (-3.3%E, 95% CI, -4.6 to -2.0) and sodium (-256 mg/2,500 kcal, 95% CI, -460 to -52).

3.3. HEFI-2019 scores and daily diet costs

Differences in the HEFI-2019 total score and daily diet costs across quarters of animal- and plant-based protein food intake are presented in Figure 1. Participants with lower intakes of animal-based protein foods (Quarter 1 vs. Quarter 4) had a higher HEFI-2019 total score (+4.0 pts, 95% CI, 0.9 to 7.1) and lower daily diet costs (-1.9 $CAD, 95% CI, -2.6 to -1.2). Participants with higher intakes of plant-based protein foods (Quarter 4 vs. Quarter 1) also had a higher HEFI-2019 total score (+14.6 pts, 95% CI, 12.4 to 16.9) with no difference in daily diet costs (0.0 $CAD, 95% CI, -0.7 to 0.7). Differences in HEFI-2019 component scores between extreme quarters of animal-based protein food intake and of plant-based protein food intake are shown in Supplementary Figure 1.

4. Discussion

The aim of this study was to document the food and nutrient profiles as well as diet quality and costs of dietary patterns consistent with relatively low intakes of animal-based protein foods and with relatively high intakes of plant-based protein foods among French Canadians. Participants with low intakes of animal-based protein foods and participants with high intakes of plant-based protein foods in this population had relatively high intakes of whole grains, plant-based proteins foods and PUFA as well as low intakes of animal-based proteins foods, SFA and sodium. Furthermore, participants with high intakes of plant-based protein foods had high intakes of vegetables, fruits and MUFA and low intakes of refined grains, processed meats, other foods not recommended in the CFG-2019 and free sugars. Participants with low intakes of animal-based proteins foods had

TABLE 5 Usual food and nutrient intakes across quarters of plant-based protein food intake in French-speaking adults from Québec, Canada[1,4].

	Q1 (Reference)	Q2	Q3	Q4
Plant-based protein foods, RA/2,500 kcal				
Mean (SE)	0.2 (0.0)	0.5 (0.0)	1.1 (0.1)	2.2 (0.1)
Range	(0.0–0.3)	(>0.3–0.8)	(>0.8–1.4)	(>1.4–11.7)
	Mean (SE)[3]	Difference vs. Q1 (95% CI)[2,3]		
Vegetables and fruits, RA/2,500 kcal	3.6 (0.1)	0.7 (0.5, 0.9)	1.2 (0.9, 1.5)	1.8 (1.2, 2.4)
Refined grains, RA/2,500 kcal	2.3 (0.1)	−0.2 (−0.4, −0.1)	−0.4 (−0.6, −0.2)	−0.5 (−0.8, −0.2)
Whole grains, RA/2,500 kcal	0.9 (0.1)	0.3 (0.3, 0.4)	0.6 (0.4, 0.7)	0.9 (0.7, 1.1)
Animal-based protein foods, RA/2,500 kcal	4.9 (0.2)	−0.3 (−0.5, −0.1)	−0.5 (−0.9, −0.1)	−0.8 (−1.4, −0.2)
Processed meats, RA/2,500 kcal	0.7 (0.1)	−0.2 (−0.2, −0.1)	−0.3 (−0.4, −0.2)	−0.4 (−0.5, −0.2)
Other foods, RA/2,500 kcal	5.8 (0.2)	−0.6 (−0.9, −0.3)	−1.0 (−1.5, −0.5)	−1.5 (−2.2, −0.8)
MUFA, % energy intake	12.3 (0.2)	0.4 (0.1, 0.6)	0.6 (0.3, 1.0)	1.0 (0.5, 1.6)
PUFA, % energy intake	6.5 (0.1)	0.5 (0.3, 0.6)	0.8 (0.6, 1.1)	1.4 (0.9, 1.8)
SFA, % energy intake	12.6 (0.2)	−0.6 (−0.9, −0.3)	−0.9 (−1.4, −0.5)	−1.5 (−2.2, −0.7)
Free sugar, % energy intake	13.8 (0.4)	−1.3 (−1.9, −0.7)	−2.2 (−3.1, −1.3)	−3.3 (−4.6, −2.0)
Sodium, mg/2,500 kcal	3,648 (66.1)	−120 (−200, −39.1)	−187 (−316, −57.0)	−256 (−460, −52.4)

[1]Usual food and nutrient intakes are based on the National Cancer Institute's multivariate method. All values were estimated using linear regression models adjusted for age and sex.
[2]Difference vs. the reference quarter corresponds to the regression coefficient in the linear regression models (see section "2. Materials and methods").
[3]SE and 95% CI are calculated using 200 bootstrap resamples.
[4]MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Q, quarter; RA, reference amount; SFA, saturated fatty acids.

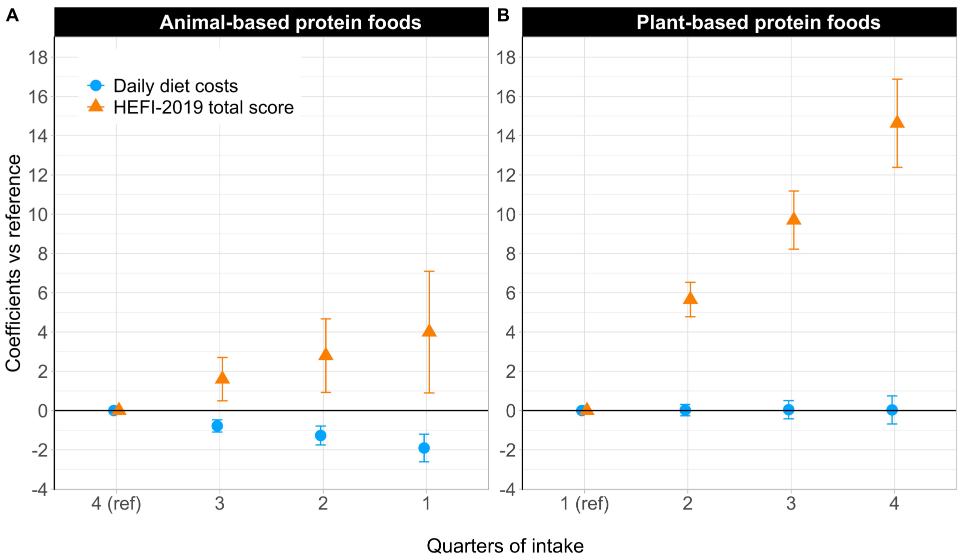

FIGURE 1
(A) Differences in the HEFI-2019 total score and daily diet costs across quarters of animal-based protein food intake. The HEFI-2019 total score and daily diet costs for the reference quarter (Q4) of animal-based protein food intake were 41.7 pts (SE, 0.8) and 13.7 $CAD per 2,500 kcal (SE, 0.2), respectively. **(B)** Differences in the HEFI-2019 total score and daily diet costs across quarters of plant-based protein food intake. The HEFI-2019 total score and daily diet costs for the reference quarter (Q1) of plant-based protein food intake were 36.3 pts (SE, 0.6) and 12.7 $CAD per 2,500 kcal (SE, 0.2), respectively. The regression coefficients scaled on the y-axis in both panels represent the differences in the HEFI-2019 total score (points) and daily diet costs ($CAD/2500 kcal) compared to the quarter of reference. Dietary intake data and costs standardized to 2,500 kcal were modeled using the National Cancer Institute's multivariate method to reflect usual intakes. SE and 95% CI were calculated using 200 bootstrap resamples. HEFI-2019, Healthy Eating Food Index-2019; Ref, reference.

high intakes of other foods not recommended in the CFG-2019 and of free sugars. Finally, participants with low compared to high intakes of animal-based protein foods had a 4.0-point higher HEFI-2019 score and lower daily diet costs, while participant with high compared to low intakes of plant-based protein foods had a 14.6-point higher HEFI-2019 score with no difference in daily diet costs.

Findings from our study are partially consistent with previous studies comparing the dietary patterns of low vs. high meat-eaters. For example, a recent study in the Netherlands reported that intakes of nuts and seeds were higher in men and women with a lower consumption of meat (26). However, and unlike our own observations, Dutch participants with a relatively low meat consumption compared to those consuming more meat had higher intakes of vegetables and refined grains (26). In the United Kingdom, adults with low meat consumption were found to consume more whole grains, soy, legumes, nuts, seeds, vegetables and fruits, and less refined grains, fried foods, alcohol and sugar sweetened beverages compared to regular meat-eaters (27), partially confirming our observations regarding the dietary patterns of low vs. high animal-based protein food consumers.

Previous studies have also examined the dietary patterns associated with different intakes of plant-based proteins. Aggarwal and Drewnowski reported that a greater consumption of plant-based proteins was associated with higher intakes of fruits and vegetables, and with lower intakes of solid fats and added sugars, which is consistent with data from the present study (28). Along with a greater consumption of plant-based protein foods, vegetarians and vegans have also been reported to consume more fruits, vegetables and whole-grain foods, and less refined grains, processed meats and fried foods than regular meat eaters (29, 30), which is consistent with data from the present study. Finally, French-Canadian adults who reported consuming more plant-based protein foods also had higher intakes of PUFA and MUFA, and lower intakes of SFA and sodium than those who consumed little plant-based protein foods, consistent with previous findings (30, 31).

The literature suggests that healthy dietary patterns generally cost more than unhealthy dietary patterns, regardless of the diet quality index used, including the HEFI-2019 (24, 32–35). In the present study, participants with a relatively lower intake of animal-based protein foods (Quarter 1 vs. Quarter 4 in this population) had a better diet quality (+4.0 points in the HEFI-2019 total score) at lower daily diet costs. This agrees with a recent study having shown that the intake of animal proteins *per se* was negatively associated with diet quality and positively with diet costs (28). On the other hand, we found that participants with a diet characterized by higher vs. lower amounts of plant-based protein foods (Quarter 4 vs. Quarter 1) had a more pronounced difference in diet quality (+14.6 points in the HEFI-2019 total score) at no additional daily diet cost. This marked increase in the HEFI-2019 is consistent with the better diet quality associated with vegetarian diets (30, 36) and with the replacement of animal-based protein foods by plant-based protein foods (37). Moreover, diets with more energy from plant-based protein have been previously associated with a better diet quality with minimal increase in daily diet costs (28).

The present findings provide perspectives on the differences in dietary intakes and quality that may be expected as dietary recommendations increasingly advocate for a reduction in the consumption of animal-based protein foods and an increase in the consumption of plant-based protein foods. First, only a small proportion of participants were categorized into both the low quarter of animal-based protein food intake and the high quarter of plant-based protein food intake, indicating that these are quite distinct dietary patterns. The differences observed between high compared with low plant-based protein food dietary patterns suggest that high plant-based protein food consumers may be more prone to consider health or nutrition concerns when choosing foods, as observed among vegetarian adult populations (38, 39). Second, the quarter of the population with the highest consumption of plant-based protein foods, and with the highest HEFI-2019 score, still reported consuming approximately 4 RA (or "servings") of animal-based protein foods per day. This indicates that a slight increase in the consumption of plant-based protein foods may be sufficient to observe a marked improvement in the overall quality of the diet of French Canadians at no additional cost, without a drastic reduction in the consumption of animal-based protein foods or even its exclusion from the diet. This supports the potential acceptability of adopting healthier and more sustainable protein-related dietary patterns in this population as it does not require major changes in the diet.

This study has several strengths including the use of an age- and sex-representative sample of French-speaking adults in each of the five pre-selected most populated administrative regions of the province of Quebec. Another strength is the use of the NCI multivariate method to account for random errors affecting dietary intake data measured by repeated 24 h recalls and thus, the ability to generate usual dietary intakes and daily diet costs rather than data on "any given day." Characterizing dietary patterns based on both high/low animal- and plant-based protein food intake, rather than just one or the other, is original and another strength. The use of the HEFI-2019, a validated index reflecting adherence to the most recent recommendations on healthy food choices in Canada, as a proxy of diet quality is also a strength. Limitations also need to be addressed. First, the data used are from 2015 to 2017, which may not represent the current dietary patterns of French Canadians since data from industrialized countries suggest a slight but ongoing decrease in animal-based food intakes and an increase in plant-based food intakes in recent years (40, 41). Secondly, there are some limitations specific to the food price database used. For example, food prices were not available by type of store, season, geographic location, or other demographic factors, and do not represent the lowest price available. Moreover, by using Nielsen food price data, we assumed that all foods and beverages were bought from grocery or big box stores, and food waste was not considered. Thirdly, results cannot be generalized to all other populations given the relatively high education and income of the study sample. Finally, the lack of information on the environmental impact of the documented dietary patterns of French Canadians does not allow us to have a full overview of their sustainability.

In conclusion, data from this cohort of French-speaking Canadian adults suggest that a transition toward dietary patterns characterized by lower amounts of animal-based protein foods may reasonably improve diet quality at lower daily diet costs in this population. However, shifting to a diet with more plant-based protein foods may be even more effective to enhance diet quality at no additional cost. These data suggest that promoting the adoption of plant-based dietary patterns, without full exclusion of animal-based protein foods, is promising and should continue to be one of the key strategies in dietary guidelines to achieve healthier and more sustainable dietary patterns. Strong public health initiatives may be required to facilitate the adoption of such dietary patterns at the population level. Additional research on the environmental impact of dietary patterns with higher amounts of animal- or plant-based protein foods among French Canadians and in other populations is needed to better assess and compare their sustainability.

Data availability statement

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

Ethics statement

This study involving human participants was reviewed and approved by Research Ethics Committees of Université Laval (ethics number: 2014-271), Centre Hospitalier Universitaire de Sherbrooke (ethics number: MP-31-2015-997), Montreal Clinical Research Institute (ethics number: 2015-02), and Université du Québec à Trois-Rivières (ethics number: 15-2009-07.13). The participants provided their written informed consent to participate in this study.

Author contributions

GR, JR, VP, SD, SL, and BL designed research. GR and DB performed statistical analysis. GR and BL wrote the manuscript. BL had primary responsibility for final content. All authors read and approved the final manuscript.

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Conflict of interest

BL had received funding from Health Canada (completed in 2021). DB had been a casual employee of Health Canada (2019–2020).

The remaining 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.2023.1148137/full#supplementary-material

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Sustainable nutrition and the case of vegetable oils to match present and future dietary needs

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Sustainable nutrition represents a formidable challenge for providing people with healthy, nutritious and affordable food, while reducing waste and impacts on the environment. Acknowledging the complexity and multi-dimensional nature of the food system, this article addresses the main issues related to sustainability in nutrition, existing scientific data and advances in research and related methodologies. Vegetable oils are epitomized as a case study in order to figure out the challenges inherent to sustainable nutrition. Vegetable oils crucially provide people with an affordable source of energy and are essential ingredients of a healthy diet, but entail varying social and environmental costs and benefits. Accordingly, the productive and socioeconomic context encompassing vegetable oils requires interdisciplinary research based on appropriate analyses of big data in populations undergoing emerging behavioral and environmental pressures. Since oils represent a major and growing source of energy at a global level, their role in sustainable nutrition should be considered beyond pure nutritional facts, at the light of soil preservation, local resources and human needs in terms of health, employment and socio-economic development.

KEYWORDS

palm oil, non-communicable disease, cardiovascular disease, diets, sustainable nutrition, saturated fats, complexity science, sustainable development goals

Introduction

Traditional food science is unable to fully define the sustainability of foods in a world where the concept of healthy nutrition is the dominant paradigm in food consumption. The old paradigm arose after the second world war, when conditions of famine were the main concern. Afterwards, the health aspects of foods became the leading concern, particularly in Northern and richer countries, but without considering the emerging need of environmental preservation. Diets that promote adequate nutrition, physical health and environmental sustainability are an aspiration of many (1) and major determinants in the goals of global sustainable development and of the One Health approach (2). Sustainable nutrition encompasses various systems in food production and consumption by simultaneously addressing nutrient adequacy; ecosystem stability; food affordability, availability and safety;

waste and loss reduction and sustaining human health in the frame of primary and secondary prevention (3–6).

Because of its complex nature, sustainable nutrition requires a holistic view to interpret all the critical elements along the food chain, from production contexts and impacts to such consequences of consumption as nutrient provision, health benefits and dietary preferences (7). The scientific challenge is to analyze the interactions between these different elements and present them in a way that most consumers can grasp. Moreover, consumers' knowledge of food choices is currently derived from producers, traders, governments and campaigning organizations, each one with their own interests and the final result of consumers' confusion (8). Therefore, the complex concepts of sustainability should be translated into simpler information driving consumers to better evaluate their choices. International exchange of knowledge, with spillovers of agricultural technology and production patterns across countries (9), should ensure an easier access to foods that meet individual consumer needs and health expectations while simultaneously addressing global sustainability and the One Health concept. We have selected the case of vegetable oils, an important source of fat and energy (6), as a relevant example of sustainability in nutrition. A diverse international panel of experts across medicine, nutrition, food science and food environment were assembled to review the nutritional sustainability of vegetable oils.

The case of vegetable oils

Vegetable oils represent a major component of the food system, an important source of energy and an important economic commodity for producers. While in economically advantaged parts of the world there is overconsumption of energy-yielding food components, around 800 million people worldwide were undernourished in 2020 (10). Fats provide 25–30% of daily energy in high income settings (11) and are an affordable food for undernourished people who need increased energy intake (6). Since the 1980s, the global use of vegetable oils has increased across various industrial and consumer segments. The land used for oil crops grew from 114 million hectares (Mha) in 1961 to 332 Mha in 2020 (12), corresponding to $\sim$ 23% of all cropland worldwide (notice that this excludes maize as an oil producing crop). Oil palm, soybean, sunflower seed and rapeseed together account for more than 80% of all the sources of vegetable oil production, with cotton, groundnuts, olive and coconut comprising most of the remainder (8) (Figure 1). Palm oil is principally produced in Indonesia and Malaysia, soy bean oil in South America, sunflower seed oil in Ukraine, Russia, USA and China and rapeseed oil in China, Canada and some European countries (Figure 1). These crops, including soybean (127 Mha planted area) and maize (202 Mha planted area), are also used as animal feed. The global gross production value of oil crops (not including maize) was estimated at US$ 335 billions in 2020 (12). The predicted growth in vegetable oils production because of the rising demand is an environmental concern, because crop expansion has been often associated with tropical deforestation or loss of other natural ecosystems (e.g., woodland savanna, natural grassland) as well as with disruption of biodiversity and other ecosystem values. On the positive side, vegetable oil production can locally lead to higher incomes, generate labor employment and reduce poverty among farms as well as non-farm households (13).

Since the end of the 1980s dietary recommendations have consistently discouraged the intake of animal fats but also of such plant oils rich in saturated fats such as palm and coconut oils (14). The saturated fats supplied by all plant oils and also by other sources can be hydrolyzed to increase their content of polyunsaturated fat, thus leading to the conversion to trans-fatty acids (TFAs) that epidemiological data unequivocally link to an increased risk of cardiovascular disease (15). On the other hand, shorter-chain saturated fats have not been associated with high serum cholesterol levels (6). The 2020 Dietary Guidelines for Americans (DGAs) still support the recommendation of replacing saturated with unsaturated fats, broadly indicating that $<$10% of total energy should be provided by saturated fat intake but without disentangling the varied effects of saturated fats on serum cholesterol levels. Newer epidemiological data, at odds with these still current recommendations and guidelines on fat consumption, indicate that reducing the intake of saturated fatty acids and replacing them with carbohydrates may be associated as well to adverse effects on blood lipids. By the same token, replacing saturated with unsaturated fats did improve some cardiovascular risk markers but worsened others (16). Furthermore, it must be kept in mind that compounds with potential negative health effects are produced during oil processing, beyond the simple content of saturated fatty acids in vegetable oils (17).

A critical review of the role of dietary saturated fatty acids on cardiovascular disease concluded that the composition of the whole diet is more strongly associated to a lower risk of cardiometabolic disease, than any single nutrient (18). Accordingly, it is being recognized that dietary patterns align not only with healthier outcomes but also with environmental sustainability, consistent with the One Health approach (19). Plant-based dietary patterns commonly defined as healthy—such as the Mediterranean diet, the New Nordic diet, the Japanese diet—translate into different food pyramids but with a shared basis made by vegetables, fruits, whole grains, legumes, nuts and seeds. These diets provide, beyond local sustainability, a wide spectrum of antioxidants with anti-inflammatory properties. Accordingly, healthy dietary pyramids follow indications on vegetable oil consumption inclusive of both tradition and local geographic characteristics, ranging from a relevant role in the Mediterranean diet to a less evident emphasis in the Nordic diet (with milk derived products as a major source of fats) and no clear mention in the Japanese diet, on the whole based on low-fat foods and dressings (e.g., soy). Within these heterogeneous contexts, fats in general and vegetal oils specifically should now be looked at as a complex matrix of multiple nutrients, included in different recipes based on local culinary cultures, and with distinct tradeoffs and synergies among the sustainable development goals of Zero Hunger (SGD 2), Good Health and Wellbeing (SDG 3), and, among others, Life on Earth (SDG 15), Climate Action (SDG 13), and Responsible Production and Consumption (SDG 12) (5). This is the complexity that exemplifies and defines Sustainable Nutrition.

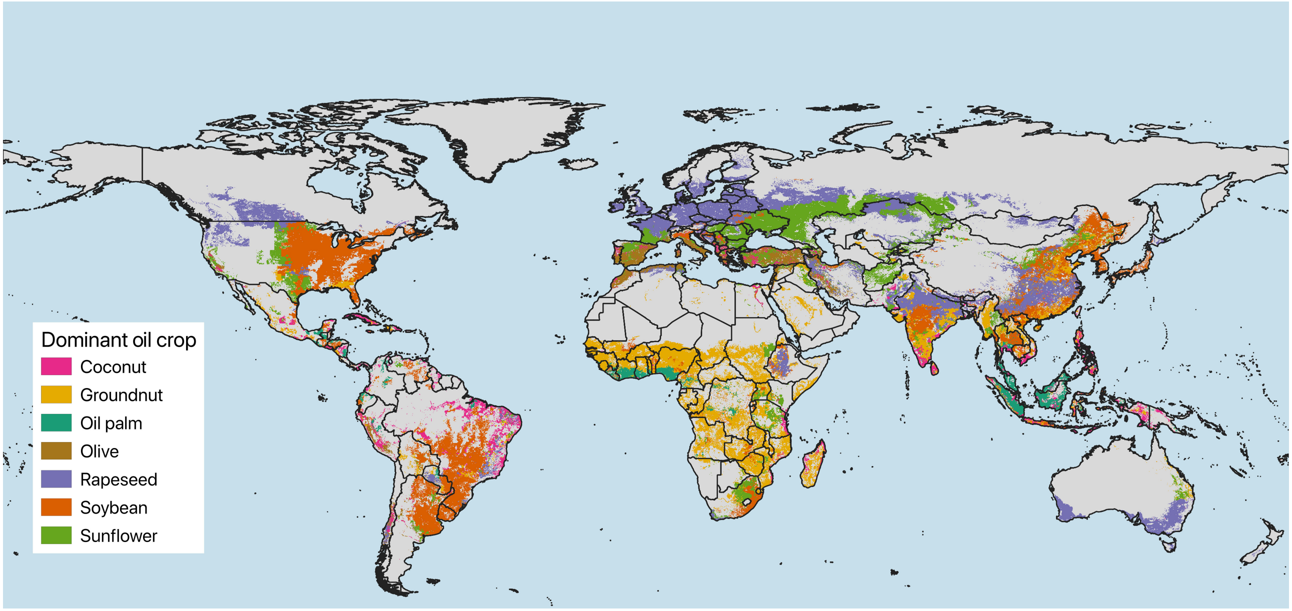

FIGURE 1
Global map showing the dominant oil crops per grid cell (8). See also text (page 2), for additional information.

Environmental and socio-economic impact of vegetable oils

Vegetable oils have been the focus of public media for decades due to controversies on their environmental impacts. In particular, palm oil has been associated with tropical deforestation, often featuring the iconic orangutans (*Pongo* spp.) (20). Other oil crops have generally received less attention, notwithstanding that the total areas allocated to many of them exceed those used for oil palm (8). For example, the oil palm produces $\sim$ 36% of global vegetable oils on 8.6% of the land allocated to oil crops, while soybean produces 26% of oils on 39% of land. Furthermore, the production of groundnut and cottonseed employs relatively large land areas but yields small amounts of oil. Indeed, all oil crops have environmental impacts, ranging from biodiversity impact when their expansion displaces natural ecosystems to water depletion as well as to nitrogen and phosphorus pollution. What is clear is that land requirements vary significantly for different oil crops, with some crops producing much more oil per unit area than others (8). Reducing the land areas allocated to oil production is generally auspicious from an environmental perspective. However, environmental issues are not the only concern pertaining to oil crops, because expansion of crop lands often entails social costs, especially in parts of the world where land or labor rights are weakly defended. On the other hand, there are also benefits, because people living in oil producing areas are able to obtain labor income. Locally produced oils can also provide an affordable source of edible fat to local people, particularly relevant when poor or undernourished (6).

Understanding the impacts of vegetable oil production requires high-resolution and accurate maps showing where these crops are grown. These maps have been produced at a regional scale for soybean, rapeseed and sunflower, but only oil palm and coconut have been accurately mapped at a high resolution and global scale (21, 22). Without these maps it is actually difficult to determine the true impact of the expansion of oil crops on natural ecosystems or other measures of environmental impact (e.g., biodiversity, water pollution, soil health). While palm oil has been associated with tropical deforestation in Indonesia and Malaysia (8) and soybean expansion to loss of forest and woodland savanna in South America (23), environmental impacts have been less well-characterized for other oil crops. There are, for instance, few or no comprehensive data on groundnut and deforestation in Africa, on the water footprint of cottonseed, olive and soybean nor on the relative impacts on biodiversity of pesticides and fertilizers employed for different crops. Similarly, in the frame of a broader sustainable development, there is only limited understanding of how vegetable oil production contributes to different and important development goals (for instance, reducing poverty and hunger, respecting people's rights, preserving the environment). Complex systems and big data analyses might lead to a better understanding on how these issues relate to each other and how they vary under different production systems, i.e., different crops and different scales of production spanning from subsistence-based agroforestry and small-holder plantations to industrial-scale initiatives. Indeed, the production systems may be more relevant for interpreting pathways of sustainability than the individual crops grown in these systems. Vegetable oils are to a significant extent interchangeable (24), and sustainable outcomes are determined more by the production systems than by the oil crops themselves.

In the next few years, the global demand for vegetable oil is expected to further rise alongside a growing human population and related demand for easily available food. Thus, we need to know where oil crops can expand and increase productivity with the least environmental impact. Can this be done by increasing yields on existing lands, or allocating new lands toward oil production?

Crops (or indeed production systems) able to better meet a broad range of sustainable development goals should be considered as primary choices. Further research should therefore investigate where crops are grown and how and which are the local synergies and tradeoffs between different socio-economic and environmental goals in the frame of the different production systems. New analytical tools for analyzing these complex scenarios are warranted to clearly visualize and quantify the impact of the different choices for crop production.

Bridging nutrition and environmental impact

For too long the impacts of nutrition and food consumption have been considered separately from food production processes and related environmental and social impacts. Methods that integrate dietary impacts on human health and natural and socio-economic environments by using consistent and compatible metrics (25) should be developed with the goal to produce health relevant data on the impact of diets, individual foods or food groups such as vegetable oils, as well as to reflect as much as possible the related changes for health rather than just meeting single nutrient recommendations (26, 27). On the environmental side, impacts on a life cycle basis, covering the entire supply chain and including both agricultural production and food processing, should also be assessed. An holistic assessment should also include the role of climate change, land and water use impact, eutrophication on freshwater and marine ecosystems as well as fine particulate matter pollution associated with ammonia emissions.

This assessment effort should be based on the same health metrics used for dietary impacts. Inventory dataset for agriculture processes have recently been developed and are now available for more than 400 food products and ingredients. In particular, the World Food LCA Database and its integration in Ecoinvent (28), the Agribalyse database (29) or the Agri-footprint database (30) cover specific data sets for vegetable oils, including coconut, linseed, maize, olive, palm, peanut, rapeseed, sunflower oil and also margarine. These data enable us to identify the key processes and inputs of these oils on the agricultural production chain. Substantial progress has also addressed the environmental impacts, after comprehensive assessment methods such as ReCiPe or Impact World+ enabled to assess damages on the ecosystem (31). On the nutrition side, the comprehensive Global Burden of Disease (GBD) study systematically analyzed data on nutritional epidemiology, showing that dietary risks play a major role in disease incidence and prevalence and that these risks can be compared by means of the health-based metric DALYs (disability adjusted life *years*), already used for other aspects of environmental impact such as for instance particulate air pollution (32, 33). DALYs measures the potential reduction in life expectancy due to early mortality (Years of Life Lost, YLL), as well as the reduction of a healthy life associated with Years Lived with Disability (YLD), expressed using as a disability metric the YLL equivalent. Fifteen risk factors were identified by the GBD study to be associated with nutrients or food groups. Beneficial risk includes milk, nuts and seeds, fruits, fibers and two fatty acid families, i.e., omega-3 fatty acids from seafood and polyunsaturated fatty acids (PUFAs: linoleic acid, LA, and alpha-linolenic acid, ALA) mainly supplied by vegetable oils. GBD risk factors also identified health damage associated with processed meat, red meat, sugar-sweetened beverages (reflected on body mass index), sodium (reflected on blood pressure) and TFAs (33).

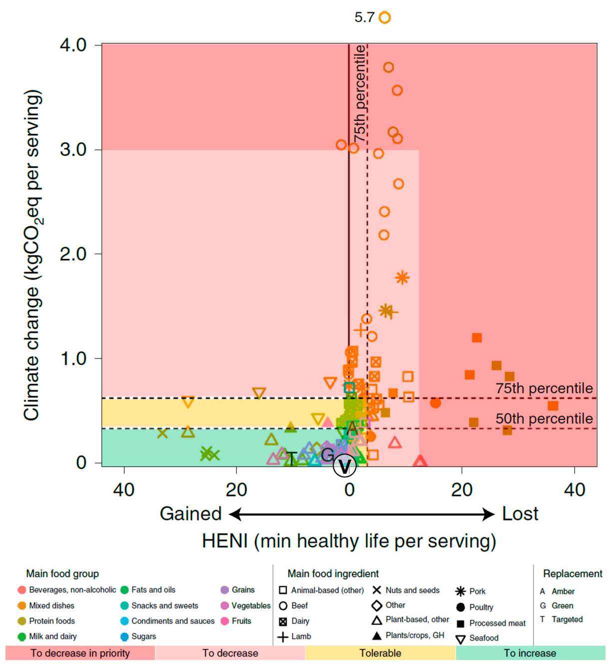

FIGURE 2
Environmental vs. nutritional impacts for 167 foods representative for the US diet. Nutritional impact of mostly consumed foods (Health Nutrition Index = HENI, $N = 168$) in the US diet as a function of their global warming impacts per serving. Adapted from (34). The circled V denotes average of vegetable oils to the other letters representing consumption-weighted food mixes of the amber-tolerable (A) and green—to increase (G) zones, and a targeted mix of the most nutritionally beneficial foods from both zones (T).

On the whole, nutrition and environmental impacts have been put together in the DALY metric (33, 34), bringing the GBD population-oriented study to the food item level. By analyzing the composition of 5,800 food items in the frame of fifteen GBD risk factors, a Health Nutrition Index (HENI) was produced expressing the impacts of various foods in marginal minutes of life lost or gained within the context of the entire diet. For example, one hot dog leads to 36 min of life lost, whereas a serving of nuts provides 25 additional minutes of healthy life. In comparison, vegetable oils are close to neutral together with grains, fruits and vegetables, between 1 min gained and 2 min lost per serving size (Figure 2).

Comparing the HENI scores with eighteen different environmental metrics that include carbon footprint, water use and air pollution-induced health damages enables us to analyze trade-offs between dietary and environmental performances. Figure 2 shows that, as estimated by the GBD ranking, vegetable oils (identified by V) relative to other foods, both per serving and per kcal, are placed at the low end of carbon and land use while being close to neutrality for dietary impacts on health, together with grains, vegetables and fruits (Figure 2). The Results also suggest that the consumption of beef, pork and lamb meats should be reduced in order to limit the carbon footprint and that processed meat and sweetened sugar beverages should be reduced with high priority from a dietary perspective. What the HENI scores cannot address yet is the relative impacts of different vegetable oils but this should become possible in the near-future.

This modern approach follows the present development of analytical systems that, based on the machine learning approach (artificial intelligence), allow for the extraction of the main effective factors from background noise. For consumers, the environmental impact of food processing and cooking as well as the incidence of these processes on the nutritional quality and nutrient availability should be defined. For vegetable oils more detailed data and information are needed to improve knowledge on the role of individual fatty acids and their combinations and interactions, since current studies often focus on a single dietary factor at a time. Such approaches as survival random forest to large databases containing both dietary intakes of individuals (e.g., European Prospective Investigation into Cancer and Nutrition-EPIC or National Health and Nutrition Examination Survey-NHANES database) and their mortality and morbidity status (e.g., the US National Death Index, NDI) should be employed to address the holistic issue of vegetable oils from crops to consumers with the transversal effects on socio-economical impact, even though it is warranted that data collection occurs over longer time periods (35, 37).

Next frontiers for innovation and nutrition

Geography originally determined which fats and oils were included in the diet: butter, lard and beef tallow in Northern Europe, North and South America, contrasting with olive, sesame, sunflower seed and soybeans oils in Southern Europe and the rest of the world (35). Unsaturated fatty acid can be converted to a saturated fatty acid by bubbling hydrogen through a heated vegetable oil in a closed vessel. This discovery of hydrogenated vegetable oils, which makes them more solid and spreadable, started an ongoing debate about which fats and oils are "healthy" and which fatty acids are "sustainable". Adding complexity to the debate on fatty acids is the consideration that fats and oils come from both animal and plant sources.

Previous sections emphasized how data collection is a crucial issue of the path connecting nutrition with health and sustainability within an holistic, complex and multidisciplinary perspective capable to rigorously assess vegetable oils as well as other major dietary components in terms of their impact on health and on such societal and environmental aspects as land allocation to different oil crops and cultivars as well as the impact on the quality of life of local populations and biodiversity preservation. Despite many attempts to homogenize databases, existing data are scarce, heterogeneous and often unreliable (36). Not all the countries (indeed a small fraction of them) provide at least partial food composition tables for their food products and most of them are derived from the analysis of a limited number of samples, a further confounder being the analysis time frame (7). The fat issue needs a fast update, in the frame of complex systems-based views aimed to emphasize novel evidence-based dietary patterns matching sustainability with the goal to overcome the present energetic crisis. To make effective dietary recommendations in order to change food composition and dietary choices, the cultural heritage must also be taken into account, as well as the stability of dietary habits of the same type of food in each country.

Conclusion

Fats play an important role in the transition to sustainable diets as they are a concentrated energy source that will be needed to future food security (37). Each plant oil can be accessed for its nutritional attributes and ability to be sustainably produced. To accomplish this goal, experts in nutrition, health, agronomy, food production, economy, sociology and governments need to work together with the common goal of feeding the world sustainably in the next future.

There are still knowledge gaps in the concept of sustainable nutrition and how consumers can be provided with metrics that they can easily use for their dietary decision making. Vegetable oils show all the steps of the complex chain connecting agriculture to health across molecular to societal and global environmental scales, including issues such as soil exploitation, climate change impact, role of environmental stressors of the food production processes, health impacts of different foods and the cultural context of nutrition. High resolution data should focus on where crops are grown, needs for fertilizers, environmental impacts and local benefits as indicators of social and economic development. Accordingly collective and global efforts are warranted not only by the scientific community but also by governments, food industry and global health systems in order to build the scientific basis for the introduction of a new paradigm beyond classic dietary patterns, in the frame of a wider holistic approach to individual and global health.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

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The remaining 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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Valorization of spent barley grains: isolation of protein and fibers for starch-free noodles and its effect on glycemic response in healthy individuals

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Spent barley grains (SBG) were valorized into a spent barley protein and fibers (SBPF) ingredient. The ingredient was utilized to formulate SBPF-derived starchless noodles with a negligible glycemic response (GR) in healthy individuals, a significant reduction of 93.16% (SD = 8.07) postprandial GR after consumption when compared to conventional starch-based noodles. Their nutritional content, visual analog scale, textural property, and palatability were also evaluated. The SBPF-derived starchless noodles showed comparable hardness and springiness to the conventional starch-based noodles, but their cohesiveness and chewiness were improved. There was no significant difference in appetite and hunger ratings between the two types of foods. The overall palatability ratings for both foods were comparable. The SBPF-derived noodles were a source of nutrients (such as protein and fibers). This study has considerable potential for the development of functional food and food as medicine industries.

KEYWORDS

spent barley protein and fibers (SBPF), valorization, functional foods, food as medicine, circular economy

1. Introduction

The global barley production is 147.05 × 10^6 tons in the 2021/2022 crop year, reducing from around 160.53 × 10^6 tons in 2020/2021 (Shahbandeh, 2022). Meanwhile, the pandemic and geopolitical tensions seriously impact the safety of the food supply chains (Aday and Aday, 2020; Jagtap et al., 2022). Thus, there is an urgent need to ensure food sustainability and security. Pristine barley grains provide fermentable sugars to yeasts during beer brewing (Kok et al., 2019), and 20 kg SBG is generated per 100 L beer production (Mussatto et al., 2006). The global beer production was 1.91 × 10^{11} L in 2022 (Chee, 2022), which was equivalent to 38.2 × 10^6 tons SBG. The SBG contains water insoluble protein, husk residues, pericarp, and seed coat within the grains (Townsley, 1979). The barley grain has been approved as a functional food by the U.S. Food and Drug Administration since 2006, and regular barley consumption reduces blood cholesterol and controls cardiovascular diseases (Geng et al., 2022). The β-Glucan, arabinoxylans (AX), and phenolic compounds within the barley significantly improve human immunity, and provide reliefs for type 2 diabetes, stroke, hypertension, and cardiovascular diseases (Maheshwari et al., 2019; Tosh and Bordenave, 2020; Zannini et al., 2022).

The SBG is made of 70% fibers, 20% protein, and 10% fats (Reshamie et al., 2018). The SBG fibers include approximately 17% cellulose, 28% non-cellulosic polysaccharides, mainly AX, β-Glucan, and 28% lignin (a poly-phenolic macromolecule) (Mussatto et al., 2006). The SBG protein contains essential and non-essential amino acids including phenylalanine, lysine, tryptophan, histidine, methionine, alanine, glycine, proline, and serine (Mussatto et al., 2006). Cookies made of 20% SBG showed increments of protein, lysine, and fibers by 55, 90, and 220%, respectively (Özvural et al., 2009). The SBG lowers the sweetness and increases the shelf-life of sourdough and bread (Plessas et al., 2007; Stojceska and Ainsworth, 2008; Szwajgier et al., 2010; Waters et al., 2012). The phenolic extracts from the SBG used in fruit beverages show better ferric reducing antioxidant power (FRAP) activity (Celus et al., 2007). The SBG is nutritious and not a food waste. There is a possibility for SBG valorization to meet the nutritional needs of the aging population.

A practical method to produce the SBPF ingredient is developed and reported in this article (Figure 1). The SBPF ingredient is exploited to create a brand new representative functional food in noodle form (SBPF-derived noodles). The appearance, texture, and GR of the SBPF-derived noodles are investigated and compared with the property of conventional starch-based noodles. The SBPF-derived noodles have the potential to replace conventional starch-based noodles as a protein and fibers rich Asian staple food with negligible GR. The SBPF-derived noodles address the perennial challenge faced by diabetics. The starchless noodles can be palatably and tastily prepared without blood glucose elevation.

2. Materials and methods

2.1. SBG valorization, SBPF ingredient, and SBPF-derived noodles preparation

The dry SBG was obtained from a local malt production site, and placed in an electric superfine powder machine (Horus Industry, China) and ground continuously into powder with a stainless-steel blade at 6,000 rpm. The SBPF ingredient was obtained in powder form within 3 h. Subsequently, the SBPF ingredient was mixed with vital wheat gluten, konjac powder, premium quality fine salt, sodium carbonate, and water to form a paste. The paste was directly extruded by a stainless-steel manual noodle maker (Sailnovo, Malaysia) into water to form the SBPF-derived noodles. The noodles were pasteurized and vacuum packed and could be served directly without cooking. All the abovementioned items were food-grade and purchased from Phoon Huat Pte Ltd, Singapore. The control of this study was starch-based noodles (ready to eat) procured from a local vendor (Hokkien noodle round, FORTUNE brand, Singapore). The frozen vegetables were purchased from Watties, New Zealand. Sesame oil was from Pagoda, Singapore, and the light soy sauce was acquired from Tai Hua, Singapore.

2.2. Nutritional content analysis and physical tests

2.2.1. Nutritional content analysis

The SBPF ingredient and SBPF-derived noodles were sent to the Setsco lab (Setsco Services Pte Ltd) for nutritional content analysis. Nutritive values including energy, protein, dietary fibers, carbohydrates, fat, moisture, and ash of the samples were determined according to BCTD/FC/IHM068/2018 Rev(2), BCTD/FC/IHM123/2013 Rev(1), AOAC official method No. 985.29 (2005), BCTD/FC/IHM068/2018 Rev(2), AOAC official method No. 996.06 (2008), AOAC official method No. 950.46 (2008) and AOAC official method No. 920.153 (2005), 930.30 (2005), and 923.03 (2005), respectively. The nutritional information for starch-based noodles, vegetables, sesame oil, and soy sauce was acquired from their respective product nutritional information panels.

2.2.2. Characterization of SBPF-derived noodles and starch-based noodles

A texture analyzer (TA/TX-plus, Stable Micro System, Surrey, UK) with a 5 kg load cell was employed to perform the texture measurements. Hardness (g), springiness (mm), cohesiveness, and chewiness (g × mm) were analyzed respectively. Texture profile analysis was performed and a P35 cylinder probe was used (with pre-test, test, and post-test speeds at 2, 1, and 2 mm/s, respectively). The target mode was set at 70% strain, the trigger type was auto, and the trigger force was 5 g. The measurements were conducted fifteen times.

2.3. *In vivo* GR studies

The study aimed to compare the GR of each participant after consuming the SBPF-derived and conventional starch-based noodles, respectively. The *in vivo* studies followed previous publications regarding GR tests (Jang et al., 2017; Crummett and Grosso, 2022) and were performed using the Singapore Accreditation Council's (SAC) FFT-2010-0001A Functional Food Testing Scheme, based on ISO/IEC 17025:2017 and Technical Note FFT 01. Ethical approval for GR testing was sought from an independent ethics committee before conducting the GR tests. The ethical approval reference number for this study was TP-IRB Ref: IRB170102. The details of the GR testing protocol were explained to the subjects and informed consent was obtained. Fifteen (15) healthy volunteers (19–60 years old) were recruited into the study based on the following inclusion and exclusion criteria in Supplementary Table 1. The two types of test foods were assessed separately in each subject on separate days (with a washout period of at least 2 days).

The 100 g SBPF-derived and starch-based noodles were prepared in the same manner. Briefly, both were warmed up in 500 ml of hot water (approximately 90°C) in <1 min. Then, they were mixed with 50 g cooked vegetables, 6 g sesame oil, and 6 g light soy sauce for GR tests (Table 1). The food was served with 250 ml water to the test subjects. The subject's capillary fasting samples

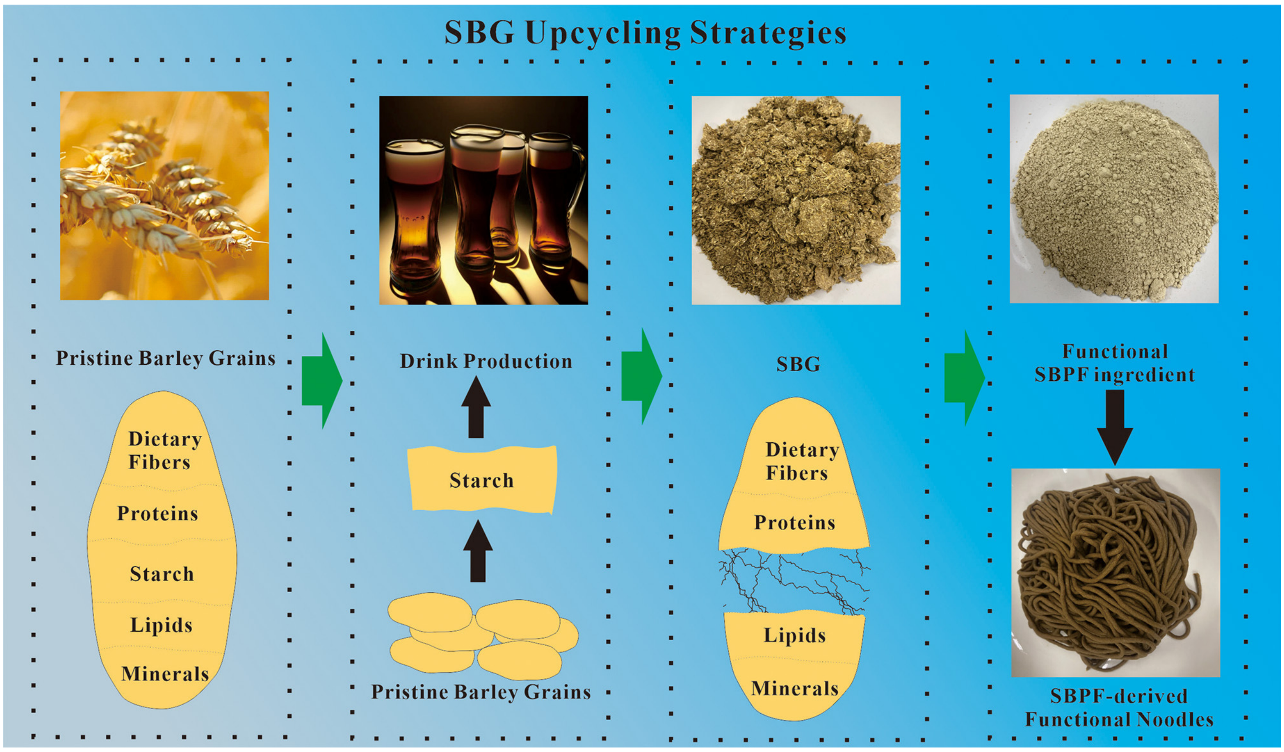

FIGURE 1
Flowchart of the collection of SBG, extraction and purification of SBPF ingredient and the representative SBPF-derived noodle production.

were obtained by a finger prick after ensuring an overnight fast of 10–14 h. They consumed the test food within 12 min and remained seated. Their blood samples were obtained at 15, 30, 45, 60, 90, and 120 min after food consumption. The participants' capillary blood samples were analyzed by calibrated YSI 2300 Stat Plus Glucose and Lactate analyzer. Analysis of test data was conducted by evaluating incremental areas under the curve (AUC) for the two samples and plotted in a graph. The equation used to determine the GR difference between starch-based and SBPF-derived noodles is:

$$\textit{The GR difference}\ (\%) = \left(1 - \frac{AUC_{SBPF-derived\ noodles}}{AUC_{starch-based\ noodles}}\right) \times 100$$

2.4. Overall palatability rating

The overall palatability ratings of the starch-based and SBPF-derived noodles were analyzed using the 7-point hedonic scale (Supplementary Figure 1).

2.5. Visual analog scale

A visual analog scale (VAS) questionnaire was provided to the participants. Appetite and other sensations were assessed using 100 mm VAS (Supplementary Figure 2). Eight variables were questioned. On each 100 mm line, an appetite (hunger, satisfaction, fullness, and desire to eat) sensation was paired with the opposing sensation, (for example, "hungry" and "not hungry" or "full" and "not full"). To determine "prospective consumption," the participants were asked questions including "How much do you think you can eat?" and the analog scales were administered at each time point when blood samples were obtained, namely fasting, 15, 30, 45, 60, 90, and 120 min after the consumption of the test foods. The individual response was measured (in mm) and then the average value ± SD was reported.

2.6. Statistical analysis

The statistical significance between data sets was calculated using Student's *t*-test, and $p < 0.05$ is considered statistically significant. All the tests were conducted at least in triplicate.

3. Results

3.1. Nutritive value of SBPF ingredient and SBPF-derived noodles

Spent barley protein and fibers ingredient yielded energy at 280 kcal per 100 g, and contained 29.1 g protein, 45.9 g fibers, 6.6 g carbohydrates, and 15.3 g total fats (8.2% of the total fats were polyunsaturated fats). Meanwhile, 100 g SBPF-derived noodles provided 32 kcal energy, 4 g protein, 6.6 g fibers, 0.85 g carbohydrates, and 1.42 g fats. The 100 g conventional starch-based noodles provided 180 kcal energy, 5.3 g protein, 1.1 g fibers, 1.6 g

TABLE 1 Nutritive values of SBPF ingredient and SBPF-derived noodles, results are expressed as mean.

	Starch-based noodles (100 g)	SBPF-derived noodles (100 g)	Vegetables (50 g)	Oil (6 g)	Soy sauce (6 g)
Energy (kcal)	180	32.0	26.9	11.7	0.8
Protein (g)	5.3	4.0	1.4	0	0.4
Dietary fiber (g)	1.1	6.6	1.8	0	0
Carbohydrates (g)	35.9	0.9	4.0	0	0.4
Fat (g)	1.6	1.4	0.2	6.0	0

TABLE 2 Textural analysis of starch-based and SBPF-derived noodles: hardness (g), springiness (mm), cohesiveness, chewiness (g × mm), and average palatability ratings; results are expressed as mean ± standard deviation.

	Starch-based noodles	SBPF-derived noodles	*p* value
Hardness (g)	1,714 ± 392.90	1,745.05 ± 361.28	0.824
Springiness (mm)	0.90 ± 0.06	1.42 ± 1.00	0.065
Cohesiveness*	0.50 ± 0.03	0.54 ± 0.02	0.002
Chewiness (g × mm)*	773.18 ± 207.08	1,248.80 ± 720.64	0.026
Palatability	5.55 ± 0.85	4.95 ± 1.49	0.347

*$p < 0.05$.

fat, and 35.9 g carbohydrates (Table 1). The nutritive values of 50 g vegetables, 6 g sesame oil, and 6 g soy sauce were included in the table. The amounts of energy and macronutrients contained in the test meals can be calculated.

3.2. Textural parameters of starch-based and SBPF-derived noodles

The textural properties of starch-based and SBPF-derived noodles could be seen in Table 2. There was no significant difference in hardness ($p = 0.824$) and springiness ($p = 0.065$) of the two representative foods. Interestingly, significant differences were observed in cohesiveness ($p = 0.0024$) and chewiness ($p = 0.026$) measurements.

3.3. GR studies

The average age of the 15 healthy participants (9 women and 6 men) was 40 years old (SD = 13.74). Their average BMI was 21.1 kg/m^2 (SD = 2.17). The average 120 min GR responses of the participants for equal volumes of the two test foods were shown in Figure 2A. The fasting blood glucose concentration of each participant before food testing was evaluated as a control. The consumption of the starch-based noodles increased the mean blood glucose concentration from 4.09 mM (SD = 0.42, baseline level) to 6.11 ± 0.54 mM (48.8% higher than the baseline value) within the first 30 min. In addition, 2 h later, the blood glucose concentration gradually declined to 4.91 mM (SD = 0.91, 20% higher than the fasting baseline value). On the contrary, intake of SBPF-derived noodles did not increase blood glucose level significantly, the value was constant for 4.15 mM (SD = 0.32) for the first 30 min, and then at 4.06 mM (SD = 0.29) after 2 h. Moreover, the starch-based noodles consumption brought significant blood glucose fluctuations of the test subjects at 15, 30, 45, 60, 90, and 120 min after food consumption, when compared with the values at the same time after SBPF-derived noodles consumption (Figure 2B). It worth mentioning that the intake of SBPF-derived foods did not cause a significant increase in blood glucose values within 120 min. The p values are 0.18, 4.6×10^{-4}, 5.06×10^{-11}, 7.2×10^{-10}, 4.74×10^{-8}, 4.4×10^{-7}, and 9.7×10^{-4} for blood glucose levels at 0, 15, 30, 45, 60, 90, and 120 min, respectively, after the test subjects consumed starch-based and SBPF-derived noodles. Meanwhile, the p values of blood glucose fluctuations are 1.06×10^{-5}, 9.89×10^{-14}, 1.37×10^{-11}, 1.16×10^{-12}, 1.34×10^{-10}, and 1.29×10^{-5} at 15, 30, 45, 60, 90, and 120 min, respectively.

The AUC calculation of individual participants was shown in Figure 2C box plot, the AUC spread in the participants consuming SBPF-derived noodles was narrower than that of the starch-based noodles ($p = 3.46 \times 10^{-9}$), and one unusual case (outlier) could be found in the participants consuming SBPF-derived noodles. The AUC values between consumptions of starch-based noodles and SBPF-derived noodles showed no intersection. Furthermore, the individual GR difference was plotted in the Box and Whisker graph in Figure 2D, the average GR difference was 93.16% (SD = 8.06). In addition, the upper extreme, upper quartile, median, lower quartile and lower extreme were 99.61, 98.82, 95.81, 90.15, and 84.64%, respectively. It was noticeable that two participants were having unusual GR differences (two outliers). There were 12 out of 15 (80%) participants in the interquartile range (IQR) of the GR difference Box and Whisker plot.

3.4. Overall palatability rating and visual analog scales

The overall palatability ratings for both foods were comparable (Table 2), and the average palatability ratings were 5.54 (SD = 0.85) and 4.95 (SD = 1.49) for starch-based noodles and SBPF-derived noodles ($p = 0.347$) individually. The average fasting, 60- and 120-min VAS ratings for four appetite variables of the starch-based, and SBPF-derived noodles were shown in Table 3. The participants

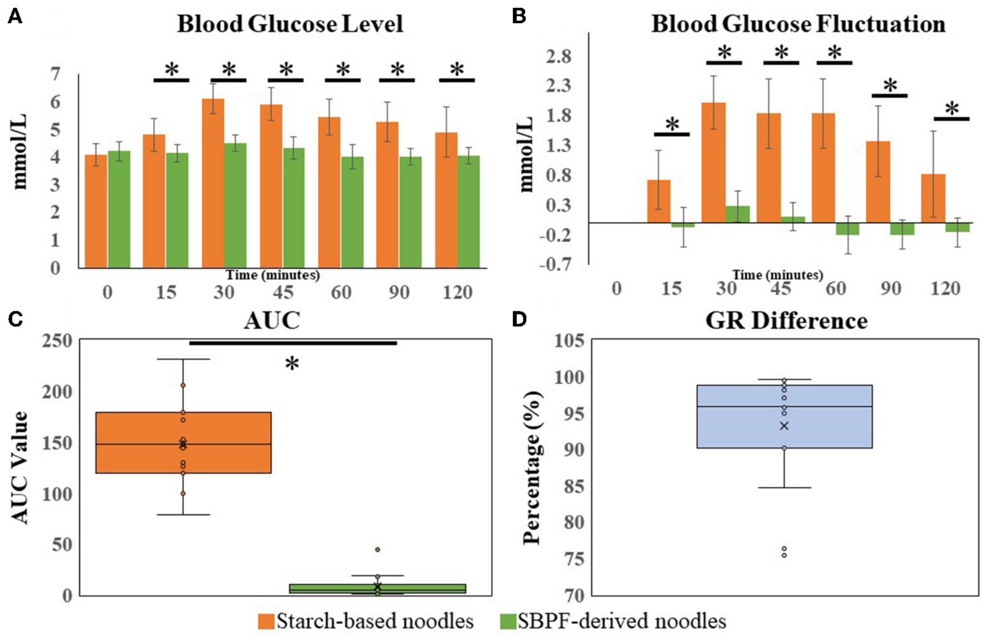

FIGURE 2
The average blood glucose concentrations **(A)** and fluctuations of blood glucose concentrations **(B)** before and after consuming the starch-based (orange) and SBPF-derived (green) noodles; Box and Whisker plots of the participants' AUC values after consuming starch-based noodles (orange) in contrast to the values of SBPF-derived noodles (green) within 2 h **(C)**; and GR difference within the participants after consumption of starch-based and SBPF-derived noodles in 2 h **(D)**, $^{*}P < 0.05$.

preferred to eat more SBPF-derived noodles immediately after fasting. There was no significant difference in appetite and hunger ratings between the two types of foods at 60 and 120 min after consumption in all subjects.

4. Discussion

The SBPF ingredient inherits the functional components from the pristine barley grain except for the starch (as illustrated in Figure 1 and Table 1). The addition of the SBPF ingredient not only increases the food's protein and dietary fiber content but also lowers the overall content of carbohydrates vs. starch-based foods (Townsley, 1979; Mussatto et al., 2006; Stojceska and Ainsworth, 2008). Our team utilized the SBPF ingredient to form the SBPF-derived noodles and evaluated its nutritional content, textural parameters, GR, VAS, and overall palatability rating. The palatability, texture, and taste aspects especially make it an attractive alternative staple for diabetics due to the significant GR reduction (93.16% less GR, SD = 8.06). Meanwhile, AX, β-glucan, and lignin are three major functional dietary fibers within SBG and the SBPF ingredient. The AX enters the large intestine and is fermented by colonic microflora to show prebiotic activity (Lu et al., 2000), and it increases bulk viscosity, slows gastric emptying and gastrointestinal motility, the blood glucose and insulin responses are subsequently delayed (Lu et al., 2000). The β-glucan reduces cholesterol and sugar levels in the blood upon consumption (Geng et al., 2022). The lignin prolongs the survival of bifidobacterial versus glucose as a substrate (Niemi et al., 2013). Moreover, the SBPF ingredient provides a rich combination of protein and minerals including hordeins, glutelins, globulins, albumins, phosphorus, calcium, and magnesium (Celus et al., 2006; Waters et al., 2012).

The textural analysis has been performed to evaluate the samples (Table 2), and the tensile test is used to elongate the sample and test the force required to break the sample. The texture profile analysis employs a double compression test to mimic the chewing of food. There is no significant difference between the data on hardness and springiness. Hardness describes the necessary forces to achieve a given deformation, the participants use a comparable force to break and crush the noodles during eating. The springiness measurements indicate the two representative noodles have comparable rates to return to their respective undeformed condition after force removal. The SBPF-derived noodles show better cohesiveness and chewiness, which is probably caused by the addition of dietary fibers, vital wheat gluten, and konjac powder (Lin and Huang, 2008; Barak et al., 2014; Liu et al., 2021).

In vivo GR study was conducted using starch-based noodles as control food (not any other control food like glucose or white bread). It is practical to compare the GR of SBPF-derived functional noodles with the starch-based noodles, and it will be helpful to ascertain that the SBPF-derived functional noodles have lower GR and will be an ideal choice for health-conscious consumers who wish to consume noodles without any blood glucose spike. In Figure 2A, the blood glucose level spikes from 4.09 mM (SD = 0.42, fasting baseline) to 6.11 mM (SD = 0.54) within 30 min upon intake of the starch-based food, then gradually reduces to 4.91 mM (SD = 0.91) within 120 min. The consumption of SBPF-derived noodles flattens the blood glucose curve without any spike. Furthermore, the fluctuations in blood glucose levels are illustrated in Figure 2B. Consumption of the starch-based noodles boosts the blood glucose level by 2 mM from its basal level within 30 min

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TABLE 3 Average VAS ratings (in mm) at fasting, 60 min, and 120 min in 15 participants; results are expressed as mean ± standard deviation.

Variable(s)	Fasting		60 min		120 min	
How hungry do you feel?						
Starch-based noodles	54.1 ± 29.9	$p = 0.58$	30.7 ± 23.6	$p = 0.17$	37.7 ± 33.4	$p = 0.42$
SBPF-derived noodles	51.1 ± 39.2		24.2 ± 16.3		39.3 ± 28.2	
How satisfied do you feel?						
Starch-based noodles	27.2 ± 17.9	$p = 0.21$	70.2 ± 20.4	$p = 0.29$	58.1 ± 30.2	$p = 0.48$
SBPF-derived noodles	21.6 ± 15.9		62.4 ± 24.7		53.4 ± 30.7	
How full do you feel?						
Starch-based noodles	24.9 ± 19.7	$p = 0.27$	72.9 ± 18.2	$p = 0.16$	60.5 ± 30.0	$p = 0.33$
SBPF-derived noodles	19.6 ± 19.5		61.5 ± 25.3		51.1 ± 31.1	
How much do you think you can eat?						
Starch-based noodles	54.5 ± 27.5*	$p = 0.01$	35.5 ± 20.8	$p = 0.29$	47.7 ± 26.9	$p = 0.46$
SBPF-derived noodles	72.5 ± 21.1*		32.0 ± 17.9		49.0 ± 25.3	

*$p < 0.05$.

and the value remains peaked for another 30 min. However, the blood glucose fluctuations upon intake of SBPF-derived noodles are inconspicuous, and negligible when compared with the blood sugar levels after starch-based noodle consumption. The data from AUC and GR difference in Figures 2C, D is in line with the blood glucose analysis. The average AUC is 150.96 (SD = 38.81) for participants after consuming starch-based food, but 5.48 (SD = 4.98) for the same participants consuming SBPF-derived noodles. The participants show negligible GR after consumption of SBPF-derived noodles (narrow range of AUC Box and Whisker plot), while intake of starch-based noodles cause significant GR variations (long range of AUC Box and Whisker plot). Moreover, the GR difference value indicates 93.16% (SD = 8.06) GR reduction upon SBPF-derived noodles consumption and 12 out of 15 (80%) participants in the IQR of the GR difference Box and Whisker plot. The data concentrates on the IQR area indicating less variability and high repeatability of negligible GR for the participants after consuming SBPF-derived noodles. There are two outliers in the GR difference box plot, they might come from the personal physical variations, as we can exclude the data entry, measurement errors, sampling problems, and other unusual conditions. The SBPF-derived and the conventional starch-based noodles have comparable palatability (Table 2), and they have comparable VAS (Table 3). Interestingly, the participants consider eating more SBPF-derived noodles when they are fasting, and the starchless noodles provide less energy and carbohydrates with a larger volume of dietary fibers. Moreover, the participants indicate comparable fullness upon finishing both types of noodles in 60 and 120 min, respectively. Considering the two test foods show comparable values of sensory evaluations, and texture properties, the SBPF-derived noodles can be a green and functional replacement for conventional starch-based noodles. All the data endorses the effects of SBPF-derived noodles on controlling blood glucose (negligible GR). The SBPF-derived noodles provide nutrients and have enormous potential in empowering blood sugar control for people with diabetes, metabolic and body weight concerns, without the need for overbearing constraints in food intake. The inclusion of SBPF ingredient at high levels (>20%) is bound to have a greater positive impact on the nutritional levels of the end products but comes with costs, such as impacting the final product's texture, volume, color, and thus, sensorial characteristics and ultimate consumer acceptance (Lynch et al., 2016). The SBPF functional ingredient is herein utilized to produce functional noodles with negligible GR, and it is an ideal candidate for functional food preparation and health promotion. There is still technical progress that can be achieved to ensure and even improve the quality of the products. For example, the productivity of the SBPF functional ingredient can be improved with new milling methods and the consistency of nutritional content from various SBG batches must be ensured. Our team is constantly working on quality of the SBPF ingredient related foods, and major improvements could be reported in the future.

5. Conclusion

The SBG protein and fibers are valorized into SBPF functional ingredient, which can be applied to the preparation of functional noodles/foods with negligible GR. The SBPF-derived functional noodles show optimistic consumer acceptance and provide fiber and protein rich nutrition. The SBPF ingredient and SBPF-derived noodles and foods are especially suitable for consumers with blood sugar and body weight concerns, and potentially attractive alternative staple foods for people with diabetes. Our food waste upcycling strategies may resolve food sustainability and security concerns, and provide a source of nutrients to address nutritional needs without further planetary damage.

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 Singapore Accreditation Council's (SAC) FFT-2010-0001A Functional Food Testing Scheme, based on ISO/IEC 17025:2017 and Technical Note FFT 01. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PS: conceptualization, methodology, validation, formal analysis, writing—original draft, writing—review and editing, and visualization. RN: sample preparation, investigation, and writing—original draft. PV: characterization of SBPF-derived noodles and starch-based noodles. SL: writing—review and editing. KB: conceptualization, methodology, validation, formal analysis, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

PS and RN were employed by Kosmode Health Singapore Pte. Ltd.

The remaining 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/fsufs.2023.1146614/full#supplementary-material

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Plant to animal protein ratio in the diet: nutrient adequacy, long-term health and environmental pressure

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Background: Animal and plant protein sources have contrasting relationships with nutrient adequacy and long-term health, and their adequate ratio is highly debated.

Objective: We aimed to explore how the percentage of plant protein in the diet (%PP) relates to nutrient adequacy and long-term health but also to environmental pressures, to determine the adequate and potentially optimal %PP values.

Methods: Observed diets were extracted from the dietary intakes of French adults (INCA3, n=1,125). Using reference values for nutrients and disease burden risks for foods, we modeled diets with graded %PP values that simultaneously ensure nutrient adequacy, minimize long-term health risks and preserve at best dietary habits. This multi-criteria diet optimization was conducted in a hierarchical manner, giving priority to long-term health over diet proximity, under the constraints of ensuring nutrient adequacy and food cultural acceptability. We explored the tensions between objectives and identified the most critical nutrients and influential constraints by sensitivity analysis. Finally, environmental pressures related to the modeled diets were estimated using the AGRIBALYSE database.

Results: We find that nutrient-adequate diets must fall within the ~15–80% %PP range, a slightly wider range being nevertheless identifiable by waiving the food acceptability constraints. Fully healthy diets, also achieving the minimum-risk exposure levels for both unhealthy and healthy foods, must fall within the 25–70% %PP range. All of these healthy diets were very distant from current typical diet. Those with higher %PP had lower environmental impacts, notably on climate change and land use, while being as far from current diet.

Conclusion: There is no single optimal %PP value when considering only nutrition and health, but high %PP diets are more sustainable. For %PP>80%, nutrient fortification/supplementation and/or new foods are required.

KEYWORDS

healthy dietary patterns, nutrient adequacy, environmental footprints, diet optimization, plant-based diets

Introduction

Historical and current nutritional transitions are coupled with changes in the relative contribution of dietary animal and plant proteins. This has been studied from hunter-gatherers to post-agricultural societies (1), from traditional diets that enabled thriving civilizations to diets in post-industrialized countries (2, 3), and more recently, in Western countries with the emerging trend toward more plant-predominant diets.

Changes in plant and animal protein intake raise classic nutritional questions. One in particular concerns the possible risk of some nutrient shortage with diets too low in animal protein, since animal protein foods contribute significantly to the intake of indispensable nutrients like iron, calcium and vitamin B12, whose overt deficiencies have various adverse health consequences (such as anemia and higher risk of osteopotosis) (4, 5). However, plant proteins are also important for the intake of fiber and some indispensable nutrients (like vitamins B9 and C), which modulate short and long-term disease risk, and are also lower in saturated fats that are excessively consumed (6, 7). Beyond the relationship to nutrient adequacy, animal/plant proteins and their packages largely affect the metabolome and the microbiota and physiological functions that are crucial for long-term health (6, 8–10). Accordingly, there have been many contrasting associations reported recently between plant and animal protein intake and mortality, especially regarding cardiovascular diseases (6, 11, 12).

More globally, plant (such as legumes, nuts and whole grains) and animal (such as red and processed meats) protein sources have heterogeneous relationships to nutrient adequacy (13) and to long-term health regarding cardiovascular diseases (9, 14–16) and cancers (17, 18). There is indeed a challenge for food-based dietary guidelines to point out what proportions of plant and animal protein foods should be recommended (19, 20). However, the plant to animal protein ratio remains a poor, summarizing descriptor of dietary patterns, since two diets with the same plant to animal protein ratio can actually be very different (9). There is thus a need to analyze the overall proportion of plant protein in the diet in view of the related dietary profiles and their nutritional adequacy and healthiness.

Furthermore, current interest in the proportions of plant and animal proteins in the diet also stems from their differential association with environmental pressures, in particular greenhouse gas emissions (GHGe) and land use (21–24). Altogether, the plant to animal protein ratio in the diet appears central to the sustainability of the food systems (21, 25). This has implications for dietary guidelines that aim to encompass both human and planetary health (26–28).

Thus, the literature lacks an analysis of what proportion of plant protein in the diet (%PP, the percentage of plant protein in total protein intake) is adequate and, even further, what proportion is optimal from a unified nutrition and health perspective that also considers the impact on other aspects of sustainability. We hypothesized that %PP could be safely increased well beyond its current low level, but will certainly be limited by a too-low level of animal protein. We also hypothesized that, rather than an optimal value, there may be a relatively wide range of %PP values that would be similarly adequate, when considering only human nutrition and health. Here, using advanced diet modeling and optimization, we studied whether an optimal %PP value can be identified when taking into account the reference values for nutrients and the disease burden risks for food categories. We characterized modeled diets that departed as little as possible from prevailing diets at all levels of adequate %PP values for nutrient adequacy and long-term health to identify nutritional issues (i.e., limiting nutrients) and dietary levers (i.e., effective foods). We furthermore estimated the environmental pressures associated with modeled diets along the whole range of adequate %PP values.

Materials and methods

Input of dietary data

The data used for this study were extracted from the French Individual and National Study on Food Consumption Survey 3 (INCA3) conducted in 2014–2015. The INCA3 survey is a representative cross-sectional survey of the French population; its method and design have been fully described elsewhere (29). Males aged 18–64 years ($n = 564$) and pre-menopausal females aged 18–54 years ($n = 561$), not identified as under-reporters, were included in the present study; the final sample contained 1,125 adults (Supplementary Figure S1).

Dietary data were collected by professional investigators assisted by a standardized and validated dietary software (GloboDiet) from three unplanned, non-consecutive, 24 h dietary recalls spread over a three-week period (two weekdays and one weekend day). Portion sizes were estimated using validated photographs (29), and the nutrient contents of different food items came from the 2016 food composition database operated by the French Information Centre on Food Quality (CIQUAL) (30). Mixed foods were broken down into ingredients and then gathered into 45 food groups (Supplementary Table S1). For each sex, the nutrient content of each food group was calculated as the mean nutrient content of food items constituting the food group weighted by their mean intake by the sex considered, as previously described (31). All dietary data (food group consumption and nutrient content) relate to the total population of each sex (including non-consumers).

Multi-criteria diet optimization under constraints

Using multi-criteria optimization, we identified modeled diets (i.e., modeled consumptions of the 45 food groups) with a minimal long-term health risk and a minimal departure from the observed diet (taking into account cultural acceptability and inertia), under constraints that would ensure adequate nutrient intakes and remain within current consumption limits. In this context, we investigated the role of %PP to identify its adequate range of variations and to

Abbreviations: ANSES, French Agency for Food, Environmental and Occupational Health and Safety; CIQUAL, French Information Centre on Food Quality; DALYs, Disability-adjusted life-years; *DD*, Diet departure criterion; GBD, Global Burden of Diseases; GHGe, greenhouse gas emissions; *HR*, Health risk criterion; INCA3, Third Individual and National Study on Food Consumption French Survey; %PP, Percentage of plant protein in the diet; TMREL, Theoretical minimum risk exposure level.

characterize the dietary, nutritional and environmental consequences of these variations.

This non-linear optimization problem was performed using the NLP solver of the OPTMODEL procedure of SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Optimization was implemented at the population level but in males and females, separately. The optimized diets of males and females were then averaged to derive optimized diets for the adult population.

Objectives

The main optimization objective was to minimize the long-term health risk of the modeled diet, as assessed by the Health Risk (*HR*) criterion. The *HR* criterion was set to target the dietary recommendations from the Global Burden of Diseases (GBD) based on epidemiological studies about the associations between consumption of different food groups and risk of chronic diseases (32). The *HR* criterion thus aimed to limit the consumption of three unhealthy food groups or categories (red meat, processed meat and sweetened beverages), while promoting that of six healthy food groups or categories (whole grain products, fruits, vegetables, legumes, nuts and seeds, and milk) until their minimum risk exposure levels (TMREL) were reached. According to the most recent (2019) estimates from the GBD, TMREL values were 0 g/d for red meat, processed meat and sweetened beverages, and 150, 325, 300, 95, 14.5 and 430 g/d, respectively, for whole grain products, fruits, vegetables, legumes, nuts and seeds, and milk (32). In our study, the *HR* criterion was thus expressed and minimized as:

$$\min HR = \sum_{i=1}^{3}\left(\frac{\mathrm{Opt}(i)}{\mathrm{Max}(i)} \times \frac{\mathrm{DALYs}(i)}{\mathrm{DALYs}(\mathrm{all})}\right) + \sum_{j=1}^{6}\left(\max\left[\frac{\mathrm{TMREL}(j)-\mathrm{Opt}(j)}{\mathrm{TMREL}(j)};0\right] \times \frac{\mathrm{DALYs}(j)}{\mathrm{DALYs}(\mathrm{all})}\right)$$

where i denotes the food groups to be decreased (red meat, processed meat and sweetened beverages), j denotes the food groups to be increased (whole grain products, fruits, vegetables, legumes, nuts and seeds, and milk), Opt(i) and Opt(j) are the optimized consumptions of food groups i and j, respectively (in g/d), Max(i) is the upper limit of consumption of food group i (in g/d), TMREL(j) is the TMREL value of food group j (in g/d), DALYs(i) and DALYs(j) are the disability-adjusted life-years (DALYs) associated with excessive or insufficient consumptions of food groups i and j, respectively (in y), and DALYs(all) is the sum of all DALYs(i) and DALYs(j). The Max values used were the maximal recommended consumption of unhealthy foods in line with the French dietary guidelines (33): 71 g/d for red meat, 25 g/d for processed meat and 263 g/d (corresponding to the average portion size) for sweetened beverages intake. The TMREL and DALYs values used were issued from the most recent (2019) estimates from the GBD (32) adapted to our study context (by using sex-specific and French DALYs values, Supplementary Table S2).

We also evaluated how the modeled diets deviated from current diets, in order to consider inertia to changes in food consumption, which is one way to account for social/cultural acceptability. The Diet Departure (*DD*) criterion was defined as the sum of the squares of the differences between observed and optimized food group consumption, standardized by their observed standard deviations, as previously explained (31). *DD* was thus expressed and minimized as:

$$\min DD = \sum_{k=1}^{n}\left[\frac{\mathrm{Obs}(k)-\mathrm{Opt}(k)}{\mathrm{SD}(k)}\right]^2$$

where k is the number of food groups (n = 45), Obs(k) and Opt(k) are, respectively, the observed and optimized consumption of food group k (in g/d) and SD(k) is the current standard deviation of the consumption of food group k.

Constraints

During diet optimization, the total energy intake was constrained to stay within ±5% of its observed value. Thirty-five nutritional constraints were applied to ensure adequate nutrient intake in the male and female populations (Supplementary Table S3), based on the most recent reference values from the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) (34). We did not consider any constraints for vitamin D, because its reference value is known to be much too high to be reached by a non-fortified diet alone (31, 33). As the absorption of iron and zinc is dependent on dietary factors, the requirements were based on bioavailable iron and zinc calculated from the dietary intake using equations that predict their absorption (35–37), as detailed in a previous study by our group (31). This previous study had demonstrated that current recommendations regarding bioavailable iron and zinc are very constraining when trying to model healthier diets, these recommendations being much higher than current intakes (e.g., there is a current iron-deficiency anemia prevalence of 4.1% in French women) (31). Therefore, like in this previous study, we used threshold values lower than current reference values. They correspond to a deficiency prevalence of 5%, because such flexibility enables the identification of diets that are apparently healthier overall, with a better balance in DALYs due to less cardiometabolic disease, despite a higher prevalence of iron-deficiency anemia (31). In addition, to take into account the slightly lower digestibility of plant *vs* animal proteins regarding the nutritional constraint on protein requirement, a 5% penalty was applied to protein intake from plant protein food items, as previously described (38). As the intake of individual amino acids is generally adequate when the protein intake is sufficient in a varied diet (39), only protein requirements were considered in the model constraints, but we have *a posteriori* verified that modeled diets also contained adequate intakes of indispensable amino acids by using a database of the amino acid composition of food groups (Supplementary Information Text S1).

Moreover, some acceptability constraints were applied to the food group consumption (Supplementary Table S4). Acceptability constraints aimed to keep the food group intakes within the range of observed intakes, by bounding each food group intake between its 5th and 95th percentile of observed consumption in males and females separately. We did not do this for the unhealthy food groups or categories (red meat, processed meat and sweetened beverages), for which a dietary constraint with an upper limit was already defined according to the French dietary guidelines. Another exception was made for some healthy food groups (legumes and milk) that had 95th percentile values slightly lower than TMREL values, and for which the upper limit has thus been raised to their TMREL values.

Optimization strategy

We firstly aimed to determine the range of adequate %PP values in the diet that would ensure nutrient adequacy with a minimal long-term health risk. This first problem of identifying the adequate %PP range was addressed by optimizing the *HR* criterion under all the nutritional and acceptability constraints, with an additional constraint on %PP that was iteratively parameterized according to a grid search. This grid search constraint forced the %PP value to be equal to x%, with x% varying from 0 to 100% by steps of 5% (or even 1% at the edges of the adequate %PP range). As this problem was often non-uniquely identifiable, leading to different solutions with slightly distinct dietary patterns but similar *HR* values (especially for the intermediate %PP values that allowed for a variety of food group combinations with a similarly null *HR* value), we choose to systematically select the dietary solution that was the most acceptable *a priori*, based on the lowest departure from the current diet. According to the hierarchical method in multi-criteria optimization (40), this second problem of diet selection was addressed in a second stage. This time it was done by optimizing the *DD* criterion under the constraint that *HR* was equal to its previously identified minimal value, always under all the nutritional and acceptability constraints, and the grid search constraint on %PP covering its previously identified adequate range.

Limiting nutrients and contribution of food groups to their intake

We conducted a dual value analysis to better characterize the tensions between %PP, nutrient adequacy and long-term health. We reported the dual values associated with the %PP equality constraint and the nutritional constraints during *HR* optimization (obtained during the first problem solving, as explained above), which represent the potential *HR* gain if the limiting bound (lower or upper) of the considered constraint was relaxed by one unit. In order to compare the relative influence of nutrients, their dual values were standardized to represent the potential *HR* gain if the limiting bound was relaxed by 10%, to classify nutrients from the most limiting (higher absolute standardized dual value) to the least limiting (lowest absolute standardized dual value).

For the most limiting nutrients in the different modeled diets (i.e., nutrients with the most active constraints), we studied contributions of different food groups to intake of that particular nutrient in each modeled diet identified for each adequate %PP value (i.e., in the modeled diets resulting from the second problem solving, as explained above).

Sensitivity analysis

We also conducted a sensitivity analysis to assess the influence of some constraints of particular interest. We thus compared the results obtained when requiring the deficiency prevalence to be ≤1% rather than ≤5% (main model) in the nutritional constraints for bioavailable iron and zinc (their alternative threshold values are given in Supplementary Table S3), and when removing or not (main model) all the dietary and acceptability constraints on food group intakes.

Diet environmental impacts

Finally, to assess environmental pressures related to the observed and modeled diets, we used the French agricultural life cycle inventory database AGRIBALYSE® v3.1; its methodological approach (summarized in Supplementary Information Text S2) has been described elsewhere (41–43). In particular, we evaluated the food-related GHGe (in kg CO_2eq, with the non-CO_2 GHGe included and weighted according to their relative impact on warming), land use (referring to the use and transformation of land, dimensionless), water use (relating to the local scarcity of water, in m^3 water deprivation) and fossil resource use (use of non-renewable fossil resources such as coal, oil, and gas, in MJ), together with a single environmental footprint score (dimensionless) that aggregated 16 indicators (44).

Results

Range of adequate %PP values and identified tensions between %PP, nutrient adequacy and long-term health

The adequate %PP range compatible with nutrient adequacy was 16–82% in males and 16–77% in females, and only the 25–70% %PP range was additionally compatible with a minimal health risk (*HR* criterion) for both sexes (Table 1). In this narrower range, a null *HR* value was attained by the removal of unhealthy foods (red meat, processed meat and sweetened beverages) and an increase in healthy foods (whole grain products, fruits, vegetables, legumes, nuts and seeds, and milk) up to or above their TMREL values (32).

Among the %PP equality constraint and the nutritional constraints, none were found limiting for *HR* minimization over the 25–70% %PP range (Table 2). The %PP equality constraint was limiting only for %PP values lower than 25% (strongly) and higher than 70% (more moderately, due to the lower *HR* impact of the milk decrease for the highest %PP values than of the red meat increase and whole grain product decrease for the lowest %PP values). Nutrients identified as increasingly limiting as %PP decreased below 25% were fiber, sugar (excluding lactose), saturated fatty acids and atherogenic fatty acids (lauric, myristic and palmitic acids). As %PP decreased below 25%, it was hence increasingly challenging to maintain sufficient intake of fiber and non-excessive intakes of sugar and fatty acids (as shown by the opposite sign of their dual values), which resulted in dietary solutions of increasingly degraded *HR* values. Nutrients that were identified as increasingly limiting when %PP increased above 70% were iodine, sodium, vitamin B2, calcium, EPA + DHA, vitamin A and α-linolenic acid in both sexes together with vitamin B12 in males and bioavailable iron in females. As %PP increased above 70%, it was increasingly challenging to maintain sufficient intakes of these nutrients and a non-excessive sodium

TABLE 1 Range of adequate values of the percentage of plant protein in the diet (%PP) and corresponding minimal values of long-term health risk (*HR* criterion) in French males and females.

	Males							Females					
	Observed diet	Modeled diets						Observed diet	Modeled diets				
%PP	33%	16%	20%	25–70%	75%	80%	82%	34%	16%	20%	25–70%	75%	77%
HR value	0.983	0.602	0.180	0.000	0.004	0.024	0.049	0.736	0.516	0.065	0.000	0.039	0.052
HR components:													
Risk of excessive intake of unhealthy foods:													
Red meat	0.236	0.211	0.065	0.000	0.000	0.000	0.023	0.207	0.347	0.000	0.000	0.000	0.000
Processed meat	0.213	0.021	0.000	0.000	0.000	0.000	0.000	0.126	0.000	0.000	0.000	0.000	0.000
Sweetened beverages	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.002	0.013
Risk of insufficient intake of healthy foods:													
Whole grain products	0.213	0.195	0.000	0.000	0.000	0.000	0.000	0.157	0.090	0.000	0.000	0.000	0.000
Legumes	0.111	0.127	0.067	0.000	0.000	0.000	0.000	0.053	0.056	0.042	0.000	0.000	0.000
Fruits	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.093	0.000	0.000	0.000	0.000	0.000
Vegetables	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
Nuts & seeds	0.039	0.048	0.048	0.000	0.000	0.000	0.000	0.019	0.023	0.023	0.000	0.000	0.000
Milk	0.022	0.000	0.000	0.000	0.004	0.024	0.026	0.037	0.000	0.000	0.000	0.037	0.039

Values of the HR criterion and its individual components when optimizing HR under all the nutritional, dietary and acceptability constraints and an additional constraint imposing the %PP value according to an iterative grid search. Results are reported for the %PP values allowing model convergence (i.e., nutrient-adequate diets) for each sex (16–82% and 16–77% %PP ranges in males and females, respectively). Within these %PP values, those outside the 25–70% range have non-null HR values because of excessive intake of unhealthy foods and/or insufficient intake of healthy foods.

intake. The other nutrients (n = 20, those not shown in Table 2) were never limiting over the adequate %PP range, including, of note, protein.

Sensitivity analysis showed that being more demanding for bioavailable iron and zinc (i.e., constraining their deficiency prevalence at ≤1% rather than ≤5% as in the main model) resulted in slightly restricting the adequate %PP range on the right (16–79% in males and 16–70% in females), and the %PP range ensuring a null *HR* value on both sides (30–65% in males and 35–45% in females) (data not shown). Conversely, when suppressing all the food group consumption limits (i.e., all the dietary and acceptability constraints) from the model (Supplementary Table S5), the range of adequate %PP values was expanded on both sides (8–94% in males and 8–92% in females), as was the %PP range ensuring a null *HR* value (16–86% in males and 16–84% in females), but consistently with the same limiting nutrients as in the main model (in particular, insufficient fiber intake for excessively low %PP values, or insufficient intakes of vitamin B12, iodine and EPA + DHA for excessively high %PP values).

Modeled diets

All the modeled diets identified (Figure 1; Supplementary Figure S2) were very distant from the current typical French diets, with departure values (*DD* criterion) equal to or greater than twice the standard deviation observed in the population. Furthermore, most of the modeled diets with a null *HR* value (i.e., in the 25–70% %PP range) were all about equally distant from the observed diets, with close *DD* values (differing by less than 20%) in the 35–65% %PP range and similar *DD* values (differing by less than 5%) in the 45–60% %PP range.

Although the energy intake remained relatively stable between modeled and observed diets (by construction), the total intakes of both animal-based and plant-based foods were increased in the 25–70% %PP range, notably owing to the important increases in milk, fruits and vegetables up to or above their TMREL values (Supplementary Table S6; Supplementary Figure S3). Regarding plant products, all the modeled diets exhibited dramatic increases in fruits and vegetables, whole grain products and legumes and nuts. Regarding animal products, red and processed meats were readily removed as %PP increased. These meats were replaced by poultry and eggs, which transiently increased, the modeled diets then being meat-free from PP% = 60%. Dairy and seafood were the only remaining animal products at the right end of the adequate %PP range (Supplementary Figure S3).

Over the entire adequate %PP range, including meat-free diets, the intakes of protein and of each indispensable amino acid were always much higher than their 98% safe intake thresholds (Figure 2; Supplementary Table S7; Supplementary Figure S4).

Contributions of food groups to limiting nutrient intakes

Regardless of their %PP value, all the modeled diets were nutrient-adequate, in contrast with observed diets (Supplementary Table S8).

As %PP increased, it was increasingly difficult to maintain sufficient intakes of bioavailable iron, vitamins B12, B2 and A, and

TABLE 2 Dual values of the active constraints identified during minimization of the long-term health risk (*HR* criterion) in French males and females[1].

	Males						Females				
	Modeled diets						Modeled diets				
%PP	16%	20%	25–70%	75%	80%	82%	16%	20%	25–70%	75%	77%
%PP constraint[2]	−0.246	−0.081	NS	0.003	0.004	0.037	−0.166	−0.042	NS	0.004	0.017
Nutrients that are more limiting as %PP increase[3]											
Iodine	NS	NS	NS	0.002	0.005	0.058	NS	NS	NS	0.006	0.043
Sodium	NS	−0.023	NS	−0.002	−0.005	−0.041	NS	NS	NS	−0.006	−0.027
Bioavailable iron	NS	NS	NS	NS	NS	NS	NS	0.096	NS	0.014	0.027
Vitamin B12	NS	NS	NS	0.002	0.004	0.035	NS	NS	NS	NS	NS
Vitamin B2	NS	NS	NS	NS	0.001	0.006	NS	NS	NS	NS	0.021
Calcium	NS	NS	NS	NS	0.001	0.009	NS	NS	NS	0.001	0.013
EPA + DHA	NS	NS	NS	0.001	0.001	0.007	NS	NS	NS	0.002	0.009
Vitamin A	NS	NS	NS	0.001	0.002	0.009	NS	NS	NS	0.004	0.001
α-linolenic acid	NS	NS	NS	0.001	0.001	0.001	NS	NS	NS	0.001	0.004
Nutrients that are more limiting as %PP decrease[3]											
Fiber	0.435	0.136	NS	NS	NS	NS	0.332	0.077	NS	NS	NS
Sugar excluding lactose	−0.125	−0.044	NS	NS	NS	−0.012	−0.106	−0.030	NS	NS	−0.002
Saturated fatty acids	−0.095	−0.074	NS	NS	NS	NS	−0.085	−0.036	NS	NS	NS
Atherogenic fatty acids	−0.083	−0.001	NS	NS	NS	NS	−0.094	−0.006	NS	NS	NS

[1]Dual values when optimizing *HR* under all the nutritional, dietary and acceptability constraints and an equality constraint imposing the percentage of plant protein in the diet (%PP) value according to an iterative grid search. Results are reported for the %PP values allowing nutrient-adequate diets (16–82% and 16–77% %PP ranges in males and females, respectively), which includes those also allowing a null *HR* value (25–70% %PP range in both sexes). NS, not significant (<0.0001).
[2]For the %PP equality constraint, dual values represent the potential effect on *HR* of the relaxation by one unit of the limiting bound, with positive (negative) values if the lower (upper) bound is limiting (e.g., for %PP = 82% in males, the dual value indicates that there would be a potential *HR* gain of 0.037 if %PP was decreased from 82 to 81%).
[3]For nutritional constraints, dual values have been standardized to represent the potential effect on *HR* of the relaxation by 10% of the limiting bound, to classify the nutrients from the most limiting (higher absolute value) to the least limiting (lowest absolute value). Limiting nutrients have a positive (negative) dual value if their lower (upper) bound is limiting (e.g., for %PP = 82% in males, the dual value for iodine indicates that there would be a potential *HR* gain of 0.058 if the lower bound for iodine intake was decreased by 10%, from 150 to 135 μg/d). Only nutrients with an active constraint (i.e., with a non-null dual value) for at least one %PP value are presented here. For nutrients not presented in this table, dual values were always equal to zero, meaning that compliance with these constraints was not limiting.Atherogenic fatty acids, lauric and myristic and palmitic acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

iodine and calcium, owing to the decreases in the animal products that were their main contributors (red meat, dairy products and eggs) (Supplementary Figure S5). The EPA + DHA and α-linolenic acid intakes, which are largely insufficient in the observed diets, were made sufficient in all the modeled diets by increases in their main contributors, respectively, seafood and added fats, with difficulties to maintain them sufficient for the highest %PP values (Supplementary Figure S5). The sodium intake, which is dramatically excessive in the observed diets, was reduced to its upper limit in all the modeled diets as a result of removing processed meat and reducing refined grain products, with difficulties to maintain sodium not excessive for the highest %PP values, due to increases in some starch and miscellaneous foods (Supplementary Figure S5). Conversely, as %PP decreased, it was increasingly difficult to maintain a sufficient intake of fiber and non-excessive intakes of sugar and saturated fatty acids, due to the meat and dairy increases (Supplementary Figure S6).

Environmental impacts of modeled diets

Across modeled diets, GHGe gradually decreased as %PP increased until %PP = 70%, where the GHGe were ~ 50% lower than with the observed diet (Figure 3). Similar trends were observed for land use and, to a lesser extent, fossil resource use (Supplementary Figure S7), with 40% and ~ 20% decreases, respectively, from the observed to the modeled diet with %PP = 70%. In contrast, water use was ~25–50% higher for the null-*HR* modeled diets than for the observed diet, due to their very high levels of fruits and vegetables that were by far the most water-demanding food groups (Supplementary Figure S7). Overall, at the level of the single environmental footprint score that aggregated 16 indicators, the same trend was observed as for GHGe, with a 37% decrease in this aggregated score from the observed to the modeled diet with %PP = 70% (Supplementary Figure S7).

Discussion

Gathering all nutritional information over a large spectrum that covered nutrient reference values and long-term health risks, our study formally establishes ranges of plant protein proportion (%PP) for nutrient-adequate and healthy diets. One major finding is that there is no optimal %PP value, as we found a spectrum of similarly healthy diets over the 25–70% range. However, diets in the upper end were associated with substantially lower GHGe and overall environmental impact.

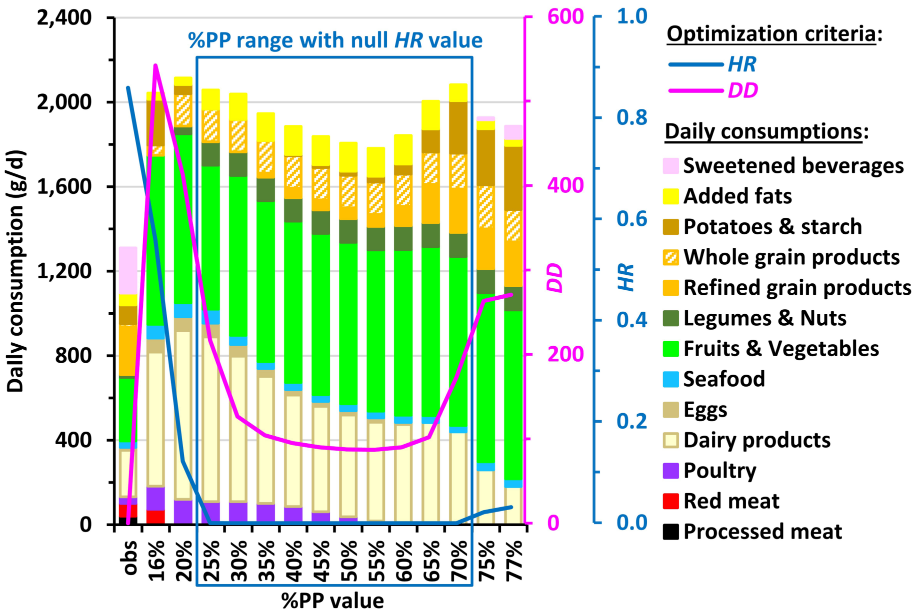

FIGURE 1
Daily food category consumption in the observed diets (obs) and modeled diets obtained by long-term health risk (*HR*) and diet departure (*DD*) minimization under imposed percentage of plant protein in the diet (%PP) in French adults. Results are reported for all the adequate %PP values ensuring nutrient adequacy (16–77%), which includes those also ensuring a null *HR* value (25–70%). The Bar charts represent the cumulative consumptions of food categories (black axis on the left) and the curves represent the *HR* and *DD* values (blue and pink axes on the right, respectively). For clarity, the 45 modeled food groups are not represented here but grouped into broader categories that are included in *HR* (such as red and processed meats) or represent other protein sources (such as poultry and seafood). Consumption of water, hot beverages, alcohol and miscellaneous foods are not shown for clarity. Details about food grouping and consumptions of food categories not shown here are given in Supplementary Tables S1, S6, respectively.

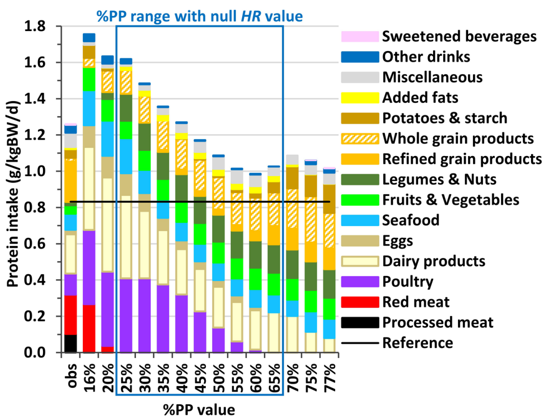

FIGURE 2
Contribution of food categories to protein intake in the observed diets (obs) and modeled diets obtained by long-term health risk (*HR*) and diet departure minimization under imposed percentage of plant protein in the diet (%PP) in French adults. Results are reported for all the adequate %PP values ensuring nutrient adequacy (16–77%), which includes those ensuring also a null *HR* value (25–70%). Sections inside the bars represent the contributions of food categories to protein intake (in g of protein/kg of BW/d). See Supplementary Table S1 for the detailed composition of food categories.

A wide dietary %PP range, from ~15 to 80%, was found compatible with providing all nutrients in adequate amounts. Our results do not agree with those of Vieux et al., who recently argued that %PP must be <50% to ensure nutritional adequacy (45). However, in this diet optimization study, solutions with %PP >50% were rejected not because of their true intrinsic inability to meet nutrient requirements but because of an incorrect problem formulation, as we recently pointed out (46). Furthermore, by not analyzing how the constraints considered affected the results and by not identifying the limiting nutrients, this work was not informative about the nutritional barriers to increasing %PP, which was our concern here along with its other health and environmental impacts. In our study, as shown by sensitivity analysis, the wide %PP range identified as compatible with nutritional adequacy was slightly restrained by the considered constraints for food acceptability, whereas the nutritional issues identified remained broadly the same with or without these constraints. From a nutritional viewpoint, no diet with %PP < ~15% was able to provide enough fiber and non-excessive amounts of saturated fatty acids, while also satisfying all constraints for nutrient intakes and food acceptability. In these too-low %PP diets, inadequate fiber intake was due to insufficient consumption of whole grains, legumes, and nuts, which were the most critical plant protein sources with intakes below their minimum-risk exposure levels. More interestingly, given the ongoing dietary transition, we could not find diets with %PP > ~80% that would provide sufficient amounts of a large set of nutrients, particularly iodine, vitamin B12 (in males), bioavailable iron (in females), calcium and EPA + DHA. These nutrients are considered to be at issue in vegetarian diets (except calcium and iodine in lacto-ovo-vegetarian), notably calcium and B12 in predominantly plant-based diets (47, 48). From a dietary viewpoint,

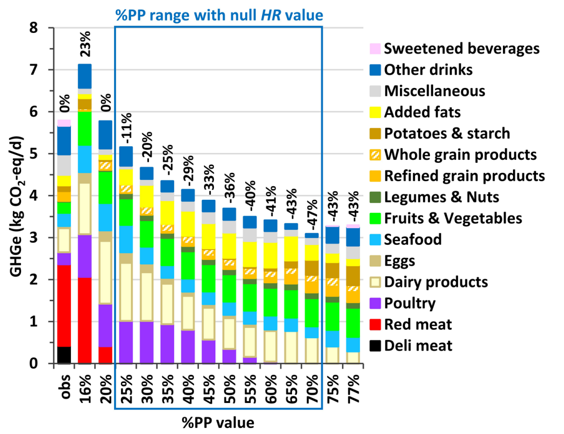

FIGURE 3
Greenhouse gas emissions (GHGe) associated with the observed (obs) and modeled diets obtained by long-term health risk (*HR*) and diet departure minimization under imposed percentage of plant protein in the diet (%PP) in French adults. Results are reported for all the adequate %PP values ensuring nutrient adequacy (16–77%), which includes those ensuring also a null *HR* value (25–70%). Sections inside the bars represent the contributions of food categories to GHGe (in kg CO_2-eq/d), and values above the bars represent the relative deviation in GHGe from its observed value (in %). See Supplementary Table S1 for the detailed composition of food categories.

as shown when approaching the critical value of %PP = 80%, dairy appeared to be key to preventing iodine and calcium shortages. Seafood, meanwhile, appeared critical to providing EPA + DHA (with oily fishes as the main source) as well as iodine and B12. Milk and seafood were the last remaining animal products at the highest %PP values, confirming their importance as healthy, nutrient-dense protein sources (49). Healthy plant protein sources such as legumes and nuts apparently could not replace milk or seafood. This is because they actually reached their upper allowed intake very early (as soon as %PP = 25% for legumes), which indicates that they constitute an effective dietary lever. However, even when removing all food intake limits (in sensitivity analysis), it remained impossible to obtain 100% plant-based diets because of the same nutritional issues (insufficient intakes of vitamin B12, iodine and EPA + DHA). Our findings do not indicate that vegetarian (without seafood) or vegan diets (without seafood and dairy) cannot be nutritionally adequate. It means that solutions for diets that are entirely or almost entirely plant-based should rely on additional food products than those presently consumed by the general population, including fortified foods (50–52). This warrants further studies about the potential of new foods to extend the limit of the %PP range identified as adequate here.

Within the wide range of nutrient-adequate %PP values, we did not find a single optimal diet, but a large range of diets with %PP from 25 to 70%. These diets were all optimal when considering their health value, because their food consumptions complied with minimum-risk exposure levels. These consisted of no red meat and high levels of fruits and vegetables, whole grains, legumes, nuts and milk, in line with dietary guidelines (53). Modeled healthy diets were variations of this pattern, which explains why they were also similarly distant from current diets. Within this healthy pattern spectrum, the increase in %PP was predominantly related to the decrease in total and animal proteins. This occurred mostly in poultry and eggs and, to a lesser extent, dairy. This finding aligns well with the current spectrum of observed diets, with plant-based diets being higher in plant protein but especially low in total and animal protein (54, 55). This could simply be ascribed to the higher protein density in animal protein sources compared to plant protein sources. Also, the nutrients identified as limiting at the borders of the healthy %PP range appear to be related to the nutrient density of animal vs. plant protein sources when expressed relative to protein density. However, the dietary protein amount was never limiting, even at the highest %PP levels. There is a growing consensus that the protein package and not the protein *per se* are important to the question of plant to animal protein ratio in the diet (6, 9). Likewise, indispensable amino acid amounts were well above reference values based on requirements. It is usually considered that dietary proteins, and in particular plant proteins tend to complement each other, because dietary proteins are not low in the same amino acids (56, 57). Lysine, which is the most critical amino acid, and is specifically low in grains is not limiting in the diet if grains are not the main source of protein in the diet (39). In real diets, composed of a mix of different types of proteins that complement each other, sufficient amounts of protein appear to guarantee sufficient amounts of amino acids (22, 55, 58).

Distance from the prevailing diets is often used in diet modeling to take into account so-called cultural acceptability (59–61), also referred to as dietary inertia (62). In this study, healthy diets in the 35–65% %PP range departed rather similarly from the prevailing diets, which are still at ~35% %PP. This confirms that the plant to animal protein ratio is, by itself, a poor descriptor of diet characteristics, and so blanket statements about the right %PP are not warranted. Given that modeled healthy diets ranging from 35% %PP (the level of the current diets in Western countries) to 65% %PP were all very distant from current diets, our study also shows that overcoming dietary inertia is required for healthy diets, irrespective of the plant to animal protein target ratio (63).

The GHGe and overall composite score for environmental pressures were lower for healthy modeled diets than observed diets, and all the more as %PP increased. A large body of literature has reported that diets which are more plant-based are associated with lower environmental pressure, and vice versa, whether diets were modeled (48, 61, 64, 65), observed (66–68) or composite (25). However, until our study, this relationship had not yet been shown according to %PP in healthy diets. In our setting, %PP was strongly associated with the environmental impact of healthy diets. As compared to the prevailing diets, lower GHGe and composite score are firstly explained by the removal of total red meat in all healthy diets, red meat accounting for ~1/3 of the pressure in prevailing diets. This is in line with the literature that points to red meat and associated sustainability concerns (65, 69). Finally, we found that other environmental pressures (land use and fossil resource use), except water use, had similar patterns of change, in line with the literature (21, 48). The general relationship between %PP and environmental pressure can mostly be ascribed to the fact that animal sources are rich in protein, and that livestock breeding is associated with higher resource use, higher land use, and higher

GHGe (23, 70–72). Nevertheless, further investigation of the relationship between %PP and environmental impacts would require prioritizing the minimization of environmental impacts over that of diet departure. Therefore, we cannot rule out the possibility that moderate %PP diets, if well-designed, may have as low environmental impacts as high %PP diets, at the cost of a larger diet departure.

This study has some limitations. We modeled diets according to changes in intakes of food groups, based on the present food repertoire and current intake levels in the population. Food grouping is critical in diet modeling (33), and food diversity and composition can change rapidly in Western countries, as seen by recent changes (73). A similar limitation applies to the assessment of a diet's environmental impacts, for which also we did not consider variations related to food production systems (74, 75). Nevertheless, we used a classical food grouping, which helps represent dietary patterns at an appropriately high level of detail. We also believe that using standard/traditional foods in modeling provides a good starting point to evaluate the situation before considering changes in the food offer or food composition. Our study uses sources of information as background parameters, including references/targets for nutrients and food categories. Clearly, there are many uncertainties in this regard (33). Nonetheless, we believe that a strength of our study is our use of a conceptual framework that aggregates most of the state of the art knowledge in nutrition.

To conclude, we identified that the range of equally optimal %PP values for nutrition and health is wide (25–70%), and that all of these healthy diets deviate greatly from prevailing diets. From a public health perspective, there is no unique, optimal %PP value when considering nutrition and health alone. However, significant changes in current eating habits are nonetheless required to achieve healthier diets. The focus should therefore shift from protein *per se* to what is carried with protein (i.e., the nutrient package), the overall health value of the food groups that convey protein, as well as the efforts needed to move away from current Western dietary patterns (22). Moreover, in the higher end of the adequate %PP range, modeled healthy diets have a lower environmental impact and are thus more sustainable than other healthy diets. Thus, in current and future dietary transitions, environmental pressures appear to be a more direct determinant than health objectives to justify increasing %PP levels. At %PP > ~80%, changes in food repertoire diversity, food composition, nutrient enrichment or nutrient supplementation are required for fully nutrient-adequate diets. Finally, the adequate %PP range may be narrower in some populations, such as the elderly, who may have higher protein requirements than the general adult population, and this would deserve further study.

Preprint

A manuscript was deposited as a preprint, to MedRxiv (10.1101/2022.05.20.22275349). The copyright holder for this preprint is the author, with all rights reserved and no reuse allowed without permission.

Data availability statement

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

Author contributions

HF and FM designed the research, wrote the first draft of the manuscript, and had primary responsibility for the final content. HF conducted the research and analyzed the data. AD, EP, FM, JW, J-FH, and EK-G provided methodological support and help with interpretation of the results. All authors provided critical comments on the manuscript, and read and approved the final manuscript.

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Conflict of interest

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The remaining 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1178121/full#supplementary-material

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Evaluation of sustainable and healthy eating behaviors and adherence to the planetary health diet index in Turkish adults: a cross-sectional study

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Aim: The Planetary Health Diet Index (PHDI) is a relatively new index, and studies about its relationship with eating behaviors, nutritional status, and obesity in adults are very limited. For this reason, in this study, sustainable healthy eating behaviors of individuals and compliance of their diets with PHDI were evaluated.

Methods: This cross-sectional study was conducted with 1,112 adults (70.1% women and 29.9% men with mean age = 28.7 years, SE = 9.47). Study data were obtained with the face-to-face interview method via a questionnaire including sociodemographic characteristics, anthropometric measurements, the Sustainable and Healthy Eating (SHE) Behaviors Scale, and 24-h dietary recall. PHDI was evaluated for adherence to EAT-Lancet Commission recommendations.

Results: The average PHDI total score was 41.5 points. Higher SHE Behaviors Scale and PHDI scores were observed in participants with a duration of education above 8 years ($p < 0.05$). Those with lower SHE Behaviors Scale and PHDI scores were more likely to be obese ($p < 0.001$). The total PHDI score was positively associated with fiber, vitamin E, potassium, and folate, and negatively associated with pyridoxine and calcium ($p < 0.05$). The total SHE Behaviors Scale score was positively associated with carbohydrates, fiber, and potassium and negatively associated with pyridoxine, calcium, and energy ($p < 0.05$). A one-unit increase in SHE Behaviors Scale total score resulted in a 5,530 unit (95%CI: 4.652; 6.407) increase in PHDI total score and a one-unit increase in duration of education (years) resulted in a 0.660 unit (95%CI: 0.403; 0.918) increase in PHDI total score. Furthermore, a one-unit increase in Body Mass Index (BMI) (kg/m^2) resulted in a – 0.218 unit (95%CI: −0.424; −0.013) decrease in PHDI total score.

Conclusion: The participants' PHDI index scores were low; therefore, the adherence to the EAT-Lancet recommendation was low which might be associated with obesity. Clinical studies evaluating the effects of adherence to sustainable diets on adequate and balanced nutrition and health outcomes are recommended.

KEYWORDS

planetary health diet, sustainable diets, obesity, healthy eating behaviors, sustainability

1. Introduction

The United Nations predicts the world's population will grow to 9.7 billion in 2050 (1). The Food and Agriculture Organization (FAO) estimates that by 2050, food production must expand by at least 62% to meet the needs of the rising global population (2). In recent years, the development of sustainable food systems has led to changes in traditional agricultural production systems, affecting human diets (3). In addition to these developments and changes, the definition of a healthy diet has been examined and revised to incorporate planetary health principles. According to the Food and Agriculture Organization and the World Health Organization (WHO), sustainable healthy diets are "dietary patterns that promote all aspects of an individual's health and wellbeing; have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable" (4). In this line, the EAT-Lancet Commission on "Healthy Diets from Sustainable Food Systems" (EAT-Lancet) suggested the "Planetary Health Diet." This reference diet is based on the food system's environmental and human health effects. These guidelines are built on a diet rich in vegetables, greens, fruits, and whole grains and low in meat, fish, eggs, refined cereals, and tubers (5).

Plant-based diets consist of fresh and lightly-cooked foods, and the energy required for plant-based food production is substantially less than for meat preparation. Moreover, plant-based diets result in a smaller environmental footprint, which enhances the quality of soil, water, and air, thereby enhancing the health of all living organisms. Consuming seasonal fruits and vegetables in plant-based diets reduces energy consumption and promotes sustainable resource management (6). In addition, plant-based diets are energy-efficient diets. They have a smaller effect on climate change than animal-based diets (7). In order to meet the rising *per capita* demand of a growing population by 2050, the meat industry would need to increase production by 50–73% (8). However, this situation seems unlikely to be sustained.

According to the EAT-Lancet report, adopting the recommendations for a healthy and sustainable diet might prevent 11 million deaths annually (5). The main goal of the EAT-Lancet diet is to enhance population and environmental health. The report demonstrates that this reference diet is nutritionally balanced and has a low ecological impact (9). Studies evaluating adherence to EAT-Lancet guidelines in various scenarios and countries are very interesting. One study found an inverse relationship between the EAT-Lancet diet score and ischemic heart disease and diabetes (10). In the Swedish population, high adherence to the EAT-Lancet diet was associated with a decreased risk of incident diabetes among people with different genetic risks (11). Based on the EAT-Lancet diet, Cacau et al. recently proposed the Planetary Health Diet Index (PHDI), which consists of 16 components that score proportionally and consider all EAT-Lancet food groups in addition to energetic density (12).

PHDI was associated with higher overall dietary quality and lower greenhouse gas emissions (12, 13). According to a study, the Brazilian population showed low adherence to a healthy and sustainable dietary pattern and seems far from meeting the EAT-Lancet recommendations (14). Furthermore, this study showed that women, the elderly, those who are overweight or obese, and those living in urban areas had higher scores in the PHDI (14). However, another study showed that higher adherence to the PHDI may decrease obesity indicators (9). A recent study showed that obesity may affect the feasibility of a sustainable environment (15).

The PHDI is a relatively new index, and there is limited data on its relationship with eating behavior, nutritional status, and obesity in adults. In this study, sustainable healthy eating behaviors of individuals, compliance of their diets with PHDI, and the factors affecting them were evaluated.

2. Methods

The convenience sampling method was used for data collection. The study data were obtained with face-to-face interviews via a questionnaire prepared by the researchers. A total of 1,215 potential participants were reached personally or invited by e-mail to take part in the study at the Erzurum Technical University Department of Nutrition and Dietetics. However, 103 of the participants did not complete the questionnaire, were unwilling or unable to provide informed consent, and had severe acute or chronic diseases. Therefore, this cross-sectional study was conducted with 1,112 adults (70.1% women and 29.9% men with mean age = 28.7 years, SE = 0.34) between September 2022 and February 2023 in Erzurum (one of the metropolitan cities in the east of Turkiye). The inclusion criteria were meeting the age criteria (19–64 years), not having chronic health conditions or psychological disorders, consented to participate, and not following a special diet or eating model. The exclusion criteria were the inability or reluctance to complete the questionnaire, being pregnant or breastfeeding, not meeting the age requirement, and following a special diet or eating model. To carry out this research, "Ethics Committee Approval" was received from the Erzurum Technical University Ethics Committee (number of meetings: 8, decisions: 5, date: 29.08.2022). The research was carried out following the Declaration of Helsinki. Informed consent was obtained from the participants.

The questionnaire included sociodemographic characteristics, anthropometric measurements, the Sustainable and Healthy Eating (SHE) Behaviors Scale, and 24-h dietary recall. The dietary energy and nutrient intakes were evaluated using the Nutrition Information System (BeBiS) program (version 9). Height and body weight measurements were self-reported. By dividing the body weight by the square of the height, the Body Mass Index (BMI) was calculated. Participants with a BMI below 18.50 kg/m^2 were classified as underweight, with a BMI in the range of 18.50–24.99 kg/m^2 were classified as normal, with a BMI in the range of 25.0–29.99 kg/m^2 were classified as overweight, and those with a BMI ≥30.0 kg/m^2 were classified as obese (16).

2.1. Sustainable and healthy eating behaviors scale

The Sustainable and Healthy Eating (SHE) Behaviors Scale was developed by Żakowska-Biemans et al. to assess adults' self-reported sustainable and healthful eating behaviors (17). Koksal et al. conducted the Turkish adaptation, validity, and reliability study of the scale (18). Cronbach-α values of the scale and its subscales ranged from 0.764 to 0.912. This scale comprises 32 items and 7 factors. The items are rated on a seven-point Likert scale (never to always). The seven factors are quality labels, seasonal food and avoiding food waste, animal welfare, meat reduction, healthy and balanced diet, local food, and low fat. The mean of all the factor values is considered when calculating the overall scale score. Factor scores are calculated by taking the average of the scores (between 1 and 7 points) given to the items in that factor. In calculating the total scale score, the average of the scores given to all factors is taken (score range 1 to 7) (18). Increasing results on both the overall and the subscales indicate an increase in sustainable and healthy eating behaviors (18).

2.2. The planetary health diet index

In the present study, 24-h dietary recall (for 1 day) of the participants was taken by the researchers. Energy and nutrient intakes were evaluated using the Nutrition Information System (BeBiS) program (The Food Code and Nutrient Data Base, BLS II.3, 1999, version 9.0).

The Planetary Health Diet Index (PHDI) was developed from the EAT Lancet Commission's dietary recommendations (12). The daily energy intake for the EAT-Lancet Commission's dietary recommendations was set at 2,500 kcal. Each component can receive a maximum of 10 or 5 points, resulting in a PHDI score between 0 and 150 (12). PHDI is calculated based on recommendations for various dietary energy intakes and assessed based on how much each food group contributes to the total energy. The PHDI uses a gradual scoring system and is an energetic density index. This diet is based on 16 food components (adequacy component: nuts and peanuts, legumes, fruits, vegetables, and whole cereals; optimum component: eggs, fish and seafood, tubers and potatoes, dairy, and vegetable oils; ratio component: dark green vegetables/total ratio and red vegetables/total ratio; moderation component: red meat, chicken substitutes, animal fats, and added sugars) (12, 13). The calculation of the PHDI is explained in detail in relevant studies (9, 12); please see Supplementary File 1.

2.3. Data analysis

The Statistical Package for the Social Sciences (version 23.0) software was used for data analyses. The variables were evaluated using visual (histogram and probability graphs), and skewness and kurtosis (from −1 to 1) to determine whether or not they were normally distributed. Data were evaluated with descriptive statistics such as mean, standard error (SE), number, and percentage. Participants' SHE Behaviors Scale total and subgroup scores and BMI values (kg/m^2) were given according to PHDI score quartiles. Chi-square analysis was used to compare qualitative data and detect differences between groups. For comparison, the T-test, the Mann–Whitney U test, One-Way ANOVA, or the Kruskal Wallis test were used in independent groups. For post-doc analysis, Bonferroni correction was applied for multiple pairwise comparisons. The Pearson correlation analysis was used for the relationship between variables.

Furthermore, linear regression was used to determine factors related to the PHDI score to explain the relationships between observable associations. SHE total score, BMI, and duration of education (years) were selected as predictors, and the model was adjusted for age and sex (0 for women and 1 for men). The results were interpreted with 95% confidence.

3. Results

Of the total 1,112 participants (mean age = 28.7 years, SE = 9.47) enrolled in the study, 779 (70.1%) were women and 333 (29.9%) were men. More than half of the participants were not working (69.9%) and were single (64.6%). The duration of education of most of the participants (87.0%) was over 8 years (mean duration of education was 13.6 years, SE = 3.60). In this cross-sectional study, obesity indices were also examined. Accordingly, a total of 613 (55.1%) participants (mean BMI = 24.0 kg/m^2, SE = 4.50) were in the normal BMI (kg/m^2) group, and 407 (36.6%) were in the overweight/obese group.

In this study, the average PHDI total score was 41.5 points, with a total score that can range from 0 to 150. A descriptive analysis of PHDI components is presented in Supplementary File 2.

In the total of participants, the working (mean = 4.0, SE = 0.05) and married (mean = 3.8, SE = 0.05) groups had higher scores on the SHE Behaviors Scale compared to the not working (mean = 3.6, SE = 0.03, $p < 0.001$) and single group (mean = 3.6, SE = 0.03, $p < 0.05$). Higher scores on the SHE Behaviors Scale and higher PHDI scores were also observed in participants with a duration of education above 8 years (mean = 3.6, SE = 0.02, $p < 0.001$; mean = 43.1, SE = 0.48, $p = 0.001$, respectively). Those with lower SHE Behaviors Scale and PHDI scores were more likely to be obese ($p < 0.001$; Table 1).

Table 2 presents SHE Behaviors Scale total and subgroup scores, and BMI values (kg/m^2) according to PHDI quartiles. Those with the highest PHDI quartile (Q4: 51.99–98.96 points) had the highest quality labels score (mean = 3.9, SE = 0.07; $p < 0.05$) and seasonal food score (mean = 4.4, SE = 0.6; $p < 0.001$) compared to Q1 group. The participants in the highest quartile had higher healthy and balanced diet scores (mean = 4.7, SE = 0.07) compared to Q1 (mean = 4.1, SE = 0.07), Q2 (mean = 4.3, SE = 0.07) and Q3 (mean = 4.3, SE = 0.07) quartiles and higher local food scores (mean = 3.3, SE = 0.08) compared to Q1 (mean = 2.9, SE = 0.07) and Q3 (mean = 2.9, SE = 0.07) group ($p < 0.001$). Meat reduction (mean = 3.4, SE = 0.08; $p < 0.05$), animal welfare (mean = 3.9, SE = 0.08; $p = 0.001$), and low fat (mean = 4.9, SE = 0.08; $p < 0.001$) subscores were the highest in the Q4 group. SHE Behaviors Scale total scores were statistically different between all PHDI quartiles ($p < 0.001$). The participants with the highest PHDI scores tended to have the lowest BMI values (mean = 23.5, SE = 0.22 kg/m^2; $p < 0.001$) compared to Q1 group.

Table 3 presents the correlations between PHDI, SHE Behaviors Scale and subgroups, and BMI. There was a positive correlation between the SHE Behaviors Scale and PHDI ($r = 0.374$, $p < 0.001$). All SHE Behaviors Scale subgroups positively correlated with the PHDI scores ($p < 0.001$). Body mass index values (kg/m^2) were negatively correlated with PHDI and SHE Behaviors Scale scores ($r = -0.159$, $r = -0.130$, $p < 0.001$, respectively). Furthermore, quality labels and seasonal food and avoiding food waste subscales scores correlated negatively with BMI values (kg/m^2; $p < 0.001$).

Table 4 presents the association between PHDI and SHE behaviors scale scores, and nutrients. The total PHDI score showed a positive association with fiber (g), vitamin E (mg), potassium (mg), and folate (µg). It was negatively associated with pyridoxine (mg) and

TABLE 1 Sustainable and healthy eating (SHE) behaviors scale and planetary healthy eating index (PHDI) total scores of participants according to their sociodemographic characteristics.

	n (%)	SHE behaviors scale total score		PHDI total score	*p**
		Mean ± SE	*p*§	Mean ± SE	
Sex					
Women	779 (70.1%)	3.7 ± 0.35	0.178	41.6 ± 0.59	0.559
Men	333 (29.9%)	3.6 ± 0.05		41.0 ± 0.80	
Working status					
Yes	335 (30.1%)	4.0 ± 0.05	<0.001**	42.5 ± 0.80	0.126
No	777 (69.9%)	3.6 ± 0.03		40.9 ± 0.57	
Educational duration					
< 8 years	145 (13.0%)	3.2 ± 0.11	<0.001**	30.3 ± 1.46	0.001**
> 8 years	967 (87.0%)	3.6 ± 0.02		43.1 ± 0.48	
Marital status					
Married	394 (35.4%)	3.8 ± 0.05	0.011*	39.2 ± 0.88	0.001*
Single	718 (64.6%)	3.6 ± 0.03		42.7 ± 0.56	
BMI classification					
Underweight	92 (8.3%)	3.6 ± 0.09[a]		41.8 ± 1.59[a]	
Normal	613 (55.1%)	3.8 ± 0.03[a]	<0.001**	43.2 ± 0.61[a]	<0.001**
Overweight	300 (27.0%)	3.8 ± 0.04[a]		41.7 ± 0.83[a]	
Obese	107 (9.6%)	2.9 ± 0.13[b]		30.1 ± 1.98[b]	

§Difference between SHE total score according to the groups, *Difference between PHDI total score according to the groups. Data are given as numbers and (*n*) and percent (%). *$p < 0.05$, ** $p < 0.001$. [a,b] Same letters within a column indicate no differences according to pairwise comparisons.

TABLE 2 SHE subgroup scores, SHE total score, and body mass index (BMI) values (kg/m^2) of participants according to PHDI score quartiles.

	Q1 (*n* = 278) (0–30.56)	Q2 (*n* = 278) (30.56-40.61)	Q3 (*n* = 278) (60.61-51.99)	Q4 (*n* = 278) (51.99-98.96)	*p*
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Quality labels	3.6 ± 0.67[a]	3.6 ± 0.06[ab]	3.6 ± 0.06[b]	3.9 ± 0.07[b]	0.004*
Seasonal food	4.0 ± 0.07[a]	4.2 ± 0.06[ab]	4.2 ± 0.06[ab]	4.4 ± 0.06[b]	0.002*
Healthy & balanced diet	4.1 ± 0.07[a]	4.3 ± 0.07[a]	4.3 ± 0.07[a]	4.7 ± 0.07[b]	<0.001**
Local food	2.9 ± 0.08[a]	3.0 ± 0.07[abc]	2.9 ± 0.07[b]	3.3 ± 0.08[c]	0.001**
Meat reduction	3.0 ± 0.08[a]	3.2 ± 0.07[a]	3.1 ± 0.07[a]	3.4 ± 0.08[b]	0.014*
Animal welfare	3.4 ± 0.08[a]	3.6 ± 0.07[a]	3.7 ± 0.08[a]	3.9 ± 0.08[b]	<0.001**
Low fat	4.4 ± 0.08[a]	4.5 ± 0.08[a]	4.6 ± 0.07[a]	4.9 ± 0.08[b]	0.001**
SHE Behaviors Scale total score	3.2 ± 0.07[a]	3.8 ± 0.05[b]	3.8 ± 0.04[c]	4.1 ± 0.05[d]	<0.001**
BMI (kg/m^2)	24.9 ± 0.32[a]	23.6 ± 0.26[b]	24.0 ± 0.25[ab]	23.5 ± 0.22[b]	<0.001**

Data are given as mean ± standard error and number (*N*) and percent (%). *$p < 0.05$, **$p < 0.001$. [a, b, c] Same letters within a row indicate no differences according to pairwise comparisons.

calcium (mg; $p < 0.05$). The total SHE Behaviors Scale score showed a positive association with carbohydrate (g), fiber (g), and potassium (mg) and was negatively associated with pyridoxine (mg), calcium (mg), and energy (kcal; $p < 0.05$).

A one-unit increase in SHE Behaviors Scale total score resulted in a 5,530 unit (95%CI: 4.652; 6.407) increase in PHDI total score, and a one-unit increase in duration of education (years) resulted in a 0.660 unit (95%CI: 0.403; 0.918) increase in PHDI total score. Furthermore, a one-unit increase in BMI (kg/m^2) resulted in a − 0.218 unit (95%CI: −0.424; −0.013) decrease in PHDI total score.

4. Discussion

To the best of our knowledge, this study is the first to evaluate sustainable healthy eating behaviors of individuals and compliance of

TABLE 3 Correlation (r) of PHDI, SHE behaviors scale, and BMI.

	PHDI total score	SHE behaviors scale	Quality labels	Seasonal food and avoiding food waste	Healthy & balanced diet	Local food	Meat reduction	Animal welfare	Low fat	BMI
PHDI total score										
SHE Behaviors Scale	0.374**									
Quality labels	0.118**	0.635**								
Seasonal food and avoiding food waste	0.125**	0.609**	0.588**							
Healthy & balanced diet	0.158**	0.635**	0.627**	0.570**						
Local food	0.108**	0.535**	0.433**	0.371**	0.305**					
Meat reduction	0.099**	0.511**	0.338**	0.319**	0.273**	0.390**				
Animal welfare	0.118**	0.603**	0.519**	0.470**	0.480**	0.416**	0.347**			
Low fat	0.138**	0.552**	0.433**	0.503**	0.551**	0.216**	0.285**	0.485**		
BMI (kg/m²)	−0.159**	−0.130**	−0.121**	−0.156**	0.056	−0.026	0.067	0.074	0.062	

*BMI, body mass index; SHE, Sustainable Healthy Eating. *$p<0.05$, **$p<0.001$.

their diets with PHDI in Turkish adults. This research showed the participants' PHDI index scores were low; therefore, the adherence to the EAT-Lancet recommendation was low. High BMI was determined to be associated with low PHDI scores and SHE Behaviors Scale scores. Furthermore, high education level positively related to SHE Behaviors Scale and PHDI scores. Individuals with higher SHE Behaviors Scale scores also had higher PHDI scores. When the diets of the individuals were examined, dietary energy was not found to be associated with the SHE Behaviors Scale score and PHDI score. However, the PHDI score was positively correlated with fiber, vitamin E, potassium, and folate and negatively correlated with calcium and pyridoxine. SHE Behaviors Scale scores were positively correlated with dietary carbohydrate, fiber, and potassium intake but negatively correlated with pyridoxine and calcium intake, similar to the PHDI score.

This research showed that the mean PHDI score is 41.5, corresponding to the Q2 quartile when evaluated according to the PHDI quartile distributions. In a study conducted in Brazil, PHDI index scores were comparable to ours (45.9 points), and population compliance with EAT-Lancet recommendations was low (14). However, in a study conducted by Cacau et al., the mean PHDI score was 60.4 (12). In our research, adherence to EAT-Lancet recommendations was low, depending on the PHDI scores.

According to the World Health Organization (WHO) report, Turkiye reports the highest obesity rate for adults in Europe (32.1%), with the rate in the rest of Europe at 23.3% (19). In this study, 36.6% of the participants were overweight or obese. The increasing prevalence of obesity in our country and globally is alarming, as are its adverse effects on environmental health and sustainability. Unhealthy eating habits that cause obesity do not comply with planetary health principles (20). It is stated that obesity is associated with low sustainable and healthy eating behaviors and low sustainability of diet (9, 15). A study revealed that overweight individuals have low Sustainable Diet Index scores (21). In a study evaluating obesity outcomes of adherence to PHDI, individuals with high adherence to the PHDI had lower BMI ($\beta=-0.50$; 95%CI: −0.73; −0.27) and waist circumferences ($\beta=1.70$; 95%CI: −2.28; −1.12) values (9). However, another study showed that overweight/obese individuals had higher PHDI scores (14). This study determined that high BMI was associated with low PHDI and SHE Behaviors Scale scores. Our regression model concurs with this finding; as a result, a decrease in BMI is associated with an increase in the PHDI total scores (Table 5). Therefore, more studies are needed on the effects of obesity on the sustainable environment and the effects of sustainable diets on obesity prevalence.

Another significant result of our research is a positive relationship between education level, sustainable and healthy eating behaviors, and PHDI scores. Our study showed that a one-unit increase in the duration of education (years) resulted in a 0.660 unit (95%CI: 0.403; 0.918) increase in PHDI total score ($p<0.05$). A study reported that increased duration of education was positively associated with increased awareness of reducing individual ecological footprint (15). It is crucial to provide

TABLE 4 Association between PHDI scores and SHE behaviors scale scores and nutrients.

	PHDI scores					SHE behaviors scale				
	β	*t*	95% CI		*p*	*β*	*t*	95% CI		*p*
(Constant)		37.073	39.310	43.704	<0.001		50.190	3.521	3.808	<0.001
Energy (kcal)	0.052	0.024	−0.074	0.075	0.981	−4.437	−1.986	−0.010	0.000	**<0.001**
Protein (g)	0.111	0.316	−0.272	0.377	0.752	0.725	1.962	0.000	0.042	0.057
Fat (g)	−0.121	−0.123	−0.699	0.616	0.902	2.035	1.981	0.000	0.085	0.050
Carbohydrate (g)	−0.282	−0.242	−0.340	0.265	0.809	2.448	2.026	0.001	0.040	**0.048**
Fiber (g)	0.213	2.720	0.073	0.451	**0.007**	0.045	0.518	−0.010	0.017	**0.043**
Vitamin A (μg)	0.031	0.460	−0.001	0.001	0.646	−0.047	−0.636	0.000	0.000	0.604
Vitamin D (μg)	0.028	0.862	−0.067	0.172	0.389	−0.005	−0.147	−0.008	0.007	0.525
Vitamin E (mg)	0.111	2.870	0.043	0.229	**0.004**	−0.022	−0.543	−0.008	0.004	0.883
Vitamin K (μg)	0.034	0.966	−0.004	0.012	0.334	0.020	0.544	0.000	0.001	0.587
Thiamine (mg)	0.121	1.424	−1.340	8.436	0.155	0.049	0.562	−0.224	0.404	0.587
Riboflavine (mg)	0.043	0.387	−3.487	5.203	0.698	0.204	1.516	−0.074	0.578	0.575
Niacin (mg)	−0.139	−1.641	−0.308	0.027	0.101	−0.139	−1.518	−0.020	0.003	0.130
Vitamin B_5 (mg)	−0.120	−1.519	−1.536	0.196	0.129	−0.196	−2.328	−0.126	−0.011	0.129
Pyridoxine (mg)	−0.184	−2.542	−6.240	−0.803	**0.011**	−0.097	−1.257	−0.294	0.064	**0.020**
Vitamin B_{12} (μg)	−0.066	−0.918	−0.287	0.104	0.359	−0.029	−0.377	−0.016	0.011	0.706
Vitamin C (mg)	−0.061	−1.726	−0.017	0.001	0.085	−0.015	−0.402	−0.001	0.000	0.688
Sodium (mg)	−0.002	−0.054	−0.001	0.001	0.957	−0.059	−1.696	0.000	0.000	0.090
Potassium (mg)	0.318	4.395	0.002	0.007	**<0.001**	0.224	2.942	0.000	0.000	**0.003**
Calcium (mg)	−0.142	−2.779	−0.012	−0.002	**0.006**	−0.198	−3.255	−0.001	0.000	**0.001**
Magnesium (mg)	−0.102	−1.055	−0.031	0.009	0.292	−0.155	−1.267	−0.003	0.001	0.205
Iron (mg)	−0.016	−0.198	−0.485	0.396	0.843	0.174	0.967	−0.042	0.016	0.375
Zinc (mg)	−0.007	−0.092	−0.450	0.410	0.927	−0.048	−0.580	−0.036	0.019	0.562
Folate, total (μg)	0.145	2.104	0.001	0.025	**0.036**	0.056	0.781	0.000	0.001	0.405
Phosphorus (mg)	0.206	1.172	−0.003	0.000	0.241	0.174	0.967	0.000	0.001	0.334

The bold values are indicates significant at $p < 0.05$.

TABLE 5 Multiple linear regression model for the prediction of planetary healthy eating index (PHDI) total score.

	PHDI				
Independent variables	B	SE	CI 95%	*p*	Adjusted R^2
SHE total score	5.530	0.447	4.652; 6.407	<0.001**	0.169
Education duration	0.660	0.131	0.403; 0.918	<0.001**	
BMI (kg/m²)	−0.218	0.105	−0.424; -0.013	0.030*	

a. Dependent variable: Planetary Healthy Eating Index (PHDI). b. Predictors: (Constant), Sustainable and Healthy Eating (SHE) Behaviors Scale; Body mass index (BMI); education duration (years); Adjusted for: Sex (0-women, 1-men); and age (years), $*p < 0.05$, $**p < 0.001$.

education on sustainable and healthy eating behaviors and the environmental effects of diet. In this study, the increase in the scores obtained from the SHE behaviors scale was also positively associated with the increase in the PHDI scores. In line with these results, education is essential for sustainable environmental health (22).

According to a study, higher PHDI scores were associated with higher overall dietary quality and lower greenhouse gas emissions (12). In the results of our study, no relationship was found between dietary energy, protein and PHDI scores. Cacau et al. also found no association between dietary energy, dietary protein, and PHDI scores (12). Another important result in our study concerns dietary fiber. It is noteworthy that dietary fiber positively affects scores regarding nutrients. Dietary fiber draws attention to its significant functionality in non-communicable diseases, especially obesity (23). Sustainable diets are rich in vegetables, greens, fruits, and whole grains and low in meat, fish, eggs, refined cereals, and tubers. For this reason, the results of this research support that sustainable and healthy eating behaviors and high adherence to PHDI increase dietary fiber intake. There is also a concern that sustainable diets may adversely affect the intake levels of some nutrients (iron, retinol, vitamin B12, etc.) due to recommendations to reduce animal-derived food consumption (24). In European adolescents, higher PHDI scores were associated with a greater intake of nutrients predominantly from plant-source foods, such as vegetable protein, vitamin E, and folate, and with a lower intake of nutrients predominantly from animal-source foods (25). In this study, it was determined that daily intake levels of dietary calcium and pyridoxine from foods of primarily animal origin were negatively associated with high SHE Behaviors and PHDI scores, whereas vitamin E, potassium, and folate from foods of mostly plant origin were positively associated with high SHE Behaviors and PHDI scores. Cacau et al. reported similar results for pyridoxine and its association with PHDI scores. They also revealed that carbohydrates, polyunsaturated fats, fiber, vitamins C, A, E, and K, thiamine, folate, iron, phosphorus, potassium, zinc, selenium, magnesium, and copper are positively associated with PHDI. Saturated fat, total fat, cholesterol, monounsaturated fat, riboflavin, niacin, vitamin B5, and B12 were negatively associated with PHDI scores and emphasized that this is an expected result (12). Although there were relationships between some nutrients and PHDI scores in the current study, there was no relationship with all macro and micro nutrients as in the study by Cacau et al. (12). In their study, the mean PHDI scores were higher than our result. In this case, the generally low adherence of our population to PHDI might be reflected in the current result.

A study evaluating the effects of sustainable diets on healthy nutrition determined that daily dietary protein intake remained sufficient in high-income and middle-income countries, while it was below the recommended amounts in low-income countries (20). Intake of micronutrients increased, especially in high- and middle-income countries where significant amounts of animal-based foods were replaced with plant-based ones. When animal-based foods were replaced entirely with plant-based foods, baseline low levels of vitamin A, folate, iron, potassium, and fiber exceeded recommended values. However, calcium, pantothenate (vitamin B5), and vitamin B12 fell below recommended values in high-income and middle-income countries. In low-income countries, when a small amount of animal-based foods were replaced with plant-based foods, they were insufficient to increase potassium and vitamin A adequately, and riboflavin and calcium did not reach the recommended values (20). The associated financial burden is another crucial consideration when altering dietary habits to promote sustainability. In this regard, Hirvanen et al. noted that the EAT-Lancet estimates could not be met in low-income countries; for example, in South Asia, the reference diet will cost more than 1.5 times the average *per capita* household income per day. The authors also point out that fruit, vegetables, and animal products are among the most expensive food groups in the world (26). Currently, governmental initiatives to ensure food security are of utmost importance (27). Turkiye is in the upper middle-income country class (28). Our study did not find any statistical difference between the PHDI scores according to the working status ($p > 0.05$). However, the SHE Behaviors Scale score was higher in the working group. A study in India reported that even the wealthiest 5% of the population had unhealthy eating habits, low consumption of protein-rich food, fruits, and vegetables, and overconsumed processed foods (26). Concerning this, relevant government policies need to raise the public's awareness about nutrition.

5. Limitations and strength

When evaluating the study findings, the following limitations must be considered. First, the research was conducted as a cross-sectional study in Erzurum, Turkiye (one of the metropolitan cities in the east of Turkiye). This cross-sectional study cannot determine a cause-and-effect relationship but evaluates the relationship between the measured variables. Second, nutritional habits differ between countries and even regionally. The study sample may not reflect Turkiye in terms of mean age, sex, and obesity prevalence. Consequently, it is essential to repeat the research in other regions/cities nationwide. Third, the participant's weight and height were obtained from self-reports. Finally, the food consumption record could have been taken for at least three consecutive days instead of one. The strengths of the study are as follows: being an important research for evaluating the relationship between obesity and planetary health with a large sample size and being one of the first studies to evaluate individuals' sustainable healthy eating behaviors, compliance of their diets with PHDI, and the factors affecting them in Turkish adults. Furthermore, to calculate the index, dietary intake was gathered using a 24-h recall, considered more accurate than a food frequency questionnaire.

6. Conclusion

The concept of a sustainable diet is relatively novel, and there are very few studies evaluating adherence to sustainable diets. To the best of our knowledge, this study is the first study to evaluate sustainable healthy eating behaviors of individuals, compliance of their diets with PHDI, and the factors affecting them in Turkish adults. This research showed that the participants' PHDI index scores were low; therefore, the adherence to the EAT-Lancet recommendation was low. Assessment of participants' diet quality will be beneficial in interpreting low adherence to PHDI. Low adherence to PHDI and low sustainable and healthy eating behaviors may be associated with obesity. Our findings indicate that education level can have a significant impact on sustainability. It is considered that sustainable and healthful nutrition education is necessary for environmental health sustainability. While adherence to a sustainable diet increases the intake of some nutrients, namely, those of animal origin, decrease. Clinical studies evaluating the effects of adherence to sustainable diets on adequate and balanced nutrition and health outcomes are recommended.

Data availability statement

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

Ethics statement

Ethical permission was obtained from the Erzurum Technical University Ethics Committee (Number of Meetings: 8, Decisions: 5, Date: 29.08.2022). The study was carried out in accordance with the principles outlined in the Helsinki Declaration. Informed consent was obtained from the participants. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MSM-Ç: conceptualization, methodology, formal analysis, writing–original draft, writing–review, and editing. OB:

conceptualization, data curation, methodology, writing–original draft, writing–review, and editing. BK-B: conceptualization, data curation, methodology, writing–original draft, writing–review, and editing. EK: conceptualization, methodology, writing–review, and editing, and supervising. The authors have approved the final version submitted.

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.2023.1180880/full#supplementary-material

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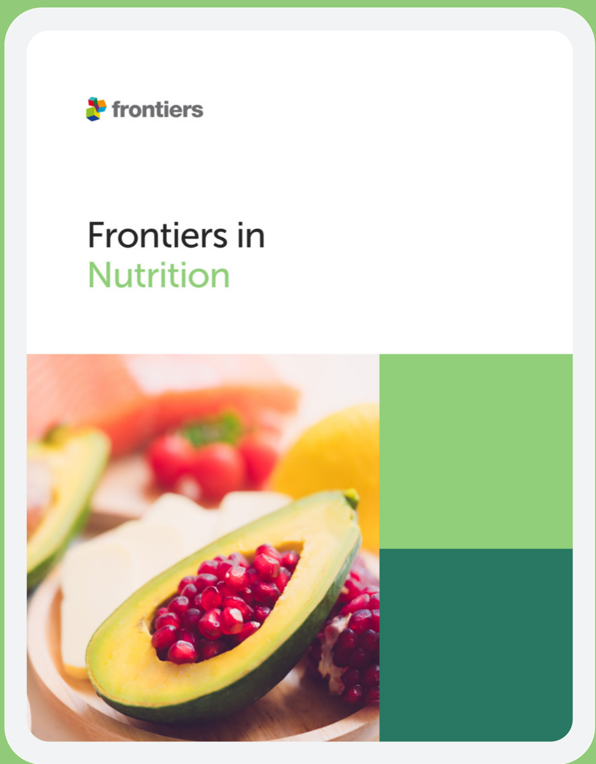

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