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Key nutrition and hydration insights for public health and policy

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Key nutrition and hydration insights for public health and policy

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Types of Nutrition Knowledge, Their Socio-Demographic Determinants and Their Association With Food Consumption: Results of the NEMONIT Study

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Objectives: To investigate nutrition knowledge in the German population, its determinants and its association with food consumption.

Methods: Data were obtained from the NEMONIT study (2014/15, $n = 1{,}505$, participants' age: 22–80 years). Nutrition knowledge was measured using the consumer nutrition knowledge scale (CoNKS) in a computer-assisted telephone interview. Two 24-h recalls were conducted to assess food consumption, which was evaluated using the Healthy Eating Index-NVS II.

Results: Areas for knowledge enhancement were the understanding of health benefits of fruit and vegetable consumption, the concept of a balanced diet and saturated fatty acids. Nutrition knowledge was higher among females, younger and high socio-economic status participants. Correlations between nutrition knowledge and a favorable diet were significant but low. Analyses of types of nutrition knowledge yielded similar results for procedural knowledge and knowledge on nutrients but not for knowledge on calories.

Conclusions: Areas for knowledge enhancement were identified, but an increase in nutrition knowledge alone seems unlikely to result in large improvements of dietary behavior.

Keywords: nutrition knowledge, types of knowledge, food consumption, dietary recommendations, healthy eating, NEMONIT

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INTRODUCTION

The factors influencing an individual's food choice are numerous and include, amongst others, habits, practical skills, cultural or environmental factors as well as motives like taste, convenience or price (1–3). Nutrition knowledge might also be one of several factors influencing food choice. Given the abundance of possibilities for food choices in Western societies, a certain level of knowledge might be necessary to make a healthy selection. Since food consumption of Western populations is often not in line with dietary recommendations (4–7), knowledge dissemination and nutrition education are common–but usually not evaluated–strategies of public health initiatives aiming to change dietary behavior.

As reported in a systematic review by Spronk et al. (3), it is well documented in international studies that nutrition knowledge is influenced by age, sex, and socio-economic status. However, the direction of the relationship between age and nutrition knowledge was contradictory across studies. While Hendrie et al. (8) observed a positive relationship, Dickson-Spillmann and Siegrist (9) and Dickson-Spillmann et al. (2) observed a negative relationship and Parmenter et al. (10) reported a curvilinear relationship, where middle-aged groups had a higher knowledge than younger and older participants. It should be noted though that the studies differ substantially in how they defined the age groups, thus limiting comparability. With regard to the association of nutrition knowledge and food consumption the results are also inconclusive. Although the review by Spronk et al. (3) shows that the majority of studies found significant associations between higher nutrition knowledge and healthier food consumption, especially higher fruit and vegetable consumption, these associations were rather weak.

A limitation of previous studies is the challenging measurement of nutrition knowledge. The General Nutrition Knowledge Questionnaire (GNKQ) has been well studied, validated, and adapted to different populations (11–15), but is far too long to be used as one of many instruments in a population-wide survey. Although many recent studies address the development and validation of questionnaires for specific nutrition knowledge areas (especially sports nutrition knowledge) or specific populations (e.g., adolescents, athletes) [e.g., (16–19)], only few validated instruments exist to measure nutrition knowledge in large population surveys.

Instruments that cover different types of nutrition knowledge are also rare (3). Some authors argue that a differentiation between declarative nutrition knowledge and procedural nutrition knowledge might be a promising approach for future research (2, 3, 20). Declarative knowledge is factual knowledge ("knowing that") while procedural knowledge is knowledge of skills and strategies ("knowing how") (2). Dickson-Spillmann et al. (2) developed and validated a short scale on nutrition knowledge (consumer nutrition knowledge scale—CoNKS) which encompasses both types of knowledge.

In Germany, the National Nutrition Survey (NVS) II (7) enquired single aspects of nutrition knowledge and confirmed that women, younger persons, and persons with a higher socio-economic status have higher nutrition knowledge and that persons with higher nutrition knowledge eat more fruit and less meat and alcoholic drinks (21, 22). However, to our knowledge, a psychometrically validated instrument has not been applied previously in a population-based survey in Germany. Additionally, most previous studies did not distinguish between different types of knowledge.

Therefore, the aims of the current study were to investigate the declarative and procedural nutrition knowledge in the German population, its determinants and its associations with food consumption based on the NEMONIT study (23) and using the CoNKS (2) as a validated and comparable measurement.

METHODS

Study Design and Participants

This cross-sectional analysis is based on the last survey year data of the NEMONIT study (2014/15). NEMONIT was designed as a longitudinal study to assess changes in food consumption and nutrient intake in Germany. Besides the repeated measurement of food consumption, the annual surveys also included questions on specific nutritional topics (e.g., nutrition knowledge) allowing further cross-sectional analyses. A detailed description of NEMONIT has been published previously (23).

The NEMONIT sample (n ∼2,000) was recruited from participants of the German National Nutrition Survey (NVS) II (7) who confirmed their interest in taking part in further surveys. Over the course of the survey years, the sample underwent minor changes due to attrition so that in the final NEMONIT round of 2014/2015, a total of 1,572 participants with an age range of 22–80 years were interviewed. In this analysis, participants of whom data of the computer-assisted telephone interview (CATI) and two 24-h recalls were available, were included ($n = 1,508$). Among those, three respondents with more than two missing items in the nutrition knowledge questions were excluded from the analysis resulting in a total sample of $n = 1,505$.

Socio-Demographic Characteristics and Lifestyle Factors

Information on socio-demographic characteristics and lifestyle factors was obtained in CATIs. Socio-demographic characteristics included sex, age, school education (highest school-leaving qualification, recoded to years spent in school), and socio-economic status (SES). SES was an index based on three characteristics (participants' education, net household income and employment status of the principal earner of the household; possible range: 3–25 points) and categorized into low, medium, or high (21).

With regard to lifestyle factors, questions on specific diets (e.g., vegetarian diet, dieting), self-rated healthiness of the own diet, self-rated health status, and smoking status were included. Body mass index (BMI) was calculated based on self-reported body weight and height and categorized according to the cut-off points provided by the World Health Organization (WHO) (24). Physical activity was assessed by asking participants to state their mild, moderate, and vigorous physical activity in h/week. After bisecting hours of mild activity and doubling hours of vigorous activities, all activities were summed up as single measure for overall physical activity per week. This figure was compared to the WHO recommendation for physical activity (25) and categorized into inactive (0 h), active below recommendations (>0 and <2.5 hours) and active in agreement with the recommendations (≥2.5 h).

Nutrition Knowledge

Nutrition knowledge was measured using an adapted version of the consumer nutrition knowledge scale (CoNKS) by Dickson-Spillmann et al. (2). This scale aims to measure nutrition knowledge in proximity to consumers by using well-known instead of scientific terms and includes both, declarative and procedural nutrition knowledge questions. In a Swiss sample it showed a good validity when compared to the General Nutrition Knowledge Questionnaire (GNKQ), the most widely used and validated questionnaire of nutrition knowledge, and in terms of subgroup differences (2). With only 20 questions (GNKQ: 89), the CoNKS can be regarded a short and efficient instrument to measure nutrition knowledge in larger population surveys. Therefore, it was considered suitable for the use in NEMONIT. Nevertheless, some adjustments of the scale were regarded necessary. These included the usage of food terms which are more common in Germany (e.g., "Emmental" instead of "Gruyère") and the replacement of three items. Those items compared the healthiness of two alternatives (e.g., "Pasta with tomato sauce is healthier than pasta with mushroom and cream sauce"), which contradicts the view that it is the overall choice of foods and dishes, which makes a diet healthy or unhealthy rather than a food or dish *per se*. They were replaced by self-developed items comparing the nutrients of foods (**Table 1**). However, the item comparing the healthiness of brown and white sugar was kept, since in this case two variants of the same product are compared but consumers are expected to often misinterpret brown sugar as healthier due to the lower degree of product processing.

The nutrition knowledge items were asked in randomized order during the CATI at the end of the second 24-h recall. For scale construction, items were recoded with correct answers taking the value "1" and incorrect answers, "don't know" answers and missing values taking the value "0." Nutrition knowledge (CoNKS Total) was calculated as the sum of the 20 items, yielding a range of 0 to 20 points. Internal consistency of the scale was measured with Cronbach's Alpha ($\alpha = 0.59$).

To analyse different types of knowledge, three subscales were formed based on content considerations: one scale for procedural nutrition knowledge (7 items, hence 0–7 points), one scale for declarative knowledge on nutrient contents (7 items, 0–7 points) and one scale for declarative knowledge on calorie content (6 items, 0-6 points) (**Table 1**). This approach differs from the validation study by Dickson-Spillmann et al. (2) where only one overall scale was used. The authors designed this

TABLE 1 | Items of the adapted version of the consumer nutrition knowledge scale (CoNKS) and response behavior of the participants in the NEMONIT study 2014/15[a], sorted by subscales and percentage of correct responses.

Item	True/False	% of participants with		
		correct answer	wrong answer	don't know/missing
Procedural nutrition knowledge (0–7 points)				
If you have eaten high-fat foods, you can reverse the effects by eating apples	F	91	4	5
A healthy meal should consist of half meat, a quarter vegetables and a quarter side dishes	F	78	21	1
Fat is always bad for your health; you should therefore avoid it as much as possible	F	71	28	1
A balanced diet implies eating all foods in the same amounts	F	69	29	1
For a healthy nutrition, dairy products should be consumed in the same amounts as fruit and vegetables	F	64	31	4
Brown sugar is much healthier than white sugar	F	60	31	9
To eat healthily, you should eat less fat. Whether you also eat more fruit and vegetables does not matter	F	55	42	2
Declarative nutrition knowledge on nutrients (0–7 points)				
Oily fish (salmon, mackerel) contain healthier fats than red meat	T	85	9	6
Lentils contain only few useful nutrients, therefore their health benefit is not great	F	81	8	10
[b]Meat sausage contains more salt than cream cheese	T	76	16	9
[b]Fruit muesli contains more fiber than cornflakes	T	73	17	10
Skimmed milk contains fewer minerals than full-fat milk	F	64	24	12
The health benefit of fruit and vegetables lies alone in the supply of vitamins and minerals	F	64	33	3
[b]Dairy products contain more saturated fats (fatty acids) than vegetable oils	T	37	44	19
Declarative nutrition knowledge on calories (0–6 points)				
If cream is whipped it contains less calories than in its liquid form	F	91	5	4
Bacon contains more calories than ham	T	80	15	4
Fat contains fewer calories than the same amount of fiber	F	79	11	11
The same amount of beef steak and chicken breast contains equally many calories	F	66	23	11
A sandwich with mozzarella contains as many calories as the same sandwich with Emmental/Swiss cheese	F	64	21	15
The same amount of sugar and fat contains equally many calories	F	60	25	15

[a]*NEMONIT study, survey year 2014/2015 (n = 1,505).*
[b]*replaced items.*

TABLE 2 | Socio-demographic and lifestyle characteristics of NEMONIT study 2014/15[a] participants.

	n	% (Mean, SD)
Sex		
Men	638	42.4
Women	867	57.6
Age		
Mean		(56.8)
SD		(14.2)
22–34 years	130	8.6
35–50 years	370	24.6
51–64 years	501	33.3
65–80 years	504	33.5
School education		
Up to 9 years	364	24.2
10 years	503	33.4
12 or 13 years	638	42.4
SES		
Mean		(15.0)
SD		(3.5)
Low	170	11.3
Medium	768	51.0
High	567	37.7
Specific diet		
Vegetarian (incl. pesco-vegetarian) diet	47	3.1
Dieting (e.g., to lose weight or due to an illness)	106	7.0
Self-rated nutritional behavior		
Very healthy	111	7.4
Predominantly healthy	1,237	82.2
Less healthy/not healthy	155	10.3
Missing	2	0.1
HEI-NVS II		
Mean		(67.9)
SD		(10.0)
Good (>88 points)[b]	26	1.7
In need of improvement (>55 and ≤88 points)[b]	1,326	88.1
Poor (≤55 points)[b]	153	10.2
Body mass index		
Mean		(26.0)
SD		(4.6)
Underweight	16	1.1
Normal weight	681	45.2
Preobese	587	39.0
Obese	221	14.7
Self-rated health		
Good	1,163	77.3
Moderate	301	20.0
Poor	39	2.6
Missing	2	0.1
Physical activity		
Inactive	441	29.3
Active, below recommendations	391	26.0
Active, in agreement with recommendations	654	43.5
Missing	19	1.3
Smoking		
Smoker	165	11.0
Occasional smoker	42	2.8
Ex-smoker	521	34.6
Non-smoker	777	51.6

(Continued)

TABLE 2 | Continued

[a]NEMONIT study, survey year 2014/2015 (n = 1,505); SES, socio-economic status (index combining participants' education, net household income, and employment status of the principal earner of the household); HEI-NVS II, Healthy Eating Index of the German National Nutrition Survey II; [b]55 points ≙ 50% of total points, 88 points ≙ 80% of total points.

scale to measure both, declarative and procedural knowledge, but did not distinguish the knowledge types in their analysis. Dickson-Spillmann et al. (2) did not theoretically distinguish between declarative knowledge on nutrient and calorie content either. However, we assumed that knowledge on nutrients and knowledge on calories could be different types of nutrition knowledge. Since the three subscales were based on content considerations, the results are exploratory in nature.

Food Consumption and Diet Quality

Food consumption (g/d) was assessed with two 24-h recalls conducted on randomly drawn non-consecutive days (at least 1 week apart) by phone using the software EPIC-Soft (26) (renamed GloboDiet in 2014) as described in detail by Gose et al. (23). Energy and nutrient intakes were calculated based on the German Nutrient Database (BLS, version 3.02) (27).

Diet quality was evaluated using the Healthy Eating Index-NVS II (HEI-NVS II) adapted to 24-h recalls. The HEI-NVS II compares ten components of food consumption or nutrient intake [e.g., "fruit/fruit products," "meat/meat products"„ "fat (% of energy intake)"] with food-based dietary guidelines of the German Nutrition Society (28) and national reference values for nutrient intake (29). It can take values from 0 to 110 points, whereby higher scores indicate a better agreement with the recommendations. Further information on the HEI-NVS II can be obtained from Gose et al. (23) and Wittig and Hoffmann (30).

Data Analysis

Descriptive statistics are provided as means with standard deviations (SD) for metric variables and percentages for categorical variables. Since nutrition knowledge was not normally distributed, differences in nutrition knowledge between groups were tested using Mann–Whitney U-test or Kruskal–Wallis test as appropriate. Spearman's Rho correlations were calculated to examine the association of nutrition knowledge and its subscales with food consumption and HEI-NVS II. Multiple linear regressions were used to examine whether associations were independent from socio-demographic factors (sex, age in years, SES index).

Data analysis was performed using SAS 9.3 (SAS Institute, Inc.) and level of significance for all analyses was set at $P < 0.05$ (two-sided).

RESULTS

Sample

Table 2 shows the socio-demographic characteristics and lifestyle factors of the study sample. The study sample includes a higher proportion of females than males. Participants had a mean age of 57 years and the majority achieved higher school education and were assigned to the medium SES class. The percentage of self-defined vegetarians and pesco-vegetarians was 3 and 7% of the participants were dieting. The majority rated their nutritional behavior as predominantly healthy while the HEI-NVS II indicates that there is need of improvement in the diet of a large majority of the participants (**Table 2**).

Nutrition Knowledge

On average, items of the nutrition knowledge scale were answered correctly by 70% of the participants. All except one item were answered correctly by more than half of the participants, indicating relatively easy items (in terms of scale construction). Participants' mean was 14.1 points for the CoNKS Total (SD 3.0, IQR 4), 4.9 points for the subscale procedural knowledge (SD 1.6, IQR 2), 4.8 points for knowledge on nutrients (SD 1.4, IQR 1), and 4.4 points for knowledge on calories (SD 1.2, IQR 2) (**Table 3**). The distribution of scores was skewed to the left in all scales (data not shown).

Some areas for knowledge enhancement can be identified when looking at the single items (**Table 1**). First, the health benefits of fruit and vegetable consumption do not seem to be sufficiently well-known to the general public. Forty two percentage of the participants assumed the following statement to be correct: "To eat healthily, you should eat less fat. Whether you also eat more fruit and vegetables does not matter." Additionally, about one third of the participants thought the statement "The health benefit of fruit and vegetables lies alone in the supply of vitamins and minerals" to be true. Second, deficits in knowledge concerning the meaning of a balanced diet became apparent. About one third of the participants thought the following statements to be true: "A balanced diet implies eating all foods in the same amounts" and "For a healthy nutrition, dairy products should be consumed in the same amounts as fruit and vegetables." Furthermore, about one fifth of the participants thought that "A healthy meal should consist of half meat, a quarter vegetables and a quarter side dishes." Notably, the questions on a balanced diet and on the health benefits of fruit and vegetables received only few "don't know" answers, compared to questions on specific nutrients and calories. This means that participants were confident about their knowledge in this area, although they more often gave wrong answers than in other areas. Third, the question on saturated fatty acids in dairy products vs. vegetable oils revealed large uncertainties in this area (37% correct answers).

Socio-demographic Characteristics and Nutrition Knowledge

Nutrition knowledge differed significantly between socio-demographic groups (**Table 3**). Women had a higher nutrition knowledge than men, except for knowledge on calories. Nutrition knowledge was higher in younger age groups and in groups with higher school education and higher SES. The results were confirmed in multiple linear regressions (data not shown).

Lifestyle Factors and Nutrition Knowledge

Nutrition knowledge was higher among individuals following a vegetarian diet, having a normal weight and being physically active (**Table 4**). This also applied to the subscales of procedural knowledge and knowledge on nutrients but not to the scale measuring knowledge on calories (except for sports activities).

The significant results of the bivariate analysis were generally confirmed in multiple linear regressions controlling for sex, age, and SES (data not shown).

Nutrition Knowledge and Food Consumption

Nutrition knowledge was positively associated with the consumption of cereals/cereal products, vegetables, fruit/fruit products, and dairy products and negatively with the consumption of potatoes/potato products and meat/meat products (**Table 5**). However, correlations were rather weak with values between −0.14 and 0.12. Very similar results were observed when analyzing procedural knowledge and knowledge on nutrients separately. Knowledge on calories, however, was not associated with the consumption of cereals/cereal products, vegetables, fruit/fruit products, or meat/meat products.

In multiple linear regressions, the associations between nutrition knowledge and its subscales with consumption of vegetables, fruit/fruit products, dairy products, and meat/meat products were largely confirmed.

Nutrition Knowledge and Diet Quality

With increasing values on nutrition knowledge, respondents also had increasing values on the HEI-NVS II (Spearman's Rho correlation coefficient: $r_s = 0.16$, $p < 0.001$). This also applied to the subscales procedural knowledge ($r_s = 0.15$, $p < 0.001$) and knowledge on nutrients ($r_s = 0.16$, $p < 0.001$), but not to knowledge on calories ($r_s = 0.02$, $p = 0.440$). However, correlations were rather weak and in a simple linear regression model, nutrition knowledge explained only 3% (procedural knowledge: 2%, knowledge on nutrients: 3%) of the variance in HEI-NVS II (data not shown).

Similar to nutrition knowledge, HEI-NVS II was higher among women and among groups with higher school education and higher SES. However, HEI-NVS II increased with age while nutrition knowledge decreased with age. Therefore, multiple linear regressions were performed again to examine whether the association between nutrition knowledge and HEI-NVS II was independent of sex, age, and SES (**Table 6**). The results confirmed an independent association between nutrition knowledge and HEI-NVS II. However, they also showed that SES was no longer a significant predictor of HEI-NVS II when nutrition knowledge was entered into the model. Therefore, nutrition knowledge may partly mediate the effect of SES on healthy eating.

TABLE 3 | Nutrition knowledge (CoNKS Total and subscales) by socio-demographic group[a] in participants of the NEMONIT study 2014/15[b].

	CoNKS total[c]		Procedural knowledge[d]		Knowledge on nutrients[d]		Knowledge on calories[e]	
	Mean	***P***	**Mean**	***P***	**Mean**	***P***	**Mean**	***P***
Total sample	14.1		4.9		4.8		4.4	
Sex		<0.001		<0.001		0.006		0.689
Males	13.6		4.6		4.7		4.4	
Females	14.4		5.1		4.9		4.4	
Age groups		<0.001		<0.001		<0.001		<0.001
22–34 years	15.3		5.6		5.0		4.7	
35–50 years	14.7		5.2		5.0		4.5	
51–64 years	14.4		5.0		4.9		4.5	
65–80 years	13.0		4.3		4.5		4.2	
School education		<0.001		<0.001		<0.001		<0.001
Up to 9 years	12.5		4.1		4.2		4.1	
10 years	14.2		4.9		4.8		4.4	
12 or 13 years	15.0		5.3		5.1		4.5	
SES class		<0.001		<0.001		<0.001		<0.001
Low	12.2		4.1		4.1		4.1	
Medium	13.9		4.8		4.7		4.4	
High	14.9		5.3		5.1		4.5	

[a] *Mann–Whitney U-test (variables with two levels) or Kruskal–Wallis test (variables with more than two levels).*
[b] *NEMONIT study, survey year 2014/2015 (n = 1,505); SES, socio-economic status; CoNKS, consumer nutrition knowledge scale.*
[c] *CoNKS Total scale ranges from 0 (no question answered correctly) to 20 (all questions answered correctly).*
[d] *CoNKS subscale procedural knowledge and knowledge on nutrients each range from 0 (no question in the respective subsection answered correctly) to 7 (all questions in the respective subsection answered correctly).*
[e] *CoNKS subscale knowledge on calories ranges from 0 (no question in this subsection answered correctly) to 6 (all questions in this subsection answered correctly).*

DISCUSSION

This analysis of nutrition knowledge in adults based on NEMONIT and using an adapted version of the CoNKS showed several key results:

(1) Areas for knowledge enhancement could be observed in the assessment of the health benefits of fruit and vegetable consumption, in the understanding of the concept of a balanced diet as well as regarding the knowledge on saturated fatty acids.
(2) Nutrition knowledge was higher among individuals who were female, younger, had higher SES or showed a more health conscious lifestyle.
(3) Nutrition knowledge was positively associated with a favorable food consumption.
(4) Analyses of subscales of nutrition knowledge yielded similar results for procedural nutrition knowledge and knowledge on nutrients but not for knowledge on calories.

Areas for Knowledge Enhancement

In accordance with the results from Dickson-Spillmann et al. (2), the present results indicate that the health benefits of fruit and vegetable consumption do not seem to be sufficiently well-known to the public. This is unexpected since in Germany, as in many other countries, large efforts have been made to promote the consumption of fruit and vegetables, which is below the official recommendations. Hence, improved strategies are needed to communicate the (numerous) advantages of fruit and vegetables and to increase knowledge as basis for intention and action to promote their consumption.

Also in agreement with Dickson-Spillmann et al. (2), the present results showed that knowledge concerning the composition of a healthy and balanced diet could be improved. Much educational work has been done in this area, too. Among others, food circles or pyramids are a common way to present the principles of a balanced diet and are usually well disseminated and known to the public (31, 32). However, consumers seem to have difficulties to keep in mind, interpret, and apply these principles (33, 34). Nutrition education in this area might benefit from providing more common and practically relevant examples.

Additionally, knowledge on saturated fatty acids seems relatively low. This result reinforces international findings ascertaining knowledge deficits with regard to types of dietary fats (35, 36). Types of fat, their health implications and their sources could be another focus of nutrition education initiatives.

Although this research identified some important areas to address in nutrition education, it simultaneously indicates that an increase in nutrition knowledge alone will not substantially improve dietary behavior (see below).

Associations of Socio-demographic and Lifestyle Factors With Nutrition Knowledge

That nutrition knowledge is higher in women, normal weight and physically active persons as well as among those with higher

TABLE 4 | Nutrition knowledge (CoNKS Total and subscales) by nutrition and health behaviour[a] in participants of the NEMONIT study 2014/15[b].

	CoNKS Total		Procedural knowledge		Knowledge on nutrients		Knowledge on calories	
	Mean	***P***	**Mean**	***P***	**Mean**	***P***	**Mean**	***P***
Vegetarian (incl. pesco-vegetarian) diet		<0.001†		<0.001†		<0.001†		0.604
Yes	16.1		6.1		5.6		4.5	
No	14.0		4.9		4.8		4.4	
Dieting (e.g., to lose weight or due to an illness)		0.016		0.036		0.131		0.083
Yes	13.3		4.5		4.6		4.2	
No	14.1		4.9		4.8		4.4	
Healthiness of diet		0.182		0.105		0.173		0.923
Very healthy/ predominantly healthy	14.1		4.9		4.8		4.4	
Less healthy/ not healthy	13.8		4.7		4.7		4.4	
Subjective health status		0.096		0.044		0.200		0.646
Very good/ good	14.2		4.9		4.8		4.4	
Moderate/ poor/very poor	13.8		4.7		4.7		4.4	
Body mass index		<0.001†		<0.001†		<0.001†		0.655
Normal weight	14.6		5.2		5.0		4.4	
Underweight, overweight	13.7		4.7		4.7		4.4	
Sport activities		<0.001†		<0.001†		<0.001		0.022
Active	14.3		5.0		4.9		4.4	
Inactive	13.5		4.6		4.6		4.3	
Smoking status		0.079		0.052		0.059		0.355
Non-smoker/ex-smoker	14.1		4.9		4.8		4.4	
Smoker/occasional smoker	13.7		4.7		4.6		4.4	

[a]*Mann–Whitney U-test.*
[b]*NEMONIT study, survey year 2014/2015 (n = 1,505); CoNKS, consumer nutrition knowledge scale.*
†*Significant difference confirmed in multiple linear regression analyses controlling for sex, age in years, and socio-economic status.*

socio-economic status (or its indicators such as education or employment status) has already been observed in a number of earlier studies and was discussed previously (8, 10, 36–39).

The relationship between age and nutrition knowledge, however, was contradictory across studies (2, 8–10). Here, similar to Dickson-Spillmann et al. (2), age was negatively associated with nutrition knowledge. The CoNKS assessed knowledge based on insights and recommendations of the last years, e.g., to correctly reject the item "Fat is always bad for your health; you should therefore avoid it as much as possible" one must recognize that nowadays a distinction according to the type of fat is made. Older respondents might have more difficulties to obtain the necessary information and to integrate these into their already well-established concept of a healthy nutrition. Given the contradictory results on the association between age and nutrition knowledge, more research might be needed to examine if people in different age groups or stages of live have access to, understand and are able to practically implement the knowledge necessary to choose a healthy diet.

In this study, self-defined vegetarians (including pesco-vegetarians) compared to non-vegetarians had a higher nutrition knowledge. Up to now, there are only few and inconsistent studies on differences in nutrition knowledge among vegetarians and non-vegetarians (40). But Hoffman (40) argues that vegetarians often become "nutrition educators" since they are regularly confronted with nutrition-related questions, like where to get protein or iron in a vegetarian diet. However, the results of the present study should be interpreted with caution due to the low proportion of vegetarians.

Associations of Nutrition Knowledge With Food Consumption and Diet Quality

Participants with higher nutrition knowledge ate more favorable (e.g., vegetables, fruit/fruit products) and less unfavorable foods (e.g., meat/meat products) and showed a higher diet quality overall. Although significant associations in the expected direction were observed, the correlations between nutrition knowledge and food consumption were low in this study, also when compared to the validation study of the CoNKS (2). The results support those findings observing only a weak relationship between nutrition knowledge and dietary behavior (3). This

TABLE 5 | Association between nutrition knowledge (CoNKS Total and subscales) and food consumption (g/d)[a] in participants of the NEMONIT study 2014/15[b].

	CoNKS Total		Procedural knowledge		Knowledge on nutrients		Knowledge on calories	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
Bread	0.00	0.958	−0.03	0.227	0.03	0.227	−0.00	0.984
Cereals and cereal products	0.11	<0.001	0.14	<0.001	0.07	0.006	0.02	0.394
Potatoes and potato products	−0.06	0.029	−0.05	0.046	−0.00	0.860	−0.07	0.004[†]
Vegetables[c]	0.09	<0.001[†]	0.11	<0.001[†]	0.11	<0.001[†]	−0.04	0.173
Fruit and fruit products	0.10	<0.001[†]	0.10	<0.001[†]	0.09	<0.001[†]	0.01	0.630
Milk, dairy products, and cheese	0.12	<0.001[†]	0.07	0.009	0.13	<0.001[†]	0.06	0.023
Eggs	0.01	0.590	−0.01	0.635	0.03	0.300	0.02	0.514
Meat, meat products	−0.14	<0.001[†]	−0.14	<0.001[†]	−0.11	<0.001[†]	−0.05	0.070
Fish, fish products, and seafood	0.03	0.194	0.03	0.259	0.05	0.077	−0.01	0.588

[a] *Spearman's Rho correlations.*
[b] *NEMONIT study, survey year 2014/2015 (n = 1,505); CoNKS, consumer nutrition knowledge scale.*
[c] *Including vegetable products, mushrooms, and pulses.*
[†] *Significant association confirmed in multiple linear regression analyses controlling for sex, age in years, and socio-economic status index.*

indicates that an increase in nutrition knowledge alone seems unlikely to provoke large improvements in dietary behavior. Dietary behavior is complex and influenced by a number of different factors (41).

Types of Nutrition Knowledge

The separate analysis of procedural knowledge and knowledge on nutrients provided similar results as the analysis of nutrition knowledge in total. Knowledge on calories, however, seems to be a different kind of knowledge. Research on different types of knowledge is rare, but Grunert et al. (42) also observed that knowledge on the calorie content of foods was unrelated to knowledge on dietary recommendations and sources of nutrients. For future studies it might be helpful to examine the construct of nutrition knowledge and its dimensions more closely to get a better understanding of which types of knowledge might be relevant for a healthy dietary behavior in the population.

According to our results, knowledge on calories does not seem helpful in making healthy food choices. Contrary to what we would theoretically expect, it was not associated with BMI either. Knowledge on the caloric content of macronutrients, foods and meals might be too technical to be translated into a diet with adequate energy intake. Our results suggest that it might be advisable to include more information on, for e.g., the contribution of a meal to a balanced diet, in the commonly used media rather than just information on calories.

Strengths and Limitations

This study has several strengths. First, it explored nutrition knowledge based on a large sample of the German adult population. This allowed using multivariate analyses to examine group differences and associations independent from socio-demographic factors. Second, the study used a scale which showed a good ability to distinguish between nutrition-literate and lay respondents. Although some essential modifications were made, low associations between nutrition knowledge and dietary behavior are unlikely to result from incapacity of the scale to distinguish between participants with different grades of nutrition knowledge. The measurement also allowed investigating both declarative and procedural nutrition knowledge. Low associations therefore cannot be attributed to a mere assessment of declarative knowledge, which was assumed by some authors to have a lower relevance for dietary behavior (2, 3). Third, food consumption was assessed with two 24-h recalls, which is in accordance with the requirements of the European Food Safety Authority regarding collection of national food consumption data (43). Fourth, the healthy eating index was calculated from actual food consumption as an outcome variable to represent compliance with dietary guidelines. This was supposed to be an outcome more closely related to nutrition knowledge, but still associations prove to be low.

Some limitations of the study also need to be considered. First, the study sample was biased toward women, older persons and persons with a higher SES (23) and respondents who took part in NEMONIT for several years might be especially interested in nutrition topics. Based on this sample, nutrition knowledge might be overestimated and the discussed deficits in nutrition knowledge might be higher in the general population.

Second, the measurement of nutrition knowledge includes some uncertainties. Although nutrition knowledge was measured using a previously validated instrument, Cronbach's Alpha, which is used to assess the internal consistency of a scale, was low ($\alpha = 0.59$). The value could not be substantially increased by deleting an item and the correlations between some items were very low. This could indicate that nutrition knowledge is a heterogeneous construct with different dimensions. Another

TABLE 6 | Associations of socio-demographic characteristics and nutrition knowledge (CoNKS Total and selected subscales) with HEI-NVS II[a] in participants of the NEMONIT study 2014/15[b].

	Unstandardized regression coefficient	P
Model: socio-demographic characteristics only (Adj. R^2 = 0.024)		
Female sex	2.986	<0.001
Age (in years)	0.055	0.003
SES index	0.177	0.019
Model: socio-demographic characteristics and nutrition knowledge (CoNKS Total) (Adj. R^2 = 0.051)		
Female sex	2.391	<0.001
Age (in years)	0.080	<0.001
SES index	0.023	0.760
CoNKS Total	0.620	<0.001
Model: socio-demographic characteristics and CoNKS subscale procedural knowledge (Adj. R^2 = 0.043)		
Female sex	2.407	<0.001
Age (in years)	0.077	<0.001
SES index	0.065	0.400
Procedural knowledge	0.924	<0.001
Model: socio-demographic characteristics and CoNKS subscale knowledge on nutrients (Adj. R^2 = 0.051)		
Female sex	2.630	<0.001
Age (in years)	0.064	<0.001
SES index	0.052	0.500
Knowledge on nutrients	1.289	<0.001

[a]Multiple linear regression analysis with HEI-NVS II as dependent and socio-demographic characteristics and nutrition knowledge (CoNKS Total), respectively, CoNKS subscales procedural knowledge and knowledge on nutrients as independent variables.
[b]NEMONIT study, survey year 2014/2015 (n = 1,505); SES, socio-economic status; CoNKS, consumer nutrition knowledge scale; HEI-NVS II, Healthy Eating Index of the German National Nutrition Survey II.

possible explanation for a low Cronbach's Alpha would be a very homogenous sample. As previously mentioned, NEMONIT respondents might consistently have a higher interest in nutrition topics. Nutrition knowledge in the sample was high with a relatively low standard deviation. This might restrict the ability of the study to find large associations between nutrition knowledge and dietary behavior.

Finally, it is important to note that no causal relationships can be implied from the cross-sectional analysis.

CONCLUSION

The present study identified areas for knowledge enhancement in the assessment of the health benefits of fruit and vegetable consumption, in the understanding of the concept of a balanced diet as well as regarding the knowledge on saturated fatty acids. These topics might be most relevant for future nutrition education efforts. However, this study also supports a number of previous studies observing significant but weak associations between nutrition knowledge and dietary behavior. This indicates that an increase in nutrition knowledge through nutrition education alone is unlikely to provoke large improvements in dietary behavior. From health and sustainability literature, it is well-known that knowledge is usually not directly translated into action. Instead, behavior is complex and influenced by a number of different factors. Research should find ways to address the complexity of dietary behavior and to identify the most important factors that need to be addressed to improve dietary behavior of the population.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because according to the regulations for the use of NVS II- and NEMONIT-study data, the datasets of the NEMONIT study are only available for universities, public and/or publicly funded scientific research institutions. Furthermore, a general essential prerequisite is the pure scientific use of the data, excluding any commercial use of the data and of the derived results. Requests to access the datasets should be directed to the corresponding author, ingrid.hoffmann@mri.bund.de.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FK analyzed and interpreted the data and drafted the manuscript. IH was involved in data interpretation and manuscript preparation. EC initiated and conceptualized the research, was involved in data interpretation and manuscript preparation and was responsible for the final content. All authors read and approved the final version of the manuscript.

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Investigating the Effectiveness of an Educational Escape Game for Increasing Nutrition-Related Knowledge in Young Adolescents: A Pilot Study

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Objective: As a pilot trial under the Games of Food consortium, this study assessed the effectiveness of an educational escape game alongside a self-study method as a nutrition knowledge intervention. Furthermore, this study explored the use of an escape game as an educational tool for young adolescents.

Materials and Methods: Altogether three schools participated, one from Finland and two from the UK. Baseline questionnaires assessing knowledge were administered before intervention day. Participants from each class were randomly allocated by the researchers into either the escape game condition, where participants played a nutrition education escape game with a focus on plant-based protein sources, or the self-study condition, where participants received an educational leaflet with identical content. In addition to the knowledge post-assessment, the educational escape game condition answered an enjoyment and intrinsic motivation questionnaire to evaluate the game experience. Paired *t*-tests were used to determine significant changes within intervention conditions and ANCOVA was used to estimate the differences in knowledge.

Results: The participants were 130 children (11–14 years), divided into educational escape game ($n = 68$) and self-study ($n = 62$) conditions. Both the educational escape game (20.7 vs. 23, $p < 0.001$) and self-study (21.1 vs. 23.1, $p = 0.002$) had improved overall knowledge scores. No significant differences in gained knowledge existed between groups. Of the educational escape game participants, 60% reported the game as mostly enjoyable and 46% reported added use and value for learning.

Conclusion: The educational escape game condition was comparable to the self-study method for nutrition education in adolescents. However, since the educational escape game provides an enjoyable experience that may enhance intrinsic motivation to promote learning and possible behavior change, the use of escape games for nutrition education warrant further investigation.

Keywords: escape game, educational game, game-based learning, nutrition education, nutrition knowledge, school-aged children

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INTRODUCTION

The contribution of diet to health and wellbeing is irrefutable. A healthful diet is particularly important for young adolescents, who are in a crucial pubertal phase of development and growth (1). Though many adolescents do not follow recommended dietary guidelines (2–4), many exceed protein intake recommendations and have high consumption of animal-based protein, which has been associated with higher weight status (5). Lasting diet patterns and food preferences are formed during childhood, therefore targeting youth at an age where autonomy in food choice is reachable would further promote healthy dietary habits into adulthood (6, 7). Effective intervention strategies to reverse the growing trends in childhood obesity and improve diet quality remain high priorities in public health (8).

Increasing nutrition knowledge and interest are typical strategies for nutrition intervention and important prerequisites for eliciting diet-related behavior changes (9–13), though their impact may be weak (11). Activities that improve food literacy, which encompasses nutrition knowledge, have shown to be effective in improving dietary behaviors among adolescents (12, 13). Nutrition knowledge can include a plethora of different concepts, not all of which are relevant for adolescence in the general population. Previous studies have varied in their inclusion of nutrition knowledge concepts and methods of measuring change in knowledge (8, 12–14), highlighting the need for further investigation to explore how different interventions can support different concepts of nutrition knowledge in varied age groups. Educational games have shown success in health promotion, with meta-analyses finding positive effects in knowledge, attitude, behavior, and biological indicators from board games (15, 16), as well as digital games (16, 17). Though many educational game results go unpublished (18), studies evaluating specific games for nutrition education have shown improvement in game-related knowledge immediately (19, 20) and up to 1 year after gameplay (21) among young adolescents. Game-based education may enhance learning opportunities by positively affecting motivation and interest of participants (18, 22, 23). A recent review found that game-based education, especially those with compelling and immersive storylines, can have an impact on children's eating behaviors (16).

Escape games are once-played, team-based games that physically immerse players in a narrative to reach an ultimate goal (typically escape), by solving standard game mechanics within time-pressured environments. The challenges of an escape game make for memorable and rewarding game-play, which are elements particularly suited for behavior change directives (16, 24).

Escape games have become popular over the last decades, with educational escape games recently gaining more traction. Nevertheless, only a handful of studies on the effectiveness and efficacy of educational escape games exist, and all have been conducted in adult populations. Studies have reported both increased (25–27) and decreased (28) knowledge scores after gameplay. Furthermore, all studies lack comparison with traditional or passive learning methods, such as self-study. Remarkably, the handful of existing adult studies utilizing educational escape games all report positive feedback in learning and enjoyment (25–29).

The lack of consistent evidence of the effectiveness of educational escape games as mean to increase in knowledge warrants further research. Though commercialized applications of (digital and physical) educational escape games in primary and secondary education classrooms exist, to this day there are no peer-reviewed publications on either nutrition interventions or school-aged children regarding the effectiveness of educational escape games.

The aim of this intervention trial was to pilot the assessment and compare the effectiveness of the educational escape game, Zombie Attack, with self-study material for increasing short-term nutrition knowledge of 11–14 year old children. Another aim was to identify if an educational escape game was an appropriate pedagogical tool for school-aged children.

MATERIALS AND METHODS

This binational pilot study was conducted in 11–14 year old children from Helsinki, Finland and Reading, United Kingdom (UK) as a part of a larger project, Games of Food (www.gameoffood.com). Intervention materials were designed by the partners of the Games of Food consortium, which comprises of researchers and developers from Technion Institute in Israel, University of Warsaw in Poland, University of Reading in UK, University of Helsinki in Finland, and the European Food Innovation Commission (EUFIC) in Belgium. The project was financially sponsored by EIT Food, a subset of the European Institute of Innovation and Technology (EIT) funded by the Horizon 2020.

Two school classes from Finland were recruited based on teacher interest and an invitation to participate was provided for every subsequent student and guardian. The participants from Finland consisted of an eighth (12–13 years old) and ninth (13–14 years old) grade class from a school specializing in physical education. Two schools from UK, with classes from seventh (11–12 years old) and eighth (12–13 years old) grades participated. All participants were randomly allocated within their classes to participate either in the educational escape game or the self-study condition. In Finland, students conducted the intervention conditions in separate rooms, with only one team in the educational escape game condition room at a time. In the UK, intervention conditions for each class were conducted simultaneously within a large gymnasium, where students were spatially separated. Background data, as well as baseline knowledge, were assessed prior to intervention periods. The background questionnaire inquired whether or not participants had previous experience with escape rooms or any dietary restrictions, along with basic demographic information, including age, gender, and date of birth.

All students that had provided written informed consent and written parental informed consent were eligible to participate. The study was reviewed by the Ethical Review Board in the Humanities and Social and Behavioral Sciences of the University of Helsinki (statement 10/2019) and the University Research Ethics Committee of the University of Reading (statement 19/20).

Interventions

Both educational escape game and self-study material presented key information on healthy diet with a focus on plant-based protein intake. Educational content centered around nine nutrition topics: (1) food pyramid, (2) macronutrients, (3) food energy calculation, (4) protein recommendations, (5) essential amino acids, (6) complete proteins, (7) complementary proteins, (8) functions of protein in the body, and (9) sustainable sources of protein-rich foods. Nutrition researchers from the University of Helsinki reviewed the content for nutrition education.

Educational Escape Game Condition

The escape game Zombie Attack is a portable game designed to engage players with a post-apocalyptic storyline that is meant to create a sense of alarm and urgency during gameplay. The players are encouraged to work together as a team to save themselves and humanity by learning about a healthy and sustainable diet, as it is the only repellent against the zombie attacks. Each of the nine linear puzzles focuses on one aforementioned nutrition topic and plays a role in the elaborate storyline. Puzzle mechanics incorporate matching, padlocks, cipher, videos, and puzzles that require players to calculate energy, identify correct food sources, read nutrition labels, and evaluate recipes. Zombie Attack was developed in English and later translated into the Finnish language. Posters and infographics unrelated to the puzzle mechanics were placed around the team to maximize exposure to the nutrition education content throughout the gameplay.

Teams of 3–5 players were drawn within the participating classes. The interventions were allocated for 1 h. Study researchers supervised the gameplay and provided technical advice to teams only when necessary for game progression.

Self-Study Condition

The participants in the self-study condition were each provided material in leaflet form with the same nutritional messages as in the educational escape game. In Finland, each participant individually read the material of the intervention. In the UK, participants were individually given copies of the leaflet to read alone, but were allowed to sit and discuss in groups of 3–5 children.

Outcome Measures

Nutrition Knowledge

A questionnaire assessing knowledge concerning nutrition concepts was administered ∼1 week before the intervention sessions (baseline questionnaire), and immediately repeated after the intervention (post questionnaires). Questionnaires were created based on previously validated questionnaires from the General Nutrition Knowledge Questionnaire-revised (30) and a nutrition knowledge questionnaire for young athletes (31). Questions selected were, if necessary, altered to reflect the learning objectives relevant to healthy diet that were appropriate for the age group participating in the study with emphasis on plant-based proteins. The knowledge questionnaire consisted of 41 nutrition-related multiple choice and true or false questions. Knowledge scores were evaluated by summing 1 point for every correct answer and none for "unsure" or incorrect answers. The knowledge questionnaire was divided by topic for sub-analysis based on the centralized topics of the nutrition knowledge questionnaire: protein (14 questions), sugar (8 questions), fiber (9 questions), and reduced disease risk (10 questions).

Intrinsic Motivation

All educational escape game participants received an additional post intervention questionnaire to evaluate the game. The 14-item questionnaire, adapted from the intrinsic motivation inventory (IMI) (32), consisted of two 7-point Likert-scale (1 = not at all true, 7 = very true) domains based on the original IMI interest/enjoyment and value/usefulness subscales. This two-part questionnaire was used to assess enjoyment and intrinsic motivation, the latter which was defined as the usefulness and added value to one's self as perceived from the escape game.

Statistical Analyses

Participants who completed baseline questionnaires and post knowledge questionnaires were used in the analysis, with the exception of the intrinsic motivation questionnaire analysis that included four additional participants who completed the educational escape game intervention without baseline data. Chi-square tests (categorical variables) and t-tests (continuous variables) were used to test significance between intervention conditions in baseline data. Paired t-tests were used to determine significant changes within intervention conditions. Differences in knowledge between intervention conditions were compared using analysis of covariance, which adjusted for baseline knowledge, age, and gender. Further adjustment for previous participation in an escape game and adhering to a special diet did not appreciably change the results. Internal consistency of knowledge subdomains, enjoyment, and intrinsic motivation were analyzed by Cronbach's alpha. Two-tailed $P < 0.05$ were considered statistically significant. All data was analyzed using free statistical software R (R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/).

RESULTS

A total of 159 school-aged children were enrolled in the trial, of which 82% completed baseline and post-nutrition knowledge questionnaires (**Figure 1**). Of those present for both baseline and intervention days ($n = 130$), the mean age was 13.3 years (SD 1.2) and 81% were girls without significant differences between baseline nutrition knowledge, previous escape game experience, or adherence to a special diet between the educational escape game and self-study intervention conditions (**Table 1**).

Over half of the study participants were from UK (65%), with mean age (12.4 ± 0.4) and knowledge score (18.9 ± 6.8) in UK considerably lower than the age (14.8 ± 0.6) and knowledge score (24.5 ± 4.9) among participants from Finland. From UK, 98% of participants were girls, and in Finland 50%. From participants that completed both baseline and post questionnaires, 2.5% of

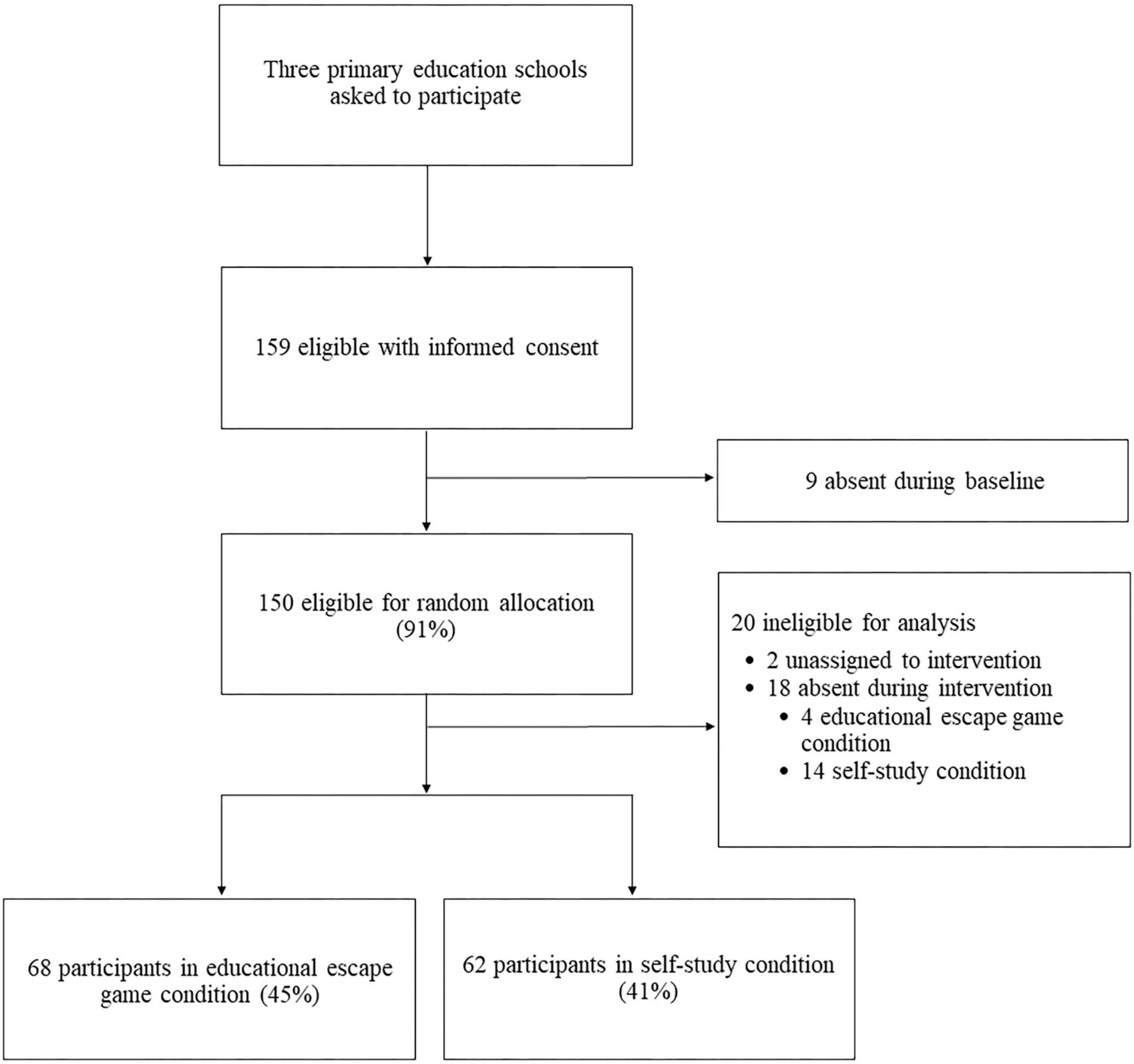

FIGURE 1 | Flow diagram of study participants.

knowledge questions in baseline and 0.8% in post questionnaires were left unanswered.

Improvement between baseline and post intervention nutrition knowledge average score significantly improved within both educational escape game [2.27 points, 95% CI (0.96, 3.57), $p < 0.001$] and self-study [2.03 points, 95% CI: (0.81, 3.25), $p = 0.002$] conditions. However, there was no significant difference in overall nutrition knowledge gained between the two intervention conditions (**Table 2**). Analysis of knowledge subdomains observed the participants in the educational escape game condition to have less improvement in post-intervention questions related to protein compared to the self-study condition participants, with differences between conditions estimated as −0.82 points [95% CI: (−1.56, −0.08), $p = 0.03$]. Conversely, educational escape game participants had a better improvement in fiber-related questions compared to the participants in the self-study condition, with differences between conditions estimated as 0.63 [95% CI: (0.09, 1.17), $p = 0.02$]. No differences between conditions were observed in sugar and reduced disease risk knowledge subdomains. Internal reliability of knowledge subdomains was moderate in sugar ($\alpha = 0.63$) and fiber ($\alpha = 0.69$), and moderately high in protein ($\alpha = 0.77$) and reduced risk of disease ($\alpha = 0.80$).

TABLE 1 | Baseline data by intervention condition.

	Educational escape game ($n = 68$)	Self-study material ($n = 62$)	Total ($n = 130$)
	total *N*(%) or mean ± SD	total *N*(%) or mean ± SD	total *N*(%) or mean ± SD
Age (years)	13.24 ±1.24	13.16 ± 1.20	13.25 ± 1.22
Gender			
Girls	55 (82.1%)	57 (81.4%)	105 (80.8%)
Boys	12 (17.9%)	13 (18.6%)	25 (19.2%)
Country			
United Kingdom	38 (64.2%)	48 (68.6%)	84 (64.6%)
Finland	24 (35.8%)	22 (31.4%)	46 (35.4%)
Participants with previous escape game experience	18 (27.3%)	8 (12.9%)	25 (19.2%)
Participants with a special diet	12 (18.2%)	19 (30.2%)	29 (22.3%)
Baseline nutrition knowledge[1]	20.69 ± 6.55	21.07 ± 7.07	20.87 ± 6.78

[1] *Number of correct answers from 41-question nutrition knowledge questionnaire.*

TABLE 2 | Comparison of mean baseline and post-intervention knowledge scores in educational escape games and self-study intervention conditions.

Knowledge outcome	Number of Questions	Educational escape game ($n = 68$)		Self-study material ($n = 62$		Estimated difference[1] (95% CI)	*P*-value
		Baseline	Post-intervention	Baseline	Post-intervention		
Overall nutrition knowledge	41	20.69 ± 6.55	22.96 ± 7.09	21.07 ± 7.07	23.09 ± 7.63	0.08 (−1.63, 1.78)	0.93
Sugar	8	4.77 ± 1.53	5.78 ± 1.92	4.86 ± 1.34	5.79 ± 1.79	0.05 (−0.49, 0.60)	0.85
Fiber	9	4.10 ± 1.79	4.84 ± 1.87	4.44 ± 1.96	4.40 ± 1.99	0.63 (0.09, 1.17)	**0.02**
Protein	14	6.54 ± 2.69	7.06 ± 2.78	6.15 ± 2.73	7.57 ± 3.06	−0.82 (−1.56, −0.08)	**0.03**
Reduce disease risk	10	4.84 ± 2.26	6.12 ± 2.61	5.24 ± 2.58	6.23 ± 3.03	0.18 (−0.54, 0.89)	0.63

[1] *Estimated difference between adjusted means of educational escape game and self-study post intervention scores. ANCOVA model adjusted for baseline knowledge, gender, and age. Statistically significant ($p < 0.05$) values are in bold.*

All educational escape game participants ($n = 72$) were able to escape within the allocated hour and generally reported high (mean ± SD = 5.8 ± 1.1) enjoyment with questions from a Likert 7-point scale, with mean interquartile range between 5.1 and 6.8. A majority (60%) of participants reported the game as mostly or completely enjoyable and 32% somewhat enjoyable. Questions assessing intrinsic motivation from usefulness and value found respondents to perceive the game as beneficial (5.5 ± 1.2), with mean interquartile range between 4.4 and 6.5. Slightly less than half (46%) of the participants reported the game as mostly or completely useful and valuable, followed by 44% reporting the game as somewhat useful and valuable (see **Table 3**).

DISCUSSION

This pilot study explored the effectiveness of nutrition education tools in young adolescents comparing an educational escape game with traditional self-study learning method. The results found that both educational escape game and self-study intervention conditions showed significant improvements in short-term knowledge when comparing baseline and post-intervention nutrition knowledge assessments, though no appreciable differences were found between the two conditions.

The results of this study corroborate the findings from previous studies gamifying health education. A meta-analysis found the positive effect size of health promotional board games on knowledge large (Cohen's $d = 0.82$, 95%CI: [0.15, 1.48]) and on behavior moderate [Cohen's $d = 0.38$, 95% CI (0.07, 0.69)] (15). Much like the present study, a recent study on school-aged children that piloted the digital game "Fit Food Fun" with traditional teaching techniques found both gaming and traditional methods to be effective tools for short-term improvement in nutrition-related knowledge (20).

Unlike the present study, the ETIOBE Mates digital game was reported to have had higher improvement in nutrition-related knowledge scores among young adolescents when compared to a leaflet control condition after 2-weeks of

TABLE 3 | Assessment of enjoyment and intrinsic motivation questionnaire items of all educational escape game condition participants (*n* = 72).

Domains and questions[1]	Not true	Somewhat true	True	Mean score SD	Cronbach's Alpha
Enjoyment total	8%	32%	60%	5.81 ± 1.09	0.88
I enjoyed doing this activity very much	7%	31%	62%	6.14 ± 1.03	
This activity was fun to do	3%	42%	55%	6.01 ± 1.24	
I thought this was a boring activity (answer reversed)	3%	20%	77%	6.35 ± 1.17	
This activity did not hold my attention at all (answer reversed)	10%	21%	69%	5.98 ± 1.56	
I would describe this activity as very interesting	7%	43%	50%	5.77 ± 1.27	
I thought this activity was quite enjoyable	13%	30%	57%	5.86 ± 1.45	
While I was doing this activity, I was thinking about how much I enjoyed it	15%	37%	48%	5.23 ± 1.70	
Intrinsic motivation total	10%	44%	46%	5.45 ± 1.24	0.91
I believe this activity could be of some value to me	10%	49%	41%	5.38 ± 1.37	
I think that doing this activity is useful for learning about nutrition and health	14%	37%	49%	5.44 ± 1.66	
I think this is important to do because it can teach me about nutrition but also allows me to have fun	7%	41%	52%	5.75 ± 1.39	
I would be willing to do this again because it has some value to me	8%	36%	56%	5.74 ± 1.44	
I think doing this activity could help me to think about what foods I choose to eat	15%	47%	38%	5.20 ± 1.63	
I believe doing this activity could be beneficial to me	8%	51%	41%	5.49 ± 1.42	
I think this is an important activity	8%	45%	47%	5.54 ± 1.41	

[1]*Based on Likert scale 1–7 (1 = not at all true, 4 = somewhat true, and 7 = very true). 1–3 = not true, 4–5 = somewhat true, and 6–7 = very true.*

intervention (19). A cluster-randomized controlled trial of 20 schools studied the effects of the health promotional board game, Kaledo, in school-aged children when exposed to short gameplay once a week for 20-weeks. Regarding nutrition knowledge assessment, researchers found sustained improvement 6-months and 18-months post intervention compared to a non-treatment control among adolescents in middle school (21). These controlled intervention trials suggest that gamed-based learning may be able to provide additional learning opportunities when reinforcing traditional teaching methods.

The results from this pilot study begin to address gaps in the literature by investigating escape games as educational tools in school-aged children and as nutrition intervention tools. There are only a few studies measuring the effectiveness of educational escape games, none of which study adolescents or children. Preliminary results presented in a conference observed the Zombie Attack educational escape game to increase measured nutrition-related knowledge in 228 adults across five countries (26). A diabetes management escape game for pharmacy students ($n = 74$) found significant improvement in knowledge comparing pre and post assessment scores (25). A more recent escape game trial with nursing students found improvement in most knowledge questions after gameplay, though statistical significance was not calculated (27). On the other hand, a study on 63 pharmacy students reported that participants had a lower score in knowledge after the post intervention assessment, even though 96% stated they felt the game facilitated learning and improved their clinical skills. However, the authors hypothesized that the observed decrease in knowledge was due to misplaced incentive, as pre-assessment results weighed for about a third of the class grade and post-assessment results were unsubstantial (28).

The participants of the educational escape game condition reported to having enjoyed the game and generally perceived the game as beneficial and of value for them. This coincides with all descriptive studies on escape games, which report tremendously positive feedback for enjoyment and their perceived potential to facilitate learning and motivation (27, 29, 33–38). It is theorized that the increased intrinsic motivation with gaming stems from immersion, high concentration, challenges and accomplishments throughout the gameplay experience (17, 22), which are quintessential qualities of escape games. Teaching tools that stimulate intrinsic motivation and are enjoyable have the potential to facilitate further learning, which is particularly useful in school settings where various educational approaches can be utilized. Moreover, there is evidence that active modes of education (e.g., interactive game-based learning) are more effective than passive modes (e.g., self-study) and that active education is generally more suitable for teaching (39).

The intervention content had a focus on promoting healthy and sustainable plant-based protein sources, which may have influenced the overall positive gain in knowledge. A study on adolescents across Europe observed interventions that promoted healthy habits were more accepted than those that discouraged unhealthy dietary habits (40). The present intervention attentively promoted and educated participants on healthful plant-based protein sources and their role in environmental sustainability, which can be more impactful to youth motivation in comparison to future compromised health status. A double-blind randomized controlled trial found that, in eighth graders, autonomy, and social justice fueled healthful choices more than personal health implications (41). This indicates that interventions focusing on sustainability and planetary health may be most appropriate for peaking motivation and eliciting behavior changes in adolescents. Furthermore, it suggests that the dietary intervention programs for adolescents should differ compared to adults, with less emphasis on personal health gain and more on providing opportunity for autonomous choices and increased environmental and societal health.

Subdomain analysis of nutrition knowledge topics found that the educational escape game condition did not significantly improve knowledge in protein-related questions, where the self-study intervention condition did. Considering the intervention materials had a focus on plant-based protein sources, the effectiveness of internalizing key messages may be muddled in gameplay in comparison to self-study methods, where the main messages are readily identifiable. Prospective educational escape game designers should consider repetition of highlighted objectives from the overarching themes throughout the gameplay. Future studies on educational escape games should investigate if the simulated panic in storyline hinders the internalization of key messages. Moreover, learning mechanisms should be studied in more detail to understand which types of puzzles result in the most effective learning outcomes.

Strengths of this study include the binational and comparative design, the random allocation by class, and the homogeneity between intervention conditions. Questionnaires were adapted from previously validated questionnaires and, though not validated for the current study population, the nutrition knowledge questionnaire produced similar baseline results to a questionnaire validated for 12–14 year old adolescents aged 12–14 in Italy (14). Bias regarding classroom heterogeneity was alleviated by collecting data for both interventions from every classroom. Therefore, any baseline differences between classrooms were likely equally distributed between the intervention conditions. In addition, the escape game Zombie Attack had been pretested on adults and the results were used to adjust the prototype and remove inconsistencies. Likewise, the escape game has already shown positive results for effectiveness in knowledge assessment with adult populations in preliminary pilot studies (26).

When compared to other game-based interventions, a general limitation to an educational escape game is that the intervention can only be administered once, due to the unrepeatable nature of escape games. This pilot intervention study has many additional limitations. For instance, these intervention results cannot be extrapolated to long-term gains in nutritional knowledge, since only results from short-term knowledge change were measured. This pilot sample size was rather small in number, with 18% of participants with missing the baseline or intervention data. Initially, teachers were invited to participate in the study, introducing a selection bias, as participating classrooms may have more motivated teachers inclined to trial innovative learning techniques. Likewise, selection bias may also be present in students and parents, as only those with written parental consent were permitted to participate. Though participants were advised not to discuss interventions during the intervention day, it is not possible to completely rule out cross-contamination outside the monitored intervention conditions. Moreover, since UK class participants conducted the interventions in the same area and time, cross-contamination between and within intervention conditions was possible.

Country specific differences are also noteworthy. The Finnish study sample was about a year older than the intervention mean and the participants all attended school with a physical-education focus, which likely contributed to the higher mean assessment results in Finland compared to UK. Unlike in Finland, the UK sample consisted mostly of girls (98%) due to the proportion of girls in the participating classrooms, and, thus, strongly skewed the overall study population to be overrepresented by girls (81%). Future trials should consider a more gender-equivalent study population, as young adult and adolescent females may have more interest and motive for healthy eating compared to males (42). Likewise, the participants from Finland may not have been representative of typical baseline nutrition knowledge for that age, as they attended a school specialized in physical education and, therefore, may have more knowledge on health-related topics. Lastly, it is notable that nutrition knowledge may vary due to cultural differences between Finland and the UK. However, the lack of difference in overall gained knowledge between the two countries suggests that these underlying differences in population should not affect potential for positive knowledge improvement.

This binational pilot study found the use of an educational escape game as sufficient as traditional self-study methods for teaching school-aged children about nutrition. Both intervention conditions showed improvement in nutritional knowledge between baseline and post-interventions. These findings provide evidence that game-based teaching techniques can offer comparable intervention strategies for improving diet-related knowledge compared to traditional, self-study teaching methods. This study also found educational escape games to be an effective and encaptivating tool for nutrition education in school-aged children. Since this pilot study was unable to assess behavioral changes and long-term retention of gained knowledge, future intervention trials are needed to evaluate the potential benefits of educational escape games in both nutrition knowledge and dietary behavior change.

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 Ethical Review Board in the Humanities and Social and Behavioral Sciences of the University of Helsinki (statement 10/2019) and the University Research Ethics Committee of the University of Reading (statement 19/20). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

NM, HV, and MF acquired the data and conducted the research. AA analyzed the data and drafted the manuscript. NM, HV, MM, MG, and MF critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Sources of Added Sugars Intake Among the U.S. Population: Analysis by Selected Sociodemographic Factors Using the National Health and Nutrition Examination Survey 2011–18

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Recent estimates of added sugars intake among the U.S. population show intakes are above recommended levels. Knowledge about the sources of added sugars contributing to intakes is required to inform dietary guidance, and understanding how those sources vary across sociodemographic subgroups could also help to target guidance. The purpose of this study was to provide a comprehensive update on sources of added sugars among the U.S. population, and to examine variations in sources according to sociodemographic factors. Regression analyses on intake data from NHANES 2011–18 were used to examine sources of added sugars intake among the full sample ($N = 30,678$) and among subsamples stratified by age, gender, ethnicity, and income. Results showed the majority of added sugars in the diet (61–66%) came from a few sources, and the top two sources were sweetened beverages and sweet bakery products, regardless of age, ethnicity, or income. Sweetened beverages, including soft drinks and fruit drinks, as well as tea, were the largest contributors to added sugars intake. There were some age-, ethnic-, and income-related differences in the relative contributions of added sugars sources, highlighting the need to consider sociodemographic contexts when developing dietary guidance or other supports for healthy eating.

Keywords: added sugars, food sources, sociodemographics, NHANES, US

INTRODUCTION

Recommendations on the intake of added sugars are generally used to inform population-wide dietary guidance. The Institute of Medicine has suggested a maximum daily limit of 25% of calories from added sugars (1). More recent recommendations are lower, with the World Health Organization issuing a guideline of <10% of calories per day from “free sugars” (inclusive of both added sugars and sugars naturally present in 100% fruit juice) and a conditional recommendation for a further reduction to <5% (2). The 2020–2025 Dietary Guidelines for Americans recommend limiting foods and beverages higher in added sugars, and that a healthy dietary pattern limits added sugars to <10% of calories (3).

Estimates of added sugars intake for the U.S. population show that recent intake levels are above current recommendations; however, they are lower than previous intakes: population-wide (2+ years) daily average added sugars intake was approximately 18% of calories in 1999 to 2000 (4) compared to 13% among individuals 1+ years in 2015 to 2016 (5). However, examining added sugars intake levels without considering the sources of added sugars is not sufficient to inform dietary guidance. Furthermore, understanding how added sugars in the diet may vary across sociodemographic subgroups could also help to develop more targeted dietary guidance.

Sources of added sugars and sociodemographic factors have been examined in conjunction with added sugars intake. Such investigations have been carried out for all age groups across the U.S. population using NHANES 2009–12 (6) and for younger age groups using NHANES 2011–14 (7, 8); and results from these studies have shown that sweetened beverages and sweet bakery products are the top two dietary sources of added sugars. Based on later NHANES 2013–16 data, sweetened beverages remain the top source for most age groups (9). However, recent studies of added sugars sources and sociodemographic factors other than age and gender are limited. Factors such as ethnicity and income have been examined previously (data from 1994 to 2010) but mainly in children (10–12) or other specific cohorts (13, 14).

The purpose of the present study was to provide a comprehensive update on sources of added sugars intake among the U.S. population using NHANES 2011–18 and to examine variations in added sugars sources according to the selected sociodemographic factors: age; gender; ethnicity; and, income.

MATERIALS AND METHODS

Data Source and Participants

The health and nutritional status of the U.S. population is monitored regularly through the NHANES, a cross-sectional survey conducted by the Centers for Disease Control and Prevention National Center for Health Statistics (15). The survey sample is selected through a clustered stratified multistage sampling design, with periodic oversampling of select population groups, and is representative of the non-institutionalized civilian resident population. Data collection for the dietary interview component of NHANES, called What We Eat in America (WWEIA), is conducted by the U.S. Department of Agriculture (USDA) Food Surveys Research Group (FSRG) (16). Dietary interviews are conducted by trained interviewers using the Automated Multiple Pass Method: for children 2–5 years, diet interviews are conducted with a proxy; those 6–11 years receive help from a proxy; and, individuals 12+ years complete the interviews themselves. The first 24-h dietary recall interview is conducted in person and a second 24-h recall is conducted by telephone 3 to 10 days later on a subsample of participants. Details of the NHANES survey design and dietary data collection procedures are reported elsewhere (15, 16).

We used data from four cycles of NHANES (2011–12, 2013–14, 2015–16, and 2017–18) to provide large enough sample sizes for specific groups. Analyses were conducted of added sugars sources among all age groups (2–8, 9–18, 19–50, 51–70, and 71+ years) and by gender, ethnicity, and income for those 2–18 and 19+ years.

NHANES 2011–18 procedure and protocols (#2011–17, #2018–01) were reviewed and approved by the NCHS Research Ethics Review Board. Ethical review and approval were waived for this study, due to the use of secondary data. NHANES obtained written, informed consent for all adult participants.

Added Sugars Intake and Sources

The Food Patterns and Equivalents Database (FPED), developed by the USDA FSRG, converts foods and beverages reported in the 24-h recalls into food pattern equivalents corresponding to those in the Dietary Guidelines for Americans (17). The "added sugars" food pattern component is comprised of caloric sweeteners (syrups, sugars, and others) using the definition of added sugars as "sugars that are added to foods as an ingredient during preparation, processing, or at the table; added sugars do not include naturally occurring sugars such as lactose present in milk and fructose present in whole or cut fruit and 100% fruit juice" (17), which are similarly defined for the nutrition labeling of food (18). While this definition of added sugars has been stable over time, fruit juice concentrates not diluted to single strength juices have been designated as added sugars since 2011–12, which may affect added sugars values for foods such as snack bars, ready-to-eat (RTE) cereals, baby foods, and fruit spreads. More detail on determining added sugars content of foods is provided in FPED documentation (17).

We determined added sugars intake using the FPED for each NHANES cycle. Day 1 intake data were used as these data were collected in person; whereas day 2 intakes were collected by telephone, and a validation of this method, to our knowledge, has never been reported. Furthermore, a single day of intake is sufficient for providing an accurate estimate of population mean intake (19), which was required for our analyses. Mean added sugars intake as a percentage of total calories was calculated for each age group (2–8, 9–18, 19–50, 51–70, and 71+ years) and for two overall age groups (2–18 and 19+ years) using the population ratio method, which required summing the daily added sugars intake for all individuals in a particular age group, and then dividing by the sum of daily calorie intake for the same individuals. The population ratio method was used because it provides an unbiased estimate of population intakes when using 1 day of intake data (20).

In order to facilitate analyses of population food intakes, USDA uses a food grouping scheme for WWEIA, in which foods and beverages are grouped according to their similar nutrient content and common use in the diet; and individual food categories can be combined into larger food groups if required for analytical purposes (21). We analyzed sources of added sugars based on the USDA/WWEIA food categories (**Table 1**). Within each of the food categories and larger food groups, mean added sugars contribution expressed as grams was determined, and then percentage of total daily added sugars intake was calculated using the population ratio method; food sources were then ranked from highest to lowest.

TABLE 1 | Breakdown of food groups into types of foods (categories[a]) that provide added sugars.

Food group	Food category
Breads, Rolls, Tortillas	Yeast breads; Rolls and buns; Bagels and English muffins; Tortillas
Candy	Candy: containing chocolate; not containing chocolate
Coffee and Tea	Coffee; Tea
Fats and Oils	Butter and animal fats; Margarine; Cream cheese, sour cream, whipped cream; cream and cream substitutes; Mayonnaise; Salad dressings and vegetable oils
Flavored Milk	Flavored milk: whole; reduced fat; lowfat; non-fat
Other Desserts	Ice cream and frozen dairy desserts; Pudding; Gelatins, ices, sorbets
Quick Breads and Bread Products	Biscuits, muffins, quick breads; Pancakes, waffles, French toast
Ready-to-Eat Cereals	RTE[b] cereal: higher sugar (>21.2 g/100 g); lower sugar ($\leq$21.2 g/100 g)
Sugars	Sugars and honey; Sugar substitutes; Jams, syrups, toppings
Sweet Bakery Products	Cakes and pies; Cookies and brownies; Doughnuts, sweet rolls, pastries
Sweetened Beverages	Soft drinks; Fruit drinks; Sport and energy drinks; Nutritional beverages; Smoothies and grain drinks
Yogurt	Yogurt: regular; Greek

[a]*What we eat in America food categories (21).*
[b]*RTE, ready-to-eat.*

Statistical Analyses

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC, USA), and weighting factors provided by NHANES were applied to adjust for the complex survey sampling design (primary sampling units and strata), non-response rates, and oversampling of certain subgroups (day 1 sample weights). Food sources contributing at least 2% to total daily added sugars intake based on NHANES 2011–12, the reference year, were considered for analysis. Linear regression analyses were used to compare mean added sugars intake from food sources in 2017–18 vs. 2011–12 for each age group, examining both the WWEIA food categories and the larger food groups. Means $\pm$ standard errors (SE) were used to present results, which is how NHANES data are typically presented. Given the large sample size used for these analyses and to help ensure extremely small differences were not deemed significant, a more conservative p-value of $p < 0.01$ was selected.

Additionally, the combined sample (2011–18) was stratified by gender, ethnicity (using the groups as self-defined in NHANES as Asian, Black, Hispanic, and White), and income [household poverty income ratio (PIR) of low, medium, and high (PIR<1.35, 1.35$\leq$PIR$\leq$1.85, and PIR>1.85, respectively)]. These PIR categories were chosen because similar percentages are used by the U.S. government to determine eligibility for federal programs, such as nutrition assistance and school lunch programs, with the higher values having higher socioeconomic status. Added sugars sources were then examined by gender for each age group, and by ethnicity and PIR for the two overall age groups (2–18 and 19+ years). Again, food sources contributing at least 2% to total daily added sugars intake based on NHANES 2011–12 (the reference year) were considered for analysis and regression analyses were used to compare mean added sugars intake from the WWEIA food categories sources in 2017–18 vs. 2011–12 for each age group using $p < 0.01$.

RESULTS

Using data from all four cycles of NHANES (2011–18), after exclusions for unreliable dietary data (n = 5,548) as determined by the USDA FSRG, pregnant or lactating females (n = 359), and kilocalories = 0 (n = 3), the final sample size was 30,678. Distributions of sociodemographic characteristics among the final sample are shown in **Table 2**. The final sample size using data from NHANES 2011–12 was 7,862, and from NHANES 2017–18 was 7,035.

In 2017–18, the population mean intake (SE) of added sugars as a percentage of calories was 12.7% (0.3), corresponding to a mean of 67.8 g/day (1.6). Intakes were highest among adolescents and teens 9–18 years at 14.3% (0.3), and lowest among older adults 71+ years at 11.3% (0.4), corresponding to a mean of 73.1 g/day (1.8) and 53.8 g/day (1.5) of added sugars, respectively.

Children 2–8 Years

The top two sources of added sugars among children in 2017–18 were sweetened beverages and sweet bakery products, contributing 22.8 and 19.2%, respectively, to total daily added sugars intake (**Table 3**). Candy, RTE cereals, and other desserts ranked number three to five, and all together two-thirds (66%) of daily added sugars intake came from the top five food groups. The contribution from sweetened beverages was significantly lower in 2017–18 compared to 2011–12, while the contribution from sweet bakery products was significantly higher; however, they remained the top two sources of added sugars. The contribution from sugars was significantly lower in 2017–18 compared to 2011–12, and it fell in rank from number six to number eight.

Within the sweetened beverages food group, fruit drinks and soft drinks accounted for almost all of the added sugars,

TABLE 2 | Distribution of sociodemographic characteristics for the combined NHANES 2011–18 analytical sample.

Age and gender[a] (total sample *N* = 30,678)					
Age	***n***	**% (SE)[b]**	**Gender**	***n***	**% (SE)**
2–8 years	4,759	9.40 (0.28)	Male	2,408	51.86 (1.01)
			Female	2,351	48.14 (1.01)
9–18 years	6,154	13.77 (0.31)	Male	3,075	50.14 (1.05)
			Female	3,079	49.86 (1.05)
19–50 years	10,286	42.34 (0.71)	Male	5,122	51.61 (0.65)
			Female	5,164	48.39 (0.65)
51–70 years	6,649	25.57 (0.58)	Male	3,251	47.51 (0.77)
			Female	3,398	52.49 (0.77)
71+ years	2,830	8.92 (0.32)	Male	1,415	43.95 (1.07)
			Female	1,415	56.05 (1.07)
Ethnicity (total sample *N* = 29,177[c])					
Age		**Ethnicity**		***n***	**% (SE)**
2–18 years (*n* = 10,143)		Asian		1,053	4.67 (0.46)
		Black		2,736	13.79 (1.35)
		Hispanic		3,356	23.91 (1.92)
		White		2,998	51.90 (2.49)
19+ years (*n* = 19,034)		Asian		2,282	5.53 (0.50)
		Black		4,528	11.41 (0.98)
		Hispanic		4,734	15.06 (1.18)
		White		7,490	64.53 (1.71)
Income (total sample *N* = 28,055[c])					
Age		**PIR[d]**		***n***	**% (SE)**
2–18 years (*n* = 10,082)		Low		4,588	35.29 (1.70)
		Medium		1,278	11.54 (0.69)
		High		4,216	53.17 (1.86)
19+ years (*n* = 17,973)		Low		6,123	24.26 (0.97)
		Medium		2,226	9.94 (0.45)
		High		9,624	65.80 (1.21)

[a] *Values for gender are within each age group.*
[b] *SE, standard error.*
[c] *Total sample sizes for ethnicity and income are different and lower than the total sample size for age and gender due to missing information for some individuals (ethnicity and/or income not reported).*
[d] *PIR, poverty income ratio; low (PIR<1.35), medium (1.35 ≤ PIR ≤ 1.85), high (PIR > 1.85).*

with fruit drinks contributing slightly more than soft drinks (**Supplementary Table 1**). The significantly lower contribution from fruit drinks in 2017–18 compared to 2011–12 accounted for the lower contribution from sweetened beverages over this time, while soft drinks consumption remained the same. A decline in the contribution from jams, syrups, toppings in 2017–18 compared to 2011–12 accounted for the lower contribution from sugars over this time.

Adolescents and Teens 9–18 Years

The top two sources of added sugars intake among adolescents and teens in 2017–18 were sweetened beverages and sweet bakery products, contributing 33.5 and 14.3%, respectively, to total daily added sugars intake (**Table 4**). Compared to the results in younger children, the top two sources were the same; however, sweetened beverages contributed more, and sweet bakery products contributed less to added sugars intake among those 9–18 years. Candy, coffee and tea, and RTE cereals ranked number three to five, while all together nearly two-thirds (62%) of daily added sugars intake came from the top four food groups. The contribution from sweetened beverages was significantly lower in 2017–18 compared to 2011–12; however, it remained the number one source of added sugars, while RTE cereals went up in rank from number seven to number five in 2017–18.

Within the sweetened beverages food group, soft drinks and fruit drinks accounted for almost all of the added sugars, with soft drinks contributing twice as much as fruit drinks (**Supplementary Table 2**), in contrast to their fairly equal contributions among children. Within the coffee and tea food group, tea accounted for almost all of the added sugars. Unlike the results in children, declines in the contributions from both soft drinks and fruit drinks in 2017–18 compared to 2011–12 accounted for the lower contribution from sweetened beverages

TABLE 3 | Food group sources[a] and ranking of added sugars as a percentage of total daily added sugars intake among children 2–8 years, NHANES 2017–18 ($n = 914$) compared to NHANES 2011–12 ($n = 1,436$); values are mean (standard error) based on first day dietary recall.

Food group	2017–18		2011–12		*P*-value[b]
	% Added sugars from food group	Rank	% Added sugars from food group	Rank	
Sweetened beverages	22.81 (1.63)	1	29.31 (1.48)	1	0.0032
Sweet bakery products	19.18 (1.03)	2	15.41 (0.81)	2	0.0040
Candy	9.52 (1.03)	3	7.04 (0.92)	4	0.0739
Ready-to-eat cereals	7.23 (0.65)	4	6.62 (0.40)	5	0.4239
Other desserts	7.08 (0.76)	5	7.73 (0.95)	3	0.5897
Flavored milk	6.14 (0.72)	6	6.25 (0.76)	7	0.9140
Coffee and tea	3.46 (1.08)	7	2.89 (1.03)	9	0.6979
Sugars	3.34 (0.38)	8	6.29 (0.93)	6	0.0031
Quick breads and bread products	2.96 (0.57)	9	2.29 (0.55)	10	0.3964
Yogurt	2.14 (0.27)	10	3.63 (0.56)	8	0.0163
Total daily added sugars intake[c]	53.77 (2.34) g/day		61.83 (1.37) g/day		

[a]Those contributing at least 2% to total daily added sugars intake in 2011–12 (the reference year).
[b]From linear regression analysis comparing 2017–18 to 2011–12; $p \leq 0.01$ considered significant.
[c]Provided as reference to convert percentages to gram equivalents.

TABLE 4 | Food group sources[a] and ranking of added sugars as a percentage of total daily added sugars intake among adolescents and teens 9–18 years, NHANES 2017–18 ($n = 1,345$) compared to NHANES 2011–12 ($n = 1,549$); values are mean (standard error) based on first day dietary recall.

Food group	2017–18		2011–12		*P*-value[b]
	% Added sugars from food group	Rank	% Added sugars from food group	Rank	
Sweetened beverages	33.52 (1.27)	1	40.17 (1.60)	1	0.0011
Sweet bakery products	14.33 (1.19)	2	13.12 (0.91)	2	0.4193
Candy	7.44 (0.82)	3	5.95 (1.10)	4	0.2766
Coffee and tea	6.83 (0.86)	4	7.12 (1.20)	3	0.8466
Ready-to-eat cereals	6.79 (0.56)	5	5.17 (0.44)	7	0.0230
Other desserts	6.55 (0.89)	6	5.81 (1.08)	5	0.5976
Sugars	5.07 (0.69)	7	5.35 (1.01)	6	0.8219
Flavored milk	2.56 (0.25)	8	2.68 (0.35)	8	0.7745
Total daily added sugars intake[c]	73.13 (1.76) g/day		83.75 (2.63) g/day		

[a]Those contributing at least 2% to total daily added sugars intake in 2011–12 (the reference year).
[b]From linear regression analysis comparing 2017–18 to 2011–12; $p \leq 0.01$ considered significant.
[c]Provided as reference to convert percentages to gram equivalents.

over this time. Also in 2017–18, soft drinks contributed twice as much to added sugars intake among those 9–18 years compared to those 2–8 years, and the combined contribution of soft drinks, fruit drinks, and tea was greater among those 9–18 years at 35.9% compared to 24.0% for those 2–8 years.

Adults 19–50 Years

The top two sources of added sugars among younger adults in 2017–18 were sweetened beverages and coffee and tea, contributing 37.7 and 10.4%, respectively, to total daily added sugars intake; sweet bakery products ranked number three at 10.3%, contributing almost the same to added sugars intake as coffee and tea (**Table 5**). Compared to the results in adolescents and teens, sweetened beverages made a bigger contribution to added sugars intake, while sweet bakery products made a smaller contribution. Sugars and candy ranked number four and five, and 65% of added sugars intake came from the top four food groups. The only significant difference in 2017–18 compared to 2011–12 was a decline in the contribution from breads, rolls, tortillas to below 2%, but their ranking remained the same at number eight.

Within the sweetened beverages food group, soft drinks, fruit drinks, and sport and energy drinks accounted for almost all of the added sugars, with soft drinks contributing five times as much as fruit drinks or sport and energy drinks, both of which contributed fairly equal amounts (**Supplementary Table 3**). The top five food categories in rank order were soft drinks, tea, sugars and honey, fruit drinks, and sport and energy drinks, and combined they accounted for almost half (46%) of added sugars intake. The contribution from soft drinks to added sugars intake was over two-fold higher than that observed in children, while

TABLE 5 | Food group sources[a] and ranking of added sugars as a percentage of total daily added sugars intake among adults 19–50 years, NHANES 2017–18 (n = 2,241) compared to NHANES 2011–12 (n = 2,669); values are mean (standard error) based on first day dietary recall.

Food group	2017–18		2011–12		*P*-value[b]
	% Added sugars from food group	Rank	% Added sugars from food group	Rank	
Sweetened beverages	37.72 (2.15)	1	42.44 (1.47)	1	0.0702
Coffee and tea	10.36 (1.10)	2	8.89 (0.66)	3	0.2509
Sweet bakery products	10.26 (0.84)	3	12.02 (0.67)	2	0.1014
Sugars	6.68 (0.61)	4	6.70 (0.55)	4	0.9787
Candy	6.28 (0.74)	5	4.36 (0.30)	6	0.0173
Other desserts	3.55 (0.49)	6	4.46 (0.72)	5	0.2924
Ready-to-eat cereals	2.90 (0.20)	7	3.26 (0.27)	7	0.2828
Breads, rolls, tortillas	1.53 (0.13)	8	2.12 (0.16)	8	0.0042
Total daily added sugars intake[c]	72.33 (2.69) g/day		83.60 (2.29) g/day		

[a] *Those contributing at least 2% to total daily added sugars intake in 2011–12 (the reference year).*
[b] *From linear regression analysis comparing 2017–18 to 2011–12; $p \leq 0.01$ considered significant.*
[c] *Provided as reference to convert percentages to gram equivalents.*

similar to the results in children, and adolescents and teens, the contribution from fruit drinks declined significantly in 2017–18 compared to 2011–12.

Adults 51–70 Years

The top two sources of added sugars among older adults in 2017–18 were sweetened beverages and sweet bakery products, contributing 28.3 and 14.6%, respectively, to total daily added sugars intake (**Table 6**). Sugars, coffee and tea, and candy ranked three to five, and almost two-thirds (61%) of added sugars came from the top four food groups. There was a significant decline in the contributions from RTE cereals and breads, rolls, tortillas in 2017–18 compared 2011–12, with both dropping down one rank to number eight and nine, respectively, while the contribution from breads, rolls, tortillas also fell below 2%.

Within the sweetened beverages food group, soft drinks accounted for approximately 75.4% of the added sugars, with the rest coming mainly from fruit drinks (**Supplementary Table 4**). The combined added sugars contributions from all categories of beverages, including soft drinks, fruit drinks, sport and energy drinks, and tea was 36.1%, more than twice as much as the contribution from the sweet bakery products food group.

Adults 71+ Years

In contrast to all the other age groups, the top source of added sugars in 2017–18 among older adults 71+ years was sweet bakery products at 20.7% of total daily added sugars intake, while sweetened beverages ranked second at 17.7% (**Table 7**). Other desserts, sugars, and candy ranked number three to five, and almost two-thirds (63%) of added sugars intake came from the top five sources. Similar to the other adult age groups, the contribution from breads, rolls, tortillas declined significantly in 2017–18 compared to 2011–12.

Within the sweet bakery products food group, cakes and pies contributed the most to added sugars, followed closely by cookies and brownies, and next by a relatively small contribution from doughnuts, sweet rolls, pastries (**Supplementary Table 5**). Within the sweetened beverages food group, soft drinks accounted for the majority (70.1%) of added sugars; however, the added sugars contribution from soft drinks was the second lowest of all the age groups (with the lowest being among children 2–8 years). Also, fruit drinks made a very small contribution, which declined significantly in 2017–18 compared to 2011–12. A significantly lower contribution from yeast breads in 2017–18 compared to 2011–12 accounted for the lower contribution from breads, rolls, tortillas over this time.

Gender

Using data from the combined sample (2011–18), rankings of food group sources of added sugars were similar between males and females among all ages, with only some variation in their percentage contributions. In general, males tended to have higher added sugars contributions from soft drinks compared to females, but their ranking was the same (data not shown).

Ethnicity

Using data from the combined sample (2011–18), individuals 2–18 years had a mean intake (SE) of added sugars that ranged from a low of 48.0 g/day (1.4) among Asians to a high of 72.2 g/day (1.5) among Whites (**Table 8**). Among adults 19+ years, added sugars intake ranged from a low of 39.9 g/day (0.9) among Asians to a high of 78.9 g/day (1.6) among Blacks (**Table 9**).

For the two overall age groups (2–18 and 19+ years), sources of added sugars were similar across ethnic groups, with sweetened beverages and sweet bakery products as the top two sources (data not shown); however, some differences across ethnic groups emerged within these food groups.

Among children, adolescents, and teens 2–18 years, soft drinks ranked number one for all ethnicities except Blacks (**Table 8**). Fruit drinks ranked first among Blacks, second among Hispanics and Whites, and fourth among Asians. Compared to an average

TABLE 6 | Food group sources[a] and ranking of added sugars as a percentage of total daily added sugars intake among adults 51–70 years, NHANES 2017–18 (n = 1,776) compared to NHANES 2011–12 (n = 1,559); values are mean (standard error) based on first day dietary recall.

Food group	2017–18		2011–12		*P*-value[b]
	% Added sugars from food group	Rank	% Added sugars from food group	Rank	
Sweetened beverages	28.29 (2.38)	1	28.76 (1.52)	1	0.8680
Sweet bakery products	14.61 (1.11)	2	14.94 (1.03)	2	0.8300
Sugars	9.18 (0.62)	3	8.72 (0.88)	3	0.6709
Coffee and tea	8.78 (1.54)	4	8.52 (1.90)	4	0.9145
Candy	6.28 (0.73)	5	7.07 (0.74)	5	0.4476
Other desserts	6.15 (0.70)	6	6.88 (0.94)	6	0.5346
Fats and oils	3.26 (0.54)	7	2.74 (0.27)	9	0.3902
Ready-to-eat cereals	2.17 (0.25)	8	3.39 (0.32)	7	0.0028
Breads, rolls, tortillas	1.91 (0.20)	9	3.16 (0.17)	8	<0.0001
Total daily added sugars intake[c]	67.85 (3.13) g/day		61.82 (2.49) g/day		

[a]*Those contributing at least 2% to total daily added sugars intake in 2011–12 (the reference year).*
[b]*From linear regression analysis comparing 2017–18 to 2011–12; $p \leq 0.01$ considered significant.*
[c]*Provided as reference to convert percentages to gram equivalents.*

TABLE 7 | Food group sources[a] and ranking of added sugars as a percentage of total daily added sugars intake among adults 71+ years, NHANES 2017–18 (n = 759) compared to NHANES 2011–12 (n = 649); values are mean (standard error) based on first day dietary recall.

Food group	2017–18		2011–12		*P*-value[b]
	% Added sugars from food group	Rank	% Added sugars from food group	Rank	
Sweet bakery products	20.65 (1.35)	1	21.09 (1.62)	1	0.8348
Sweetened beverages	17.71 (1.22)	2	18.19 (1.27)	2	0.7840
Other desserts	9.46 (1.16)	3	9.54 (1.01)	4	0.9572
Sugars	7.89 (0.67)	4	11.13 (1.51)	3	0.0499
Candy	7.04 (1.63)	5	5.99 (0.79)	5	0.5624
Coffee and tea	6.15 (0.94)	6	5.82 (1.41)	6	0.8471
Ready-to-eat cereals	4.14 (0.37)	7	4.42 (0.57)	7	0.6765
Fats and oils	3.07 (0.51)	8	2.56 (0.29)	9	0.3878
Breads, rolls, tortillas	2.56 (0.23)	9	3.57 (0.22)	8	0.0013
Fruits	2.05 (0.75)	10	2.29 (0.40)	10	0.7806
Total daily added sugars intake[c]	53.81 (1.47) g/day		53.24 (2.01) g/day		

[a]*Those contributing at least 2% to total daily added sugars intake in 2011–12 (the reference year).*
[b]*From linear regression analysis comparing 2017–18 to 2011–12; $p \leq 0.01$ considered significant.*
[c]*Provided as reference to convert percentages to gram equivalents.*

of 30.1% among all ethnicities, the contribution to added sugars intake from soft drinks and fruit drinks combined was higher among Blacks (36.3%) and lower among Asians (18.2%). Within the sweet bakery products food group, cookies and brownies accounted for the majority of added sugars, while their ranking was different: second among Asians; third among Hispanics and Whites; and, fourth among Blacks.

Similar to the results in younger individuals, among adults 19+ years, soft drinks were the number one source of added sugars for all ethnicities. Compared to an average of 25.1% among all, the contribution to added sugars intake from soft drinks was higher among Hispanics (31.6%) and lower among Asians (17.0%) (**Table 9**). Fruit drinks varied in both ranking and added sugars contribution among ethnic groups, ranking second among Blacks and Hispanics, sixth among Asians, and seventh among Whites; and they contributed 12.6, 8.0, 4.9, and 3.7% to added sugars intake, respectively.

Income

Using data from the combined sample (2011–2018), individuals 2–18 years had a mean intake of added sugars that was fairly consistent across PIR groups (**Table 10**); however, among adults 19+ years, mean intake (SE) was lowest in the high PIR group at 65.3 g/day (1.0), and highest in the low PIR group at 80.4 g/day (1.7) (**Table 11**).

For the two overall age groups, sources of added sugars were similar across PIR groups, with sweetened beverages and sweet bakery products as the top two sources (data not shown); however, some differences across PIR groups emerged within these and other food groups.

TABLE 8 | Sources of added sugars among children, adolescents, and teens (2–18 years) overall and from four ethnic groups, NHANES 2011–18: food categories[a] and ranking by added sugars as a percentage of total daily added sugars intake; values based on first day dietary recall.

Food category	All individuals (n = 10,913)[b]		Asian (n = 1,053)		Black (n = 2,736)		Hispanic (n = 3,356)		White (n = 2,998)	
	Mean (SE)[c]	Rank	Mean (SE)	Rank	Mean (SE)	Rank	Mean (SE)	Rank	Mean (SE)	Rank
Soft drinks	18.60 (0.61)	1	10.47 (1.29)	1	15.42 (0.77)	2	20.18 (0.86)	1	19.38 (0.92)	1
Fruit drinks	11.49 (0.47)	2	7.69 (0.84)	4	20.84 (0.90)	1	12.86 (0.74)	2	8.66 (0.58)	2
Cookies and brownies	6.72 (0.24)	3	9.32 (0.78)	2	6.26 (0.38)	4	7.34 (0.45)	3	6.64 (0.39)	3
RTE[d] cereal, higher sugar (>21.2 g/100 g)	5.54 (0.20)	4	5.40 (0.68)	6	6.07 (0.39)	5	6.39 (0.34)	4	5.00 (0.27)	7
Tea	5.18 (0.51)	5	4.24 (0.76)	9	3.60 (0.45)	10	4.41 (0.54)	7	5.93 (0.81)	4
Candy not containing chocolate	5.12 (0.25)	6	5.06 (0.53)	8	6.32 (0.41)	3	4.58 (0.33)	6	5.02 (0.38)	6
Ice cream and frozen dairy desserts	4.88 (0.25)	7	7.51 (0.96)	5	4.06 (0.28)	7	4.28 (0.33)	8	5.05 (0.39)	5
Cakes and pies	4.45 (0.32)	8	8.22 (1.42)	3	4.49 (0.64)	6	5.01 (0.66)	5	4.01 (0.49)	8
Jams, syrups, toppings	3.57 (0.20)	9	3.82 (0.60)	10	4.00 (0.39)	8	2.52 (0.26)	10	3.86 (0.34)	9
Doughnuts, sweet rolls, pastries	3.43 (0.18)	10			3.62 (0.34)	9	3.06 (0.21)	9	3.65 (0.28)	10
Candy containing chocolate	2.85 (0.24)	11	5.26 (1.05)	7					3.21 (0.38)	11
Sport and energy drinks	2.83 (0.23)	12	1.26 (0.52)	13	2.24 (0.29)	11			3.11 (0.38)	12
Total daily added sugars intake[e] (g/day)	67.53 (0.83)		48.0 (1.4)		70.86 (1.38)		60.63 (1.29)		72.21 (1.51)	

[a]Those contributing at least 2% to total daily added sugars intake among all individuals (2–18 years); empty cells represent a food category contributing <2%.
[b]Sample size for all individuals is larger than the total of the sample sizes for all ethnic groups due to missing information for some individuals (ethnicity not reported).
[c]SE, standard error.
[d]RTE, ready-to-eat.
[e]Provided as reference to convert percentages to gram equivalents.

TABLE 9 | Sources of added sugars among adults (19+ years) overall and from four ethnic groups, NHANES 2011–18: food categories[a] and ranking by added sugars as a percentage of total daily added sugars intake; values based on first day dietary recall.

Food category	All individuals (n=19,765)[b]		Asian (n = 2,282)		Black (n = 4,528)		Hispanic (n = 4,734)		White (n = 7,490)	
	Mean (SE)[c]	Rank	Mean (SE)	Rank	Mean (SE)	Rank	Mean (SE)	Rank	Mean (SE)	Rank
Soft drinks	25.10 (0.61)	1	16.96 (1.49)	1	25.52 (0.82)	1	31.61 (1.24)	1	23.67 (0.79)	1
Tea	8.20 (0.47)	2	6.17 (0.80)	4	8.27 (0.52)	3	5.97 (0.55)	3	8.60 (0.64)	2
Cakes and pies	5.81 (0.24)	3	5.89 (0.67)	5	6.30 (0.51)	4	5.25 (0.54)	5	5.83 (0.36)	3
Fruit drinks	5.61 (0.28)	4	4.88 (0.50)	6	12.63 (0.48)	2	7.99 (0.61)	2	3.73 (0.34)	7
Cookies and brownies	5.07 (0.16)	5	6.67 (0.53)	3	4.83 (0.24)	6	4.28 (0.25)	6	5.26 (0.24)	4
Sugars and honey	5.04 (0.16)	6	8.54 (0.53)	2	5.25 (0.29)	5	5.96 (0.30)	4	4.50 (0.22)	6
Ice cream and frozen dairy desserts	4.20 (0.19)	7	4.21 (0.36)	7	2.94 (0.20)	8	2.82 (0.24)	9	4.83 (0.26)	5
Sport and energy drinks	3.34 (0.23)	8	1.99 (0.42)	13	2.90 (0.44)	9	4.13 (0.49)	7	3.35 (0.29)	9
Candy containing chocolate	3.23 (0.17)	9	3.60 (0.40)	8	2.63 (0.21)	10			3.70 (0.25)	8
Candy not containing chocolate	2.52 (0.16)	10	2.47 (0.45)	10	3.45 (0.28)	7				
Jams, syrups, toppings	2.40 (0.14)	11	2.39 (0.38)	11	1.99 (0.15)	11	1.58 (0.17)	11	2.69 (0.20)	10
RTE[d] cereal, higher sugar (>21.2 g/100 g)	2.33 (0.08)	12	1.92 (0.30)	15			1.92 (0.22)	10	2.44 (0.11)	11
Doughnuts, sweet rolls, pastries	2.29 (0.11)	13	2.85 (0.30)	9			3.20 (0.21)	8		
Total daily added sugars intake[e] (g/day)	69.29 (0.84)		39.95 (0.94)		78.88 (1.59)		68.08 (1.27)		69.72 (1.16)	

[a]Those contributing at least 2% to total daily added sugars intake among all individuals (19+ years); empty cells represent a food category contributing <2%.
[b]Sample size for all individuals is larger than the total of the sample sizes for all ethnic groups due to missing information for some individuals (ethnicity not reported).
[c]SE, standard error.
[d]RTE, ready-to-eat.
[e]Provided as reference to convert percentages to gram equivalents.

Among children, adolescents, and teens 2–8 years, soft drinks and fruit drinks ranked first and second among all PIR groups; however, their contributions to added sugars intake varied (**Table 10**). Compared to an average of 30.1% across all PIR groups, the contribution to added sugars intake from soft drinks and fruit drinks combined was higher in the low and medium PIR groups (33.7 and 32.0%, respectively) and lower in the high PIR group (26.9%). While the contribution from other sweetened beverages (tea, and sport and energy drinks combined) was highest in the low PIR group.

TABLE 10 | Sources of added sugars among children, adolescents, and teens (2–18 years) overall and across income strata, NHANES 2011–18: food categories[a] and ranking by added sugars as a percentage of total daily added sugars intake; values based on first day dietary recall.

Food category	All individuals (n = 10,913)[b]		Low (PIR[c] < 1.35) (n = 4,588)		Medium (1.35≤PIR≤1.85) (n = 1,278)		High (PIR>1.85) (n = 4,216)	
	Mean (SE)[d]	**Rank**	**Mean (SE)**	**Rank**	**Mean (SE)**	**Rank**	**Mean (SE)**	**Rank**
Soft drinks	18.60 (0.61)	1	19.68 (0.95)	1	20.43 (1.20)	1	17.38 (0.84)	1
Fruit drinks	11.49 (0.47)	2	14.01 (0.89)	2	11.58 (1.08)	2	9.56 (0.55)	2
Cookies and brownies	6.72 (0.24)	3	6.41 (0.33)	4	5.72 (0.55)	4	7.10 (0.35)	3
RTE[e] cereal, higher sugar (>21.2 g/100 g)	5.54 (0.20)	4	6.70 (0.33)	3	6.44 (0.51)	3	4.46 (0.27)	7
Tea	5.18 (0.51)	5	5.56 (0.99)	5	5.64 (1.09)	5	4.95 (0.65)	6
Candy not containing chocolate	5.12 (0.25)	6	4.85 (0.32)	6	4.91 (0.55)	6	5.30 (0.44)	5
Ice cream and frozen dairy desserts	4.88 (0.25)	7	3.88 (0.29)	8	4.22 (0.42)	8	5.82 (0.41)	4
Cakes and pies	4.45 (0.32)	8	4.69 (0.49)	7	4.04 (0.63)	9	4.36 (0.49)	8
Jams, syrups, toppings	3.57 (0.20)	9	3.13 (0.35)	9	3.61 (0.51)	10	4.00 (0.31)	9
Doughnuts, sweet rolls, pastries	3.43 (0.18)	10	2.90 (0.25)	10	3.14 (0.51)	11	3.78 (0.29)	10
Candy containing chocolate	2.85 (0.24)	11	2.48 (0.27)	11	4.40 (1.06)	7		
Sport and energy drinks	2.83 (0.23)	12	2.40 (0.30)	12			3.24 (0.40)	11
Total daily added sugars intake[f] (g/day)	67.53 (0.83)		66.52 (1.34)		68.02 (2.29)		68.99 (1.4)	

[a] *Those contributing at least 2% to total daily added sugars intake among all individuals (2–18 years); empty cells represent a food category contributing <2%.*
[b] *Sample size for all individuals is larger than the total of the sample sizes for all PIR groups due to missing information for some individuals (income not reported).*
[c] *PIR, poverty income ratio.*
[d] *SE, standard error.*
[e] *RTE, ready-to-eat.*
[f] *Provided as reference to convert percentages to gram equivalents.*

TABLE 11 | Sources of added sugars among adults (19+ years) overall and across income strata, NHANES 2011–18: food categories[a] and ranking by added sugars as a percentage of total daily added sugars intake; values based on first day dietary recall.

Food category	All individuals (n = 19,765)[b]		Low (PIR[c] < 1.35) (n = 6,123)		Medium (1.35≤PIR≤.85) (n = 2,226)		High (PIR > 1.85) (n = 9,624)	
	Mean (SE)[d]	**Rank**	**Mean (SE)**	**Rank**	**Mean (SE)**	**Rank**	**Mean (SE)**	**Rank**
Soft drinks	25.10 (0.61)	1	32.07 (1.05)	1	28.21 (1.77)	1	21.36 (0.76)	1
Tea	8.20 (0.47)	2	9.56 (0.77)	2	8.27 (0.81)	2	7.76 (0.55)	2
Cakes and pies	5.81 (0.24)	3	4.77 (0.39)	5	5.28 (0.75)	4	6.46 (0.40)	3
Fruit drinks	5.61 (0.28)	4	7.06 (0.47)	3	6.46 (0.68)	3	4.73 (0.40)	6
Cookies and brownies	5.07 (0.16)	5	4.15 (0.22)	7	5.14 (0.55)	5	5.47 (0.24)	4
Sugars and honey	5.04 (0.16)	6	5.82 (0.32)	4	5.03 (0.45)	6	4.62 (0.22)	7
Ice cream and frozen dairy desserts	4.20 (0.19)	7	2.81 (0.22)	8	3.90 (0.39)	7	4.84 (0.27)	5
Sport and energy drinks	3.34 (0.23)	8	4.58 (0.54)	6	3.25 (0.54)	9	2.75 (0.23)	10
Candy containing chocolate	3.23 (0.17)	9	2.31 (0.18)	9	3.32 (0.49)	8	3.68 (0.27)	8
Candy not containing chocolate	2.52 (0.16)	10	2.24 (0.23)	10			2.74 (0.25)	11
Jams, syrups, toppings	2.40 (0.14)	11	1.74 (0.23)	13	2.00 (0.31)	11	2.83 (0.20)	9
RTE[e] cereal, higher sugar (>21.2 g/100 g)	2.33 (0.08)	12	2.19 (0.19)	11	2.56 (0.26)	10	2.38 (0.12)	12
Doughnuts, sweet rolls, pastries	2.29 (0.11)	13	2.14 (0.18)	12			2.25 (0.12)	13
Total daily added sugars intake[f] (g/day)	69.29 (0.84)		80.41 (1.7)		71.57 (1.82)		65.32 (0.99)	

[a] *Those contributing at least 2% to total daily added sugars intake among all individuals (19+ years); empty cells represent a food category contributing <2%.*
[b] *Sample size for all individuals is larger than the total of the sample sizes for all PIR groups due to missing information for some individuals (income not reported).*
[c] *PIR, poverty income ratio.*
[d] *SE, standard error.*
[e] *RTE, ready-to-eat.*
[f] *Provided as reference to convert percentages to gram equivalents.*

Compared to the results in younger individuals, among adults 19+ years, soft drinks ranked the same at number one across all three PIR groups; however, tea ranked second (**Table 11**). Percent contributions to added sugars intake from each beverage varied across PIR groups, with the highest contributions from soft drinks and tea in the low PIR group, and the lowest contributions from each in the high PIR group. Compared to an average of 25.1% across all PIR groups, the contribution to added sugars intake from soft drinks was higher in the low PIR group (32.1%) and lower in the high PIR group (21.4%). Fruit drinks varied in both ranking and added sugars contribution across PIR groups; they ranked third in the low and medium PIR groups and sixth in the high PIR group, and contributed 7.1 and 6.5% to added sugars intake in the low and medium PIR groups, respectively, and 4.7% in the high PIR group.

DISCUSSION

Using data from NHANES 2011–18, the results of this study provide a comprehensive update on the sources of added sugars in the American diet and a detailed examination of variations in added sugars sources according to selected sociodemographic factors. We estimated daily average added sugars intake at 12.7% of total calories based on NHANES 2017–18 data, which is similar to the estimate of 13% from a recent analysis using NHANES 2013–16 data (9). In looking at sources, we found the majority of added sugars in the diet came from a few sources, with sweetened beverages and sweet bakery products as the top two contributors, consistent with other studies both in the U.S. over the years 2001 to 2016 (6, 8, 9, 11, 22, 23) and in other countries (24–29). Looking further into sociodemographic factors, we also saw that the list of top added sugars sources was generally similar across age, ethnicity and PIR groups; however, there were differences in their relative contributions among these sociodemographic groups.

Our examination of added sugars sources among children, adolescents, and teens revealed distinct patterns between the younger and older age groups. Soft drinks became a bigger contributor to added sugars intake among those 9–18 years compared to younger children (2–8 years), replacing fruit drinks as the number one source and contributing twice as much to added sugars intake. The top sources of added sugars also changed with age: from two food groups, sweetened beverages (mainly fruit drinks) and sweet bakery products (cookies and brownies) among younger children, to only beverages, as sweetened beverages (mainly soft drinks and to a lesser extent, fruit drinks) and tea among adolescents and teens; and these patterns are consistent with other NHANES analyses (6, 7, 23, 30). A general shift in influence from parents and other caregivers to peers occurs in the transition from childhood to adolescence (3), and so a greater degree of parental control and family influence specifically on children's diets compared to that on the diets of adolescents and teens may explain the differences in added sugars sources that we observed in between the younger (2–8 years) and older (9–18 years) age groups. Research has shown that teens consume more sweetened beverages, and are more likely to skip breakfast and family dinners, which are behaviors that have been associated with poorer diet quality among them compared to younger children (31).

Similarly, we observed a distinct pattern in added sugars sources among adult age groups. Sweetened beverages predominated as the number one source of added sugars among adults 19–50 years and 51–70 years, mainly due to soft drinks, while sweet bakery products was the number one source among adults 71+ years. This difference is consistent with other analyses of NHANES data (23) and analyses of added sugars intakes in other countries (32, 33) and could be explained by the distinct lifestyles that characterize the working years of adulthood vs. retired older adults and the elderly. The oldest group (71+ years) represents individuals living in their retirement years, who are no longer confined by the daily routines and restrictions of working, and have more leisure time, and different social activities and contacts (34), all of which could influence dietary habits. A shift from mainly sweetened beverages to sweet bakery products as sources of added sugars may thus reflect a more leisurely routine to eating that comes with retirement, whether it is eating alone or congregating with others for socialization.

Our examination of the U.S. population stratified by ethnicity (Asians, Blacks, Hispanics, Whites) revealed that added sugars sources were similar across these groups, suggesting that ethnicity may not be an influence. Sweetened beverages and sweet bakery products were the top two sources of added sugars among all four ethnicities in both age groups, 2–18 years and 19+ years, with soft drinks being the top source; and this pattern is consistent with an analysis of earlier NHANES data (11). The one exception to soft drinks as the number one source of added sugars was observed in younger Black individuals (2–18 years), in which fruit drinks was the top source, contributing about twice as much to added sugars intake compared to the other three ethnic groups. A similar finding has been demonstrated previously using NHANES 2003–06 (11) and also more recently using NHANES 2013-16 (9), indicating a persistent pattern. Among Black adults (19+ years), fruit drinks also ranked higher (number two) and contributed more to added sugars intake compared to the other ethnic groups, suggesting fruit drinks is a common choice for this segment of the population. In contrast, added sugars intake from sweetened beverages (soft drinks and fruit drinks) was lowest among Asians of any age compared to the other three ethnic groups. This finding is supported by other research comparing added sugars sources among children (4–13 years) in the U.S., China, and Mexico, which showed Chinese children had the lowest added sugars intake overall and the lowest contributions from soft drinks and fruit drinks (35). Our findings together with those of others suggest a potential cultural basis for the observed differences in added sugars sources among Asians and the other three ethnic groups.

Similar to our findings among ethnic groups, our examination of the U.S. population stratified by income (low, medium, high PIR) revealed that added sugars sources were similar across PIR groups. Sweetened beverages and sweet bakery products were the top two sources of added sugars for all PIR groups among both younger individuals (2–18 years) and adults (19+ years). Furthermore, regardless of income, soft drinks and fruit drinks were the top contributors to added sugars intake among younger individuals, while soft drinks and tea were the top contributors among adults. However, some income differences between these two age groups emerged, with fruit drinks as the highest contributor to added sugars intake among younger individuals in the lowest PIR group, and soft drinks as the highest contributor among adults in the lowest PIR group; and this finding is consistent with a previous analysis using NHANES 2003–06, which also demonstrated a higher contribution to added sugars intake from fruit drinks among younger individuals (2–18 years) in the lowest income group (11). Research on the economics of food choices has demonstrated that added sugars are one of the lowest cost sources of dietary energy (36), and to the extent that fruit drinks and soft drinks are relatively inexpensive sources of energy, this differential may partly contribute to their greater prominence in the diets of lower income individuals. As we found fruit drinks were the top source of added sugars among Black children, income-related patterns in fruit drinks consumption may also be related to ethnicity, given Black children tend to be over-represented in the lowest income stratum (37).

Our study of added sugars sources has some strengths and limitations. One strength is our findings can be generalized to the U.S. population because our analysis is based on nationally representative data. Furthermore, NHANES data provide a rich source of information on sociodemographic variables; and by combining data from several cycles, we were able to conduct a rigorous examination of added sugars sources across segments of the population defined by ethnicity and income. We were also able to analyze added sugars sources within disaggregated age groups; for example, we separated younger individuals into two age groups, 2–8 and 9–18 years, and adults into three age groups, 19–50, 51–70, and 71+ years, allowing us to observe differences that may emerge with transitions into different life stages. Another strength of our study compared to others which required the derivation of added sugars values from algorithms (24, 25) is that our estimates of added sugars intake were based on values from the USDA FPED specific to each NHANES cycle. We also conducted and compared analyses on three different measures of added sugars sources (grams, teaspoon equivalents, and percentage of total daily added sugars intake) and results were consistent across all measures, providing confidence in the validity of our estimates.

As with any analysis of self-reported dietary intake data, our results are subject to error from recall bias and underreporting. To date, no ideal method for adjusting for underreporting effects has been identified or widely adopted (38); thus, no adjustments were applied in our analyses, consistent with the approach of other studies of added sugars sources (6, 8, 9, 11, 22–29, 39), and thereby facilitating comparisons of results across studies. Nonetheless, given that sweets and desserts are more prone to underreporting compared to other foods (40), added sugars intakes and contributions from these foods reported in our study may have been underestimated. Additionally, investigations of underreporting in NHANES samples have shown that it is more likely among older age groups (adolescents and teens vs. children, and older vs. younger adults), among Blacks compared to Whites, and among lower PIR groups (41, 42); and these differences could have contributed to the added sugars results we observed among groups stratified by these characteristics. However, with the exception of older adults, our results showed higher levels of added sugars intake among those groups in which underreporting is more likely (adolescents and teens, Blacks, and lower PIR groups), suggesting that the differences we observed were real and not simply an artifact of underreporting.

In conclusion, regardless of age, ethnicity or income, sweetened beverages and sweet bakery products were the top two sources of added sugars among the U.S. population in 2011–18. More specifically, sweetened beverages, including soft drinks and fruit drinks, as well as tea, were the largest contributors to added sugars intake. There was some variation in the types of beverages and their relative contributions across age, ethnicity, and income groups, highlighting the need to consider particular sociodemographic contexts when developing dietary guidance. For example, dietary guidance related to young children could consider their consumption of fruit drinks as a top contributor to added sugars intake, while guidance for adolescents and teens, and younger adults could consider soft drinks as the top contributor. Likewise, further examination of the factors underlying ethnic- and income-related differences in added sugars sources would contribute to a better understanding of the differences and help to target dietary guidance or other supports for healthy eating.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. The data used in this study are openly available in the website of the National Health and Nutrition Examination Survey: NHANES Questionnaires, Datasets, and Related Documentation; https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

ETHICS STATEMENT

The NHANES study procedures are approved by an institutional ethics review board, and documented consent is obtained from NHANES participants.

AUTHOR CONTRIBUTIONS

VF, PG, MS, LR, and LD: conceptualization and writing—review and editing. VF: methodology and formal analysis.

LR and LD: writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

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Integrative Strategies for Preventing Nutritional Problems in the Development of Children in Brazil

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Child healthcare has been a priority subject in several programs and public policies developed over the decades. However, initiatives implemented seem insufficient to overcome the challenges regarding the integral development and improvement of the nutritional status of children in Brazil. The initial developmental stages of a child include pregnancy, breastfeeding, and complementary feeding, which are determinants in future aspects of health and nutritional status. Therefore, the strategies addressing problems during these three periods of life have the potential to positively impact the promotion of healthy eating habits and food security throughout life. Developing countries with huge dimensions and vast inequalities, like Brazil, are marked by differences in regional, cultural, and social contexts that may hinder the implementation of programs and policies with a broad scope. Extensive operational and professional costs, in addition to time-consuming activities that are necessary to apply, monitor, and evaluate interventions may jeopardize the proper assessment of programs and policy goals, generating the inefficiency and waste of resources in the health system. Thus, programs and policies aimed at creating and modifying habits should consider an intersectoral action within local contexts, involving health professionals, universities, policy managers, and the community. Therefore, this article aimed to discuss the preliminary conception of an integrated approach of decentralized strategies to promote healthy eating habits and food security of children in Brazil.

Keywords: nutrition, infant feeding, public policies, nutritional strategies, child development

INTRODUCTION

Child healthcare has been a priority in several programs and public policies developed over recent decades, contributing to important changes in indicators of child health, and development. However, although these initiatives reinforce the prioritization of children in the broad scope of healthcare, they are still insufficient in dealing with challenges relevant to the integral development and improvement of the nutritional status of children from 0 to 60 months of age (1).

Throughout decades, diverse public policies and health programs have been implemented and extinguished in Brazil due to the lack of resources, absence of evidence regarding the effectiveness, or political disagreements (2). One of the main problems regarding the discontinuity of health programs or abrupt changes in the orientation of public health policies refers to a low adherence of the population to the recommendations of health professionals, considering the doubts raised, and confusion generated due to the instability of government actions (3).

The quality and frequency of the prenatal follow-up are still limited; the prevalence and duration of breastfeeding are still challenging, although there are several policies, programs, and recommendations available for guidance; similarly, feeding practices in first years of life are considerably far from guidelines. Since 1981, the National Breastfeeding Incentive Program has tried to induce a set of actions related to promotion, protection, and support of breastfeeding. In this context, over the years, the policies in Brazil have focused on the process of expansion of strategies that involve a wide range of actions developed in hospitals and primary healthcare, such as the Baby-Friendly Hospital Initiative (*Iniciativa Hospital Amigo da Criança*) and human milk banks (*Bancos de Leite Humano*). Another intervention performed in a primary care is the National Strategy for the Promotion of Breastfeeding and Healthy Complementary Food in the Primary Health Care System—*Estratégia Amamenta Alimenta Brasil* (EAAB), which seeks to promote, protect, and support breastfeeding until ≥2 years of age, being exclusively in the first 6 months of life and complemented with healthy foods from 6 months (1). However, even with such efforts over the last four decades, the prevalence of exclusive breastfeeding in Brazilian infants under 6 months of age is only 45.7% (4). Therefore, the evolution in important indicators of the health of a child still faces obstacles in the current context, including the high proportion of premature and low-birth-weight infants, one of the leading causes of preventable deaths in the first year of life (5). In addition, the low quality of food intake from complementary feeding has contributed to the increase of overweight and obesity among young children, concurrently with a high prevalence of nutritional deficiencies, a condition called "hidden hunger." The current context of coronavirus disease 2019 pandemic has further intensified these problems, with important consequences for the quality of nutrition of children and child development. A recent study found that 59.4% of Brazilian households reported that they have experienced some level of food insecurity, as they did not have the adequate quantity and quality of food or as the concern that there would be a shortage of food compromises their choices (6).

The recent evidence shows that maternal feeding plays a leading role in the long-term health of a baby. After birth, exclusive breastfeeding up to 6 months of life is a decisive factor in the health of children and may impact adulthood. Finally, the period of food introduction may shape the quality of the diet, eating habits, and relationship with foods throughout life of children. This triad of periods involved in the initial aspects of child development—pregnancy, breastfeeding, and complementary feeding—is determinant of future aspects of the health and nutritional status of an individual. Therefore, the strategies addressing these important periods can positively impact the promotion of healthy eating habits and food security throughout life.

Thus, the aim of this study was to discuss the preliminary conception of a master plan to tackle the obstacles in the triad of periods decisive for child development—pregnancy, breastfeeding, and complementary feeding—through an integrated approach of decentralized strategies to promote healthy eating habits and food security of children in Brazil.

PROBLEMS

The Beginning of Everything: The Role of Adequate Nutrition From Pregnancy to Postpartum

The role of maternal nutrition influences the important aspects in child development. In this context, a maternal health status marked by nutritional deficiencies, poor diet quality, and presence of modifiable risk factors, such as smoking, may reflect on the health conditions from conception throughout life.

The availability and quality of access to health services and prenatal care are relevant for promoting healthy maternal nutrition, and its distribution throughout Brazil is still fragile. The adequacy and frequency of a prenatal follow-up comprise a challenge to overcome due to the low coverage by qualified professionals, resulting in barriers to regular access, and consequent prioritization in care, exclusively for pregnant women who are at a high risk.

Although there is a broad recognition of relevant themes involving child health and feeding in healthcare facilities, informational approaches are insufficient to solve the lack of maternal support. The rates of breastfeeding and early weaning, for example, still represent a challenge, even considering constant guidance in services and development of policies and programs for the promotion of breastfeeding since the decade of 1970s. In this sense, acknowledging the problem may be an insufficient stimulus for adopting practical actions in daily life, which may not be enough to promote changes in habits and improve indicators of interest, such as exclusivity and duration of breastfeeding.

The Moment of Transition: Complementary Feeding in the Formation of Future Healthy Habits

Another fundamental moment in the formation of healthy eating habits among children comprises the process of introduction of complementary feeding, which should occur after 6 months of life. It is also a period of intense palate training that intensifies with the addition of new consistencies, colors, and flavors, and, simultaneously, it is a moment of a higher risk for exposure to inadequate foods with low nutritional value.

The practices of complementary feeding in Brazil are often marked by early introduction and high intake of inappropriate foods, especially nutritionally deficient items with a high content of added sugar, sweets, biscuits, and calories. Most children receive milk of cows prematurely, in replacement or complementarily to breast milk, being consumed by ∼80% of children between 12 and 60 months of age. This results in excessive protein intake, which tends to reduce the consumption of other non-dairy foods and may cause critical nutritional deficiencies including iron, vitamin A, and fiber, among others (7).

Numerous factors influence the quality of diet supplied in the first months of life, thus, guidance on the nutritional value, and healthy food choices may be insufficient to tackle the problem. Issues, such as the access and availability of quality food in the neighborhood, permissive parental style, active family participation in planning and encouragement of appropriate eating practices, and early exposure to electronic equipment, are challenges that need to be recognized and addressed for the development of multifaceted strategies that incorporate the complexity of these factors in promoting healthy eating during childhood.

The Abyss of Communication: When Recommendations of Health Professionals Are Not Heard

Historically, vertical educational strategies are the cornerstone of policies for encouragement of breastfeeding, promotion of adequate infant feeding, and follow-up of pregnant and post-partum women. However, these strategies have been unsuccessful in converting knowledge into practices in daily life of the target audience. These educational "microinterventions" are ineffective and usually present high direct and indirect costs due to the need for time-consuming activities of health professionals, particularly in primary healthcare, like the Family Health Strategy (FHS) in Brazil, designed to provide primary healthcare for individuals during regular household visits through interdisciplinary teams.

It is important to acknowledge that food consumption represents more than a simple biological act to fulfill the need for survival, being also a social practice. Therefore, the strategies aimed to promote adequate eating habits should consider the complexity of determinants involved in food choices, going beyond the concept of healthy food or its role in the health of an individual, which comprises an approach exclusively or predominantly based on the physiological context of food.

The household environment, the lifestyle choices of parents, and the intrafamilial relationships play prominent roles in infant feeding, generally determining the nutritional quality and quantity of food, food preferences, time, and the interval between meals and eating practices and behaviors of a child. Therefore, the cultural, economic, and psychosocial backgrounds of the family are relevant to drive the formation of positive experiences with an impact on infant feeding and, potentially, throughout life.

POTENTIAL SOLUTIONS AND ALTERNATIVES

Fragmenting to Enlarge When Local Glance Becomes Part of Global Solution

Developing countries with huge dimensions and vast inequalities, like Brazil, are marked by differences in regional, cultural, and social contexts that may hinder the implementation of programs and policies with a broad scope. Extensive operational and professional costs, in addition to time-consuming activities that are necessary to apply, monitor, and evaluate the interventions, may jeopardize the proper assessment of programs and policy goals, generating inefficiency, and waste of resources in health system.

The decentralization of planning, implementation, and evaluation of initiatives may be an alternative to overcome these obstacles. The primary strategy within the decentralized approach would rely on creating small-scale "sentinel centers" directed for tangible interventions, allowing to generate knowledge and scientific evidence using a limited number of local resources. These sentinel centers would comprise the center for action of the pediatrician, linked to other health services, universities, or municipalities, in different regions of the country.

In addition to the partnership with universities distributed in different regions, empowerment and stimulation of active participation of policy managers, along with the engagement of the community, are necessary for the relevant and effective performance of these monitoring centers. A strategy that may integrate this approach with the potential for success is the initiative "Mayor Friend of the Child," from the Abrinq Foundation (*Fundação Abrinq* or Foundation of the Brazilian Association of Toys Manufacturers), which is responsible for engaging policy managers in the implementation of actions and public policies aimed to promote and ensure rights of children and adolescents in Brazilian municipalities[1]. Therefore, a pilot program could explore the potential of municipalities already committed and dedicated to actions of interest in the subject toward the implementation of sentinel centers based on public-private partnerships. This collaborative process can contribute to the actions and activities of programs already implemented in the health system, like EAAB, which could enhance the expected results from these strategies and improve the health indicators of the population.

Regional centers located in different parts of the country would comprise a network of facilities adopting immediate and site-specific preventive interventions adapted to local context, including hundreds of health professionals in a single project to monitor food and nutritional status of children. A short-term follow-up measure would encompass centers throughout the country that collect and organize the data into a central unit. The actions would be remotely monitored and assessed to comprise the results comparing diverse local interventions in a practical way. Targeted strategies could be developed in each of the sentinel centers, contributing to identify successful strategies, according to the following proposals.

Strategy 1: Antenatal Childcare in Health Services

Childcare is the task for pediatricians who focus on monitoring the life of children in the primary healthcare. Considering recent recommendations on the early start

[1]Fundação Abrinq. Programa Prefeito Amigo da Criança. [Online] *Fundação Abrinq*. Available at: https://www.fadc.org.br/o-que-fazemos/programa-prefeito-amigo-da-crianca (accessed October 19, 2020).

of intervention for promoting the health of children, encompassing the period before conception (8), called preconception childcare, the pediatrician would participate in the counseling process of the couple who intends to have children (9), to ensure that the conception occurs more healthily. However, considering that it is not easy to engage individuals in preconception childcare, it would be essential to involve parents in the follow-up by a pediatrician at least during the gestational period using protocols of antenatal childcare (10).

Recommendations regarding the frequency of consultations and approaches in antenatal childcare include attendance of a pediatrician in consultations with the pregnant mother, preferably accompanied by the father, at least once every trimester of pregnancy. The expanded follow-up proposal should include approaches focusing on the health of a child, from monitoring the fetal growth, and preparing for immunizations to guidance on maternal nutrition, including the strategies for prevention of obesity and malnutrition and supplementation with micronutrients (11). The encouragement of breastfeeding would be one of the main focuses of the intervention, as it would be possible to prepare the family through training for the father and mother, focusing on the importance of breastfeeding and on strategies for it to be successful.

The proposal in the first strategy would be the implementation of antenatal childcare programs in a sample of primary healthcare facilities in different parts of the country (12), through a pilot project in which pediatricians would make three visits with pregnant women, one every quarter. The measurement of health outcomes like prevalence and duration of exclusive breastfeeding and adherence to immunization programs would be performed throughout a minimum period of 1 year in order to evaluate the effectiveness of the intervention.

Strategy 2: Continuous Training in Complementary Feeding for Professionals of the FHS

Health professionals become promoters of healthy eating when they can translate technical concepts into practical and accessible language among the community they assist (13). The Brazilian FHS provides an opportunity for dissemination of healthy lifestyles throughout local communities, considering its focus on the improvement of basic health practices including prevention and early detection of health problems through household visits and recurrent interactions with families (14). The conviviality allows the family members to spread changes in habits, e.g., good practices in food preparation, hygiene, and cleaning.

Thus, the proposal of the second strategy is based on a process of permanent education on complementary feeding for FHS professionals, adopting a hybrid format of conceptual activities and training in action with the following aims:

- Expansion of knowledge on complementary feeding;
- Improvement in communication skills of professionals regarding complementary feeding, using the tool developed in the project[2]. Experiences that Feed (in Portuguese, *Experiências que Alimentam*);
- Improvement of food and nutrition education practices; and
- Training health professionals to conduct meaningful conversations in health education.
- Development of a digital model of the tool aforementioned.

Strategy 3: Reduction of Inequalities in Nutritional Quality of Diets With Focus on the Family

Considering that healthy and unhealthy behavioral patterns are developed and maintained within the family, it serves as a basic social context and reference point for the development of behavioral characteristics. The utilization of the Internet through mobile, mainly the smartphones, has been a viable alternative to online cable connections in Brazil.

Approximately, 1,127 million Brazilians have access to mobile Internet and instant messaging applications like "WhatsApp," adopted by 93% of the population[3]. In order to build on the potential of information and communication technologies for the dissemination of knowledge, the proposal in the third strategy is based on the expansion of face-to-face interventions through continuous training based on technological channels. The actions developed should include:

- Adaptation of mobile technologies to send messages on healthy habits, considering cultural aspects of each region and availability of regional foods;
- Policies to encourage food consumption directed to protect health, including subsidies and social support for regional foods (15);
- Guidance from community leaders toward family engagement through messages about healthy eating, e.g., salt, sugar, and fat reduction in food items available for the local population; and
- Applying the principles in the Dietary Guidelines for the Brazilian population (16) to promote healthy eating habits, such as encouraging culinary practices in the family environment and recommending the consumption of natural or minimally processed foods, such as fruits, vegetables, grains, and pulses.

Strategy 4: Social Participation and Centrality of Individuals for Assertive Communication of Health Professionals

The social context plays a significant role in the choice of strategies adopted in the feeding of children. Food consumption practices are originated from the experiences constructed from sociocultural conditions and scientific and popular knowledge of each period. The social network and the community bond

[2]Project directed to promote the dialogue between family members and health professionals about complementary feeding, based on the support and development of facilitation materials through collaborative construction, theory of change, and design thinking, for 24 months. Available at: Centro de Recuperação e Educação Nutricional. Relatório Final do projeto "Experiências que alimentam". CREN—Centro de Recuperação e Educação Nutricional, 2019.

[3]Statista. Internet usage in Brazil: statistics & facts. In: Internet: demographics & use. Statista Research Department; 2020. Available at: https://www.statista.com/topics/2045/internet-usage-in-brazil/ (accessed January 26, 2020).

are also determinants in the formation of childcare practices, especially concerning breastfeeding, and infant feeding.

Often, professional guidance and follow-up are replaced by the advice of other family members who underwent the same experience, like mothers, or grandmothers. It is important to emphasize that these situations should not be excluding alternatives, but rather should be complementary.

Working jointly with the community, including community leaders and community health workers, might be a strategy that encourages the protagonism of individuals and community recognition of health promotion spaces. Some initiatives to expand the role of community for health promotion may include:

- Creation of spaces for monitoring of child health and development in an articulated network of health professionals, including an opportunity for socialization between mothers and pregnant women, clarification of doubts, and guidance in groups from community experiences;
- Community radio or podcast projects as space for listening and speaking with community participation, turning into an information tool shared by health professionals and community members as active guests and participants; and
- Use of technologies for recording and dissemination of contents produced, e.g., radio, that may be used later in other moments for training sessions and dissemination of information in waiting rooms of healthcare facilities. Community health workers can be important actors in bringing community and strategies closer together, acting in capturing and encouraging participation and in disseminating material within the community itself. Materials can be used, for example, during home visits as a "product of the community itself," a communication mechanism produced "along with" the people and not only "for" the people.

The estimation of costs related to the operationalization of the activities implemented in each sentinel center would allow the selection of cost-effective strategies in terms of the social value per resources invested and projection of conditions required for expansion of coverage and dissemination of activities to other locations (*scaling-up*), mainly through the incorporation of social participation in the evaluation processes (17).

The assessment of direct healthcare costs would be based on the measurement of resources required for operationalization of each activity, followed by the pricing of each resource unit employed, through the six stages as follows (18):

(1) Identification of procedures performed during each activity of the sentinel center, through the application of a semi-structured questionnaire to interview health professionals involved in its execution;
(2) Identification of human resources, equipment, and materials employed for each procedure performed;
(3) Estimation of units of time dedicated by human resources and equipment or units of other inputs spent for each service comprising each procedure performed;
(4) Investigation of monetary values of resources involved in each procedure performed (salaries per hour of human resources, price and duration of equipment, and unit prices of other inputs required);
(5) Calculation of direct costs of each procedure performed through the multiplication of units of each resource by its monetary value; and
(6) Estimation of total direct costs of each activity implemented in the sentinel center by summing up the direct costs of each procedure involved in its execution.

Finally, using the information on health outcomes obtained from each activity performed, it would be possible to estimate the cost-effectiveness ratios of each strategy proposed, allowing the comparison of actions implemented in specific local contexts to select the best alternatives for dissemination in health policies and programs at the municipal level in the different regions of the country. Furthermore, the adoption of an incremental approach in the redirection of actions with lower impacts on the health of children may avoid traumatic ruptures in public health activities, generating greater consistency, continuity, and reliability in health policies and programs (13).

FROM PLANNING TO IMPLEMENTATION: WAYS TO FOSTER PREVENTION DURING CHILDHOOD IN BRAZIL

The manuscript presented the preliminary conception of a master plan to tackle deficiencies in the triad of periods decisive for child development (i.e., pregnancy, breastfeeding, and complementary feeding) through a series of decentralized strategies that comprise a national framework toward the promotion of healthy eating habits and food security of children in Brazil, a developing country with large territory marked by substantial socioeconomic inequalities.

The first stage in the implementation of the strategies in the country should be based on the operationalization of a pilot study in the five regions of the country, namely, North, Northeast, South, Southeast, and Middle-West. The pilot study would be designed to encompass the strategies previously described into a set of "sentinel centers" selected for the analysis of feasibility, costs, and health outcomes that may be obtained in the initiative prior to the full implementation of the master plan.

The idea of the pilot study would be to perform a small-scale preliminary evaluation of the requirements, strengths, and potential frailties of the master plan, in order to incorporate the improvements to the initiative design prior to investment for its execution throughout the country. The pilot study would be conducted through collaboration among at least five major public universities in Brazil (minimum of one university from each region), coordinated by the Brazilian Ministry of Health in cooperation with the State and Municipal Secretaries of Health responsible for healthcare provision within the area selected for operationalization of the "sentinel centers."

The "sentinel centers" in the pilot study would be established on priority areas of the five regions that would be selected based on the following criteria:

(1) Analysis of socioeconomic, demographic, and health indicators referring to child health and food security;
(2) Availability of infrastructure, material, and human resources within the structure of the public sector directed to primary healthcare;
(3) Engagement and consent to participation in the study from the community and the stakeholders from the public and private sector at a local level.

In order to allow the comparison of results obtained by the "sentinel centers" established in the pilot study, we proposed that there should be at least 10 "sentinel centers" (two for each region) that would be implemented in Brazilian municipalities with comparable characteristics across regions (i.e., five small-scale low-income municipalities with the scarcity of infrastructure, material, and human resources, marked by poor performance in outcomes of the child health, and five medium-scale to medium-income municipalities with minimum infrastructure, material, and human resources, marked by intermediate performance in outcomes of the child health).

The operationalization of the strategies at the local level would be executed by local stakeholders with the support of state-level resources and initial coordination of the Brazilian Ministry of Health. The "sentinel centers" established in the pilot study would be assessed by multidisciplinary teams of researchers from the universities involved throughout the first year of implementation. At the end of each 4-month cycle of the pilot study, the results of the strategies proposed would be assessed and compared across "sentinel centers" and regions, in order to propose changes that may improve health outcomes obtained in the initiative.

Following the pilot study, the second stage of the implementation refers to continuous dissemination of "sentinel centers" to other Brazilian municipalities that will be selected according to the priority using the criteria previously proposed in the pilot study. The third and final stages of implementation would be based on the recruitment of other municipalities wishing to engage in the initiative in the subsequent periods, based on the dissemination of the results in local workshops performed by policymakers, street-level bureaucracy, health professionals, and university researchers, following a structure similar to the annual meeting of the National Council of State Secretaries of Management (*Conselho Nacional de Secretários de Estado da Administração*)[4].

Staggered adhesion of Brazilian municipalities would allow to maintain the assessment of impacts of the initiative on child health, based on the statistical analyses of the data obtained throughout the process of implementation, using quasi-experimental design approaches and qualitative analysis of comparative case studies. Thus, eventual failures and setbacks could be addressed by evaluation of problems identified, followed by a redesign of the intervention at the local level, and dissemination of the experience to other "sentinel centers" through the annual meeting. The succession of action monitoring/evaluation redesign in the "sentinel centers" would be continuous to allow a permanent adaptation of the initiative to the local circumstances and to the community needs, potentially establishing a cycle that allows adaptation, and evolution of the public policy.

[4]https://gestaoeplanejamento.com/pt/instituicoes/consad

CONCLUSION

Why challenges experienced for almost a century in the country remain on the political agenda due to the absence of adequate and permanent solutions? The change in health behaviors depends on several individuals, social, and environmental factors, thus, the programs and policies aimed at the creation and modification of habits should consider an intersectoral action within local contexts, involving health professionals, universities, policy managers, and the community.

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.

AUTHOR CONTRIBUTIONS

MF, CN-d-A, MPA, RMF, and FMS contributed to the conception and design of the study. LDB wrote the first draft of the manuscript. All authors wrote sections of the manuscript and contributed to manuscript revision, read, and approved the submitted version.

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Associations Between Nutritional Deficits and Physical Performance in Community-Dwelling Older Adults

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Background: Whether multiple nutritional deficiencies have a synergic effect on mobility loss remains unknown. This study aims to evaluate associations between multi-nutritional deficits and physical performance evolution among community-dwelling older adults.

Methods: We included 386 participants from the Multidomain Alzheimer Preventive Trial (MAPT) (75.6 ± 4.5 years) not receiving omega-3 polyunsaturated fatty acid (PUFA) supplementation and who had available data on nutritional deficits. Baseline nutritional deficits were defined as plasma 25 hydroxyvitamin D < 20 ng/ml, plasma homocysteine >14 µmol/L, or erythrocyte omega-3 PUFA index ≤4.87% (lower quartile). The Short Physical Performance Battery (SPPB), gait speed, and chair rise time were used to assess physical performance at baseline and after 6, 12, 24, 36, 48, and 60 months. We explored if nutrition-physical performance associations varied according to the presence of low-grade inflammation (LGI) and brain imaging indicators.

Results: Within-group comparisons showed that physical function (decreased SPPB and gait speed, increased chair rise time) worsened over time, particularly in participants with ≥2 nutritional deficits; however, no between-group differences were observed when individuals without deficit and those with either 1 or ≥2 deficits were compared. Our exploratory analysis on nutritional deficit-LGI interactions showed that, among people with ≥2 deficits, chair rise time was increased over time in participants with LGI (adjusted mean difference: 3.47; 95% CI: 1.03, 5.91; $p = 0.017$), compared with individuals with no LGI.

Conclusions: Accumulated deficits on vitamin D, homocysteine, and omega-3 PUFA were not associated with physical performance evolution in older adults, but they determined declined chair rise performance in subjects with low-grade inflammation.

Clinical Trial Registration: [https://clinicaltrials.gov/ct2/show/NCT00672685], identifier [NCT00672685].

Keywords: vitamin D, homocysteine, omega-3 fatty acids, physical performance, inflammation

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INTRODUCTION

Decline in physical performance, as measured by lower extremity function, often marks the early stage of disability in older age (1, 2). It is crucial to identify modifiable factors, such as nutritional risk factors, and their underlying biological mechanisms leading to impaired mobility in older individuals. Indeed, several blood-based nutritional markers, such as homocysteine, vitamin D, and omega-3 polyunsaturated fatty acids (PUFAs), have gradually become the focus of research and clinical interventions (3, 4). Hyperhomocysteinemia (HHcy) has been associated with faster physical impairment, such as in walking test and chair rise test, in several longitudinal studies (5–7). Vitamin D deficiency has been cross-sectionally associated with poor physical performance (8–10); however, similar associations were not discovered in longitudinal studies (11, 12). Although the literature on omega-3 PUFAs is mixed, some studies have found increased omega-3 PUFAs was associated with low risk of mobility disability (13), poor Short Physical Performance Battery (SPPB) score, and slower gait speed over time (14).

Considering that the accumulation of deficits can be related with the ability of an individual to respond to stressors (15), it is possible that combined deficiencies in homocysteine, vitamin D, and omega-3 PUFAs would work synergistically to determine physical performance over time. This concept had been supported by the findings of a previous study, which have indicated that an increasing number of nutritional deficits were associated with faster cognitive decline (16). In another study, the nutritional index, which was constructed with 41 nutrition-related parameters from anthropometric, plasma, and nutrient intake measurements, had shown a stronger prediction of frailty and mortality risk compared with single nutritional parameters separately (15). Furthermore, there is a lack of studies investigating underlying mechanisms behind multi-nutritional deficits and physical impairment. Indeed, several physiological deteriorations that drive age-related mobility loss (17), including changes in the central nervous system (CNS) and chronic inflammation, have shown intimate associations with these nutritional markers (18, 19). For instance, HHcy can promote inflammation (20). Higher circulating levels of omega-3 were associated with larger hippocampal volume (21); on the other hand, smaller brain volumes were observed in people with low vitamin D status (22). Therefore, exploring the interactions between nutritional deficits and physiological alterations (i.e., chronic inflammation and CNS changes) might allow us to understand better their shared biological pathways leading to mobility decline.

The main objective of this study was to investigate the associations between multi-nutritional deficits (i.e., vitamin D deficiency, HHcy, and low omega-3 PUFA index) and physical performance in community-dwelling older adults over 5 years. In addition, we explored if the nutrition-physical performance associations varied according to the presence of low-grade inflammation (LGI) and brain imaging indicators.

METHODS

Design and Ethical Statement

This observational study used data from Multidomain Alzheimer Preventive Trial (MAPT), whose details have been described in previous publications (23, 24). Briefly, MAPT was a multicenter, 3-year randomized controlled trial that aimed to evaluate the protective effect of omega-3 PUFA supplementation and multidomain lifestyle interventions (exercise advice, cognitive training, and nutritional counseling), combined or alone, on cognitive decline in community-dwelling older adults (23). No significant effect of the interventions on cognitive function (24) or muscle strength (25) was found over 3 years. In this secondary analysis, 5-year follow-up data (3-year intervention plus an additional 2-year observation period after the end of interventions) were retrieved. The MAPT study was registered at ClinicalTrials.gov (no: NCT00672685), was approved by the French Ethical Committee located in Toulouse (CPP SOOM II), and was authorized by the French Health Authority. All the participants had signed informed consent.

Study Population

The MAPT study enrolled 1680 dementia-free adults aged $\geq$70 years, recruited from 13 memory centers in France and Monaco between 2008 to 2011, presenting at least one of the following conditions: spontaneous memory complaint, limitations in one instrumental Activities of daily living (such as disability in using telephone and transportation.), or slow gait speed ($\leq$0.8 m/s). In this study, 840 subjects who received the intervention of omega-3 supplementation and 454 subjects without nutritional markers measurement at baseline were not included; we finally considered data from 386 subjects into this study.

Measures

Definition of Nutritional Deficits

Three nutritional markers were used to define nutritional deficits: plasma 25-hydroxyvitamin D [25(OH)D], plasma homocysteine, and erythrocyte membrane omega-3 PUFA concentration. Details of nutritional marker assessment are described in **Supplementary Materials**. Nutritional deficits were determined at baseline according to the clinical cutoffs below: (1) vitamin D deficiency, if 25(OH)D $<$20 ng/ml (26); (2) HHcy, if homocysteine $>$14 μmol/L (27); (3) low omega-3 PUFA index, defined as omega-3 index (28) [% docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA)] below the lower quartile of study population ($\leq$4.87%) (16). The participants were then classified into three groups based on the counting of nutritional deficits: no deficit, 1 deficit, and $\geq$2 deficits.

Physical Performance

Three outcomes of physical performance were evaluated in this study: 4-m usual pace gait speed (in m/s), 5-repetition maximal chair rise time (in s), and Short Physical Performance Battery (SPPB) (29) score. The SPPB consisted of a walk test, a chair rise test, and a standing balance test with three challenging positions; each component was scored ranging from 0 (inability to complete the test) to 4 (best performance). The overall SPPB score was calculated by summing the three component results (ranging from 0 to 12, higher score indicates better performance) (29). All the measurements were assessed at baseline, and after 6, 12, 24, 36, 48, and 60 months of follow-up.

Low-Grade Inflammation (LGI)

In this study, 293 of the 386 participants had C-reactive protein (CRP) measured at baseline, 6- and 12-month visits, using immunoturbidity according to standard protocols. LGI (dichotomous variable) was defined as having at least two consecutively high CRP values (3–10 mg/L) between baseline and the 12-month visit, according to previous studies (30–32). Participants we could not categorize as LGI or non-LGI (e.g., people with CRP value >10 mg/L in the intermediate 6-month measurement) were excluded. Finally, we included 267 participants in the exploratory analysis.

Magnetic Resonance Imaging (MRI) Variables

Several MRI variables that had been reported to be associated with impaired mobility (18, 33, 34) were retrieved: total gray matter volume (cm^3), hippocampal volume (mm^3) and white matter hyperintensity (WMH) volume (cm^3). Total intracranial volume (TICV) was also collected for model adjustment. The acquisition protocol for brain MRI has been detailed elsewhere (23) and in **Supplementary Materials**.

Confounders

Several confounding variables were selected: age, sex, MAPT intervention groups (i.e., multidomain intervention alone or placebo), level of education (ordinal), and body mass index (BMI; kg/m^2). We also controlled the baseline physical activity status using a dichotomous variable (active or inactive) based on low physical activity component in the Fried's frailty criteria (35). In the analysis for MRI variables, adjusted models included the confounders mentioned above as well as TICV.

TABLE 1 | Baseline characteristics of the study population[1].

	Total population (*N* = 386)	Number of nutritional deficits[2]			
		No deficit (*N* = 84)	1 deficit (*N* = 153)	≥2 deficits (*N* = 149)	*p*-value[3]
Age (years)	75.6 (4.5)	74.8 (4.1)[a]	75.0 (4.3)[b]	76.7 (4.8)[a,b]	0.001
Sex (female)	263 (68.1%)	66 (78.6%)	111 (72.6%)	86 (57.7%)	0.002
MAPT groups					
Multidomain intervention	191 (49.5%)	40 (47.6%)	77 (50.3%)	74 (49.7%)	0.922
Placebo	195 (50.5%)	44 (52.4%)	76 (49.7%)	75 (50.3%)	
Education (*n* = 376)					
No diploma or primary school certificate	80 (21.3%)	13 (15.9%)	30 (19.9%)	37 (25.9%)	0.407
Secondary education	122 (32.4%)	28 (34.1%)	47 (31.1%)	47 (32.9%)	
High school diploma	58 (15.4%)	12 (14.6%)	29 (19.2%)	17 (11.9%)	
University level	116 (30.9%)	29 (35.4%)	45 (29.8%)	42 (29.3%)	
Body mass index (kg/m^2) (*n* = 385)	26.1 (3.9)	25.1 (4.0)[a]	25.9 (3.9)	26.8 (3.8)[a]	0.003
CDR status: 0.5 (mild cognitive impairment)	155 (40.2%)	27 (32.1%)	64 (41.8%)	64 (43.0%)	0.234
Physical status: inactive (*n* = 384)	53 (13.8%)	5 (6.0%)	22 (14.5%)	26 (17.5%)	0.051
Nutritional risk factors					
Vitamin D (ng/ml)	23.2 (12.5)	30.8 (12.2)[a,b]	25.2 (12.7)[a,c]	16.9 (8.8)[b,c]	<0.001
Deficiency (<20 ng/ml)	163 (42.2%)	0 (0%)	57 (37.3%)	106 (71.1%)	<0.001
Homocysteine (μmol/L)	15.64 (5.27)	11.32 (1.41)[a,b]	15.31 (4.88)[a,c]	18.41 (5.32)[b,c]	<0.001
Hyperhomocysteinemia (>14 μmol/L)	220 (57.0%)	0 (0%)	85 (55.6%)	135 (90.6%)	<0.001
Omega-3 index (%)	5.86 (1.44)	6.61 (1.13)[a]	6.21 (1.25)[b]	5.08 (1.41)[a,b]	<0.001
≤4.87 (lower quartile)	98 (25.4%)	0 (0%)	11 (7.2%)	87 (58.4%)	<0.001
Physical performance					
SPPB score, 0–12 (*n* = 378)	10.7 (1.7)	10.9 (1.5)	10.7 (1.7)	10.5 (1.8)	0.156
Gait speed (m/s) (*n* = 383)	1.08 (0.25)	1.10 (0.25)	1.08 (0.25)	1.06 (0.25)	0.590
Chair rise time (s) (*n* = 367)	11.6 (3.9)	10.4 (2.4)[a]	11.6 (3.9)	12.3 (4.5)[a]	0.004

[1]*Values presented in number (%) for categorical variables or mean (standard deviation) for continuous variables; CDR, clinical dementia rating scale; MAPT, Multidomain Alzheimer Preventive Trial; SPPB, Short Physical Performance Battery.*

[2]*Cutoff of nutritional deficits: Vitamin D < 20 ng/ml, homocysteine> 14 μmol/L, omega-3 index ≤ lower quartile (≤4.87%).*

[3]*P-value based on ANOVA or Chi-square test across groups; [a,b,c]same letters indicate significant differences between groups (p < 0.05).*

TABLE 2 | Linear mixed-effect regressions examining cross-sectional associations between nutritional deficits[a] and physical performance at baseline.

	Unadjusted model			Adjusted model[b]		
	β	95% CI	*p*-value	*β*	95% CI	*p*-value
Outcome: SPPB score (0–12)						
Nutritional deficits						
No deficit	Ref.	–	–	Ref.	–	–
1 deficit	−0.20	−0.60, 0.19	0.318	−0.16	−0.53, 0.21	0.391
≥2 deficits	−0.59	−0.99, −0.20	0.004	−0.33	−0.71, 0.05	0.089
Outcome: gait speed (m/s)						
Nutritional deficits						
No deficit	Ref.	–	–	Ref.	–	–
1 deficit	−0.01	−0.07, 0.05	0.703	−0.01	−0.06, 0.05	0.906
≥2 deficits	−0.06	−0.12, −0.01	0.041	−0.02	−0.08, 0.04	0.525
Outcome: chair rise time (s)						
Nutritional deficits						
No deficit	Ref.	–	–	Ref.	–	–
1 deficit	0.70	−0.22, 1.61	0.137	0.60	−0.28, 1.49	0.182
≥2 deficits	1.58	0.66, 2.50	0.001	0.99	0.07, 1.90	0.036

CI, confidence interval; Ref, reference group; SPPB, Short Physical Performance Battery.
[a]Cutoff of nutritional deficits: vitamin D < 20 ng/ml, homocysteine > 14 μmol/L, omega-3 index ≤ lower quartile (4.87%).
[b]Adjustments for age, sex, Multidomain Alzheimer Preventive Trial (MAPT) groups, education, body mass index, and physical activity status.

Statistical Analysis

Baseline characteristic comparisons across the nutritional deficit groups were performed by Chi-square test for categorical variables and ANOVA for continuous variables. Linear mixed-effect regressions, including a random effect at participant level and a random slope on time, were conducted to evaluate the cross-sectional and longitudinal associations between nutritional deficits and physical performance outcomes.

A series of exploratory analyses were conducted to explore the roles of LGI (among 267 subjects with available CRP data) and imaging markers (among 164 subjects with MRI measures) in the association between nutritional deficits and physical performance. We first performed logistic regressions to examine the association between nutritional deficits and LGI. Then, an interaction term by LGI and nutritional deficits was introduced into the same mixed-effect models for main analysis; only assessments of the outcomes performed at 12 months and after were considered for this analysis. For imaging markers, cross-sectional associations with nutritional deficits were tested by linear mixed-effect regressions (with random intercept for the center effect). Longitudinal analysis considering the interaction effect (MRI variable × nutritional deficits) on physical performance was examined by linear mixed-effect regressions (three-level nested model, with the participants nested into the center); for participants who received MRI scans at 6-month visit and 12-month visit, measurements of physical performances before MRI scans were excluded from the analysis. Statistical significance was defined as $p < 0.05$; the *p*-values of between-group mean differences are presented after false discovery rate correction (36). All the statistical analyses were performed with Statistical Analysis Software (SAS) version 9.4 (Cary, NC, USA).

RESULTS

Among the overall 386 participants, 21.8% ($n = 84$) had no nutritional deficit at baseline, 39.6% ($n = 153$) presented 1 deficit, and 38.6% ($n = 149$) presented ≥2 deficits. Participants with more nutritional deficits tended to be older and male, and to present with higher BMI (**Table 1**). At baseline, compared with those without any deficit, having ≥2 deficits was associated with longer chair rise time, i.e., poor chair rise performance (**Table 2**). No cross-sectional associations were found with SPPB score or gait speed.

After 5 years of follow-up, decreased SPPB score and gait speed, and increased chair rise time were observed among the participants with ≥ 2 nutritional deficits (**Table 3**). *P*-values for linear trend for within-group change in physical performance outcomes were all significant (*p* for trend <0.001). However, no significant between-group differences were discovered for the changes in SPPB, gait speed, and chair rise time when individuals without deficit and those with either 1 or ≥2 deficits were compared (**Table 3**).

In the logistic regression for LGI and nutritional deficits, people with ≥2 deficits had higher likelihood of having LGI (adjusted OR = 2.53; 95% CI: 1.01 to 6.33; $p = 0.006$; **Supplementary Table 1**), compared with those without deficits. Significant interaction effects by LGI and nutritional deficits on chair rise time were observed in the linear mixed-effect regression. Among people with ≥2 deficits, the adjusted mean difference in chair rise time over 5 years

TABLE 3 | Linear mixed-effect regressions examining variation in physical performance over 5 years according to nutritional deficits[a].

	Within-group 5-year change from baseline β (95% CI); *p*-value	*P* for trend	Between-group difference	
			Unadjusted model β (95% CI); *p*-value[c]	Adjusted model[b] β (95% CI); *p*-value[c]
Outcome: SPPB score (0–12)				
Nutritional deficits		<0.001		
No deficit,	−0.43 (−0.86, 0.01); 0.057		Ref.	Ref.
1 deficit,	−0.47 (−0.84, −0.11); 0.011		−0.05 (−0.62, 0.52); 0.871	−0.03 (−0.59, 0.53); 0.921
≥2 deficits)	−0.80 (−1.17, −0.42); <0.001		−0.37 (−0.95, 0.20); 0.411	−0.23 (−0.80, 0.33); 0.843
Outcome: gait speed (m/s)				
Nutritional deficits		<0.001		
No deficit,	−0.09 (−0.16, −0.02); 0.010		Ref.	Ref.
1 deficit,	−0.05 (−0.11, 0.01); 0.052		0.03 (−0.05, 0.12); 0.879	0.03 (−0.06, 0.11); 0.813
≥2 deficits)	−0.08 (−0.14, −0.03); 0.005		0.01 (−0.08, 0.09); 0.879	−0.01 (−0.10, 0.08); 0.813
Outcome: chair rise time (s)				
Nutritional deficits		<0.001		
No deficit,	0.77 (−0.08, 1.62); 0.075		Ref.	Ref.
1 deficit,	0.46 (−0.24, 1.17); 0.198		−0.31 (−1.41, 0.80); 0.585	−0.18 (−1.26, 0.90); 0.739
≥2 deficits)	1.13 (0.39, 1.86); 0.003		0.36 (−0.77, 1.48); 0.585	0.40 (−0.71, 1.50); 0.739

CI, confidence interval; Ref, reference group; SPPB, Short Physical Performance Battery.

[a]*Cutoff of nutritional deficits: vitamin D < 20 ng/ml, homocysteine >14 μmol/L, omega-3 index ≤ lower quartile (≤4.87%).*

[b]*Adjustments for age, sex, Multidomain Alzheimer Preventive Trial (MAPT) groups, education, body mass index, physical activity status, and time interactions.*

[c]*P-value adjusted for multiple comparisons using the Benjamini-Hochberg procedure.*

between those with and without LGI (reference group) was 3.47 s (95% CI: 1.03, 5.91; $p = 0.017$), indicating that LGI reinforced the impact of ≥2 deficits on worsening chair rise performance (**Supplementary Table 2**). On the other hand, no association between imaging markers and nutritional deficits was found (**Supplementary Table 3**). There was no evidence of any significant interaction between each imaging marker and nutritional deficits on physical performances in the linear mixed-effect models (**Supplementary Table 4**).

DISCUSSION

To our knowledge, this is the first study to investigate the associations between accumulated nutritional deficits and physical performance in community-dwelling older adults. We discovered that presenting two or more nutritional deficits (i.e., vitamin D deficiency, HHcy, and low omega-3 PUFA index) was cross-sectionally associated with poor chair rise performance at baseline. We did not observe associations of combined nutritional deficits with mobility decline over 5 years; however, our exploratory analysis found that the association of nutritional deficits with chair rise performance could vary according to LGI status, with a more pronounced increase in chair rise time (worse performance; 0.69 s more per year) among older adults with ≥2 deficits and LGI compared with their non-LGI counterparts.

The relationship between the nutritional markers investigated in our study and physical performance has mixed findings in the literature (8–13). In this study, although the between-group differences did not reach significance, within-group changes for all the three physical performance outcomes showed higher overtime declines as the number of deficits increased (p for trend <0.001). Noteworthy, after the 5-year follow-up, more than half of the participants with ≥2 nutritional deficits became octogenarians whose mobility tends to decline faster than in younger people (17). On the other hand, our exploratory analysis found that LGI, an important mechanism implicated in both aging (37) and mobility disability (17, 38), contributed to this accelerated decline of physical performance in older individuals with combined nutritional deficits. This finding suggests that both the presence of nutritional deficits and chronic inflammation contribute to physical impairment. Indeed, omega-3 PUFAs and HHcy had been proposed to affect mobility outcomes through inflammatory pathway. Omega-3 PUFAs can suppress chronic inflammation, further inhibiting muscle catabolism (39); HHcy can lead to inflammation by causing reactive oxygen species accumulation and pro-inflammatory cytokine secretion (4, 20). Although vitamin D is well-known for its metabolic roles in muscle synthesis and bone formation (3), the recent evidence had suggested it has immunomodulatory effects by regulating both innate and adaptive immunity (40). On the other hand, it is plausible that LGI status is independent of the presence of nutritional deficits, but that their joint effect enhances the detrimental impact on physical function. For example, accelerated muscle catabolism caused by inflammation (41), combined with muscle weakness caused by vitamin D deficiency (10), can lead to faster decline in overall muscle function and physical performance.

Our cross-sectional and exploratory analyses only observed significant associations between nutritional deficits and chair rise performance, suggesting nutritional deficits would affect physical performance through a muscle quality-related mechanism rather than changes in the central nervous system. This is also supported by our findings related to MRI indicators, which showed no significant interaction between brain volumes and nutritional deficits on changes in physical performance over time. Compared with gait speed, a functional vital sign (42) relying on complex movement controls with executive function involved (43), chair rise test is a more specific measure of muscle function (44), strongly determined by muscle mass and power in older adults (45, 46). Another possible explanation for the limited findings on change in SPPB is that only a few participants of this study had mobility limitation at baseline, with 6% having SPPB ≤ 7 (1) and about 20% having SPPB ≤ 9 (47). Although it is possible that people who started having mobility limitations would decline faster in mobility (48), the associations of nutritional deficits with mobility limitation in mobility-impaired individuals require further investigations.

A number of strengths should be mentioned in our study. We evaluated multiple nutritional deficits, assessed by three blood-based biomarkers, and several measures of physical performance in older adults over 5 years. In addition, we explored the potential role of inflammatory and neuroimaging markers in nutrition-physical performance associations. However, some limitations are worth mentioning. First, this is an observational study with data retrieved from a randomized controlled trial. Even though MAPT multidomain intervention did not affect physical performance (25), our results need to be interpreted cautiously, since the exercise advice and nutritional counseling part of the multidomain intervention could have modified the nutritional markers overtime. In order to minimize this bias, MAPT group allocation was added as a confounder in the models. Residual confounding may not be excluded, since some other potential confounders, such as nutritional supplementation (except for omega-3 PUFAs), inadequate dietary protein intake, and smoking and drinking habits (12, 49), were not available. Finally, the MAPT study enrolled a sample of community-dwelling older adults at risk of cognitive decline, which might affect the generalizability of our findings to other populations.

To conclude, this study did not observe prospective associations between combined nutritional deficits (vitamin D deficiency, HHcy, and low omega-3 index) and overtime mobility decline in community-dwelling older adults. However, different trajectories of chair rise performance were observed among people with two or more deficits, once the presence of chronic, low-grade inflammation was considered. Future studies that will investigate nutritional deficits and physical impairment focused on older adults with different conditions characterized by LGI, including subjects with mobility limitation, could shed light on this topic.

DATA AVAILABILITY STATEMENT

Data described in the article, code book, and analytic code will be made available upon request pending application and approval. Requests to access these datasets should be directed to Wan-Hsuan Lu, wan-hsuan.lu1@univ-tlse3.fr.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by French Ethical Committee located in Toulouse (CPP SOOM II). The patients/participants provided their written informed consent to participate in this study.

MAPT/DSA GROUP

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AUTHOR CONTRIBUTIONS

BV conceived the MAPT study. W-HL and PSB designed current research. J-FM managed image data. W-HL performed statistical analysis, drafted the manuscript, and had a primary responsibility for the final content. All authors contributed to the article and approved the submitted version.

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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.2021.771470/full#supplementary-material

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Enterprise Food Fraud in China: Key Factors Identification From Social Co-governance Perspective

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Food fraud not only exacerbates human public health risks but also threatens the business development of food and related industries. Therefore, how to curb food fraud effectively becomes a crucial issue for governments, industries, and consumers. Previous studies have demonstrated that enterprise food fraud is subject to joint influences of factor at various hierarchical levels within a complex system of stakeholders. To address enterprise food fraud, it is necessary to identify the key such factors and elucidate the functional mechanisms, as well as systematic analysis of the interrelationships among clusters and factors. Hence, we grounded on a social co-governance perspective and investigated the food fraud key influencing factors and their interrelationships in an emerging food market – China, by using the DEMATEL-based analytic network process (DANP). Results showed that the identified key cluster was government regulation, social governance, and detection techniques. Four other key factors were also identified, including government regulatory capability and penalty intensity, expected economic benefits, maturity of market reputation mechanism, and transparency of supply chain. Policy implications from the social co-governance perspective for China and similar economies are discussed finally.

Keywords: food fraud, business ethics, social co-governance, safety and quality, DEMATEL-based ANP

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INTRODUCTION

Food fraud is a collective term used to encompass the deliberate substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging or false/misleading statements made about a product for economic gain (1–5). With the escalation in incidents, scope, and harm, research on food fraud has increased in recent years (6, 7). In recent years, food fraud has become a serious and challenging issue for worldwide society (8–10). Food fraud grows obstacles for food safety regulation and the food industry (11, 12). It also increases human health risks (13), hinders development of the food market/industries, and causes trust issues among stakeholders, including food producers and dealers, consumers, trading partners, and regulatory authorities (14). Food fraud can also be more difficult to expose and can carry greater threat than conventional food safety issues (15).

For example, one of the most notorious food fraud cases worldwide was the discovery in 2008 of the illegal addition of melamine, an industrial raw material, to infant milk powder in China, which negatively impacted 300,000 infants, six of whom died (16, 17). The company in question is a large enterprise group with a history of over 50 years and total assets of nearly RMB 2 billion (as of the end of 2007). In order to reduce costs, the enterprise involved used water and melamine in the milk to counterfeit. This is an example of food fraud as 'commercial enterprise crime' carried out by producers in the food supply chain. The discovery in 2013 of the addition of horsemeat to certain products in many European countries is another example of significant food fraud (18). Such incidents have led governments and relevant organizations in various countries to step up food safety regulation (19). For instance, In China, the government has repeatedly restructured its food safety regulatory bodies, reformed regulatory rules and practices, and promulgated the highly stringent *Law on Food Safety* protocols, which are targeted at effective regulation (20, 21).

Previous food safety regulations, however, are not designed to curb deliberate misconduct and are therefore not effective at addressing intentional food fraud (22, 23). Existing systems (and research on them) only focused on the compliance of food producers with food safety control systems, particularly the *Hazard Analysis Critical Control Point* (HACCP) system, to minimize the microbial, chemical, and physical risks incurred during food production (24, 25). Food fraud is a deliberate behavior of a food producer (generally) and will include attempts to evade supervision and regulation (11). Lord et al. (26) emphasizes that food fraud constitutes a crime and generally occurs along the supply chain of ordinary food, similar to other criminal activities.

Existing food fraud research is heavily weighted toward food science, packaging and labeling, and legal areas of knowledge discovery (27). Enterprise food fraud is a business behavior performed under certain conditions. Moving forward, this requires a business decision-making perspective to further study the problem of food fraud in food companies (13). So far, Van Ruth et al. (23), Levi et al. (28), Meerza et al. (29–31) and other studies have initially discussed how various factors affect the fraud behavior of food companies. We have a clearer understanding of the fraudulent decision-making behavior of food companies and laid the foundation. For instance, Meerza et al. (31) studied the Optimal Policy Response to Food Fraud and found that under different circumstances, strict monitoring and enforcement and increased certification costs will have different effects on companies' food fraud behaviors. However, the existing research only analyzes the influence of various factors on the fraud behavior of food companies. So, among these factors, which ones are the key factors? What are their interrelationships between factors? Existing research ignores these important issues. According to our knowledge, there is currently no literature report that identifies the key factors that affect the food fraud behavior of companies and analyzes the internal relationships between the factors.

In addition, enterprise food fraud is not only influenced by the action of certain individual factors, but also joint, organizational actions of a complicated system of clusters (cluster is a factor sets formed by different factors of the same class) at different hierarchical levels and among factors within a cluster (32). However, studies on the correlations among clusters and factors that motivate enterprise food fraud and how such clusters and factors jointly influence enterprise food fraud remain limited.

Heeding to such thoughts of the gaps in the literature, we argue that studies need to explore key factors of food fraud from more systematic and holistic theoretical lens and methodology, such as the social co-governance perspective and the DEMATEL-based analytic network process (DANP) method proposed here. Social co-governance theory for food safety emphasizes on "social participation for the collective pursuit of food safety" (33). As compared with traditional governance approaches for food issues, social co-governance stresses more on the wide and collective efforts from a diverse set of stakeholders, which ensures better informational transparency/symmetry, risk and cost sharing capacity, and resource richness (34). Such relationship also stresses social contract beyond economic ones (35). For methodological concern, Huang et al. (36) and Wu et al. (37) found the DANP to be an effective approach for studying the correlations among factors and (more usefully) the inter- and intra-clusters (factor sets) relationships at different hierarchical levels.

From the analysis of the development history of food fraud, the problem of food counterfeiting exists in any country in the world with varying degrees. And food fraud often occurs in the highly competitive food market. China has a highly competitive and relatively mature food market, which is similar to the food markets in the United States and the European Union. However, about 50% of food safety incidents in China are caused by food fraud, which is the result of the combination of complex factors such as the huge return from food consumption market, the large number of food enterprises with insufficient integrity, and the weakness of food supervision in China, etc., which might be different from the United States and the European Union but similar to most developing countries. Therefore, using China as the research object to study food fraud is reasonably significant and is of positive value in understanding the causes of food fraud/counterfeiting in similar economies' contexts with potential measures that might be taken by the whole society.

LITERATURE REVIEW

Extant Literature

Production View

Information asymmetry between producers and consumers creates an adverse choice for oversupply of low-quality, unsafe products (38, 39). Traditional food regulation is dominated by the command-control type of intervention. In most developed countries, food safety regulation has focused on the imposition of standards that specify how food products should be produced and/or their final safety level (40). However, since the 1990s, food operators have frequently been given more responsibility to monitor food safety (41). For example, the UK Food Safety Act (1990) encourages food companies to establish private food safety control measures to ensure the quality and safety of food produced and sold (42). The EU Food Hygiene Regulations, implemented on January 1, 2006, require all food producers and operators to have food safety control measures to prove that they are managing food safety in their businesses. In United States, food safety control measures, such as HACCP, become a category of food safety regulation (43).

Food risks may be caused by malpractice of suppliers who exploit the fact that their production processes and resulting product properties cannot be directly observed by buyers (44). However, current food safety regulations aim to deal with unintentional food safety incidents such as microorganisms, physics and chemistry, rather than deliberately deceiving people. Besides, the design of food safety control measures from a production view could not take into account deliberate fraud. So, malicious intent is the blind spot of current food safety law (2). Food fraud often occurs outside the authorized supply chain and usually involves the addition of unsupervised substances (13). The fraudsters can design and manufacture adulterated materials based on the nature of the adulterated product, thereby escaping existing food safety controls (45). Therefore, existing food safety control measures are not effective against food fraud (22, 23).

Criminology View

Food fraud is crime-committed by producers and operators in the food supply chain to make full use of the opportunities of crime (26). In order to successfully implement food fraud, fraudsters actively seek for the opportunity and actively avoid detection using their technical expertise (46). In terms of the nature of food fraud, the collapse of the security of the entire food supply chain depends on a single factor, the criminal (45). Since food fraud is caused by conscious intelligent human opponents, food fraud is a crime and the crime prevention related theories have been applied in research (3, 4, 47, 48). The routine activity theory sees crime as the outcome of the convergence in time and place of (1) motivated offenders and (2) suitable targets in (3) the absence of capable guardians (23). Food fraud and other types of corporate crime have similar characteristics. In accordance with the routine activity theory, it is necessary to study the factors affecting the food fraud vulnerability from three aspects: opportunities, motivations and control measures, and develop a food fraud vulnerability assessment tool (23). From the view point of the Criminology, food fraud vulnerability assessment tools should be used, identify potential weaknesses of food systems, and to effectively prevent food fraud (49, 50). Nonetheless, if (as the present study does) the food fraud is defined as a commercial enterprise crime, then we need to extend such a crime prevention theory to designing organizational and institutional prevention strategies to enhance the integrity of the food system (26).

Social –Co-governance Perspective

Based on the reviews above, we found that the production viewpoint does not take into account the deliberate characteristics of food fraud, so it is ineffective to control food fraud. Although the criminological viewpoint makes up for the above shortcomings of production viewpoint, and places its emphases on preventing food fraud through prevention. However, under the background of rapid development of food production technology and increasingly internationalization and complexity of food supply chain, it is far from enough to rely solely on the strength and resources of enterprises and governments to prevent food fraud. With the purpose to reduce costs and improve the effectiveness of food safety regulation, the new collaborations between public authorities and food operators in monitoring food safety has been developed (34, 40, 51). But, establishing a better food economy with sustainable development needs the efforts of all stakeholders and the integration of relevant resources. Involvement of all stakeholders to work together helps to improve the practicability of decision-making and reduce the burden on participants (33). Therefore, food safety risk governance must introduce the participation of consumers, non-governmental organizations and other social forces to guide the whole society to co-govern together (52).

In theory, social co-governance is rooted in the theory of cooperative governance. In the late 20th century, the role orientation of "super nanny" of the government in western countries' welfare systems resulted in many disadvantages, such as expansion of functions, overstaffed institutions and inefficiency, which caused public discontent due to inadequate governance of environmental protection, market monopoly, food safety and other issues (33). In order to solve the problems of fragmentation and decentralization of government governance, the theory of social governance, which emphasizes the multi-dispersed subjects to reach a multilateral interactive cooperative network, began to emerge at the end of the 20th century (53). As an important stakeholder of food safety, the media, employees, consumers and other social entities can also play an important role in preventing food safety risks. Social co-governance of food safety is a concept aimed at strengthening the partnership among the government, enterprises and social entities. The concept of social co-governance has become a practice in many countries. In the EU, governments, enterprises, social organizations, and citizens are actively involved in food safety governance. In China, the Food Safety Law of the People's Republic of China on October 1, 2015 established social co-governance as an important criterion for food safety risk governance. According to this, both theory and practice require that the discussion of the governance of food fraud be extended to the main constituents of a society.

In sum, the governance of food fraud with production and criminological views is mainly from the two main entities of the enterprise and the government, respectively. This paper expands the research scope to a pluralistic social groups (i.e., enterprise, government, consumers, and other stakeholders) based on the theory of social co-governance. A major reason is that the social co-governance theory suggests that stakeholders such as the consumers, social organizations or the other relatively neglected actors can also play an important role in ensuring food safety and in preventing fraud, posing a powerful complement to government governance and corporate self-discipline (33). To this end, based on our review of the perspective of social co-governance, this paper proposes five dimensions and 12 factors that may affect the corporate food fraud behavior (**Table 1**).

TABLE 1 | Key clusters and factors influencing enterprise food fraud.

Target	Cluster	Factor
Enterprise food fraud	Enterprise characteristics (D_1)	Enterprise scale (C_{11})
		Enterprise business ethics (C_{12})
		Manager's awareness of social responsibility (C_{13})
	Economic benefits and technical hardness of food fraud (D_2)	Expected economic benefits (C_{21})
		Technical hardness of food fraud (C_{22})
	Government regulation, social governance, and detection techniques (D_3)	Government regulatory capability and penalty intensity (C_{31})
		Supervision by social forces (C_{32})
		Utility of detection techniques and methodologies (C_{33})
	Market governance (D_4)	Maturity of market reputation mechanism (C_{41})
		Consumption behavior on food market (C_{42})
	Internal relationship and transparency of food supply chain (D_5)	Constraints by downstream enterprises (C_{51})
		Transparency of supply chain (C_{52})

Enterprise Characteristics Cluster

Enterprise food fraud is closely related to business scale, business ethics, and awareness of social responsibilities.

Enterprise Scale

Enterprise scale refers to the number of employees and the size of assets. Though an enterprise may engage in food fraud regardless of its scale (47), a food enterprise of smaller scale has higher risk of deliberate crime as it may choose not to recall sold products suspected of authenticity or safety problems and may ignore consumer grievances (54). For instance, Wu et al. (55) reported that small-scale enterprises are more inclined to abuse food additives. Levi et al. (28) also revealed that smaller farms are more vulnerable to risks and may resort to food fraud when facing quality uncertainty or price pressure.

Business Ethics

Business ethics refers to the integrity and ethical atmosphere within the enterprise. Business ethics are basic ethical codes that an enterprise complies with in all production and trade activities (56). Food fraud is unethical conduct (57), which is often closely related to business culture and the decision-maker's failure to stand behind the ethical bottom line (58). Business ethics is an important risk factor for corporate financial fraud (59, 60). Similarly, business ethics are key cultural factors leading to food fraud vulnerability (23). Enhancing an enterprise's business ethics imposes a positive influence on the enterprise from a cultural perspective and encourages the business to refrain from food fraud (61).

Manager's Awareness of Social Responsibility

Manager awareness refers to the attitude of managers toward the social responsibility that the enterprise should take. Social responsibilities are fundamental duties related to environmental protection, justice, and equality that an enterprise assumes while striving for maximum benefits (62). Furthermore, ensuring food safety is the most important social responsibility of a food enterprise (63). Though most enterprises understand social responsibilities of food safety for the sake of a good public image (64), the concept of social responsibility originated and evolved to promote compliance with ethics and legislation among increasing cases of non-compliance (65). Illegal conduct will decrease if an enterprise strictly adheres to its social responsibilities. A manager's awareness of these social responsibilities also influences both willingness and performance. The stronger the manager's awareness of social responsibilities, the more responsible an enterprise is in regard to food safety and food fraud misconduct (66).

Expected Economic Benefits and Technical Hardness Cluster

Food fraud is intrinsically subject to expected economic benefits and technical hardness.

Expected Economic Benefits

Although food fraud may require an input of resources, it will also undoubtedly generate benefits (67). This is why fraudsters choose to misbehave in violation of social ethics and even with the risk of punishment (68, 69). For example, Levi et al. (28) found that enterprise food fraud aims to maximize the perceived quality of low-quality products to achieve higher economic benefits. Bitzios et al. (70) also determined that foods bearing geographical indication (GI) labels usually resulted in better quality foods and higher consumer acknowledgment; furthermore, when substantial economic benefits are expected from counterfeiting ordinary food into a GI product, the enterprise exhibits a higher probability of committing food fraud.

Technical Hardness

An enterprise will be more inclined to commit fraud when it is technically easy (71). The technical hardness of fraud can be measured from both knowledge and substance aspects. On the knowledge side, a fraudster is usually a technical expert with rich knowledge of production and knows how to perform the fraud and how to evade capture (46). Furthermore, it is relatively easy to acquire the knowledge and techniques necessary for food fraud (23). On the substance side, most food fraud does not necessitate complicated equipment or other substances and the required additives are often easily available (72). For example, the infant milk powder incident in China resulted from adding melamine to milk to conceal that it had been diluted with water (16). Melamine is an ordinary and easily accessible chemical and its addition to the milk did not require any complicated techniques.

Government Regulation, Social Governance, and Detection Techniques Cluster

Food fraud is subject to impact from government regulatory capability and penalty intensity, social supervision, and utility of detection techniques and methodologies.

Government Regulatory Capability and Penalty Intensity

Food fraud rampancy is closely related to inefficient government regulation (73). In China, the deficiency in food safety supervision and control, as well as the fragmentation of regulatory agencies, has resulted in poor regulation (47) as well as increased opportunities for fraudulent behavior. In addition, the government sampling inspection system is based on conventional empirical methodologies, information, and knowledge (i.e., what additives may be supplemented to food), and often cannot identify fraud based on the latest developments (74, 75). Furthermore, there is no punitive "joint examination" on similar enterprises. Therefore, the food quality sampling inspection system itself does not effectively deter food fraudsters (76). In addition, relatively moderate punishment coupled with high expected economic benefits does not constitute an effective deterrent, thus resulting in the high risk of food fraud (54).

Supervision of Social Forces

Media, consumers, employees, and social organizations can help alleviate food fraud (33). For example, Peng et al. (77) found that food safety scandals disclosed by the media can lead to a decline in sales and damage to the brand's reputation, which might reversely help correct the misconducts of food producers. However, companies may be under the expectation that food fraud will not be discovered, which can induce enterprise misconduct. Traditionally, China is a institution-driven market economy with limited participation by civil society (78). If the general public identify and report fraud cases, those committing the fraud are exposed (69) and the enterprise manager's psychological expectations may change. The "whistler" inside the enterprise can help discover the fraudulent behavior (79). Waterhouse et al. (80) indicated that employees are more aware of hidden fraud and therefore whistle-blowing is a powerful tool to prevent fraudulent activities from inside food enterprises. Li et al. (81) stated that social organizations can help to avoid the dual failure of public government power and private market power and play an irreplaceable role in supervising the operation of food enterprises.

Utility of Detection Techniques and Methodologies

A fundamental reason why food fraud is rampant is the poor utility of food testing methodologies, which are unable to detect food fraud (11). Generally speaking, food testing methodologies are based on known additives and pollutants and whether such additives are excessive compared with the prescribed threshold values (82). However, the sophistication of food and raw materials complicates both analysis and detection (67), particularly when the testing institutes do not know the additives (6). Thus, in response to enterprise food fraud, it is important to combine targeted and non-targeted testing methods (19).

Market Governance Cluster

Food fraud is also subject to influence from the maturity of the market reputation mechanism and consumption behavior on the food market.

Maturity of Market Reputation Mechanism

Good market reputation can enhance market sales (83) and can be the primary means by which an enterprise avoids market risks and achieves economic benefits (84). Therefore, market reputation constitutes a foundation of survival and benefits. Food fraud can result in severe damage to an enterprise's market reputation (85), not just for the enterprise committing the wrongdoing, but also for other enterprises in the same industry, causing heavy economic losses. For example, the 2008 melamine infant milk powder incident in China damaged the reputation of the company involved so badly that it went bankrupt in the same year. Therefore, reputation is a key market mechanism for preventing enterprise food fraud (54). For instance, a mature market reputation mechanism, whereby any enterprise food fraud is disclosed to the general public, can deter other enterprises from committing such misconduct.

Consumption Behavior on Food Market

Regulation of food systems exists to ensure safety and enhance consumer confidence in the food which they purchase and consume. However, food fraud scandals have caused consumers to be anxious and distrustful of local food products, and further stimulate distrust in food system. Consumers' awareness of food fraud incidents has reduced consumers' willingness to pay for products from companies and industries that have experienced food fraud scandals (86). Moreover, when consumers believe that there is a lack of regulatory protection, they will develop strategies to reduce the risk of food fraud to prevent the purchase and consumption of fraudulent food (54). The three main coping approaches include purchasing decision making, information searching & sharing and daily self-preservation strategies (87). These risk mitigation strategies of consumers (that is, consumer behavior) affect the food fraud behavior of companies.

Internal Relationship and Transparency Along Food Supply Chain Cluster

Mutual constraints among stakeholders and transparency along the food supply chain are also key factors influencing enterprise food fraud.

Constraints by Downstream Enterprises in the Supply Chain

Previous studies have demonstrated that downstream enterprises in the supply chain can constrain upstream enterprises by inspecting the safety and authenticity of foods or materials, thus preventing food fraud. Babich and Tang (88) and Cao et al. (89) showed that inspection and deferred payment mechanisms can prevent adulteration by suppliers and upstream enterprises.

Nevertheless, deficiencies in the constraints mechanism by downstream enterprises can also increase the probability of enterprise food fraud. Levi et al. (28) revealed that, compared with concentrated supply chains, distributed supply chains entail difficulties for downstream enterprises to impose constraints on upstream enterprises, thus raising the probability of food fraud along the supply chain.

Transparency of Supply Chain

Increasing complexity of the supply chain network can result in less visibility of the operational management of suppliers and is a key cause of food fraud (70). For example, Waterhouse et al. (80) determined that adulterated wine can reach consumers due to the non-transparent chain of supply and distribution. The melamine infant milk powder scandal in China also provides evidence that non-transparency of the upstream supply chain can lead to food fraud (61). Ensuring transparency of the supply chain can enhance food safety and quality (90). The Safe Supply of Affordable Food Everywhere (91) organization states that efforts should be made to acquire and maintain enhanced traceability information to ensure high transparency of the supply chain and minimization of food fraud.

METHODS AND DATA

Methods

Based on the existing literature, this paper has summarized some factors and clusters that influence the counterfeiting decisions of food companies, so what is the interrelationship between clusters and factors? What are the intrinsic mechanisms upon which they can influence food counterfeiting? What are the key clusters and key factors? To answer the questions, based on Hsu et al. (92) and Huang et al. (36), we applied the DANP method as follows:

Acquired the Influential Net Relationship Map With DEMATEL

Step 1 – Calculated direct relationship average matrix A. Firstly, a direct relationship matrix was generated based on the assessment results of each expert member. The average matrix $A = \left[a_{ij}\right]_{n\times n}, i, j = 1, 2, \ldots, n$ was then obtained by calculating the average of the same factor of all direct relationship matrices.

Step 2 – Calculated initial direct influence matrix D.

$$D = z \times A$$

$$z = \min\left\{1/\max_i \sum_{j=1}^{n} a_{ij}, 1/max_j \sum_{i=1}^{n} a_{ij}\right\},$$

$$\text{where} i, j \in \{1, 2, \ldots, n\}$$

Step 3 – Calculated total influence matrix T.

$$T = \left[t_{ij}\right]_{n\times n}, i, j = 1,\ 2, \ldots, n$$

where, t_{ij} is the degree of the direct and indirect influences of factor i on factor j.

$$T = D + D^2 + D^3 + \ldots + D^h = D\left(I - D^h\right)(I - D)^{-1}$$

$$\text{As } D = \left[d_{ij}\right]_{n\times n}, 0 \le d_{ij} < 1, 0 \le \sum_i d_{ij} \le 1, 0 \le \sum_j d_{ij} \le 1,$$
$$\text{when } h \to \infty, D^h = [0]_{n\times n},$$

then

$$T = D(I - D)^{-1}$$

Step 4 – Calculated the sum of each line and each column of total influence matrix T.

$$r_i = \sum_{j=1}^{n} t_{ij}$$

$$c_j = \sum_{i=1}^{n} t_{ij}$$

where, r_i is the total of the direct and indirect influences of factor i on other factors in the system and c_j is the total of the direct and indirect influences that factor j receives from other factors in the system. When $i = j$, $r_i + c_i$ is the sum of influence that factor i imposes on other factors and receives from other factors and $r_i - c_i$ is the difference of influence that factor i imposes on other factors and receives from other factors. $r_i - c_i > 0$ indicates that factor i has influence on other factors and is the cause factor in the system. $r_i - c_i < 0$ indicates that factor i is influenced by other factors and is the result factor in the system.

Step 5 – Acquired the influential net relationship map.

Calculated the Mixed Weight by Combining DEMATEL and the Analytic Network Process

Assuming each cluster has an equal degree of influence, ANP standardizes an unweighted supermatrix established by pair comparison between indicators into a weighted supermatrix. However, different clusters have different influences on enterprise food fraud. Therefore, DEMATEL can be used to determine the degree of influence of each cluster and thus normalize the ANP unweighted supermatrix to simulate real-world situations (92).

Step 1 – Acquired the unweighted supermatrix. We first divided the total influence matrix T into the T_D matrix (by cluster) and T_C matrix (by factor) based on clusters and factors in **Table 1**.

$$T_C = \begin{array}{cc} & \begin{array}{ccccc} D_1 & \cdots & D_i & \cdots & D_n \\ C_{11}\cdots C_{1m_1} & & C_{j1}\cdots C_{jm_j} & & C_{n1}\cdots C_{nm_n} \end{array} \\ \begin{array}{c} D_1 \begin{array}{c} c_{11} \\ \vdots \\ c_{1m_1} \end{array} \\ \vdots \\ D_i \begin{array}{c} c_{i1} \\ \vdots \\ c_{im_i} \end{array} \\ \vdots \\ D_n \begin{array}{c} c_{n1} \\ \vdots \\ c_{nm_n} \end{array} \end{array} & \begin{bmatrix} T_C^{11} & \cdots & T_C^{1j} & \cdots & T_C^{1n} \\ \vdots & & \vdots & & \vdots \\ T_C^{i1} & \cdots & T_C^{ij} & \cdots & T_C^{in} \\ \vdots & & \vdots & & \vdots \\ T_C^{n1} & \cdots & T_C^{nj} & \cdots & T_C^{nn} \end{bmatrix} \end{array}$$

$$T_D = \begin{bmatrix} t_D^{11} & \cdots & t_D^{1j} & \cdots & t_D^{1n} \\ \vdots & & \vdots & & \vdots \\ t_D^{i1} & \cdots & t_D^{ij} & \cdots & t_D^{in} \\ \vdots & & \vdots & & \vdots \\ t_D^{n1} & \cdots & t_D^{nj} & \cdots & t_D^{nn} \end{bmatrix}$$

We then calculated the standardized total influence matrix T_c^a.

$$T_C^{\alpha} = \begin{array}{cc} & \begin{array}{ccccc} D_1 & \cdots & D_i & \cdots & D_n \\ C_{11}\cdots C_{1m_1} & & C_{j1}\cdots C_{jm_j} & & C_{n1}\cdots C_{nm_n} \end{array} \\ \begin{array}{c} D_1 \begin{array}{c} c_{11} \\ \vdots \\ c_{1m_1} \end{array} \\ \vdots \\ D_i \begin{array}{c} c_{i1} \\ \vdots \\ c_{im_i} \end{array} \\ \vdots \\ D_n \begin{array}{c} c_{n1} \\ \vdots \\ c_{nm_n} \end{array} \end{array} & \begin{bmatrix} T_C^{\alpha 11} & \cdots & T_C^{\alpha 1j} & \cdots & T_C^{\alpha 1n} \\ \vdots & & \vdots & & \vdots \\ T_C^{\alpha i1} & \cdots & T_C^{\alpha ij} & \cdots & T_C^{\alpha in} \\ \vdots & & \vdots & & \vdots \\ T_C^{\alpha n1} & \cdots & T_C^{\alpha nj} & \cdots & T_C^{\alpha nn} \end{bmatrix} \end{array}$$

$$\text{where, } T_C^{\alpha 11} = \begin{bmatrix} t_{c11}^{11}/d_{c1}^{11} & \cdots & t_{c1j}^{11}/d_{c1}^{11} & \cdots & t_{c1m_1}^{11}/d_{c1}^{11} \\ \vdots & & \vdots & & \vdots \\ t_{ci1}^{11}/d_{ci}^{11} & \cdots & t_{cij}^{11}/d_{ci}^{11} & \cdots & t_{cim_1}^{11}/d_{ci}^{11} \\ \vdots & & \vdots & & \vdots \\ t_{cm_1 1}^{11}/d_{c1}^{11} & \cdots & t_{cm_1 j}^{11}/d_{cm_1}^{11} & \cdots & t_{cm_1 m_1}^{11}/d_{cm_1}^{11} \end{bmatrix},$$

$$d_{ci}^{11} = \sum_{j=1}^{m_1} t_{ij}^{11}, i = 1, 2, \ldots m_1.$$

Finally, we calculated the unweighted supermatrix W.

$$W = \left(T_C^{\alpha}\right)'$$

Step 2 – Calculated the weighted standardized supermatrix W^{α}.

$$T_D^{\alpha} = \begin{bmatrix} t_D^{11}/d_1 & \cdots & t_D^{1j}/d_1 & \cdots & t_D^{1n}/d_1 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ t_D^{i1}/d_i & \cdots & t_D^{ij}/d_i & \cdots & t_D^{in}/d_i \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ t_D^{n1}/d_n & \cdots & t_D^{nj}/d_n & \cdots & t_D^{nn}/d_n \end{bmatrix} = \begin{bmatrix} t_D^{\alpha 11} & \cdots & t_D^{\alpha 1j} & \cdots & t_D^{\alpha 1n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ t_D^{\alpha i1} & \cdots & t_D^{\alpha ij} & \cdots & t_D^{\alpha in} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ t_D^{\alpha n1} & \cdots & t_D^{\alpha nj} & \cdots & t_D^{\alpha nn} \end{bmatrix},$$

$$d_i = \sum_{j=1}^{n} t_D^{ij}, i = 1, 2, \ldots, n.$$

$$W^{\alpha} = T_D^{\alpha} W$$

Step 3 – Calculated the ultimate supermatrix W^*.

$$W^* = \lim_{g \to \infty} \left(W^{\alpha}\right)^g$$

Step 4 – Calculated the mixed weight as per the following formula:

$$Z = w + T \times w = (I + T)\, w$$

where, Z is the mixed weight and W is the comprehensive weight of secondary indicators.

Data

In order to ensure the data quality and quantity requirements of the DANP method, we have done following efforts. In terms of data quality, since sample's appropriateness and richness is very important (93), this paper selects qualified experts based on three criteria. First, experts are experienced and have at least 15 years of research or work experience in food safety areas. Second, experts must have an academic professorship, food industrial manager, or a government food safety governor background, in order to possess a more comprehensive knowledge structure. This determines the diversity, representativeness and breadth of the expert group, and can give a comprehensive evaluation based on the comprehensive consideration of the views and interests of different stakeholders related to food fraud. Third, experts all must be from China.

It should be noted that the research method used in this paper does not require a high number of experts to participate in the evaluation. For example, when Chiu and Tzeng (94) and Shen and Tzeng (95) used DANP (DEMATEL-based ANP) to conduct the study, the number of experts participating in the evaluation was only eight. Thus, our study refers to the literature of Chiu and Tzeng (94), Shen and Tzeng (95), Chuang and Chen (96), and Huang et al. (36), and uses the average deviation rate (or what is referred to as "errors of gap ratio") to determine the number of experts, which satisfies the number of participating experts in the evaluation process as required by the DANP method. In terms of data quantity, according to Chiu et al. (94), Huang et al. (36), Chuang and Chen (96) and Shen and Tzeng (95), this paper uses the average deviation rate to assess whether the expert size reaches theoretical saturation ($\frac{1}{n(n-1)} \sum_{i=1}^{n} \sum_{j=1}^{n} \frac{\left|a_{ij}^{p} - a_{ij}^{p-1}\right|}{a_{ij}^{p}} \times 100\%$). p is the number of experts, a_{ij}^{p} is the average effect of factor i on factor j, and n is the number of factors being affected. In this paper, a group of experts were invited to participate in the project, who come from China National Food Industry Association, China Agricultural University, Shandong Agricultural University, Jiangnan University, Jiangsu Academy of Agricultural Sciences and other institutions. Experts can express their opinions and discuss together before evaluating the relationship between the two factors. Since the opinions of the experts are expressed in terms of language rather than numerical values, when the evaluation results are finally collected, experts are required to score the pairwise relationship between the factors according to the corresponding integer values in **Table 2**.

Finally, regarding the theoretical saturation, we refer to Chuang and Chen (96) for our study. Using the average deviation rate (or "errors of gap ratio," EGR) method, we calculated that the average deviation rate of the nine experts who participated in the evaluation was 4.25% <5% (see P24 of the revised paper). This indicates that we have more than 95% confidence that there is no significant difference between the results of 9 experts and 8 experts participating in the evaluation. According to Chuang and Chen (96), it is reasonable to assume that 9 experts are close to the theoretical saturation and meet the requirement of an appropriate number of experts.

TABLE 2 | Conversion between linguistic variables and integer rank.

Linguistic variable	Corresponding integer
No (no influence)	0
VL (very low influence)	1
L (low influence)	2
H (high influence)	3
VH (very high influence)	4

RESULTS

By averaging the expert assessment results, we obtained the direct relationship average matrix A. By repeating the above step, we then obtained the initial direct relationship matrix D (**Table 3**), line sum and column sum (r_i and c_i) of total influence matrix T and of each cluster and factor (**Table 4**), and the mixed weights of the clusters and factors. Finally, we performed normalized sorting of the mixed weights to compile (**Table 5**).

DISCUSSION

Based on the calculation results obtained by the DANP method, this section identifies the interrelationships between Clusters and Factors that affect food counterfeiting and the intrinsic mechanisms that influence counterfeiting decisions of food

TABLE 3 | Initial direct relationship matrix D.

	C_{11}	C_{12}	C_{13}	C_{21}	C_{22}	C_{31}	C_{32}	C_{33}	C_{41}	C_{42}	C_{51}	C_{52}
C_{11}	0.00000	0.07609	0.08333	0.09058	0.07609	0.03261	0.02899	0.03623	0.03261	0.05435	0.07971	0.08696
C_{12}	0.06884	0.00000	0.11957	0.08333	0.03623	0.07609	0.07609	0.05435	0.10145	0.06159	0.08696	0.07971
C_{13}	0.07246	0.11594	0.00000	0.08696	0.05435	0.08696	0.08696	0.05072	0.10870	0.06522	0.08696	0.07609
C_{21}	0.07246	0.07246	0.07246	0.00000	0.10145	0.10507	0.09783	0.07609	0.10145	0.07246	0.08696	0.06884
C_{22}	0.09058	0.05797	0.05072	0.09420	0.00000	0.07609	0.06884	0.09058	0.07971	0.05435	0.09783	0.07246
C_{31}	0.08696	0.09420	0.07246	0.10870	0.09420	0.00000	0.10145	0.08333	0.09783	0.06522	0.09420	0.10145
C_{32}	0.06884	0.07971	0.07609	0.08333	0.07971	0.09783	0.00000	0.09058	0.09783	0.08333	0.08696	0.08333
C_{33}	0.06884	0.08333	0.04710	0.07609	0.10145	0.08333	0.04348	0.00000	0.06522	0.06159	0.07246	0.04348
C_{41}	0.10507	0.11232	0.08696	0.07609	0.03986	0.09783	0.09420	0.03261	0.00000	0.10145	0.07246	0.09420
C_{42}	0.02899	0.07246	0.07246	0.06522	0.05072	0.09783	0.10145	0.05797	0.10145	0.00000	0.08696	0.09420
C_{51}	0.08696	0.09058	0.06884	0.08333	0.09058	0.08333	0.06522	0.06159	0.06884	0.05797	0.00000	0.09420
C_{52}	0.07246	0.09783	0.07609	0.09420	0.07246	0.08333	0.08696	0.06522	0.09783	0.05797	0.09783	0.00000

TABLE 4 | Values of r_i, c_i, $r_i + c_i$, and $r_i - c_i$ for clusters and factors influencing enterprise food fraud.

Cluster	r_i	c_i	$r_i - c_i$	$r_i + c_i$	Factor	r_i	c_i	$r_i - c_i$	$r_i + c_i$
D_1	2.53517	2.72529	−0.19012	5.26046	C_{11}	5.11596	6.20740	−1.09144	11.32336
					C_{12}	6.35550	7.08381	−0.72831	13.43931
					C_{13}	6.67952	6.24157	0.43795	12.92110
D_2	2.62924	2.59154	0.0377	5.22078	C_{21}	6.91238	6.99331	−0.08093	13.90570
					C_{22}	6.19436	5.94982	0.24455	12.14418
D_3	2.77796	2.58851	0.18945	5.36647	C_{31}	7.38820	6.83774	0.55046	14.22594
					C_{32}	6.89975	6.40159	0.49816	13.30135
					C_{33}	5.59856	5.26934	0.32922	10.86790
D_4	2.73436	2.63741	0.09695	5.37177	C_{41}	6.80847	7.07836	−0.26989	13.88683
					C_{42}	6.31226	5.56394	0.74832	11.87619
D_5	2.72901	2.86299	−0.13398	5.59200	C_{51}	6.34861	7.02748	−0.67887	13.37610
					C_{52}	6.73675	6.69597	0.04079	13.43272

companies, and identifies the key Clusters and key Factors from three aspects.

Relationships Among Clusters and Factors That Influence Enterprise Food Fraud

The $r_i - c_i$ and $r_i + c_i$ values of each cluster and factor obtained from DEMATEL analysis are shown in **Table 4**. With reference to the plotting methods of Yang and Tzeng (97) and by use of the $(r_i + c_i, r_i - c_i)$ dataset, we obtained the influential net relationship map (**Figure 1**).

TABLE 5 | Normalized rank of mixed weights of clusters and factors influencing enterprise food fraud.

	Influence weight	Rank	Criterion	Influence weight	Rank
D_1	0.23108	2	C_{11}	0.06623	12
			C_{12}	0.08112	10
			C_{13}	0.08373	7
D_2	0.17232	5	C_{21}	0.09111	2
			C_{22}	0.08121	9
D_3	0.24903	1	C_{31}	0.09245	1
			C_{32}	0.08634	5
			C_{33}	0.07024	11
D_4	0.17293	4	C_{41}	0.09057	3
			C_{42}	0.08236	8
D_5	0.17464	3	C_{51}	0.08541	6
			C_{52}	0.08922	4

Figure 1 depicts the direct relationships among five clusters that influence enterprise food fraud, i.e., enterprise characteristics (D_1), economic benefits and technical hardness of food fraud (D_2), government regulation, social governance, and detection techniques (D_3), market governance (D_4), and internal relationship and transparency of food supply chain (D_5). The direct influence of cluster D_3 on D_4, D_2, D_5, and D_1 can be expressed as $D_3 \rightarrow \{D_4, D_2, D_5, D_1\}$. Similarly, the direct influence of cluster D_4 on D_2, D_5, and D_1 can be expressed as $D_4 \rightarrow \{D_2, D_5, D_1\}$; the direct influence of cluster D_2 on D_5 and D_1 can be expressed as $D_2 \rightarrow \{D_5, D_1\}$; and the direct influence of cluster D_5 on D_1 can be expressed as $D_5 \rightarrow \{D_1\}$.

Figure 1 also shows the direct influence relationship among factors within the same cluster. For example, cluster D_1 encompasses three interrelated factors, i.e., enterprise scale (C_{11}), business ethics (C_{12}), and manager's awareness of social responsibilities (C_{13}). The direct influence of C_{13} on C_{12} and C_{11} can be expressed as $C_{13} \rightarrow \{C_{12}, C_{11}\}$ and the direct influence of C_{12} on C_{11} can be expressed as $C_{12} \rightarrow \{C_{11}\}$. The direct influence relationship among factors within each of the other four clusters can be expressed in the same way as cluster D_1.

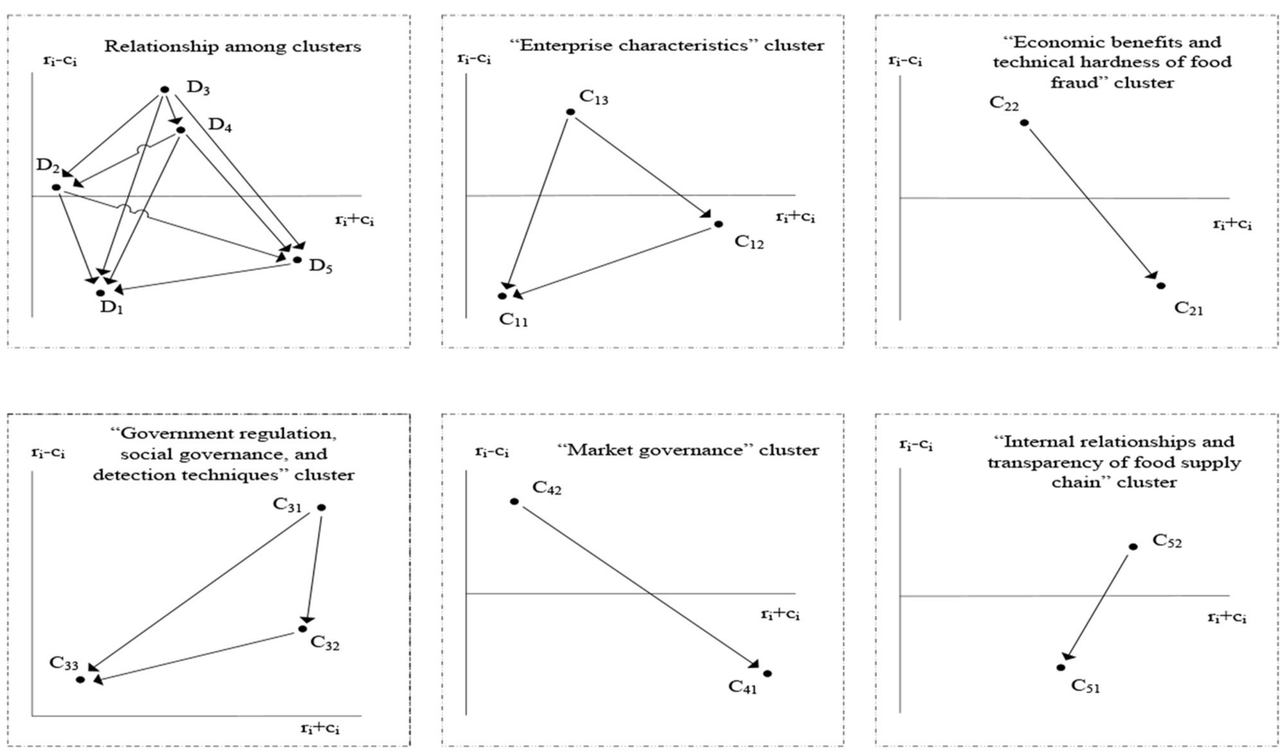

FIGURE 1 | Influence relationship net map among clusters and factors.

Intrinsic Mechanism of How Various Clusters and Factors Influence Enterprise Food Fraud

The $r_i - c_i$ values in **Table 4** were used to determine by what intrinsic mechanism the clusters and factors influence enterprise food fraud. Firstly, at the cluster level, D_3, D_4, and D_2 were identified as cause clusters based on their positive $r_i - c_i$ values, with each influencing other clusters in the system to a certain degree. In addition, D_1 and D_5 were identified as result clusters based on their negative $r_i - c_i$ values, with both influenced significantly by other clusters in the system. Therefore, the five clusters interacted intrinsically, such that clusters D_3, D_4, and D_2 directly and/or indirectly influenced clusters D_1 and D_5, and ultimately enterprise food fraud. This intrinsic mechanism can help us understand the causes of food fraud. In developed countries, the lack of detection technology is an important cause of food fraud (6). However, the **Figure 1** shows that in China, the lack of government governance is highly related to insufficient supervision of social entities, but not for the reasons of governance approaches. This result might also apply to, and have implications for, other developing countries.

At the factor level, seven factors were identified as cause factors based on their positive $r_i - c_i$ values, with each imposing significant influence on other factors in the system to varying degrees. These factors included consumption behavior on food market (C_{42}), government regulatory capability and penalty intensity (C_{31}), supervision by social forces (C_{32}), manager's awareness of social responsibility (C_{13}), technical hardness (C_{22}), utility of detection techniques and methodologies (C_{33}), and transparency of supply chain (C_{52}). The other five factors were identified as result factors based on their negative $r_i - c_i$ values, with each influenced significantly by other factors to varying degrees. These factors included enterprise scale (C_{11}), business ethics (C_{12}), constraints by downstream enterprises (C_{51}), expected economic benefits (C_{21}), and maturity of market reputation mechanism (C_{41}). In summary, the factors interacted and influenced the fraudulent behavior of food enterprises intrinsically, with C_{42},C_{31}, C_{32},C_{13},C_{22},C_{33}, and C_{52} directly and/or indirectly influencing C_{11}, C_{12}, C_{51}, C_{21}, and C_{41}, and ultimately enterprise food fraud. From a supply perspective, an in-depth understanding of the unethical behavior of companies pursuing profits in the supply chain can help us understand the food fraud behavior of companies (1). However, this intrinsic mechanism further reveals the particularity of the causes of Chinese food fraud from the perspective of demand. As in **Table 4**, C_{42}′s $r_i - c_i$ value is the largest, indicating that the consumption behavior of the food market, especially the food literacy of consumers, provides a market space for food fraud. This may also be an important reason why food fraud in rural China is more serious than in urban areas.

In addition to the above, another major advantage of the DANP method is that when a result factor emerges, the decision-maker can determine what has caused the issue by examining the cause factors. Take the internal relationships and transparency of the food supply chain (D_5) cluster as an example. **Table 4** shows that constraints by downstream enterprises (C_{51}) was the only result factor in this cluster, whereas transparency of supply chain (C_{52}) was the cause factor. Loose constraints on upstream enterprises by downstream enterprises on the supply chain may be due to inadequacy of supply chain transparency. Similarly, low manager awareness of social responsibilities may be due to small scale or poor business ethics of the enterprise. High expected economic benefits from food fraud may be due to the low technical hardness of fraud. These inferences conform to what occur in the real world and may provide essential references for the government in stipulating and enforcing relevant policies.

How to Identify the Clusters and Factors Influencing Enterprise Food Fraud

Based on the internal relationships among clusters and factors and the intrinsic mechanism of how they influence enterprise food fraud, we used the mixed weights in **Table 5** to further identify the key clusters and factors that influence enterprise food fraud.

Results demonstrated that the government regulation, social governance, and detection techniques (D_3) cluster had an influence weight of 0.24903, and thus was a key cluster ranking first among the five clusters, as also seen in **Figure 1**. Furthermore, D_3 had the maximum $r_i - c_i$ value, which did not differ significantly from that of D_5. This implies that, as a key cluster, D_3 significantly influenced the other clusters and played a dominant role in the system. Therefore, based on the mixed weights, the DANP results were consistent with those obtained using DEMATEL. The results showed that the relationships between dimensions and real-world considerations are more significant than any single dimension. This also reveals the importance of establishing a system of social co-governance (implemented by improving all dimensions but not any single one) that is jointly supervised by the government and social entities in China.

Secondly, factors with a mixed weight > 0.09 in **Table 5** were identified as key factors that influence the food fraud behavior of enterprises. Government regulation and penalty intensity (C_{31}) was deemed a key factor based on its first-ranked mixed weight of 0.09245. This is consistent with the conclusions of Lord et al. (47) and Kendall et al. (54). The expected economic benefits (C_{21}) and maturity of market reputation mechanism (C_{41}) were also deemed as key factors with mixed weights of 0.09111 and 0.09057, ranking second and third, respectively. These findings are supported by Charlebois et al. (68). Transparency of supply chain (C_{52}) was also determined to be a key factor, with a mixed weight of 0.08922 (close to 0.09), ranking fourth in the system. This result is supported by Bitzios et al. (70).

The key factors identified above are consistent with previous studies, thus providing preliminary proof that the DANP method is applicable and the conclusions of the study are reliable. Furthermore, to verify the applicability of the DANP method, we compared the key factors identified by DEMATEL and DANP analyses, which showed consistent conclusions. Previous studies have generally identified key factors by the magnitude of the r_i+c_i values obtained using DEMATEL (55). As seen in **Table 4**, the first four key factors ranked by the DEMATEL $r_i + c_i$ values were government regulation and penalty intensity, expected economic benefits from fraud, maturity of market reputation mechanism, and transparency of supply chain. These results agree with the conclusions obtained using the mixed weight magnitudes from DANP (**Table 5**). Therefore, it is reasonable to believe that the four key factors proposed by this paper are accurate. Thus, we found the DANP method to be applicable in the identification of key factors that influence enterprise food fraud behavior.

In addition to the four key factors above, six other factors, namely supervision by social forces (C_{32}), constraints by downstream enterprises (C_{51}), manager's awareness of social responsibility (C_{13}), consumption behavior on food market (C_{42}), technical hardness of food fraud (C_{22}), and enterprise business ethics (C_{12}), had mid-rank mixed weights ranging from 0.8 to 0.9, and were thus deemed to be secondary key factors. Two further factors, namely utility of detecting techniques and methodologies (C_{33}) and enterprise scale (C_{11}), ranked last in the system and were therefore deemed to be non-key factors. As seen from most food safety incidents in China, food fraud is primarily uncovered by simple detection. Thus, the utility of detection methodologies is not directly related to enterprise food fraud. Furthermore, although it is generally recognized that enterprise scale can influence fraudulent behavior (28, 55), this was not supported in the current study. It is possible that food fraud occurs frequently in China and enterprises can commit food fraud regardless of enterprise scale. Therefore, food fraud may not be necessarily associated with enterprise scale.

POLICY IMPLICATIONS AND CONCLUSIONS

Policy Implications

In a complex system encompassing multiple stakeholders, we found that enterprise food fraud was subject to joint influences by multiple clusters. Government regulation, social governance, and detection techniques was the key cluster. Furthermore, government regulatory capability and penalty intensity, expected economic benefits from fraud, maturity of market reputation mechanism, and transparency of food supply chain were the four key factors. We further determined the intrinsic mechanism of fraudulent behaviors of food enterprises and demonstrated that the DANP method is effective at identifying key clusters and factors that influence enterprise food fraud.

The current research was based on participation of a group of experts and was conducted within the context in China's food systems. One common attribute is that all of the experts have deep care and understanding of policy making regarding food fraud. Thus, the results could have profound policy implications from the social co-governance perspective for China and similar economies. First, Fraudulent behavior depends not only on expected economic benefits but also on expected cost (i.e., probability of getting caught and the penalty if they are caught cheating). Among them, the probability of being caught is determined by factors such as the effectiveness of detection techniques and methods (i.e., utility of detection Techniques and methodologies), and the supervision of social forces. The punishment after being caught is determined by factors such as Government Penalty Intensity and Maturity of Market Reputation Mechanism. Due to the major attractive effect of expected economic benefits of committing food frauds for enterprises, the government should be increased penalty of getting caught, so that the economic costs of food fraud are increased to a level sufficient to change the psychological expectation for economic return of food fraud. From a social co-governance perspective, not only the government should exercise such a penalty system. Business partners (e.g., suppliers or buyers), for example, could exercise such penalty method by contract; while end consumers could exercise such penalty by collective actions of refusing purchases (98, 99). In addition, in addition to strengthening supervision and sampling and improving the level of detection technology, it is also necessary to actively promote internal employees to provide food fraud clues.

Second, a regulation mechanism based on individual person's and an enterprise's life-long, public credit should be established. Food enterprises should be rated by credit levels and regulation should differ for the different levels, including punitive measures and close-out mechanisms against credit-losing enterprises. With such system, all stakeholders could see the credit and collectively perceive the credibility of a food enterprise.

Third, priority should be given to criminal liabilities. In parallel with behavioral and property punishments, confinement should be stressed, i.e., administrative detention of the responsible persons. By eliminating no or weak enforcement and limited economic penalties in substitution for stronger criminal liabilities, a lasting system-based mechanism and legislative environment will be established to ensure that food enterprises are unable to or do not wish to commit food fraud.

Fourth, the market reputation mechanism should be leveraged to control food fraud by disclosing food fraud information in a widespread manner through public media.

Fifth, a food traceability system should be established, and the food supply chain should have due transparency. Government authorities should establish and popularize food traceability systems and ensure food enterprises maintain continuous records to create reliable information flow along the supply chain, thus allowing food production processes and destinations to be monitored, food fraud to be identified by tracking, and recall to be ordered when necessary. These measures will, in turn, encourage food enterprises to maintain compliance in business operation.

Sixth, although the food fraud vulnerability assessment tools are still in their infancy, its full impact remains to be seen. However, over time, food fraud vulnerability assessment tools can be used to ensure the food supply chain. Play an active role in integrity (100). China should also actively promote and encourage companies to implement food fraud vulnerability assessments. This is also an important part of social co-governance.

Conclusions

This paper adopts the DANP approach to make up for the deficiencies of existing studies that do not examine the key factors (cluster) and the interrelationships between factors (cluster) that influence food enterprises' food fraud decisions from the perspective of social co-governance and business decision making, thus contributing to an in-depth understanding of the causes of food fraud by food enterprises and to the formulation of targeted. The study contributes to the understanding of the causes of food fraud in food companies, and to the formulation of targeted measures to change the decisions of food companies and reduce food fraud at source.

Theoretically and practically, a social co-governance perspective extends the scope of governance to a multiple-agent level. That is, not just the producer enterprise is the focus of fraud prevention, but all stakeholders become the ones being governed by all of other social actors. The system design thinking of a food fraud governance should be a dominant logic to cover all government needs, whether which social actor is the one who is governing or governed.

What needs to be explained is the government regulatory capability and penalty intensity. Government has two instruments to control food fraud: (1) certification and (2) monitoring and enforcement system (31). The major reason for not discussing about the certification in the scope of the present study are: First, this paper is based on China's information. In China, the government's approach to countering food fraud is mainly government supervision and punishment, not certification. Second, in China, the government still needs to continuously improve the average product quality level in the market. When the government wants to increase the average product quality in the market while combating food adulteration, strict monitoring and enforcement is more effective than increasing certification costs (31). Therefore, this article will not discuss certification issues for the time being.

Additionally, in China, both legal food producers who have obtained food production licenses and a large number of illegal food producers who have not obtained licenses (such as illegal workshops) may engage in food fraud. The enterprise in this article refers to a legal food producer who has obtained a food production license. At the same time, we believe that the research conclusions are also applicable to illegal food producers who have not obtained a license to a certain extent.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

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

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Digital Technology, Knowledge Level, and Food Safety Governance: Implications for National Healthcare System

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Exploring the intrinsic relationship between digital technology and the efficiency of food safety supervision contributes to a better understanding of the role of digital technology in food safety supervision and how to maximize its influence. This study employed sample data from 31 regions in China between 2015 and 2017 for an empirical analysis of the correlation between the two and to examine the moderating effects of the knowledge levels of food producers and consumers. The results show that the development of digital technology contributes to enhancing the efficiency of food safety supervision. In this process, the higher the knowledge level of consumers, the greater the positive promotional effect of digital technology. On the contrary, when the knowledge level of producers is higher, it is not conducive to enhancing the effect of digital technology on the efficiency of food safety supervision. The author holds the view that this is related to the fact that employees in the food production and manufacturing industry have insufficient moral and legal knowledge. This not only limits the effect of digital technology on enhancing the efficiency of food safety supervision, but also opens the door to illegal production for some unprincipled producers. The policy implications are that digital technology should be used to improve food safety supervision, the moral and legal knowledge of food producers should be improved, and consumers should be encouraged to use digital technology more in the pursuit of food safety. Implications for national healthcare system would be also discussed in our paper.

Keywords: digitalization, food safety supervision efficiency, knowledge level of producers, knowledge level of consumers, food safety regulation

INTRODUCTION

As a fundamental factor in quality of life, food safety is crucial to people's lives and health. It is a matter of societal concern and something that governments find difficult to regulate. The lack of integrity of some food producers who are driven by self-interest and inadequate government supervision mean that the maintenance of food safety has become a global problem. According to the World Health Organization, approximately 600 million people suffer from foodborne diseases each year, of whom 420,000 die, resulting in a loss of 33 million healthy life years[1] For example, 48 million people in the United States contract diseases from eating contaminated food every year, of which 128,000 are hospitalized and 3,000 die. The economic loss caused by foodborne diseases is approximately as high as 93.2 billion USD (1). Food safety issues have gravely affected human life and health and have caused great harm to society and the global economy.

To improve the quality of food safety supervision, an adequate accountability mechanism must first be built so that local governments, regulatory agencies, and manufacturers can have clear concepts of their roles within the accountability system, exert corresponding influence, and form a joint force. At the same time, it is necessary to improve food safety supervision with the intelligent use of technology and data. Few studies have explored the issues of digital technology and food safety supervision; therefore, we are unable to fully understand the role that digital technology plays in this process. This impedes the improvement of digital technology and its application to food safety supervision. To fill the gap left by existing research, this study intended to answer the following two questions: First, how does digital technology affect food safety supervision? Second, do the knowledge levels of producers, consumers, and direct stakeholders in food safety influence the effect of digital technology on the efficiency of food safety supervision?

It is certain that this study can help managers realize the key influencing factors to improve the efficiency of food safety supervision, so as to improve the efficiency of food safety supervision and maintain the health of consumers. Of course, this paper describes the impact of digital technology on improving the efficiency of food safety supervision, and emphasizes that digital technology plays an important role in reducing the occurrence of events damaging consumers' health.

THEORETICAL BASIS AND RESEARCH HYPOTHESES

Food safety supervision usually refers to the oversight over food production and processing by the government and regulatory bodies (2). Efficiency can be considered as the ratio of output to input, including three categories: technical efficiency, allocation efficiency, and efficiency of scale (3). According to these definitions of food safety supervision and efficiency, for the purpose of this study, food safety supervision efficiency is defined as the ratio of the relationship between the cost invested by the government and food safety regulatory agencies in the process of implementing food safety regulatory actions to the regulatory output (i.e., regulatory results). Existing research on food safety supervision mostly applies the cost–benefit approach in the analyses. For example, Herman et al. (4) found that to decide on whether and how to implement food safety regulations, the cost–benefit approach is essential. Traill and Koenig (5) took the British government as the subject of research and introduced a cost-benefit approach that can be used to evaluate the efficiency of food safety supervision thoroughly. In addition, Millstone et al. (6) opined that the government should supervise food supply and improve the efficiency of food production supervision to ensure food safety. Han and Yan (7) believe that, considering the finiteness of regulatory resources and the problems of regulatory costs, a regulatory model that is led by the government and supported by enterprises should be constructed.

Digital technology generally consists of technologies such as blockchain, big data, cloud computing, and artificial intelligence. The biggest advantage of its application is that it can improve the overall economic efficiency of society. The problems of plane connection include excessive nodes and low efficiency. Digital technology can build a more direct and efficient network, breaking the plane connection between enterprises and enterprises, people and people, and between people and materials (8). Giacomo et al. (9) believe that a structure with amplified natures of multidimension and interaction will be established in the future through digital technology. The end-to-end interactive connection mode in this structure will eliminate intermediate nodes and further improve interactions and cooperation between subjects. In addition, the blockchain based on the digital technology will strengthen the trust of consumers and customers, maintain a state of low cost and high efficiency in economic operation, and drive the rapid development of society. The application of digital technology has formed a trend, especially for traditional industries such as food production. To break through the production frontier and improve product quality, the support of digital technology is indispensable.

Digital Technology and Food Safety Supervision Efficiency

As the food supply chain continues to lengthen, regulators and consumers lack sufficient and accurate information to trace the authentic sources of food or locate the origins of food quality problems in time. Digital technology can bring about various approaches to improve the status of food safety supervision (10, 11), including the tracking of food safety information, improvement of relevant laws and regulations, and the enhancement of the spread of food safety knowledge.

The development of digital technology is conducive to the tracking of food safety information. In an ideal situation, the monitoring of food quality would involve the whole process without the loss of any information. Whole-process monitoring is difficult to achieve against the background of large-scale food production, distribution, and sales. However, with the development of digital technologies such as wireless sensing and the Internet of Things, food safety supervision can go beyond the constraints of the existing workforce and material resources and entail a system as close as possible to the real-time monitoring of food production and distribution. Cranfield

[1] Information extracted from the food safety data published by the World Health Organization: https://www.who.int/news-room/fact-sheets/detail/food-safety.

et al. (12) applied radio frequency technology and blockchain technology to construct a conceptual framework of a traceable system of the food supply chain and analyzed the advantages and disadvantages of the application of digital technology and its approaches. Awuor et al. (13) further proposed a food safety emergency plan based on digital technology, linking all information in the food supply chain, and established an efficient and reliable execution environment so that when food safety issues occur, core problems can be traced rapidly and losses can be minimized. Shinwell and Defeyter (14) took meat as an application scenario for the construction of a model of a digitisation system and performed functional verification. Digital technology improves the efficiency of food safety supervision by government departments by realizing information tracing.

The development of digital technology is conducive to the improvement and application of relevant laws and regulations. Consumers can only obtain food safety information from publicly available data in the food market, which means that information access is asymmetric. Consumers will usually be unaware of safety risks if producers and processors deliberately conceal information about it. In addition, the shortfalls in the timeliness and accuracy of information disclosure of food safety supervision by government departments lead to a situation in which most consumers cannot obtain dynamic information of food inspection on time, which increases the safety risks. Because of identification difficulties and inaccurate judgments, loopholes in relevant laws and regulations may be exploited when these safety issues occur. Reeder et al. (15) believe that digital technology will significantly promote the informatization level of the food industry, increase the transparency of food safety information and the supervision efficiency of administrative agencies, and is a powerful tool for social co-governance. From a legal perspective, Marcotrigiano et al. (16) explored how to introduce digital technology into the supervision of food safety.

On the one hand, digital technology improves the efficiency of food supervision and reduces management costs. On the other hand, it reduces the rent-seeking behavior of government departments and protects consumer rights in some countries. By improving relevant laws and regulations, digital technology improves government departments' food safety supervision efficiency.

The development of digital technology is conducive to spreading knowledge about the safe production and consumption of food. The governments of various regions in China have formulated regulations on food quality standards, food contaminants, pesticide residue limits, and specifications of food hygiene practices. Although the overall level of the standard system is slightly lower than the average international level, the technical level and hardware conditions of Chinese food producers and processors do not meet the standard in many cases, and this is one of the factors that reduces the efficiency of food safety supervision. Digital technology closely connects all links in the food production and processing chain by building a network platform that combines the online and offline worlds and promotes the continuous circulation of advanced production and processing technologies within the food industry (17). It can increase the degree of codification of high-quality production and processing knowledge, making this easier to be disseminated and accepted (18). Digital technology can also strengthen the tacit understanding between the business partners of the food production and processing chain; therefore, any technical requirements of each link can be satisfied by matching suppliers in time (19). In addition, consumers can learn more about food safety through digital technology and promptly report food safety issues to producers and regulators during consumption (20). Digital technology improves the efficiency of food safety supervision of government departments through the spillover of knowledge in food safety production and consumption.

H1: There is a positive correlation between digitalization and the efficiency of food safety supervision.

Producer Knowledge Level

In some cases, high-level technology and equipment are needed for the safe production of food. With a higher knowledge level, a producer can master the corresponding operating technology and equipment more easily. As the level of knowledge increases, the cognitive levels of producers will increase and help them to select production technology behaviors that are safer (21, 22). In addition, not only does the accumulation of knowledge help producers to promote technology in the safe production of food and improve their legal awareness of food safety, but it also indirectly loosens the financial constraints and risk constraints faced by producers, creates the necessary conditions for producers to adopt advanced and new technology, and in turn contributes to improving the degree of safety of production technologies. From the perspective of producers, Demazeau et al. (23) examined the factors influencing high-quality production by dairy farmers and found that factors including education level, whether there is technical guidance, and the degree of understanding in food safety knowledge significantly affect safety behaviors of feeding and disinfection. The promotion of production technology and knowledge level is one of the main characteristics in the digital transformation of the industry. A higher knowledge level of the producer leads to greater exertion of the effect of digital technology on the supply side of food. First, it accelerates the circulation and acceptance of safe production technology of food. Second, it strengthens producers' awareness of the safe production of food, prevents food safety hazards by eliminating the root causes, and improves the efficiency of food safety supervision.

H2: A higher knowledge level of food producers strengthens the effect of digital technology on improving the efficiency of food safety supervision.

Consumer Knowledge Level

Food safety issues ultimately affect the lives and health of consumers. In the process of food safety supervision, consumers play a feedback role, that is, to reflect consumer experience to the market and to regulatory authorities, actively or passively. Barakabitze et al. (24) believe that the higher the consumers' knowledge level, the better their ability to seek out high-quality products in the market, while having a higher awareness of food safety and risk prevention. When consumers encounter food safety issues, they are inclined to disclose such problems publicly or to protect their consumer rights by legal means. Yan et al. (25) also investigated the topic from the consumers' perspective. The study examined the effect of education level on preference for food attributes. The results showed that consumers with higher education and income levels have a stronger ability to obtain information and attach more importance to high-quality labels certified by international agencies. The two studies mentioned show that as the education level increases, consumers' awareness of safe consumption will increase. Moreover, this indicates that when consumers' education level is low, their food safety awareness is also at a low level, and it impedes the effect of digital technology on food safety supervision. Conversely, a higher knowledge level of the consumer leads to a greater effect of digital technology on the consumption side of food. The main manifestation is that a high knowledge level of consumers is conducive to the feedback and supervision effect of digital technology on the end consumer market, thereby enhancing food safety supervision efficiency.

H3: A higher knowledge level of consumers strengthens the effect of digital technology on improving the efficiency of food safety supervision.

VARIABLE SELECTION AND DATA COLLECTION

Food Supervision Efficiency (Regul_Effic)

Extensive research has been conducted on the evaluation indicator systems of food safety supervision efficiency. For instance, Khayyam et al. (26) believe that the frequency of supervision actions and random inspection can provide a complete reflection of the scale of investments in food safety supervision. The study selected the supervision frequency, random inspection rate of food safety, and rate of administrative punishment as input indicators, and selected the rate of food poisoning and qualified rate of a product as output indicators. Chen et al. (27) selected the average penalty amount and the intensity of the random inspection of food as input indicators and selected the qualified rate of randomly inspected products as an output indicator. The ratio between input and output was used to judge the level of food safety supervision. Kang et al. Kang et al. (28) determined the food safety situation by looking at the number of food poisoning incidents, the number of people affected by food poisonings, the number of deaths from food poisoning, and the qualified rate of random inspected products.

Zhang et al. (29) and Zhang and Song (30) highlighted two shortcomings in the existing methods. First, the effect of food safety supervision funding and other auxiliary supervision equipment on the efficiency of supervision is not duly being considered. Second, the effect of low-quality output on the efficiency of food safety supervision is not duly being considered. These studies have built more comprehensive evaluation indicator systems but have not conducted empirical analyses.

TABLE 1 | Explanation of the evaluation indicators of food safety supervision efficiency.

	Indicator	Method of calculation
Input	Level of regulatory funding investment	(Food safety affairs expenditures/total public safety affairs expenditures) ×100%
	Intensity of random inspections	(Total inspected batches/total population of the region) ×100%
	Intensity of administrative punishment	(Total amount of penalty concerning food safety/number of food safety violations) ×100%
Output	Food safety qualified rate	(Qualified batches in random inspections/Total inspected batches) ×100%

Based on the above research, this study constructed an input–output indicator system for food safety supervision efficiency, as shown in **Table 1**.

Among the above indicators, food safety is a part of public safety affairs, and investment in the supervision of food safety improves supervision efficiency. Sufficient funding of food safety supervision is the key to ensuring the improvement of food safety supervision efficiency; random inspection of products is a direct approach to improve food safety. Generally speaking, the greater the intensity of random inspection per capita, the greater the effect of warnings and food safety supervision. The intensity of random inspection of food is represented by batches inspected per one thousand people in food supervision; the greater the intensity of administrative punishment, the greater the warning and deterrent effect to food producers, which is conducive to promoting the improvement of food production quality. The intensity of administrative punishment is represented by the average amount of the penalty for each food safety violation. The output of food safety supervision refers to the effect of the supervision actions of the food safety supervision departments. The qualified rate of food in random inspection is selected as the output indicator. The higher the qualified rate, the more effective the supervision.

Now that we have explained the input and output indicators, the super-efficiency data envelopment analysis (DEA) method was employed in this study to calculate the efficiency of food safety supervision. This is because first, DEA is a non-parametric method used to evaluate the relative effectiveness of decision-making units (DMUs) under a multi-inputs and multi-outputs mode; and second, when a traditional DEA model is used to calculate the relative efficiency of the DMUs, effective DMUs cannot be further differentiated and compared. To overcome this shortcoming, studies such as Cook et al. (31) and Li et al. (32) proposed and improved the super-efficiency DEA model based on the traditional model so that effective DMUs can also be ranked and compared. The basic idea of the super-efficiency DEA model is that when a DMU is being evaluated, it is excluded from the set of DMUs. As its frontier remains unchanged, the overall efficiency of invalid DMUs is the same as that of the traditional DEA model. For effective DMUs, as its production frontier shifts backwards, the efficiency value obtained will be greater than the measured value in a traditional DEA model, that is, >1. The form of the linear programming is shown in Formula (1) and Formula (2):

$$\min\left[\theta-\tau\left(\sum_{i=1}^{m}S_i^-+\sum_{r=1}^{p}S_r^+\right)\right] \tag{1}$$

$$s.t.\begin{cases}\sum_{j=1,j\neq k}^{n}a_jx_{ij}+S_i^-=\theta x_{ik},\ i=1,2,\ldots,m\\ \sum_{j=1,j\neq k}^{n}a_jx_{rj}-S_i^+=y_{rk},\ r=1,2,\ldots,p\\ a_j\geq 0, j=1,2,\ldots,n\end{cases} \tag{2}$$

θ represents the supervision efficiency under constant returns to scale; S_i^- and S_r^+ are the slack variables, representing the reduced input and increased output, respectively, x_{ij} and y_{rk} represent the input variables and output variables of the model, respectively, a_j is the weight vector of the input factors in the DMU. When $\theta < 1$, it indicates that the supervision of the DMU is not effective; when $\theta \geq 1$, it indicates that the supervision of the DMU is effective, and the greater the value of θ, the higher the efficiency.

Thirty-one provincial administrative units in China from 2015 to 2017 were taken as the objects of research in this study[2], while the super-efficiency DEA model was employed to evaluate the supervision efficiency of DMUs. The software EMS 1.3 was used to calculate the super-efficiency value of food safety in each region during 2015, 2016, and 2017.

Digitalization Level (Digit)

The mean value of indicators included the average rate of fixed broadband ports, mobile phone penetration rate, and mobile Internet penetration rate (33), and the ratio of investment in the telecommunications industry to total investment (34) was selected to represent the level of regional digitalization. These four indicators reflect to a certain extent the regional digital access level, equipment level, application level, and industry development level, respectively. Among these, the average rate of fixed broadband ports, mobile phone penetration rate, and mobile Internet penetration rate are good indicators to represent the level of digital services in a region. To provide a better reflection of the level of digital development in each region, the ratio of industry investment to total industry investment was further adopted as the expression thereof.

Producer Knowledge Level (Produ_Edu) and Consumer Knowledge Level (Consu_Edu)

Previous studies have shown that the level of education is positively correlated with the individual's cognitive level and knowledge learning ability [e.g., (35–37)]. The higher the level of education, the easier it is to accept the technical products represented by digitization. From the perspective of managers, some studies believe that managers' education level significantly affects the foresight of enterprise strategy (38). Therefore, we can think that the knowledge level of producers and consumers is positively correlated with their education level. In view of this, the knowledge level of food producers in this study is represented by the mean value of the composition ratio of employees in the agriculture, forestry, animal husbandry, and fishery industries with a bachelor's degree or above, and the composition ratio of employees in the food manufacturing industry with a bachelor's degree or above (39). The knowledge level of consumers is expressed by the average education level of the fixed population in each region (40).

Control Variables

To minimize the potential impact on the results of time-varying regional characteristic variables, control variables such as per capita GDP (Per_GDP), population density (Pop_Dens), and food industry output value (Output_Val) of various countries (41, 42) were added to the calculation to remove the impact of non-critical factors on the efficiency of food safety supervision. In addition, time-fixed effects were used to control the impact of time-varying unobservable factors on the results at the macro level. Individual fixed effects controlled the impact of unobservable factors that are not time varying on the regional-level results.

This study incorporated the direct effects of the level of digital technology on the efficiency of food safety supervision and the moderating effects of knowledge level into the same research framework while considering control variables such as per capita GDP, population density, and food industry output value. On this basis, the direct and indirect effects of digital technology and knowledge level on the efficiency of food safety supervision were examined. The model is shown in Formula (3). Here, α_i represents the parameters to be estimated, and ε_i, σ_t, and ω_{it} are the individual fixed effect, time fixed effect, and random error term, respectively. The meaning of the remaining symbols is the same as above.

$$\begin{aligned}Regul\_Effic_{it} =\ & \alpha_0+\alpha_1 Digit_{it}+\alpha_2 Consu\_Edu_{it}\\ &+\alpha_3 Produ\_Edu_{it}+\alpha_4 Digit_{it}Consu\_Edu_{it}\\ &+\alpha_5 Digit_{it}Produ\_Edu_{it}+\alpha_6 Per\_GDP_{it}\\ &+\alpha_7 Pop_D ens_{it}+\alpha_8 Output\_Val_{it}+\varepsilon_i+\sigma_t+\omega_{it}\end{aligned} \tag{3}$$

Data Source

The data required for this study are all secondary data. (1) The data needed for the calculation of the food supervision efficiency can only be obtained from the official websites of China's provincial Food and Drug Administrations or the Bureau of Statistics website[3]. As the responsible authorities of many regions have not published the latest statistical data or migrated the previous data to new websites, data collection was difficult. Moreover, the statistical calibers and methods of food safety supervision indicators in different regions are inconsistent, leading to problems that included the ineffectiveness of collected

[2]Considering the completeness and accessibility of the data, in this study, the data of 31 regions from 2015 to 2017 were used. As the data in Hong Kong, Macau, and Taiwan were insufficient, these regions were not included in the research.

[3]For example, the data in Beijing comes from the information published on the website of the Beijing Municipal Medical Products Administration (http://yjj.beijing.gov.cn/yjj/xxcx/zlgg/yp30/index.html).

TABLE 2 | Descriptive statistics.

Variable	Obs.	Mean	St. dev.	Min.	Max.
Regul_Effic	93	1.0402	0.5778	0.262	3.426
Digit	93	2.4062	4.5873	0.006	23.5763
Consu_Edu	93	0.089	0.0524	0.04	0.304
Produ_Edu	93	0.0307	0.0294	0.002	0.16
Per_GDP	93	3.3428	1.7624	1.0971	8.4277
Pop_Dens	93	4.2708	6.5533	0.024	37.54
Output_Val	93	7.4339	8.0309	0.0658	44.5199

TABLE 3 | Variance inflation factor test.

Variables	VIF
Digit	3.18
Consu_Edu	7.45
Produ_Edu	9.60
Per_GDP	6.51
Pop_Dens	1.97
Output_Val	3.48
Mean	5.36

TABLE 4 | Digital technology, knowledge level, and food supervision efficiency.

Variable	OLS-Fe	POLS	OLS-Fe	OLS-Re
Digit$_{it}$	0.09759***	0.00018	**0.09228****	0.01157
	(6.88)	(0.01)	**(2.05)**	[0.56]
Consu_Edu$_{it}$	9.04392*	2.66346	9.27435**	−3.99166
	(1.97)	(0.41)	(2.42)	[−0.86]
Produ_Edu$_{it}$	11.8633**	0.72685	10.34224*	4.06119
	(2.24)	(0.08)	(1.90)	[0.74]
Digit$_{it}$ × Consu_Edu$_{it}$		0.01219	**2.09364****	0.21780
		(0.01)	**(2.30)**	[0.27]
Digit$_{it}$ × Produ_Edu$_{it}$		4.60956*	**−1.46757**†	1.95973†
		(1.85)	**(−1.56)**	[1.53]
Per_GDP$_{it}$	0.19829**	0.12972†	0.20084**	0.30255***
	(2.22)	(1.46)	(2.20)	[2.96]
Pop_Dens$_{it}$	−0.37289*	−0.04525**	−0.55757**	−0.07381***
	(−1.85)	(−2.31)	(−2.52)	[−2.91]
Output_Val$_{it}$	−0.01553*	0.01153	−0.00692	0.00398
	(−1.81)	(0.55)	(−0.75)	[0.33]
Cons	2.08533**	0.62881**	2.74456***	0.26921
	(2.23)	(2.27)	(3.03)	[0.93]
Time fixed effect	Yes	No	Yes	No
Regional fixed effect	Yes	No	Yes	No
Hausman test				23.21*** <0.0057>
$Q(p)$-stat			0.79 <0.374>	
IS-stat			1.63 <0.444>	
R^2	0.7399	0.1073	0.7577	0.6721
Obs	93	93	93	93

*Note: †, *, **, *** indicate that the parameter estimators are significant at the levels of 0.15, 0.10, 0.05, and 0.01, respectively; values in () are the t-values, values in [] are the z-values, and values in <> are the p-values.*
Bold values represent the final reference results.

data. Therefore, to ensure data accuracy, this study used data from 2015, 2016, and 2017 as the research sample to reduce the impact of such problems as missing samples and inconsistent statistical calibers on the data quality. (2) The sample data of explanatory variables, adjustment variables, and control variables were mainly derived from the China Statistical Yearbook, China Population and Employment Statistical Yearbook, and China's Fixed Asset Investment Statistical Yearbook[4]. The descriptive statistics of the sample data obtained are shown in **Table 2**.

RESULTS ANALYSIS AND ROBUSTNESS TEST

The test results of the variance inflation factor (VIF) showed no or weak collinearity problems in the model (as shown in **Table 3**). However, it was expected that adding interaction terms will aggravate the collinearity problems of the model. Regarding existing research methods (43), the core explanatory variables in this study were centralized to reduce the overall collinearity problems of the model, and robust estimation methods were also used to avoid possible heteroscedasticities in the model. On this basis, a fixed-effects model was used to test H1–H3, and the results are shown in **Table 4**.

The coefficients of determination in **Table 4** show that the explanatory power of random effects (OLS-Re) and fixed effects (OLS-Fe) to the model are better than those of the pooled least squares method (POLS). The results of the Hausman test show that the parameter estimators obtained from fixed effects are better than the random effect estimation results, which further proves that the parameter estimators of fixed effects are relatively accurate and reasonable. In addition, the statistics of Q(p)-stat and IS-stat show that the null hypothesis 'there is no autoregression or serial correlation in the model' cannot be significantly rejected, which means that there is no spurious regression problem in the model. The above analysis indicates preliminarily that the regression results are reliable and stable to a certain extent.

The fixed effects regression results show that the parameter estimator of digital technology is 0.09228, and the result is significant at the 5% confidence level, indicating that the level of regional digital technology is positively correlated with the efficiency of food safety supervision, and H1 is confirmed. The parameter estimator of the interaction term of the consumer knowledge level is 2.09364, and the result is significant at the 5% confidence level, indicating that consumer knowledge level has a positive adjustment effect on the improvement of food safety supervision efficiency driven by digital technology and thereby supporting H3. The positive adjustment effect increases as the consumer knowledge level increases, as shown in **Figure 1**. The parameter estimator of the interaction term of the producer knowledge level is −1.46757. Although the value only reaches

[4]All yearbook data comes from China's economic and social big data platform (https://data.cnki.net/?al=f).

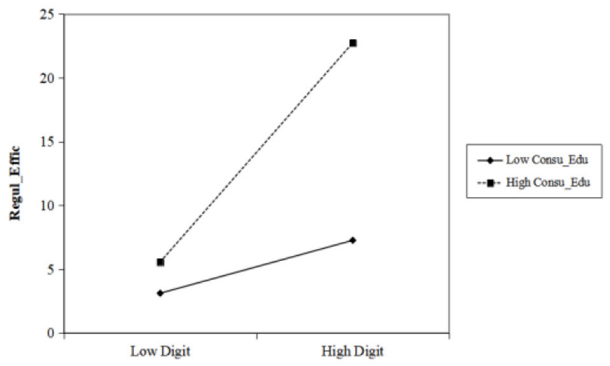

FIGURE 1 | Adjustment effect of consumer knowledge level.

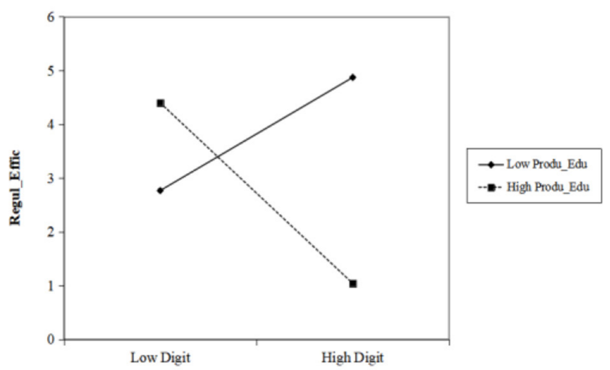

FIGURE 2 | Adjustment effect of producer knowledge level.

TABLE 5 | Endogenous test.

Variable	2SLS	2SLS
$Digit_{it}$	0.18117*** (11.64)	0.20864** (1.79)
$Consu\_Edu_{it}$	13.74281*** (3.49)	10.52923* (1.34)
$Produ\_Edu_{it}$	4.69192† (2.06)	17.28182* (1.70)
$Digit_{it} \times Consu\_Edu_{it}$		0.62396† (1.31)
$Digit_{it} \times Produ\_Edu_{it}$		−2.00417† (−1.41)
Per_GDP_{it}	0.27711** (2.63)	0.25826*** (2.29)
Pop_Dens_{it}	−0.57782* (−1.68)	−0.55472* (−1.55)
$Output\_Val_{it}$	−0.02689** (−2.60)	−0.03529** (−2.25)
Cons	1.03869 (0.74)	0.93208 (0.59)
Time fixed effect	Yes	Yes
Regional fixed effect	Yes	Yes
Hausman test	0.43 <0.9999>	0.61 <0.9999>
R^2	0.8287	0.8353
Obs	93	93

*Note: †, *, **, *** indicate that the parameter estimators are significant at the level of 0.15, 0.10, 0.05, 0.01 respectively; values in () are the t-values, values in <> are the p-values.*

the 15% confidence level, its significance is not to be ignored, as the result indicates that producer knowledge level has a negative marginal effect on the improvement of food safety supervision efficiency driven by digital technology, and H2 is not proven (**Figure 2**). A possible reason is that the education of employees in the food production industry only focuses on technical and business capabilities but has moral and legal education deficiencies. Thus, digital technology might become a tool for some producers who are driven by self-interest to produce food that does not meet the required safety standards, thereby reducing food safety supervision efficiency.

Endogenous problems may occur due to measurement errors or the omission of crucial control variables in the model, leading to inaccurate parameter estimation results. The problems were tested with instrument variables estimation in this study. The similarities and differences between the original parameter estimation results and the parameter estimation results under instrumental variable conditions were compared to determine whether the research conclusion is robust and reliable. The idea of pursuing instrumental variables comes from Porta et al. (44) and Rajan and Zingales (45). When the instrumental variable is difficult to find, the lagged independent variable can also be used as the instrumental variable. Therefore, we attempts to use the digital technology level lagging 1 period as an instrumental variable and uses the two-stage least squares method to test the endogenous problem of the model.

In the process of regression, the dependent variable in the first stage is the digital technology level, while the independent variable is the instrumental variable. Then, the predicted value of the digital technology level in the first stage is brought into the model as an independent variable for regression. The endogenous test results are shown in **Table 5**. First, the F statistics in the first stage show the correlation between the instrumental variable and the explanatory variable, which indicates a strong instrumental variable. Second, the two columns of parameter estimates represent the results of the second-stage parameter estimation, without the adjustment effect and with the adjustment effect, respectively. The parameter estimator of each model is consistent with the results obtained in **Table 4** in terms of the magnitude of the value and significance. Again, the Hausman test results show that, in the case of this model, there is no significant difference between the results of the two-stage least squares method and the fixed-effect estimation method. In other words, it is impossible to reject the null hypothesis 'there is no estimation bias caused by significant endogenous problems in the model'. Therefore, there are grounds to believe that the model designed in this study and the results obtained are reliable and robust.

To enhance the reliability of our conclusions, a robustness test was conducted after the endogeneity test. Drawing on Heydari et al. (46), we found that digital technology is the basis for promoting informatization development and for improving the

TABLE 6 | Robustness test results.

Variable	OLS-Fe	OLS-Fe
$Digit_{it}$	0.10362*** (6.09)	0.09615** (2.20)
$Consu\_Edu_{it}$	6.86016† (1.53)	6.15503* (1.67)
$Produ\_Edu_{it}$	10.85996** (2.06)	8.58882* (1.72)
$Digit_{it} \times Consu\_Edu_{it}$		2.44427*** (2.78)
$Digit_{it} \times Produ\_Edu_{it}$		−1.53999† (−1.54)
Per_GDP_{it}	0.10353 (0.64)	0.06206 (0.42)
Pop_Dens_{it}	−0.28222 (−1.43)	−0.48052** (−2.23)
$Output\_Val_{it}$	−0.02063* (−1.90)	−0.01243 (−1.18)
Cons	1.99785** (2.12)	2.81957*** (3.19)
Time fixed effect	Yes	Yes
Regional fixed effect	Yes	Yes
R^2	0.7553	0.7800
Obs	93	93

*Note: †, *, **, *** indicate that the parameter estimators are significant at the level of 0.15, 0.10, 0.05, 0.01 respectively; values in () are the t-values, values in <> are the p-values.*

application level of informatization. Therefore, Heydari et al. measured the level of regional digitalization from the perspective of informatization investment and used regional informatization density, that is, the ratio between a region's investment in informatization and gross domestic product, as the proxy variable of digitalization. informatization investment usually refers to information and communication technology (ICT) investment, which can be divided into hardware investment and software investment. In this study, fixed investment in electronic information manufacturing was chosen to represent ICT investment in hardware, while fixed investment of the whole society in information transmission, computer services, and the software industry was chosen to represent ICT investment in software. The sum of the two is the total investment in the digital construction of a region.

The first and second columns in **Table 6**, respectively, represent the parameter estimation results of no interaction item and interaction item added after replacing the sample value. Compared with the results in **Table 4**, the conclusions presented by the robustness test did not change; hence, the empirical conclusions in this study can be considered robust and reliable.

DISCUSSION

Summary

Food safety issues have been a focus of attention in various countries. As digital technology gradually penetrates the food industry, what impact does it have on food safety supervision efficiency? How does the adjustment effect of producers' and consumers' knowledge levels influence the relationship between digital technology and food safety supervision efficiency? Answering the two questions above contributes to a fuller understanding of the role of digital technology in food safety supervision and how to maximize the influence of digital technology in improving the efficiency of food safety supervision. Unfortunately, existing literature that focuses on the intrinsic relationship between digital technology and food safety supervision efficiency are limited. To fill the gap left by existing research, this study employed sample data from 31 regions of China between 2015 and 2017 for the empirical analysis of the correlation between the two. At the same time, the moderating effects exerted by the knowledge level of producers and consumers were examined, and the following conclusions were made: The development of digital technology contributes to enhancing the efficiency of food safety supervision. In this process, the higher the knowledge level of consumers, the greater the positive promotional effect of digital technology; on the contrary, when the knowledge level of the producer is higher, it is not conducive to enhancing the effect of digital technology on the efficiency of food safety supervision. The author holds the view that this is related to the fact that employees in the food production and manufacturing industry have insufficient moral and legal knowledge. Not only does this limit the effect of digital technology on enhancing the efficiency of food safety supervision, but this also opens the doors to new ways of illegal production for some unprincipled producers.

Research Significance

Food safety supervision is not only an important method to protect people's health, but also a prerequisite for maintaining national stability. This is because food is the most basic material condition for people's survival. Food safety issues are related to human life, survival and continuity, and food safety issues usually cause unnecessary burden to a regional medical system. Therefore, in the context of knowledge economy, in order to maintain the public health safety of food, on the one hand, we need to improve the efficiency of food safety supervision and food hygiene quality with the help of digital technology to reduce the occurrence of malignant public health safety events; On the other hand, improve consumers' food safety knowledge and health defense knowledge, improve food producers' food safety production knowledge, and increase the punishment for manufacturers endangering food hygiene and safety.

Overall, this paper studies the relationship between food safety supervision efficiency, digital technology, consumers' and producers' knowledge level of food safety, and tries to interpret the potential relationship between food safety supervision efficiency and public health from the perspective of digitization and knowledge. It is a meaningful research for reducing the occurrence of public health security events and alleviating the pressure of the medical system.

Policy Implications

In view of the results of this study, the author recommends that food safety supervision work should first apply digital technology to improve supervision efficiency actively. Secondly, increase the moral and legal knowledge of employees in the food production and manufacturing industries through specialized training, regular education, and setting up typical examples. Capitalize on the positive effect of digital technology on food production and processing and reduce the negative effects. Finally, encourage consumers to make use of digital technology to assist the regulatory authorities in supervising food safety issues and to regulate the production behavior of producers in as many ways as possible.

Limitation and Future Research

Due to restricted data availability, this study only employed data from China as an example in the empirical analysis and did not include data from other countries or regions. This limitation will be addressed in follow-up research.

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/s.

AUTHOR CONTRIBUTIONS

All authors undertook research, writing, and review tasks throughout this study. All authors have read and agreed to the published version of the manuscript.

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Fish Nutritional Value as an Approach to Children's Nutrition

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Fish is a relatively cheap and accessible source of animal protein for human consumption even in rural communities. It is critical for global food and nutrition security, and its consumption continues to increase. As a highly nutritious food, fish consumption is highly recommended for children and expectant mothers for normal growth and development. The present paper explores the nutritional value of fish as approach to nutrition in children and its benefits. The findings reveal that fish is a valuable source of essential amino acids (EAA) and polyunsaturated fatty acids (PUFAs) that play important physiological functions for maintenance and development of fetuses, neonates, and infant brains. Therefore, it could be a valuable tool in the fight against food insecurity and malnutrition. However, fish and fish products are also highly susceptible to contamination by various organic and inorganic compounds that threaten public health. Particularly, heavy metals and biogenic amines (BAs) have shown adverse effects when contaminated fish is consumed, and the effects in children have been worse. Hence, while fish consumption is highly recommended for children's nutrition, the safety and quality of the product should always be checked to safeguard public health.

Keywords: aquatic food, omega-3, nutrition, malnutrition, brain development, PUFAs, physiological functions

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INTRODUCTION

Fish is consistently among the most commonly used and low-cost dietary sources of animal protein for most people worldwide (1, 2). It is a valuable source of essential nutrients, especially high-quality protein and fats (macronutrients), vitamins, and minerals (micronutrients) that make a vital contribution to the world's food and nutrition security (3). As a food product, fish is of greater importance in developing countries where it accounts for 75% of the daily animal protein, referred to as "rich and poor food" as an important companion (4, 5). Compared with other animal protein sources, fish is readily available even in poorer communities at a relatively cheaper price. Furthermore, fish production through aquaculture is considered sustainable and the most efficient way to produce high quality proteins for human consumption (6–8).

In children, inadequate intake of dietary protein could lead to serious health consequences, including stunted growth and poor development (9). Hence, the protein component of the human diet is very crucial and an important area of focus when it comes to malnutrition due to its physiological functions (9). Globally, malnutrition remains a major problem and it is estimated that 47 million children suffer from stunting, due to the lack of micronutrients of vitamin A, iron, and iodine, which is a source of public health concern in the world (10). Its consequences include nutritional blindness, impaired learning capabilities, poor growth, and increased morbidity and mortality rates (4). In many developing countries, malnutrition is a major risk for sickness and death in children (11). This is mainly driven by lack of access to high quality food products. Fisheries and aquaculture programs can address and mitigate issues of malnutrition in the world by increasing the access to fish (12, 13) due to its nutritional value. Therefore, increasing fish production could increase the access to fish products and improve the nutritional status in children which has the potential to end malnutrition and food insecurity.

Recently, the number of studies exploring the importance of fish consumption in children have increased. This is because fish has been recognized as an important source of high quality animal protein required for bodybuilding and other physiological functions in children compared to adults (14). Besides, during childhood stage, the provision of adequate protein intake is very crucial for the overall growth and development into adulthood. As fish is tender and easily digested than meat, its consumption in children would be an excellent source of calcium and fluorine essential for the development of strong bones and teeth (15). Furthermore, the consumption of fish, particularly oily fish, is essential for optimal development of the brain and neural system of the children, as omega-3 fatty acids in the form of docosahexaenoic (DHA) rather than alpha-linoleic acid (ALA) are required for optimal brain development (16). Unfortunately, much of the existing studies on fish have had a bias toward its economic importance and merely as a food item, while paying less attention to its nutritional value, particularly in children nutrition. Understanding the benefits associated with fish consumption is very cardinal for promoting the consumption of fish as it is often the cheapest source of animal protein in marginalized communities to improve the nutritional status normal development in children. This study, therefore, aimed to synthesize existing studies on the nutritional value of fish, including the benefits and risks associated with its consumption in children.

GLOBAL OVERVIEW OF FISH CONSUMPTION

Fish is very crucial to a nutritious diet in many areas across the world and it provides about 3.3 billion people with almost 20% of their average per capita intake of animal protein. As the global population increases, potential nutritional concerns are raised, and fish represents an important source of animal protein. For this reason, global fish for human consumption is projected to increase by 16.3% indicating that 90% of the fish being produced will be utilized for human consumption by the year 2029 (17). In 2018, fish accounted for about 17% of the total animal protein and 7% of this was animal protein consumed globally (3). The consumption of fish and the fish products has experienced major changes in the past decades. The world evident per capita fish consumption has been increasing steadily from an average of 12.5 kg in the 80's to 14.4 kg in the 90's and reaching 20.5 kg in 2017 (18). This expansion in the consumption have been driven not only by the increase in production but also by the nutritional standards it has shown to provide to the people, reduced waste, better utilization, improved distribution channels, and increased demand (3). Therefore, the increase in the consumption globally is an indication that the health benefits of fish consumption are manifold and well-understood from both scientific and nutritional perspectives. This also means that fisheries and aquaculture will continue to play a very crucial role in meeting the animal protein demands of the global population, with aquaculture being the dominant supplier (**Figure 1**).

The Chemical Composition of Fish

Fish contains 18–20% protein, and contains eight essential amino acids including sulfur containing lysine, methionine, and cysteine (12). It provides easily digestible protein of high biological value that is important for the growth and development of the body, the maintenance and repair of worn out tissues and for the production of enzymes and hormones necessary for many of the body's processes, it's contains less fat than red meat (19).

The fat content ranges from 0.2 to 25%, especially polyunsaturated fatty acids (PUFAs), which are essential for the proper growth of children and are not associated with the occurrence of cardiovascular disease (20). Fats also contribute to the energy supply and aid in the adequate absorption of vitamins K, D, A, and E (21). Fish is a vital source of vitamins **Figure 2**, especially vitamins A and D of the fats, as well as thiamine, riboflavin, and niacin (vitamins B1, B2, and B3) (22). Vitamin A found in fish is more available in the body compared to plant foods and is essential for normal vision and bone growth, also, fatty fish contains more vitamin A than the lean types (22). Vitamin D, found in fish liver and oils, is essential for bone growth because, it is essential for calcium absorption and metabolism (23). Energy metabolism requires thiamin, niacin, and riboflavin (23). Fresh fish provides a small amount of vitamin C, which is essential for wound healing, maintaining the integrity of tissue, and assisting in the absorption of iron in the nervous system (22).

Phosphorus, calcium, iodine, iron, selenium, fluorine, and zinc are among the minerals found in fish and are extremely "bioavailable," ensuring that they are readily consumed by the organism (24). Iron is critical for the formation of hemoglobin in the blood, which would be responsible for distributing oxygen across the body (24). Calcium is critical for the development and mineralization of bones, as well as the normal operation of tissues and the central nervous. It also plays a significant role in the clotting of blood (25). When young fish are consumed with their bones, the phosphorus, calcium, and fluorine consumption is greatest (25). Zinc is needed for growth performance, function of immune system and the maintenance of healthy skin (26). Iodine, found in aquatic food, is necessary for hormones that control body metabolism, growth and proper behavioral development in children (22). Fish clearly provides more to people's diets than just high-quality protein (27). As a result, fish can be a staple to every diet, avoiding starvation which, make these nutrients readily accessible to absorption by organs.

In recent decades, as people's concerns about their health have grown, so has their concern about fats (28). Fatty acids are molecules consisting of one glycerol and three fatty acids that serve as a source of energy in our body and are deposited in the

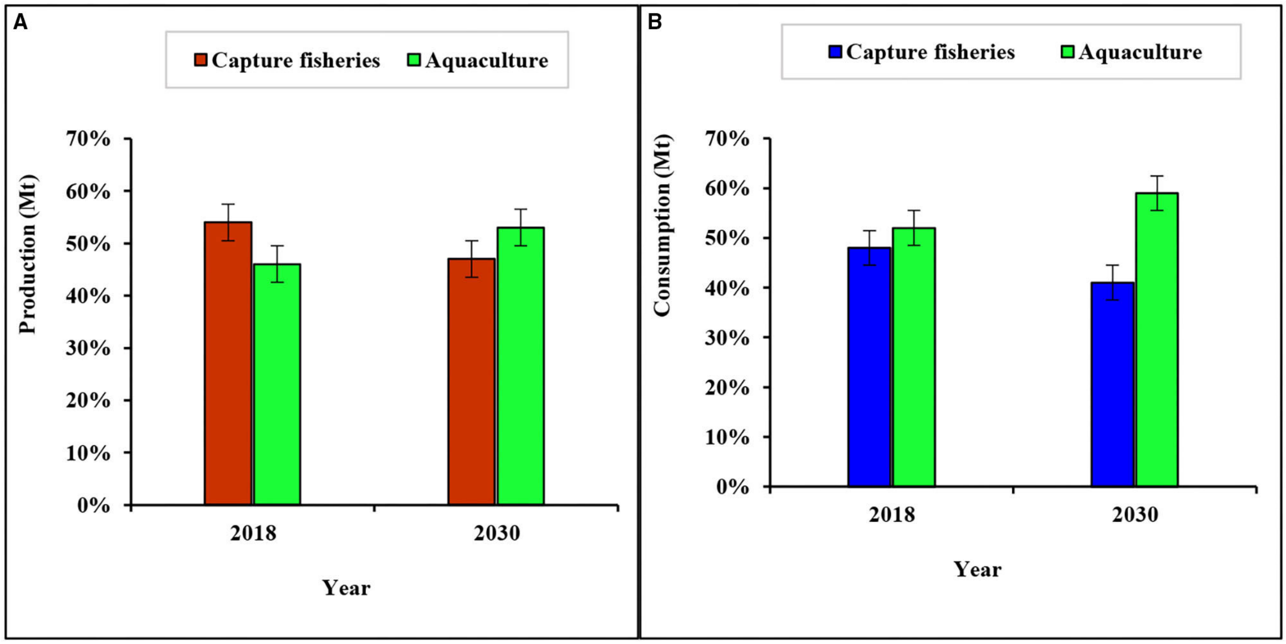

FIGURE 1 | Global fish **(A)** production in 2018 and projected production in 2030, and **(B)** consumption in 2018 and projected consumption in 2030, from capture fisheries and aquaculture. Source: Adapted from FAO (3).

meat, muscles and liver (29). When fat is ingested, lipase breaks it down into one glycerol and three fatty acids, while a few fatty oils molecules are pass through intact through the intestine (30). The ingested fat is at initial storage in the liver, the muscular or subcutaneous inner layer and then broken down as needed to provide energy (31).

Furthermore, saturated and unsaturated fatty acids are distinguished by the presence or absence of an intramolecular double bond (20, 32). Saturated fatty acids are found in animal oils and are harmful to an individual's health, while unsaturated fatty acids are found in vegetable oils and are beneficial to an individual's health (21, 33). As a result, polyunsaturated fatty acids (PUFAs), such as omega-3s, have received significant attention (12). There are several medications and able to pay foods on the market, and the number of products aimed at children has risen as well (34). However, since there is concern about indiscriminate and excessive PUFA intake, it is critical to understand the correct use of PUFAs (35). As a result, the purpose of this study is to investigate at the fundamental concepts, kinds of PUFAs, physiological mechanisms of action of PUFAs, and PUFA consumption in children.

Polyunsaturated Fatty Acid Types

Animal oils, like pork, and butter, are rich in saturated fatty acids. Fish oil is an unsaturated fatty acid similar to animal oil (36). Unsaturated fatty acids make up the majority of vegetable fats, although saturated fatty acids like those found in coconut and palm oils are also present (37).

The persistent stability of saturated fatty acids causes them to harden and become white at cold temperatures. It is easy to store and does not easily strip when exposed to heat or pressure (38). Saturated fatty acids are problematic because they contribute to a number of circulatory and vascular issues (39). Saturated fatty acids, that harden at low temperatures, can cause atherosclerosis, angina, and stroke by raising cholesterol levels, and they can also alter blood flow (40). Some saturated fatty acid such as myristic acid C14/0, stearic acid C18/0, monounsaturated fatty acid as palmitoleic acid C16/1, Oleic acid C18/1 (41).

Unsaturated fatty acids on the other hand do not solidify and exist in liquid form at low temperatures due to their structural instability; they strip quickly when heat or pressure is applied, and spoil quickly (42). Unsaturated fatty acids are recognized to offer a variety of health benefits (43). Unsaturated fatty acids—linolenic acid (ALA) C18/3, linoleic acid C18/2, arachidonic acid (AA) C20/4; n−6, eicosapentaenoic acid (EPA) C20/5; n−3, and docosahexaenoic acid (DHA) C22/6; n−3 are those that have a physiologically vital role for children (44). The location of the first omega double bond, the carbon atom at the end of the carbon chain (the CH3 radical) within the fatty acid molecular structure, is used to classify unsaturated fatty acids (45). The essential fatty acids ALA, linoleic acid, and AA are required for optimal growth and health, but are not produced in animals' bodies, so they are classified as essential fatty acids (46). Wild fishes have higher levels of omega-3 PUFAs than farmed fish (47). Cold-water fishes contents contain greater amounts of long chain n3 PUFAs, which aid in their adaptation to the cold temperature (47). Aquatic oil, can provide EPA, Docosapentaenoic acid (DPA), DHA, and arachidonic acid (ARA), that can be used immediately in the body for regular physiological processes (20, 48). Omega-chain fatty acids are also known as unsaturated fatty acids (49).

Omega-3 (n-3), omega-6 (n-6), and omega-9 are all good examples (n-9) (50). Fish oil (for example, Sardine (10.14 EPA;

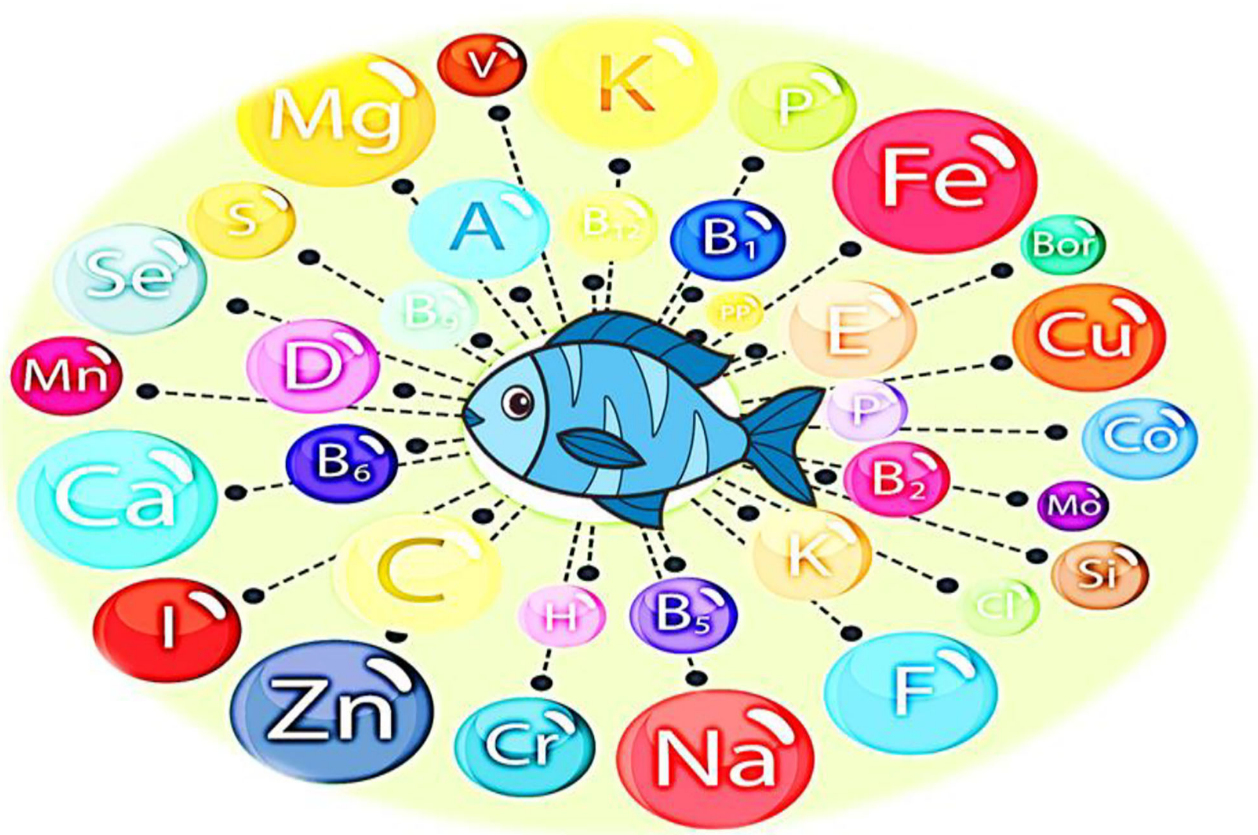

FIGURE 2 | Fish as a vital source of vitamins and minerals for children.

10.66 DHA), Menhaden (13.17 EPA; 8.56 DHA), Salmon (13.20 EPA; 18.23 DHA), Cod liver (9.90 EPA; 10.97 DHA), Herring (6.27 EPA; 4.21 DHA) and Fish such as Caviar, black and red (2.74 EPA; 3.80 DHA), values are g/100 g. (51). Vegetable oils (such as perilla oil, flaxseed oil, soybean oil, and canola oil) are both high in Omega-3. ALA, EPA and DHA are the nutritionally necessary omega-3 fatty acids (52, 53).

Animals have a limited ability to synthesize EPA and DHA (long-chain fatty acids) from ALA because they cannot produce omega-3 fatty acids (short-chain fatty acids) (54, 55). Grape seed oil, soybean oil, corn oil, sunflower oil, and cottonseed oil are high in omega-6 fatty acids (56, 57). Linoleic acid, linoleic acid, and AA are all omega-6 fatty acids (58). Oleic acids belong to the class of omega-9 fatty acids, and make up more than 80% of olive oil (59). Lard, palm oil, and sesame oil also contain omega-9 fatty acids (60).

PUFAs' Physiological Mode of Action

PUFAs are necessary fatty acids that are not produced in animals' bodies but are required for optimal growth and health (61, 62). When PUFAs are deficient, a variety of symptoms can develop, therefore PUFAs are regarded medically essential (63). The positive health activities of DHA and EPA, which are the materials designated as omega-3 functionality, are particularly well-established (47, 55). EPA improves blood circulation and lowers cholesterol levels in the blood (low-density lipoprotein, LDL) (64, 65). DHA is a fatty acid present in brain tissue and the optic nerve, which assists to rejuvenate brain cells and improve brain function, **Figure 3** (66–68). Omega-3 fatty acids, on the other hand, have anti-thrombotic, anti-arrhythmic, and anti-inflammatory properties, whereas omega-6 fatty acids are known to promote inflammation and thrombus development (69). DHA has been the most common n-3 LC-PUFA in the human nervous system (NS) (5 g in the human brain, 15% total fatty acids (FAs) (70). Additionally, DHA phospholipids have a great level of functional flexibility, which may be a key feature of a range of biochemical characteristics, including cognitive activities, acyl chain order, phase behavior, and synapse transmission locations of the synaptosomal cytoplasmic membrane in the brain has been preserved throughout growth and maintenance G protein-coupled signaling which leads to altered gene expression (71, 72). DHA also serves as an extra-and intracellular transcription factor, and blood DHA levels have been related to improved neurological development and visual in children (70, 73). EPA can stimulate the regeneration process of remyelination and cure multiple sclerosis of the central nervous system (CNS), after toxic damage to CNS oligodendrocytes (74). PUFAs' supplementation can help children with attention problems

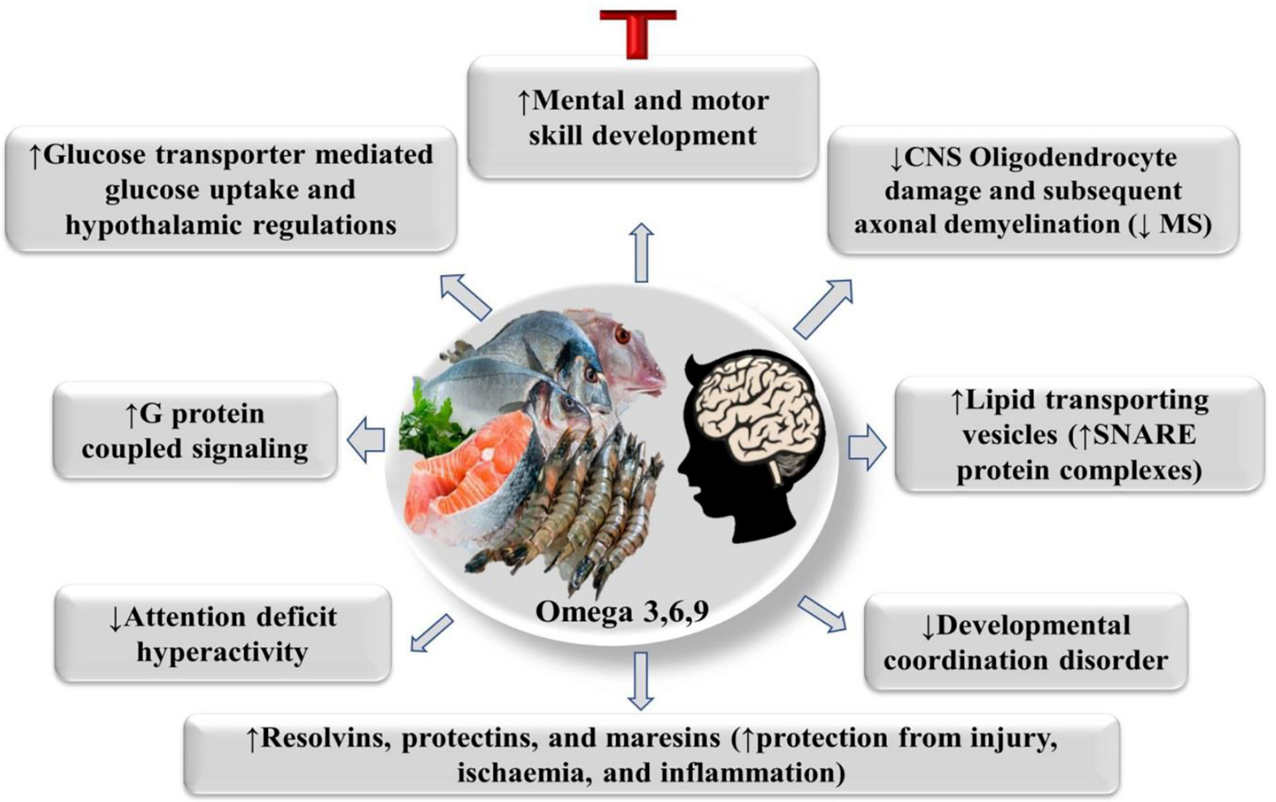

FIGURE 3 | PUFAs' physiological mode of action on brain health. CNS, Central nervous system, G protein-coupled signaling (leading to altered gene expression).

enhance their brain development and reading abilities (70, 75).

Consumption of PUFAs by Children

Omega-3 fatty acids are essential for children's health. Infants that were fed powdered formula with high grades of omega-3 fatty acid had better eye-hand coordination, attention, and social skills, as well as higher Intelligence Quotient (IQ) test results (76–78). It was also discovered that consuming long-chain polyunsaturated fatty acids (LCPUFAs) during pregnancy lengthened the pregnancy and lowered the frequency of premature deliveries (79, 80). According to Olsen et al. (81), children of moms who took fish oil during pregnancy had a lower risk of developing asthma in their teenage years. According to certain studies, powdered formula containing omega-3 helps preterm babies' growth and cognitive development (82, 83). The studies described above aren't conclusive; however they might be a cause to consume omega-3 that contains DHA or EPA. As a result, DHA and EPA are included in many powdered formulas now on the market. Furthermore, breast milk is the best supply of omega-3 (84), however it is heavily influenced by the diet that mothers consume (84). Nevertheless, according to a recent meta-analysis on omega-3 fatty acids, there is insufficient data to establish if LCPUFAs consumption during pregnancy aids cognitive or visual development (85).

Food Fish Quality and Safety

The quality and safety of food products determines the protection of public health, social stability as well as the food and nutrition security (2, 86). Fish is vulnerable to contamination by pollutants such as heavy metals that threaten their safety for human consumption. Heavy metals are classified as elements having a high atomic weight and a density of at least five times greater compared to that of water and are present in nature from the earth's crust (87–90). Despite numerous heavy metal elements present in nature, mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) are considered the most toxic elements and threat to public health. The widespread contamination of heavy metals in aquatic environment results mainly from anthropogenic activities including agricultural, industrial (such as mining), medical, and domestic applications (87, 90). Fish accumulate heavy metals by uptake through the gills and the skin when in contact and can bioaccumulate and bio-magnify them to toxic levels for human consumption (91, 92). However, the risks associated with consuming fish depends on the levels of contamination. The Food and Agriculture Organization (FAO) has set limits within which fish contaminated with heavy metals is considered safe for consumption (**Table 1**). As indicated, some elements could be toxic even at low levels while others at higher levels. Invariably, the consumption of fish and fish products contaminated with

TABLE 1 | Recommended values of some heavy metal elements by the food and agriculture organization FAO (106).

Heavy metal	Value (wet weight)	Value (dry weight)
Cr	0.15–1.0 ppm	0.65–4.35 ppm
Zn	30.0 ppm	130.43 ppm
Mn	1.00 ppm	4.35 ppm
Fe	100.00 ppm	434.78 ppm
Co	0.04–0.26 ppm	0.17–1.13 ppm
Cu	30.00 ppm	130.43 ppm
Se	1.00 ppm	4.35 ppm
Hg	0.50 ppm	2.17 ppm
Pb	0.50 ppm	2.17 ppm
Ni	80.00 ppm	347.82 ppm
As	1.00 ppm	4.35 ppm

heavy metals at levels beyond safe limits could have adverse effects in humans. However, children are more vulnerable due to their low body weight and behavior. For example, exposure to Pb in children could cause learning deficit, intelligence quotient (IQ) lowering, and severe damages in the brain and kidneys (93). Consuming excess Cd levels in fish products could result in kidney failure and bones softening, as well as prostate cancer in males (94, 95). Consumption of As in food products above safe level causes cardiovascular diseases, developmental anomalies, hearing defects, carcinoma, and hematologic disorders (96, 97). Hg is known to cause permanent damage to the central nervous system in children (98, 99). Effects such as heart function alteration, leukemia, kidney damage, neurocognitive defects and neuromotor disabilities have been reported in children exposed to Hg in sea food (100–102). Besides, Hg could affect children during any stage of development including maternal exposure particularly from methyl mercury (MeHg) species (87). Exposure to Cr could affect the functions of the heart, hematological parameters, kidneys, liver, and the central nervous system (103). Therefore, it is suggested that regular monitoring of heavy metals accumulation levels in aquatic environments and fish be conducted to safeguard public health (104, 105).

Fish, also being a perishable product, is vulnerable to fermentation and decomposition resulting in biogenic amines (BA) that threaten fish safety for consumption. BA refer to toxicants non-volatile amines resulting from amino acids decarboxylation (107). They are produced either by proteolytic activities of certain microorganisms or naturally during the metabolism of related precursor amino acids (108). However, in fish, histamine (HIS), cadaverine (CAD), and putrescine (PUT) are the only biogenic amines of concern when it comes to food safety and quality control (107). HIS is a monoamine produced from histidine precursor amino acid *via* a one-step decarboxylation reaction (108). CAD is a diamine produced from lysine and putrescine *via* a decarboxylation reaction (109). PUT is also a diamine but is produced either through a single-step decarboxylation from agmatine and ornithine, or indirectly after arginine hydrolysis (110). Although BA, at their physiological levels, play important roles in various cells process such as cell growth, gene expression, and tissue repair (111, 112), their ingestion at higher levels, although unlikely, could pose serious health hazards like symptoms of histamine poisoning including anaphylaxis, hypertension, nervous manifestation, and even death (113). Besides, Doeun et al. (114) reported that CAD and PUT could give way to gastric cancer during its conversion into carcinogenic N-nitroso compounds by microorganisms present in the digestive tract. Furthermore, in Europe, consumption of fish containing elevated levels of BAs was associated with widespread cases of intoxication (European Food Safety Authority EFSA (115) Therefore, it is very important that fish products are screened for BAs before administered for consumption to safeguard public health. This can be done 2-fold: maintaining high level of hygiene during fish harvesting, storage, processing and distribution to consumers, and by controlling total mesophilic (TMC) and total psychrophilic (TPsC) bacterial counts in fish products. El-Ghareeb et al. (109) observed a positive correlation between total BAs and TMC, suggesting that microorganisms play a major role in contaminating fish products with BAs.

Contextualizing Research on Children and Food Marketing

To ensure the growth, health and development of children to their full potential, adequate nutrition during infancy and early childhood is essential. The most effective interventions to improve child health is through optimal infant and young child feeding practices (116). Poor diets will drive malnutrition in early childhood and millions of children are eating too little of what they need, and millions are eating too much of what they do not need which is the main risk factor for the global burden of diseases (117). According to Unicef (117) malnutrition, which is an umbrella term for both excess consumption of nutrients (overnutrition), inadequate consumption of nutrients (undernutrition) or micronutrient deficiency ("hidden hunger"), is primarily caused by a suboptimal diet. The burden of child undernutrition remains a global threat, with 21.3 percent of children under the age of 15 years stunted, 6.9 percent wasted and 340 million suffering from micronutrient deficiencies (3). It is reported by (118) and (119) that in addition to contributing to greater dietary diversity and boosting the micronutrient intake of women of reproductive age, the consumption of aquatic foods in the first 1,000 days of life from conception to a child's second birthday is associated with positive birth outcomes, a better nutrient composition of breastmilk, reduced stunting and a decline in the prevalence of severe acute malnutrition. It is also attested that eating fish early in life can promote positive behavioral and mental health outcomes and prevent certain allergies, such as asthma, eczema, and allergic rhinitis (41).

Aquatic foods, especially aquatic animals, have long been valued as a rich source of animal protein and thus, considered a key constituent of nutritious diets (120) but the policies on aquatic foods tend to focus primarily on production, economic efficiency, resource management, environment and climate issues whilst paying less attention to value chains and the contribution of aquatic foods to people's nutrition and health. Ahern et al.

(121) recommended that aquatic foods are part of the solution to building resilient food systems and sustainable healthy diets for all, but for this to be fully achieved, they need to be available, accessible, affordable and desired. The nutritional value and health benefits of the fishes are unrecognized and undervalued. Despite a lot of benefits in the health of humans particularly the children, some people are still unaware of these benefits (122). The contribution of capture fisheries for instance to diet quality is poorly understood in most contexts, particularly where small-scale fisheries remain undocumented and overlooked in both fisheries and food system development (123, 124) hence limiting the nuanced assessment of fisheries contribution to diet quality of children under 12 years of age which is the critical age at which interventions have the greatest long-term effects for growth and health (117, 125). In contrast, Crona et al. (126) and HLPE (127) records that fish and other aquatic foods are gaining attention for their potential to efficiently provide two fundamental components of sustainable, nutritious food systems. Fish from inland fisheries are an important source of animal source foods (ASF) in monotonous diets for children in the sub-Saharan Africa and Asia (128), especially in land-locked African countries such as Malawi (129, 130) and Zambia (131). Therefore, there is a need to realize the importance of fish for human nutrition, in addition to its role in reducing poverty and hunger. This will ensure a greater impact by improving the nutritional status of children.

CONCLUSION

The nutritional benefits of fish consumption in children has been reviewed. Our findings show that fish is an important animal protein source and its consumption is likely to increase over the coming years. This will be driven primarily by population increase and the demand for healthy and high-quality protein for human nutrition. The polyunsaturated fatty acids (PUFAs) that are highly present in fish play an important physiological role in the growth and development of fetuses, newborns, and children's brains. As a result, they should be provided in the diets of children for normal development. Besides, *in situations* including auto-immune illnesses diseases, PUFAs have been found to enhance blood flow, minimize chronic inflammation and decrease coronary artery disease.

AUTHOR CONTRIBUTIONS

SM and KN wrote the draft manuscript. MA-T reviewed the manuscript. HK designed the study and wrote and formulated the objectives, as well as reviewed the manuscript. All the authors read and approved the final manuscript.

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Trends in Nutritional Biomarkers by Demographic Characteristics Across 14 Years Among US Adults

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Background: Understanding trend in nutritional status is crucial to inform national health priorities to improve diets and reduce related diseases. The present study aimed to analyze trends in the concentrations of all measured nutritional biomarkers and their status among US adults across 14 years.

Methods: Trends on the concentrations of nutritional biomarkers and nutritional status evaluated by the prevalence of deficiency, inadequacy, excess, and dyslipidemia were analyzed among US adults in 7 cross-sectional National Health and Nutrition Examination Surveys (NHANES 2003–2016) and by age, sex, race/ethnicity, and socioeconomic status.

Results: A total of 38,505 participants (weighted mean age of 47.2 years, 51.4% women) were included in the present study. Across 14 years, increased trends were found in red blood cell (RBC) folate, serum vitamin B_{12}, vitamin D and albumin, the prevalence of iodine deficiency, vitamin B_6 inadequacy, and hypophosphatemia, whereas decreased trends were observed in serum vitamin E, phosphorus, total calcium, total protein, apolipoprotein B (Apo B), low-density-lipoprotein cholesterol (LDL-C), triglyceride (TG), total cholesterol (TC), blood lead, cadmium, mercury, and the prevalence of vitamin C deficiency, vitamin D inadequacy, iodine excess, and dyslipidemia with elevated LDL-C, TC, TG, and lowered HDL/LDL. Non-Hispanic blacks (NHB) and participants with low socioeconomic status were accounted for the poor nutritional status of most biomarkers compared to their comparts.

Conclusion: Most nutritional biomarkers and their status were improved among US adults from 2003 to 2016, but some specific populations should be paid much attention to improve their nutritional status, especially for NHB and participants with low socioeconomic status.

Keywords: nutritional biomarkers, nutritional status, temporal trend, US adults, demographic characteristics

INTRODUCTION

Diet and nutritional status play an important role in the prevention and management of leading causes of death and non-communicable diseases (1). In the United States, dietary risks accounted for more than 500,000 deaths per year and more than 5% of risk-attributable of cardiovascular diseases (CVDs), neoplasms, diabetes, diet-related cancers, obesity, etc. (2). Understanding their trends is crucial to inform national health priorities to improve diets and reduce the risk of diet-related diseases.

Self-reported dietary data is one of the main methods for assessing dietary intake, however, subjective recall poses a great challenge for obtaining an accurate evaluation of diet and nutritional status (3). Besides dietary intake data, nutritional biomarkers would provide less error, more proximal, and objective assessment of diet and nutritional status reflecting a combination of dietary intakes and supplements consumption and thus, they were strongly recommended in nutritional epidemiology (4, 5). The trends of several nutritional biomarkers have been reported in some studies, such as blood folate (6), serum vitamin C (7), B_{12} (8), and urinary iodine (9) in the National Health and Nutrition Examination Surveys (NHANES 1988–2010, 1988–2004, 1988–2006, and 2001–2012, respectively), serum 25-hydroxyvitamin D [25(OH)D] in the NHANES (1998–2014) and Canadian Multicentre Osteoporosis Study (CaMos 1997–2007) (10, 11), blood lead, cadmium, and mercury in Korea NHANES (KNHANES 2005–2011) (12), serum lipid profiles in the National Center for Health Statistics (NCHS; 1998–2010), and Coronary Artery Risk Development in Young Adults (CARDIA, 1985–2011) study (13, 14). However, limited evidence is available on the trends of all possible measured nutritional biomarkers at the population level and specific subgroups, which would help to provide important guidance to improve the nutritional status of the American population.

In the present study, data from 7 consecutive cycles of the NHANES (2003–2016) were employed to analyze trends in the concentrations of a total of 24 nutritional biomarkers and their status including the prevalence of deficiencies, insufficiencies, or excesses among US adults.

MATERIALS AND METHODS

Study Population

The NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the US, which was conducted by the NCHS/Center for Disease Control and Prevention (CDC) (15). The survey is unique in that it combined interviews and physical examinations. The interview included demographic, socioeconomic, dietary, and health-related questions. In addition, a direct standardized physical examination, such as body measurements, phlebotomy, and urine collections, was carried out in a mobile examinations center (MEC) (16). The present analysis focused on data from 6 2-year survey cycles of NHANES (2003–2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012, 2013–2014, and 2015–2016). After excluding subjects aged ≤20 years and pregnant women, a total of 38,505 subjects were examined in the present study. Additionally, the numbers of subjects for each measured nutritional biomarker are inconsistent, which are summarized in **Supplementary Table S1**.

All respondents provided their written informed consents. The NHANES protocols were approved by the NCHS Research Ethics Review Board.

Laboratory Methods

Serum vitamin A, B_{12}, C, E, 25(OH)D, folate [both serum and red blood cell (RBC)], calcium, iron-status indicators [iron, ferritin, transferrin saturation (TS), and erythrocyte protoporphyrin (EP)], potassium, sodium, phosphorous, total protein, albumin, apolipoprotein B (Apo B), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), plasma vitamin B_6, blood lead, cadmium, mercury, urine arsenic, and iodine were examined in the NHANES. Not all biomarkers were measured in each examined survey. The methods of assay for each biomarker and the survey years are described in **Supplementary Table S2**. As per NCHS recommendations, the fractional polynomial regression equation was applied to convert the Bio-Rad (BR) Quanta Phase II radio-assay blood folate results to equivalent values to match the microbiological assay (MA) blood folate (6). For serum 25(OH) D, calibrated harmonized data were used to adjust for differences in assay methodology as recommended by CDC using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) equivalent data for correct interpretation of trends in 25(OH) D data (17). It is to be noted that serum 25(OH)D was considered as the best level of vitamin D since it can be a relatively comprehensive reflection of systemic vitamin D reserves from food and endogenous skin synthesis through sunlight, and the LC-MS/MS method in the present study was considered as the best way to get correct levels of it among the current laboratory methods (18, 19). For LDL-C, when the values of TC are less than or equal to 400 mg/dl, the Friedewald calculation was employed: LDL-C = TC – [HDL-C + TG/5] (20).

Blood samples were collected and stored using standardized methods for each survey, followed by subsequent processing and using appropriate laboratory methods to quantify the biomarkers. The NHANES quality assurance and quality control (QA/QC) protocols met the 1988 Clinical Laboratory Improvement Act mandates. QA activities prior to data collection included equipment calibration and laboratory staff training, and QC activities during the collection included automated software editing, data analysis, and analytical processing by technicians. The quality control coefficients of variation (CV) for each biomarker in survey years are shown in **Supplementary Table S3**.

Assessment of Subgroup Variables

Race-ethnicity was divided into Mexican American (MA), non-Hispanic white (NHW), non-Hispanic black (NHB), and others (includes race/ethnicity other than MA, NHW, and NHB,

TABLE 1 | Characteristics of study participants in the National Health and Nutrition Examination Survey (2003–2016)[a].

Characteristic	2003–2004 $N = 4848$	2005–2006 $N = 4687$	2007–2008 $N = 5888$	2009–2010 $N = 6170$	2011–2012 $N = 5522$	2013–2014 $N = 5722$	2015–2016 $N = 5668$	*P*-trend[b]
Age groups (y)								
Aged 20–39 y	1,551 (38.1)	1,633 (36.9)	1,864 (37.0)	2,037 (36.5)	1,920 (35.8)	1,909 (35.7)	1,904 (35.6)	0.130
Aged 40–59 y	1,396 (39.0)	1,484 (39.7)	1,870 (39.1)	2,060 (38.6)	1,811 (38.2)	1,972 (37.2)	1,863 (36.2)	0.018
Aged ≥60 y	1,901 (23.0)	1,570 (23.4)	2,154 (23.8)	2,073 (24.9)	1,791 (26.0)	1,841 (27.1)	1,901 (28.2)	0.001
Sex								
Women	2,430 (51.4)	2,300 (51.0)	2,978 (51.4)	3,164 (51.4)	2,782 (51.7)	2,964 (51.5)	2,921 (51.5)	0.661
Race								
Non-Hispanic white	2,591 (72.2)	2,359 (72.1)	2,752 (69.7)	2,959 (68.1)	2,030 (66.5)	2,452 (65.9)	1,849 (64.0)	0.009
Non-Hispanic black	962 (11.1)	1,087 (11.5)	1,219 (11.3)	1,116 (11.4)	1,445 (11.5)	1,165 (11.4)	1,189 (11.4)	0.640
Mexican American	937 (7.7)	910 (7.8)	1,013 (8.2)	1,126 (8.5)	735 (8.7)	765 (9.2)	986 (8.8)	0.361
Others	358 (9.0)	331 (8.7)	904 (10.9)	969 (12.0)	1,312 (13.3)	1,340 (13.5)	1,644 (15.9)	<0.001
Socioeconomic status[b]								
High	1,257 (38.9)	1,400 (42.3)	1,455 (41.2)	1,472 (39.6)	1,420 (39.5)	1,550 (39.2)	1,348 (42.3)	0.038
Medium	2,617 (53.3)	2,427 (50.9)	3,005 (49.9)	3,227 (52.2)	2,868 (51.7)	3,033 (53.0)	2,982 (51.0)	0.822
Low	619 (7.8)	553 (6.7)	837 (9.0)	798 (8.3)	696 (8.7)	655 (7.7)	652 (6.7)	0.732
Use of supplements								
Use	2,496 (53.5)	2,263 (53.6)	2,682 (48.9)	2,878 (49.5)	2,688 (51.6)	2,878 (53.7)	2,951 (55.9)	0.152
Not use	2,339 (46.4)	2,417 (46.3)	3,201 (51.0)	3287 (50.5)	2,830 (48.3)	2,842 (46.3)	2,715 (44.1)	0.152

[a]*Values are presented as mean (SE) for continuous variables and n (%) for categorical variables; race and ethnicity were self-reported. Percentages were adjusted for NHANES survey weights.* [b]*Calculated by using the linear regression model.*

including multiracial) based on the self-reported data of the participants on race and Hispanic origin.

The socioeconomic status was defined according to educational attainment (EA) and a poverty income ratio (PIR), participants were classified into high (more than 12 completed years of EA and PIR ≥ 3.5), low (less than 12 years of EA and a PIR < 1.30), and medium (others) (21).

Dietary supplements (yes/no, NHANES 2007–2016) were obtained through a family interview. Frequency, duration, and dose of dietary supplements were collected in the past 30 days. More detailed information on the type of supplements is described in **Supplementary Table S4**.

Assessment of Nutritional Status

The reference concentrations or cutoff points for each nutrient were employed to evaluate nutritional biomarkers status, such as deficiency, insufficiency, excess, and dyslipidemia (**Supplementary Table S5**).

Statistical Analysis

Sample weights, which accounted for planned oversampling of some groups and adjust for non-response and non-coverage to ensure nationally representative estimates, were incorporated in all analyses (22). Arithmetic means and SE were used to describe the mean of serum concentrations of vitamin A, 25(OH) D, potassium, sodium, phosphorus, total calcium, Apo B, HDL-C, LDL-C, and TC. Geometric means (SEs) were used to describe mean serum concentrations of vitamin B_6, B_{12}, C, E, folate, iron-status indicator, lead, cadmium, mercury, arsenic, iodine, and TC, since symmetric distributions for these biomarkers were obtained after logarithm transformation of their original skewed distributions. Percentages (SE) were calculated to estimate deficiency, insufficiency, or excess of biomarkers, and dyslipidemia. SEs used to calculate 95% CIs were estimated by using Taylor series linearization, a design-based approach that accounts for the sample design (23).

Linear trends and quadratic trends of the concentrations and percentages of deficiencies, inadequacies, excesses, and dyslipidemia for each nutritional biomarker were estimated using a linear regression model by treating survey year and the square of survey year as a continuous variable, respectively. General linear regression and ANOVA were employed to assess the variability in subgroups (age, sex, race/ethnicity, and socioeconomic status). Student-Newman-Keuls (SNK) was applied for pairwise comparison when statistically significant variability was observed.

All analyses were performed by using SPSS 24.0 (SPSS Inc., Chicago, IL, USA), and graphics were finished by using R project 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria). Two-sided $p < 0.05$ was considered to be statistically significant.

RESULTS

Participant Characteristics

A total of 38,505 US adults (18,966 men, 19,536 women) were included in the present analysis. The basic characteristics of the participants are described in **Table 1**. From 2003 to 2016, the percentage of elderly adults aged ≥60 years was increased from 23.0 to 28.2% (p-linear trend = 0.001), while the percentage of adults aged 40–59 years was decreased from 39.0 to 36.2% (p-linear trend = 0.018). The proportion of NHW was declined

TABLE 2 | Trends of nutritional biomarkers among US adults in the National Health and Nutrition Examination Survey (2003–2016).

Characteristics	2003–2004	2005–2006	2007–2008	2009–2010	2011–2012	2013–2014	2015–2016	*P*-linear[b]	*P*-quadratic
Vitamins									
Vitamin A (umol/L)	2.10 (0.02)	2.13 (0.02)	–	–	–	–	–	0.312	–
Plasma vitamin B_6 (nmol/L)[a]	43.78 (2.18)	50.21 (1.35)	51.71 (1.64)	48.87 (1.18)	–	–	–	0.724	0.006
Serum folate (ng/mL)[a]	17.32 (0.35)	17.57 (0.37)	16.58 (0.43)	16.01 (0.23)	17.27 (0.33)	17.43 (0.30)	16.60 (0.35)	0.334	0.103
RBC folate (ng/mL)[a]	467.84 (5.97)	497.28 (6.17)	502.11 (11.58)	468.92 (7.06)	471.52 (9.66)	511.33 (8.48)	507.69 (8.18)	0.017	0.310
Vitamin B_{12} (pg/mL)[a]	461.21 (8.72)	471.95 (7.69)	–	–	529.66 (6.27)	524.81 (4.40)	–	<0.001	0.201
Vitamin C (umol/L)[a]	42.70 (1.82)	44.57 (1.01)	–	–	–	–	–	0.363	–
25-hydroxyvitamin D (nmol/L)	62.40 (1.65)	60.64 (1.08)	67.11 (1.00)	67.59 (1.39)	70.69 (1.58)	69.59 (1.31)	–	<0.001	0.406
Vitamin E (umol/L)[a]	29.39 (0.46)	27.37 (0.31)	–	–	–	–	–	<0.001	–
Minerals									
EP (umol/L)[a,c]	0.98 (0.02)	0.99 (0.02)	–	–	–	–	–	0.623	–
Ferritin (ug/L)[a,c]	42.84 (1.35)	38.83 (1.34)	36.52 (1.01)	39.87 (0.97)	–	–	–	0.134	0.002
Iron (umol/L)[a,c]	13.08 (0.17)	12.68 (0.27)	–	–	–	–	–	0.182	–
TS (%)[a,c]	20.65 (0.33)	20.00 (0.38)	–	–	–	–	–	0.172	–
Blood lead (ug/dL)[a]	1.54 (0.04)	1.43 (0.02)	1.39 (0.04)	1.24 (0.03)	1.10 (0.03)	0.98 (0.04)	0.93 (0.03)	<0.001	0.063
Blood cadmium (ug/L)[a]	0.38 (0.01)	0.37 (0.01)	0.38 (0.02)	0.36 (0.01)	0.34 (0.01)	0.30 (0.01)	0.29 (0.01)	<0.001	0.002
Blood total mercury (ug/L)[a]	0.98 (0.07)	1.06 (0.08)	0.95 (0.05)	1.04 (0.04)	0.86 (0.04)	0.81 (0.03)	0.81 (0.03)	<0.001	0.117
Potassium (mmol/L)	4.01 (0.01)	3.97 (0.01)	3.98 (0.01)	3.99 (0.01)	3.94 (0.01)	4.02 (0.02)	3.96 (0.01)	0.323	0.171
Phosphorus (mmol/L)	1.23 (0.005)	1.23 (0.003)	1.22 (0.004)	1.21 (0.003)	1.21 (0.002)	1.24 (0.005)	1.19 (0.008)	0.001	0.487
Sodium (mmol/L)	139.17 (0.07)	138.99 (0.13)	139.23 (0.16)	139.36 (0.09)	138.85 (0.08)	139.78 (0.11)	138.70 (0.15)	0.694	0.052
Total calcium (mmo/L)	2.39 (0.003)	2.37 (0.004)	2.35 (0.007)	2.36 (0.003)	2.35 (0.003)	2.36 (0.003)	2.34 (0.002)	<0.001	0.006
Urinary iodine (ng/mL)[a]	142.13 (3.90)	149.38 (5.56)	155.02 (4.39)	137.66 (4.78)	129.00 (5.02)	128.20 (4.96)	124.71 (4.73)	0.001	0.019
Urinary total arsenic (ug/L)[a]	8.44 (0.63)	9.81 (0.72)	8.44 (0.62)	10.15 (0.77)	7.08 (0.53)	6.49 (0.49)	6.33 (0.46)	<0.001	0.003
Protein									
Total protein (g/L)	71.92 (0.23)	70.90 (0.22)	71.39 (0.13)	71.27 (0.18)	71.01 (0.21)	70.29 (0.20)	70.25 (0.22)	0.002	0.084
Albumin (g/L)	42.75 (0.12)	42.41 (0.13)	42.62 (0.06)	42.80 (0.13)	43.13 (0.09)	42.77 (0.08)	43.67 (0.12)	<0.001	0.001
Lipids									
Apo B (mg/dL)	–	101.13 (1.46)	93.27 (0.73)	90.77 (0.80)	90.06 (0.95)	90.15 (0.64)	93.79 (0.69)	<0.001	<0.001
HDL-C (mg/dL)	53.87 (0.39)	54.35 (0.34)	51.85 (0.51)	53.01 (0.43)	52.83 (0.51)	53.03 (0.29)	55.52 (0.75)	0.200	<0.001
LDL-C (mg/dL)	116.90 (1.10)	115.21 (1.24)	115.80 (0.80)	115.94 (0.92)	115.23 (0.97)	111.45 (0.89)	113.00 (1.03)	0.001	0.411
TC (mg/dL)	201.57 (0.73)	198.57 (0.78)	197.18 (0.81)	195.98 (0.91)	195.22 (0.98)	189.33 (0.85)	192.39 (1.33)	<0.001	0.348
TG (mg/dL)[a]	122.55 (2.40)	117.41 (1.94)	114.37 (2.23)	107.65 (1.62)	111.87 (3.31)	98.28 (2.52)	107.32 (1.12)	<0.001	0.107

SE, Standard Error; EP, Erythrocyte Protoporphyrin; TS, Transferrin Saturation; Apo B, apolipoprotein B; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; TC, Total Cholesterol; TG, Triglyceride. Nutrients were measured in serum unless otherwise stated. [a]*Geometric mean.* [b]*Calculated by using the linear regression model.* [c]*Among 4,909 women's aged 20–49 years.*

from 72.2 to 64.0% (p-linear trend = 0.009), whereas other races/ethnicities was increased from 9.0 to 15.9% (p-linear trend < 0.001). In addition, the proportion of participants with high socioeconomic status was increased from 38.9 to 42.3% (p-linear trend = 0.038). But no evident changes were observed for other characteristics.

Trends of Nutritional Biomarkers for Vitamins

From 2003 to 2016, the increased trends were observed for the concentrations of vitamin B_{12}, 25(OH)D and RBC folate, being from 461.21 to 524.81 pg/ml (p-linear trend < 0.001), 62.40 to 69.59 nmol/L (p-linear trend < 0.001), and 467.84 to 507.69 ng/ml (p-linear trend = 0.017), while decreased trend was found for vitamin E, being from 29.29 to 27.37 μmol/L (p-linear trend < 0.001). The inverse U-shaped trend was found for the concentration of vitamin B_6 (p-quadratic trend = 0.006). No statistically significant trends were found for other vitamins concentrations (**Table 2**).

In subgroup analysis, inverse U-shaped trends were observed in all subgroups except for participants aged ≥60 years, men, and participants with high socioeconomic status for plasma vitamin B_6 (p-linear trend = 0.189, 0.569, and 0.199, respectively; **Supplementary Figures S1A1–A4**). The increased trends were observed in all subgroups for vitamin B_{12} (**Supplementary Figures S1B1–B4**), except for vitamin

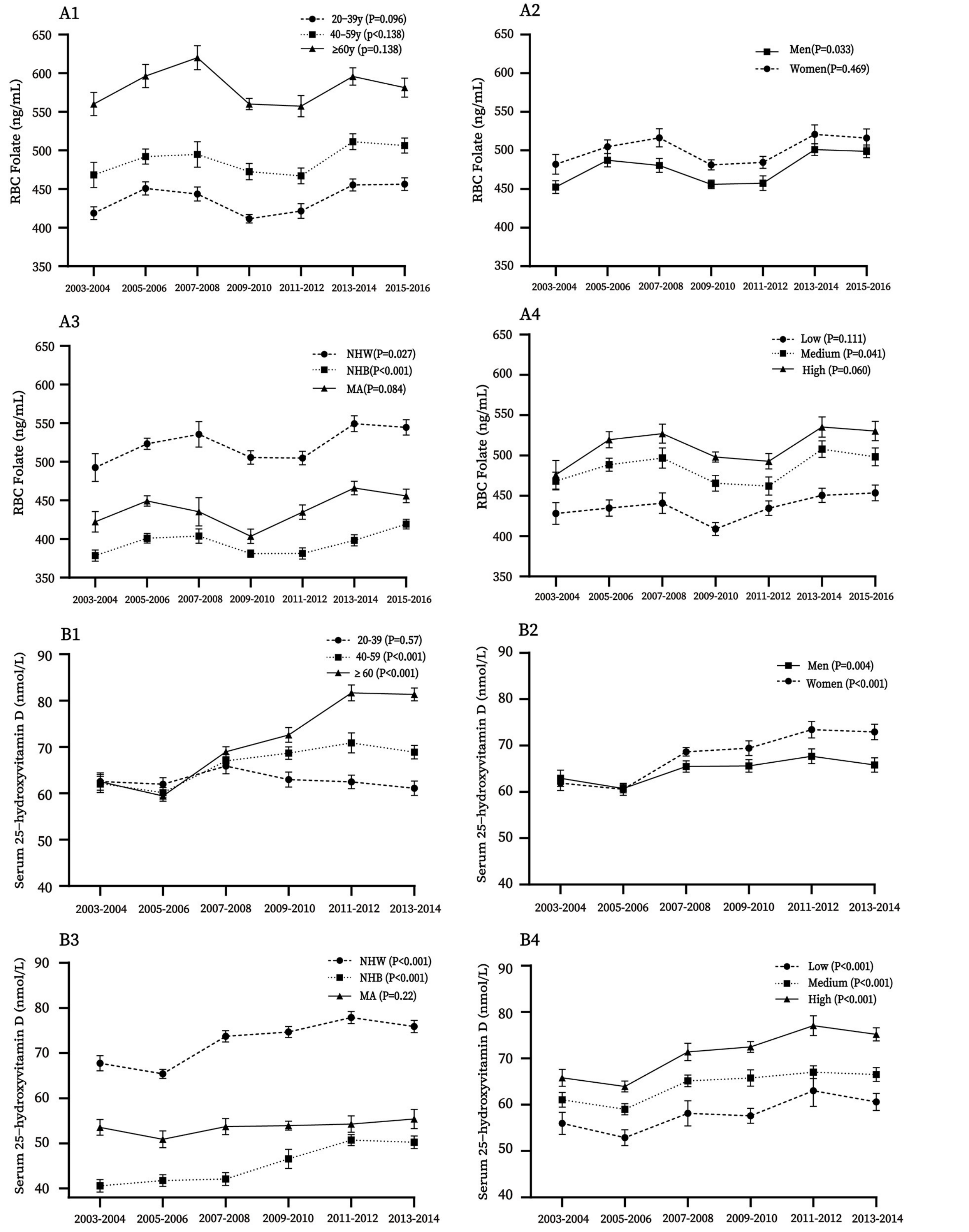

FIGURE 1 | Trends of concentrations of RBC folate **(A1–A4)** and 25-hydroxyvitamin D **(B1–B4)** by age, sex, race-ethnicity, and socioeconomic status (SS) among US adults from 2003 to 2016. MA, Mexican American; NHW, non-Hispanic white; NHB non-Hispanic black.

TABLE 3 | Trends of prevalence (SE) of deficiencies, insufficiencies, excesses, and dyslipidemia for nutritional biomarkers among US adults in the National Health and Nutrition Examination Survey (2003–2016)[a].

Characteristics	2003–2004	2005–2006	2007–2008	2009–2010	2011–2012	2013–2014	2015–2016	*P*-trend[b]	*P*-quadratic trend
Deficiencies (%)									
Vitamin A	0.3 (0.1)	0.3 (0.1)	—	—	—	—	—	0.834	—
Vitamin B_6	19.6 (1.5)	12.7 (0.9)	10.1 (0.9)	13.9 (0.9)	—	—	—	0.001	<0.001
Vitamin B_{12}	2.0 (0.3)	1.9 (0.4)	—	—	1.7 (0.2)	1.8 (0.2)	—	0.095	0.231
Serum folate	0.6 (0.2)	0.2 (0.1)	0.0 (0.0)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0.055	0.005
RBC folate	1.0 (0.5)	0.6 (0.3)	0.1 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	0.059	0.189
Vitamin C	8.5 (1.1)	5.7 (0.7)	—	—	—	—	—	0.032	—
25-hydroxyvitamin D	7.7 (1.2)	5.2 (0.7)	6.4 (0.9)	6.9 (0.9)	5.6 (0.8)	5.8 (0.8)	—	0.335	0.738
Vitamin E	0.7 (0.2)	0.5 (0.1)	—	—	—	—	—	0.137	—
Iron	11.8 (1.3)	13.5 (0.7)	15.2 (0.9)	15.2 (1.3)	—	—	—	0.055	0.138
Iodine	31.2 (1.9)	31.4 (1.4)	30.1 (1.2)	33.9 (1.3)	36.9 (1.5)	40.7 (1.8)	41.8 (1.9)	<0.001	0.031
Phosphorus									
Hypophosphatemia	0.3 (0.1)	0.6 (0.1)	0.6 (0.1)	0.8 (0.1)	1.2 (0.2)	0.6 (0.1)	1.1 (0.1)	0.002	0.849
Potassium									
Hypokalemia	3.4 (0.3)	4.4 (0.2)	4.0 (0.4)	3.7 (0.4)	5.7 (0.7)	3.6 (0.3)	6.4 (0.5)	<0.001	0.023
Sodium									
Hyponatremia	1.5 (0.2)	2.5 (0.5)	2.6 (0.5)	1.8 (0.3)	3.2 (0.5)	1.9 (0.4)	2.3 (0.4)	0.376	0.081
Hypoproteinemia	0.4 (0.6)	0.9 (0.7)	0.5 (0.6)	0.6 (0.5)	1.0 (0.9)	1.5 (0.5)	1.6 (0.4)	0.008	0.125
Hypoalbuminemia	1.9 (0.2)	2.2 (0.1)	1.9 (0.1)	1.7 (0.1)	1.5 (0.2)	1.8 (0.2)	1.2 (0.2)	0.090	0.807
Insufficiencies (%)									
Vitamin B_6	11.7 (1.3)	15.7 (0.8)	15.3 (0.6)	16.0 (0.5)	—	—	—	0.005	0.053
Serum folate	1.7 (0.6)	2.3 (0.6)	4.2 (0.5)	4.5 (0.4)	2.1 (0.4)	2.2 (0.2)	3.2 (0.3)	0.239	<0.001
RBC folate	0.1 (0.6)	0.0 (0.4)	0.3 (0.1)	0.1 (0.0)	0.3 (0.1)	0.3 (0.1)	0.1 (0.1)	0.141	0.320
25-hydroxyvitamin D	22.9 (2.0)	23.9 (1.8)	19.8 (1.2)	18.9 (1.2)	19.0 (1.9)	18.8 (1.2)	—	0.002	0.573
Excesses (%)									
Iodine	36.6 (1.3)	37.2 (1.0)	37.9 (0.8)	32.4 (1.7)	31.8 (1.9)	29.1 (1.9)	27.6 (1.8)	<0.001	0.101
Potassium									
Hyperkalemia	0.8 (0.1)	0.2 (0.1)	0.3 (0.1)	0.5 (0.1)	0.6 (0.2)	0.9 (0.3)	0.6 (0.2)	<0.001	0.023
Sodium									
Hypernatremia	0.4 (0.1)	0.3 (0.1)	0.9 (0.3)	0.5 (0.1)	0.4 (0.1)	1.2 (0.2)	0.2 (0.1)	0.198	0.005
Elevated blood lead	3.4 (0.2)	2.7 (0.3)	2.9 (0.3)	2.1 (0.3)	2.2 (0.6)	1.7 (0.5)	1.2 (0.2)	<0.001	0.697
Elevated blood cadmium	15.2 (0.7)	13.1 (0.9)	12.8 (1.1)	11.8 (0.7)	12.4 (0.9)	8.8 (1.1)	9.5 (0.9)	<0.001	0.996
Elevated blood mercury	11.2 (1.3)	10.7 (0.8)	9.6 (1.3)	12.0 (0.8)	9.5 (1.6)	8.6 (0.8)	7.9 (1.1)	<0.001	0.583
Dyslipidemia									
Elevated Apo B	—	12.8 (1.5)	6.5 (0.5)	5.9 (0.7)	4.8 (0.8)	6.0 (0.6)	5.1 (1.1)	0.047	<0.001
Lowered HDL-C	28.0 (1.0)	26.0 (1.0)	33.8 (1.3)	32.6 (0.9)	28.9 (1.7)	31.1 (1.2)	28.8 (1.4)	0.269	0.003
Elevated LDL-C	31.8 (1.5)	31.5 (1.5)	30.1 (1.0)	30.3 (1.2)	31.1 (1.1)	26.9 (1.2)	29.4 (1.0)	0.022	0.936
Lowered HDL-C/LDL-C	38.6 (1.6)	34.1 (1.4)	37.1 (1.2)	37.2 (1.2)	36.1 (1.5)	32.2 (1.5)	32.6 (1.4)	0.003	0.316
Elevated TC	47.5 (1.3)	44.2 (1.0)	43.1 (1.0)	42.3 (1.1)	42.3 (0.9)	36.0 (1.3)	38.8 (1.4)	<0.001	0.619
Elevated TG	33.1 (1.5)	30.1 (1.3)	30.2 (1.0)	25.0 (1.1)	26.5 (2.0)	23.3 (1.2)	22.0 (1.0)	<0.001	0.681

SE, Standard Error; Apo B, apolipoprotein B; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-density Lipoprotein Cholesterol; TC, Total Cholesterol; TG, Triglyceride. [a]Serum measurement otherwise stated. [b]Calculated by using the linear regression model.

D among participants aged 40–59 years (p-linear trend = 0.57; **Figures 1A1–A4**), RBC folate among men, NHW, NHB, and participants with medium socioeconomic status (p-linear trend = 0.033, 0.027, <0.001, and 0.041, respectively; **Figures 1B1–B4**, **Supplementary Table S6**). Participants aged 20–39 years, NHB, and participants with low socioeconomic status for vitamin B_6, folate, and 25(OH)D, men for serum and RBC folate, 25(OH)D, women for vitamin B_6 were accounted for the lowest concentrations compared to their comparts, respectively (**Supplementary Table S7**).

In addition, the decreased trends were observed in the prevalence of vitamin C deficiency and vitamin D inadequacy, from 8.5 to 5.7% (p-linear trend = 0.032), and 22.9 to 18.8% (p-linear trend = 0.002), whereas an increased trend was found in the prevalence of vitamin B_6 insufficiency, from 11.7 to 16.0 (p-linear trend = 0.005). The U-shaped trends were observed for the vitamin B_6 and serum folate deficiencies (p-quadratic trend < 0.001 and = 0.005, respectively), and an inverse U-shaped trend was found for serum folate insufficiency (p-quadratic trend < 0.001). No statistically significant trends were observed for the prevalence of vitamin A, B_{12}, D, and E deficiency, RBC folate deficiency, and insufficiency (p-linear trend = 0.834, 0.095, 0.335, 0.137, 0.059, and 0.141, respectively; **Table 3**).

Trends of Nutritional Biomarkers for Minerals

From 2003 to 2016, decreased trends were apparent in the concentrations of serum calcium, phosphorus, blood lead, cadmium, and mercury, from 2.39 to 2.34 mmo/L (p-linear trend < 0.001), 1.23 to 1.19 mmo/L (p-linear trend = 0.001), 1.54 to 0.93 μg/dl (p-linear trend < 0.001), 0.38 to 0.29 μg/dl (p-linear trend < 0.001), and 0.98 to 0.81 μg/dl (p-linear trend < 0.001), whereas no statistically significant trends were found for the concentrations of potassium and sodium (p-linear trend = 0.323 and 0.694, respectively). The inverse U-shaped trends were found for the concentrations of ferritin, urinary iodine, and total arsenic (p-quadratic trend = 0.002, 0.019, and 0.003, respectively; **Table 2**).

In subgroup analysis, the decreased trends were found in all subgroups for blood lead (**Figures 2A1–A4**), cadmium (**Figures 2B1–B4**), serum calcium (**Supplementary Figures S2A1–A4**), and phosphorus (**Supplementary Figures S2B1–B4**; all p-linear trend < 0.05) and were not found among participants aged ≥ 60 years, Mexican American, and participants with low socioeconomic status for blood mercury (p-linear trend = 0.263, 0.134, and 0.437, respectively) (**Supplementary Figures S3A1–A4**). Inverse U-shaped trends were found in all subgroups for urinary arsenic (all p-linear trend < 0.05; **Supplementary Figures S3B1–B4**), except for NHB for urinary iodine (p-linear trend = 0.84; **Supplementary Figures S4A1–A4**). In addition, participants aged ≥ 60 years, non-Hispanic blacks, and participants with low socioeconomic status for blood lead, cadmium, and mercury, women for blood cadmium, and men for blood lead and mercury accounted for the highest concentrations compared with their comparts. For urinary iodine, participants aged 40–59 years, women, NHB, and participants with high socioeconomic status accounted for the lowest concentrations compared with their comparts, respectively. The concentrations of nutritional biomarkers for each mineral by demographic variables are described in **Supplementary Table S7**.

In addition, the decreased trends were found for iodine excess, hyperkalemia, elevated lead, cadmium, and mercury, from 36.6 to 27.6% (p-linear trend < 0.001), 0.8 to 0.6% (p-linear trend < 0.001), 3.4 to 1.7% (p-linear trend < 0.001), 15.2 to 9.5% (p-linear trend < 0.001), and 11.2 to 7.9% (p-linear trend < 0.001), while the increased trends were observed for the prevalence of iodine deficiency and hypophosphatemia, from 31.2 to 41.8% (p-linear trend < 0.001) and 0.3 to 1.1% (p-linear trend = 0.002). The inverse U-shaped trends were observed for hypokalemia and hypernatremia (p-quadratic trend = 0.023 and 0.005, respectively; **Table 3**).

Trends of Nutritional Biomarkers for Protein and Lipids

Across 14 years, trends of serum total protein, Apo B, TC, TG, LDL-C were decreased from 71.92 to 70.25 g/L (p-linear trend = 0.001), 101.13 to 93.79 mg/dl (p-linear trend < 0.001), 201.57 to 192.39 mg/dl (p-linear trend < 0.001), 122.55 to 107.32 mg/dl (p-linear trend < 0.001), and 116.90 to 113.00 mg/dl (p-linear trend = 0.001), while serum albumin concentration increased from 42.75 to 43.67 g/L (p-linear trend < 0.001), respectively. The U-shaped trend was found in the serum LDL-C concentration (p-quadratic trend < 0.001; **Table 2**). In subgroups analysis, the decreased trends were observed in all subgroups for serum Apo B (**Supplementary Figures S4B1–B4**), TC (**Figures 3A1–A4**) and TG (**Figures 3B1–B4;** all p-linear trend < 0.05), except for participants aged 20–39 years and participants with high socioeconomic status for serum total protein (p-linear trend = 0.133 and 0.202, respectively; **Supplementary Figures S5A1–A4**). In addition, statistically significant linear trends were found in participants aged ≥ 60 years, men, NHW, and participants with medium socioeconomic status for LDL-C (p-linear trend < 0.001, = 0.001, 0.002, and 0.010, respectively (**Supplementary Figures S6A1–A4**).

The increased trends were observed in all subgroups for albumin (all p-linear trend < 0.05; **Supplementary Figures S5B1–B4**), but the U-shaped trends were not found in participants aged 40–59 years and Mexican American for HDL-C (p-quadratic trend = 0.157 and 0.192, respectively; **Supplementary Figures S6B1–B4**, **Supplementary Table S6**). Participants aged ≥60 years, women for serum total protein and albumin, NHW, and participants with high socioeconomic status for total protein, NHW, and participants with low socioeconomic status for albumin, participants aged 20–39 years, men, Mexican American, and participants with low socioeconomic status for HDL-C accounted for the lowest concentrations compared with comparts, respectively. Participants aged 40–59 years and Mexican American for Apo B, LDL-C, TC, and TG, men for Apo B, TC, and TG, participants with high socioeconomic status for LDL-C and TC, participants with low socioeconomic status for Apo B and TG accounted for the highest concentrations compared with comparts, respectively (**Supplementary Table S7**).

In addition, there were significantly decreased trends for the prevalence of dyslipidemia with elevated LDL-C, TC, TG, and lowered HDL-C/LDL-C from 31.8 to 29.4% (p-linear trend = 0.022), 47.5 to 38.8% (p-linear trend < 0.001), 33.1 to 22.0% (p-linear trend < 0.001), and 38.6 to 32.6% (p-linear trend = 0.003), but increased trend for hypoproteinemia being from 0.4 to 1.6 (p-linear trend = 0.008). The inverse U-shaped trends were found for the prevalence of elevated Apo B and lowered HDL-C, respectively (p-quadratic trend < 0.001 and = 0.003; **Table 3**).

Sensitivity Analysis

When the trends analysis was conducted among participants with and without dietary supplements, most of the above

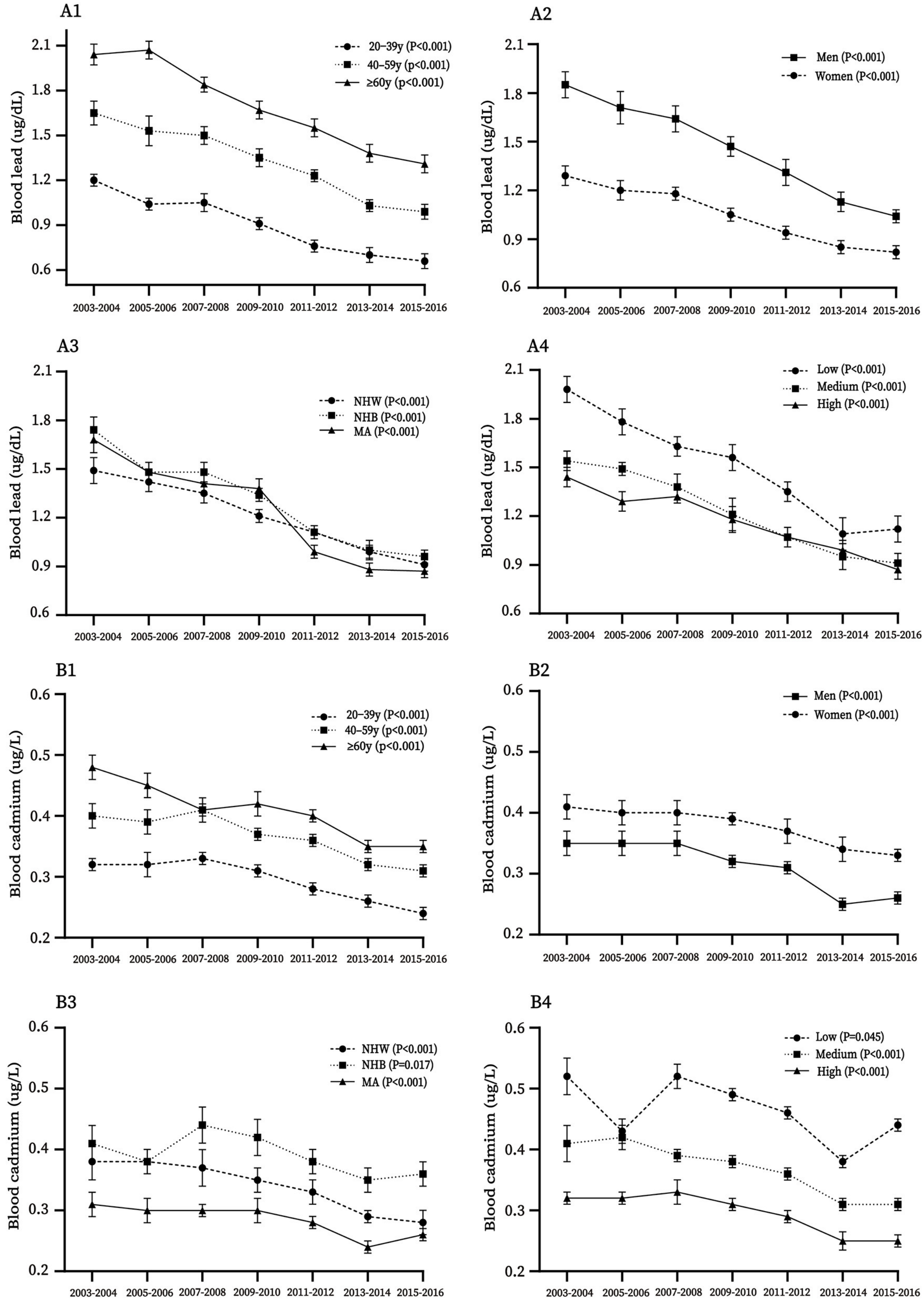

FIGURE 2 | Trends of concentrations of blood lead **(A1–A4)** and cadmium **(B1–B4)** by age, sex, race-ethnicity, and socioeconomic status (SS) among US adults from 2003 to 2016. MA, Mexican American; NHW, non-Hispanic white; NHB, non-Hispanic black.

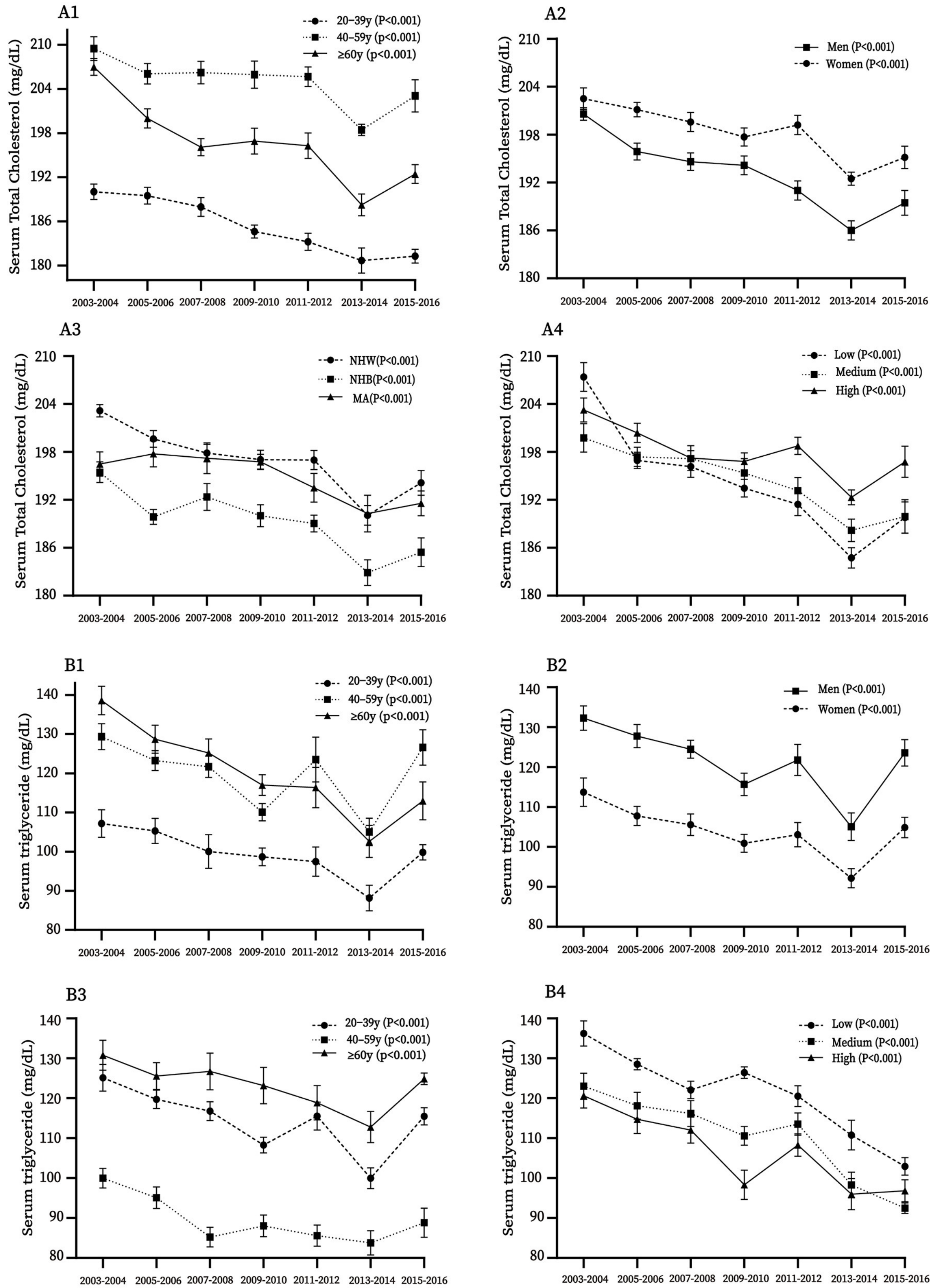

FIGURE 3 | Trends of concentrations of serum total cholesterol **(A1–A4)** and triglyceride **(B1–B4)**, by age, sex, race-ethnicity, and socioeconomic status (SS) among US adults from 2003 to 2016. MA, Mexican American; NHW, non-Hispanic white; NHB non-Hispanic black.

significant results remained (**Supplementary Table S8**), except that the previous increased trends of serum 25(OH)D and folate concentrations turned not to be statistically significant among participants without dietary supplements (p-linear trend = 0.152 and 0.884, respectively). In addition, compared to participants without dietary supplements, higher concentrations of vitamin B6, B12, folate, 25(OH)D, total protein, HDL-C, and urinary iodine, and lower concentrations of blood lead, cadmium, mercury, and TC were observed among participants with dietary supplements (**Supplementary Table S9**).

DISCUSSION

Based on data from the nationally representative US surveys across 14 years (2003–2016), most nutritional biomarkers of US adults were improved, but some specific populations should be paid much attention to improve their nutritional status, especially for NHB and participants with low socioeconomic status. To our knowledge, this is the first study that reported the comprehensive evaluation of the trends of nutritional biomarkers and their status among US adults.

Trends of overall diet quality from 1999 to 2016 and dietary nutrients from 2003 to 2016 among US adults have been investigated by Rehm et al. and this study, respectively (24, 25). The analyzed data in these two studies were all from a 24-h dietary recall interview. Besides these, a total of 24 nutritional biomarkers were measured in the NHANES, which provided data on evaluating diet and nutritional status with less error and objective assessment. However, evidence on assessing the trends of all nutritional biomarkers measured in NHANES is limited. In the present study, most nutritional biomarker status was improved from 2003 to 2016, which adds further strength to the previous data that diet quality and dietary nutrients were improved among US adults (24, 25).

It is to be noted that although most nutritional biomarkers were improved among US adults, participants aged 40–59 years, men, NHB, and participants with low socioeconomic status should be paid more attention to due to their relatively poor status of nutritional biomarkers. These results were in line with previous reports for these specific populations (24, 25). The improved nutritional status could be achieved by giving different recommendations for a specific nutrient.

Iodine, cadmium, mercury, LDL-C, HDL-C, TG, and TC should be the main nutrients to be improved for participants aged 40–59. These adults should be encouraged to intake more seafood particularly rich in iodine and iodized salts (26, 27), fewer food crops, shellfish containing cadmium (28), and predatory fish with much methylmercury (29). In addition, they should intake more dietary fiber, red yeast rice, hawthorn fruit, garlic, and seaweed but less processed meat and cream, which could efficiently lower serum LDL-C, TG, and TG concentrations and increase the HDL-C concentration (30, 31). Furthermore, in order to decrease the LDL-C/HDL-C ratio among men, increasing serum HDL-C and decreasing LDL-C concentrations would be the main means, which could be attained by encouraging them to intake more fish and their products, nuts, and nutraceuticals containing phytosterols and isoflavones, and less fried food (32, 33).

Vitamin B_6 and D, mercury, potassium, and iodine would be the key nutrients to be improved for NHB. More foods rich in vitamin B_6 and vitamin D, such as eggs, chicken, fish, beans, and nuts, exposing adequate sunlight, fresh fruits, vegetables, which are generally good sources of potassium (34, 35), and seafood particularly rich in iodine (36), but less predatory fish with much methylmercury (29), should be recommended for them. Vitamin B_6 and D, cadmium, lead and iodine, HDL-C, TG, and HDL-C/LDL-C ratio were all needed to be better for participants with low socioeconomic status. The suggestions stated above should be applied to them as well. Moreover, government and non-governmental organizations allocated funds for them and educated them on nutrition and health may be effective in improving their nutritional status (37, 38). In particular, this vulnerable population should be the focus of attention because of their relatively poor nutritional status.

Besides the above nutrients, serum concentrations of total calcium, proteins, and vitamin E may need to be paid attention to based on their decreased trends. For total calcium, the mean concentrations from 2003 to 2016 were 2.37 nmol/L for men, which was lower than the 2.81 nmol/L for Indian men and 2.42 nmol/L for men in the literature review (39, 40). In order to improve the nutritional status of calcium, men should be recommended to intake more dairy products involving milk, yogurt, and cheese, which are the best dietary source for calcium (41). Although the decreased trend was observed for total calcium for women, the mean concentration for it across 14 years was 2.35 nmol/L, which was comparable to the 2.31 nmol/L for women in the literature review (39, 42). In addition, the mean concentration of serum proteins was 71.11 g/L across 14 years, which was lower than the 74.80 g/L in the Korean cross-sectional study and 74.90 g/L in the Coronary Artery Risk Development in Adults (CARDIA, 1985–2011) study (43, 44). US adults should be encouraged to intake more seafood and plants, such as whole grains, legumes, and nuts, but fewer animal foods, such as unprocessed red meats and processed meats, to improve nutritional status for protein (24). Furthermore, the vitamin E concentration decreased from 29.39 to 27.37 μmol/L, but either of them was higher than the mean of 24.71 μmol/L in the NHANES (1999–2002) (45). Meanwhile, the prevalence of vitamin E deficiency was $<1\%$ in the present study, which was consistent with the result in the Second National Nutrition Report for US Population (46). Therefore, this slight decrease may not need to raise concerns.

In particular, dietary supplement products are widely used to maintain health and improve nutritional status in the US (47), and 52.3% of participants reported that they have eaten dietary supplements in the present study. As expected, nutritional status for all biomarkers was better among participants using dietary supplements than it among participants without supplements. However, some randomized controlled trials reported adverse outcomes for dietary supplements, in which additional nutrient intake from supplements may lead to intakes above the tolerable upper intake level (UL), especially for those nutrients that are fortified in foods (48). Although no concentrations above UL were observed in the present study, using dietary supplements should be cautious and it is better to follow medical guidance.

The present study has some merits to mention. The trends of nutritional biomarkers were measured from large nationally representative US surveys across 14 years. In addition, all biomarkers were measured by the CDC Nutrition Biomarker Laboratory, which has a well-established quality architecture based on laboratory policy and procedure manuals to ensure high-quality data. Of course, several limitations existed in the present study. First, differences in laboratory instruments, methods, and staff may influence the actual or measured concentrations of biomarkers, but the converted regression equations and equivalent data were used to match differences in laboratory methods in the NHANES survey years (3). Second, urinary concentrations of sodium and potassium are considered the gold standard measurements but they were not available and instead of serum concentrations in the NHANES. Third, the iron-status indicators were limited by only measured among women 12–49 years in the NHANES.

CONCLUSION

In summary, most nutritional biomarkers were improved among US adults from 2003 to 2016. But further improvements of some nutrients are needed for the specific population especially for NHB and populations with low socioeconomic status, such as vitamin B_6 and vitamin D, lead, cadmium, mercury, iodine, potassium, LDL-C, TG, TC, and HDL-C.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.cdc.gov/nchs/nhanes/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Center for Health Statistics (NCHS) Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW contributed to the conceptualization and design of the study, supervised the data collection, statistical analyses, initial drafting of the manuscript, and reviewed and revised the manuscript. WW conceptualized and designed the study, completed the statistical analyses, drafted the initial manuscript, and reviewed and revised the manuscript. FZ, LW, and SH assisted with the data interpretation, reviewed, and revised the manuscript. All authors read and approved the final manuscript.

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Does Mothers' Awareness of Health and Nutrition Matter? A Case Study of Child Malnutrition in Marginalized Rural Community of Punjab, Pakistan

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Maternal nutritional awareness might reduce the risk of malnutrition in children. This study assesses the impact of mothers' nutritional and health awareness (MNHA) on the nutritional status of pre-school children in rural South Punjab. Using a proportionate purposive simple random sampling technique we collect data with the help of a self-administered questionnaire on height, age, the weight of children, and socio-economic profile from 384 rural households in one of the marginalized districts of Punjab. The study applied the binary logistic regression model to compute the probability of malnutrition. The results indicate that malnutrition was high in the district (the prevalence rate for underweight is 46.1%, for stunting 34.83%, and for wasting is 15.49%). Around 91.84% of malnourished children belonged to the low MNHA category compared to medium (5.61%) and high (2.55%) MNHA categories. The results further show that the prevalence of moderate and severe stunting, wasting, and underweight in low MNHA categories was much higher with large differences compared to both medium and high MNHA categories. The binary logistic regression results depict that, across the household deprivation index (HDS), the odds of a child becoming malnourished were lower in households HDS-2 category (OR = 0.02, 95% CI: 0.01–0.89), and odds were also lower in households HDS-3 category (OR = 0.001, 95% CI: 0.001–0.16). Similarly, across the scores of MNHA index, the odds of malnutrition were lower among the children of those mothers who had medium MNHA (OR = 0.04, 95% CI: 0.002–1.24), and also the probability of child malnutrition was lower among the children of mothers who had high MNHA (OR = 0.008, 95% CI: 0.002–0.29). The study urges that well-resourced, targeted, and coordinated health and nutritional education and awareness programs are required to tackle malnutrition.

Keywords: health and nutritional awareness, household deprivation, malnutrition, Pakistan, odds ratio

INTRODUCTION

Malnutrition is a multifaceted problem as it is one of the most significant public health concerns of the Pakistani government. Malnutrition is still high in Pakistan, about 38% of under 5-year children are stunted, 23% are under-weight and 7% are wasted (PDHS, 2017–18). It is of vast interest as to why malnutrition remains high in Pakistan. In the light of previous researches, it is fact that child malnutrition cannot be tackled without understanding its accurate causes. Most of the literature on causes of malnutrition depicts that household poor socio-economic/wealth status is a main unseen cause behind child malnutrition (1–6). It is acknowledged that mothers' education (formal and on nutrition) is an important factor even after controlling wealth or socioeconomic variables (1). Previous Literature highlighted that mothers' education is highly associated with child development (7–10). It is argued that child care especially feeding, food serving in-home, medical needs against illness mostly depend on mothers, so educated mothers can raise their children more healthily. Mothers having nutrition knowledge can be healthily raised their children by providing a balanced diet to them (11, 12).

Poor nutritional awareness and education of mothers have been identified as one of the major causes of child malnutrition in many studies (13–17). There is a consensus that low nutritional awareness in mothers and household socioeconomic deprivation are the main risk factors of child malnutrition. To find the answer, whether mothers' poor nutritional and health awareness in the socioeconomically deprived segment of society is contributing to children's nutritional status or not is the main concern of the research. First of all, there are limited researches on the impact of MNHA on child malnutrition, particularly in those women who getting education informally in Pakistan. Secondly, in the context of Pakistan, it remains still unclear the nature of the relationship between the nutritional knowledge of illiterate mothers and their children's nutritional status. Thirdly, the researchers in Pakistan assessed the relationship between mothers' education (school or formal education) with malnutrition and mothers' nutritional awareness in the case of only food items. It remains uncertain what type of information and education a mother should have about the health and nutrition of their children. The mother must be given enough awareness not only regarding the type of food items given to the child but also the knowledge of screening malnutrition and diseases for protection. This present study covers these gaps.

This study constructed a mother's nutritional and health awareness index (MNHA) based on previous literature considering all the dimensions and themes regarding health and nutritional education and awareness. The research identified five components for the measurement of MNHA; 1- Immunization, 2- Food diversity, 3- Fertility, 4- Diagnosis of malnutrition, 5- awareness of nutrition. If the mother is aware of the above five dimensions of the MNHA then it could be hypothesized that malnutrition can be reduced to a large extent along with better socio-economic status. So, the question of this research is whether MNHA enlarges the role of socioeconomic status in malnutrition reduction or not. District Rahimyar Khan is one of the largest districts of Southern Punjab which shows a true representation of rural Punjab, having about 78.5% population living in rural areas (18). Most of its population is living with poor socio-economic status facing difficulties to meet their ends. Rahimyar Khan is included among one of the high malnutrition districts in the Punjab province with a very low literacy rate (19). In the district, child mortalities are high especially in rural areas. According to Punjab Development Statistics, this district stood fourth in Punjab in high rates of infants and under-five mortalities (20). Hence to see the impact of MNHA in socioeconomically marginalized rural society in Punjab, we selected the Rahimyar Khan district in Punjab.

CONCEPTUAL FRAMEWORK

This study followed the conceptual framework of Victora (21). The distribution of variables in this framework is in three group types: socio-economic reasons, intermediate factors containing maternal and environmental issues, and proximal or individual aspects. In short, pre-school children's nutritional status may be affected by these factors (21). The conceptual framework of the study is given below in (**Figure 1**).

METHODS

Study Area, Sampling, and Data Collection

This study collected the primary data through a self-administered questionnaire using a proportionate purposive simple random sampling technique from households in rural areas of district Rahimyar Khan. The sample was allocated proportionately among four sub-districts [(1)-KhanPur, (2)-Liaquatpur, (3)-Rahimyar Khan, and (4)-Sadiqabad]. The design of the sample was established based on the probability proportional to the size (PPS) in all four tehsils of district Rahimyar Khan. The sampling frame consisted of all rural households in the district. The first stage was a stratified random sampling of rural clusters [village; also called Union Councils (UCs) in every tehsil/subdivision]. The rural households for the survey were chosen randomly through the lady health worker register record.

During the survey, if there is more than one family in one premises or joint families in one house, then the study considered them nuclear if they make food independently. The anthropometric measurements were taken by lady health workers as they were trained enough before assigning the task of anthropometric measurements. We used MUAC tape, a weight machine, measurement tape for collecting data on the height, weight, along with the age of children and their mothers.

Data was gathered during 3 months in the study area from November 2017 to January 2018. After the approval of the district health officer, mothers and their close families were informed in local languages (Punjabi and Saraiki) through lady health workers about the nature of the study 1 week before to seek their verbal consent and willingness to participate in the study. All mothers from 384 households agreed to participate voluntarily in the study, and mothers gave their verbal consent during the pre-interview meeting. Written consent was not sought as the

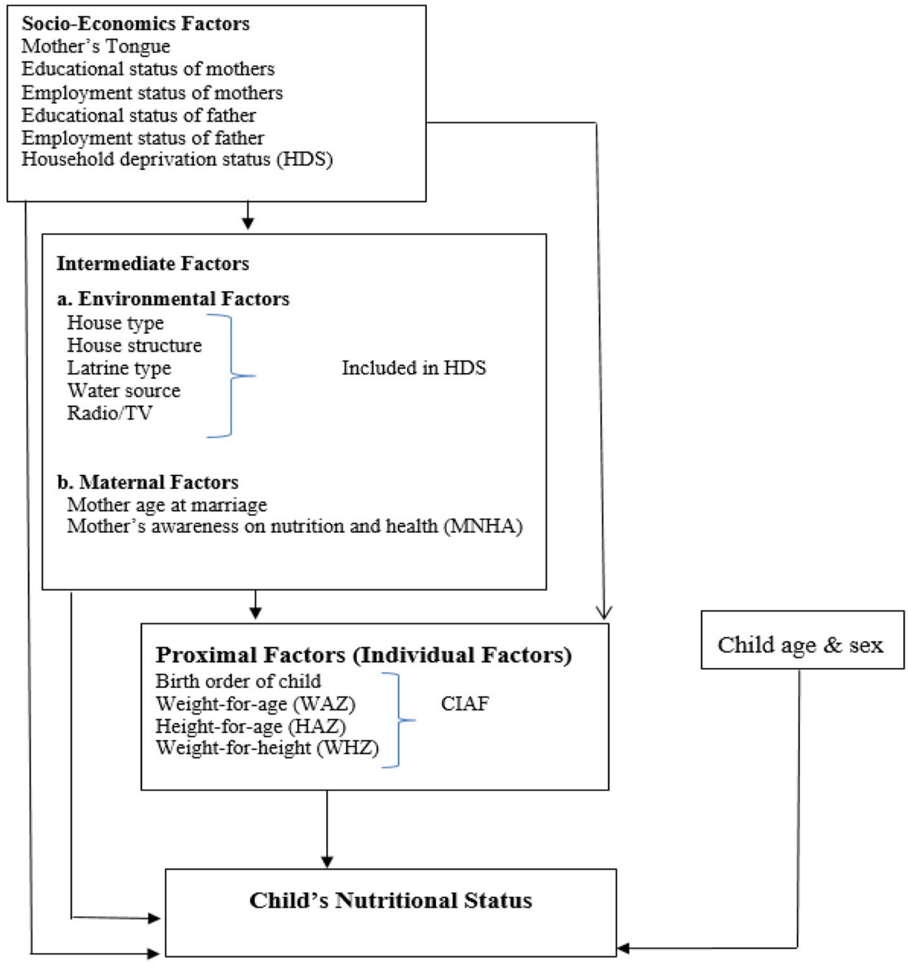

FIGURE 1 | Conceptual framework for determinants of child malnutrition. Victora et al. (21).

majority of the mothers (74%) had no formal education and they were also reluctant due to their cultural bounds. The sample size that came to be (n = 384 households) using Raosoft calculator, keeping the confidence interval at 5% and confidence level as 95%. The detail about sample size calculation is given below:

$$\text{Sample size} = Z^2 * (p) * (1 - p)/c^2$$

Where:

Z = Z value (e.g., 1.96 for 95% confidence level).
p = Percentage picking a choice, expressed as decimal (0.5 used for sample size needed).
c = Confidence interval, expressed as decimal (e.g., 0.04 = ± 4).
Sample size = $(1.96)^2$ *(0.5) * (1-0.5) / $(0.05)^2$ = 384.16 = 384.

The detail about the proportional allocation of n=384 in twelve Union Councils is given below in (**Table 1**):

$$NI = n * Ni/N$$

The formula for each UC sample calculation = Population of UC 1, 2, 3/total Population of 3 unions councils*sample size of tehsil.
NI = Number of sampled respondents in each union council.
I = Number of UCs in study area i.e., 1, 2, 3…., 12.
n = Total sample size.

Outcome Variable

The study dichotomized the dependent variable (CIAF) into two categories: "1" if a child is malnourished otherwise use "0" if a child is not malnourished. According to CIAF classification, children are divided into seven groups which are as follows: A: no failure, B: stunted only, C: wasting only, D: underweight only, E: stunted and underweight, F: wasting and underweight, and

TABLE 1 | Proportional allocation of sample size ($n = 384$) distribution from Tehsils to Union Councils.

Tehsil name with allocated sample	Name of UCs with population	Approximate sample from Tehsil to Union Council
Khanpur	Bagho Bahar (Pop = 24,349)	N1 = No. of respondent's in UC1 = 24349/90811*96 = **26**
96	Azeem Shah (Pop = 32,876)	N2 = No. of respondent's in UC2 = 32876/90811*96 = **34**
	Kotla Pathan (Pop = 33,586)	N3 = No. of respondent's in UC3 = 33586/90811*96 = **36**
Liaquatpur	Ghooka (Pop = 39,367)	N4 = No. of respondent's in UC4 = 39367/126573*81 = **25**
81	Shadani (Pop = 40,990)	N5 = No. of respondent's in UC5 = 40990/126573*81 = **26**
	Trinda Gurgaij (Pop = 46,216)	N6 = No. of respondent's in UC6 = 46216/126573*81 = **30**
Rahimyar Khans	Bahishti (Pop = 32,870)	N7 = No. of respondent's in UC7 = 32870/111793*115 = **34**
115	Sonak (Pop = 45,423)	N8 = No. of respondent's in UC8 = 45423/111793*115 = **46**
	Chak No. 84/P (Pop = 33,500)	N9 = No. of respondent's in UC9 = 33500/111793*115 = **35**
Sadiqabad	Kot Sanger Khan (Pop = 31,543)	N10 = No. of respondent's in UC10 = 31543/88105*92 = **33**
92	Muhammad Pur (Pop = 31,269)	N11 = No. of respondent's in UC11 = 31269/88105*92 = **32**
	Roshan Bhet (Pop = 25,293)	N12 = No. of respondent's in UC12 = 25293/88105*92 = **27**
Total = 384	**Union Councils = 12**	***n* = 384**

Authors. Bold values shows the sample size in tahsils and each union councils.

last is G: stunting, wasting, and underweight. CIAF estimates the overall presence of malnutrition in children. The total measure of child malnutrition prevalence is calculated by combinations of all groups except group A. Outcome variable (CIAF) was based on three indices; HAZ, WHZ, and WAZ which is stated by WHO child growth standards guidelines (2009) followed by anthropometric measurements (22).

Independent Variables

This study follows the conceptual framework of Victoria for the choice of possible determinants of child malnutrition (21). The independent variables for the analysis included in the study were the gender (male, female), age (0–5 years), birth order of child (1, 2–3, 4–5, and $\geq$6), educational status of mother and father (illiterate, primary, middle, matric, intermediate and higher), mother working status (working or not working), father working status (Government job, private job, own business, daily wages/labor, and agriculture), mother's tongue or ethnicity (Saraiki or Punjabi), Mother's age at marriage (<18, 18–25, and >25 years), household deprivation status (HDS-1, HDS-2, HDS-3), and MNHA (Low, Medium, High).

This study used the household deprivation status index of Srinivasan and Mohanty (23, 24). In the HDS index, 6 variables are used which are as follows: (1)- household has a mud house type or have cemented type, (2)- some landholding by the household or not, (3)- electricity facility is available in the house or not, (4)- within the residence or household is drinking facility available or not, (5)- any one member in the household is literate or not, (6)- keeping T.V, radio or newspaper in the house or not. The variables are in binary form. Adding of these six variables shows the total scores and the range of scores is 0–6. Those having none of any items from six possessions or have only 1 or 2 items, includes in HDS-1 and are called "moderate deprivation (MD)" that shows deprived segments of the population. "Just above the deprivation (JAD)" indicates those which have 3 possessions included in HDS-2. In HDS-3, those who have 4 or 6 items, it indicates "well-above the deprivation (WAD)." The HDS is not a direct measure for economic conditions of the household like total expenditure, per capita income, or living standard index, however, it extends the measure to depict the household above of the three dimensions as deprived (23, 24).

Development of Health and Nutrition Awareness Index

We developed the MNHA index based on previous studies (11–15, 25–40) searched from google scholar and Pubmed. We considered only those studies which tested the relationship between mothers' nutritional and health-related attitude/awareness and malnutrition. The main focus remained on studies that used mothers' nutritional and health-related education/attitude/awareness variables or indexes, and further, they related with malnutrition of child. We found ∼25 articles specifically on the relationship of mothers' nutritional and health awareness with malnutrition. Following keywords for literature search were used: (1) nutritional awareness and malnutrition; (2) nutrition education and malnutrition; (3) health knowledge and malnutrition; (4) mothers health awareness and malnutrition; (5) mothers' nutritional knowledge and malnutrition; (6) mothers nutritional and health education and malnutrition; (7) mothers nutrition and health awareness and malnutrition.

Most of the studies were concluding that although general formal education of mothers is an important factor the mother's nutritional or health-related education/attitude/awareness has much significant role in reducing malnutrition in children. During literature on different mothers' nutritional and health awareness/attitude studies, the authors noticed that information in most of the researches in their index or mothers nutritional awareness/attitude variables were related to food consumption/food diversity, diagnosis of malnutrition, or malnutrition-related care, immunization and infection-related information, fertility or birth intervals link with adverse health, and awareness about nutrition. After searching relevant literature we discussed it with a few experts of the field for validation. In their opinion, the awareness tool must be easy to understand, short, and simple for local mothers. Therefore, the study combined only five basic questions to build the MNHA index covering the most important aspects of health and nutrition.

The study developed the MHNA index, which is a key independent variable of this study. This index consists of five questions regarding attitudes and awareness of mothers on health and nutrition which are as follows: Are you aware of the immunization programs? Does complete immunization save children from infections? Do you feel that milk/egg/meat/vegetables are essential for that food item of children? Do you feel that continuous birth interval adversely affects a child's overall health? Do you have any idea about the underweight problem of children? Have you noticed this in the case of your children? Do you know what makes the children weak?

The index is in the binary form; if the answer of the mother to the question is "Yes" it assigns a value equal to 1 otherwise 0 if the answer is "No". Further, this index is categorized into three groups; low, medium, and high awareness of mothers on nutrition. The value range for the low MNHA group is from 0 to 2; for the medium MNHA, the value is 3 only, and for the higher MNHA group the range of value is 4–6. The high value shows that mothers have good knowledge of child's health and nutrition.

Statistical Analysis

The surveyed data was in three files. Household records and children's record files were merged for analysis. Before testing the relationship between CIAF and explanatory variables, the data were cleaned by all outliers. From the data, the Z-scores that were outside the WHO flags were also skipped. For analysis study included 316 children while 201 children Skipped from analysis out of a total of 517 under-five children because of over range (<5 and $> +5$). Cross-tabulations were measured as descriptive statistics to get the percentage of the occurrence of CIAF (malnutrition) concerning each explanatory variable. The logistic regression technique was applied to assess the influence of socioeconomic factors on malnutrition. Analysis of data was taken on statistical package STATA 15.

Binary Logistic Regression

To evaluate the connection between MNHA and children nutritional status this study employs the logistic regression method which measures the probability of malnutrition in two ways ("1" if a child is malnourished otherwise use "0" if a child is not malnourished), by hypnotizing that malnutrition of children depends on many proximate factors including household socio-economic, maternal and child characteristics. Malnutrition was assessed by the CIAF, a measure that is based on wasting, stunting, and being underweight. To assess the association among malnutrition and a set of explanatory variables binary logistic regression was used as this regression estimates the probability of outcome variable (CIAF) conditioned on many proximate indicators. The model specification and reduced form of binary logistic regression are shown below:

$$P\,(Yi = 1|X_{1i}, X_{2i} \ldots, Xkn) = F\,(\beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \ldots. + \beta_n X_{kn})$$

CIAFi = f (Individual factors, Environmental factors, Maternal Factors, Socio-Economic Factors, εCIAF).

In this equation, y_i denote indicators of child malnutrition i as dependent variable (CIAF); X shows explanatory variables; β's are coefficients of interest, which explain the degree of association with dependent variable CIAF; εCIAF is a random error assumed with covariates in the reduced form which shows nutrition outcome function can be uncorrelated. Here Y is a binary outcome variable (CIAF) and (Yi = 1) implies that the child is malnourished, and (Yi = 0) represents child is not malnourished, X = (X_{1i}, X_{2i}..., Xkn) are independent variables, and xi is the observed value of the independent variable for observation i.

RESULTS

Profile of the Respondents

Most of the mothers (76.53%) were without any formal education in the district and 84.18% among them belonged to the 18–25 years age group at marriage. Around 58% of households had an annual income of <50,000 Pakistani Rupees, while 26% of households' annual income was <100,000 Pakistani Rupees. Around 91% of households belonged to the HDS-1 (2.5%) and HDS-2 (88.78%) category-the most deprived segment of the society. Around 79% head of the households (fathers) were daily wagers, laborers, or agricultural employment. Around 92% of mothers were poor in MNHA. A total of 517 Under-Five children were sampled for the study, out of which 286 (56%) were male children and 231 (44%) were female children.

Descriptive Statistics

The prevalence of malnutrition was very high in the district (the prevalence rate for underweight is 46.1%, for stunting 34.83%, and for wasting is 15.49%). **Table 2** indicated the percentage of the occurrence of CIAF in a child concerning different socio-economic factors. The descriptive results in **Table 2** showed that 47.45% male and 52.55% female children were malnourished. Around 76.53% of malnourished children belonged to those households where mothers had no education, while 79.08% of malnourished children belonged to those houses in which fathers were daily wagers/labor or engaged in agricultural work. Mothers who were not employed had 98.47% of malnourished children and the households belonging to the HDS-2 category which is the deprived segment of the society had 88.78% malnourished children in their houses. This suggests that child malnourishment is due to MNHA, as well as, unemployment and poverty. Mothers who have low MNHA scores had 91.84%, malnourished children. The descriptive results showed that malnutrition prevalence rates were high among those households who were deprived and their females were mostly illiterate and their mothers had low MNHA scores. Around 87.24% of malnourished children were Saraiki ethnic group.

TABLE 2 | Descriptive analysis describing the association between different socioeconomic characteristics over CIAF (child malnutrition) (*N* = 316).

Variables	Categories	Frequencies	Percentages	*P*-Values
Sex of child	Male	93	47.45	0.140
	Female	103	52.55	
Age of child (in months)	0–12	19	9.69	0.000***
	13–24	26	13.27	
	25–36	60	30.61	
	37–48	51	26.02	
	49–60	40	20.41	
Birth order number	Birth order 1	52	26.53	0.079*
	2 or 3	79	40.31	
	4 or 5	41	20.92	
	6 or above	24	12.24	
Mother's age at marriage	<18 years	22	11.22	0.559
	18–25 years	165	84.18	
	>25 years	9	4.59	
Mother's education	No education	150	76.53	0.788
	Primary	22	11.22	
	Middle	14	7.14	
	Matric	6	3.06	
	FA & Higher	4	2.04	
Father's education	No education	135	68.88	0.403
	Primary	35	17.86	
	Middle	7	3.57	
	Matric	16	8.16	
	FA & Higher	3	1.53	
Mother's working status	Working	3	1.53	0.012***
	Not-working	193	98.47	
Father's working status	Govt. job	8	4.08	0.133
	Private job	8	4.08	
	Own business	25	12.76	
	Daily wages/Labor & Agriculture	155	79.08	
Mother's tongue/ethnicity	Punjabi	25	12.76	0.114
	Saraiki	171	87.24	
Household deprivation status	HDS-1	5	2.55	0.084*
	HDS-2	174	88.78	
	HDS-3	17	8.67	
Nutritional and health awareness of mothers	Low	180	91.84	0.008***
	Medium	11	5.61	
	High	5	2.55	

*Authors' estimation [Significance level: *** if P < 0.01, ** if P < 0.05, * if P < 0.1].*

Table 3 illustrates the association among all three indices of child nutritional status with MNHA. According to the weight for age classification (underweight), moderate underweight in all the three groups which are Low MNHA, medium MNHA, and high MNHA was 22.68, 2.23, and 0%, while the severe underweight prevalence in all three groups in pre-school children was 43.12, 1.86, and 1.11%, respectively. For classification of height for age (stunting), moderate stunting prevalence in all the three groups was 32.58, 1.87, and 0.37%, while this rate of prevalence for severe stunting in all three groups among pre-school children was 32.58, 0.76, and 1.49%, respectively. Furthermore, in weight for height classification (wasting), prevalence rates of moderate wasting in all the three groups were 23.94, 4.22, and 1.41%, while in case of severe wasting in all three groups in pre-school children was 12.68, 2.82, and 0%, respectively. The moderate and severe underweight, stunting, and wasting prevalence rates in the low MNHA category were much higher compared to medium and high MNHA categories.

Logistic Regression Estimates

The logistic regression estimates for CIAF were displayed in (**Table 4**). The logistic results for age of children depicted that age of children from 25 to 36 months was associated with the higher odds of malnutrition among pre-school children in district Rahimyar Khan (OR = 8.68, 95% CI: 2.83–26.61). Birth order/interval of children 4–5 years was accompanied with lower likelihoods of malnourishment (OR = 0.44, 95% CI: 0.21–0.94). Mothers who were not engaged in any employment or job had higher odds of malnutrition among their under-five children (OR = 3.31, 95% CI: 0.31–35.69). Children who belonged to Saraiki families had lower chances of becoming malnourished as compared to Punjabi counterparts (OR = 0.39, 95% CI: 0.15–0.98). Across the HDS scores, the odds of children becoming malnourished were lower in the HDS-2 households' category (OR = 0.02, 95% CI: 0.01–0.89), and odds were also lower in the HDS-3 households' category (OR = 0.001, 95% CI: 0.001–0.16). Similarly, across the scores of MNHA index, the odds of malnutrition were lower among the children of those mothers who had medium MNHA (OR = 0.04, 95% CI: 0.002–1.24), and also the probability of child malnutrition was lower among the children of mothers who had high MNHA (OR = 0.008, 95% CI: 0.002–0.29).

DISCUSSION

This study assessed the impact of maternal health and nutritional awareness (MNHA) along with household socioeconomic deprivation status (HDS) on child malnutrition status in a marginalized rural Punjab district of Pakistan. The results of the study depicted that the prevalence rate of underweight is 46.1%, stunting 34.83%, and wasting is 15.49%. The study findings showed that the age of a child, birth order of the child, ethnicity, and mother working status have a significant association with child malnutrition. Furthermore, the results of main policy variables highlighted that MNHA along with HDS largely contributed to child malnutrition. The study results further demonstrated that across the household deprivation index scores, the odds of a child becoming malnourished was lower in the HDS-2 households' category (OR = 0.02, 95% CI: 0.01–0.89), and odds were also lower in the HDS-3 households' category (OR = 0.001, 95% CI: 0.001–0.16). As the household deprivation in basic amenities of life reduced or in other words increased in basic amenities of life from HDS-2 to HDS-3, it reduced the probability of malnutrition in children as compared to HDS-1 (as HDS-1 shows that household has nothing or one or two basic amenities of life).

TABLE 3 | Descriptive analysis illustrating the association between mother's nutritional and health awareness (MNHA) and anthropometric indicators.

Indicators	Mother's nutritional and health awareness				
	Categories	**Normal**	**Moderate**	**Severe**	**Total**
Underweight	MNHL-1 (Low)	71 (26.39%)	61 (22.68%)	116 (43.12%)	248 (92.19%)
	MNHL-2 (Medium)	2 (0.74%)	6 (2.23%)	5 (1.86%)	13 (4.83%)
	MNHL-3 (High)	5 (1.86%)	0 (0%)	3 (1.11%)	8 (2.97%)
	Total	**78 (29%)**	**67 (24.91%)**	**46.10 (24.91%)**	**269 (100%)**
Stunting	MNHL-1 (Low)	69 (25.84%)	87 (32.58%)	87 (32.58%)	243 (91.02%)
	MNHL-2 (Medium)	4 (1.49%)	5 (1.87%)	2 (0.76%)	11 (4.12%)
	MNHL-3 (High)	8 (3%)	1 (0.37%)	4 (1.49%)	13 (4.86%)
	Total	**81 (30.34%)**	**93 (34.84%)**	**93 (34.83%)**	**267 (100%)**
Wasting	MNHL-1 (Low)	31 (43.66%)	17 (23.94%)	9 (12.68%)	57 (80.28%)
	MNHL-2 (Medium)	6 (8.45%)	3 (4.22%)	2 (2.82%)	11 (15.49%)
	MNHL-3 (High)	2 (2.82%)	1 (1.41%)	0 (0%)	3 (4.23%)
	Total	**39 (54.93%)**	**21 (29.58%)**	**11 (15.49%)**	**71 (100)**

Authors' estimation. Bold values shows the total frequency and percentage in cross tabulation by each section.

TABLE 4 | Binary logistic regression analysis results for CIAF (child malnutrition) and its correlates.

Variables	**Categories**	**Coefficients**	**Std. Err.**	**Odd. Ratios**	**95% CI**
Sex of child (female-reference)	Male	−0.358	0.1972	0.699	[0.402, 1.215]
Age of child (0 to 12 months-reference)	13–24 months	0.898	1.4558	2.455	[0.768, 7.848]
	25–36 months	2.1609	4.9607	8.679***	[2.831, 26.607]
	37–48 months	0.1272	0.5104	1.136	[0.471, 2.740]
	49–60 months	−0.1117	0.4129	0.894	[0.362, 2.211]
Birth order number (birth order 1-reference)	2 or 3	−0.1995	0.2986	0.819	[0.401, 1.674]
	4 or 5	−0.8183	0.1689	0.441**	[0.208, 0.934]
	6 or above	−0.0578	0.4821	0.944	[0.347, 2.568]
Mother's age at marriage (<18 years-reference)	18–25 years	−0.7169	0.2891	0.488	[0.153, 1.558]
	>25 years	1.3364	4.9544	3.805	[0.297, 48.83]
Mother's education (illiterate-reference)	Primary	−0.263	0.8542	0.769	[0.087, 6.786]
	Middle	−0.4636	0.7462	0.629	[0.062, 6.434]
	Matric	−1.5127	0.2932	0.220	[0.016, 2.989]
Father's education (illiterate-reference)	Primary	−0.2082	0.8851	0.812	[0.096, 6.876]
	Middle	0.7817	2.6304	2.185	[0.207, 23.126]
	Matric	1.7912	7.0102	5.996	[0.606, 59.293]
	FA & Higher	2.2146	14.356	9.158	[0.424, 197.75]
Mother's working status (working-reference)	Not-working	1.1969	4.0156	3.309**	[0.307, 35.685]
Father's working status (govt. job-reference)	Private job	−1.1423	0.5235	0.319	[0.0128, 7.948]
	Own business	−1.969	0.2173	0.139	[0.007, 2.949]
	Daily Wage/ Labor & Agriculture	−1. 087	4.0149	2.966	[0.208, 42.123]
Mother's tongue/ethnicity (punjabi-reference)	Saraiki	−0.949	0.1834	0.387**	[0.153, 0.979]
Household deprivation status (HDS-1-reference)	HDS-2	−3.835	0.0409	0.022**	[0.005, 0.879]
	HDS-3	−6.6539	0.0032	0.001***	[0.001, 0.155]
Nutritional and health awareness of mothers (low MNHA-reference)	Medium	−3.2933	0.0664	0.037**	[0.002, 1.237]
	High	−4.7759	0.0154	0.008***	[0.002, 0.294]
The overall significance of the model					
No. of observations = 306			Prob>Chi^2 = 0.0000		
LR Chi^2 (26) = 80.09			Pseudo R^2 = 0.1982		

**References: odd ratios; p-values; confidence intervals.*
*Significance level: *** if $P < 0.01$, ** if $P < 0.05$, * if $P < 0.1$.*
Authors' estimation.

The results showed that in low MNHA categories the stunting, wasting, and underweight prevalence rates were much higher compared to both medium and high MNHA categories. The logistic regression depicted that, across the scores of MNHA, the odds of malnutrition were lower among the children of those mothers who had medium MNHA (OR = 0.04, 95% CI: 0.002–1.24), and also the probability of child malnutrition was lower among the children of mothers who had high MNHA (OR = 0.008, 95% CI: 0.002–0.29). Childcare depends on the mother's knowledge and education, especially on health and nutrition. Mother takes better nutritional care of a child if she was more aware of signs and causes of nutritional deficiency and further requirements of nutrition rather than the educated mothers.

Studies in Asia depicted MNHA as a curtailed indicator for child malnutrition. The study in rural Bengal endorsed that if parents showed nutritional ignorance, the frequency of malnutrition among their children remained high (25). A study in Indonesia established a maternal nutritional knowledge index based on five components (knowledge in nutrition, knowledge of micronutrients, label reading and numeracy, food measure skill, and grouping food in groups) and found that double burden of malnutrition was among the children of low maternal nutritional literacy households (26). Another study in urban Indonesia used maternal nutritional knowledge for obese mothers based on three dimensions such as macronutrients knowledge, household food measure skills, grouping food into categories skill and concluded that maternal knowledge has increased the self-efficacy in mothers and provided improvements in children growth behaviors (27). A study in Iran used food and nutrition knowledge assessment index using 60 items based on six dimensions such as knowledge on nutrition and food, functional skills, interactive skills, advocacy, critical investigation of information, and skills on reading a food label and concluded that nutrition knowledge level among senior-high-school students was low while academic performance and socioeconomic status were significant predictors (28).

Studies from Pakistan and India also highlighted the impact of MNHA on malnutrition. A study conducted in different areas of Pakistan (Quetta and Tandojam) used nutrition education intervention based on the counseling of mothers. The intervention was based on breastfeeding and food items and there was a significant reduction in malnutrition after this intervention (29). Another study in Pakistan developed mothers' knowledge, perceptions, and knowledge on health based on eight components awareness on child weight and height gain, food type, number of times a day child feed, knowledge on solid foods, ORS or boiled water, cleanliness, maintaining contact with lady health workers, and breastfeeding practices and found that mothers' awareness and literacy was a significant predictor of better nutritional outcomes (30).

Because a mother is the prime provider of a child's nutritional needs and care, the chances of malnutrition are reduced in mothers who have high MNHA (31–33). Study in Andhra Pradesh, India assessed the mothers' knowledge, perceptions, and attitude on malnutrition based on 23 questions on child feeding and found that only 35 percent of mothers showed a positive attitude toward child feeding and 33 percent of mothers have practiced proper feeding and nutritional knowledge (34). Another study in India assessed the knowledge and attitude of mothers regarding dietary and malnutrition prevention based on 30 multiple choice questions found that mothers with ∼56 percent adequate knowledge and dietary practices helped in the prevention of malnutrition among their children (35).

Studies in Africa verified the importance of nutritional and health-related awareness of mothers with malnutrition. A study in Mozambique, East Africa designed the maternal health awareness index based on responses from mothers regarding malnutrition cases, symptoms, and screening. It found that mothers could somehow be able to screen only serve malnutrition while they could not detect the early signs of stunting and undernutrition (36). Another study in west Africa, in Niger, composed mothers' perceptions and awareness on malnutrition index based on five components mainly protein deficiency, nutrients deficiency in the body, lack of right food type, deficiency of carbohydrates, and breastfeeding, and found that mothers' awareness on malnutrition had a significant association with malnutrition (37). A study from Niger also used mother nutritional related knowledge index based on five components (such as colostrum knowledge of the mother, breastfeeding knowledge, knowledge on using ORS for diarrhea prevention, knowledge on immunization of child, knowledge on family planning) and concluded that the current mothers' knowledge on nutrition is not sufficient to reduce malnutrition (38). A study in Madagascar, East Africa, used mothers' health knowledge (focusing on knowledge of food, knowledge on nutrition and malnutrition, experiences on food, knowledge on services for malnutrition, and breastfeeding) concluded that maternal health knowledge significantly contributed to the reduction of SAM cases (39). A study in Cameroon, Central Africa used mothers' nutritional knowledge index based on nine questions related to breastfeeding, food items, pre-lacteal liquids, and use of palm cooking oil and found that only seven percent of mothers breastfeed their children according to WHO guidelines, nutritional education has improved dietary habits (40).

A study in India highlighted that HDS significantly impacted child nutrition status as deprivation in basic amenities increased the malnutrition prevalence in children (24). The HDS index in India found that more than half of truly poor households had at least one child underweight or stunted in their houses as compared to non-poor counterparts (41). Literature from Pakistan indicated that households' poor socioeconomic status or poverty was a major contributing factor in child malnutrition (2–6). With the improvement in social and economic status, families have more resources to provide their children with food and nutrition as well as proper medication in case of any disease.

It is assumed that there could be a trade-off between the working status of women and child care. If women are engaged in proper employment, then child care and development especially child feeding may be affected due to less time given to the child because of employment (42). But on the other hand, the working status of women independently contributes toward children's nutrition. Women's income increases the household's resources to buy food and nutrition and afford basic amenities of life. The results of the study show that mothers who were not engaged in any employment or job had higher odds of malnutrition among their under-five children (OR = 3.31, 95% CI: 0.31–35.69). The results of a study revealed that working women belonging to the household of the first two quintiles (poorest and poorer) of the wealth index were not contributing to the nutrition of the children while in the third quintile (medium) of wealth index, the working status of women contributed to the nutritional prestige of children in Pakistan (43). It may be concluded that women's employment should be at that level that can support the socio-economic status of the household, but on the other hand, child care should not be suffered.

The logistic results of the study in district Rahimyar Khan showed that the birth order of children 4–5 years was accompanied by lower probabilities of malnutrition (OR = 0.44, 95% CI: 0.21–0.94). However, it might not have a linear relation with the increase in birth order. It may be a reflection of fact that most of the parents were fulfilling the prime food requirements of their children because in rural areas natural food items (milk, fruits, and vegetables, etc.) are cheap and easy to access. A study in Nepal depicts that birth interval $<$24 months was significantly associated with severe acute malnutrition (44). Likewise, a study in Pakistan illustrated that higher birth order significantly increased the odds of stunting (45). Also, another study in Pakistan showed that the probability of child mortality decreased with greater birth intervals (46).

The food and care requirements of a child can vary with age. The study results revealed that the age of children from 25 to 36 months was associated with the higher odds of malnutrition among pre-school children (OR = 8.68, 95% CI: 2.83–26.61). As the child's age increased, the probability of malnutrition also increased. It reflects that most of the parents could not fulfill the nutrition requirements of their child with increasing age or due to bad health and water facilities children failed to recover quickly. The results of the study indicated that the ages of 13–24 and 25–36 months have a significant and positive association with child malnutrition which means children aged 0–36 months have a greater risk of malnutrition than those above 36 months. The findings in Pakistan and Bangladesh are in line with our results in which the age of the child is positively associated with child malnutrition (34, 47).

The results of ethnicity in the current study illustrated that children who belonged to Saraiki ethnicity had lower chances of becoming malnourished as compared to Punjabi counterparts (OR = 0.39, 95% CI: 0.15–0.98). The outcome reflected that in the total sample the rate of malnutrition prevalence in Punjabi's children is higher than the Saraiki children because the average total income of Punjabis was less than the average total income of Saraiki households in district Rahim Yar khan. Income could be a factor to increase malnutrition in Punjabi children. A study in Nepal regarding ethnicity shows that Dalit children were more underfed as compared to Brahmin children (48). In another study, the ethnicity variable was also significantly impacting the nutritional status of children in Vietnam (49).

CONCLUSION

This study investigates the association between mothers' nutritional and health awareness and child nutritional status in a marginalized district of Punjab province of Pakistan. Results reveal that health and nutritional awareness in mothers strongly contribute to child malnutrition especially when households are socioeconomically deprived. It implies that these two factors have a syndemic relationship as they mutually reinforce each other. Therefore, both factors need to be compositely dealt with to tackle child malnutrition side by side. As this district has remained marginalized in terms of socioeconomic conditions there is a strong need to create income-generating opportunities along with social security nets to end the deprivation. Additionally, one of the best ways to raise awareness on health and nutrition might be Lady Health Workers (LHWs) program with proper training because they are often in close contact with pregnant and lactation women.

LIMITATIONS OF STUDY

Due to the financial and logistical limitations, the study only covered one of the most deprived districts of Punjab and restricted its sample size to 384 households.

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 Graduate Research Management Council (GRMC) in its 6th meeting through the Department of Health Economics at Pakistan Institute of Development Economics (PIDE). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MS and FA designed the study and drafted the manuscript. YC, FA, SR, JG, NM, UR, MQ, RS, and RM were involved in manuscript revision. All authors have contributed equally in approving this manuscript to its final version.

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REVIEW
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Dairy as a Source of Iodine and Protein in the UK: Implications for Human Health Across the Life Course, and Future Policy and Research

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This narrative review summarizes key concepts in dairy nutrition for supporting human health throughout the life course. Milk and dairy products have been a staple component of our diet for thousands of years and provide a wide range of important nutrients that are otherwise difficult to obtain from dairy-free diets. In this review, we provide a broad perspective on the nutritional roles of iodine and dairy protein in supporting human health during pregnancy and early life, childhood and adolescence, mid- and later-life. New methodologies to identify biomarkers of dairy intake *via* high-throughput mass spectrometry are discussed, and new concepts such as the role of the food matrix in dairy nutrition are introduced. Finally, future policy and research related to the consumption of dairy and non-dairy alternatives for health are discussed with a view to improving nutritional status across the lifespan.

Keywords: dairy, iodine, protein, pregnancy, sarcopenia

INTRODUCTION

The clinical implications of dairy nutrition encompass somatic growth and development, neurological and cognitive development, and cardiometabolic health, including risk and incidence or cardiovascular disease (CVD), type 2 diabetes and sarcopenia. Hence, the nutritional value of dairy products is relevant across the lifespan. The over-arching aim of this narrative review is to summarize the state-of-the-art regarding the myriad of health implications associated with dairy intake across the life course, and how this science could be used to help shape future policy. The review is organized into three sections: (i) pregnancy, childhood and adolescence, (ii) mid life, and (iii) older age. While milk and dairy products provide a variety of nutrients that are important across the life course, for example calcium and vitamin B12, this review focuses on the health implications of the constituent protein and iodine content of milk and dairy products. We focus on these nutrients because they are of current concern from both the perspective of population deficiency risk, and because there are implications for these nutrients when plant-based milk-alternatives are used as a substitute to cows' milk, a practice that is becoming increasingly common in many countries, including the UK. For information on the health impact of other dairy-derived macro- and micronutrients, such as calcium, we refer the reader to other publications (1, 2). In this review, we also focus on new methodologies for assessment of dairy intake and considerations for future research into dairy nutrition and health, including vitamin D fortification.

DAIRY IN PREGNANCY, CHILDHOOD, AND ADOLESCENCE

Iodine

Milk and dairy products are the primary dietary source of iodine in the UK and many other European countries (3), especially where iodised salt is not mandatory or widely available. Iodine is essential for thyroid hormone production [tri-iodothyronine (T_3) and thyroxine (T_4)] which are crucial for brain and neurological development during pregnancy and early life. Iodine deficiency disorders include goiter (thyroid enlargement), impaired neurological development, and negative effects on child growth and development (4, 5). Iodine deficiency during pregnancy has considerable implications for the developing child and the iodine-intake recommendation is higher for pregnant/lactating women than adults according to WHO (250 vs. 150 μg/day for non-pregnant adults), although in the UK recommendation is the same (140 μg/day) (6, 7), as it is assumed that iodine stores are maximized prior to pregnancy, which can be used to meet additional demands (7). Emerging evidence suggests that even mild-to-moderate iodine deficiency during pregnancy may be negatively associated with child cognition and behavior (8, 9). This observation underpins the need for follow up interventional studies in pregnant women that utilize milk and dairy products as a vehicle to reverse iodine deficiency and improve the cognitive health of new-borns.

Cow's milk has a naturally low iodine concentration but is a rich source of iodine through standard farming practices such as the addition of iodine salts to cattle feed and use of iodine-based disinfectants (10–12). Iodophors mainly increase iodine concentration in milk through skin absorption of the iodine from topical application to teats (either pre or post milking) (13) and to a lesser extent through residues on the teat that transfer to the milk, especially if the teats are not dried sufficiently (14). In some countries, iodophors have been replaced with other disinfectants, and this is one explanation suggested for the decline in milk iodine concentrations in those countries [e.g. Australia and New Zealand (15, 16)]. Iodophors continue to be permitted and used in the UK, and in other countries. Indeed, a recent study in Switzerland found that teat dipping with iodine-containing disinfectants significantly increased milk-iodine concentration (17).

There is considerable variability in the iodine content of milk between countries, which is likely a result of differences in farming practice (12). UK milk has a relatively high iodine concentration compared with many other countries (12) at 427 μg/L (18). In the UK, a glass of milk (200 ml) provides 85 μg of iodine, constituting ∼57 and 34% of the WHO recommended iodine intake for adults (150 μg/day) and pregnant women (250 μg/day), respectively (19). Seasonal variation in milk-iodine concentration exists whereby winter milk has a higher iodine concentration than summer milk (11, 20). This is a result of greater reliance on mineral-fortified feed during winter months when cattle are housed indoors rather than grazing on pasture (12), and possibly also related to goitrogenic components of feed being lower in winter milk (i.e., reducing competition with iodine and allowing greater excretion into milk). In the UK, and other European countries, studies have previously found also demonstrate that organic milk is lower in iodine content than conventional milk (∼40% lower) (21, 22), largely as a result of restrictions on mineral fortified feed and higher goitrogen components of feed; however organic milk is still a good source of iodine in the UK, with a concentration of 241 μg/L and more recent UK research suggests that there is no overall difference in iodine concentration between organic and conventional milk (23), likely because of changes in organic farming methods. However, these sources of variation mean that milk is an inconsistent source of iodine, and the value assigned in food tables may be inaccurate, making it difficult to assess iodine intake and to provide dietary recommendations for optimal iodine intake.

Milk and dairy products have been shown to be determinants of iodine status in pregnant women. For example, in a study in three European countries [the Netherlands, Spain and the UK (24)], of all food groups, "milk and dairy products" was the only food group that was positively associated with iodine status. A UK study demonstrated that milk (out of a number of investigated dietary factors) was significantly associated with iodine status in pregnant women (25) (median iodine-to-creatinine ratio in women with milk intake 140 vs. 280 ml/day: 72 vs. 150 μg/g), whereas seafood intake was not (median iodine-to-creatinine ratio in women with seafood intake 2 vs. 2 portions/wk: 107 vs. 99 μg/g). The seasonal variability in milk-iodine concentration (18) translates to iodine status in pregnancy, with higher iodine status in winter months and the greatest difference between seasons in those who consumed the most milk (25). This observation underpins the need for a reliable and constant source of iodine so that individuals are not affected by underlying changes in milk-iodine concentration associated with farming practice.

Milk and dairy consumption contribute 51% of total iodine intake in UK children aged 4–10 years. A relatively high milk intake in children is one possible explanation for the iodine-sufficiency observed in UK children (4–10 years) in the National Diet and Nutrition Survey [NDNS (3)] and other UK studies (26). In contrast, there have been reports that 27% of girls aged 11–18 years have a low iodine intake (3) and pregnant women are classified as deficient (25). Given that milk intake, and thus iodine status, is higher in children than adults in countries where milk is an important iodine source is problematic for population monitoring. This issue stems from the WHO recommendation that population iodine status is monitored through assessment of school-aged children, however, this is likely to lead to an overestimation of population iodine status if a child's iodine intake (skewed by milk intake) is not representative of other population groups, such as women of childbearing age (27). Indeed, women of childbearing age are susceptible to low iodine intake and cross-sectional studies of adolescent girls (14–15 years) in the UK (n = 737) (28) and Ireland (n = 903) (29) demonstrate a positive association between milk intake and iodine status. In those studies, other dietary sources of iodine (such as fish) were not significantly associated with iodine status. Although fish (e.g., cod, haddock) is a rich natural source of iodine (30), in countries, such as the UK, these iodine sources make a relatively small contribution to population iodine intake [10 vs. 34% for milk and dairy products for adults 19–64 years (3)] because of their relatively infrequent consumption.

Historically, in the UK there was a concern that high consumers of milk would be at risk of excess iodine intake. This concern was especially true for young children, who have a higher milk intake than adults and who may exceed the Tolerable Upper Limit (TUL) for iodine from milk intake alone [TUL: 250 μg/d for 7–10 yr olds (31)]. Therefore, in the 1980s and 1990s, the milk-iodine concentration of UK milk was monitored by the (then) Ministry of Agriculture Fisheries and Food (MAFF), and estimated exposure was calculated for different age groups (32). Since then, the regulation for the permitted iodine content of cattle feed has been revised over concerns of potential toxicity of iodine from milk (33); the maximum concentration has been reduced from 40 mg/kg dry matter, to 5 mg/kg (33). The health implications of excess iodine intake in children are relatively unexplored but iodine excess can lead to both hyper- and hypothyroidism and should be avoided, especially in those with previous history of iodine deficiency (34).

Protein

The consumption of adequate amounts of high-quality protein during childhood and early adolescence is crucial for optimizing growth and development (35). The role of milk consumption for growth and maturation in children is widely recognized in both developing and developed countries (36). For example, a trial in Bangladesh compared the growth of children in households which kept dairy cows with those that did not (37). The study reported that children in dairy cow owning households had increased height-for-age Z scores (+0.52 standard deviations) during the rapid growth period of 6- to 23-months of age compared with children in households without dairy cows. Moreover, children aged 0–11 months from the households with dairy cows were 21.7% less likely to be breastfed than those in households without cows. These findings suggest that easy access to cows' milk can substantially reduce the incentive for mothers to adopt breast feeding.

The benefit of milk consumption on linear growth during childhood is now understood to be primarily attributed to the casein fraction stimulating the secretion of hepatic insulin-like growth factor 1 (IGF-1) (38). Consistent with this notion, a recent study in pre-pubescent (8 years old) boys demonstrated that 7 days of casein administration increased serum IGF-1 concentrations to a greater extent than whey, with whey eliciting a greater insulin response (38). Moreover, the report of Locatelli et al. (39) highlights the mechanistic role of IGF-1 in the longitudinal growth of bone and skeletal maturation, as well as bone mass development and its remodeling in adult life. The regulation of bone length is linked to changes in chondrocytes of the proliferative and hypertrophic zones of the growth plate (40) and it now understood that activation of the Peroxisome Proliferator- Activated Receptor gamma (PPARγ) regulates changes in hepatic IGF-1 secretion and gene expression resulting from dietary protein intake (41). Allied with growth hormone (GH), IGF-1 mediates bone mass accretion by reducing collagen degradation and increasing the recruitment of osteoblastic cells and deposition of the collagen matrix (42). Furthermore, *in vitro* studies using chondrocytes have provided evidence that GH directly stimulates the formation of immature precondrocytes, whereas IGF-1 stimulates cells at a later stage of maturation. Hence, IGF-1 exerts a direct action on osteoblast function which is potentiated by the presence of GH and IGFBP-3 (insulin-like growth factor binding protein-3) (39). Taken together, these data provide important mechanistic insight regarding how dietary protein and related amino acids stimulate the hepatic secretion of IGF-1 and promote bone health during childhood.

Prepubertal males and females are in a state of constant growth, increasing stature by on average ∼5 and 8.3 cm/y, respectively (43). Furthermore, the velocity of weight gain increases from 3 to 9 kg per year during puberty and almost entirely consists of lean mass. Underlying this growth in stature particularly during puberty is a chronic positive net muscle protein balance whereby rates of muscle protein synthesis (MPS) exceed muscle protein breakdown (MPB) (44). Traditionally, protein quality for muscle health is determined by the interplay of two factors, namely the digestive properties (and subsequent absorption kinetics) (45) and amino acid profile of an isolated intact protein source (i.e., whey, casein, soy protein). Collectively, these factors determine the bioavailability of protein-derived amino acids to the muscle for stimulation of MPS (46). Regarding amino acid composition, a complete profile of all indispensable (also known as "essential") amino acids (IAA) is required to provide the building blocks necessary for MPS. Of the IAA, leucine serves as a key anabolic signal by activating the mechanistic target of rapamycin complex one pathway, leading to greater protein translational efficiency and ultimately increased MPS rates (47). Hence, it is generally accepted that rapidly digested protein sources that are rich in leucine content and contain a full complement of all IAA provide the highest quality protein. This has led to the general consensus that animal-derived protein sources are of higher quality than plant-derived protein sources. The potency of cow's milk to stimulate MPS in children aged 7–11 yrs has been shown by Karagounis et al. (48). In this study protein doses of ≥7 g at breakfast resulted in the attenuation of overnight protein losses in the subsequent 9 h. Furthermore, a protein dose-dependent increase in net muscle protein balance was observed, highlighting the importance of (milk-derived) protein intake with breakfast.

DAIRY IN MID-LIFE

Iodine

Maintaining sufficient iodine levels during midlife is clinically relevant, especially for women of childbearing age who need to maximize thyroidal stores of iodine prior to pregnancy (49). Iodine stores can be used to maintain thyroid hormone production during periods of suboptimal iodine intake and increasing evidence suggests that long-term iodine sufficiency prior to pregnancy is preferable to an abrupt increase in iodine intake at the onset of pregnancy (50). Adults need to maintain thyroid hormone production through adequate iodine intake, not only to prevent thyroid enlargement (and eventually goiter) but also as iodine deficiency (especially moderate-to-severe) is associated with certain types of thyroid cancer (51) and hypercholesterolemia (52).

While data presented in the NDNS suggest that adults exhibit an adequate overall iodine status, a significant proportion of adult women have low iodine intake (i.e., intake below the Lower Reference Nutrient Intake) (53). In the UK, dairy consumption contributes 34% of total iodine intake in adults (19 to 64 years), and a positive association has been observed between milk intake and iodine status in women of reproductive age (54), underpinning the importance of milk as a source of iodine at this life stage. Dairy products provide an effective means to raise iodine status in women of childbearing age. Support for this notion is provided by a 12-week randomized controlled trial in women of childbearing age in Northern Ireland (55) that reported a higher iodine status in the intervention group (who were provided with additional milk) after 6 and 12 weeks compared with the control group (who continued their usual milk intake). Indeed, milk intake was 340 vs. 130 ml/day at 6 weeks and 260 vs. 120 ml/day at 12 weeks in the intervention and control group respectively) (55).

Protein

The role of protein nutrition in maintaining skeletal muscle mass and strength is crucial for healthy aging (56). Numerous studies have demonstrated an acute stimulation of MPS with protein ingestion, with a graded protein dose-MPS response relationship observed up to 20–30 g of ingested high-quality protein (57). Most studies investigating the MPS response to protein ingestion have focussed on isolated protein sources such as whey, casein and soy. In these studies, the milk protein fractions (whey and casein) were shown to elicit a greater MPS response compared with plant derived proteins (58). Nevertheless, most individuals consume dietary protein in whole foods. In this regard, milk has been shown to promote muscle accretion to a greater extent than soy proteins when consumed after resistance exercise (59). Moreover, consuming an isonitrogenous dose of whole milk elicited a similar MPS response in middle-aged men when compared with whey protein, despite greater digestion rates and leucine availability with whey protein ingestion (60). A study by Burd et al. investigated the digestion and absorption kinetics, and subsequent MPS response, following the consumption of milk and beef in healthy young (18–35 y) men. Both protein sources increased the MPS response after resistance exercise, with milk eliciting a greater MPS response during the early postprandial phase (61).

A series of recent studies have investigated the response of MPS to the ingestion of alternative protein sources. For instance, a recent study demonstrated that ingesting a 35 g bolus of intact or hydrolysed wheat protein initiated a robust increase in circulating essential amino acid concentrations, however failed stimulate myofibrillar protein synthesis rates above basal values (62). Instead, a 60 g bolus of wheat protein was required to stimulate an increased response of MPS in older adults. Moreover, the ingestion of 30 g of corn protein (63) and 70 g of mycoprotein (64) was recently demonstrated to increase the stimulation of MPS in healthy young males. Taken together, these findings have implications for future dietary guidelines that might, besides supporting optimal nutritional guidelines, also support a more sustainable future for protein nutrition.

Whereas most studies have explored the amount of protein necessary to maximally stimulate MPS, a study by Mitchell et al. (60) investigated the minimal dose of milk protein concentrate required to enhance the anabolic signaling response to a bout of resistance exercise, concluding that 9 grams of milk protein was sufficient to enhance signaling proteins related to muscle protein anabolism. These studies highlight the capacity for dairy proteins, particularly milk and its respective protein fractions (whey and casein), to stimulate MPS to the same extent as isolated protein sources. Studies evaluating the efficacy of protein (dairy) nutrition to maintain skeletal muscle mass specifically during mid-life are scarce, but, to date, underscore their importance for healthy aging.

DAIRY IN LATER-LIFE (65 YEARS+)

Iodine

Most studies that have investigated the impact of iodine deficiency on human health outcomes have been conducted in children and young adults; hence, data on the impact of iodine status in later life are currently limited. However, it has been hypothesized that long-term iodine deficiency may have a detrimental effect on cognitive function and brain volume in older age. In this regard, a study of 189 individuals in the Lothian Birth cohort demonstrated an association between low iodine intake (mainly related to low consumption of dairy products) and inner brain atrophy (65). However, there are inherent challenges with estimating iodine intake over many years, including fluctuations in milk-iodine content, meaning that these results are hypothesis-generating and require exploration in future research using a prospective-study design.

Protein

A decline in skeletal muscle mass, strength and function (sarcopenia) is observed with advancing age and presents a clinical threat to independence by reducing mobility and increasing the risk of falls, fractures and hospitalization (66). Hence, the preservation of skeletal muscle mass is critically important for healthy aging. The causal mechanisms that underpin sarcopenia are clearly multifactorial, but ultimately stem from a chronic period of negative muscle protein balance (67). Contributing to this negative net muscle protein balance with age is the phenomenon termed "anabolic resistance" that describes the reduced capacity for older adults to mount a "youthful" MPS response to a meal-like (20–30 g) quantity of ingested protein (68). Hence, identifying high-quality protein sources that can stimulate a robust increase in MPS is crucial in mitigating sarcopenia and its associated morbidities.

Based on the superior quality of animal-derived protein sources, and in particular dairy, the postprandial MPS response to ingesting 20 or 40 g of whey protein (69) exceeded that of soy protein (70) in healthy older adults. Moreover, a recent study demonstrated a greater response of MPS to ingesting intact micellar casein compared with a dose-matched quantity of whole wheat; the most abundant plant-based protein in the diet comprising ∼25% of total protein intake (62). The superior MPS response to the ingestion of milk proteins was primarily attributed to the more favorable IAA and leucine content of intact whey or casein compared with soy or wheat. Moreover, milk proteins are the only protein sources that exhibit a higher constituent IAA composition than human skeletal muscle, whilst boasting a complete IAA profile. In contrast, soy and wheat proteins are deficient in one or more IAA, namely methionine and lysine, and have lower leucine concentrations, rendering their IAA profile inferior to that of dairy proteins. Accordingly, based on typical dietary intake patterns across Europe (71) and North America (72), dairy holds a prominent position as a readily available and commonly consumed protein-rich food source for older adults in combating the threat of sarcopenia.

Specific protein intake guidelines for older individuals are currently lacking. The RDA of 0.8 g/kg/day for protein intake has recently been challenged and an alternative protein intake of 1.0–1.2 g/kg/day has been proposed (73). Furthermore, as a consequence of age-related anabolic resistance, the required per meal-protein dose is greater in older compared with younger individuals (74). To maximally stimulate MPS with each meal, older individuals require ∼0.4 g/kg body mass of protein, whereas younger individuals require only 0.31 g/kg body mass of a high-quality protein (75). In the context of a mixed meal, a higher protein dose is likely necessary to maximally stimulate MPS. To aid the development of a framework for healthy aging, protein intake guidelines should be conceptualized not only in an age-specific but also in a meal-specific (breakfast, lunch, dinner and snacks) manner.

Whereas numerous studies have demonstrated a robust stimulation of MPS in response to ingesting an isolated intact protein source (i.e., whey, casein or soy protein), relatively few studies have compared the postprandial MPS response to different protein-rich foods. Moreover, limited information exists regarding how various food components modulate this process. This gap in knowledge has led to the recent emergence of evidence indicating a biological role for the food matrix in determining the anabolic capacity of commonly consumed protein-rich foods (76). As such, ingesting whole milk immediately following exercise resulted in greater muscle uptake of amino acids compared with an isonitrogenous dose of fat-free milk in young adults (77). These data suggest that an unidentified component or mechanism within the dairy matrix is able to increase the bioavailability of amino acids for MPS stimulation.

DAIRY NUTRITION ACROSS THE LIFE COURSE–FUTURE RESEARCH AND IMPLICATIONS FOR POLICY

Research

Since there is considerable variation in the iodine content of milk in the UK, for instance because of season or organic farming (22, 23), further research is warranted to understand the factors that influence the iodine concentration of milk and how these may be manipulated to increase the reliability of milk as a source of iodine. For example, no data exist regarding the effect of key aspects of organic feed (such as white clover) on milk-iodine concentration, or whether the seasonal effects seen in the past are as pronounced today with the increasing practice for year-round housing for dairy cows on large farms. Furthermore, seaweed is starting to be used in farming (e.g., to reduce greenhouse gas emissions), either given to cows or used as a fertilizer on grassland; seaweed use in the dairy industry has been shown to increase milk-iodine concentration (78, 79) but further research on ways to incorporate it without risking iodine excess is required.

There also is a need for research to establish a reliable biomarker of individual iodine status so that risk of iodine deficiency can be established, both for future research studies and for clinical practice. Currently, the preponderance of evidence relies on urinary iodine concentration (which can be corrected for urinary creatinine concentration), but this is an imperfect marker on an individual basis and limits the exploration of associations between dietary intake and iodine status. Thus, establishing a more reliable biomarker, or combination of existing biomarkers (such as UIC with thyroglobulin measures) to provide a more robust assessment of long-term intake in individuals, would enable the impact of milk and dairy products on iodine status to be better understood, as well as identifying potential risk of deficiency in those who are not consuming iodine-rich foods.

With regards to protein, future research is warranted to elucidate the interaction between protein and other macronutrients on muscle metabolism. A study by Elliot et al. (77) showed a trend for increased uptake of the IAA phenylalanine and threonine when whole milk was provided after resistance exercise as opposed to fat-free milk and an isocaloric fat-free milk. These results are indicative of an interaction between the nitrogen utilization of the ingested protein and the other nutrients. The importance of the food matrix on MPS has been confirmed by van Vliet et al. that demonstrated a superior stimulation of MPS after resistance exercise when whole eggs, as opposed to egg whites only, were consumed (80). Therefore, future studies should investigate the effects of mixed nutrient meal ingestion on MPS rather than isolated protein sources.

Policy–Milk Alternatives

Given that milk and dairy products are the primary source of iodine in many countries, including the UK, any policy that includes a shift away from milk and dairy products [e.g., based on the EAT Lancet report (81)] must consider adequate iodine intake and possibly alternative sources of iodine. Over the past 40 years, milk consumption in the UK has decreased, as demonstrated by data on the purchase of milk (82) and while the consumption of cow's milk exceeds other types of milk, the popularity of plant-based milk-alternative products (e.g., soya, almond, oat) has increased (83). For example, data from the US (84), Norway (85) and the UK (86) show that unless fortified with iodine, these milk-alternatives have a low iodine content compared with cow's milk (i.e., 7.3 vs. 438 μg/kg) (86). Most milk-alternative drinks on the UK market are not fortified with iodine (unlike calcium), although this may change in the future as more companies add iodine to their products.

The low iodine content of unfortified milk alternative products, coupled with the positive trend in consumption, might be particularly concerning in countries with limited availability of iodised salt, such as the UK. Indeed, in a study that extracted data from the UK NDNS (between 2014 and 2017; when most milk alternatives on the market were not iodine-fortified), individuals who exclusively consumed milk-alternative drinks had a significantly lower iodine intake (94 vs. 129 μg/day) and iodine status (measured by the median urinary iodine concentration (UIC): 79 vs. 132 μg/L) than cow's milk consumers, suggesting that consumers of milk alternatives were not replacing the iodine elsewhere in the diet (87). The results are meaningful from a public health perspective, as those who consumed cow's milk were classified as iodine-sufficient according to the WHO criterion (median UIC 100 μg/L), whereas the exclusive consumers of milk alternatives were classified as iodine deficient.

Changes in food patterns are likely to affect iodine status in the UK, particularly in specific population groups that are more likely to avoid and/or substitute cow's milk for milk alternatives (i.e., women of childbearing age, vegans, or individuals with a milk allergy) (83). Hence, it may be considered presumptuous to assume that milk alternatives are viable substitutes in the context of meeting dietary iodine recommendations across the life course, particularly in countries without an iodised salt policy. The nutritional content and micronutrient fortification of more recently emerging milk-alternative dairy products (e.g., cheese, yogurt, cream alternatives) and their impact on population nutritional status is unclear and should also be considered.

Manufacturers of plant-based alternative milk drinks (and other products sold as alternatives to dairy) should be encouraged to fortify their products with an appropriate amount of iodine. Indeed, the British Dietetic Association have recently launched a campaign to raise awareness about iodine and to ask for a commitment for iodine fortification from manufacturers of milk alternatives (88). The iodine content of these products should be similar to the (average) iodine concentration of milk so that consumers are not put at risk of iodine deficiency. Fortification should be with potassium iodide/iodate, rather than a seaweed-based ingredient in order to ensure reliable iodine concentration and avoid risk of excessive iodine.

In recent years, advocates of commonly consumed animal-based protein sources, including dairy, have been challenged by a social movement attributing, in part, the increase in worldwide greenhouse gas emissions (GHGE) to livestock production (89). Consistent with this idea, on a per gram basis, plant-derived proteins are associated with lower GHGE (<4 kg of carbon dioxide per kg of edible weight) than most animal-derived protein sources, including dairy (90). However, as outlined above, the reduced potency of plant-based proteins to stimulate MPS means that, in theory, a considerably larger amount of plant-based protein food is required to support the maintenance of muscle mass with aging. Furthermore, most plant proteins are high in fiber which impairs the digestive properties of the protein source, and are deficient in at least one IAA (91). Accordingly, if GHGE are expressed relative to the amount of food required to satisfy daily IAA requirements, the benefits of a plant-based protein diet over an animal-derived protein diet, including dairy, may become less clear-cut than is often espoused (92).

If milk and dairy products are not consumed (for various reasons), public-health messages need to ensure alternative sources of key nutrients are clearly signposted. In the case of iodine, other dietary sources would include fish and eggs, or appropriately fortified milk-alternative products. Although seaweed is a rich source of iodine, intake of brown seaweeds and supplements (e.g., kelp) can lead to excess iodine intake and therefore should be avoided as an iodine source (93).

Potential Fortification of Milk and Milk Alternatives With Vitamin D

Milk and dairy products are not rich sources of Vitamin D. In this regard, UK cows' milk has trace concentrations (94) or 0.06 μg/100 ml according to recent analysis of whole milk in Northern Ireland (95). In contrast to iodine, milk-alternative drinks are often fortified with vitamin D, at approximately 0.75 μg/100 ml, which means that they are a better source of Vitamin D than cows' milk in the UK. In the US and Canada milk is fortified with vitamin D to a concentration of 0.875–1.125 μg/100 ml) (96). A recent study that modeled (using NDNS data) the impact of vitamin D fortification of milk at varying concentrations (1 μg/100 ml, 1.5 μg/100 ml or 2 μg/100 ml) showed that fortification had the greatest effect in children (1.5–3 years) with predicted vitamin D intake increasing to 4.8, 6.2, and 7.6 μg/day, respectively (95) [the vitamin D recommendation for is 10 μg/day (400 IU) (97)]. These data suggest that vitamin D fortification of milk could be a strategy to address vitamin D deficiency. This is significant as Vitamin D is essential for bone formation through its role in intestinal calcium absorption (98) and low vitamin D status can lead to rickets in children and a subsequent increased risk of osteoporosis in later life. At the beginning of the 20th century, around 80% of children in North America and Europe had severe rickets but was essentially eradicated by the late 1930s due to food fortification with vitamin D and provision of cod liver oil to children (99). Today, a sub-optimal vitamin D status is widespread in children and adults and it is of concern that cases of rickets are again increasing in the US (100), UK (101) and worldwide (102, 103) and vitamin D deficiency is similar for older adults as children, teenagers and middle-aged adults (104).

Maintaining adequate vitamin D levels (>50 nmol/L) in older individuals is important for bone health, muscle maintenance and several other health-related outcomes. Low plasma levels of 25(OH)D are associated with a higher risk of osteoporosis and is a key underlying factor for fractures in women and men aged >50 yrs (105). A study by Sahni et al. examined the association between dairy consumption and bone loss in older individuals and examined whether these associations were modified by vitamin D supplementation (106). The findings revealed a protective effect of dairy consumption on bone mineral density in those individuals who supplemented their diet with vitamin D. Taken together, these findings highlight the potential role of fortified dairy-derived vitamin D on bone health.

Recent evidence also suggests a potential role for vitamin D in the maintenance of muscle mass with advancing age (107, 108). A meta-analysis investigating the impact of dairy-derived protein and vitamin D supplementation demonstrated a positive effect of protein on body weight, whereas vitamin D facilitated small, but physiologically relevant improvements in physical performance in frail, inactive older individuals (109). Whilst acknowledging the value of fortifying dairy products with vitamin D, this intervention might not be adequate to fulfill daily vitamin D needs. Therefore, additional food sources, such as eggs (110) and orange juice (111) are often fortified in vitamin D and could contribute to overall adequate vitamin D status.

NEW CONCEPTS AND METHODOLOGIES IN DAIRY RESEARCH

The accurate measurement of dairy intake represents a distinct challenge for epidemiological studies investigating the association between dairy intake and cardio-metabolic health across the life course. In this regard, identifying biomarkers of dairy intake is generally accepted to be an important strategy to overcome the limitations of traditional dietary assessment methods.

Biomarkers of Dairy Fat

Several potential biomarkers of dairy fat have been explored including individual fatty acids such as odd-chain saturated pentadecanoic (C15:0) and heptadecanoic (C17:0) acids, along with ruminant trans-fatty acids such as trans-palmitoleic acid (t16:1n-7). Limited studies have assessed the relationship between reported dairy intake and C15:0 or C17:0 circulating and adipose tissue levels, and consistently favor C15:0 as the better biomarker of dairy fat compared to C17:0 (112). Moreover, circulating trans-palmitoleic acid was strongly correlated with whole-fat dairy products in a large American prospective study (113). However, these circulating fatty acids may respond to non-dairy dietary sources (e.g., fish, meat), endogenous synthesis and other nutrient intakes such as dietary fiber (114). Thus, the reliability of these results in the context of varied study populations and dietary patterns remains unclear.

Biomarkers of Dairy Proteins

The limited number of studies that have explored potential biomarkers for dairy protein have produced mixed results. In a randomized controlled cross-over trial (115), 47 participants consumed meat-derived protein, dairy products or grain. Investigators identified potential urinary and plasma biomarkers for meat- and grain-derived protein but not for dairy protein. Potential biomarkers of whey protein (a globular protein isolated from whey) and casein (a major component of cheese) protein intake were identified in several other randomized cross-over studies, including linear dipeptides and γ-glutamyl conjugates (116). Nevertheless, these trials were small-scale, and more validation studies are warranted to confirm these results. In addition to dairy fat and protein biomarkers, other potential dairy biomarkers relate to gut microbiota, which could potentially provide signatures of specific dairy product intake. However, a paucity of studies has been conducted to evaluate the gut microbiota as a "biomarker" of dairy intake, and more prospective cohort studies or randomized trials are warranted.

Untargeted Approaches to Identify Novel Biomarkers: Metabolomics and Lipidomics

Odd-chain fatty acids and trans-16:1n-7 have been consistently associated with dairy consumption and offer promising biomarkers of dairy fat intake. However, their limitations suggest a need for identifying novel biomarkers. Contrary to the candidate biomarker approach, the exponential increase in the use of metabolomics and lipidomics over the last few decades due to the development of high-throughput methods including proton nuclear magnetic resonance (H-NMR) and mass spectrometry (MS) offers a promising hypothesis-free approach to identify novel nutritional biomarkers (117). Metabolomics is the study of low-molecular weight metabolites (usually <1,500 Da) (117). Several studies have assessed the metabolomic profiles of blood (118–121), urine (122–126) or feces (125) samples, using MS (118–120, 122, 123) or H-NMR (121, 123–126), in a trial (119–126) or observational (118) study design among healthy adults, patients (120, 121) or children (124). More recently, Drouin-Chartier et al. (127) investigated numerous ($n = 385$) plasma metabolites in relation to dairy consumption among participants from the PREDIMED intervention study ($n = 1,833$) and a confirmatory cohort of 4,932 participants from the PREDIMED study year 1, the Nurses' Health Study, the Nurses' Health Study II, and the Health Professionals Follow-Up Study. This study identified 38 metabolites associated with total dairy consumption declared in food frequency questionnaires [Pearson correlation, r (95%CI) = 0.37 (0.33–0.40)] and 30 metabolites associated with intakes of reduced fat dairy [r = 0.24 (0.19–0.30)]. Among the metabolites measured, higher plasma levels of C14:0 sphingomyelin and C34:0 phosphatidylethanolamine, together with reduced levels of γ-butyrobetaine, were consistently associated with higher dairy intake. Furthermore, authors observed an inverse association between a score based on identified dairy-related metabolites and incident risk of type 2 diabetes in the PREDIMED baseline cohort [HR per 1 SD increment of metabolite score = 0.76 (0.63–0.90)], but not among the follow-up PREDIMED cohort or the US cohorts investigated, providing new evidence on potential biomarkers to study the mechanisms underlying the relationship between dairy nutrition and type 2 diabetes.

Whilst these studies have successfully utilized untargeted approaches to identify metabolite signals for dairy consumption, several limitations warrant consideration. As such, the inclusion of patient populations (120, 121) limits the generalisability and the identification of unspecific biomarkers of dairy consumption (122–126). The use of metabolomics for dairy consumption biomarker discovery is promising but still in its infancy, and the identified metabolites thus far still have their limitations. Therefore, further metabolomics studies are needed using a combination of metabolites in order to contribute toward the discovery of novel and more specific biomarkers of dairy consumption.

Food Matrix Effect

The health and nutritional properties of foods are conventionally assessed according to their individual nutrient composition. This approach associates one nutrient with one health effect, e.g., the diet-heart hypothesis. According to this hypothesis, saturated fat foods, such as dairy, are often associated with higher cholesterol levels. However, a growing body of evidence suggests that postprandial responses are strongly affected by the food matrix (128), which is the arrangement of the food constituents and their interactions at the multiple length scales (129), e.g., from the molecular to the physical structure. This matrix is created naturally or modified by processing and can affect nutrient bioavailability and, subsequently, the metabolic responses.

Dairy products often provide the predominant dietary source of saturated fat. Historically, dietary fat intake has been linked with an increased risk of cardiovascular disease (CVD) resulting from elevations in serum concentrations of cholesterol and low density lipoprotein cholesterol (LDL-C) (130). This association has led to many dietary guidelines proposing a restriction of saturated fat intake to <10% of total energy intake (131). Despite being a major source of saturated fatty acids, there is now a substantial body of evidence from prospective studies and associated meta-analyses that suggest the overall intake of dairy foods is not associated with an increased risk of CVD. Indeed, a recent study reported a reduced risk of stroke and type 2 diabetes (132). However, there is an increasing body of evidence that supports the idea that specific fatty acids, rather than total saturated fatty acids, may exacerbate the adverse effects on CVD risk (133).

A key feature of any food source is the possible influence of the so-called food matrix that can modulate the amount of fat digested and absorbed. This notion was elegantly demonstrated by Hjerpsted et al. (134). In this study, 23 participants underwent two 6-week crossover periods whereby a proportion of their habitual dietary fat intake was replaced by either cheese or butter, both of which provided 80 and 36 g/day of total fat and saturated fatty acids, respectively. The calcium content of cheese and butter was 834 and 19 mg/100 g, respectively. Relative to baseline, cheese intake was not associated with an increase in serum total cholesterol or LDL-C concentrations, whereas the butter diet was associated with an increase in both lipids. Cholesterol and LDL-C concentrations in the cheese diet were 5.7 and 6.9% respectively lower than the butter diet. Further evidence for the food matrix effect can be found in a review by Thorning et al. (128).

Several mechanisms have been proposed to underpin the health benefits of manipulating food matrix. For instance, studies that provided a hard cheese resulted in an increased fecal fat and calcium excretion. This observation stemmed, at least in part, from the saponification reaction in the gastro-intestinal tract between calcium and fatty acids that resulted in the production of largely indigestible soaps. In addition, several studies have demonstrated increased fecal bile acid excretion that may be linked with their absorption onto amorphous calcium phosphate. An increased fecal bile acid excretion is indicative of reduced enterohepatic recycling of bile acids. Hence the liver synthesizes bile acids from cholesterol, and reduces bile acid recycling that may lead to reduction in circulating cholesterol concentrations (128). Finally, there is evidence that in some dairy foods the milk fat globule membrane can protect the dairy fat from digestion and absorption (135).

CONCLUSIONS

Milk and dairy products provide important nutrients across the life course, some of which (e.g., iodine) are likely underappreciated by the consumer. Milk and dairy products (as a rich source of dietary iodine) intake should remain a staple component of the diet for pregnant/lactating women, given that iodine deficiency during pregnancy is associated with cognitive implications for the developing child. However, excessive iodine intake may be of concern in those who consume high quantities of iodine-rich milk (e.g., winter UK milk), and hence it is important to continue to monitor milk-iodine concentration, especially in relation to changes in the iodine content of cattle feed. Milk proteins, and specifically the casein fraction, are fundamental to supporting musculoskeletal growth and development during childhood and adolescents, mediated primarily by the secretion of IGF-1. With advancing age (mid and later life), dairy proteins play a prominent role in maintaining skeletal muscle mass and strength *via* the stimulation of MPS. Likewise, maintaining a sufficient iodine status with advancing age is particularly relevant given the emerging link between iodine deficiency and brain health in older age. Moving forward, it is critically important that policy and practice reflects the role that milk and dairy products play in the provision of important nutrients for metabolic health in humans across the life course.

AUTHOR CONTRIBUTIONS

All authors were involved in conceptualization, writing and editing of the narrative review and have read and agreed to the published version of the manuscript.

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Increasing Parental Knowledge About Child Feeding: Evaluation of the Effect of Public Health Policy Communication Media in France

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Background: Unhealthy eating behaviors are risk factors for non-communicable diseases. Parents largely influence the development of eating behaviors during childhood through their feeding practices. Parental feeding practices in line with recommendations are more likely to turn into healthier outcomes in children. From a public health perspective, it should be first ascertained whether providing parents with recommendations about child feeding is a useful approach for increase parental knowledge. Recently, the French health authorities developed a brochure covering updated child feeding recommendations. The present study aims to evaluate the short-term effects of reading this brochure on parental knowledge about child feeding, distinguishing knowledge accuracy and certainty.

Methods: A brochure containing updated child feeding recommendations for 0–3 years old was developed by the French public health agency. A representative sample of French parents ($n = 400$) was targeted to complete an online questionnaire (T0) comprising 30 statements regarding child feeding. For each statement, parents indicated whether it was true/false and how certain they were of their answer (4-point scale). After receiving and reading the brochure, the same parents completed the same questionnaire 3 weeks later (T1). Accuracy (number of correct answers) and certainty (number of mastered answers: correct answers given with the maximal degree of certainty) were compared at T1 vs. T0 using paired t-tests. Knowledge evolution based on parental age, parity and education level was tested with linear models.

Results: A total of 452 parents responded at T0 and T1 and were considered for analysis. Between T0 and T1, the number of correct answers [median 22–25, $t_{(451)} = 17.2$, $p \leq 0.001$] and mastered answers [median 11–17, $t_{(451)} = 18.8$, $p \leq 0.001$] significantly increased. The median of the difference between T1 and T0 was larger for mastered than for correct answers. The observed evolution in knowledge was independent of parental age, parity or education level.

Conclusions: A brochure containing child feeding recommendations has the potential to increase the accuracy and, to an even greater degree, the certainty of parental knowledge. This increase was observed even for younger or less educated parents.

Keywords: public health nutrition, child feeding, knowledge assessment, feeding guidelines, nutrition education

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INTRODUCTION

Childhood obesity is a major public health concern worldwide, threatening the health of children, especially in Western societies. In France, in 2013, 12% of children under the age of six were overweight (1); moreover, in 2017, 18% of adolescents were overweight, and 5% were affected by obesity. More of concern, between 2009 and 2017, the prevalence of childhood obesity rose (2), despite the deployment of public health campaigns starting in 2001 to target nutrition and physical activity (3). The benefits of establishing healthy eating behaviors from early childhood are countless in terms of non-communicable disease prevention. In fact, previous studies have demonstrated that health-promoting nutritional practices in the first 1,000 days of life can positively impact future health (4). For example, the introduction of vegetables can be boosted during the early years of a child through the application of specific feeding practices, fostering their acceptance later on (5). Furthermore, food preferences established early in life track into adulthood and are the basis for pursuing the maintenance of a healthy diet (6). Parental feeding practices influence the development of children's eating habits and preferences (7, 8). Those practices have an impact on shaping the child's risk of developing diet-related, non-communicable chronic diseases, such as obesity.

International and national feeding guidelines aim to facilitate the familiarization of parents with evidence-based best practices and guide them in the feeding process; however, two main problems might arise. First, from a public health perspective, it is difficult to always maintain the recommendations in line with the latest scientific evidence. Some countries may have incomplete feeding recommendations due to a lack of regular updating (9). Consequently, public health stakeholders may face challenges in terms of institutional time required for national nutrition and health policies renovation. For instance, in France, the communication material used to spread the official child feeding recommendations is not recent (2004), despite new guidelines covering feeding children 0–3 years of age having recently been published, but not yet adapted toward lay public dissemination (10). Second, having updated guidelines does not automatically leads to knowledge increase and changes in behavior. Care should be given in bridging the existing gap between the evolution of scientific knowledge and the transfer of this evidence-based knowledge in a timely manner into public health policies and actions (e.g., communication campaigns, interventions on the environment). Ultimately, this should be beneficial for promoting healthy behaviors changes within the population.

Feeding practices of French parents do not always meet official recommendations. A cross-sectional study conducted in 2013 with a sample of 1,184 children under the age of 3 years increased focus on the low prevalence and duration of breastfeeding (11). Regarding the introduction of solid foods, the same study showed that 54% of infants were introduced to solid foods between 4 and 6 months of age, but younger mothers still struggled with achieving the initiation of complementary feeding within the recommended time frame (11). Low breastfeeding duration was observed in another French cohort and was shown to be linked to a variety of factors. Those factors included the fact that mothers had been breastfed themselves, and a high rate of maternal professional activities (12–14). Data from the French study Epifane (a nationwide birth cohort) confirmed that socioeconomic characteristics can affect the concordance of complementary feeding behavior with national recommendations. In fact, parents in more disadvantaged situations were more likely to follow less strictly the guidelines (15). Similar results emerged from studies conducted in other European countries, with the introduction of solid foods occurring earlier than recommended by national guidelines (16). Parental cultural and sociodemographic characteristics (e.g., lower socioeconomic status and education level), or other markers of unhealthy lifestyle (e.g., maternal smoking), often predicted the early start of complementary feeding (16–18).

A recent integrative review showed that European parents' knowledge about child feeding, particularly complementary feeding (e.g., when to first start the introduction of solid foods), is far from optimal (19). In contrast, an online quantitative survey conducted in the UK evaluated parents' understanding of feeding guidelines, reporting high knowledge of the recommendations (20). Providing information is one means to increase knowledge, but increasing knowledge is not sufficient to predict a change toward healthier behaviors (21). Nevertheless, knowledge remains one of the main components of the theoretical domains framework to achieve behavior change when implementing health interventions (22). Therefore, understanding information and comprehending recommendations remain important steps in the early-stage process of eliciting lifestyle changes through behavior modifications. One major challenge is to define the best strategy to educate parents toward feeding practices that encourage the adoption of healthy eating behaviors in children. Many different theories have been applied to explain parent feeding practices, including ecological systems theory (23) and social cognitive theory (24). Nevertheless, little is known about the knowledge that might drive parents to adopt a certain practice. Metacognition theories explore how the "feeling of knowing" can mediate controlled vs. automatic processes, implying that a difference might exist in applying knowledge that we might be more or less conscious to have acquired (25). A study performed by Bruttomesso et al. found that, in order to better characterize knowledge, subjects can be asked to indicate their degree of certainty for each answer (26). A similar approach has been used by Norman and Furnes to measure confidence rate when answering questions (27). To the best of our knowledge, no similar research has been done in the context of understanding parental perception of child feeding guidelines, highlighting a gap in the literature. It is hypothesized that being certain of one's own (correct) knowledge can make one more inclined to apply it (26). In this context, when evaluating knowledge, it seems important to understand both the correctness (accuracy) and the degree of certainty.

In France, public health stakeholders have been preparing a new communication strategy regarding infant and young child feeding before its field implementation. A first step was the renewing the official child feeding guidelines (10, 28). Then two quantitative studies were performed to understand French parents' and pediatricians expectations on communicating about child feeding information (29, 30). Among the support measures employable for public health dissemination purposes, paper documents have been shown to continue to play a role: 44% of parents expressed using them when seeking child feeding information (30). For 59% of pediatricians, paper brochures appeared to be the most effective tool for grabbing parents' attention when advising them about feeding during consultations (29). Santé publique France (the public health agency of the French Ministry of Health) then developed a paper brochure containing the latest child feeding recommendations, as an attempt to spread new child feeding recommendations efficiently to parents. Parents qualitatively tested this brochure (focus groups and individual interviews). In this context, it was judged essential to characterize whether this brochure could make it possible to increase parental knowledge accuracy and degree of certainty. In addition to evaluating the global effect of reading the brochure, this approach should also make it possible to identify which, if any, of the specific topics the parents were more uncertain about. Within this framework, considering the existing gap in child feeding knowledge evaluation, the present study aimed to evaluate: (1) how much French parents know about child feeding (accuracy and certainty of knowledge); (2) whether the brochure containing new official child feeding recommendations could contribute to increasing parental knowledge (accuracy and certainty) about child feeding; and (3) whether knowledge evolution related to reading the brochure would depend on parental sociodemographic characteristics. A secondary objective was to explore parental attitudes toward the content of the brochure.

MATERIALS AND METHODS

The Updating of Child Feeding Recommendations and the Development of a Brochure Targeted to Parents

In France, official recommendations for feeding children ages 0–3 years have been updated and published recently by ANSES (the French national agency for Food, Environmental and Occupational Health Safety) under the supervision of the Ministry of Health (10). In October 2020, the High Council of Public Health released a report reflecting these benchmarks (28). As of follow-up in 2019–2020, the content for a paper brochure intended for the final users of this communication strategy, the parents, had been developed by the French public health agency, Santé publique France. The intent was to make the recommendations relating to feeding children ages 0–3 years as accessible as possible to parents. In the present study, the document that was sent to the parents was a draft version of the final brochure, and it comprised just the core text, without subsequent graphical adaptations. The brochure contained 11 pages plus a table that summarized the recommendations for introduction of each food group based on the age of the child. The brochure was divided into ten chapters. Five chapters addressed the topic of feeding based on the age of the child (milk feeding, complementary feeding until 3 years of age when the child eats like the whole family); three chapters covered specific topics in line with parental feeding practices (e.g., responsive feeding); one chapter addressed physical activity and sleeping; and one chapter summarized foods not suitable for children. The version of the brochure (in French) used for this study is presented as **Supplementary Material**.

Participants, Questionnaire, and Study Procedure

The recruitment was done via an agency (Panelabs – MIS Group) composed of a panel of participants from all around France. We could not run a power calculation to set the population size in the absence of a previous study on this topic, but a targeted sample size of 400 was defined *a priori*, as it was considered large enough for our purposes based on previous studies conducted by Panelabs on similar subpopulations. The recruiting agency aimed to initially include a sample of 500 parents to account for potential drops out (see **Figure 1** for the flowchart of participants). The targeted population comprised French parents of children between 0 and 3 years of age. Specific prerequisites to participate were: (1) having a child < 4 years old and (2) not residing in one of the following French departments: 21, 52, 70, 71 or 39 (to avoid biases due to the implementation of another study in these areas). The representativeness of the sample was ensured by the quota sampling method, which was applied to the study population (parents of 0–3 years old children living in France) on the following variables: age of the parent; profession of the household reference person (i.e., the parent with the highest salary); living area (urban vs. rural); and primiparous or multiparous parent. The general population census was used to identify the quotas within our study population and for data calibration (31).

To collect the data, two online questionnaires were prepared by the researchers and were administered online to the participants via the web system of the recruitment agency (Made in Surveys) at two different time points. The first online questionnaire (T0) comprised an initial part to collect

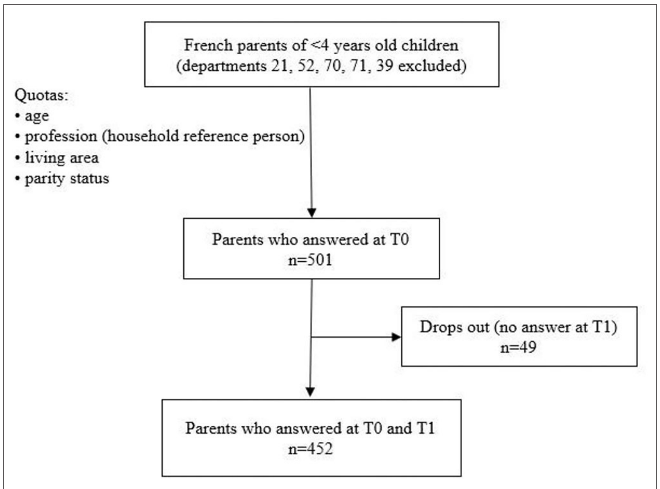

FIGURE 1 | Flowchart of the participants.

demographic data and a second part made up of 30 items in the form of declarative sentences to evaluate the child feeding knowledge of parents (see detailed content below and **Table 1** for the items). The second questionnaire (T1) was made up of the same 30 items plus some questions to gather other information regarding the attitudes of parents toward the brochure (i.e., usefulness of the content and parental self-efficacy regarding following the recommendations of the brochure). The questionnaire was checked for accuracy and appropriateness by two experts working at Santé publique France in October 2020, to ensure that all the topics of the brochure were covered by the questionnaire. At the same time, it was also certified that all the novel aspects of the recommendations were covered by the items; to check for this, the items were classified as new, old or reformulated notions (in comparison to the previous guidelines) and checked by two of the authors. The questionnaires were pretested by the recruitment agency with 41 participants. After the pretest the questionnaire was sent to all the other participants. Quality of the answers was ensured by the fact that all of the questions required a mandatory answer, therefore there was no risk for uncompleted questionnaires.

The T0 questionnaire was sent to the participants on November 13th, 2020. The exact date of completion was registered for each participant. Immediately after completion the participants received an email with a PDF version of the brochure, and the paper version of the brochure was also sent to each participant by regular mail. The last answers to T0 were obtained on November 24th, 2020, and by November 25th, all the paper brochures were sent to the participants. The T1 questionnaire was then sent by December 8th, 2020 to all the participants who answered at T0; in this way, it was estimated that all the parents had at least 2 weeks to read the brochure before completing the second questionnaire. The last answers to T1 were obtained on January 4th, 2021.

Measures

Demographics

Parents were asked to report how many children they had and specify the sex and the date of birth of each child to ensure a precise calculation of each child's age. Parents were also asked to report their own sex, age, living area, employment status, number of persons in the household, number of years living in France, whether French was their mother tongue, level of education and perception of their financial status. In addition, to describe the feeding history of their youngest child, parents were asked to report whether the youngest child was born preterm, had medical problems that could have impacted his or her diet (medical conditions: gastroesophageal reflux disease, cow's milk protein allergy, nasogastric intubation or congenital abnormalities of the digestive tract), was breastfed (if yes, for how long), and had started complementary feeding (if yes, what was the frequency of given commercial and homemade baby foods).

Parents' Knowledge (Accuracy and Degree of Certainty)

The questionnaire items exploring parental knowledge were in line with the content of the brochure, covering the ten chapters. The 30 items were developed to ensure that all of the chapters of the brochure were covered, and special attention was given to those recommendations that differed from previous guidelines. In fact, 17 of the 30 items covered aspects that were not addressed in the previous recommendations (32) and were thus considered new. Following results from a previous survey, it was ensured that all the content that parents were looking for was also covered (30). The content can be categorized into one of the following six areas: (1) breastfeeding/milk feeding (four questions); (2) age and modalities of introduction of food groups and different textures (three questions); (3) feeding strategies (four questions); (4) child behavior and parental feeding practices (six questions); (5) recommended foods (nine questions); and (6) children's cues (four questions). The questionnaire (list of items) is shown in **Table 1**. For each item, participants were asked to indicate whether it was true or false and to score their degree of certainty on a four-point scale (absolutely not sure, rather not sure, rather sure, very sure).

Parental Attitudes Toward the Content of the Brochure

In the second part of the T1 questionnaire, parents were asked their opinion regarding the content of the brochure. They were asked to indicate whether they found the content to be useful, easy to understand, to answer their questions and whether the topics that they considered interesting were well covered in the brochure using a 4-point Likert scale, which ranged from strongly agree to strongly disagree. Parents were also asked whether, in the prior 2 weeks, they had sought information regarding child feeding anywhere other than in the brochure and, in the case of an affirmative answer, in which media they did so. Finally, parents were asked to answer using the same 4-point Likert scale whether they agreed on three self-efficacy statements regarding following the recommendations contained in the brochure. In particular, they were asked to indicate whether: (1) they were willing to follow those recommendations; (2) it would be difficult to follow the recommendations without the support of their partner and family; and (3) it would be difficult to follow the recommendations if their friends were not following the same recommendations. These three self-efficacy statements were formulated in accordance with a validated questionnaire to assess maternal attitudes toward infant feeding (33).

Ethical Consideration

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. Participants voluntarily agreed to participate in the study, gave their informed consent to take part in the study (by ticking a box on the first page of the questionnaire), and were compensated for their participation according to the criteria of the recruiting agency. The compensation was set at 3.80 euros for all finalized respondents (who went to the end of the process, with T1 completed at 100%). This amount was credited to the participant's account on the website of the recruiting agency. This study was approved by the institutional review board (IRB00003888, IORG0003254, FWA00005831) of the French

TABLE 1 | Items exploring parental knowledge about child feeding.

Q	Q code	Item*	True	False
1	Only milk < 4 m	Until the age of 4 months, a baby should be given only milk, nothing else.	X	
2	Growup milk 6–12 m	Growing-up milk is suitable for babies aged 6 to 12 months.		X
3	Almond milk	Almond milk is suitable for the baby's needs, as long as it is fortified with calcium.		X
4	Milk alternance	From 1 year, it is possible to give alternately "growing-up milk" and whole UHT cow's milk.	X	
5	All foods 4–6 m	All foods can be given between the 4 and 6 months of the child [vegetables, meat, fish, fruits, eggs, pulses (lentils, beans, chickpeas), starches including whole starches (pasta, rice, semolina, bread), dairy products, unsalted nuts (almonds, hazelnuts, walnuts)] in the order you want but adapting the texture to the child's age.	X	
6	New textures 6–8 m	Between 6 and 8 months, most babies are able to swallow smooth purees without any problem and are ready to eat new textures.	X	
7	Drinks	At the start of complementary feeding, it is advisable to start giving the child different types of drinks such as fruit juice or plant-based milks (i.e., almond milk).		X
8	Food refusal	If a child does not like a food after 2 or 3 tries, there is no point in continuing to offer him that food.		X
9	Reward	It is advisable to offer small rewards (toys, desserts, etc.) to encourage the child to finish all the vegetables on his plate.		X
10	Veg variety	It is important to give the child a taste of a wide variety of vegetables, varying the recipes.	X	
11	Veg diet only	It is possible to offer a vegetarian or vegan diet to children under 3 years old.		X
12	Finish food	If a child does not finish what he has on his plate, it is good to force him to finish because he needs to eat everything to be healthy.		X
13	Screen < 3 y	Exposing a child under the age of 3 to any screen (TV, tablet, smartphone) is not recommended.	X	
14	Moving	It is advisable to encourage the baby to move, especially with games, from 6 months of age.	X	
15	Force	If a child refuses a food, he should not be forced to eat it.	X	
16	Growth chart	The best way to tell if a child is eating well and getting enough is to follow the growth chart during visits to the doctor.	X	
17	Family food	When the child comes to the table with his family, he can eat just like everyone else.		X
18	Pulses 2/w	From the age of 1 it is advisable to offer the child pulses (lentils, chickpeas, beans) at least twice a week.	X	
19	Fats	Fats (such as a teaspoon of oil) should always be added to homemade preparations and store-bought foods if they do not contain it.	X	
20	Raw milk	Raw milk products and raw milk cheeses may be offered to children under 3 years old.		X
21	Juices	Fruit juices are one of the foods that must be introduced into the child's diet at the start of complementary feeding.		X
22	Water only	The only recommended drink (other than milk) for a child up to 3 years old is water.	X	
23	Salt	It is recommended to add salt to "homemade" foods.		X
24	Whole starch	It is possible to introduce whole starch foods (pasta, rice, semolina, bread) and pulses (lentils, beans, chickpeas) in the child's diet from the start of complementary feeding.	X	
25	Nut powder	Unsalted nut powder (almonds, walnuts, hazelnuts) can be added in a puree or a compote.	X	
26	Allergens	To avoid the risk of allergy, the main food allergens (such as eggs and peanuts) should not be introduced at the start of complementary feeding.		X
27	Tasks division	Parents decide what and when to eat while the child decides how much to eat.	X	
28	Neophobia 2 y	It is normal for children to begin to refuse new tastes or new textures around the age of 2.	X	
29	Wake up	If the baby falls asleep on the bottle, wake him up to finish all the contents of the bottle.		X
30	Bottle to sleep	Leaving a bottle in your baby's bed or leaving him in front of the TV are good strategies to let him falling asleep.		X

**Items translated from French to English. The correct answer is indicated for each item. Beside answering True/False to each item, parents had also to indicate how sure they were about their answer, choosing between: absolutely not sure, rather not sure, rather sure, very sure.*

Institute of Medical Research and Health, and a study registration was performed by the relevant data protection service.

Statistical Analysis

For all statistical analyses, R version 3.6.1 was used (34). Frequencies, percentages and medians with interquartile ranges (IQRs) were used to describe the results. The statements regarding parental attitudes requiring an answer on a 4-point Likert scale were considered discrete and were dichotomized as "agree" (grouping the two positive answers) or "disagree" (grouping the two negative answers) for the analysis. An answer was considered correct when parents gave the right true/false value and mastered when parents gave the correct answer with a higher degree of certainty. A global score of knowledge was calculated by summing the number of correct answers of all the questions (range, 0–30). The same global score was also calculated for mastered answers. The difference in the number of correct answers between T0 and T1 (T1–T0) was considered to define the evolution of knowledge accuracy; knowledge certainty evolution was defined by the difference in the number of mastered answers between T0 and T1 (T1–T0). Paired *t*-tests were performed to determine whether the mean difference in correct and mastered answers between T0 and T1 was significant. McNemar's tests were calculated to check whether the proportion of correct answers (and mastered answers) differed significantly for each individual item between T0 and T1. The effect of selected sociodemographic characteristics (parent age, parity and education level) on knowledge (accuracy and certainty) at T0 and evolution between T0 and T1 was tested with a linear model. One model per variable was run to verify the effect of each characteristic on the number of correct and mastered answers at T0 and on the difference in correct and mastered answers between T0 and T1.

For each question, the proportion of correct answers was compared to 0.5, the chance level, through tests based on the normal approximation of the binomial distribution. Two kinds of tests were performed. First, a unilateral test was performed at T0 to detect recommendations opposite to the common belief. The alternative was a probability lower than 0.5, and rejection of the null hypothesis was an indication of disagreement with the general conviction. Second, another unilateral test was performed at T1 to detect questions that clearly ought to be reworked. The alternative was a probability higher than 0.5, and no rejection of the null hypothesis was an indication of response at chance level. When appropriate, the χ^2 test was used to determine whether the relationship between parental attitudes toward the content of the brochure and sociodemographic characteristics was statistically significant. Significance was set at $p < 0.05$.

TABLE 2 | Characteristics of the sample of French parents who responded to the survey at T0 and T1 ($n = 452$).

Characteristics		*N*	%
Responding parent's characteristics			
Gender	Female	365	81
	Male	87	19
Age	<35 years old	253	56
	35 years old and more	199	44
Education level[a]	<A level	87	19
	≥A level	365	81
Socioprofessional category of the interviewed parent[b]	Low	211	47
	High	146	32
	No occupation/retired	95	21
Parity	Primiparous	152	34
	Multiparous	300	66
Younger child characteristics			
Prematurity	Yes	40	9
	No	412	91
Ever breastfed	Yes	295	65
	No	157	35
Complementary feeding started	Yes	413	91
	No	39	9
Having problems that could affect the diet	Yes	26	6
	No	426	94
Household characteristics			
Self-perception of financial situation[c]	Good	266	59
	Difficult	186	41
Living area	Rural	191	42
	Urban	261	58
Household composition (median = 4 people)	Single parents with children	29	10
	Couple with children	423	90

[a] *A level corresponds to the diploma obtained after completion of upper secondary school (equivalent to 12 years of formal education in France).*
[b] *High (liberal profession, entrepreneur, executive or higher intellectual profession), intermediate or low (laborers and clerks) or no occupation/retired (including also students).*
[c] *Parents were classified as having a good financial situation when they perceived they were comfortable or okay with it. The other parents were classified as being in a difficult financial situation when they had a perceived uncomfortable situation imposing to pay attention to their budget, or making it difficult to reach the end of the month or forcing them to take out debts.*

RESULTS

Characteristics of the Study Sample

The characteristics of the study sample are described in **Table 2**. A total of 501 parents responded at T0, but only 452 parents also responded at T1 and were considered for the analysis (**Figure 1**).

Evolution of Knowledge Accuracy and Degree of Certainty Between T0 and T1

In our sample of parents, knowledge accuracy and knowledge certainty significantly improved after reading the brochure. In fact, the number of correct answers increased from 22 (IQR = 4) at T0 to 25 (IQR = 5) at T1, and the number of mastered (certain) answers increased from 11 (IQR = 9) to 17 (IQR = 10) (**Figure 2**). Paired t-tests showed that both differences were significant [correct answers: $t_{(451)} = 17.15$, $p < 0.001$; mastered answers: $t_{(451)} = 18.81$, $p < 0.001$]. At T1, the variability in the number of mastered answers (ranging from 0 to 30) was higher than the variability in the number of correct answers (13–30).

Figure 3 shows the distribution of answer evolution between T0 and T1. For 75% of parents, the number of correct answers increased, and for more than 75% of parents, the number of mastered answers increased.

No significant effect of the parents' sociodemographic characteristics (age, education level and parity status) was found on the evolution of knowledge between T0 and T1. At T0, an

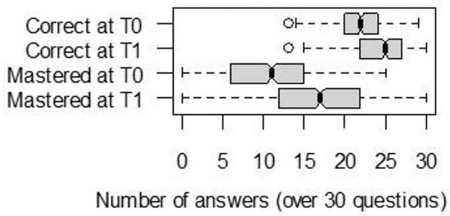

FIGURE 2 | Median, lower and upper quartiles, of correct and mastered answers, over the 30 questions about complementary feeding answered by $n = 452$ parents at T0 and T1.

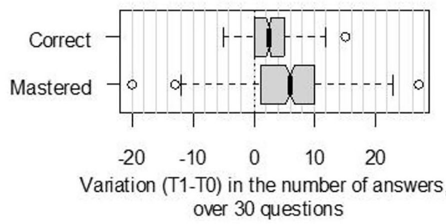

FIGURE 3 | Variation in the number of correct and mastered answers, between T0 and T1 (before/after reading the brochure), for $n = 452$ French parents who rated the correctness of 30 questions (true/false), and rated their degree of confidence for each answer (mastered = correct answer given with the maximal degree of confidence, on a 4-pt scale).

effect of education on the proportion of mastered answers was observed. Parents with a higher education level had a significantly higher mean of mastered answers at T0 than did parents who had fewer years of formal education (mean difference = 1.4; t-value = 2.14; $p = 0.03$). The effects of other sociodemographic characteristics on knowledge accuracy or degree of certainty at T0 were not significant (all p-values > 0.05).

Knowledge Accuracy and Certainty at T0 and T1 for Each Question

Figure 4 shows the evolution of knowledge between T0 and T1 for each of the 30 questions. The proportion shown for each question is equal to the proportion of participants answering correctly (or correctly with the higher degree of certainty) to this specific question. Questions are ranked according to the number of correct responses at T0, from bottom to top. At T0, the proportion of correct answers ranged from 20 to 100%, with a proportion higher than 50% for most questions (90% of them) and was significantly lower than the level of chance (<0.5) for q5, q24, q25, and q26. The proportion of correct answers increased significantly between T0 and T1, except for q6, q8, q10, q13, q17, q23, and q30. For questions q10, q13, and q30, the proportion of correct answers was already close to 100% and thus could not increase. At T1, the probability of a correct answer was not significantly higher than 0.5 for q5, q17 or q26. The lowest proportion of correct answers at T1 was detected for q26 (allergens). The proportion of mastered answers significantly increased between T0 and T1 for all the questions.

Parental Perception of the Content of the Brochure

The parents were on average very satisfied with the content of the brochure. The majority (98%) agreed that the content of the brochure was useful, easy to understand and answered their questions. Moreover, 98% of parents found that all the themes that they considered interesting were covered by the content of the brochure. Thirty-two percent of parents revealed that in the weeks prior to completing the questionnaire at T1 they looked for information on child feeding by means other than the brochure. Among those parents using other sources, the most popular ways to search information were via the internet (in particular websites on childcare, 37%), health care professionals (especially pediatricians, 28%) and parents' personal networks (grandparents, 19%, and friends, 16%). Regarding the three self-efficacy statements, a majority of parents (98%) declared they would try to follow the advice and recommendations contained in the brochure, but for 29% of them it would be difficult without the support of their partner and family. For 10% of parents, it would be difficult to follow the recommendations if their friends were not following the same ones. Whether parents would follow the recommendations did not differ according to parents' sociodemographic characteristics (age, parity, education level).

DISCUSSION

The main objective of this study was to evaluate the short-term effect of reading a brochure containing child feeding recommendations on the accuracy and the degree of certainty of French parents' knowledge. The results showed that knowledge accuracy, but especially knowledge certainty increased after parents read the brochure. At completion of the questionnaire, the parents with higher education levels were more certain of their correct knowledge compared to parents who had fewer years of formal education. For most of the questions, the proportion of correct answers significantly increased, as did the proportion of mastered answers for all questions. A secondary aim of the study was to evaluate parental attitudes toward the brochure. There was clear evidence that almost all parents were satisfied with the content of the brochure. Nevertheless, one-third of them sought child feeding information between T0 and T1 *via* means other than the brochure. The majority of parents were positive about following the recommendations of the brochure, but for one-third, it would be difficult to do so without the support of their partner and family. For some parents, following the advice of the brochure would be easier if their friends would also be willing to do so.

Our findings indicated that French parents already have a good level of knowledge regarding child feeding regardless of their age and parity; they scored high (median 22/30) even before having read the updated recommendations provided in the brochure. This may be seen as a result of the public health policy, first developed in 2001 regarding the National Program on Nutrition and Health (3). However, at T1, parents did not answer better than chance to q5 (all foods 4–6 m), q17 (family food) or q26 (allergens), meaning that sentences regarding

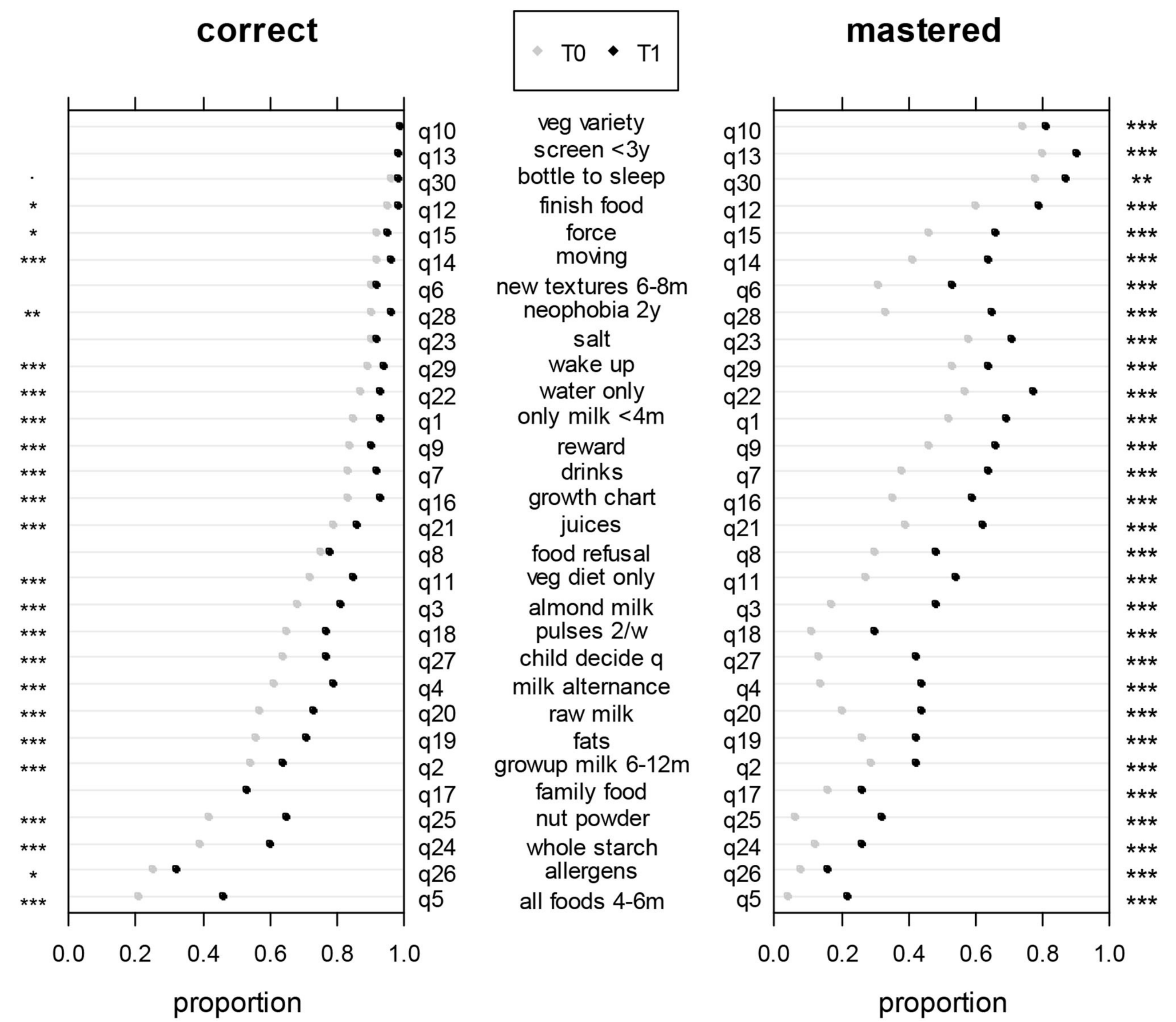

FIGURE 4 | Proportions of correct answers (on the left) and mastered answers (on the right) at T0 (gray dot) and T1 (black dot) for each question. The asterisks indicate whether the increase between T0 and T1 was significant (McNemar's tests). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

those topics in the brochure might need reformulation or that parents might need more time or other consistent advice to gain more confidence with these recommendations. Parents with lower education levels, despite similar knowledge accuracy to parents with higher education levels, were less certain of their knowledge before reading the brochure. This interesting result could suggest that lack of confidence may interfere with parents' attitudes and actual knowledge and undermine the ability of parents with lower education to apply their knowledge regarding child feeding (35), thereby often displaying feeding practices less compliant with recommendations (15–18). In fact, higher education is often associated with better knowledge performance (36, 37). Knowledge accuracy significantly increased between T0 and T1 for all questions, except for q6 (new textures 6–8 m), q8 (food refusal), q17 (family food) and q23 (salt). For q26 (age of introduction of allergens), there was a significant increase, but the proportion of correct answers was still low after reading the brochure. For those questions, a reformulation of the related recommendations in the brochure might be advised to improve comprehension. Interestingly, the evolution in knowledge certainty was equivalent regardless of education level, which reveals that the design of the brochure was appropriate to reach its goal to inform all parents, regardless of their age, parity or education level, about best practices in child feeding.

This work introduces a novel approach to evaluate the effect of reading a brochure containing child feeding recommendations on the degree of knowledge certainty in parents. From the results, we highlighted an increase in both accuracy and certainty, but the increment of certainty was higher. Self-efficacy is defined as the belief in one's own capacity to perform a task or behavior (38), and it can transfer to different domains, including parents' ability to feed their child. According to Bandura's theory applied to the parenting domain, parents need to judge themselves efficacious in their parenting role to be successful and skillful in performing tasks related to that role (for example, feeding) (39). Also demonstrated in a study examining parental knowledge on child development is that the relationship between parental self-efficacy and proficiency in a given behavior is moderated by knowledge. In fact, when knowledge is high, self-efficacy and parenting competences (such as feeding) are positively associated (40). Parental self-efficacy and knowledge can both play a role in predicting parental behavior related to feeding, but this has not been extensively studied. Conrad et al. explored how accounting for both parental self-efficacy and knowledge could predict maternal behavioral competence (41). This study showed that, when there was high confidence, mothers who had more knowledge (vs. less) had more positive interactions with their children (41). Being certain in one's own knowledge might also strengthen parenting self-efficacy and contribute to the prediction of specific behaviors, but this aspect was not investigated in the present study, and further research is required to explore this point.

Evidence suggests that even if parents might have some basic nutritional knowledge and are aware of guidelines, they may still struggle to implement proper feeding practices due to factors, such as inconsistent and conflicting advice (42, 43). In fact, conflicting advice can create doubts as to whether your knowledge is the most up to-date or correct. A qualitative study conducted in Australia highlighted the perception of mothers that "everyone gives you advice" (43). Mothers can be influenced by their personal network in making decisions related to the introduction of solids, but they also do their own research, which may accentuate the perception of being surrounded by conflicting information (43). Parents' knowledge in relation to feeding is a fundamental basis on how to empower them to provide their children healthy foods and diets and favor healthy eating behavior. Strictly related to knowledge, there is the concept of health literacy, which is considered a health determinant and defined as "the degree to which individuals can obtain, process, and understand the basic health information and services they need to make appropriate health decisions" (44). While high health literacy levels might favor the adoption of healthier lifestyles (45), there is rising interest in a newer and more feeding-related concept, known as food literacy. Food literacy is defined as a set of food-related knowledge and skills that enables people to improve their own health by making informed choices about food and nutrition (46). This might be extended to parents making feeding choices for their children.

From a purely public health institutional perspective, the fact that the brochure was very welcomed by parents is an important achievement. The development of this kind of material requires a huge amount of time and the involvement of many different stakeholders (Minister of Health, research and public health institutions). In such processes, it is paramount to ensure proper vulgarization of messages that otherwise would not be fully understood from those that are the first recipients, in this case the parents. Despite the high satisfaction with the content of the brochure, 32% of parents declared they looked for information via other means. There might be different reasons for which parents still did so, but this was not explored in this study. First, since some topics were new, some recommendations may have surprised parents who needed to search for confirming information. Second, it is normal that some people need to double check information: a given piece of advice found on the internet may be more likely to be followed if it is also confirmed by a doctor. Third, not all the information needed by all parents can be present on a paper brochure, which has the purpose of giving information in a direct and synthetic way. For example, parents of premature children or children presenting with certain medical conditions might not find what they are looking for regarding their children's needs (30). Finally, some parents might still find it time-consuming to go and check one specific piece of information on the brochure, passing by all ages before finding what they needed. Parents might find it easier just to ask one specific question to a doctor or on an internet search engine, as examples. According to our present results, the most popular ways to search for information were via the internet (in particular websites on childcare), health care professionals (especially pediatricians) and parents' personal networks. This is in accordance with other studies (11, 30, 47) placing those three sources as the most used by parents when seeking child feeding information. It is important to simplify access to consistent information for parents from different and officially recognized sources.

Even if the majority of parents declared they would try to follow the advice and recommendations contained in the brochure, one-third of them thought it would be difficult to do so without the support of their partner and family. This aspect might limit the transition from knowledge to behavior. Parents' confidence in their role can also be defined as infant care self-efficacy, and it can impact the belief that parents can provide adequate and good care for their babies. Self-efficacy is also defined as one's judgment of how effectively one can deal with a designated situation. Self-efficacy influences people's thinking, feelings, motivations and actions (48). One of the sources to evaluate self-efficacy is verbal persuasion from others that for infant care translates into reinforcement from others (e.g., family, friends) (49). In fact, maintaining self-efficacy beliefs can be easier for those in whose capacities their significant others (e.g., family, friends) believe, thereby strengthening their belief to be doing well in the parent role (48). From our results, it can be deduced that parents may have low self-efficacy in relation to the verbal persuasion aspect. If encouragement from family and partner is lacking, this might exacerbate doubts that can lead to suboptimal care for the baby (49). More specific investigation is needed, using appropriate validated questionnaires to explore all four sources of information involved in the construction of parental self-efficacy [positive enactive mastery experiences, vicarious experiences, verbal persuasion and appropriate physiological and affective state (48)] regarding following recommendations.

This study is part of the brochure deployment process in order to validate the ability of the brochure to convey child feeding recommendation messages (before the national dissemination of the brochure). However, strengths and limitations must be considered alongside these results. First, the choice between different degrees of certainty that participants had to perform might have been impacted by the participants' individual capacity to estimate their own knowledge. In fact, estimating one's own knowledge is a task that people are not used to performing, and this kind of task often requires some training, which was not performed for our study. Additionally, one may raise the fact that knowledge is not always transformed into practice. Increasing knowledge may contribute to change behavior but further long-term studies are necessary to evaluate effective practices of parents. Even if, according to the knowledge-attitudes-behavior model, knowledge can impact attitudes and reflect on behavior, Eccles et al. tested multiple theoretical models trying to explain clinical behaviors, and, from their results, it appeared that knowledge was not predictive of behavior (21). However, in France, recent studies have shown that guidelines for feeding practices are generally followed in practice. In fact, a quantitative study including a sample of 600 parents showed that the majority adhered to recommendations on the introduction of solid foods (50), but other topics, such as milk feeding, were still not well integrated into parents' practices. Further studies will investigate the effect of the information contained in the brochure and whether the newly introduced recommendations will be integrated into parental feeding practices. The primary strength of our study lies in the novelty of its approach in the field of public health guideline evaluation. To the best of our knowledge, this is the first time that material intended for the general public has been evaluated for both knowledge accuracy and certainty before national dissemination. This study was included in a timely manner in the evaluation process of the brochure. The results allowed public health stakeholders to consider final adjustments about how information was given and organized in the brochure to be disseminated.

CONCLUSIONS

Our results showed that, after reading a brochure containing the newly updated guidelines regarding child feeding, parents' knowledge increased. The knowledge increased both, in terms of accuracy and degree of certainty, despite a good level of knowledge at baseline, even for younger or less educated parents. The participants were generally satisfied with the content of the brochure, even if some of them expressed that they might experience some insecurity in following the recommendations without the support of their close family. Some parents felt the need to gather information from sources other than the brochure. From the perspective of programming a national plan for the dissemination of new child feeding recommendations it can be useful to provide parents with the same official information via differing sources (internet, health care professionals). This will contribute to avoiding rising doubts about how to perform optimal feeding practices and will make parents even more certain about their knowledge.

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 Review Board (IRB00003888, IORG0003254, and FWA00005831) of the French Institute of Medical Research and Health. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SDR was a major contributor in writing the manuscript, the study design, conception of the questionnaires, and finalized the questionnaires. SN, PD, and CS contributed and validated the methodology and critically edited the questionnaires. SDR and CC analyzed the data. All authors critically reviewed and commented on subsequent drafts of the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.782620/full#supplementary-material

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Households' Food Insecurity and Their Association With Dietary Intakes, Nutrition-Related Knowledge, Attitudes and Practices Among Under-five Children in Gaza Strip, Palestine

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The present study aimed to determine the households' food insecurity and their association with dietary intakes, nutrition-related knowledge, attitudes and practices (KAP) among under-five children in the Gaza Strip, Palestine. This cross-sectional study was conducted in 2021 among a representative sample of under-five children. A total of 350 children and their proxy were selected from all Gaza strip governorates, using a cluster random sampling method. The Radimer/Cornell food-security scale was used. A 24-h dietary recall was employed for dietary intakes assessment. Nutrition-related KAP for feeding under-five children, anthropometric measurements, and demographic-socioeconomic characteristics were obtained with an interview-based questionnaire. Statistical analysis was performed using SPSS version 25. The children from food-insecure households had a high prevalence of moderate underweight (30.4%), stunting (32.8%), wasting (9.6%), and acute undernutrition (30.4%). Between food-insecure and food-secure groups there were significant differences in weight, height/length, mid-upper arm circumference, weight-for-age and mid-upper arm circumference z-scores, underweight, acute undernutrition, intakes of protein, fat, vitamin D, zinc, continued breastfeeding, having nutrition-related adequate knowledge, having nutrition-related positive attitudes, and achieved minimum dietary diversity score ($P < 0.05$ for all). Additionally, about (56.0%) food-insecure households have inadequate nutrition-related knowledge, (77.6%) have nutrition-related negative attitudes, and (95.2%) did not achieve a minimum dietary diversity score. In conclusion, the children from food-insecure households had a high prevalence of moderate underweight, stunting, wasting, and acute undernutrition. Moreover, low economic status, poor dietary intakes, low levels of nutrition-related KAP, and lack of dietary diversity could contribute to the high levels of food insecurity among under-five children.

Keywords: anthropometric measurements, dietary intakes, food insecurity, nutrition-related knowledge, under-five children

INTRODUCTION

Food insecurity is a significant nutritional issue worldwide and is commonly found in low- and middle-income countries (1, 2). Since 2014, the global incidence of moderate or severe food insecurity has steadily increased, with the projected increase in 2020 equaling the preceding 5 years combined (3). In addition, in 2020, it is estimated that 22.0 % (149.2 million) of children under 5 years of age were affected by stunting, 6.7 % (45.4 million) were suffering from wasting, and 5.7 % (38.9 million) were overweight (4). Furthermore, it was projected that 119 million children under five would be stunted in 2030 in the 135 low- and middle-income countries (5). In the Gaza Strip, Palestine, over 68 % of households (about 1.3 million people) are severely or moderately food-insecure, according to the preliminary findings of the latest Socio-Economic and Food Security Survey carried out in 2018 (6). Also, stunting (10.3%) remains the most prevalent form of undernutrition among children under 5 years, followed by underweight (2.5%) and wasting (2.4%) (7). Moreover, a nutrition needs assessment was conducted in the most vulnerable areas in the Gaza strip, indicating that only 14% of children under 5 years of age had a minimum acceptable diet (8).

Food insecurity exists when people do not have adequate physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and preferences for an active and healthy life (9). It is a global problem, and it is related to macro-and micronutrient deficiencies and lack of dietary diversity (10). Food insecurity has the potential to be harmful to individuals of any age, but it can be especially devastating to under-five children (11). Children who experience food insecurity may suffer from poor health and nutritional deficiencies (12). Inadequate nutrition can permanently alter children's brain architecture and stunt their intellectual capacity, affecting children's learning, social interaction and productivity (13). Children struggling with food insecurity may be at greater risk for stunted development, anemia, asthma, oral health problems, and hospitalization (14). Overall, food insecurity is linked with a poorer physical quality of life, preventing children from fully engaging in daily activities (15, 16). Effective solutions to child food insecurity require addressing the immediate food needs of individual households as well as the underlying economic factors contributing to local food insecurity (17, 18).

On the other hand, having physical and economic access to food on their own is insufficient to ensure that people are food-secure and well nourished. It is essential that people understand what constitutes a healthy diet, mainly what nutrition-related health issues affect their communities and how to address these through food-based approaches, and know how to best use their resources. They should also have positive attitudes toward nutrition, foods and closely related health issues to perform optimal dietary and feeding practices that ensure their nutritional wellbeing and that of their families (19, 20). In the present study, the Food and Agriculture Organization of the United Nations (FAO) questionnaire for assessing nutrition-related knowledge, attitudes and practices (KAP) feeding children younger than 5 years was used (21).

Additionally, nutrition-related knowledge and attitudes are necessary for dietary changes toward a healthier dietary pattern. For that reason, nutrition-related KAP is one of the key factors to achieving households' food and nutritional security. To the best of our knowledge, no study has ever investigated this association among under-five children in the Gaza Strip, Palestine. Therefore, the present study aimed to determine the households' food insecurity status and their association with dietary intakes, nutrition-related KAP among under-five children.

METHODS

Study Design

This cross-sectional community-based study was conducted in 2021 among a representative sample of under-five children in the Gaza strip governorates. A total of 350 children and their proxy were selected from all Gaza strip governorates, using a cluster random sampling method. Households having at least one child (male or female), aged < 5 years, and living with his/her mother in the same household, and mothers and fathers aged ≥ 18 years and having under-five children were included in the present study. On the contrary, households without under-five children, under-five children with disabilities or chronic disease, preterm infants ($<$37 weeks), infants of diabetic mothers, and under-five children who have a history of complications during delivery (aspiration or trauma) were excluded from the present study.

Study Location

The current study was conducted in the households of the Gaza strip, Palestine. The estimated population of the Gaza strip is about 2,106,745 million (22). The Gaza strip is divided into five governorates: North-Gaza, Gaza, Middle-Area, Khanyounis, and Rafah; with a population density of 19.3, 34.9, 14.4, 19.1, and 12.2%, respectively (23).

Sample Size and Sampling Technique

In the present study, the representative sample size was calculated using the following formula (24).

$$Sample\ size\ (n) = \frac{Z^2_{1-\alpha/2}\, P(1-P)}{d^2} \tag{1}$$
$$= \frac{(1.96)^2\ (0.30)\ (1-0.30)}{(0.05)^2} = 323$$

Where, $Z_{1-\alpha/2}$ = Standard normal variate (Z value is 1.96 for a 95 percent confidence level); p = Response distribution (30%); and d = Margin of error (5%).

Accordingly, the calculated sample size of the current study was 323 under-five children, to which we added 10% as an expected non-response rate. Finally, this study applied a cluster random sampling method to 350 under-five children and their proxy were responded and recruited. The sample was distributed into the five governorates of the Gaza strip based on the population density as follows: 42 from Rafah, 68 from Khanyounis, 50 from Middle-Area, 122 from Gaza, and 68 from North Gaza.

DATA COLLECTION

Interview Questionnaire

An interview-based questionnaire was used; the data was collected from the head of the household (mothers or fathers) and the under-five children by ten qualified data collectors, who were trained and prepared by the researcher. The questionnaire contains items about demographic and socio-economic characteristics of under-five children, the Radimer/Cornell food security scale (25), two non-consecutive days of the 24-h dietary recalls, anthropometric measurements, and the FAO nutrition-related KAP for feeding children younger than 5 years (26). Before data collection, a pilot study was carried out in thirty participants to enable the researcher to examine the tools of the study. The questionnaire and data collection process were modified according to the results of the pilot study.

Demographic and Socio-Economic Characteristics of Under-five Children

An individual face-to-face interview was conducted with the heads of households (mothers or fathers) to collect information about demographic and socio-economic characteristics of under-five children, including gender, date of birth, gestational age (weeks), birth weight (kg), governorate, the nature of the living area, history of any disease, history of gestational diabetes, use of medications; household monthly income (NIS), and educational level of the heads of households (mothers or fathers). The age of the children (months) was calculated from their date of birth (from birth certificates) to the day of data collection. Additionally, the used categories of educational level and household monthly income (NIS) variables in the current study were similar to which mentioned in earlier studies in Gaza strip (27, 28).

Food Insecurity Measurement

The 10-items Radimer/Cornell food security scale was used for determining the households food security status (25). The scale is a valid and reliable tool for measuring household food insecurity in a culturally diverse setting (29, 30). The households were classified by food security status as follow: (1) Household food secure: Negative answers to all hunger and food insecurity items; (2) Household food insecurity: Positive answers ("sometimes true" or "often true") to one or more hunger and food insecurity items (29, 30).

Dietary Intakes Assessment of Under-five Children

Two non-consecutive days of 24-h dietary recalls were employed to determine the quantity of macronutrients and micronutrients consumed by the under-five children. Mothers were requested to recall all beverages, number of breastfeeds, and food consumed by their children in the past 24 h. The portion sizes were estimated using a set of household measurements (i.e., plates, cups, glasses, and spoons). Dietary data from the 24-h dietary recall was processed by hand (office work) in order to calculate the net grams of foods consumed by the under-five children. This information was analyzed using the Nutritionist Pro Software version 7.1.0 (Axxya Systems, USA) (31) to determine energy (kcal) and nutrients intakes, including protein (g), carbohydrate (g), fat (g), iron (mg), vitamin A (μg), vitamin D (μg), calcium (mg), and zinc (mg).

ANTHROPOMETRIC MEASUREMENTS OF UNDER-FIVE CHILDREN

Length/Height and Weight

Recumbent length (cm) was recorded to the nearest 0.1 cm using the length board, appropriate for children under 2 years old. The mothers were asked to lay their children on their backs against the fixed headboard, compressing the hair and eyes looking straight up. In addition, the height (cm) and weight (kg) of children were measured following standard recommended procedures (32). A digital weighing scale (to the nearest 0.1 kg) (SECA, Germany) and a body meter (with the precision of 0.1 cm) (SECA, Germany) were used. The measurement for each child was carried out twice, and the average reading was documented as the final reading. Furthermore, the age, weight, and height of the children were translated into three indices: Height/length for age (HAZ), weight for age (WAZ), and weight for height/length (WHZ), which were expressed in terms of z-score using the WHO Anthro Software (Version 3, 2009) (33). Then, the under-five children were classified into moderate and severely underweight, moderate and severe stunting, and moderate and severe wasting, which mean that WAZ, HAZ, and WAZ z-scores are < -2 and < -3, respectively (32).

Mid-upper Arm Circumference

MUAC (cm) was recorded to the nearest 0.1 cm using the MUAC measuring tape. Investigators measured the MUAC at the midpoint of the arm where the measuring tape was snugged to the skin but not pressing soft tissues (34). The measurement for each child was carried out twice, and the average reading was recorded as the final reading. Then, the measurement of MUAC was used to calculate MUAC for age z-scores (MUACZ), using the WHO Anthro Software (version 3, 2009) (33). Moreover, the children were classified into moderate and severe acute undernutrition as MUACZ is < -2 and < -3, respectively (35).

Assessment of Nutrition-Related KAP

The FAO of the United Nations questionnaire for assessing nutrition-related KAP was used to conduct high-quality nutrition-related KAP surveys. The questionnaire comprises predefined questions that capture information on critical KAP related to feeding children younger than 5 years (26). In the present study, the nutrition-related KAP consists of 7-questions related to nutrition-related knowledge, 7-questions related to nutrition-related attitudes, and other questions about nutrition-related practices and dietary diversity.

Calculation of the Minimum Dietary Diversity Score

The minimum nutritional diversity indicator was calculated as the number of under-five children who received food from four or more food groups during the previous day, divided by the number of under-five children, multiplied by 100; breast milk was not included among the food groups (26).

Data Analysis

The Statistical Package for Social Science (SPSS) for Windows (version 25) was used for data analysis. Descriptive statistics were used to describe continuous and categorical variables. The chi-square test and fisher's exact test were used to determine the significant differences between categorical variables. The differences between means were tested by independent samples *t*-test. A $P < 0.05$ was considered as statistically significant.

RESULTS

A total of 350 under-five children and their proxy were included in the present study (54.8% were males, and 45.2% were females). The characteristics of under-five children were compared by household food insecurity status (**Table 1**). The results revealed that children in food-insecure households had a higher proportion (71.4%) than those in food-secure households (28.6%). The mean ages (months) for children in food-secure and food-insecure households were 34.05 ± 12.16 and 32.26 ± 13.21, respectively. There were significant differences in the governorate of residence and the nature of residence area between children in food-secure and food-insecure households (*P*-values = 0.001, and 0.046, respectively), as half of the study participants (50.2%) residing in refugee camps. In addition, a significant difference was found in monthly income (NIS) between food-secure and food-insecure households (*P*-value = 0.009).

Table 2 shows the nutritional status and anthropometric measurements of under-five children by household food security status. The study showed that under-five children from food-insecure households had a lower mean z-score for all indexes, and a higher prevalence of moderate underweight, moderate stunting, moderate wasting, and moderate acute undernutrition, than their food-secure counterparts. Furthermore, the results demonstrated that, of food-insecure under-five children, about 76.0 (30.4%) were moderately underweighted, 82.0 (32.8%) moderately stunted, 24.0 (9.6%) moderately wasted, and 76.0 (30.4%) moderately acute undernourished. Moreover, there were significant differences in weight (kg), height/length (cm), mid-upper arm circumference (MUAC) (cm), weight-for-age z-score (WAZ), mid-upper arm circumference z-score (MUACZ), underweight, and acute undernutrition between the food-insecure and food-secure groups ($P < 0.05$ for all).

Table 3 shows the energy, macronutrients and micronutrients intakes among under-five children by household food security status. For children from food-insecure households the calculated mean intake for energy (kcal) amounted to 912 ± 217, protein (g) 42.36 ± 15.57, carbohydrate (g) 105.85 ± 29.79, fat (g) 30.97 ± 10.45, iron (mg) 4.48 ± 2.48, vitamin A RAE (μg) 307.96 ± 158.99, vitamin D (μg) 4.60 ± 3.39, calcium (mg) (456.13 ± 224.65), and zinc (mg) 3.05 ± 1.21. These were generally lower than those calculated for their counterparts from the food-secure group. Moreover, the calculated difference for protein (2.66 g), fat (3.4 g), vitamin D (1.31 μg) and zinc (0.81 mg) was significant (*P*-values = 0.041, 0.032, 0.036, and 0.044, respectively).

TABLE 1 | Demographic socioeconomic characteristics of under-five children by household food-security status (n = 350).

Variables	Household food-secure (n = 100)	Household food-insecure (n = 250)	*P*-value
Age (months)			
Mean ± SD	34.05 ± 12.16	32.26 ± 13.21	0.431[a]
Gender			
Males	56.0 (56.0)	134.0 (53.6)	0.792[b]
Females	44.0 (44.0)	116.0 (46.4)	
Governorate			
North Gaza	50.0 (50.0)	18.0 (7.2)	< 0.001[b*]
Gaza	36.0 (36.0)	86.0 (34.4)	
Middle Area	8.0 (8.0)	42.0 (16.8)	
Khanyounis	4.0 (4.0)	64.0 (25.6)	
Rafah	2.0 (2.0)	40.0 (16.0)	
Living area			
City	36.0 (36.0)	90.0 (36.0)	0.046[b*]
Village	14.0 (14.0)	34.0 (13.6)	
Camp	50.0 (50.0)	126.0 (50.4)	
Household monthly income (NIS)			
≤ 2,000	76.0 (76.0)	228 (91.2)	0.009[b*]
> 2,000	24.0 (24.0)	22.0 (8.8)	
Educational level of the head of households (mothers or fathers)			
Low education	28.0 (28.0)	82.0 (32.8)	0.334[b]
High education	72.0 (72.0)	168 (67.2)	

[a]*Independent Samples t-test.*
[b]*Chi-Square Test.*
**Difference is significant at the 0.05 level (two-tailed).*
NIS, New Israeli Shekel.
Low education: Illiterate, primary, or preparatory; High education: Secondary, or university.

TABLE 2 | Nutritional status and anthropometric measurements of under-five children by household food-security status ($n = 350$).

Measurements	Household food-secure ($n = 100$)	Household food-insecure ($n = 250$)	*P*-value
Weight (kg)			
Mean ± SD	14.83 ± 3.59	12.95 ± 4.02	0.004[a*]
Height/Length (cm)			
Mean ± SD	74.72 ± 31.07	58.25 ± 40.81	0.012 [a*]
MUAC (cm)			
Mean ± SD	17.56 ± 0.93	13.62 ± 2.86	< 0.001[a*]
WAZ (z-score)			
Mean ± SD	0.51 ± 1.14	−0.51 ± 1.54	< 0.001[a*]
HAZ (z-score)			
Mean ± SD	−1.28 ± 1.52	−1.34 ± 1.75	0.834[a]
WHZ (z-score)			
Mean ± SD	0.82 ± 1.26	0.38 ± 1.63	0.086[a]
MUACZ (z-score)			
Mean ± SD	1.46 ± 0.44	−1.22 ± 1.61	< 0.001[a*]
Underweight			
Normal	100 (100)	174.0 (69.6)	< 0.001[b*]
Moderate	0.0 (0.0)	76.0 (30.4)	
Severe	0.0 (0.0)	0.0 (0.0)	
Stunting			
Normal	82.0 (82.0)	168.0 (67.2)	0.064[b]
Moderate	18.0 (18.0)	82.0 (32.8)	
Severe	0.0 (0.0)	0.0 (0.0)	
Wasting			
Normal	96.0 (96.0)	226 (90.4)	0.355[c]
Moderate	4.0 (4.0)	24.0 (9.6)	
Severe	0.0 (0.0)	0.0 (0.0)	
Acute undernutrition			
Normal	100.0 (100)	174.0 (69.6)	< 0.001[b*]
Moderate	0.0 (0.0)	76.0 (30.4)	
Severe	0.0 (0.0)	0.0 (0.0)	

MUAC, Mid upper arm circumference; WAZ, weight for age z-score; HAZ, Height/length for age z-score; WHZ, weight for height/length z-score; MUACZ, Mid upper arm circumference for age z-score.

Moderate and severely underweight, moderate and severe stunting, and moderate and severe wasting, which mean that weight for age, height/length for age, and weight for height/length z-scores are < −2 and < −3, respectively (32). Moderate, and severe acute undernutrition as MUACZ is < −2 and < −3, respectively (35).

[a]*Independent Samples t-test.*

[b]*Chi-square test.*

[c]*Fisher's Exact Test.*

**Difference is significant at the 0.05 level (two-tailed).*

TABLE 3 | Energy, macro and micronutrients intake among under-five children by food security status ($n = 350$).

Variables	Household food-secure ($n = 100$)	Household food-insecure ($n = 250$)	*P*-value
Energy (kcal)			
Mean ± SD	913 ± 177	912 ± 217	0.982
Protein (gram)			
Mean ± SD	45.02 ± 14.76	42.36 ± 15.57	0.041*
Carbohydrate (gram)			
Mean ± SD	114.94 ± 43.74	105.85 ± 29.79	0.178
Fat (gram)			
Mean ± SD	34.37 ± 12.27	30.97 ± 10.45	0.032*
Iron (mg)			
Mean ± SD	4.72 ± 2.59	4.48 ± 2.48	0.579
Vitamin A RAE (microgram)			
Mean ± SD	310.43 ± 231.01	307.96 ± 158.99	0.940
Vitamin D (microgram)			
Mean ± SD	5.91 ± 3.81	4.60 ± 3.39	0.036*
Calcium (mg)			
Mean ± SD	465.60 ± 227.54	456.13 ± 224.65	0.805
Zinc (mg)			
Mean ± SD	3.86 ± 1.19	3.05 ± 1.21	0.044*

Statistical testing using Independent samples t-test.

**Difference is significant at the 0.05 level (two-tailed).*

Vitamin A RAE, Vitamin A Retinol Activity Equivalents.

Figure 1 shows the percentage of nutrition-related adequate knowledge for feeding under-five children among household food-secure and household food-insecure. The average percentages of seven dimensions of nutrition-related adequate knowledge were obtained from the head of the household (mothers or fathers) about their under-five children. The results revealed that the highest average percentage (74.0%) of nutrition-related adequate knowledge among food-secure households were in the dimension of "consistency of meals"; while the highest average percentage (55.2%) of nutrition-related adequate knowledge among food-insecure households were in the dimension of "responsive feeding". The lowest average percentage of nutrition-related adequate knowledge among food-secure and food-insecure households was in the dimensions of "meaning of exclusive breastfeeding" and "breast milk at birth" of 52.0 and 36.8%, respectively.

Figure 2 presents the average percentages of seven dimensions of nutrition-related positive attitudes for feeding under-five children among household food-secure and household food-insecure. The data were obtained from the head of the household (mothers or fathers) about their under-five children. The dimension of "Giving a diversity of food-perceived benefits" got the highest average percentage of nutrition-related positive attitudes among both food-secure and food-insecure households of 82.0 and 59.0%, respectively. Whereas, the lowest average percentage of nutrition-related positive attitudes for feeding under-five children among food-secure and food-insecure households were in the dimensions of "continuing breastfeeding beyond 6 months-perceived barriers" and "feeding frequently-perceived benefits" of 32.80 and 27.20%, respectively.

Table 4 shows the practices and the diet diversity of under-five children by household food security status. The study indicated the presence of significant differences among food-secure and

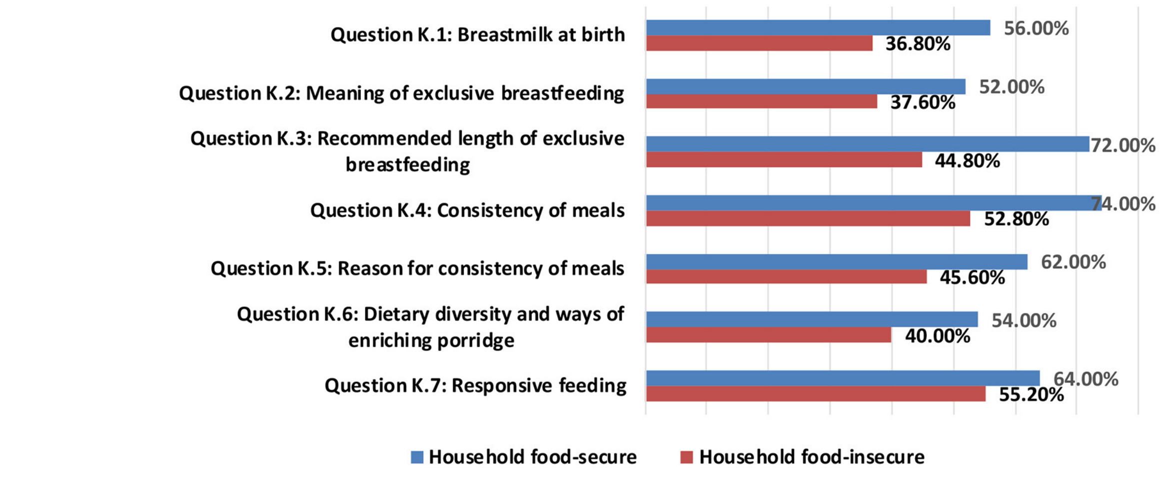

FIGURE 1 | Nutrition-related adequate knowledge among household food-secure and household food-insecure.

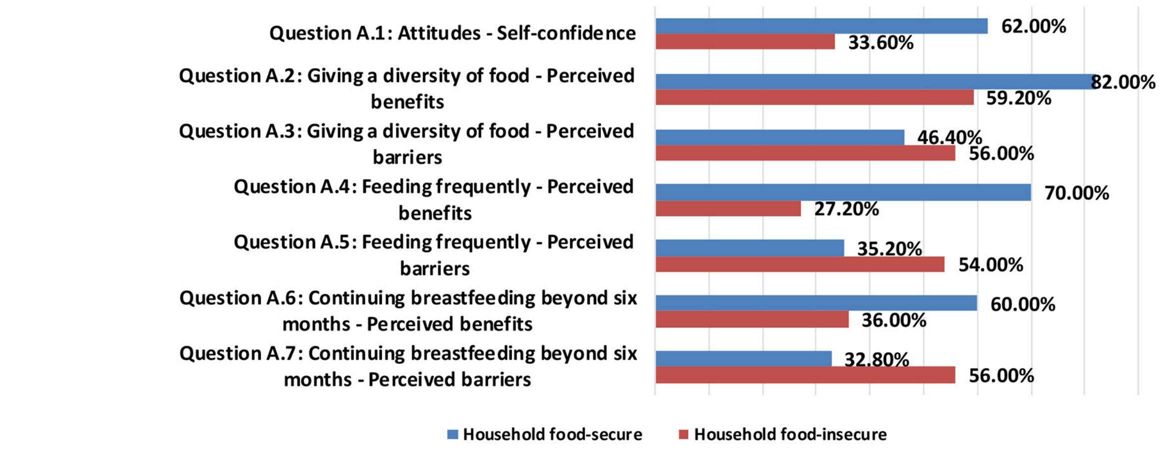

FIGURE 2 | Nutrition-related positive attitudes among household food-secure and household food-insecure.

food-insecure households regarding the dimensions of continued breastfeeding, intake of group 4: flesh foods, and group 7: other fruits and vegetables (P-value = 0.044, 0.013, and 0.011, respectively). About 72.8 and 54.0% of under-five children in food-insecure and food-secure households, respectively, do not continue breastfeeding ($P < 0.05$). In addition, 92.0 and 78.0% of under-five children in food-insecure and food-secure households do not consume group 4: flesh foods, respectively ($P < 0.05$). Furthermore, 38.4 and 20.0% of under-five children in food-insecure and food-secure households do not intake group 7: other fruits and vegetables, respectively.

Table 5 shows the overall knowledge, attitude, and achieved minimum dietary diversity score of under-five children by household food security status. Significant differences were found among food-secure and food-insecure households regarding adequate nutrition knowledge and positive attitudes for feeding under-five children and achieved minimum dietary diversity scores (P-value = 0.001, 0.003, and 0.019, respectively). More than half of food-insecure households (56.0%) have inadequate nutrition-related knowledge for feeding under-five children, while only 28.0% of food-secure households have inadequate knowledge. Furthermore, about (77.6%) of food-insecure households have nutrition-related negative attitudes for feeding under-five children, while only (28.0%) of food-secure households have negative attitudes. Most food-insecure households (95.2%) did not achieve a minimum dietary diversity score, while 84.0% of food-secure households did not achieve a minimum dietary diversity score.

DISCUSSION

The current study is the first to investigate the issue of households' food insecurity and their association with dietary intakes, nutrition-related KAP among under-five children in the Gaza strip, indicating that household food insecurity

TABLE 4 | Practices and dietary diversity of under-five children by household food-security status (n = 350).

Variables	Household food-secure (n = 100)	Household food-insecure (n = 250)	*P*-value[a]
Continued breastfeeding			
Yes	44.0 (44.0)	62.0 (24.8)	0.044*
No	54.0 (54.0)	182 (72.8)	
Don't know	2.0 (2.0)	6.0 (2.4)	
Dietary diversity			
Group 1: Grains, roots, and tubers			
Yes	44.0 (44.0)	106 (42.4)	0.842
No	56.0 (56.0)	144 (57.6)	
Group 2: Legumes and nuts			
Yes	44.0 (44.0)	90.0 (36.0)	0.327
No	56.0 (56.0)	160 (64.0)	
Group 3: Dairy products			
Yes	76.0 (76.0)	166 (66.4)	0.215
No	24.0 (24.0)	84.0 (33.6)	
Group 4: Flesh foods			
Yes	22.0 (22.0)	36.0 (8.0)	0.013*
No	78.0 (78.0)	230 (92.0)	
Group 5: Eggs			
Yes	68.0 (68.0)	71.0 (56.8)	0.115
No	32.0 (32.0)	54.0 (43.2)	
Group 6: Vitamin A fruits and vegetables			
Yes	42.0 (42.0)	74.0 (29.6)	0.082
No	58.0 (58.0)	176 (70.4)	
Group 7: Other fruits and vegetables			
Yes	80.0 (80.0)	154 (61.6)	0.011*
No	20.0 (20.0)	96.0 (38.4)	
Others: Not counted in the dietary diversity score			
Yes	22.0 (22.0)	62.0 (24.8)	0.695
No	78.0 (78.0)	188 (75.2)	
Minimum meal frequency			
Mean ± SD	2.85 ± 1.09	2.47 ± 1.25	0.053[b]

[a]*Statistical testing using Chi-Square Test.*
[b]*Independent Samples t-test.*
**Difference is significant at the 0.05 level (two-tailed).*

TABLE 5 | The overall knowledge, attitudes, and achieved minimum dietary diversity score of under-five children by household food-security status (n = 350).

Variables	Household food-secure (n = 100)	Household food-insecure (n = 250)	*P*-value[a]
Have nutrition-related adequate knowledge			
Yes	72.0 (72.0)	110 (44.0)	0.001*
No	28.0 (28.0)	140 (56.0)	
Have nutrition-related positive attitudes			
Yes	72.0 (72.0)	56.0 (22.4)	0.003*
No	28.0 (28.0)	194.0 (77.6)	
Achieved minimum dietary diversity score			
Yes	16.0 (16.0)	12.0 (4.8)	0.019*
No	84.0 (84.0)	238 (95.2)	

[a]*Statistical testing using Chi-Square Test or Fisher's Exact Test.*
**Difference is significant at the 0.05 level (two-tailed).*

was widespread among Gaza strip families. In this survey, approximately two-thirds of the under-five children were in food-insecure households. In line with the present findings, other surveys carried out in the Gaza strip stated a high prevalence of household food insecurity (6, 36). This indicates that food insecurity is a significant issue facing Palestinians in the Gaza strip. In addition, the present study showed that food insecurity is common in households with lower economic status. Consistent with this result, the UNICEF malnutrition conceptual framework reported that the poor financial situation of households negatively affects food access (37).

Our key results indicate that under-five children from food-insecure households had a higher prevalence of moderate underweight (30.4%), stunting (32.8%), wasting (9.6%), and acute undernutrition (30.4%). In addition, household food insecurity was associated with mean MUAC in children in terms of age z-scores, underweight, and acute undernutrition, but not with stunting and wasting. Studies conducted in the Gaza strip and developing countries indicated an association between household food insecurity and child underweight (38–42). Moreover, this result was in accordance with the UNICEF conceptual framework for malnutrition in developing countries; household food insecurity adversely affects the nutritional status of children by reducing the quantity and quality of food intake (37). Studies in developing countries have revealed no association between food insecurity, child wasting, and stunting (42–44), whereas other studies showed a positive relationship between food insecurity and stunting (43, 45, 46).

The current study showed that energy and macro-and micronutrients intakes, which contribute to crucial development indices of a growing child, among under-five children from food-insecure households were lower than among their food-secure counterparts. This result has been discussed and confirmed in the literature, as the poor economic status of households negatively affects the food expenditure and limits food choices to affordable options, which may not include required nutrient sufficiency for wellbeing (47–49). Moreover, this study has shown significant differences between food-secure and food-insecure households regarding having nutrition-related adequate knowledge and nutrition-related positive attitudes for feeding under-five children. More than half of food-insecure households (56.0%) have inadequate nutrition-related knowledge for feeding under-five children, while only 28.0% of food-secure households have inadequate knowledge. Furthermore, about (77.6%) of food-insecure households have nutrition-related negative attitudes, while only (28.0%) of food-secure households have negative attitudes. Nutrition-related knowledge and attitude are necessary for dietary changes toward a healthier dietary pattern. For that reason, food and nutrition-related KAP is one of the key factors to achieving household food and nutritional security (50). This study also revealed that achieved minimum dietary diversity score is significantly associated with household food security status. Most food-insecure households (95.2%) did not achieve a minimum dietary diversity score, while this this level was 84.0% for food-secure households. A Nepalese study supports this result – secondary data analysis of the Nepalese Demographic and Health Survey (51), and an Ethiopian study- Minimum dietary diversity and associated factors among children aged 6–23 months in Addis Ababa, Ethiopia (52). This can be attributed to the fact that children from food-secure households may eat various foods because their families may be more likely to afford miscellaneous foods than children from food-insecure households.

This study shares the standard limitation of cross-sectional design, challenging to make a causal association. Besides, information was collected from household heads (mothers/fathers) it was likely to have recall bias. Moreover, as the study considered only the 24-h recall method, it might not correctly reflect the exact figure of under-five children past dietary habits. Since anthropometric measurements are subject to measurement bias, we may not exclude some misclassified children's nutritional status classification. However, there was a standardization of measurement tools, intensive training of data collectors and careful supervision to overcome measurement errors and interview bias. Finally, although those diseased children were excluded, there may be some children who were not ill the week before but have lost their appetite, which may undervalue our results. However, this work should be seen as opening the door to the design of more rigorous studies on this phenomenon and alternatives that efficiently improve the capacity of under-five children caregivers.

CONCLUSION

In conclusion, the children from food-insecure households had a higher prevalence of moderate underweight, stunting, wasting, and acute undernutrition. Moreover, low economic status, poor dietary intake, low levels of nutrition-related KAP, and lack of dietary diversity could contribute to the high levels of food insecurity among under-five children. Policy makers should continue to focus attention and investments in the most appropriate combinations of interventions to mitigate food insecurity levels among under-five children in the Gaza strip, Palestine.

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 study protocol was approved by the Palestinian Health Research Council (Helsinki Ethical Committee of Research Number: PHRC/HC/961/21), University of Palestine Ethical Committee of Research, the Palestinian Ministry of Health, and Ministry of Interior. Furthermore, informed consent was obtained from each participant or their proxy. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

INFORMED CONSENT STATEMENT

Written informed consent was obtained from each participant or their proxy.

AUTHOR CONTRIBUTIONS

AE collected, analyzed, and interpreted the data and wrote the first draft of the manuscript. AE, AA-J, AA, SA, IE, and LN significantly contributed in the study design and the critical review of the manuscript. AE and AA-J remarkably contributed to the analysis and interpretation of data and the critical review of the manuscript. All authors approved the final manuscript.

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Impact of a Nutrition Education Intervention on Salt/Sodium Related Knowledge, Attitude, and Practice of University Students

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Background: Salt reduction strategies help reduce the risk of cardiovascular diseases (CVDs) by reducing high blood pressure. This study aimed to assess salt related knowledge, attitude, and practices (KAP) before and after administering an evidence-based nutrition education workshop.

Methods: Ninety non-medical university students were recruited to investigate KAP related to dietary salt intake. The KAP components were assessed before, immediately after, and 4-weeks after administering an evidence-based educational workshop and leaflet.

Results: Knowledge and attitudes related to salt improved significantly immediately post-intervention but were not fully retained after 4-weeks. Five of the 13 evaluated practices improved after 4-weeks: trying to buy low-salt foods increased from 10 to 19% ($P = 0.022$), rarely adding salt to food during cooking increased from 5 to 16% ($P = 0.019$), rarely adding salt to food at the table increased from 29 to 42% ($P = 0.011$), tried to reduce salt intake increased from 26 to 41% ($P = 0.014$), and tried to use spices to reduce salt increased from 31 to 45% ($P = 0.044$).

Conclusions: The educational intervention had a positive impact on salt-related knowledge, attitudes, and practices, but the effect was not fully retained on the long-term. Periodic educational interventions should be considered to refresh knowledge and reinforce practices.

Keywords: educational intervention, dietary salt, dietary sodium, knowledge retention, university students

INTRODUCTION

Non-communicable diseases (NCDs) cause premature death in over 15 million people between the ages of 30–70 years annually (1). Cardiovascular diseases (CVDs) contribute to most of the NCDs deaths followed by cancers, respiratory diseases, and diabetes (2). Measurable global policies for the averting of CVDs are required to reduce exposure of populations to risk factors as well as early detection and treatment at individual levels. Interventions targeting the reduction of the four main risk factors of cardiovascular diseases: tobacco use, harmful alcohol consumption, unhealthy diet, and physical inactivity, could prevent much of the morbidity and mortality caused by NCDs (3). Moreover, high dietary intake of salt/sodium is associated with elevated blood pressure and is one of the major contributors of premature deaths from cardiovascular diseases globally (4). The recent modeling from the Global Burden of Disease data estimates that globally the average salt intake in adults is 14 g per day (5).

Considering this, in 2013 the World Health Assembly (WHA) committed to nine global voluntary targets to reduce NCDs and a 30% relative reduction in a mean population intake of salt/sodium by 2025 (6). To achieve this, the action plan by the World Health Organization (WHO) recommends adults to consume <5 g of salt or 2 g of sodium per day (7). Supporting the action plan of the WHO, the World Action of Salt, Sugar and Health (WASSH), was established in 2005 to encourage sodium reduction worldwide and it currently has expert members in 100 countries (8). Countries around the world were encouraged to implement the action plan of the WHO through five key components: surveillance, product reformulation, standardized food labeling, knowledge, and environment (9). Globally there has been an increase in the number of countries implementing different approaches. But none have yet met the targeted 30% relative reduction in salt intake from baseline (10). Reformulation strategies are likely to be more effective in countries where a large proportion of dietary salt comes from packaged foods and food prepared outside the home, whereas salt substitution may be more effective in countries where there is extensive use of discretionary salt.

Current salt intakes in the Eastern Mediterranean Region (EMR) are very high with an average intake of more than 12 g per person per day, which is more than double the recommended level by the WHO (11). Countries of the Gulf Cooperation Council (GCC) started taking actions to develop framework of salt reduction action plans and some were able to achieve 20% salt reduction in bread (12). Bread is a popular staple food in the GCC countries, and it is one of the main contributors of salt in the diet (12). Salt reduction interventions in the Eastern Mediterranean region focus on awareness campaigns to assist consumers in making informed choices and product reformulation to reduce salt content in processed foods (11).

In the United Arab Emirates (UAE), NCDs account for more than 75% of deaths each year, with CVDs contributing to about half of the total deaths (13). Evidence shows that hypertension is more prevalent among adults; with a prevalence rate of 53% and 47% among the females and males, respectively, in the UAE (13). Notwithstanding, hypertension and heart disease prevalence is increasing among younger population and therefore salt reduction strategies should be addressed with serious measures (14). A recent study among adult residents of the UAE measured 24-h urinary sodium excretion and revealed that about two thirds of the participants were significantly exceeding sodium recommendation dose of a mean of ∼2,700 mg/day (15). Moreover, a study among the university students in the UAE indicated an alarming rate of 89% of the students exceeding the recommendation for dietary sodium intake with a mean dose of 3,571 mg/day (16). The Ministry of Health and prevention (MOHAP) in the UAE is leading the salt intake reduction strategies and activities focusing on product reformulation and empowering consumers with the knowledge needed to make healthier choices (17).

The available literature indicates low salt/sodium related knowledge and poor attitudes and practices toward salt (18–21). Similar findings were also reported in cross sectional studies in the UAE (15, 16). An intervention study was conducted on cardiac patients in Lebanon to assess their knowledge and attitudes toward salt/sodium consumption before and after distributing an educational leaflet, and it concluded a favorable impact on salt-related knowledge (22). Similarly, a salt reduction program implementation in 120 villages around China suggested positive effect on salt-related knowledge and attitudes of the study sample, which in turn contributed to a reduction in salt consumption (23).

That being the case, increasing the population knowledge and investing in intervention strategies related to salt reduction may assist in reducing the risk of high blood pressure and act as a preventative measure against CVDs.

Previously gathered baseline data on knowledge, attitudes and practices related to salt/sodium consumption among the university students in the UAE identified several salt-related knowledge gaps and engaging in unfavorable attitudes and practices (16). Moreover, the 24-h dietary recall revealed a high percentage of students exceeding the recommended level of dietary sodium intake (16). Based on this population specific evidence, the current educational intervention study was designed to determine the impact of a nutrition education intervention on salt-related knowledge, attitudes, and practices among the UAE university students.

MATERIALS AND METHODS

This study was approved from the University of Sharjah Research Ethics Committee (UOS-REC) reference number: REC-19-04-15-01-S. This study was conducted according to the stated principles in the Declaration of Helsinki (24). A written informed consent form was obtained from all participants.

Participant Recruitment

An experimental (one group pre-test post-test) research design was used on a sample of students from the University of Sharjah (UOS), UAE, during the academic year of 2018/2019. Non-medical students from Applied Sciences and Humanities Colleges (without prior knowledge of cardiovascular diseases) were invited to participate in the study through email invitations (n = 1,200). About 113 students, aged 18–24 years old showed interest in the study, out of which, only 90 students met the following inclusion criteria: not having a history of hypertension, coronary artery disease or heart failure and no prior education about cardiovascular diseases. Three were previously diagnosed with hypertension or cardiovascular disease (excluded due to prior knowledge of cardiovascular diseases),

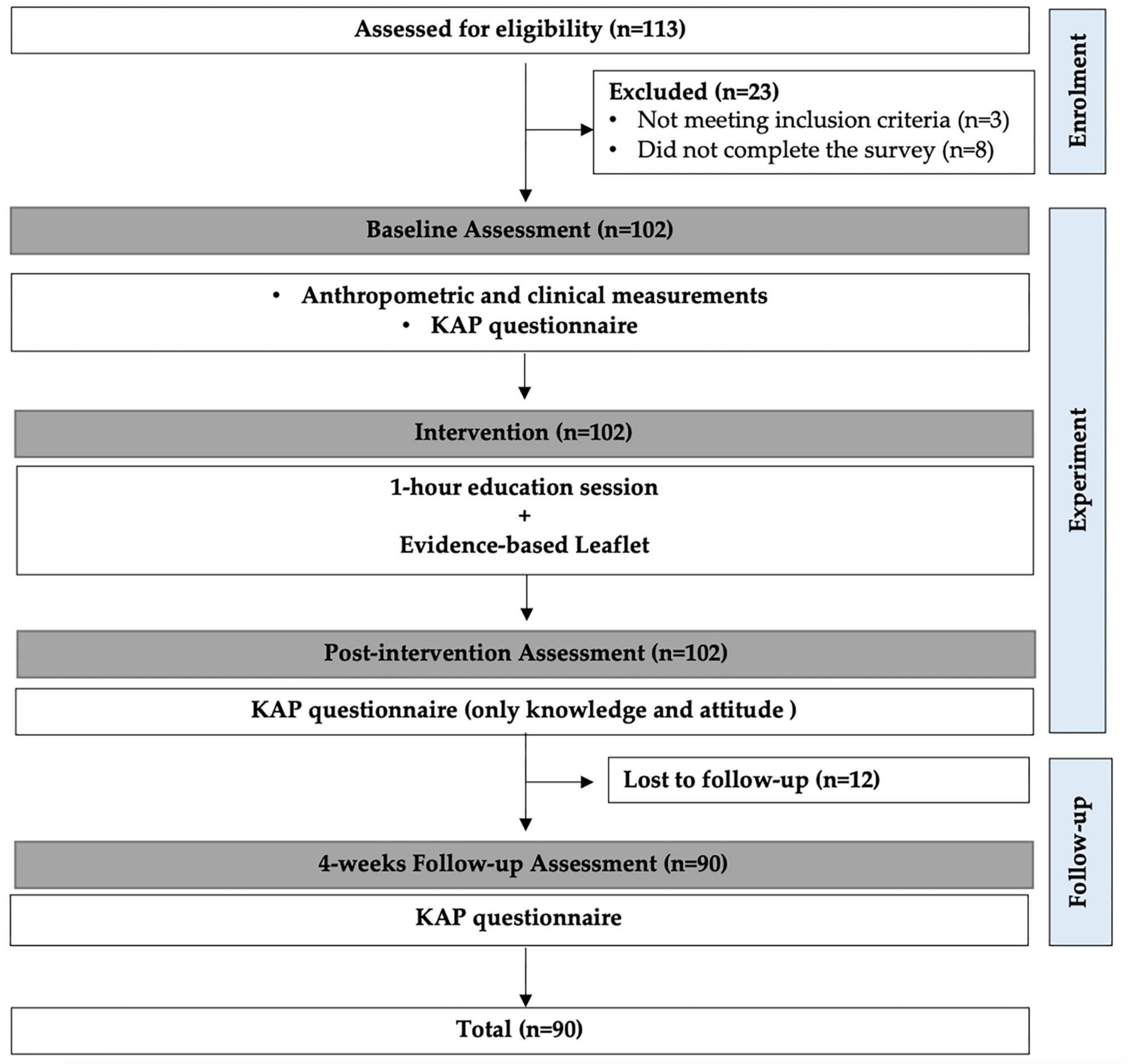

FIGURE 1 | Flow of participants and study design.

eight did not complete the study survey, and twelve were lost to follow-up (**Figure 1**).

The sample size calculation was performed using G*Power software, version 3.01 (Franz Faul, Christian-Albrechts-Universität Kiel, Kiel, Germany) (25) for the two dependent groups (McNemar test). The calculation revealed the need for a sample of 57 participants to be included in the study. The level of salt related knowledge among students was assumed to be similar to that found in a previous study conducted among UOS students (16) and the significance level was set at $p < 0.05$ and the power was 0.80. Considering drop-out factors of 20%, the sample size was inflated to be 69.

Pre-intervention Assessment

Anthropometric measurements including height and weight were determined. Height was measured without shoes and recorded to the nearest 0.5 cm. Weight was measured with light clothing on and recorded to the nearest 0.1 kg. Measurements were performed using a calibrated medical scale and stadiometer (SECA 284; Seca, Hamburg, Germany). Body mass index (BMI) was calculated by dividing weight (kg) by the height (m) squared (kg/m^2) and then classified based on WHO cutoffs (26).

A pre-intervention bilingual (English and Arabic) multicomponent, self-administered questionnaire was administered to students. It included questions on socio-demographic, knowledge, attitudes, and practices (KAP) toward salt/sodium consumption. This questionnaire was previously validated in student's population and adult population aged 20–60 years old in the UAE (15, 16). The details of the KAP questionnaire have been described elsewhere (15, 16). In brief, it included a socio-demographic section (age, gender, nationality, college, residence, and choice of meals), 29 questions on salt/sodium related knowledge, 5 questions on attitudes, and 13 questions on practices. The full version of the questionnaire is available a **Supplementary File**.

The Intervention

After completing the pre-intervention assessment, students attended an educational workshop on the importance of salt reduction. The educational material was given to participants through a single 1-h interactive session via power point presentation followed by 30 min activities to ensure involvement of the participant.

The 1-h educational presentation included information on definition of salt, differences between salt and sodium, salt intake recommendation compared to average salt consumption, food labels reading to identify low, medium, and high salt foods, strategies for salt intake reduction along with meal alternatives ideas, and information about salt related diseases.

The 30 min activities post presentation included guessing games such as estimation of sodium amount in various provided food samples, categorization of some commonly consumed dishes to low and high sodium content, and differentiation between two salad dishes: one contained salt in the dressing and the other one with spices and herbs substituting the salt. Toward the end of the session a leaflet was distributed to each participant including a summarized version of the information provided in the session.

Post-intervention Assessment

The post-intervention questionnaire was conducted immediately after the students attended the educational workshop and read the leaflet. The post-intervention assessment questionnaire included only the knowledge and attitude sections of the KAP questionnaire because salt-related behavior would require time to change. Therefore, the change in practices was assessed in the 4-week follow-up.

Four-Week Follow-Up Assessment

After 4 weeks of the intervention session, students were invited again to complete a follow-up KAP questionnaire. The KAP questionnaire included all three components; knowledge, attitudes, and practices, and was repeated to assess the long-term impact of the educational workshop and the material provided.

Data Analysis

Continuous data were expressed as mean ± standard deviation (SD), and categorical data were expressed as counts and percentages. Comparisons between baseline, immediate post-intervention, and 4 weeks post-intervention were conducted using the McNemar test. Scores for knowledge, attitude and practice toward salt were calculated based on the sum of the correct answers or positive attitudes/practices.

Knowledge scores ranged from 0 to 29 based on the number of correct answers, attitude scores ranged from 0 to 5 based on positive attitudes toward salt, and practice score ranged from 0 to 13 based on the number of positive practice responses (15, 16). Scores were calculated for all components in the pre-intervention and in the 4-weeks follow up. However, only knowledge and attitude scores were calculated immediately post-intervention. Comparison between knowledge scores at baseline, immediate post-intervention, and 4 weeks post-intervention was conducted using the paired sample *t*-test.

Participants were stratified based on based on their responses to seven of the practice questions to low-risk, moderate-risk and high-risk groups (22). These included "check food labels specifically for salt," "sodium shown on the label the affects purchasing," try to buy "low salt foods," "try to buy 'no added salt' food," "adding salt during food preparation," "using stock cubes during cooking," and "adding salt before tasting." Risk scores were calculated pre-intervention and at the 4-weeks follow-up. Participants were given 1 point if they answered "always" or "often" to adding salt during food preparation, using stock cubes during cooking, and adding salt before tasting, and 1 point if they answer "never" for any of the other four practices that lead to higher salt consumption. Consequently, participants with zero points were categorized as low risk for high salt consumption, participants with 1 point as moderate risk, and with 2 or more points as high risk (22).

TABLE 1 | Characteristics of the study participants ($N = 90$).

Characteristics		
Age (years), mean ± SD	20.72 ± 1.35	
Gender, n (%)		
Male	41	(45.6)
Female	49	(54.4)
College, n (%)		
Applied Sciences	74	(82.2)
Humanities	16	(17.8)
Residential type, n (%)		
With family	69	(76.7)
Hostel	19	(21.1)
Alone	2	(2.2)
Most meals consumed at, n (%)		
Homemade	69	(76.7)
Restaurant	21	(23.3)
BMI Classification (kg/m²), n (%)		
Underweight (<18.5)	7	(7.8)
Normal (18.5–24.9)	44	(48.9)
Overweight (25–29.9)	19	(21.1)
Obese (≥30)	20	(22.2)

TABLE 2 | Pre-intervention, post-intervention and 4-week follow-up knowledge correct responses of study participants ($n = 90$).

Variable[a]	Pre-intervention n (%)	Post-intervention n (%)	*P*-value[b]	4-weeks follow-up n (%)	*P*-value[c]
Percentage of sodium in salt (40%)	12 (13.3)	73 (81.1)	<0.001	77 (85.6)	<0.001
High salt intake may increase risk factors for					
Hypertension (Yes)	75 (83.3)	86 (95.6)	0.007	86 (95.6)	0.013
Cardiovascular diseases (Yes)	56 (62.2)	80 (88.9)	<0.001	82 (91.1)	<0.001
Diabetes (No)	48 (53.3)	62 (68.9)	0.016	57 (63.3)	0.175
Fever (No)	41 (45.6)	61 (67.8)	0.004	50 (55.6)	0.222
Water retention (Yes)	61 (67.8)	76 (84.4)	0.001	78 (86.7)	0.001
Renal diseases (Yes)	59 (65.6)	79 (87.8)	<0.001	72 (80.0)	0.029
Reducing salt intake will improve					
Health (Yes)	75 (83.3)	88 (97.8)	0.001	87 (96.7)	0.008
Blood Pressure (Yes)	80 (88.9)	87 (96.7)	0.065	88 (97.8)	0.039
Sodium content in the following foods is					
Pita bread (High)	12 (13.3)	46 (51.1)	<0.001	48 (53.3)	<0.001
Iranian bread (High)	22 (24.4)	43 (47.8)	<0.001	54 (60.0)	<0.001
Fruits (Low)	69 (76.7)	85 (94.4)	<0.001	80 (88.9)	0.019
Fresh vegetables (Low)	70 (77.8)	84 (93.3)	0.003	82 (91.1)	0.008
Canned vegetables (High)	47 (52.2)	84 (93.3)	<0.001	82 (91.1)	<0.001
Cheddar cheese (High)	66 (73.3)	88 (97.8)	<0.001	80 (88.9)	0.003
Pickles (High)	70 (77.8)	85 (94.4)	0.003	83 (92.2)	0.015
Olive oil (Low)	57 (63.3)	71 (78.9)	0.007	71 (78.9)	0.020
Basmati rice (Low)	37 (41.1)	53 (58.9)	0.009	42 (46.7)	0.522
Egyptian rice (Low)	32 (35.6)	45 (50.0)	0.053	44 (48.9)	0.065
Milk, yogurt (Low)	42 (46.7)	46 (51.1)	0.585	60 (66.7)	0.008
Salad dressing oil (High)	53 (58.9)	65 (72.2)	0.050	68 (75.6)	0.024
Ketchup (High)	57 (63.3)	74 (82.2)	0.002	76 (84.4)	0.001
Tomato paste (High)	56 (62.2)	75 (83.3)	<0.001	75 (83.3)	0.001
Red meat (Low)	27 (30.0)	47 (52.2)	0.002	36 (40.0)	0.175
Poultry (Low)	29 (32.2)	35 (38.9)	0.307	34 (37.8)	0.522
Corn flakes (High)	19 (21.1)	27 (30.0)	0.185	29 (32.2)	0.076
Chicken cubes (High)	66 (73.3)	75 (83.3)	0.064	77 (85.6)	0.035
Instant noodle (High)	71 (78.9)	86 (95.6)	0.001	80 (88.9)	0.093
Filtered water (Low)	47 (52.2)	77 (85.6)	<0.001	74 (82.2)	<0.001

[a]The correct answers are provided in brackets next to each variable; [b]Significance of post-intervention compared to pre-intervention; [c]Significance of follow-up compared to pre-intervention. The p-values indicate the results of McNemar test.

$P < 0.05$ were considered statistically significant. Data was analyzed using SPSS software (27), version 26.0 (SPSS, Chicago, IL, USA).

RESULTS

Sample Characteristics

A total of 90 students participated in the study. Key demographic variables are shown in **Table 1**. Participants' ages ranged from 18 to 24 years (mean = 20.7, standard deviation = 1.35), with a female to male ratio of almost 1:1 (54.4% females, 45.6% males). The majority of the participants were enrolled in applied science majors (82.2%), lived with their family (76.7%) and consumed most meals at home (76.7%). Almost half of the participants had normal BMI (48.9%) with the remaining classified as overweight and obese (21.1%, 22.2%) respectively.

Pre-intervention Questionnaire Responses: Knowledge, Attitude, and Practice

The number and percentage of students who answered knowledge related questions correctly is shown in **Table 2**. During pre-intervention, sodium percentage in salt was identified correctly by only 13.3% of the students. A high proportion answered correctly to higher salt intake and its relation to a disease risk factors; hypertension (83.3%), CVD (62.2%), water retention (67.8%) and renal diseases (65.6%). Similarly, most of the students knew that reducing the salt intake will improve the general health (83.3%) and improve blood pressure (89%). Most of the participants categorized the salt contact in the following

TABLE 3 | Pre-intervention, post-intervention and 4-week follow-up for attitude related responses of study participants ($n = 90$).

Attitude[a]	Pre-intervention n (%)	Post-intervention n (%)	*P*-value[b]	4-weeks follow-up n (%)	*P*-value[c]
How much salt do you think you consume (Just the right amount)	64 (71.1)	36 (40.0)	<0.001	51 (56.7)	0.029
Are you concerned about the amount of salt/sodium in the diet (Yes)	9 (10.0)	24 (26.7)	0.001	11 (12.2)	0.791
Reducing added salt to foods is important to you (Agree)	27 (30.0)	48 (53.3)	0.001	33 (36.7)	0.327
Reducing consumption of processed foods is important to you (Agree)	31 (34.4)	57 (63.3)	<0.001	39 (43.3)	0.200
Reducing your sodium intake is important to you (Agree)	19 (21.1)	51 (56.7)	<0.001	29 (32.2)	0.052

[a]Attitude was assessed based on a three-point Likert scale but only answers of "positive attitude" are presented; [b]Significance of post-intervention compared to pre-intervention; [c]Significance of follow-up compared to pre-intervention. The p-values indicate the results of McNemar test.

TABLE 4 | Pre-intervention and 4-week follow-up for practice related responses of study participants ($n = 90$).

Practice[a]	Pre-intervention n (%)	4-weeks follow-up n (%)	*P*-value[b]
Check food labels (Often)	21 (23.3)	27 (30.0)	0.286
Information on food labels affects purchasing decisions (Often)	21 (23.3)	19 (21.1)	0.824
Check labels specifically for salt/sodium content (Often)	8 (8.9)	13 (14.4)	0.227
Salt/sodium content on label affects purchasing decisions (Often)	7 (7.8)	11 (12.2)	0.424
Try to buy "low salt" foods (Often)	10 (11.1)	19 (21.1)	0.022
Try to buy "no added salt" foods (Often)	3 (3.3)	6 (6.7)	0.453
Add salt to food during cooking (Rarely)	5 (5.6)	16 (17.8)	0.019
Use Stock Cubes during cooking (Rarely)	32 (35.6)	36 (40.0)	0.557
Add salt to food at the table (Rarely)	29 (32.2)	42 (46.7)	0.011
Add salt before tasting the food (Rarely)	42 (46.7)	44 (48.9)	0.851
Did you try to reduce salt intake before (Yes)	26 (28.9)	41 (45.6)	0.014
Did you try to use spices to reduce salt (Yes)	31 (34.4)	45 (50.0)	0.044
Type of bottled water (low sodium)	18 (20.0)	29 (32.2)	0.052

[a]Answer options for practice questions included often, sometimes, and never, only answers of positive practice are presented; [b]Significance of follow-up compared to pre-intervention. The p-values indicate the results of McNemar test.

foods correctly: instant noodle (78.9%), pickles (77.8%), cheddar cheese (73.3%), chicken cubes (73.3%), ketchup (63.3%) and tomato paste (62.2%). Whereas, less than a third answered correctly for Iranian bread (24.4%), corn flakes (21.1%) and pita bread (13.3%).

Regarding attitude toward salt (**Table 3**), 71.1% of the students assumed that they consume "just the right amount" daily and only 10% reported being concerned about amount of salt/sodium in their diet. A relatively low proportion of students agreed with the statements: "reducing added salt is important to you" (30.0%), "reducing processed foods consumption is important to you" (34.4%), and "reducing your sodium intake is important to you" (21.1%).

Regarding practice habits as shown in **Table 4**, 23.3% of the students reported that they check food labels and that their purchasing decisions are affected by the information provided on the label. Moreover, <10% checked specifically for salt/sodium content and reported that their purchasing decision was affected by the salt/sodium content on the label. Only 35.6% of the students reported rarely using stock cubes during cooking. Furthermore, 67.8% reported adding salt to food at the table; moreover, 94.4% added salt during cooking. When asked about measures taken to reduce salt intake, 28.9% of the students reported trying to cut down salt before, and 34.4% of them used spices as alternatives to salt.

Post-intervention Questionnaire Responses: Knowledge and Attitude

Knowledge and attitude were reassessed immediately after the educational session. As shown in **Table 2**, there was a significant improvement in answering correctly about the percentage of sodium in salt (81.1% compared to 13.3% in the pre-intervention, $p < 0.001$). Moreover, significant increases in identifying most food items as high or low sodium food sources were evident post-intervention except for Egyptian rice, milk, salad dressing oil, poultry, corn flakes, and chicken cubes. Likewise, students were able to correctly identify the relationship of high dietary salt intake to health and disease (**Table 2**). As for attitude (**Table 3**), all five questions had significant increase in the proportion of students choosing favorable attitudes ($p \leq 0.001$).

Four Weeks Follow-Up Questionnaire: Knowledge, Attitudes, and Practice

As presented in **Table 2**, there was a significant increase in the correct answers for most knowledge related questions from baseline (pre-intervention) to the 4-weeks follow up. Percentage of sodium in salt was identified correctly by 85.6% of the students ($p < 0.001$) compared to both pre-intervention (13.3%) and immediately post-intervention (81.1%). A significantly higher proportion of students in the 4-weeks follow-up were able to relate hypertension ($P = 0.013$), CVD ($p < 0.001$), water retention ($P = 0.001$) and renal diseases ($P = 0.029$) to high salt intake when compared to the baseline. A similar significant increase was evident in correctly identifying high/low sodium food items such as pita bread, Iranian bread, canned vegetables, ketchup, tomato paste, chicken cubes and filtered water ($p < 0.001$) (**Table 2**).

The proportion of students reporting positive attitudes toward salt dropped in the 4-weeks follow-up compared to the immediate post-intervention but remained above baseline in four of the attitude questions (**Table 3**); "concerned about the amount of salt/sodium in the diet," "it is important to reduce added salt to foods," "it is important to reduce consumption of processed foods," and "reducing your sodium intake is important to you." A significantly less proportion of students reported that they think they consume "just the right amount of salt" in the 4-weeks follow-up compared to baseline ($P = 0.029$).

Regarding practice (**Table 4**), there was a significant improvement in positive practices toward salt in the 4-weeks follow-up compared to the baseline for; trying to buy low-salt food ($P = 0.022$), rarely adding salt during cooking ($P = 0.019$), rarely adding salt to food at the table ($P = 0.011$), tried to reduce salt intake before ($P = 0.014$), and trying to use spices to reduce salt ($P = 0.044$). The remaining practices showed an increase in positive responses; however, no significant improvement was found between the pre-investigation and the 4-weeks follow-up.

Knowledge, Attitude, and Practice Scoring and Risk Stratification

The average knowledge score at baseline was 16.2 ± 6.1 (possible scores: 0 to 29). It increased significantly to 22.0 ± 3.7 at the immediate post-intervention assessment ($P = 0.001$) and then declined to 21.7 ± 4.0 at the 4-weeks follow-up assessment, however remained significantly higher than baseline ($P = 0.002$) as shown in **Figure 2**. A similar trend was observed for attitude scores, where the average attitude score pre-intervention was 1.7 ± 1.2 (possible scores: 0 to 5), increased to 2.4 ± 1.5 immediately post-intervention and dropped to 1.6 ± 1.4 after 4-weeks. Moreover, the practice score increased from 2.8 ± 2.5 (possible scores: 0 to 13) pre-intervention to 3.9 ± 2.8 after 4-weeks ($P = 0.001$).

At baseline, 26.6% of the students were categorized as having high risk for salt consumption practices, however, this dropped to 14.4% at the 4-weeks follow-up assessment (**Figure 3**). Moreover, at baseline, 12.2% of the students were categorized as having low risk behavior, and this increased to 22.2% at the 4-weeks follow-up. The McNemar test showed a trend toward practice category improving from baseline to the 4-weeks follow-up assessment ($P = 0.003$).

DISCUSSION

The aim of this study was to determine the efficiency of applying evidence-based nutrition intervention on salt related knowledge, attitudes, and practices among non-medical students at the University of Sharjah using a validated multi-component questionnaire and an educational interactive session. Parameters were measured before and after the intervention to allow comparison. The results revealed that salt-related knowledge improved significantly immediately post-intervention, but it was the knowledge was not fully retained until the 4-weeks follow-up, however it remained above the baseline. Moreover, improvement in the high-risk for salt consumption group was recorded, as a shift was observed toward the moderate-risk group and a trend toward practice category improving from baseline to the follow-up was shown.

Findings at baseline showed a relatively low salt/sodium related knowledge with low tendency toward implementing salt/sodium reduction behaviors. These findings were consistent with the previous study among the UOS students (16). For example, the mean knowledge score in this study was 16.2 ± 6.1 compared to 17.2 ± 6.1 in the previous study (16). Also consistent with the results of health science students in Bangladesh on salt related knowledge (28). Moreover, more than half of the students in the current study estimated that they consume just the right amount of salt. Similarly, 62% of adults in the UAE reported consuming the right amount of sodium (15). However, the same study estimated that more than two-thirds of the participants exceeded the WHO recommendations (15). These findings suggest that perceived intake does not necessarily reflect actual intake. Most of the students were aware of the obvious adverse health effects associated with high salt intake. Similar findings were reported in several studies in the UAE, European countries and the Lebanon investigating salt related knowledge, attitude, and behavior (15, 16, 28, 29). Also, more than three in four students indicated that salt reduction would actively improve their health and blood pressure which was in contrast to a study reported among adults in Montenegro where less than half of the participants had the same indication (29).

Furthermore, findings showed that less than a quarter of the participants check food labels and use the information on food labels to guide their purchasing decisions. More

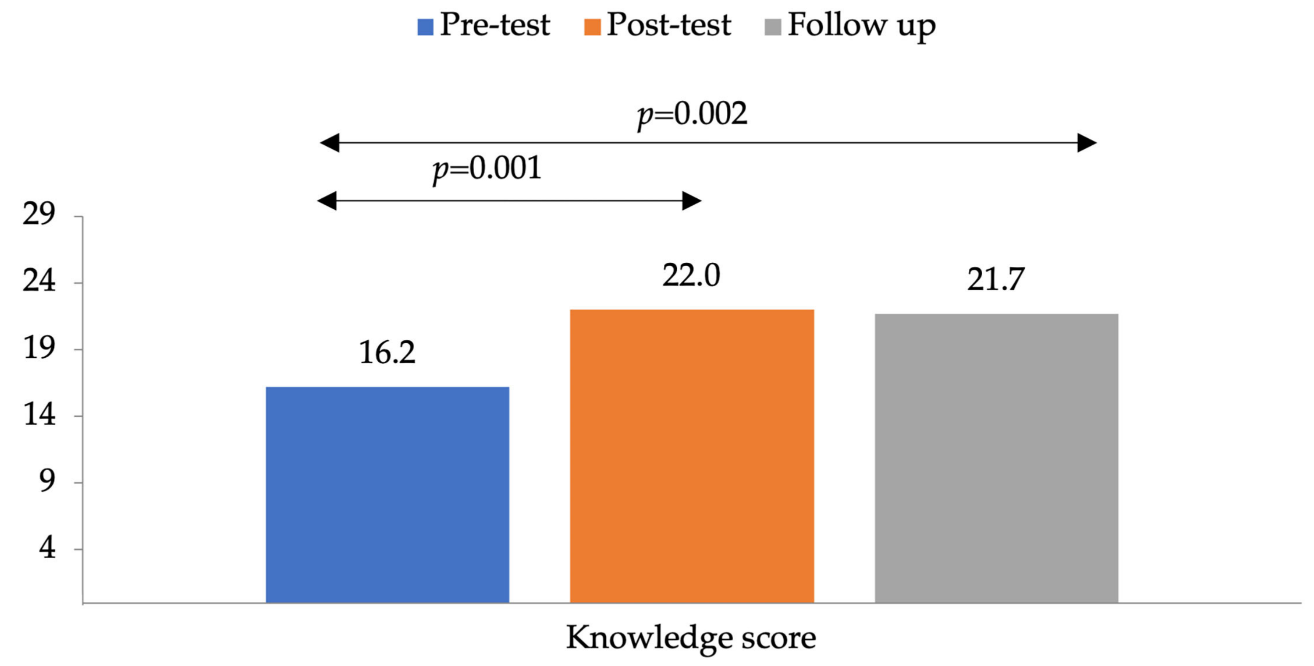

FIGURE 2 | Mean knowledge scores of pre-test, post-test, and follow up. The *p*-value indicates the statistical significance of the paired sample *t*-test.

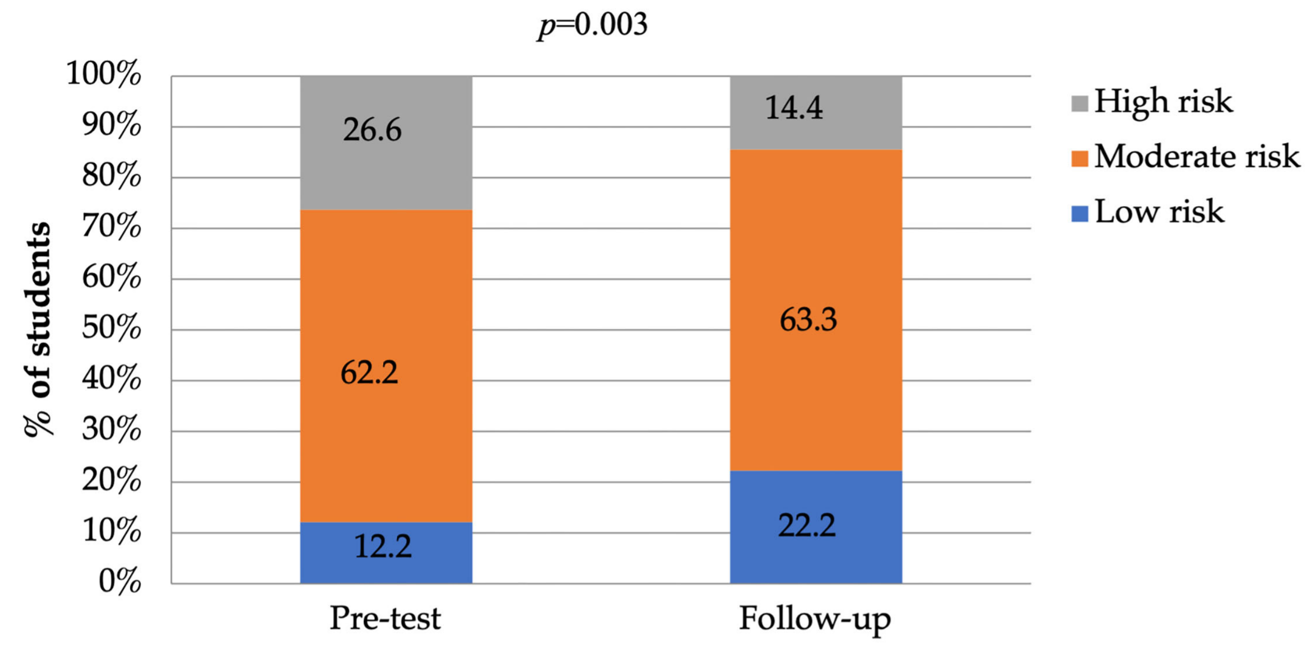

FIGURE 3 | Risk of high-salt consumption categories for student's pre-test and at 4-weeks follow up (low risk, moderate risk, and high risk). The *p*-value indicates the statistical significance of the McNemar test.

worryingly, a very small proportion of students check for salt content on the food label and use it to guide their food choices. In the previous study among UOS students only 17% reported checking food labels and 20% of students used the labels to guide their purchasing decisions (16). Findings in a study among adult consumers in Lebanon revealed much lower proportion for checking food labels than those reported in the UAE; stating that food labels are not adequately utilized by participants (30). Therefore, an awareness of the health risks associated with high salt consumption is recommended to increase salt label usage and purchases of low salt foods.

With respect to knowledge, in all questions, the percentage of correct answers of the immediate post-intervention was significantly better compared to the pre-intervention (baseline). After 4 weeks follow-up, the percentage of correct answers showed that retention of knowledge of students slightly decreased in comparison to the immediate post-intervention, however remained above baseline. A similar pattern was seen in a previous study conducted in Lebanon (22). Similarly, a cohort study conducted at Lurio University, Mozambique, evaluating knowledge retention showed a decrease in knowledge after 6 months compared to post-test (31). Despite the difference in the follow up time intervals, the general trend of decline in knowledge is consistent. Moreover, historically a similar decay in knowledge-retention over time following an educational program is shown in other studies (32, 33).

Due to the fact that knowledge is an important driver of attitudes and practices, it is expected that a decline in knowledge will be accompanied by similar drop in favorable attitude responses (34). As it is evident in our results, after 4 weeks there was a decrease in the positive attitude response compared to baseline simultaneously with the decline in knowledge, however knowledge remained above baseline. Therefore, conducting frequent periodical educational sessions along with testing knowledge has been used effectively to ensure long-term knowledge retention (35, 36). Aligning with our study, such strategy where students are educated on aspects of knowledge and followed-up must be implemented.

Several salt related practices showed significant improvements after the 4 weeks follow up, such as trying to purchase low-salt food and rarely adding salt to food at the table. However, no significant improvement was found in other salt-related practices such as using stock cubes during cooking and adding salt before tasting the food. The results are consistent to what was found in the intervention study performed in Iran, in which it was shown a significant increase in the mean and standard deviation of KAP among the intervention group, and a significant decrease in the mean salt intake (37). Nonetheless, in a study investigating the impact of community-based salt reduction program in Australia, the proportion of participants reporting salt reducing practices such as avoiding processed foods and checking food labels decreased significantly (38).

Our results indicate a trend toward an improve in the salt-related practices from baseline to the 4-weeks follow-up such as trying to buy low-salt food, rarely adding salt during cooking or at the table and trying to reduce salt intake by using spices. Evidence suggests that intervention strategies including peer group education has shown similar positive impact on salt-related practices and salt consumption. A study among adults having at least one risk factor of CVD indicated beneficial effects of peer group intervention on cardiovascular risk factors, with significant improvements in total score and more specifically on tobacco cessation (34). Moreover, a national consumer awareness campaign about the negative effect of salt on health in the United Kingdom has shown promising results as it indicated a significant decline in using salt at the table (39). Another randomized clinical trial depicted a lower dietary sodium intake among the intervention group (40).

There are several limitations to this study. The findings may not be generalized to all young adults in the UAE as our study was restricted to the non-medical major university students. Including participants who showed an interest in the study (self-selection) is likely to create a group of more motivated persons which could affect sample representativeness. In addition, the questionnaire included the use of self-reported attitudes and practices, that possibly might cause some respondent bias or misreporting of data that may not accurately reflect actual attitudes and practices. Another limitation to this study was the use of a single contact intervention due to time constraint. Hence, future studies with multiple interactions are encouraged to induce long-term benefits.

Our study showed a significant increase in knowledge and positive salt related attitude immediately post-intervention. This increase remained significant after the 4-weeks follow-up. However, there was a tendency to have a slight decline in knowledge after 4-weeks as it was only a single contact. A complementary educational method is advised to enhance retention of knowledge and probably have an impact on salt-related attitudes and practices. On the other hand, it was evident that changes in practice were not as prominent as changes in knowledge and attitude. This is possibly related to poor dietary habits and high consumption of fatty food, snacks, sugar, and fast food that is evident in this age group.

In conclusion, it is crucial to conduct several specifically designed awareness sessions and space them over time in conjunction with creative intervention programs focusing on the proper way of reading and using food and salt labels. Moreover, it is suggested to deliver nutrition related message using social media, given that this generation is most influenced by these platforms. It is also suggested to mandate at least one nutrition education course for all university students as well as ensuring the provision of a wide variety of healthy food options with low salt content at the university campus cafeteria and healthy vending machine snacks. Future research should also focus more on a long-term knowledge retention to investigate the impact of salt-reduction interventions on related attitude, practice, and overall health. Conducting similar studies on a large scale is recommended to be able to generalize results to the young adult population.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: FigShare: 10.6084/m9.figshare.15062367.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Sharjah Research Ethics Committee (UOS-REC) reference number: REC-19-04-15-01-S. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LC, MH, AJ, MM, and AA: conceptualization and methodology. LC, MM, and SS: formal analysis. LC, MH, RAD, RAR, SA, AA, SQ, and FM: investigation. LC, SS, MM, and AA: writing—original draft preparation. LC, MH, AJ, MM, RAD, RAR, SA, AA, SQ, FM, SS, LS, and AA: writing—review and editing. MM: visualization. LC: supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.830262/full#supplementary-material

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Associations of Ready-to-Eat Cereal Consumption and Income With Dietary Outcomes: Results From the National Health and Nutrition Examination Survey 2015–2018

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Background: Ready-to-eat (RTE) cereal has been associated with higher diet quality but it is not known if this association differs by income.

Objective: To investigate the association of RTE cereal with dietary outcomes in a US population stratified by income [measured using the poverty-to-income ratio (PIR)].

Methods: Data from children 2–18 years ($n = 5{,}028$) and adults 19 years and older ($n = 9{,}813$) with 24-h dietary recalls from the cross-sectional, US nationally-representative 2015–2016 and 2017–2018 National Health and Nutrition Examination Surveys (NHANES) were used in a multivariable linear model that included cereal eating status (based on day 1 24 h dietary recall), PIR category (Low-PIR <1.85; Mid-PIR 1.85–3.50; High-PIR >3.50) and their interaction. PIR is based on the ratio of the family household income to the poverty level set by the US Department of Health and Human Services and higher PIR values indicate higher household income.

Results: For children, there were positive associations between RTE cereal consumption and nutrient (e.g., iron, calcium, fiber, potassium and vitamin D, $p < 0.001$) and food group (e.g., whole grain and dairy, $p < 0.001$) intake and 2015-HEI ($p < 0.0001$) but no association with PIR or RTE cereal-PIR interaction. For adults, PIR category was positively associated with the intake of nutrients (e.g., fiber, magnesium, potassium, and vitamin C, $p < 0.001$) as was RTE cereal consumption (e.g., fiber, calcium, vitamin D, potassium, vitamin B_{12}, among others, $p < 0.001$). Both PIR and RTE cereal were positively associated with whole grain, dairy, and fruit ($p < 0.001$) and 2015-HEI ($p < 0.0001$) for adults. We also found a significant interaction between PIR and RTE cereal for adults for iron, phosphorus, B vitamins, and dairy ($p < 0.001$). RTE cereal contributed to one quarter or more of daily intake, across all age and PIR groups, for several B vitamins, iron, zinc, and whole grains. Added sugar intake was not associated with RTE cereal consumption in either children or adults.

Conclusion: RTE cereal was associated with improved dietary outcomes, including increased intake of under-consumed nutrients, increased intake of recommended food

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groups, and higher diet quality, at all income levels. This work can help inform future dietary recommendations.

Keywords: dietary outcomes, diet quality, ready-to-eat breakfast cereals, income, poverty-to-income ratio, food groups

INTRODUCTION

Ready-to-eat (RTE) cereal is a staple breakfast food with approximately one-fifth of Americans consuming RTE cereal on any given day (1, 2). RTE cereal has been shown to be an important food nutritionally by encouraging milk consumption, contributing several key under-consumed dietary components including whole grains, fiber, iron, folate, vitamin B12, and vitamin D and being associated with overall higher diet quality in the US (1–3) and other countries (4–8). It has long been relied on as a pragmatic and affordable food that can help support dietary quality and remains an important part of several federal feeding programs.

Based on data from the 2019 US census, 10.5% of all Americans, and 14.4% of children younger than 18 were living in households with income below the poverty threshold (9). The poverty-to-income ratio (PIR) is a measure of a household's income compared to the poverty threshold set by the US government. Social determinants of health, including household income, have been associated with numerous health outcomes and this relationship may be mediated by diet quality. Access to affordable, acceptable foods that promote a high-quality diet may help to offset some of the negative health consequences of lower income status. However, there is limited information available on how specific food categories, such as RTE cereal, are associated with dietary outcomes at different income levels. Understanding the associations between income, specific foods and dietary outcomes is, therefore, essential to providing compelling and pragmatic dietary guidance.

The aim of this study was to investigate the association of RTE cereal, income, measured using PIR, and their interaction with dietary outcomes, including nutrient and food group intake, diet quality and the contribution of RTE cereal to nutrient and food group intake, in American children and adults. We hypothesized that RTE cereal would be associated with higher intake of key nutrients and food groups and higher diet quality at all income levels and that there would be a significant interaction between RTE cereal intake and income on these dietary outcomes.

METHODS

Data Source and Study Participants

The National Health and Nutrition Examination Survey (NHANES) 2015–2016 (10) and 2017–2018 (11) and the Food Patterns Equivalents Database (FPED) 2015–2016 (12) and 2017–2018 (13) data were combined for these analyses. Participants aged 2 years and older, excluding women 20–44 years and older who were pregnant and lactating, with reliable (defined by NHANES) dietary data were included.

NHANES is a nationally representative survey of the US non-institutionalized population that is collected by the National Center for Health Statistics and the Centers for Disease Control and Prevention on an ongoing basis in 2-year cycles. NHANES collects demographic, dietary and health variables *via* an in-person examination and questionnaire. Dietary information was collected by a trained dietary interviewer using a 24-h dietary recall following a validated multi-pass methodology. Dietary information from children under 6 years of age was gathered using proxy interviews without the child present; for children 6–9 years of age, proxy interviews were conducted with the child present; and children 9–11 years provided information on their dietary intake with the assistance of an adult familiar with their intake. This study used de-identified publicly available data not requiring ethical review.

Ready-to-Eat Cereal Consumption and Poverty-to-Income Ratio

RTE cereal consumption was defined according to the day 1 24-h dietary recall and was identified using the "Ready-to-Eat Cereal" category in the What We Eat in America food classification approach defined by United States Department of Agriculture (USDA) for use with NHANES. Participates that reported consuming any quantity of RTE cereal were defined as "Cereal Eaters" while all other participants were "Cereal Non-Eaters." PIR is a pre-defined continuous variable in NHANES and is based on the ratio of the family household income to the poverty level set by the US Department of Health and Human Services. The higher the PIR value, the higher the household income with PIR values above 1 indicating household incomes above the poverty line and those at 1 or lower indicating household incomes at the poverty line or lower. We imputed missing PIR values (9% for children, 12% for adults) using a stepwise regression fitted separately for children and adults. We created three categories based on PIR: Low-PIR $<$1.85; Mid-PIR 1.85–3.50; and High-PIR $>$3.50. The lower cut-off is based on the eligibility criteria for some US federal food assistance programs including reduced price school meals and the Women, Infants and Children (WIC) program. The upper cut-off has been used previously to define higher-income (14).

We reported the following demographic characteristics of our stratified population: age (continuous); total energy intake (continuous); gender (dichotomous); ethnicity (categorical); overweight and obesity (categorical); smoking status (categorical); and household food security (categorical). Overweight and obesity, was defined in NHANES dataset based on adult BMI $\geq$25 kg/m^2 or child BMI above the 85th percentile for age and sex according to Centers for Disease Control growth charts. Household food security is based on the number of affirmative responses to a food security survey administered as part of NHANES questionnaire (15).

Study Outcomes

Daily food and beverage intake from the 24-h dietary recalls was converted to daily nutrient intakes in NHANES using the Food and Nutrient Database for Dietary Studies (FNDDS) 2015–2016 and 2017–2018 (16, 17). We reported results for 23 nutrients as the mean daily value. The FPED converts the foods and beverages in the FNDDS to the USDA Food Pattern food groups by disaggregating each individual food and beverage and summing the intake of each food group across the day. We reported the mean daily intake of 14 food groups and subgroups.

Diet quality was measured using the Healthy-Eating Index (HEI)-2015, developed by the National Cancer Institute, which measures how aligned an individual's daily dietary intake is with the recommendations of the 2015 Dietary Guidelines for Americans (18). Briefly, the HEI-2015 scores an individual's dietary intake, per 1,000 kcal, across 13 dimensions of nutrient and food group intake including nine adequacy components and 4 moderation components with a higher score indicating greater alignment with dietary recommendations and a maximum possible score of 100. Lastly, for RTE cereal eaters stratified by PIR categories, we calculated the percent contribution of RTE cereal to nutrient and food group intakes calculated as a ratio of the mean.

Covariates

We adjusted our models for age (continuous), total energy intake (continuous), gender, and race/ethnicity. Race/ethnicity was self-selected by participants from the following categories: Mexican American; Other Hispanic; Non-Hispanic White; Non-Hispanic Black; Other Race including multi-racial.

Statistical Analysis

SAS 9.3 (SAS Institute, Cary, NC, USA) was used for data analysis. NHANES 2015–2018 sample weight and SAS survey procedures were applied (19). We used a multivariable linear model to investigate the association between cereal eating status (cereal eaters vs. cereal non-eaters), PIR category (low-PIR, mid-PIR, high-PIR), and their interaction, adjusted for our covariates, with nutrient, food group, and diet quality outcomes. We also reported the p-values for a model that included cereal eating status, PIR as a continuous variable and their interaction in **Table S1**. We reported the lest-squared means for the six RTE cereal-PIR interaction terms from this model. We used a Bonferroni corrected p-value of 0.001 (0.05/36 nutrient and food group outcomes) for our nutrient and food group outcomes and a p-value of 0.004 (0.05/14 components & overall HEI score) for HEI-2015.

RESULTS

Demographic Characteristics

Demographic characteristics of children and adults are reported in **Table 1**. Children were most likely to be in the low-PIR cereal non-eater group (27.4%) and least likely to be in the high-PIR cereal eater group (8.4%). For children, 35.7% of cereal eaters were in the low-PIR category, 33.3% in the mid-PIR category, and 28.5% in the high-PIR category (data not shown). Adults were also most likely to be in the high-PIR cereal non-eater group (34.3%) and least likely to be classified as mid-PIR cereal eaters (4.7%) (**Table 1**). For adults, 14.9, 15.4, and 16.0% for the low-, mid-, and high-PIR categories, respectively (data not shown).

Among children, cereal eaters in all PIR categories were on average slightly younger and, for adults, cereal eaters were slightly older. Total dietary energy intake ranged across groups from 1,836 to 1,952 kcal/day for children and from 2,050 to 2,292 kcal/day. The prevalence of children in the non-Hispanic white race/ethnicity category ranged from 33.3% for children and 45.2% for adults in the low-PIR cereal non-eater group to 71.8% for children and 86.0% for adults for the high-PIR cereal eater group. The prevalence of overweight and obesity among children ranged from 26.9% (high-PIR cereal eaters) to 42.2% (low-PIR cereal non-eaters) while for adults the prevalence ranged from 66.9% (mid-PIR cereal eaters) to 74.7% (high-PIR cereal non-eaters). Smoking status among adults ranged from 3.5% for the high-PIR cereal eaters to 29.1% for the low-PIR cereal non-eaters. Lastly, household food security for children was from 34.6% (low-PIR cereal eaters) to 92.4% (high-PIR cereal non-eaters) and for adults, it ranged from 41.9% (low-PIR cereal non-eaters) to 95.8% (high-PIR cereal eaters) (**Table 1**).

Nutrient and Food Group Outcomes

For both children and adults, RTE cereal eating was associated with many of the nutrients and food groups investigated (**Table 2** for children; **Table 3** for adults). RTE cereal was associated with higher intake of carbohydrates, fiber, total sugar, calcium, iron, magnesium, phosphorus, potassium, zinc, folate, niacin, riboflavin, thiamine, vitamins A, B6, B12, C (adults only), and D, total dairy, fluid milk, total fruit (adults only), intact fruit (adults only) and whole grains. RTE cereal eating was also associated with lower intake for children and adults of total fat, saturated fat, selenium, sodium, refined grains (children only), total protein foods, and total meat, poultry, seafood, and egg intake (**Tables 2**, **3**).

For children, PIR category and RTE cereal-PIR interaction were not significant for any nutrients or food groups (**Table 3**). Results were similar when PIR was included as a continuous variable (**Table S1**). For adults, there were negative associations with PIR for carbohydrates, total sugars, added sugar, and refined grain and positive associations for fiber, protein, magnesium, phosphorus, potassium, vitamin C, fluid milk, total fruit, intact fruit, and total vegetable intake. There were significant interactions between RTE cereal eating and PIR category among adults for iron, phosphorus, riboflavin, thiamine, total dairy, and fluid milk intake (**Table 3**).

Diet Quality

RTE cereal consumption was significantly associated with higher diet quality for children and adults. For adults, but not children, PIR was positively associated with HEI (i.e., a higher PIR, meaning higher household income, was associated with better diet quality) and the RTE cereal-PIR interaction were also

TABLE 1 | Demographic characteristics for American children and adults by ready-to-eat cereal consumption and poverty-to-income ratio, National Health and Nutrition Examination Survey, 2015–2018[a].

	Children 2–18 years						Adults 19 years and older					
	Low-PIR		Mid-PIR		High-PIR		Low-PIR		Mid-PIR		High-PIR	
	RTEC eaters	Non-eaters	RTEC eaters	Non-eaters	RTEC eaters	Non-eaters	RTEC eaters	Non-eaters	RTEC eaters	Non-eaters	RTEC eaters	Non-eaters
N (% of population)	984 (17.0%)	1,767 (27.4%)	430 (9.1%)	854 (18.6%)	280 (8.4%)	712 (19.4%)	625 (5.0%)	3,616 (25.8%)	423 (4.7%)	2,389 (22.6%)	445 (7.6%)	2,314 (34.3%)
Age, mean ± SE, years	9.1 ± 0.2	10.5 ± 0.2	9.3 ± 0.3	10.5 ± 0.2	9.8 ± 0.4	10.6 ± 0.3	47.9 ± 1.2	44.5 ± 0.7	52.2 ± 1.7	48.1 ± 0.6	54.1 ± 1.0	49.0 ± 0.8
Gender, *n* (%) female	483 (49.1%)	931 (51.0%)	204 (46.9%)	418 (46.2%)	142 (52.6%)	348 (46.2%)	326 (52.1%)	1,891 (54.7%)	215 (50.4%)	1,190 (50.5%)	207 (51.0%)	1,134 (48.1%)
Energy intake, mean ± SE, kcal/day	1,840 ± 33	1,836 ± 35	1,916 ± 54	1,857 ± 34	1,839 ± 63	1,952 ± 41	2,292 ± 68	2,050 ± 24	2,286 ± 88	2,087 ± 36	2,128 ± 36	2,172 ± 28
Race/ethnicity, *n* (%) non-hispanic white	254 (36.3%)	389 (33.3%)	155 (53.7%)	311 (57.6%)	127 (71.8%)	324 (71.4%)	226 (52.3%)	1,032 (45.2%)	197 (70.3%)	757 (58.5%)	261 (86.0%)	942 (75.6%)
Overweight and Obesity, *n* (%)[b]	378 (37.8%)	733 (42.2%)	127 (29.5%)	331 (40.0%)	80 (26.9%)	186 (27.1%)	452 (70.0%)	2,636 (72.2%)	295 (66.9%)	1,178 (72.5%)	287 (67.1%)	1,671 (74.7%)
Smoking status, *n* (%) current smoker[c]	–	–	–	–	–	–	122 (20.8%)	941 (29.1%)	61 (15.9%)	433 (19.3%)	20 (3.5%)	238 (11.1%)
Household food security, *n* (%) full security[d]	337 (34.6%)	692 (38.3%)	260 (65.8%)	516 (64.9%)	253 (92.2%)	634 (92.4%)	259 (42.2%)	1,415 (41.9%)	303 (76.7%)	1,454 (63.5%)	411 (95.8%)	2,001 (90.3%)

[a]*Data are from the National Health and Nutrition Examination Survey (NHANES) 2015–2018 and are presented as mean ± standard error (SE) for continuous variables or n and percentage (%) for categorical variables, as indicated. Cereal eaters were defined as those that consumed any amount of ready-to-eat cereal on their day 1 24 h dietary recall. PIR bands were defined as Low-PIR <1.85; Mid-PIR 1.85–3.50; and High-PIR >3.50.*

[b]*Missing values for overweight and obesity prevalence ranged from 0.2% (n = 3) for high PIR children cereal eaters to 2% (n = 22) for low PIR children cereal eaters.*

[c]*Only reported in NHANES 2015–2018 for ages 12 years and older. There was 0.1% (n = 7) of data missing for low PIR adult cereal non-eaters and 0.1% (n = 2) of missing data for high PIR adult cereal non-eaters; all other categories had no missing values for smoking status.*

[d]*All PIR-cereal eating groups had some missing values for household full security ranging from 1.0% missing for high-PIR children cereal eaters to 6.0% for mid-PIR adult cereal non-eaters.*

TABLE 2 | Adjusted mean daily nutrient and food group intakes for American children by ready-to-eat cereal consumption and poverty-to-income ratio status, National Health and Nutrition Examination Survey, 2015–2018[a].

	Low-PIR		Mid-PIR		High-PIR		*P*-values[b]		
	RTEC eaters	**RTEC non-eaters**	**RTEC eaters**	**RTEC non-eaters**	**RTEC eaters**	**RTEC non-eaters**	**Cereal eating *p*-value**	**PIR category *p*-value**	**Cereal eating*PIR category interaction *p*-value**
Carbohydrate, g/d	254 ± 2	235 ± 1	254 ± 3	232 ± 2	256 ± 3	236 ± 3	<0.0001	0.65	0.68
Fiber, g/d	14.7 ± 0.2	13.3 ± 0.2	15.0 ± 0.5	14.1 ± 0.3	20.5 ± 0.8	16.7 ± 0.3	<0.0001	0.031	0.34
Total sugars, g/d	116 ± 2	103 ± 1	116 ± 3	101 ± 2	113 ± 3	103 ± 3	<0.0001	0.76	0.74
Protein, g/d	64.6 ± 0.9	66.1 ± 0.7	65.9 ± 1.4	67.2 ± 1.1	64.1 ± 1.5	66.9 ± 1.6	0.061	0.47	0.77
Total fat, g/d	68.2 ± 0.6	74.8 ± 0.5	68.1 ± 0.9	76.2 ± 0.9	68.4 ± 1.0	75.0 ± 0.8	<0.0001	0.72	0.47
Saturated fat, g/d	24.3 ± 0.3	25.5 ± 0.3	24.3 ± 0.5	26.3 ± 0.5	23.4 ± 0.6	25.5 ± 0.5	<0.0001	0.33	0.29
Calcium, mg/d	1,063 ± 19	889 ± 18	1,152 ± 41	902 ± 14	1,041 ± 28	892 ± 22	<0.0001	0.11	0.29
Iron, mg/d	18.5 ± 0.3	10.8 ± 0.2	18.0 ± 0.5	11.0 ± 0.2	18.3 ± 0.5	10.9 ± 0.2	<0.0001	0.86	0.59
Magnesium, mg/d	241 ± 2	219 ± 3	247 ± 5	223 ± 4	249 ± 7	230 ± 4	<0.0001	0.15	0.85
Phosphorus, mg/d	1,258 ± 14	1,191 ± 13	1,310 ± 28	1,212 ± 15	1,255 ± 31	1,201 ± 21	<0.0001	0.094	0.63
Potassium, mg/d	2,209 ± 29	2,023 ± 23	2,240 ± 46	2,025 ± 30	2,199 ± 43	2,100 ± 37	<0.0001	0.74	0.18
Selenium, µg/d	90.5 ± 1.5	95.9 ± 1.3	90.5 ± 2.2	98.1 ± 2.5	89.7 ± 2.8	95.5 ± 2.0	0.0001	0.78	0.83
Sodium, mg/d	2,813 ± 43	3,017 ± 24	2,804 ± 59	3,009 ± 33	2,786 ± 73	3,002 ± 57	0.0001	0.91	0.99
Zinc, mg/d	11.8 ± 0.2	8.3 ± 0.1	12.3 ± 0.3	8.3 ± 0.2	11.2 ± 0.4	8.1 ± 0.2	<0.0001	0.058	0.36
Folate, µg DFE/d[c]	746 ± 16	370 ± 8	708 ± 22	378 ± 7	692 ± 20	371 ± 11	<0.0001	0.16	0.23
Niacin, mg/d	25.2 ± 0.4	18.7 ± 0.3	24.9 ± 0.5	19.0 ± 0.4	24.4 ± 0.7	19.2 ± 0.6	<0.0001	0.93	0.35
Riboflavin, mg/d	2.29 ± 0.03	1.55 ± 0.03	2.31 ± 0.05	1.58 ± 0.03	2.16 ± 0.06	1.62 ± 0.03	<0.0001	0.35	0.03
Thiamine, mg/d	1.90 ± 0.03	1.31 ± 0.02	1.88 ± 0.04	1.33 ± 0.03	1.86 ± 0.06	1.32 ± 0.02	<0.0001	0.82	0.58
Vitamin A, µg RAE/d[d]	769 ± 15	459 ± 12	779 ± 28	485 ± 15	750 ± 35	514 ± 24	<0.0001	0.56	0.27
Vitamin B_6, mg/d	2.29 ± 0.05	1.35 ± 0.03	2.23 ± 0.06	1.38 ± 0.03	2.10 ± 0.08	1.42 ± 0.05	<0.0001	0.50	0.060
Vitamin B_{12}, µg/d	6.41 ± 0.14	3.47 ± 0.09	6.28 ± 0.16	3.43 ± 0.12	5.97 ± 0.30	3.16 ± 0.11	<0.0001	0.13	0.85
Vitamin C, mg/d	77.6 ± 3.2	71.2 ± 2.2	74.3 ± 6.8	67.5 ± 5.2	94.5 ± 8.1	79.0 ± 3.7	0.0070	0.068	0.47
Vitamin D, µg/d	7.40 ± 0.16	4.11 ± 0.15	7.17 ± 0.29	3.91 ± 0.14	6.41 ± 0.36	3.82 ± 0.15	<0.0001	0.024	0.26
Vitamin E, mg/d	7.22 ± 0.21	7.05 ± 0.16	6.94 ± 0.20	7.21 ± 0.19	8.54 ± 0.78	7.65 ± 0.27	0.37	0.13	0.16
Added sugars, tsp eq/d[e]	16.2 ± 0.4	15.5 ± 0.4	16.4 ± 0.6	14.8 ± 0.5	15.8 ± 0.6	14.4 ± 0.7	0.0065	0.39	0.54
Total dairy, cup eq/d	2.22 ± 0.05	1.66 ± 0.05	2.33 ± 0.10	1.69 ± 0.03	1.95 ± 0.10	1.57 ± 0.06	<0.0001	0.0074	0.21
Fluid milk, cup eq/d	1.56 ± 0.05	0.85 ± 0.05	1.44 ± 0.13	0.74 ± 0.06	1.29 ± 0.12	0.76 ± 0.04	<0.0001	0.10	0.36
Cheese and yogurt, cup eq/d	0.68 ± 0.03	0.78 ± 0.03	0.83 ± 0.08	0.85 ± 0.04	0.72 ± 0.06	0.74 ± 0.04	0.13	0.030	0.47
Total fruit, cup eq/d	1.12 ± 0.07	0.98 ± 0.05	1.05 ± 0.11	1.03 ± 0.08	1.28 ± 0.15	1.19 ± 0.06	0.14	0.12	0.76
Fruit juice, cup eq/d	0.41 ± 0.03	0.38 ± 0.02	0.36 ± 0.08	0.38 ± 0.06	0.35 ± 0.06	0.37 ± 0.03	0.83	0.41	0.75
Intact fruit, cup eq/d	0.71 ± 0.06	0.59 ± 0.04	0.69 ± 0.07	0.66 ± 0.04	0.93 ± 0.14	0.82 ± 0.06	0.10	0.049	0.69
Total grains, oz eq/d	6.60 ± 0.15	6.83 ± 0.12	6.59 ± 0.13	6.84 ± 0.12	7.02 ± 0.22	6.89 ± 0.15	0.31	0.33	0.41
Refined grains, oz eq/d	5.53 ± 0.16	6.20 ± 0.12	5.39 ± 0.14	6.06 ± 0.12	5.72 ± 0.22	6.15 ± 0.14	<0.0001	0.35	0.70

(Continued)

TABLE 2 | Continued

	Low-PIR		Mid-PIR		High-PIR		*P*-values[b]		
	RTEC eaters	RTEC non-eaters	RTEC eaters	RTEC non-eaters	RTEC eaters	RTEC non-eaters	Cereal eating *p*-value	PIR category *p*-value	Cereal eating*PIR category interaction *p*-value
Whole grains, oz eq/d	1.07 ± 0.04	0.64 ± 0.05	1.21 ± 0.08	0.78 ± 0.05	1.31 ± 0.09	0.74 ± 0.07	<0.0001	0.015	0.58
Total protein foods, oz eq/d	3.79 ± 0.12	4.61 ± 0.14	3.75 ± 0.23	4.86 ± 0.18	3.94 ± 0.24	4.76 ± 0.21	<0.0001	0.68	0.71
Nuts, seeds and legumes, oz eq/d	0.65 ± 0.06	0.68 ± 0.05	0.75 ± 0.12	0.87 ± 0.10	0.76 ± 0.15	0.78 ± 0.09	0.41	0.076	0.88
Meat, poultry, seafood and eggs, oz eq/d	3.14 ± 0.13	3.93 ± 0.14	3.00 ± 0.21	3.99 ± 0.18	3.18 ± 0.25	3.97 ± 0.23	<0.0001	0.94	0.86
Total Vegetables, cup eq/d	0.84 ± 0.03	0.89 ± 0.02	0.85 ± 0.05	0.88 ± 0.03	0.80 ± 0.04	0.99 ± 0.04	0.0018	0.72	0.16

[a]Data are from the National Health and Nutrition Examination Survey (NHANES) and Food Patterns Equivalent Database (FPED) 2015–2018 and are presented as mean ± standard error (SE). Cereal eaters were defined as those that consumed any amount of ready-to-eat cereal on their day 1 24 h dietary recall. PIR bands were defined as Low-PIR <1.85; Mid-PIR 1.85–3.50; and High-PIR >3.50.
[b]P-values were calculated using a multivariable linear model that included RTE cereal eating status (dichotomous), PIR status (categorical) and their interaction as the exposure and adjusted for age, gender, race/ethnicity and total energy intake. A $p < 0.001$ was considered statistically significant.
[c]DFE, Dietary folate equivalents 1 μg DFE = 1 μg naturally occurring food folate OR = 0.6 μg folic acid added in fortification.
[d]RAE, retinol activity equivalents 1 μg RAE = 1 μg retinol OR 12 μg dietary beta-carotene OR 24 μg dietary alpha-carotene or beta-cryptoxanthin.
[e]1 tsp eq. = 4.2 g of added sugar.

associated with HEI-2015, with larger gaps observed between cereal eaters and non-eaters at the mid-PIR and high-PIR categories compared to the low-PIR group (**Table 4**). For the HEI-2015 subcomponents, RTE cereal consumption, for both children and adults, was associated with a higher score (i.e., more aligned with dietary guidance) for the whole grains ($p < 0.0001$) total dairy ($p < 0.0001$), whole fruit (adults only, $p < 0.0001$), sodium ($p \leq 0.0001$), refined grains ($p \leq 0.0006$), and saturated fat ($p \leq 0.0003$) components (**Table 4**). RTE cereal eating was also associated with lower scores (less aligned with guidelines) for the total protein foods ($p \leq 0.0003$), fatty acids ($p \leq 0.0001$), and added sugar (children only, $p = 0.0024$) components (**Table 4**).

Contribution of RTE Cereal to Daily Nutrient and Food Group Intake

RTE cereal contributed ∼10% to daily energy intake across all age and PIR groups. RTE cereal contributed to one third or more of daily intake, across all age and PIR groups, for folate, iron, whole grains, and vitamins B6 and B12 (**Table 5**). Depending on age and PIR category, RTE cereal contributed to 14–21% of daily added sugar; 2–3% of saturated fat; and 6–9% of sodium intake (**Table 5**).

DISCUSSION

This is the first study, to our knowledge, to show that RTE cereal is associated with higher consumption of under-consumed nutrients, recommended food groups and diet quality, for children and adults living in low-, mid-, and high-income households. It has been hypothesized that lower income status leads to deleterious dietary intakes, but we only found significant associations between PIR category and dietary outcomes for adults and not children. For adults, RTE cereal consumption may help offset some of the deleterious associations of lower-income status with nutrient intakes. These results are consistent with a previous study that also found income was associated with dietary outcomes in adults but not children (20). These associations may be due to the contribution of RTE cereal itself to dietary intakes—for example, among RTE cereal eaters, RTE cereal contributed to over a quarter of daily folate, iron, vitamin A, B vitamins, and vitamin D intake—or due to overall dietary patterns that are associated with RTE cereal consumption.

RTE cereal consumption, in both children and adults, was associated with higher intake of whole grains and fluid milk and, for adults, intact fruit consumption. RTE cereal is one of the top sources of whole grain in the US diet (21) and it has been shown that RTE cereal is eaten with fluid milk at over 80% of RTE cereal eating occasions (1, 2). Like our previous studies, we also found that RTE cereal consumption was associated with lower intake of meat, poultry and seafood (1, 2). A recently published meta-analysis found that dietary patterns in adults that were higher in whole grains, fruits, legumes, nuts, and seeds (among other components) and lower in red and processed meat were associated with lower all-cause mortality (22). Therefore, the food groups associated with RTE cereal may lead to a more favorable overall dietary pattern and potential beneficial health outcomes.

TABLE 3 | Adjusted mean daily nutrient and food group intakes and diet quality for American adults by ready-to-eat cereal consumption and poverty-to-income ratio status, National Health and Nutrition Examination Survey, 2015–2018[a].

	Low-PIR		Mid-PIR		High-PIR		*P*-values[b]		
	RTEC eaters	**RTEC non-eaters**	**RTEC eaters**	**RTEC non-eaters**	**RTEC eaters**	**RTEC non-eaters**	**Cereal eating *p*-value**	**PIR category *p*-value**	**Cereal eating*PIR category interaction *p*-value**
Carbohydrate, g/d	278 ± 3	250 ± 2	286 ± 5	243 ± 2	264 ± 4	234 ± 2	<0.0001	<0.0001	0.027
Fiber, g/d	19.6 ± 0.5	16.4 ± 0.3	20.5 ± 0.8	16.7 ± 0.3	24.0 ± 0.5	18.1 ± 0.3	<0.0001	<0.0001	0.025
Total sugars, g/d	120 ± 2	108 ± 2	130 ± 6	103 ± 2	109 ± 4	92 ± 2	<0.0001	0.0001	0.12
Protein, g/d	78.7 ± 1.5	79.1 ± 0.8	75.0 ± 1.8	82.3 ± 0.9	85.4 ± 1.9	84.2 ± 0.9	0.097	0.0001	0.0055
Total fat, g/d	75.7 ± 1.4	82.0 ± 0.6	72.7 ± 2.0	83.9 ± 0.8	77.6 ± 1.8	84.9 ± 0.8	<0.0001	0.099	0.14
Saturated fat, g/d	26.0 ± 0.5	25.9 ± 0.3	24.3 ± 0.7	27.1 ± 0.3	24.5 ± 0.7	26.7 ± 0.3	0.0002	0.72	0.0068
Calcium, mg/d	1163 ± 20	845 ± 8	1045 ± 31	887 ± 13	1128 ± 27	885 ± 12	<0.0001	0.14	0.0012
Iron, mg/d	22.7 ± 0.4	12.4 ± 0.1	22.5 ± 0.6	12.7 ± 0.1	20.2 ± 0.5	12.9 ± 0.1	<0.0001	0.0011	0.0002
Magnesium, mg/d	322 ± 6	283 ± 4	318 ± 7	298 ± 5	366 ± 9	314 ± 4	<0.0001	<0.0001	0.016
Phosphorus, mg/d	1,476 ± 26	1,300 ± 10	1,385 ± 22	1,337 ± 13	1,519 ± 26	1,359 ± 11	<0.0001	0.0005	0.0006
Potassium, mg/d	2,812 ± 51	2,467 ± 23	2,720 ± 71	2,545 ± 25	3,034 ± 52	2,658 ± 28	<0.0001	<0.0001	0.089
Selenium, µg/d	109 ± 4	115 ± 1	100 ± 3	117 ± 1	116 ± 3	118 ± 1	0.0004	0.014	0.0055
Sodium, mg/d	3,348 ± 50	3,457 ± 41	3,121 ± 60	3,520 ± 34	3,315 ± 80	3,563 ± 40	<0.0001	0.13	0.0011
Zinc, mg/d	13.5 ± 0.3	10.1 ± 0.1	13.2 ± 0.3	10.4 ± 0.1	13.7 ± 0.4	10.7 ± 0.1	<0.0001	0.31	0.45
Folate, µg/d DFE[c]	878 ± 27	434 ± 8	865 ± 27	433 ± 6	830 ± 22	452 ± 7	<0.0001	0.55	0.074
Niacin, mg/d	31.1 ± 0.9	24.3 ± 0.3	28.8 ± 0.7	24.9 ± 0.4	28.5 ± 0.6	25.1 ± 0.4	<0.0001	0.29	0.0083
Riboflavin, mg/d	2.73 ± 0.05	1.82 ± 0.02	2.47 ± 0.07	1.92 ± 0.03	2.41 ± 0.06	1.97 ± 0.03	<0.0001	0.098	<0.0001
Thiamine, mg/d	2.18 ± 0.04	1.46 ± 0.02	2.05 ± 0.04	1.49 ± 0.02	1.91 ± 0.04	1.51 ± 0.02	<0.0001	0.016	<0.0001
Vitamin A, µg/d RAE[d]	918 ± 31	498 ± 13	935 ± 78	569 ± 17	913 ± 50	605 ± 20	<0.0001	0.21	0.19
Vitamin B_6 mg/d	3.07 ± 0.11	1.95 ± 0.06	2.67 ± 0.09	1.93 ± 0.05	2.793 ± 0.07	2.00 ± 0.06	<0.0001	0.10	0.0078
Vitamin B_{12}, µg	8.22 ± 0.59	4.12 ± 0.11	6.48 ± 0.26	4.18 ± 0.13	6.80 ± 0.30	4.22 ± 0.13	<0.0001	0.12	0.031
Vitamin C, mg/d	85.5 ± 4.5	79.7 ± 2.3	88.0 ± 5.4	77.1 ± 2.6	105.7 ± 4.9	89.4 ± 3.0	0.0006	0.0001	0.37
Vitamin D, µg/d	8.25 ± 0.24	3.68 ± 0.11	6.54 ± 0.35	3.91 ± 0.17	7.06 ± 0.50	3.98 ± 0.17	<0.0001	0.023	0.0022
Vitamin E, mg/d	8.29 ± 0.40	8.47 ± 0.17	9.15 ± 0.42	8.70 ± 0.21	10.80 ± 0.79	9.53 ± 0.18	0.11	0.0010	0.20
Added sugars, tsp eq/d[e]	16.1 ± 0.6	17.6 ± 0.5	19.6 ± 1.5	16.2 ± 0.4	13.1 ± 0.8	13.2 ± 0.5	0.42	<0.0001	0.016
Total dairy, cup eq/d	2.10 ± 0.05	1.14 ± 0.02	1.76 ± 0.08	1.22 ± 0.04	1.74 ± 0.08	1.19 ± 0.04	<0.0001	0.0004	<0.0001
Fluid milk, cup eq/d	1.60 ± 0.10	0.42 ± 0.03	1.17 ± 0.09	0.46 ± 0.04	0.89 ± 0.10	0.40 ± 0.04	<0.0001	0.0001	0.0006
Cheese and yogurt, cup eq/d	0.560 ± 0.065	0.643 ± 0.020	0.620 ± 0.065	0.678 ± 0.027	0.649 ± 0.052	0.691 ± 0.031	0.23	0.0030	0.75
Total fruit, cup eq/d	1.18 ± 0.08	0.91 ± 0.04	1.22 ± 0.09	1.97 ± 0.04	1.56 ± 0.09	1.06 ± 0.05	<0.0001	0.0009	0.13
Fruit juice, cup eq/d	0.32 ± 0.05	0.27 ± 0.02	0.29 ± 0.05	0.24 ± 0.02	0.31 ± 0.04	0.24 ± 0.02	0.033	0.79	0.96
Intact fruit, cup eq/d	0.86 ± 0.07	0.64 ± 0.03	0.92 ± 0.08	0.73 ± 0.03	1.25 ± 0.10	0.81 ± 0.04	<0.0001	0.0004	0.14
Total grains oz eq/d	7.37 ± 0.2	6.71 ± 0.1	6.88 ± 0.1	6.60 ± 0.1	6.71 ±, 0.2	6.57 ± 0.1	0.012	0.0037	0.20
Refined grains, oz eq/d	5.96 ± 0.18	6.08 ± 0.11	5.44 ± 0.14	5.87 ± 0.09	5.23 ± 0.17	5.80 ± 0.11	0.022	0.0004	0.25

(Continued)

TABLE 3 | Continued

	Low-PIR		Mid-PIR		High-PIR		*P*-values[b]		
	RTEC eaters	RTEC non-eaters	RTEC eaters	RTEC non-eaters	RTEC eaters	RTEC non-eaters	Cereal eating *p*-value	PIR category *p*-value	Cereal eating*PIR category interaction *p*-value
Whole grains, oz eq/d	1.41 ± 0.10	0.64 ± 0.04	1.44 ± 0.11	0.73 ± 0.05	1.49 ± 0.08	0.77 ± 0.04	<0.0001	0.33	0.90
Total protein foods, oz eq/d	5.53 ± 0.26	6.99 ± 0.12	5.48 ± 0.31	7.12 ± 0.13	7.02 ± 0.23	7.39 ± 0.15	<0.0001	<0.0001	0.0016
Nuts and seeds and legumes, oz eq/d	1.11 ± 0.11	1.37 ± 0.09	1.28 ± 0.17	1.30 ± 0.09	2.12 ± 0.24	1.58 ± 0.09	0.35	0.005	0.11
Meat, poultry, seafood and eggs, oz eq/d	4.42 ± 0.27	5.62 ± 0.11	4.19 ± 0.21	5.82 ± 0.10	4.90 ± 0.23	5.81 ± 0.13	<0.0001	0.13	0.12
Total vegetables cup, eq/d	1.27 ± 0.08	1.44 ± 0.03	1.43 ± 0.12	1.50 ± 0.04	1.65 ± 0.07	1.71 ± 0.06	0.099	<0.0001	0.53

[a]*Data are from the National Health and Nutrition Examination Survey (NHANES) and Food Patterns Equivalent Database (FPED) 2015–2018 and are presented as mean ± standard error (SE). Cereal eaters were defined as those that consumed any amount of ready-to-eat cereal on their day 1 24 h dietary recall. PIR bands were defined as Low-PIR <1.85; Mid-PIR 1.85–3.50; and High-PIR >3.50.*

[b]*P-values were calculated using a multivariable linear model that included RTE cereal eating status (dichotomous), PIR status (categorical) and their interaction as the exposure and adjusted for age, gender, race/ethnicity and total energy intake. A $p < 0.001$ was considered statistically significant.*

[c]*DFE, Dietary folate equivalents 1 μg DFE = 1 μg naturally occurring food folate OR = 0.6 μg folic acid added in fortification.*

[d]*RAE, retinol activity equivalents 1 μg RAE = 1 μg retinol OR 12 μg dietary beta-carotene OR 24 μg dietary alpha-carotene or beta-cryptoxanthin.*

[e]*1 tsp eq. = 4.2 g of added sugar.*

Despite numerous data, including the current study, showing overall beneficial dietary outcomes associated with RTE cereal intake, the sugar content of the RTE cereal category continues to be emphasized (21). We did find that total sugar intake was positively associated with RTE cereal consumption in children and adults. The increased total sugar intake among RTE cereal eaters may be due to the contribution of cereal itself (RTE cereal contributed 11–13% of daily total sugar intake across age and PIR groups) but also due to the higher intake of sugar containing food groups such as fruit and milk and possibly other foods. While RTE cereal did contribute to 14–18% of added sugar intake for adults and 18–21% of added sugar intake for children, added sugar was not associated with RTE cereal consumption. This could indicate compensation in added sugar intake may be taking place with non-RTE cereal eaters potentially consuming added sugar from other food categories. While RTE cereal contributes added sugar to the diet, it is also a nutrient dense food and, as this study has shown, is associated with beneficial dietary intake. Future research should further examine if not consuming RTE cereal is associated with compensatory increases in other dietary sources of added sugar and how that impacts overall dietary intake and quality.

As noted above, we found no gradient in dietary quality across our three income groups for children, but found that for adults, PIR was associated with diet quality. It is possible that children's diet quality may be more resilient in the face of food insecurity in part due to federal feeding programs such as WIC and the School Breakfast Program and National School Lunch Program. These supplemental feeding programs, run by the USDA, have nutrition standards, which have been periodically reviewed and strengthened over time to better align with the evidence-based recommendations within recent Dietary Guidelines for Americans. RTE cereal is included in the WIC package and is often served as part of subsidized school meals. A report from the USDA found that offering RTE cereal on every daily school breakfast menu was associated with a significantly higher HEI score (23) and that the children who consumed RTE cereal had higher whole grain intake and key nutrients including iron, calcium, vitamin D and fiber (24).

Diet cost, food preparation time, access to healthy foods, and nutrition knowledge are commonly cited barriers to healthy eating for those with lower incomes (25). The Thrifty Food Plan, recently released by the USDA, determined the market basket cost of a budget-conscious healthy dietary patterns and includes RTE cereal in its plan. Further work is needed that translate the recommendations within the Dietary Guidelines for Americans into pragmatic dietary advice around specific food categories with consideration to cost, accessibility, and/or ease of preparation for individuals and families facing barriers to healthy eating.

This study has several strengths including using nationally representative dietary intake data collected using rigorous methodology that is publicly available. Further, our analyses relied on detailed dietary intake collected using 24 h recalls through a rigorous validated multiple-pass approach. While 24 h dietary recalls cannot provide estimates of usual dietary intake for individuals, which would be needed studies examining health

TABLE 4 | Healthy Eating Index total score and subcomponents for children and adults by ready-to-eat cereal consumption and poverty-to-income ratio status, National Health and Nutrition Examination Survey, 2015–2018[a].

HEI component (maximum score)		Low-PIR		Mid-PIR		High-PIR		*P*-value[b]		
		RTEC eaters	RTEC non-eaters	RTEC eaters	RTEC non-eaters	RTEC eaters	RTEC non-eaters	Cereal eating *p*-value	PIR category *p*-value	Interaction *p*-value
Total vegetables (5)	Children	2.1 ± 0.1	2.2 ± 0.0	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	0.093	0.64	0.67
	Adults	2.7 ±0.1	3.0 ± 0.0	3.0 ± 0.1	3.1 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	0.15	<0.0001	0.022
Greens and beans (5)	Children	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	1.8 ± 0.2	1.6 ± 0.1	0.77	0.11	0.14
	Adults	1.4 ± 0.1	1.7 ± 0.1	1.8 ± 0.2	1.6 ± 0.1	2.5 ± 0.2	2.1 ± 0.1	0.36	<0.0001	0.0057
Total fruit (5)	Children	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	0.36	0.60	0.64
	Adults	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	0.19	0.68	0.78
Whole fruit (5)	Children	2.3 ± 0.1	2.0 ± 0.1	2.5 ± 0.2	2.3 ± 0.1	2.8 ± 0.3	2.7 ± 0.1	0.10	0.0024	0.88
	Adults	4.0 ± 0.2	1.9 ± 0.1	2.7 ± 0.2	2.0 ± 0.1	3.5 ± 0.2	2.4 ± 0.1	<0.0001	<0.0001	0.019
Whole grain (10)	Children	3.7 ± 0.1	2.1 ± 0.1	4.1 ± 0.2	2.5 ± 0.1	4.2 ± 0.2	2.4 ± 0.2	<0.0001	0.0066	0.90
	Adults	4.0 ± 0.2	1.9 ± 0.1	4.3 ± 0.3	2.2 ± 0.1	4.5 ± 0.2	2.2 ± 0.1	<0.0001	0.028	0.88
Dairy (10)	Children	7.7 ± 0.1	5.7 ± 0.3	7.6 ± 0.2	6.1 ± 0.1	7.0 ± 0.3	5.7 ± 0.2	<0.0001	0.066	0.043
	Adults	6.6 ± 0.1	3.8 ± 0.1	6.0 ± 0.2	4.1 ± 0.1	6.0 ± 0.2	4.1 ± 0.1	<0.0001	0.28	0.0008
Total protein food (5)	Children	3.3 ± 0.1	3.7 ± 0.1	3.4 ± 0.1	3.8 ± 0.1	3.4 ± 0.2	3.8 ± 0.1	0.0001	0.65	0.94
	Adults	3.9 ± 0.1	4.3 ± 0.0	3.8 ± 0.1	4.3 ± 0.0	4.3 ± 0.1	4.4 ± 0.0	0.0003	<0.0001	0.0003
Seafood and plant protein (5)	Children	1.7 ± 0.1	1.5 ± 0.1	1.7 ± 0.2	1.8 ± 0.1	1.7 ± 0.2	1.9 ± 0.1	0.43	0.13	0.10
	Adults	2.1 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	3.3 ± 0.1	2.7 ± 0.1	0.057	<0.0001	0.0035
Fatty acids (10)	Children	3.5 ± 0.2	4.3 ± 0.1	3.4 ± 0.2	4.1 ± 0.2	3.9 ± 0.4	4.5 ± 0.2	0.0001	0.23	0.91
	Adults	4.2 ± 0.2	5.4 ± 0.1	4.3 ± 0.2	5.2 ± 0.1	4.8 ± 0.3	4.1 ± 0.1	<0.0001	0.049	0.062
Sodium (10)	Children	5.5 ± 0.2	4.7 ± 0.1	5.5 ± 0.3	4.8 ± 0.1	5.6 ± 0.3	5.0 ± 0.2	0.0001	0.53	0.93
	Adults	5.0 ± 0.2	4.5 ± 0.1	5.4 ± 0.2	4.2 ± 0.1	4.8 ± 0.3	4.1 ± 0.1	<0.0001	0.17	0.044
Refined grains (10)	Children	5.5 ± 0.2	4.7 ± 0.2	5.8 ± 0.2	4.8 ± 0.2	5.9 ± 0.3	5.1 ± 0.2	<0.0001	0.013	0.83
	Adults	5.9 ± 0.2	5.5 ± 0.1	6.6 ± 0.2	5.8 ± 0.1	6.8 ± 0.2	5.9 ± 0.1	0.0006	0.0001	0.17
Saturated fat (10)	Children	5.7 ± 0.2	5.0 ± 0.1	5.5 ± 0.2	4.7 ± 0.2	5.9 ± 0.3	5.1 ± 0.2	<0.0001	0.32	0.87
	Adults	6.1 ± 0.2	6.1 ± 0.1	6.6 ± 0.2	5.7 ± 0.1	6.6 ± 0.2	5.8 ± 0.1	0.0003	0.76	0.021
Added sugar (10)	Children	6.4 ± 0.1	6.5 ± 0.1	6.1 ± 0.2	6.8 ± 0.2	6.4 ± 0.2	6.9 ± 0.2	0.0024	0.60	0.26
	Adults	6.8 ± 0.1	6.6 ± 0.1	6.3 ± 0.3	6.8 ± 0.1	7.6 ± 0.3	7.6 ± 0.1	0.41	<0.0001	0.067
Total HEI-2015 score (100)	Children	53.2 ± 0.7	48.4 ± 0.5	53.6 ± 0.9	50.1 ± 0.1	54.5 ± 0.7	50.8 ± 0.7	<0.0001	0.066	0.42
	Adults	56.1 ± 0.8	52.0 ± 0.4	58.2 ± 0.8	52.4 ± 0.5	63.5 ± 1.0	55.1 ± 0.4	<0.0001	<0.0001	0.013

[a] *Data are from the Food Patterns Equivalent Database (FPED) 2015–2018 and are presented as mean ± standard error (SE). Cereal eaters were defined as those that consumed any amount of ready-to-eat cereal on their day 1 24 h dietary recall. PIR bands were defined as Low-PIR <1.85; Mid-PIR 1.85–3.50; and High-PIR >3.50.*

[b] *P-values were calculated using a multivariable linear model that included RTE cereal eating status (dichotomous), PIR status (categorical) and their interaction as the exposure and adjusted for age, gender, race/ethnicity and total energy intake. A $p < 0.004$ was considered statistically significant.*

TABLE 5 | Percent contribution of RTE cereal to daily intake of nutrients and food groups for RTE cereal eaters, stratified by age and Poverty-to-income (PIR) category[a].

	Children 2–18 years			Adults 19 years and older		
	Low-PIR $n = 984$	Mid-PIR $n = 625$	High-PIR $n = 280$	Low-PIR $n = 625$	Mid-PIR n =4 23	High-PIR $n = 445$
Energy, %	9.9	9.0	9.2	9.8	9.8	9.4
Folate, %	58.0	53.8	50.2	56.2	56.2	51.0
Iron, %	52.9	49.7	49.7	53.2	52.4	45.4
Whole grains, %	45.5	44.3	50.9	51.3	55.0	60.5
Vitamin B_6, %	45.1	43.0	40.2	39.7	39.2	34.7
Vitamin B_{12}, %	41.7	38.5	38.8	36.9	38.3	34.5
Vitamin A, %	38.1	33.3	31.4	35.2	28.5	21.3
Thiamine, %	38.0	35.2	33.8	35.4	33.9	28.0
Niacin, %	37.6	34.7	32.3	32.7	31.0	24.6
Zinc, %	34.9	33.0	33.5	29.9	30.2	26.1
Riboflavin, %	31.2	27.7	28.1	27.6	26.5	20.1
Vitamin D, %	24.7	23.6	23.5	26.2	24.7	15.9
Added sugars, %	20.5	17.4	17.5	17.9	14.2	15.5
Fiber, %	17.2	17.5	18.9	21.7	22.8	22.0
Total grain, %	16.2	15.0	15.1	18.7	20.0	20.6
Carbohydrate, %	15.7	14.3	14.6	16.9	16.1	16.2
Vitamin C, %	14.7	13.6	11.1	13.0	11.9	7.5
Vitamin E, %	13.9	11.6	12.4	13.9	20.4	15.3
Total sugar, %	12.6	11.4	11.1	12.7	11.2	10.8
Refined grains, %	10.6	8.4	6.3	11.2	10.2	7.4
Magnesium, %	10.2	10.3	11.1	13.0	14.5	13.3
Sodium, %	9.0	8.0	7.0	8.0	7.4	5.7
Calcium, %	7.1	6.7	7.4	6.1	6.8	5.4
Phosphorus, %	6.8	6.8	8.1	8.8	10.5	10.0
Selenium, %	6.2	5.1	5.6	6.4	7.5	8.5
Protein, %	4.8	4.5	5.1	5.4	6.0	5.9
Potassium, %	4.7	5.1	5.6	6.6	7.0	6.6
Total fat, %	3.1	3.2	3.2	2.7	3.2	3.4
Nuts, seeds, and legumes, %	2.8	3.1	2.9	1.9	3.5	3.5
Saturated fat, %	2.6	2.5	2.6	2.0	2.1	1.9
Intact fruit, %	0.6	1.3	0.4	5.2	4.4	3.5
Total protein foods, %	0.5	0.6	0.6	0.4	0.8	1.1
Total fruit, %	0.4	0.9	0.3	3.7	3.4	2.9
Fluid milk, %	0.1	0.0	0.2	0.1	0.3	0.8
Total dairy, %	0.0	0.0	0.1	0.1	0.2	0.5
Fruit juice, %	0.0	0.0	0.0	0.0	0.0	0.0
Total vegetables, %	0.0	0.0	0.0	0.0	0.0	0.0
Cheese and yogurt, %	0.0	0.0	0.0	0.0	0.0	0.0
Meat, poultry, seafood, and eggs, %	0.0	0.0	0.0	0.0	0.0	0.0

[a] *Data are from the National Health and Nutrition Examination Survey (NHANES) and Food Patterns Equivalent Database (FPED) 2015–2018 and are presented as the percent contribution of nutrients and food groups from RTE cereal to daily intake calculated as a ratio of the means. Participants were RTE cereal eaters only and stratified according to age (children 2–18 years and adults 19 years and older), and poverty-to-income ratio (PIR). Cereal eaters were defined as those that consumed any amount of ready-to-eat cereal on their day 1 24 h dietary recall. PIR bands were defined as Low-PIR <1.85; Mid-PIR 1.85–3.50; and High-PIR >3.50. Nutrients and food groups (except for energy) are ranked in order from highest contribution to lowest contribution based on the children low-PIR group.*

outcomes, they do provide reliable estimates of mean usual intake for populations (26).

Despite these strengths, several limitations that should be acknowledged. First, this study used a cross-sectional observational design which limits our ability to make causal inferences. However, because our outcomes are dietary related, we wouldn't expect reverse causation, or significant lag time between exposure and outcomes in this study. Second, this study relies on self-reported dietary intake, which is well-known to include systematic bias. It is possible there may be differences in dietary reporting by PIR status; however, we did not see significant differences in calorie intake across our PIR bands, suggesting that each group reported similar amounts of food intake. It also remains possible that there is residual confounding. We did not fully explore all the possible social determinants of health and there are important intersections between race/ethnicity, immigration status, education, childhood adversity, and income with dietary intake and health status.

Identifying nutrient dense affordable food choices to help support healthy dietary patterns could be a strategy to increase diet quality across all income levels. RTE cereal was associated with improved dietary outcomes, including increased intake of under-consumed nutrients, increased intake of recommended food groups, and higher diet quality, at all income levels. This work can help inform future dietary recommendation and nutrient policy and regulations, particularly those directed toward improving the dietary intakes of lower-income Americans.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NCHS Research Ethics Review Board. The present study used publicly available NHANES data as secondary analysis, which is exempt from IRB review. Written informed consent to participate in the NHANES study was provided by the participants (for adults) or their legal guardian (for children).

AUTHOR CONTRIBUTIONS

JS and YZ designed research. NJ, JN, and NH analyzed the data and performed statistical analysis. JS wrote the article and had primary responsibility for final content. All authors have read and approved the final content.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: All authors are employees of General Mills, a manufacturer of several food categories, including ready-to-eat cereal.

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Dehydration and rehydration affect brain regional density and homogeneity among young male adults, determined *via* magnetic resonance imaging: A pilot self-control trial

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The effects of dehydration and rehydration on brain regional density and homogeneity are unknown and have been infrequently studied. In this pilot self-control study, twelve participants aged 18-25 years were recruited and the brain was scanned using magnetic resonance imaging for three tests under different hydration statuses. In three tests, urine osmolality was determined to assess hydration status. Test 1 was conducted after 12 h of overnight fasting. Test 2 was conducted in a dehydration state induced by 36 h of water deprivation. Test 3 was conducted in a rehydration state, which was induced by 1.5 L of purified water supplementation. Compared with test 1, participants under the dehydration state in test 2 had higher cerebrospinal fluid density ($p < 0.001$). Compared with test 2, participants under the rehydration state in test 3 showed an extensive increase in gray matter density in widespread brain regions, mainly involving the left middle temporal gyrus, cuneus, right thalamus, left rolandic opercula, Brodmann area 39, right precentral, left postcentral gyrus, and cingulate gyrus ($p < 0.001$); a higher white matter density in the temporal lobe, sub-lobar, and sub-gyral areas; and a lower cerebrospinal fluid density ($p < 0.001$). The multimodal, multiscale neuroimaging marker of the human brain connection—the regional homogeneity (ReHo) index—was used for evaluating the connectivity of nodes in the brain. Compared with test 1, participants in test 2 had a lower ReHo value in the right amygdala, left occiput median, right lingual, opercula part of right inferior frontal gyrus, and right precuneus ($p < 0.01$). Compared with test 2, participants in test 3 had a higher ReHo value in the right amygdala, right lingual, opercula part of the right inferior frontal gyrus, and right precuneus ($p < 0.01$). Dehydration state increased cerebrospinal fluid density, decreased brain regional homogeneity. Rehydration state increased brain gray matter and white matter density widespreadly, and increased brain regional homogeneity.

KEYWORDS

water, hydration, magnetic resonance imaging, brain structure, connectivity of nodes

Introduction

Water, as an important nutrient, is essential for the survival and development of life (1). Water plays important roles in various physiological processes, including maintaining the normal osmotic pressure and electrolyte balance of the body fluid, participating in the metabolism of the body, regulating body temperature, etc. (1). Maintaining adequate water intake is vital in ensuring normal physiological functions (1). Insufficient water intake has negative impacts on cognitive performance and physical activity, and it also increases the risk of urinary and cardiovascular diseases (2, 3). Some surveys in China about fluid intake showed that a large proportion of adults, and middle and primary school children did not drink the recommended amount of water, and about a quarter were in a dehydration state, which was judged using the standard of 24 h urine osmolality exceeding 800 mOsm/kg (4–6). According to the results of surveys on water intake in some other countries, the dehydration state is also a common phenomenon due to insufficient water intake in daily life around the world (7–9). However, not enough attention has been paid to the importance of water to health.

The water content of the brain reaches 75% of the brain mass. The water content of the brain's white and gray matter reaches approximately 70 and 82% in brain white and gray matter mass, respectively (1). The sensation of thirst is the basic instinct to acquire water to maintain a normal hydration state (24 h urine osmolality $\leq$ 800 mOsm/kg) (10). The sensation of thirst is caused by increased osmotic pressure in the internal environment (1). The hypothalamus is the receptor and regulatory center of osmotic pressure (1). When osmotic pressure rises, the hypothalamus first receives the stimulation but does not produce the sensation of thirst (1). Then, it passes through the afferent nerve to the cerebral cortex, which triggers the sensation of thirst. In the brain, the Na(+) concentration, plasma osmolality, and cerebrospinal fluid (CSF) are continuously monitored to adjust body-fluid homeostasis (11). In addition, the brain also participates in the control of water intake behaviors (1). When the human body feels thirsty, activations of the subfornical organ, orbitofrontal cortex, and pregenual anterior cingulate cortex are involved in generating a pleasant subjective sensation in response to water intake (12, 13). Thus, it is hypothesized that the brain's structure, brain regional density and homogeneity may be susceptible to changes in the hydration state induced by water intake and water loss.

A few studies have explored the effects of water intake and hydration on brain structure and function and achieved inconsistent results. In a study in 2011, 10 healthy adolescents were recruited, and a dehydration state (urine osmolality > 675 mOsm/kg) was induced by a thermal exercise protocol. The results of brain magnetic resonance imaging (MRI) in the study showed that the blood-oxygen-level-dependent (BOLD) response in the fronto-parietal area was stronger under a dehydrated state (14). It is speculated that the reason for this result was that neuronal activity was higher when dehydrated (14). In a study in 2014, the brains of ten healthy adult participants were scanned using MRI under the dehydration and rehydration states. Additionally, the dehydration state was caused by 14 h of fasting. The rehydration state was reduced after 1.5L of water supplementation. The results showed that the spinal cord cross-sectional area (CSA) decreased under the dehydration state (15). In 2005, a study was conducted among 20 healthy adults, and its results showed that the dehydration state induced after water restriction for 16 h led to a decrease of 0.55% in the brain's volume. Meanwhile, rehydration after 1.5 L of mineral water supplementation led to an increase of 0.72% in the brain's volume (16). In a study in 2016, the brains of 20 healthy adults were scanned with MRI under a dehydration state after 9 h of overnight fasting and under a rehydration state after 3L of water was consumed over 12 h (17). The results indicated that no statistical changes were found for brain total water content and brain volume under different hydration states (17). One more study in 2016 found that the dehydration state induced by exercise without replacing fluid losses reduced total brain volume among 10 sportsmen (18). There was one study that investigated the changes in brain structure under the dehydration state using voxel-based morphology, and it showed that there were associations between the decrease in gray matter (GM) and white matter (WM) volume and the dehydrated state in various brain regions (19). In a study conducted among nine physically active adult participants aged 24 years old, it was found that a dehydration state induced by exercise and heat stress with 2.8% body mass loss decreased intracranial volume, reduced subcortical gray matter volume, and expanded the ventricle and cerebrospinal fluid volumes (20). In a long-term hydration experiment, six healthy young adults 25 years old were recruited, and a dehydration state was induced in two days by water restriction to 150 mL water per day (19). Related studies are few, related studies has been summarized in **Supplementary Table 1**. It is meaningful to conduct further studies to explore the effects of the hydration state on the brain structure and functions using the method of brain magnetic resonance imaging.

The purposes of this study are, first, to analyze the effects of slowly progressive dehydration after 36 h of water deprivation on brain regional density and homogeneity using the method of MRI and, second, to explore the effects of rehydration after an adequate amount of water supplementation on brain regional density and homogeneity among healthy young adults in China. The results of MRI in this study provide more evidence about the importance of hydration. It is also meaningful to bring attention to drinking an adequate amount of water and maintaining an optimal hydration state.

Materials and methods

Participants

Twelve healthy male young adults were recruited from one college in Cangzhou, China.

The inclusion criteria were as follows: the age of participants was between 18 and 25 years; the participants were in a healthy state. The exclusion criteria were as follows: the age of participants were <18 years or >25 years; the participants have a history of smoking, habitually consume a large amount of alcohol (>20 g/day), or perform intensive physical exercise (> 6 METs); or they have diseases of the gastrointestinal tract or of the kidney, cognitive disorders, or other chronic and metabolic diseases.

Sample size calculation

Based on the formula $N = \left[\frac{(\alpha+\beta)\sigma_d}{\delta}\right]^2$, to achieve a power (1-beta) of 0.9 with alpha = 0.05, sigma_d = 1.4 and delta = 1.17, 12 subjects were required. Here, sigma_d = 1.4 and delta = 1.17 were based on reference (17). This sample size was also consistent with previous studies in which the sample size was in the range of 6-20.

Ethics

The study protocol and instruments were reviewed and approved by the Ethical Review Committee of Chinese Nutrition Society on November 10, 2015. The code of identification is CNS-2015-001. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Prior to the conduction of the study, all participants read and signed informed consent voluntarily.

Study design and procedure

The study procedure of the self-control trial is shown in **Figure 1**. During the study, volunteers were asked to not perform vigorous-intensity physical activities (e.g., running, race-walking, hiking uphill, etc.), to not smoke or drink alcohol, and to not consume any kinds of caffeine-containing beverages. All participants were monitored by research supervisors and investigators. Three MRI tests were performed, including test 1 under baseline state, test 2 under dehydration state caused by 36 hours of water deprivation, and test 3 under rehydration state after water supplementation. All tests were conducted in Cangzhou Central Hospital.

Day 1: All participants fasted overnight from 8:00 p.m. and were told to sleep no later than 11 p.m. They were required to not urinate until awaking on day 2.

Day 2: First, urine samples were collected at 8:00 a.m. in the morning using a sterile urine sample accumulator and then sent to be tested by lab technicians in the hospital. Cubital venous blood was collected and sent to determine the blood osmolality and blood glucose. Body measurement and blood pressure were also conducted. Visual analog scales on thirst were conducted, and brain magnetic resonance imaging (MRI) was performed as baseline test 1. After test 1 under the baseline state, participants could eat and drink. After 8:00 p.m., participants were required to fast without any food and water for 12 h.

Day 3: Participants could not drink any fluid and lasted for 24 h from 8:00 a.m. on day 3. Three specified solid meals were supplied to participants by a researcher at 7:00 a.m., for breakfast; at 12:00 a.m., for lunch; and at 5:30 p.m., for dinner. No other food was eaten. Fluid intake from foods was assessed using methods of weighing, duplicate portion, and laboratory analysis. Each urine sample was collected by the participants and then sent for evaluation of the 24 h urine volume by researchers. The urine osmolality of each urine sample was also determined. Participants were required to sleep no later than 11 p.m. and to not urinate until awaking on day 4.

Day 4: At 8:00 a.m., test 2 under dehydration state, the same procedure as test 1, was conducted on participants. Brain magnetic resonance imaging was performed under dehydration state caused by 36 h of water deprivation. Participants drank 1,500 mL of purified water in fifteen minutes at 8:30 a.m. and were required to drink 500 mL every 5 min. After resting for an hour, test 3 under rehydration state was performed. Brain magnetic resonance imaging was performed under rehydration state after water supplementation.

The temperature and humidity of the living environment among participants during these days were measured and noted. In the whole process of the study, participants who failed to meet

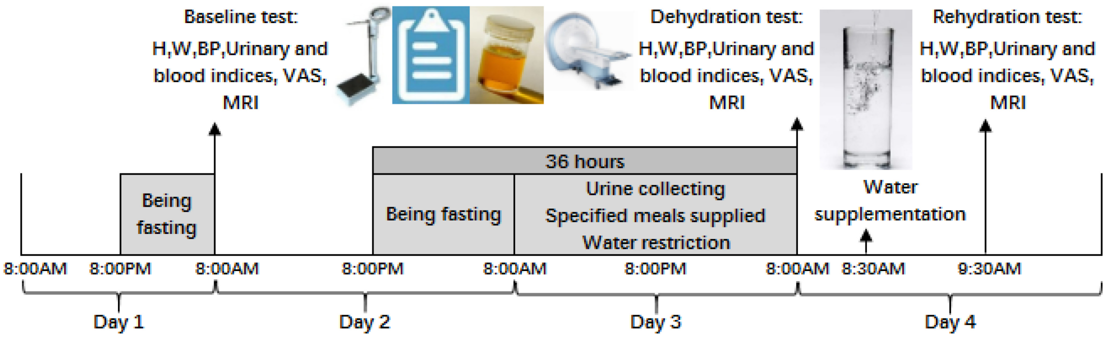

FIGURE 1
Procedure of the study. H is the abbreviation for height; W is weight; BP is blood pressure; VAS is visual analog scales; MRI is magnetic resonance imaging.

these requirements needed to let investigators know. Finally, all participants finished the study, and no one failed to meet the requirements.

Definition of hydration state

The standard for the dehydration state was the urine osmolality exceeding 800 mOsm/kg (21). Optimal hydration state was judged in accordance with the standard of urine osmolality less than or equal to 500 mOsm/kg (22). When urine osmolality was more than 500 mOsm/kg and less than or equal to 800 mOsm/kg, it can be judged as the middle hydration state (6). The rehydration state after water supplementation was defined as a urine osmolality less than or equal to 800 mOsm/kg (21).

Assessment of water intake from foods

Weighing, duplicate portion, and laboratory analysis methods were used to assess water intake from foods. See (23) for the specific steps and calculation methods.

Anthropometric measurements

Wearing light clothing and no footwear, height (H) was measured twice with 0.1 cm accuracy and weight (W) was measured twice with 0.1 kg accuracy by trained investigators using a height–weight meter (HDM-300; Huaju, Yiwu, Zhejiang, China).

Blood pressure (BP) was measured twice with 2 mmHg accuracy by a nurse with electronic sphygmomanometer (HEM-7051; Omrom, Dalian, Liaoning, China). Two measurements were conducted after 2 min intervals.

BMI (Body Mass Index) = weight (kg)/height squared (m^2).

Tests of urine biomarkers

Random spot urine samples were collected in disposable urine storage bags by participants, and then, the samples were stored at +4°C. Starting with the second voiding on day 3 and ending with the first voiding on day 4, all urine samples were collected as total 24 h urine volume on day 3. Urine volume was measured with the accuracy 0.1 kg with electronic desktop scale (YP20001; SPC; Shanghai, China). Additionally, urine osmolality was tested using an osmotic pressure molar concentration meter (SMC 30C; Tianhe, Tianjin, China).

Assessment of blood biomarkers

Cubital venous blood was also used to test osmolality and glucose of blood. Blood osmolality was tested with an osmotic pressure molar concentration meter (SMC 30C; Tianhe, Tianjin, China). Blood glucose was tested with an automatic biochemical analyzer (Cobas C501; Roche, Basel, Switzerland).

Assessment of subjective thirst sensation

Visual analog scales (VAS) are a self-rated 10 cm line designed to quantitatively measure the subjective feeling of thirst (24). The labels "not at all" and "extremely" were anchored at the beginning of the line and its end. Participants were required to draw a vertical line corresponding to their degree of thirst. The range of scores for thirst varied between 0 and 10.

Magnetic resonance imaging scans

Magnetic resonance imaging scans were administered on a 3-teslas SIGNA HDx scanner (Discovery MR 750, General

Electric; Milwaukee, WI). Participants laid flat on the scanning stage. The heads of participants were placed centrally, the mandibular was adducted, and intracranial anterior commissure and posterior commissure (AC-PC) line were as parallel as possible to the axial line. If necessary, the localization of head was realigned. Participants were required to stay awake, to close their eyes, to breathe quietly, and to plug their ears with a rubber stopper to reduce noise interference.

Scout image: First, the scout images were acquired by setting a sequence with parameters TE (time of echo) = 1.6 ms, slice number = 5, slice sickness = 7 mm, FOV (field of view) = 30 mm × 30 mm, matrix = 288 × 128, and NEX (number of excitations) = 1.

Structural MRI: Based on the scout images, structural MRI was performed in parallel with the AC-PC line. The sequence of 3D BRAVO was used with parameters TR (repetition time) = 8.2 ms, TE = 3.2 ms, slice number = 132, slice sickness = 1.2 mm, spacing = 0, flip angle = 12°, FOV = 240 mm × 240 mm, matrix = 256 × 256, NEX = 1, and bandwidth = 31.25.

Temperature and humidity

The temperature and humidity indoor and outdoor were recorded at 10:00 a.m., 2:00 p.m., and 8:00 p.m. with a temperature hygrometer by researchers during the experiment.

Analysis of structural MRI

Data processing of structural MRI was carried out on the network platform of MATLAB (2012a, MathWorks, Natick, MA, USA). VBM (voxel-based morphometry) of the T1 image was analyzed using the neuroimaging computing software SPM8 (Statistical Parametric Mapping[1]) with toolboxes of VBM8 and DARTEL. The process mainly included the following steps: (a) correction, in which the T1 images were reoriented and calibrated to ensure that the anterior commissure was the origin (0,0,0); (b) segmentation, in which the T1 images after the original point correction were segmented into GM (gray matter), WM (white matter), and CSF (cerebrospinal fluid) voxel fraction images; (c) template generation, in which the group template was generated using the DARTEL method (25) and iterated several times; (d) normalization, in which all images of participants were spatially normalized by registration to the Montreal Neurological Institute brain template (MNI152) and the voxel size after registration was 1.5 mm × 1.5 mm × 1.5 mm; and (e) smoothing, in which the smoothing kernel with 8 mm FWHM (full-width at half maximum) was used to smooth the registered GM, WM, and CSF images. The images for the location of brain regions with statistical differences between the two groups were presented by conventional axial bitmap using Software MRICron[2] and BrainNet Viewer[3].

Analysis of functional MRI in resting state (rs-fMRI)

Data processing of fMRI was also carried out on the platform of MATLAB. SPM8 software toolkit was used for data preprocessing. The processing steps were as follows: (a) the data of the first ten time points were removed to ensure data quality and magnetic balance; (b) slice timing correction, in which due to the protocols of fMRI acquisition, slices in the acquisition plane were not acquired simultaneously or sequentially and, thus, slice timing was corrected for this temporal misalignment; (c) realignment, in which realignment strategies were implemented by aligning each image in the time series to the first reference image, and the subjects were excluded if the head was translated by more than 2 mm or rotated more than 2°; (d) covariates, in which analyses were performed by treating WM, CSF, and other signals that were not related to GM as covariates; (e) normalization, in which spatial normalization of the fMRI images was carried out for the differences in anatomical structure, all images were spatially normalized by registration to the MNI152 template, and the size of the voxel after registration was 3 mm × 3 mm × 3 mm; and (f) analysis of regional homogeneity (ReHo) was performed with DPABI software[4] (26).

Statistical analysis

SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used. The mean and standard deviation (SD) were used to describe the quantitative parameters; count data (hydration state) were presented as n (percentage). The differences in brain gray matter, brain white matter, and cerebrospinal fluid among brain areas were calculated using SPM8software. The method of one-way analysis of variance (ANOVA) with replicate measures was used to compare the quantitative parameters among test 1, test 2, and test 3. Then the obtained differential brain regions are subjected to multiple comparison correction (FDR corrected). Finally, the differential brain regions corrected by multiple comparison were used as a mask for post hoc test. The significance levels were set at 0.05 ($p < 0.05$, voxel cluster $> = 10$). The classification data such as the distribution of hydration state were compared using the method of Chi-square test. When the conditions were not suitable for Chi-square test, such as the expected frequency

1 http://www.fil.ion.ucl.ac.uk/spm

2 http://people.cas.sc.edu/rorden/mricron/index.html

3 https://helab.bnu.edu.cn/

4 http://rfmri.org/dpabi

was less than 5, Fisher exact test was used for comparison and analysis. Significance levels were set at 0.05.

Results

Participants characteristics and the environment

All participants finished the study. The average age of these 12 male young adults was 20.8 years, ranging from 19.2 to 23.7 years. The height, weight, BMI, and systolic and diastolic pressures under test 1 were 176.0 ± 5.5 cm, 68.0 ± 10.9 kg, 21.9 ± 3.0 kg/m2, 114.3 mmHg, and 75.1 mmHg (**Supplementary Table 2**). However, there was statistical significance in blood glucose when compared between test 1, test 2, and test 3 (4.3 ± 0.3 vs. 4.5 ± 0.4 vs. 4.9 ± 0.2, mmol/L; $F = 11.67$, $p < 0.001$), and blood glucose among participants in test 2 was lower than that in test 3. The average temperature of day 1 to 4 was 16.2°C indoors and 20.4°C outside, and the humidity was 32% indoors and 33% outside.

Hydration state, thirst, and related urine, and blood biomarkers

Among 12 participants, the average water intake from food was 939 ± 146 ml. The 24 h urine volume of participants was 799 ± 145 ml. The void number was 5 ± 2 on day 3 (**Supplementary Table 3**). The urine osmolality was 1,004 ± 163 (mOsm/kg). Nine participants (75%) were in the dehydration state for the whole day (**Supplementary Figure 1**).

Statistically, significance was found in blood and urine osmolality and the thirst when compared among three tests. Compared with test 1, the urine osmolality and thirst scores in test 2 were higher, with statistical significance (F = 32.8, $p < 0.01$; $F = 19.62$, $p = 0.001$). Compared with test 2, participants in test 3 had a lower thirst score, urine osmolality and blood osmolality ($F = 27.64$, $p < 0.001$; $F = 100.95$, $p < 0.001$; $F = 23.31$, $p = 0.001$). There was also statistical significance in the distribution of hydration state in three tests ($\chi 2 = 31.270$, $p < 0.001$). Compared with test 1, more proportion of dehydration was found in test 2 (50 *vs* 100%). Compared with test 2, less proportion of dehydration was found in test 3 (100 *vs* 8.3%) (**Table 1**).

Changes of brain gray matter density in different hydration states

Compared with test 2, participants in the rehydration state after water supplementation in test 3 showed an extensive increase in gray matter density in widespread brain regions, mainly involving the left middle temporal gyrus, cuneus, right thalamus, left rolandic opercula, Brodmann area 39, right precentral, left postcentral gyrus, and cingulate gyrus ($p < 0.001$) (**Table 2** and **Figure 2**).

Changes in brain white matter density in different hydration states

Compared with test 2, participants in a rehydration state after water supplementation in test 3 had higher white matter density in the temporal lobe, sub-lobar, and sub-gyral ($p < 0.001$) (**Table 3** and **Figure 3**).

Changes of cerebrospinal fluid in different hydration states

Compared with test 1 for the baseline, participants in a dehydration state after 36 hours of water deprivation in test 2 had a higher cerebrospinal fluid density (voxel = 5118; T = −10.74; MNI coordinates: −24, −40.5,13.5; $p < 0.001$) (**Figure 4**). Compared with test 2, participants in a rehydration state in test 3 had a lower cerebrospinal fluid density (voxel = 5342; T = −11.07; MNI coordinates: 6, 16.5,7.5; $p < 0.001$) (**Figure 5**).

TABLE 1 Biomarkers related to the hydration state of participants.

	Test 1	Test 2	Test 3
Blood osmolality (mOsm/kg)	304.6 ± 7.1	305.7 ± 6.4	295.3 ± 7.8[#]
Urine osmolality (mOsm/kg)	803.2 ± 171.7*	1123.3 ± 65.7	387.0 ± 268.3[#]
Thirst	3.3 ± 2.2 *	6.8 ± 2.6	1.9 ± 1.5[#]
Hydration state			
Dehydration state	6 (50.0%) [a]	12 (100.0%)	1 (8.3%)
Optimal hydration state	0 (0.0%)	0 (0.0%)	9 (75.0%)
Middle hydration state	6 (50.0%)	0 (0.0%)	2 (16.7%)

*, Statistically significant differences between test 1 and test 2, $P < 0.025$. [#], Statistically significant differences between test 2 and test 3, $P < 0.025$. [a], Statistically significant differences was found in the distribution of hydration state in three tests when compared with the method of Fisher exact test.

TABLE 2 Differences in brain gray matter among participants in rehydration state in test 3 and participants in dehydration state in test 2.

Brain areas	Voxel	T	*P*	MNI coordinates		
Temporal_Mid_L (aal)	249	7.25	< 0.001	−48	−16.5	−12
Lentiform Nucleus	55	7.53	< 0.001	16.5	−1.5	−6
Cuneus	1655	11.11	< 0.001	13.5	−69	1.5
Lingual_L (aal)	141	6.12	< 0.001	−13.5	−75	3
Occipital_Mid_L (aal)	117	6.28	< 0.001	−48	−67.5	1.5
Thalamus_R (aal)	413	8.17	< 0.001	9	−12	7.5
Rolandic_Oper_L (aal)	463	15.27	< 0.001	−45	−12	7.5
Precentral Gyrus	56	7.29	< 0.001	−61.5	−4.5	13.5
Transverse Temporal Gyrus	67	6.74	< 0.001	57	−21	12
Brodmann area 39	386	9.88	< 0.001	−49.5	−67.5	22.5
Frontal_Sup_Medial_L (aal)	63	8.30	< 0.001	1.5	45	24
Cuneus_L (aal)	202	6.02	< 0.001	−9	−76.5	27
Postcentral_R (aal)	54	5.55	< 0.001	64.5	1.5	30
Sub-Gyral	192	6.58	< 0.001	−31.5	−78	21
Frontal_Inf_Oper_L (aal)	21	5.35	< 0.001	−42	6	22.5
SupraMarginal_L (aal)	24	6.31	< 0.001	−52.5	−48	27
Precentral_R (aal)	412	7.34	< 0.001	45	−15	51
Postcentral_L (aal)	767	11.19	< 0.001	−43.5	−15	36
SupraMarginal_R (aal)	143	8.88	< 0.001	51	−43.5	40.5
Parietal_Inf_L (aal)	109	5.82	< 0.001	−54	−24	37.5
Cingulate Gyrus	1186	9.10	< 0.001	9	−22.5	49.5
Precuneus	39	7.64	< 0.001	−24	−63	39
Precentral_L (aal)	80	9.25	< 0.001	−46.5	0	49.5
Inferior Parietal Lobule	40	6.54	< 0.001	−42	−54	51

MNI is the abbreviation of Montreal Neurological Institute.

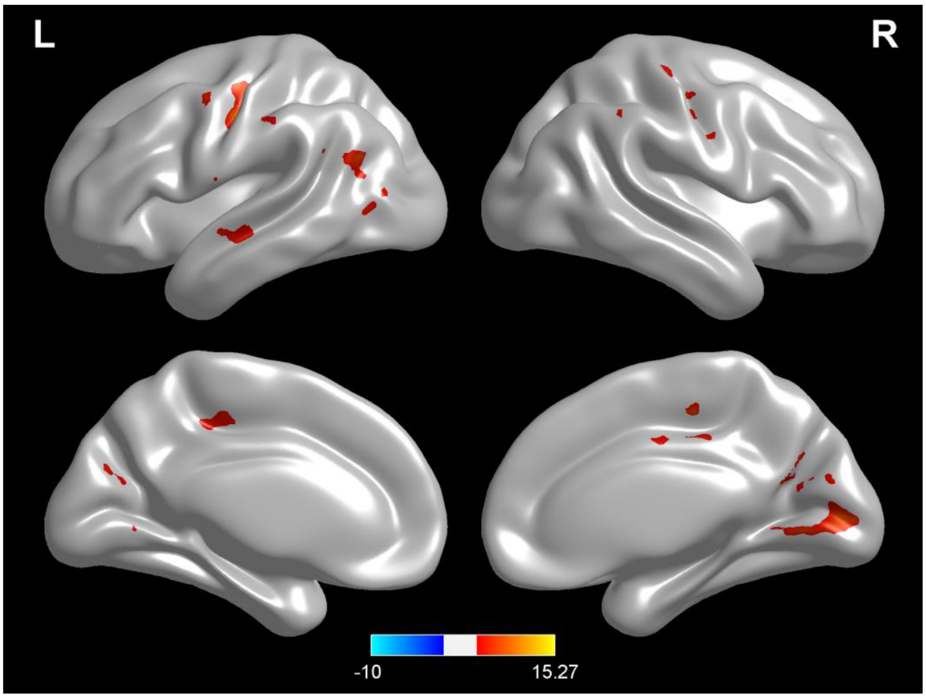

FIGURE 2
Regional changes on the voxel-based three-dimensional displayed brain gray matter among participants when compared with test 3 in rehydration state and test 2 in dehydration state. Warm colors mean that the gray matter density among participants in the rehydration test was higher than that in the dehydration test; cold colors mean that the gray matter density among participants in the rehydration test was lower than that in the dehydration test; $p < 0.05$ after false-discovery rate correction, voxel threshold of cluster >10.

TABLE 3 Differences in brain white matter among participants in the rehydration state in test 3 and participants in the dehydration state in test 2.

Brain areas	Voxel	T	*P*	MNI coordinates		
Midbrain	91	7.34	< 0.001	19.5	−21	−10.5
Temporal Lobe	796	8.44	< 0.001	−43.5	−43.5	7.5
Middle Occipital Gyrus	26	6.15	< 0.001	−28.5	−88.5	−3
Lingual_R (aal)	23	−6.26	< 0.001	12	−75	1.5
Corpus Callosum	143	7.91	< 0.001	4.5	28.5	1.5
Thalamus_L (aal)	10	6.09	< 0.001	−15	−22.5	3
Sub-lobar	503	9.01	< 0.001	6	−34.5	12
Sub-Gyral	390	7.14	< 0.001	−25.5	−51	13.5
Insula_R (aal)	57	5.99	< 0.001	30	21	15

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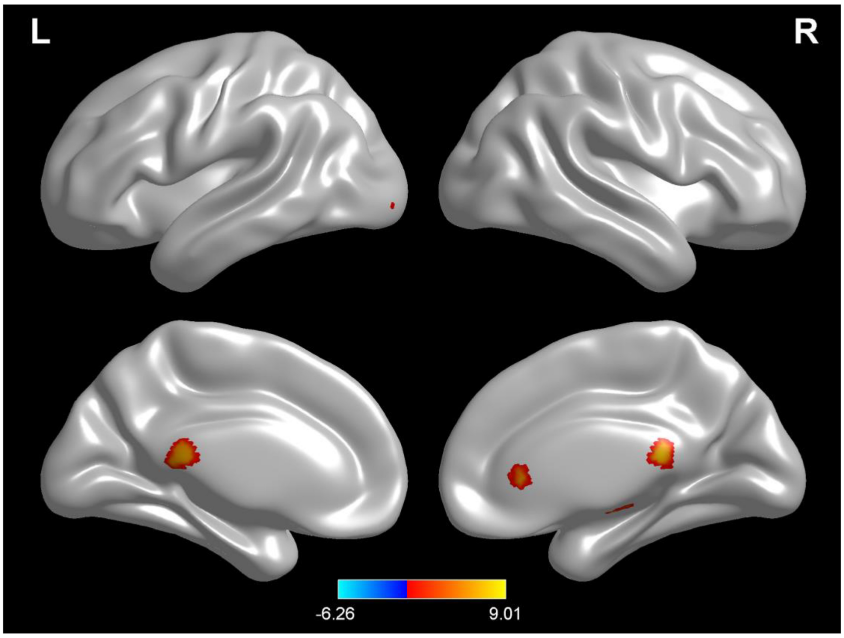

FIGURE 3
Regional changes on the voxel-based three-dimensional displayed brain white matter among participants when compared with test 3 in rehydration state and test 2 in dehydration state. Warm colors mean that the white matter density among participants in the rehydration test was higher than that in the dehydration test; cold colors mean that the white matter density among participants in the rehydration test was lower than that in the dehydration test; $p < 0.05$ after false-discovery rate correction, voxel threshold of cluster >10.

Changes in brain homogeneity in different hydration states

Compared with test 1 for baseline, participants in a dehydration state after 36 hours of water deprivation in test 2 had lower ReHo values in the right amygdala, left occiput median, right lingual, opercula part of right inferior frontal gyrus, and right precuneus and a higher ReHo value in the right supplementary activity area ($p < 0.01$) (**Table 4** and **Figure 6**).

Compared with test 2, participants in a rehydration state after water supplementation in test 3 had higher ReHo values in the right amygdala, right lingual, opercula part of right inferior frontal gyrus, and right precuneus and lower ReHo values in the left cerebellopontine area 1 and middle frontal gyrus ($p < 0.01$) (**Table 5** and **Figure 7**).

Discussion

Currently, most studies focus on the effects of Alzheimer's syndrome, ischemic brain injury, epileptic encephalopathy, and other clinical diseases on the brain's structure and the connectivity of brain nodes. However, studies about the effects of hydration on brain structure and the connectivity of brain nodes among healthy adults are scarcely reported. In the

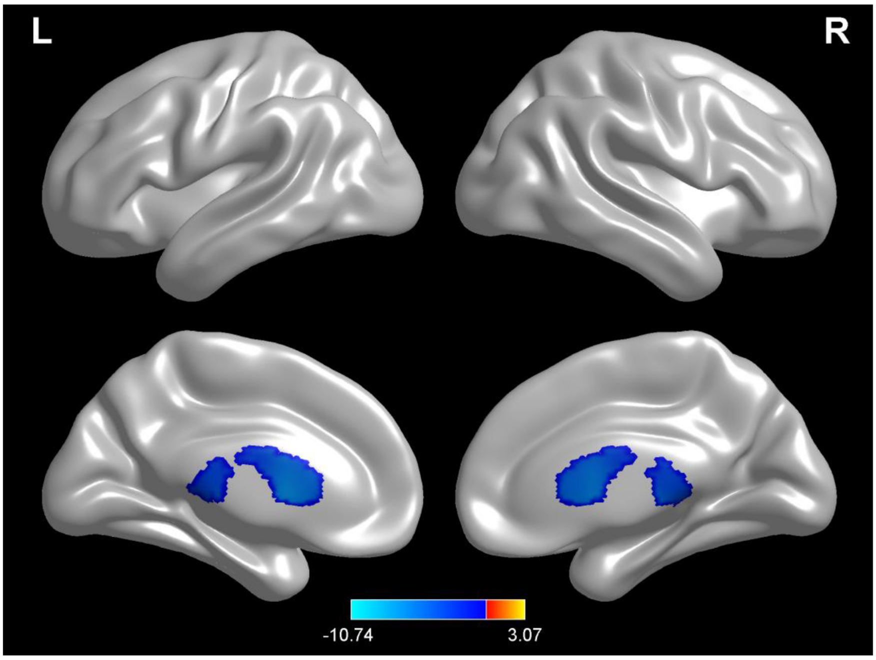

FIGURE 4
Regional changes on the voxel-based three-dimensional displayed brain cerebrospinal fluid among participants when compared with test 1 in baseline hydration state and test 2 in dehydration state. Warm colors mean that the cerebrospinal fluid density among participants in the baseline hydration test was higher than that in the dehydration test; cold colors mean that the cerebrospinal fluid density among participants in the baseline hydration test was lower than that in the dehydration test; $p < 0.05$ after false-discovery rate correction, voxel threshold of cluster > 10.

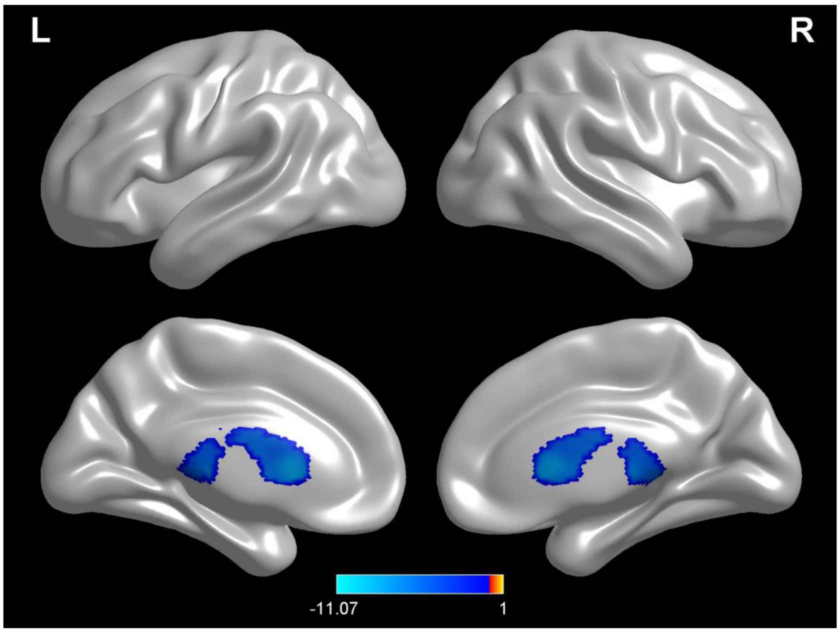

FIGURE 5
Regional changes on the voxel-based three-dimensional displayed brain cerebrospinal fluid among participants when compared with test 3 in rehydration state and test 2 in dehydration state. Warm colors mean that the cerebrospinal fluid density among participants in the rehydration test was higher than that in the dehydration test; cold colors mean that the cerebrospinal fluid density among participants in the rehydration test was lower than that in the dehydration test; $p < 0.05$ after false-discovery rate correction, voxel threshold of cluster > 10.

TABLE 4 Differences in brain regional homogeneity (ReHo) among participants in test 1 for the baseline state with participants in the dehydration state in test 2.

Brain areas	Voxel	T	*P*	MNI coordinates		
Amygdala_R (aal)	8	6.15	< 0.001	24	6	−15
Occipital_Mid_L (aal)	5	4.78	0.001	−39	−84	3
Lingual_R (aal)	10	5.45	< 0.001	6	−57	3
Frontal_Inf_Oper_R (aal)	7	10.42	< 0.001	57	12	6
Precuneus_R (aal)	20	5.74	< 0.001	6	−54	21
Supp_Motor_Area_R (aal)	5	−3.77	0.003	12	3	48

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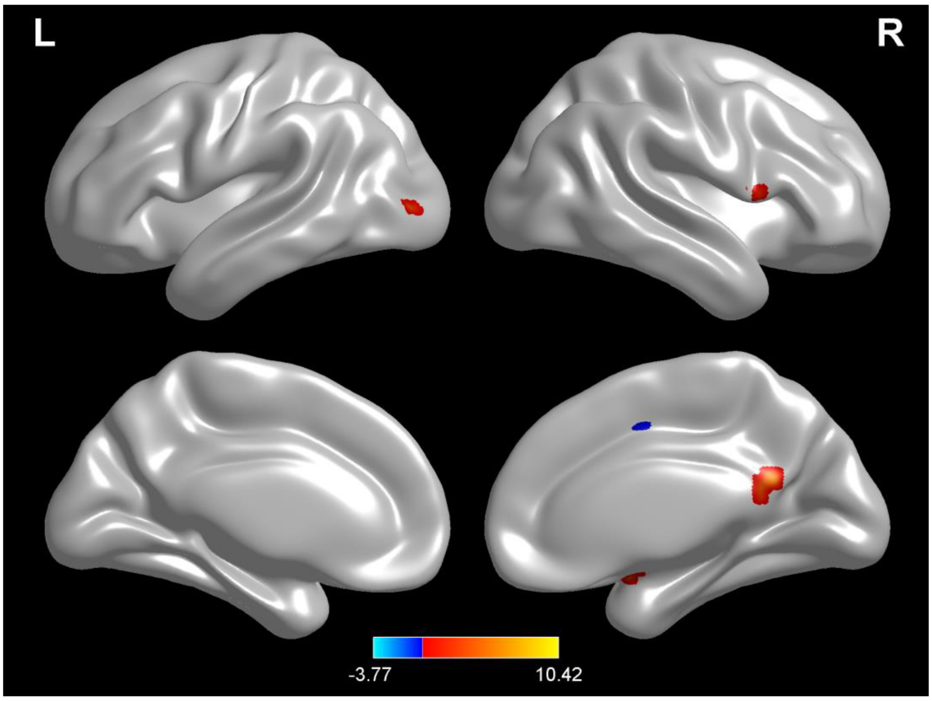

FIGURE 6
Regional changes on the voxel-based three-dimensional displayed brain regional homogeneity (ReHo) among participants when compared with test 1 in baseline hydration state and test 2 in dehydration state. Warm colors mean that the ReHo value among participants in the baseline test was higher than that in the dehydration test; cold colors mean that the ReHo value among participants in the baseline test was lower than that in the dehydration test; $p < 0.05$ after false-discovery rate correction, voxel threshold of cluster > 10.

TABLE 5 Differences in brain regional homogeneity (ReHo) among participants in test 3 for the rehydration state with participants in the dehydration state in test 2.

Brain areas	Voxel	T	*P*	MNI coordinates		
Cerebelum_Crus1_L (aal)	7	−5.91	< 0.001	−39	−60	−33
Amygdala_R (aal)	6	2.81	0.016	24	6	−18
Lingual_R (aal)	6	4.00	0.002	9	−54	6
Frontal_Inf_Oper_R (aal)	6	3.64	0.004	57	12	6
Precuneus_R (aal)	20	5.25	0.002	6	−51	21
Middle Frontal Gyrus	6	−3.49	0.005	39	6	51

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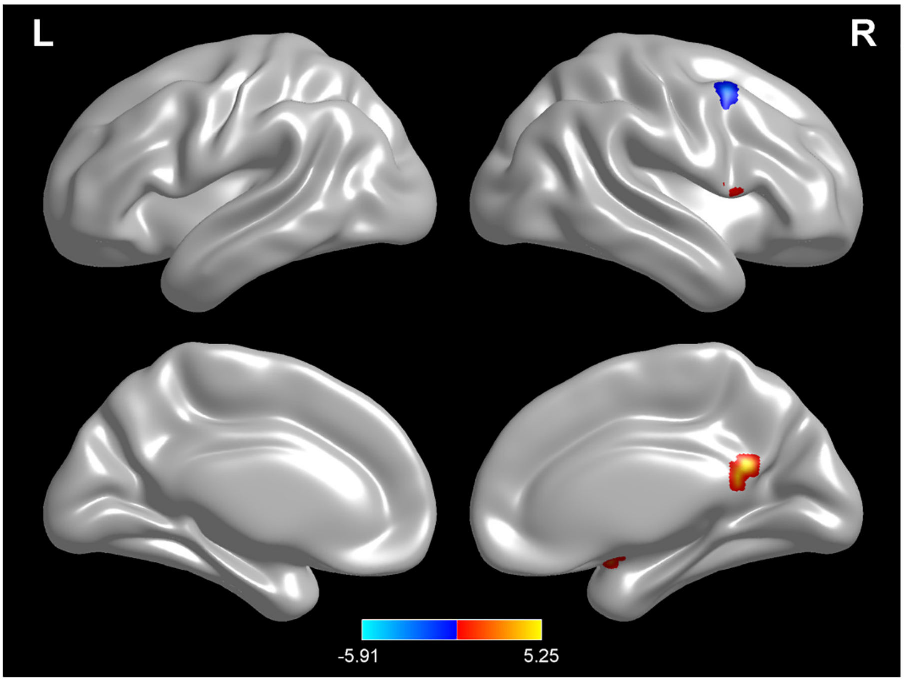

FIGURE 7
Regional changes on the voxel-based three-dimensional displayed brain regional homogeneity (ReHo) among participants when compared with test 3 in rehydration state and test 2 in dehydration state. Warm colors mean that the ReHo value among participants in the rehydration test was higher than that in the dehydration test; cold colors mean that the ReHo value among participants in the rehydration test was lower than that in the dehydration test; $p < 0.05$ after false-discovery rate correction, voxel threshold of cluster > 10.

study, the effects of hydration on brain regional density and homogeneity were measured using the method of MRI.

It was shown that dehydration increased cerebrospinal fluid density. Rehydration caused an extensive increase in gray matter density and white matter density in some specific brain regions. As one indicator of the connectivity of brain nodes, the ReHo value was also affected by the state of hydration. In China, there have been no other studies about the effects of hydration on the brain's structure and the connectivity of brain nodes. A few related studies have been conducted in some other countries. The results of one study suggested that dehydration induced by a 16 hour period of fluid restriction reduced the total brain volume, and brain volume was restored following rehydration (16). In a study conducted among sight-active men participants, it was found that dehydration with 2.9% body mass loss induced by intermittent exercise in a warm environment caused reductions in cerebrospinal fluid (27). In two other related studies, the results showed that ventricular volume changes under a hypohydration state in terms of 1.7 to 2.9% body mass loss, but brain volume did not change (28, 29). With ten trained endurance males aged 23 years old as participants, one study found that hypohydration at 3% of body mass loss induced by running on a treadmill reduced total brain volume (30). In one study, MRI scans were also conducted to explore the mechanisms of an acute dehydration state among participants, and it was found that there was an expansion of the ventricular system with the largest change appearance in the left lateral ventricle, which may induce the short-term changes of cognitive performances controlled by the brain. In another study with the method of brain MRI scans, it was suggested that blood-oxygen-level-dependent (BOLD) responses in the fronto-parietal increased and lateral ventricle were enlarged in acute dehydration induced by a thermal exercise protocol (29). However, in a study with twenty healthy volunteers, brain MRIs were scanned in three conditions: a baseline scan, a scan after hydration when consuming 3L of water over 12 h, and a scan after dehydration after overnight fasting for 9 h. Additionally, it was found that brain volume and brain total water content were not substantially affected (17). The ReHo value of regional homogeneity was usually used to evaluate spontaneous neural activity during the resting state and can be used to explore the connectivity of brain nodes and cognitive performances (31). One study demonstrated that changes in ReHo were correlated with changes in cognitive performance in some circumstances (32). The mechanism of the effects of hydration on brain structure and the connectivity of brain nodes may be explained by the following reasons. Dehydration is usually accompanied by hypovolaemia, which may cause an increase in the ventricular system volume and a reduction in brain volume (19, 28, 33, 34). Serum osmolality induced by acute dehydration could produce an osmotic gradient, resulting in an increased diffusion of intracellular water stores into extracellular space. The changes cause shrinkage of cells, particularly astrocytes, which have a vital role in the transport of water, and thus leads to ventricular system expansion (19).

Adverse health effects and related symptoms of mild and moderate dehydration in daily life often do not receive enough attention. In this study, the changes in brain regional density and homogeneity under different hydration states were discovered.

This study has some strengths and weaknesses. Referring to the method of inducing dehydration, dehydration can be induced by heat stress, fluid restriction, exercise, diuretics, or combinations of the above methods in current studies. However, some methods of inducing dehydration may affect brain regional density and homogeneity, such as heat stress and exercise. In this study, water deprivation and supplementation were used to induce changes in hydration states among participants, which may be more meaningful in exploring the effects of hydration on brain structures clearly. In addition, it is also very important to ensure the quality control during water deprivation. The osmolality of urine during the period of water deprivation was continuously monitored to explore the changing trend of hydration state and to verify the adherence of participants, which showed that the study had restricted and high quality control. In some studies, the objective physiological and biochemical indexes are not used to monitor the quality control during water deprivation. In consideration of weakness, gender differences and the effects of long-term water intervention on brain regional density and homogeneity were not studied. In addition, this is a pilot self-control trial to explore the effects of dehydration and rehydration on brain regional density and homogeneity. Randomized controlled design studies could obtain more effective results and reveal scientifically effects of hydration state on brain structure and function more clearly and accurately. In this study, only brain regional density and homogeneity was analyzed. Some other indexed such as brain volume and blood oxygen level dependent were not analyzed, more comprehensive indexes would be helpful to explore the effective of hydration on brain structure and function. Based on this pilot self-control trial, more high-quality research and analysis can be carried out in the future.

Conclusion

In summary, dehydration state increased cerebrospinal fluid density, decreased brain regional homogeneity. Rehydration state increased brain gray matter and white matter density widespreadly, and in-creased brain regional homogeneity. Maintaining a normal hydration state through sufficient water intake is helpful in maintaining brain regional density and homogeneity.

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 study protocol and instruments were reviewed and approved by the Ethical Review Committee of Chinese Nutrition Society on November 10, 2015. The code of identification is CNS-2015-001. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Prior to the conduction of the study, all participants read and signed informed consent voluntarily. The patients/participants provided their written informed consent to participate in this study.

Author contributions

NZ, SD, and GM: conceptualization. JZ: data curation. NZ: formal analysis. SD: funding acquisition. NZ and JZ: investigation. NZ, SD, and JZ: methodology and writing—original draft. NZ, JZ, SD, and GM: project administration. SD and GM: supervision. GM: writing—review and editing. All authors were involved in the manuscript revision and have approved this final version, agreed to authorship and order of authorship for this manuscript, and appropriate permissions and rights to the reported data.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.906088/full#supplementary-material

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Correlation between dietary information sources and knowledge of adequate diets in Eastern China

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Knowledge of adequate diets can improve an individual's health status. Although previous studies have identified the main resources from which Chinese people acquire dietary knowledge, it is still unclear whether information sources regarding diets (ISRDs) can increase individuals' knowledge of adequate diets (KAD) and which ISRDs are most effective in conveying KAD to the Chinese population. In this study, we interviewed 4,710 residents in Eastern China regarding their ISRDs and KAD. Descriptive statistics, ANOVA, and multivariate linear regression were used to analyze the effectiveness of different ISRDs in transmitting KAD to Chinese individuals and to determine the relationship between ISRDs and KAD. Results showed that the KAD scores of the respondents were low overall in Eastern China. Providing dietary information through expert lectures, books, newspapers, magazines, and social media could significantly improve the average KAD score of Chinese individuals. Respondents with a greater number of ISRDs were more likely to have higher KAD scores. These findings suggest that the number of ISRDs should be increased. In particular, emphasis should be placed on the role of expert lectures, books, newspapers, magazines, and social media.

KEYWORDS

dietary, information sources, knowledge, adequate diets, Eastern China

Introduction

The Chinese Nutrition Society defines an adequate diet as a diverse, balanced diet that meets the nutritional and health needs of an individual or group with different health conditions, geographical resources, habits, and beliefs (1). Consuming an adequate diet is associated with a reduced risk of death from all causes (2, 3). A recent research report from the Chinese Nutrition Society showed that consuming an inadequate diet is the leading cause of chronic disease and death in China, which accounted for 3.1 million deaths in 2017 (1).

Dietary knowledge is the basis of individual diet-related behavioral change (4, 5), and better dietary knowledge can improve Chinese individuals' health status (6, 7). Therefore, increasing the population's knowledge of adequate diets (KAD) by various means is an important way to promote a healthy diet and reduce the incidence of chronic disease and death.

Previous studies have found that television, radio, books, newspapers, magazines, and the internet are the main resources from which Chinese people acquire dietary knowledge, and that females, younger individuals, and people with higher levels of education are more likely to actively seek out nutrition and health information (8, 9). However, it is still unclear whether information sources regarding diets (ISRDs) can increase individuals' KAD, which ISRDs are most effective in conveying KAD to the Chinese population, and what sources of dietary information are preferred by different demographic groups.

Eastern China (including Jiangsu, Shandong, Anhui, Zhejiang, and Fujian Provinces and Shanghai Municipality) is a region of the country that is experiencing rapid economic development. The per capita gross regional production of Eastern China was 103,169 Chinese yuan in 2020, which is higher than the national average (10). Therefore, studies performed in Eastern China are useful for guiding decision- and policy-making in the rest of the country.

The objectives of this study were to clarify the effectiveness of different ISRDs in transmitting KAD to Chinese individuals, to determine the relationship between ISRDs and KAD, and to clarify the sources of dietary information that are preferred by different demographic groups.

Methods

Sampling

The current study was conducted in Eastern China (including Jiangsu, Shandong, Anhui, Zhejiang, and Fujian Provinces and Shanghai Municipality). Two cities were randomly selected from each province. In each selected city, two residential areas were randomly selected (four residential areas were randomly selected from Shanghai Municipality) for sampling. Two hundred residents were recruited from each residential area. Five trained research assistants conducted face-to-face interviews with the residents based on a standardized questionnaire by asking questions and taking notes. Interviews were conducted at the entrance of each residential area in the afternoon at the end of the normal work day. The average interview lasted about 10 min for each participant. Upon completion of the interview, each participant received a small gift worth about 5 Chinese yuan.

A total of 4,710 participants completed interviews, for a response rate of 98.1%. Only 90 residents refused to participate, citing lack of time or other, unstated reasons.

Ethics, consent, and permissions

The survey was conducted from March to June 2021 following ethical approval from Yangzhou University. Potential survey participants were first provided with an explanation of the study and asked for their consent to participate. Those who agreed to participate completed a face-to-face interview using a standardized questionnaire (see Appendix). The questionnaire was fully anonymous and did not collect any personal, identifying information.

Study instrument

The questionnaire was divided into two sections. The first section was designed to collect socio-economic information, including gender, age, level of education, monthly family income, and province or municipality, as well as the ISRDs that residents were exposed to. To obtain a complete picture of the ISRDs that individuals could be exposed to, we conducted a pilot survey that involved interviewing 15 residents of different ages and genders. Based on this survey, we generated a list of possible ISRDs (e.g., expert lectures, books, newspapers, magazines, television, radio, friends or relatives, the internet, social media, TikTok, and health care product sales staff). Interviewing five other residents did not yield any additional ISRDs, so we considered the ISRD list to be complete. The list was multiple-choice, and respondents were asked to indicate all their actual ISRDs.

The second part of the survey addressed KAD. To minimize respondents' time burden, and thereby improve the completion rate of the questionnaire, we selected seven main dietary knowledge items from the "The Chinese Dietary Guidelines" (2016) enacted by the Chinese Nutrition Society. To ensure that each question was easily understood, clear, and answerable, we listed daily dietary intake quantities in grams or milliliters according to "The Chinese Dietary Guidelines" (2016) and provided four possible answers to each question, only one of which was correct (see Appendix).

Data analysis

First, descriptive statistics were used to calculate the percentage of correct answers to each item in the KAD part of the questionnaire, as well as the percentages of selected and unselected ISRDs.

Second, each respondent's score (out of 100) for the KAD items was calculated, and then ANOVA was used to compare average KAD scores with each ISRD (*P-values* of <0.05 were considered significant).

Third, multivariate linear regression was employed to examine the socio-economic variables associated with the number of ISRDs, as well as both the socio-economic variables and the number of ISRDs associated with KAD scores.

SPSS 20.0 software was used to perform all of the statistical analyses described above.

Results

Study population demographics

The demographic characteristics of the study participants are presented in Table 1. Of the 4,710 respondents, 56.5% were female, and the majority of respondents were aged between 36 and 55 years (47.5%). In total, 47.3% of the study participants had an educational level of undergraduate college or above. A monthly family income of between 5,000 and 9,999 Chinese yuan was reported by the highest proportion of respondents (32.9%). The sample was representative of the demographic structure of the general population of Eastern China, as reported in the China Statistical year book (2020) (10).

TABLE 1 Respondent characteristics ($N = 4{,}710$).

Characteristics	*N*	%
Gender		
Male	2,049	43.5
Female	2,661	56.5
Age		
≤20 years	885	18.8
21–35	1,293	27.5
36–45	1,327	28.2
46–55	908	19.3
56–65	254	5.4
66–75	34	0.7
≥76 years	8	0.2
Education		
Primary or below	92	2.0
Junior high school	655	13.9
Senior high school	905	19.2
Three-year college	830	17.6
Undergraduate college	1,789	38.0
Postgraduate and above	439	9.3
Monthly family income (Chinese Yuan)		
≤5,000	1,301	27.6
5,000–9,999	1,548	32.9
10,000–19,999	1,099	23.3
20,000–39,999	464	9.9
40,000–80,000	158	3.4
≥80,001	140	3.0
Province (or Municipality)		
Jiangsu	913	19.4
Shandong	801	17.0
Anhui	953	20.2
Shanghai municipality	618	13.1
Zhejiang	709	15.1
Fujian	716	15.2

Answers regarding knowledge of adequate diets

To assess KAD levels, the study participants were asked seven questions regarding appropriate daily intake of a variety of staple foods. Regarding the seven KAD items (Table 2), daily salt intake had the highest percentage of correct answers (41.2%), followed by daily intake of vegetables (34.7%). However, the type of food eaten every day had the lowest percentage of correct answers (15.9%). Taken together, the responses indicated that, overall, KAD is low in this population.

Differences in dietary knowledge scores among different information sources regarding diets

Regarding ISRDs (Table 3), 54.9% of respondents reported that they get information about reasonable diets from books, newspapers, and magazines; 51.9% reported obtaining dietary information from social media; 49.8% reported obtaining information from friends or relatives; and only 6.6% reported revising dietary information from health care product sales staff.

Results from the ANOVA analysis showed that there were significant differences in the mean KAD score depending on whether the respondent received dietary information from expert lectures ($F = 158.55$, $P < 0.01$), books, newspapers, and magazines ($F = 118.30$, $P < 0.01$), and social media ($F = 9.49$, $P < 0.01$) compared with other sources (Table 3).

Determinants of different information sources regarding diets, number of information sources regarding diets, and knowledge of adequate diets score

The results from the logistic regression analysis (Table 4) showed that female respondents and older respondents were less likely to obtain dietary information from Expert lectures, whereas highly educated and high-income respondents were more likely to obtain dietary information from Expert lectures and Books, newspapers, and magazines. High-income respondents were less likely to obtain dietary information from Television and radio. Female, older, and high-income respondents were more likely to obtain dietary information from Friends or relatives, whereas highly educated respondents were less likely to obtain dietary information from Friends or relatives. Female and high-income respondents were more likely to obtain dietary information from Social media, whereas highly educated respondents were less likely to obtain dietary information from Social media. High-income respondents were more likely to obtain dietary information from Health care product sales staff, whereas older respondents and highly educated respondents were less likely to obtain dietary information from Health care product sales staff. On the other hand, the results from the linear regression analysis (Table 4) showed that gender, level of education, and income were significantly and positively associated with number of ISRDs, while age was significantly and negatively associated with number of ISRDs. Age, level of education, income, and number of ISRDs were significantly and positively associated with KAD score.

TABLE 2 Number of correct answers to each knowledge of adequate diets item ($N = 4,710$).

Dietary knowledge item	*N*	%
Daily use of cooking oil 25–30 g	1,539	32.7
Daily salt intake not to exceed 6 g	1,939	41.2
Daily sugar take amount not to exceed 50 g	1,243	26.4
Daily intake of milk and dairy products 300 g	1,015	21.5
Daily water intake 1,500–1,700 ml	1,276	27.1
Daily intake of vegetables 300–500 g	1,635	34.7
Eat at least 12 types of food a day	751	15.9

Discussion

The present study offers insights into ISRDs and KAD scores among residents of Eastern China, as well as into the relationship between ISRDs and KAD. In this study, we found that Expert lectures, Books, newspapers, magazines, and Social media were the most effective ISRDs for conveying KAD to residents of Eastern China, that different demographic groups prefer different information sources, and that a greater number of ISRDs correlated with higher KAD scores.

The KAD scores of the respondents in Eastern China were low overall. This is consistent with data from the China Health and Nutrition Survey (CHNS) conducted in 2015 in nine Chinese provinces and three municipalities, which reported that only 34.3% of the participants had adequate dietary literacy (6). Our findings indicate that the KAD level of residents in Eastern China has not improved significantly in recent years. This suggests that increasing the KAD of residents remains an important target for dietary intervention and education.

The survey results showed that Books, newspapers, and magazines, Social media, and Friends or relatives were the main information sources from which respondents acquired dietary knowledge. This is essentially consistent with the 2015 CHNS report (8), as well as with another survey conducted in Beijing in 2015 (9). Thus, the findings from the current study suggest that providing dietary information through Expert lectures, Books, newspapers, and magazines, and Social media significantly improves the average KAD score of Chinese individuals. In terms of the most effective ISRDs for conveying KAD to residents of Eastern China, the survey found that young respondents and respondents with a high income level were more likely to obtain dietary information from Social media, and that highly educated respondents were more likely to obtain dietary information from Books, newspapers, and magazines.

TABLE 3 Origin of information regarding diet and ANOVA analysis of knowledge of adequate diets score means among different information sources regarding diets ($N = 4,710$).

Information sources regarding diets	Group	*N* (%)	Knowledge score mean	*F*	*P*-value
Expert lectures	No	3,453 (73.3)	25.94	158.55	0.000
	Yes	1,257 (26.7)	36.86		
Books, newspapers, and magazines	No	2,126 (45.1)	24.24	118.30	0.000
	Yes	2,584 (54.9)	32.66		
Television and radio	No	2,859 (60.7)	28.27	3.54	0.060
	Yes	1,851 (39.3)	29.77		
Friends or relatives	No	2,363 (50.2)	29.38	1.79	0.181
	Yes	2,347 (49.8)	28.33		
Social media	No	2,266 (48.1)	27.61	9.49	0.002
	Yes	2,444 (51.9)	30.01		
Health care product sales staff	No	4,399 (93.4)	28.97	1.16	0.281
	Yes	311 (6.6)	27.28		

TABLE 4 Multiple regression coefficients (odds ratio, standardized β) for socio-economic variables in terms of different information sources regarding, reasonable diets (ISRDs) associated with knowledge of adequate diets (KAD) score.

Predictor variables	Logistic regression analysis regarding different information sources regarding diets						Linear regression analysis	
	Expert lectures	Books, newspapers, and magazines	Television and radio	Friends or relatives	Social media	Health care product sales staff	Number of ISRDs	KAD score
	OR	OR	OR	OR	OR	OR	β	β
Gender (female)	0.822**	1.079	1.082	1.424**	1.328**	1.234	0.058**	−0.004
Age	0.810**	1.014	1.047	1.096**	0.791**	0.697**	−0.066**	0.098**
Level of education	1.174**	1.236**	0.986	0.934**	0.977	0.837**	0.039**	0.125**
Income	1.168**	1.090**	0.947*	1.063*	1.120**	1.130**	0.076**	0.049**
Number of information sources	-	-	-	-	-	-	-	0.119**

*P < 0.05, **P < 0.01.
"-" indicates no data.
OR, odds ratio; β, standardized coefficient.

This is consistent with a survey conducted in Beijing in 2015 (9). However, our survey further revealed that female respondents were more likely to obtain dietary information from Social media, and that respondents with a high income level were more likely to obtain dietary information from Books, newspapers, and magazines. Male respondents, young respondents, and highly educated and higher income respondents were more likely to obtain dietary information from Expert lectures. Therefore, when disseminating dietary knowledge through the above information sources, more attention should be paid to elderly, low-income, and less educated individuals.

The survey results showed that female respondents, young respondents, and respondents with a high level of education were more likely to be exposed to a greater number of ISRDs. This is consistent with a previous study conducted in Beijing in 2015, which found that females, young people, and individuals with a higher level of education are more likely to actively seek out nutrition and health information (9). People who actively seek out nutrition and health information inevitably have more access to ISRDs; however, more convenient ISRDs are fundamental to improving residents' knowledge. In the current study, we found that individuals with higher income were exposed to a greater number of ISRDs, possibly because high-income groups are more likely to have access to information on healthy eating (11). Therefore, to improve residents' KAD levels, it is necessary to provide more ISRDs while increasing residents' attention to dietary nutrition and health.

Older respondents and respondents with high levels of education and higher income were more likely to have a higher KAD score. This is consistent with previous studies conducted in Chinese urban adults (12), Swiss individuals (13), Belgians (14), and Iranians (15) that found that an individual's level of nutrition knowledge mainly depends on his/her education, age, and income. While nutrition knowledge focuses more on the nutrients provided by food and their association with disease, and dietary knowledge is more concerned with the importance of an adequate diet, both dietary knowledge and nutrition knowledge emphasize the importance of healthy eating (16). In the current study, we found that respondents with a greater number of ISRDs were more likely to have a higher KAD score. This further proves that a greater number of ISRDs plays an important role in improving residents' knowledge scores.

This study also found that young respondents have more ISRDs, while older respondents have higher KAD scores. This could be because younger people have a lower perception of being at risk of chronic disease than older people, because chronic diseases primarily affect older individuals (17), thus prompting older people to pay more attention to KAD than younger people. However, further research is needed to clarify the actual reasons for this observation.

This study had several limitations. First, the cross-sectional design excluded causal inference. However, the correlation among the different variables discussed in this study suggests that the data offer a plausible explanation for cause and effect in this population. Second, this study was limited to Eastern China and therefore cannot be considered representative of residents throughout China. In the future, surveys should be conducted in an expanded study area. However, the contribution of this study to the literature is to confirm the relationship between ISRDs and KAD and to find that expert lectures, books, newspapers, and magazines and social media were most effective in conveying KAD to the Chinese population.

Conclusions

In this study, we found that residents of Eastern China have low KAD scores. According to our study, ISRDs and KAD are closely related; therefore, to improve KAD scores, the number of ISRDs should be increased. In particular, emphasis should be placed on the role of Expert lectures, Books, newspapers, and magazines, and Social media to improve KAD, and interventions should target the elderly, low-income individuals, and individuals with a low level of education.

Data availability statement

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

Author contributions

BC: conceptualization, methodology, investigation, data curation, and writing—original draft. LW: software, investigation, and writing—review and editing. FW, JP, JM, XC, MX, JK, and YT: investigation and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

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Supplementary material

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The anti-anxiety/depression effect of a combined complex of casein hydrolysate and γ-aminobutyric acid on C57BL/6 mice

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In view of a series of adverse side effects of drugs for anxiety/depression on the market at present, it is imminent to extract and develop novel anti-anxiety and depression drugs from plants and proteins (like casein hydrolysate) as adjuncts or substitutes for existing anti-anxiety and depression drugs. Consequently, this study investigated the improvement of the anxiety/depression function by the compound of casein hydrolysate and γ-aminobutyric acid (GABA) (casein hydrolysate: GABA = 4:1; CCHAA) on mice induced by chronic restraint stress-corticosterone injection. Animal experiments revealed that oral gavage administration of CCHAA significantly reversed the anxiety/depression-like behaviors. Compared to the model control group, body weights were increased after treatment with CCHAA groups [1.5, 0.75 mg/(g·d)]. As a diagnostic index of anxiety and depression, we assessed GABA and 5-HT levels in response to CCHAA ingestion. The GABA and 5-HT levels were increasingly enhanced by the CCHAA diet. In addition, histopathological changes in the hippocampus CA3 region of the anxious/depressed mice were also alleviated after the treatment with the CCHAA. Thus, the casein hydrolysate and GABA formula diets may induce beneficial effects on the mice with anxiety/depression.

KEYWORDS

behavioral tests, casein hydrolysate, GABA, 5-HT, anxiety/depression

Introduction

Anxiety and depression are widely recognized as psychiatric disorders of global concern that impair human welfare (1). Features of anxiety disorders are generally thought to include cognitive, somatic, emotional, and behavioral changes (2–4). Anxiety disorders generally come in various forms, including high blood pressure, increased heart rate, sweating, fatigue, unpleasant feelings, nervousness, irritability, and restlessness (5–7). Further, in the absence of treatment, patients would gradually develop depression and sometimes even suicidal thoughts (8). Depression was the fourth largest disease burden in the world (8, 9), and with the development of society, it has shown an increasing trend of malignant development (10, 11). Depression is typically characterized by low mood, sadness or depression, and/or loss of interest or enjoyment in activities that were previously pleasurable (12, 13).

γ-aminobutyric acid (GABA) is not only an inhibitory neurotransmitter within the central nervous system, but also a key target for drug treatment of anxiety and depression (14, 15). Although the drugs currently on the market for anxiety/depression are therapeutically effective, they can cause a range of adverse side effects, including cognitive decline, and withdrawal symptoms (16). Therefore, the development of new anxiolytic and depressive drugs derived from plants and proteins provides a novel therapeutic option in order to find an adjunct or alternative to existing anxiolytic and depressive drugs (17, 18).

Casein is a protein rich in biologically active sequences, it can be obtained by enzymatically catalyzing the hydrolysis of proteins derived from microorganisms, animals, and plants, which can release these biologically active sequences under the action of specific endonucleases (such as trypsin, etc.) and similar physiological environmental conditions, so as to obtain casein bioactive peptides with biologically active (19, 20). Casein hydrolysis released a variety of active peptides, including ACE activity inhibitory peptides, immunomodulatory peptides, casein phosphopeptides that promote calcium absorption, peptides that promote the growth of Lactococcus, antithrombotic peptides, etc. (21, 22); and the hydrolysate also contains rich free amino acids, including abundant essential amino acids and oligopeptides containing 2–6 amino acid residues (23). Interestingly, Violle et al. showed that bovine αS1-casein trypsin hydrolysate exhibited anxiolytic-like activity in rats with the conditioned defensive burial and elevated plus maze models when injected intraperitoneally (24).

Meanwhile, there is growing support for the GABA hypothesis of depression as anxiety disorders and major depressive disorders are often found to have GABAergic deficits as a common pathophysiology, and thus GABA deficiency is a hallmark of anxiety disorders and major depressive disorder (25). However, as a highly complex psychiatric disorder, the pathogenesis of anxiety/depression remain obscure (26). The above single components played a role in the treatment of anxiety/depression through different mechanisms, but there is currently no research report on the combination of these components for the treatment of anxiety/depression. In addition, our research team used whey protein hydrolyzed peptides as raw materials for the treatment of anxiety/depression in the early stage and achieved good results (27, 28). Therefore, it is interesting to investigate whether compounds containing casein hydrolysate, whey protein hydrolyzed peptides and GABA, respectively, play a role in the treatment of anxiety/depression.

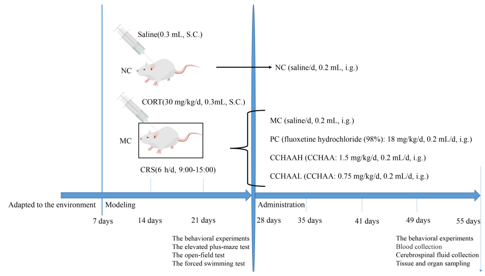
5-HT, also known as serotonin, is an important part of the biogenic aminergic neuroendocrine system (29, 30). As an important neurotransmitter in the rodent central nervous system, 5-HT is mainly involved in the regulation of various physiological activities such as cognitive function, emotional conditioning, appetite, sleep, and biological rhythms (31, 32). The 5-HT nervous system is divided into two independent nervous systems, the central 5-HT and the peripheral 5-HT, respectively (33). Peripheral 5-HT is mainly synthesized by tryptophan hydroxylase 1 (TPH1) and aromatic amino acid decarboxylase (AADC) in enterochromaffin cells, mast cells and 5-HTP cells, and stored in platelets (34). Central 5-HT is mainly synthesized by tryptophan hydroxylase 2 (TPH2) and aromatic AADC in 5-HT neurons and stored in presynaptic vesicles (35, 36). The synthesis amount of 5-HT in the center accounts for only about 5% of the total synthesis amount, and 95% of 5-HT is synthesized in the peripheral tissue of enterochromaffin cells as the main synthesis site (37). Studies have shown that both TPH1 and TPH2 dysfunction are closely related to anxiety-induced depression (38). Nevertheless, most drugs for the treatment of anxiety depression work by increasing 5-HT levels (39, 40).

This study aimed to investigate the improvement of the anxiety/depression function by CCHAA on mice induced by chronic restraint stress-corticosterone injection. The anxiety/depression-like behaviors, GABA and 5-HT synthesis, histopathological changes in the hippocampus CA3 region which is related to anxiety/depression in mice, were further studied.

Materials and methods

Materials and instruments

60 SPF grade C57BL/6 mice, 4 weeks old (21 ± 2 g) were obtained from Nanjing Junke Biological Engineering Co., Ltd. (Nangjing Jiangsu). The mice were adaptively reared for 7 days after purchase. During the experiment, the mice were fed in an environment of room temperature (25 ± 2)°C, relative humidity (50 ± 5)%, and a light/dark cycle of 12 h/12 h (every 5 cages; 320 mm × 215 mm × 170 mm), randomly provided with normal food and water with SPF level laboratory conditions. Moreover, humane care was given according to the 3R principles used in experimental animals. Corticosterone (batch number: 830F031) was purchased from Beijing Soleibo Technology Co., Ltd. (Beijing, China), and fluoxetine hydrochloride dispersible tablets (batch number: 9891A) were purchased from Eli Lilly Suzhou Pharmaceutical Co., Ltd. (Jiangsu, China). Casein hydrolysate (Lactium) was provided by Shanghai Tongyuan



SCHEME 1
Diagram of the experimental paradigm. S.C., subcutaneous injection; i.g., intragastric administration.

Food Technology Co., Ltd. GABA was provided by Nantong Licheng Biological Engineering Co., Ltd. (Nantong, China). Disposable filters, disposable syringes, 1.5 mL centrifuge tubes, 5 mL centrifuge tubes, pipettes, pipette tips, and beakers were purchased from Guangzhou Qianhui Instrument Equipment Co., Ltd. (Guangzhou Guangdong).

The animal experiments were approved by the Committee for the Care and Use of Laboratory Animals in the South China Agricultural University (IACUC No. 2018D047), and all experimental procedures were performed in compliance with the author Guidelines on Animal Ethics and Welfare for Veterinary Journals published by the International Association of Veterinary Editors for the protection of animals used for scientific purpose.

Animal experiments

Establishment of animal model of anxiety/depression

The model experiment was carried out after mice adapted to the environment for 6 days. Mice were randomly assigned to 5 experimental groups (n = 12/group) and weighed weekly. 4 groups were subjected to chronic restraint stress (6 h/d, 9:00–15:00), and CORT (CORT dissolved in normal saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80) was injected subcutaneously in the volume of 0.02 mL/g body weight (30 mg/kg/d) (27, 28, 41). Normal control (NC) mice were fasted (6 h/d, 9:00–15:00) and given normal saline subcutaneously in the volume of 0.02 mL/g body weight (27, 28, 41). Finally, after 21 days of continuous modeling, the anxiety, and depression like behaviors of mice were tested. Incidentally, the mice with anxiety and depression were selected for gavage experiment (41). Further, these procedures were all shown in **Scheme 1**.

Animal different treatments

The normal control group (NC) was treatment with saline (0.2 mL/d), the positive control group (PC) was treatment with fluoxetine hydrochloride (98%) 18 mg/kg/d (0.2 mL/d) (28, 41), the model control group (MC) was treatment with saline (0.2 mL/d), the group was treatment with CCHAA at a high dose (CCHAAH) was 1.5 mg/(g·d) (0.2 mL/d), the group for treatment with CCHAA at a low dose (CCHAAL) was 0.75 mg/(g·d) (0.2 mL/d). To be explained: the CCHAA was administered to mice by oral gavage using a combination of a 12-gauge gavage needle and a 1.0 mL syringe. In detail: First, the mouse was immobilized so that its head, neck and body were in line. The specific method is as follows: the right hand pulls the mouse's tail, the thumb, index finger, and middle finger of the left hand grab the mouse's neck scalp, and the little finger and the nameless press the mouse's tail. After grasping the mice, gavage can be performed. The specific method is as follows: the needle enters from the corner of the mouse's mouth, presses the tongue, and pushes it inward carefully against the upper jaw. The gavage volume is usually 0.01–0.02 mL/g, and the maximum gavage volume of each mouse is no more than 0.8 mL. It should be noted that the immobilization of the mouse is the most important step in the gavage administration, and it needs to move quickly to reduce the discomfort of the animal.

The elevated plus-maze test

Behavioral experiments were performed on all mice during the day after 21 days of modeling (fourth week) and 4 weeks of treatment (eighth week) (42). The ZH-DSG elevated cross maze hardware and camera system used in this experiment were purchased from Anhui Zhenghua Instrument Equipment Co., Ltd. (Huaibei, Anhui, China). The maze consists of two open arms (30 cm long × 5 cm wide) and two closed arms (30 cm long × 5 cm wide × 15 cm deep) that extend from a common central platform (5 cm long × 5 cm wide) while the entire maze was raised to a height of 80 cm. In the whole test process, a calm and stable environment was ensured to obtain accurate results. Further, each mouse was placed separately in the center of the maze to face one of the open arms, and their behavior was recorded by video for 5 min. Then, the video was analyzed, and the number of entries (OE), and time (OT) of mice entering the open arm, the number of entries (CE) and time (CT) of mice entering the closed arm during the experimental period (5 min) were recorded, which was used to evaluate the anxiety like behavior of animals. It should be noted that between each test, the maze needs to be thoroughly cleaned with alcohol so that the animals will not be affected by the smell of the previous urine and feces (41, 43).

The equation for calculating the percentage of animal open arm entry times and the percentage of animal open arm residence time is as follows:

$$OE\% = \frac{OE}{OE + CE} \times 100\% \tag{1}$$

$$OT\% = \frac{OT}{OT + CT} \times 100\% \tag{2}$$

The open-field test

The ZH-ZFT open field experiment hardware and camera system used in this experiment was purchased from Anhui

Zhenghua Instrument Equipment Co., Ltd. (Huaibei, Anhui, China). The experimental equipment is mainly composed of white PVC material, which is square box (40 cm long × 40 cm wide × 40 cm high). In addition, before each experiment or between two tests, the experimental equipment should be thoroughly cleaned with 70% ethanol aqueous solution and dried to eliminate the influence of urine and fecal odor left by the previous test. It should be noted that in order to obtain accurate results during the whole test process, it is necessary to ensure that the experiment is carried out in a quiet and stable environment. More importantly, the test also includes two stages: pre-test, in which the animal is gently placed in the top right corner of the box and allowed to adapt for 15 min. Further, a 10 min test was conducted 24 h after the end of the pre-test, in which each mouse was placed separately in the top right corner of the box again and allowed to move freely, and their movements and behaviors were also recorded *via* the digital camera system. After the experiment, the mice were immediately removed from the box and put back into the feeding cage. Finally, for video analysis, the analysis system is set to divide the box bottom into 16 equal sizes (10 cm × 10 cm) and record the time spent on each small square (28, 41). The percentage of time spent in the center was calculated skillfully by using the following formula to evaluate the anxiety like behavior of animals (42):

$$\text{Percentage of central residence time (\%)} = \frac{\text{Central residence time}}{\text{Total time}} \times 100\% \qquad (3)$$

The forced swimming test

The ZH-QPT forced swimming hardware and camera system used in this experiment were purchased from Anhui Zhenghua Instrument Equipment Co., Ltd. (Huaibei, Anhui, China). All mice were subjected to forced swimming test during the day. In order to obtain accurate results, the experiment is carried out in a quiet and stable environment with the temperature is remained at 25 ± 1°C throughout the experiment. The test was conducted twice in 2 days. In the first stage, each mouse was gently placed into a glass cylinder (diameter 10 cm × 25 cm high) filled with water (water level height of 15 cm), so that each experimental mouse was adapted to swimming for 6 min. In the second stage test, 24 h after the end of adaptive swimming, the second stage experiment was started for a total of 6 min, in which the animals were placed in the cylinder and adapted to swimming for 1 min, and then a 5-min test was started. During the test, a digital camera system was used to track and record the movement and behavior of animals, and the total duration of immobility (in seconds) was measured (28, 41). More specifically, the standard of immobility in swimming was that when the experimental mice floated, they were observed to gently stroke or move with only one foot to keep their head above the water without struggling (44). And the mice were removed from the water and put back into the feeding cage immediately after the experiment.

Determination of biochemical indexes

After the behavioral experiments, mice were killed under the anesthetic effect of chloral hydrate. At the same time of removing the mouse eyeballs, the blood was immediately collected into the EDTA tube on ice, then centrifuged in a refrigerated centrifuge with the speed of 3,000 rpm/min and the temperature of 4°C for 15 min, and finally stored at –80°C until it was used. After that according to the manufacturer's instructions (Shanghai QiaoDu Biotechnology Co., Ltd.), the contents of plasma corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and corticosterone (CORT) were determined by enzyme-linked immunosorbent assay (ELISA), and chemical colorimetry (45). Therefore, the prefrontal cortex, hypothalamus, and hippocampus were dissected accurately, weighed separately, and added with a certain amount of PBS to maintain their pH of 7.4, and snap frozen in liquid nitrogen for further use. Additionally, the specimen was thawed and still kept at a temperature of 4°C, and the supernatant was carefully collected and frozen for further use. Naturally, the double antibody sandwich method was used to determine mouse serotonin (5-HT), dopamine (DA), and γ-aminobutyric acid (GABA) levels. Firstly, the microplate was coated with purified mouse 5-HT, DA, and GABA antibodies to make solid-phase antibodies. Next, 5-HT, DA, and GABA were successively added to the micropores coated with monoclonal antibodies, and then combined with HRP labeled 5-HT antibodies to form antibody-antigen-enzyme labeled antibody complex. After thorough washing, the substrate TMB was added for color development. It should be reminded that TMB is converted into blue under the catalysis of HRP enzyme, and finally into yellow under the action of acid. In addition, the color depth was positively correlated with 5-HT, DA, and GABA in the samples. Finally, the absorbance (OD value) was measured by microplate reader at 450 nm wavelength, and the concentrations of 5-HT, DA, and GABA in mice were calculated by standard curve (41).

Histopathological examinations of hippocampus

The mice were deeply anesthetized with 4% chloral hydrate (0.1 mL/10 g intraperitoneally) and sacrificed by eyeball enucleation for blood sampling and spinal dislocation. Then, the hippocampus and hypothalamus were quickly removed on ice and rinsed with ice-cold saline solution. After absorbing water with absorbent paper, the hippocampus and hypothalamus were fixed with 4% paraformaldehyde fixative for 36 h. Next, the hippocampus and hypothalamus tissue were taken out from the fixative and placed in the dehydration box, and subsequently, the dehydration box was put into the dehydrator for dehydration with the following gradient alcohols:

Subsequently, the dehydration box was put into the dehydrator for dehydration with the following gradient alcohols: 75% alcohol dehydration for 4 h, 85% alcohol dehydration for 2 h, 90% alcohol dehydration for 2 h, 95% alcohol dehydration for 1 h, and anhydrous ethanol for 1 h, dehydration of alcohol, and benzene for 8 min, dehydration of xylene for 10 min, melted paraffin wax I at 65°C for 1 h, melted paraffin wax II at 65°C for 1 h, and melted paraffin wax III at 65°C for 1 h. The wax-soaked hippocampal and hypothalamic tissues were embedded in an embedding machine and cooled at –20°C. After the wax solidified, the wax block was taken out of the embedding frame and placed in a paraffin microtome to slice with a thickness of 4 um. The sections were floated on a spreader in 40°C warm water to flatten the tissue, and then the tissue was picked up with a glass slide and baked in a 60°C oven. After the water was dried and the wax was baked, it was taken out and stored at room temperature for later use. Immediately afterward, the slices were placed in the following reagents in sequence: xylene I for 20 min, xylene II for 20 min, absolute ethanol I for 5 min, absolute ethanol II for 5 min, 75% alcohol for 5 min, and then washed with tap water. The tissue samples were stained with hematoxylin-eosin (H & E). The slices were placed in anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, anhydrous ethanol II for 5 min, xylene I for 5 min, and xylene II for 5 min, and then sealed with transparent neutral gum. Finally, the samples were examined under an upright optical microscope (Nikon Eclipse E100, Nikon, Japan) and imaging system (Nikon DS-U3, Nikon, Japan) (41).

Data processing

One way ANOVA was performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software, and all tests were repeated three times. The analysis of the behavioral test was repeated 3 times because of errors, which need to be corrected by multiple experiments to ensure the universality and accuracy of the experiments. At the same time, it has the benefit of showing that the experiment can be repeated to show that the results are testable. Significance of differences between the means of the data in each group was assessed by using Duncan's *post-hoc* test ($p < 0.05$). And the presentation of the data was in the form of mean ± variance. In all figures, statistically significant differences between group means were expressed generally as a, b, c, for $p < 0.05$, 0.01, and 0.001, respectively.

Results and discussion

Effects of CCHAA on body weight

During the whole experimental process, the body weight changes of the mice were shown in **Table 1**. In addition, the initial body weight of the mice in each group was 21 ± 2 g, and there was no significant difference ($p > 0.05$) among the groups.

TABLE 1 Effects of several formulas on the body weight of mice.

Groups	Initial body weight (g)	Body weight at 21 days after modeling (g)	Body weight after 4 weeks of gavage (g)
NC	22.14 ± 1.28^{a}	27.62 ± 1.37^{a}	32.41 ± 2.12^{a}
MC	21.00 ± 1.82^{a}	25.00 ± 0.97^{bc}	26.69 ± 1.22^{b}
PC	21.78 ± 2.06^{a}	26.10 ± 0.71^{a}	31.57 ± 0.83^{a}
CCHAAH	20.91 ± 1.44^{a}	24.16 ± 1.49^{bc}	29.32 ± 0.73^{c}
CCHAAL	20.72 ± 0.90^{a}	24.78 ± 1.91^{bc}	28.44 ± 1.18^{c}

Different letters in the same column of data indicate significant differences between groups ($p < 0.05$).

After 21 days of modeling, the body weight of the modeling mice in each group was significantly lower ($p < 0.05$) than that of the normal group, which indicated that our modeling was successful. Further, after 4 weeks of oral gavage administration, the body weights of mice in NC, PC, CCHAA (H and L) groups were significantly higher than ($p < 0.05$) in model group mice. In short, these results suggest that each of the tested samples exhibited efficacy in alleviating anxiety-like behaviors in anxiety-depressed mice after oral gavage administration for 4 weeks.

The effects on the elevated plus-maze test

Studies have shown that the elevated plus-maze is a method for rapidly screening mice for activity and anxiety-like behaviors without requiring pre-training of animals and the establishment of complex schedules (46). After 4 weeks oral gavage administration, compared with the mice in the model group, there were significant differences ($p < 0.05$) in the number of times of entering the open arms and the percentage of time spent in open arms in each test article-treated group, and the results are listed in **Figure 1A**. It can be known that the percentage of time spent in the open arm of the NC group was significantly ($p < 0.05$) higher than that of the other treatment groups, and the percentage of time spent in the closed arms was significantly lower ($p < 0.05$) than that of the other treatment groups. Obviously, the improvement effect of the number of times of entering the open arm was in the following order: NC > CCHAAL > CCHAAH > PC > MC; Meanwhile, the improvement effect of the percentage of residence time in the open arms was in the order: NC > PC > CCHAAL > CCHAAH > MC; And the percentage improvement effect of residence time in closed arms was in the following order: NC > PC > CCHAAH > CCHAAL > MC.

In the elevated plus-maze test, the arms in the horizontal direction are closed arms, the arms in the vertical direction are open arms, and the red lines are the recorded movement

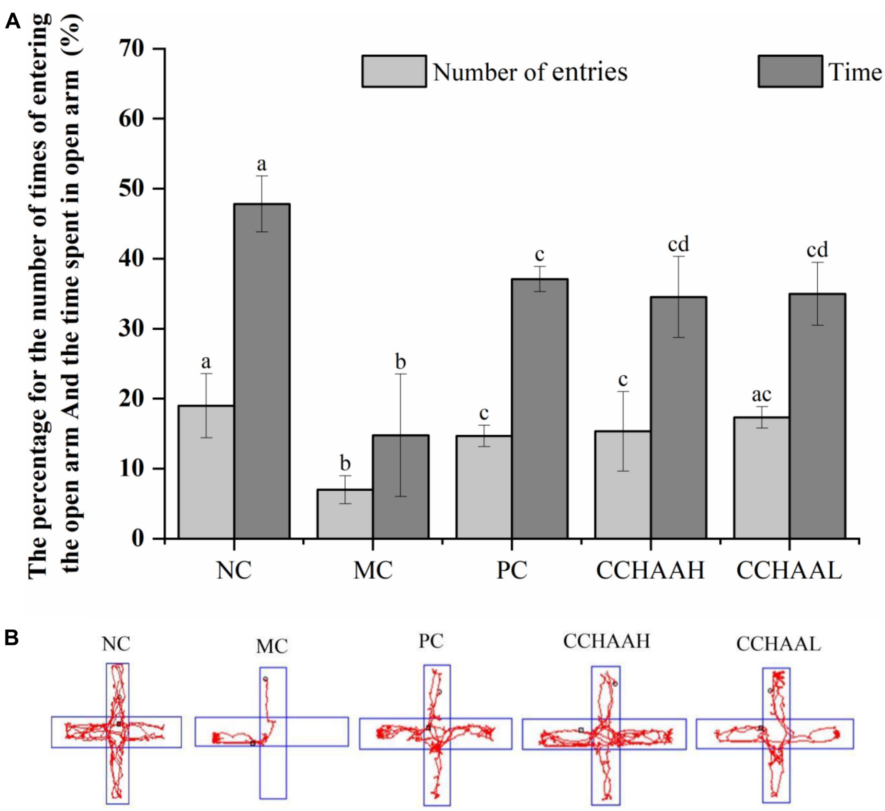


FIGURE 1
(A) The effects of CCHAA formula on the behaviors of mice in the elevated plus-maze test. Different letters indicate significant differences ($P < 0.05$). **(B)** The total movement tacked in EPM test: The red lines were the recorded motion tracks/routes of mice. The arms in the vertical direction were the closed arms, and those in the horizontal direction were the open arms.

routes of the mice (**Figure 1B**). Movement loci and maximal depth into the open arms indicated that NC mice did not exhibit anxiety-like behaviors (**Figure 1B**) (47, 48). In the MC group, less movement in the closed arm and essentially no movement in the open arm were observed, and it was observed to stay in the corner of the closed arm, exhibiting pronounced anxiety-like behavior. According to the movement loci, the performance of the mice in each test article treatment group was comparable, but the maximum depth of entry into the open arms and the number of times the mice entered the open arms varied among these groups. These results show that each test article can alleviate the anxiety-like behavior of anxiety-depressed mice after 4 weeks of oral gavage administration.

The effects on the open-field test

The open field test is a simple and widely used method for evaluating activity and anxiety-like behavior in mice (46). The results after 4 weeks of gavage-treated mice with each test article are displayed in **Figure 2A**, where the percentage of central residence time of the mice in the NC group (20.80%) was significantly higher ($p < 0.05$) than those in the other test article-treated groups. The experimental results clearly demonstrated that the percentage of central residence time (0.84%) of the mice in the MC group was significantly lower ($p < 0.05$) than that of the mice in the other test groups. Furthermore, compared with MC mice, the percentage of central residence time in PC (7.19%) and CCHAAH (5.69%) was significantly increased ($p < 0.05$). The percent improvement effect of central residence time was as follows: NC > PC > CCHAAH > CCHAAL > MC.

After 4 weeks of drug oral gavage administration, the total movement loci (within 10 min) of each group are clearly depicted in **Figure 2B**: the black dot marks indicate where the mice started the test, the black square marks indicate where the mice stopped after 10 min of testing, and the red lines are the recorded movement loci or routes of the mice (28, 41). As a result, the movement loci of the mice in the MC group were mostly away from the center (around the edges and corners). In addition, compared with the mice in the MC group, the movement loci and the central area activity loci of the mice in treatment group were significantly ($p < 0.05$) increased.

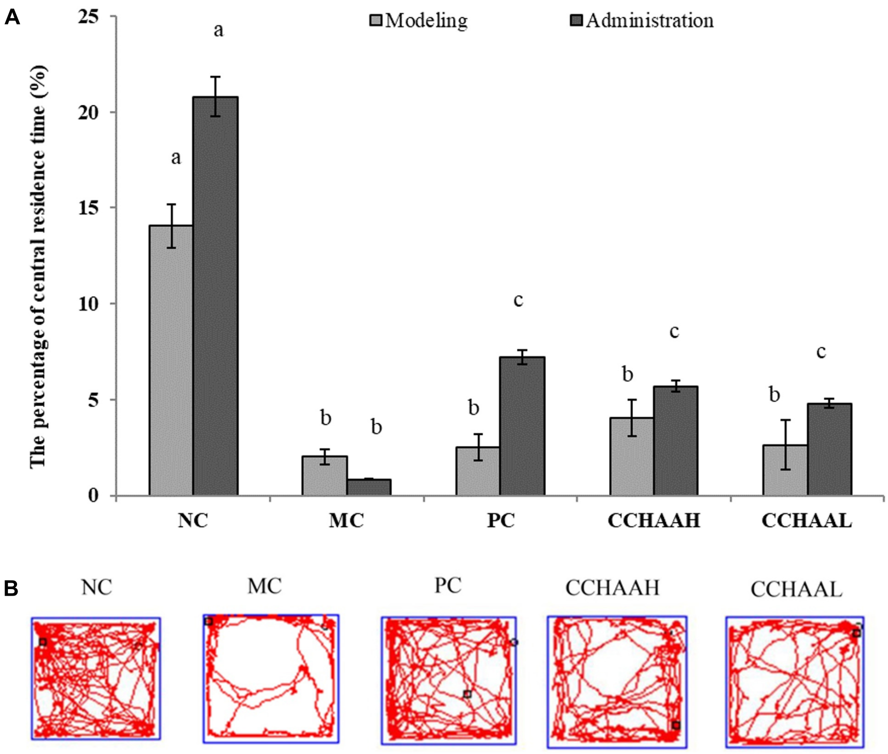


FIGURE 2
(A) The effects of CCHAA formula on the behaviors of mice in the open-field test. Different letters indicate significant differences ($P < 0.05$). **(B)** The total movement tracked in OFT: The black dots indicate where the mice started the test, with the black boxes showing where the mice stopped after the 10-min test, and the red lines were the recorded motion track/routes of mice.

In conclusion, the fluoxetine hydrochloride and CCHAA have better effects in alleviating anxiety-like behaviors of mice in the open field test.

The effects on the forced swimming test

The forced swimming test as a stress model for depression is a typical method for evaluating antidepressant drugs and depression model systems, which is widely used in basic research on stress, psychiatry, and neuropharmacology (49). The results after 4 weeks of gavage treatment of each test article are exhibited in **Figure 3A**, and the results revealed that the mice in the MC group had the longest swimming immobility time (240.08 s), which was significantly higher ($p < 0.05$) than that of the PC and CCHAA groups. Nevertheless, the swimming immobility time in the NC group was the shortest (132.97 s). Although the swimming immobility time in the NC group was slightly lower than PC and CCHAA (H and L) groups, the difference was not statistically significant ($p > 0.05$). Besides that, it has been reported that the longer the swimming immobility time of experimental animals in the forced swimming test, the more severe the depression-like behavior (50).

The total movement traces in the FST are shown in **Figure 3B**. The MC mice mostly stayed around the edges with less movements, indicating obvious depressive-like behaviors (51). Whereas the mice of the PC and the CCHAA (H and L) groups exhibited significantly ($p < 0.05$) more movement including access to the center. Accordingly, the CCHAA formula can effectively eliminate the prolonged immobility time caused by CRS-CORT in mice, thereby effectively alleviating the depression-like behaviors in mice. Consequently, according to our results, both fluoxetine hydrochloride and CCHAA reduced the swimming immobility time in mice, which indicated that CCHAA improved behavior of depression-like in anxiety/depressed mice.

The effects on the secretion of plasma hypothalamic-pituitary-adrenal axis

The dysfunction of hypothalamic-pituitary-adrenal (HPA) axis is currently recognized as one of the pathogeneses of anxiety and depression (52). Negative emotions such as anxiety and/or depression act as a stressor to stimulate the body to produce stress responses, which are felt in the cerebral cortex (53). After stimulation, the signal is transmitted to the hypothalamus, so

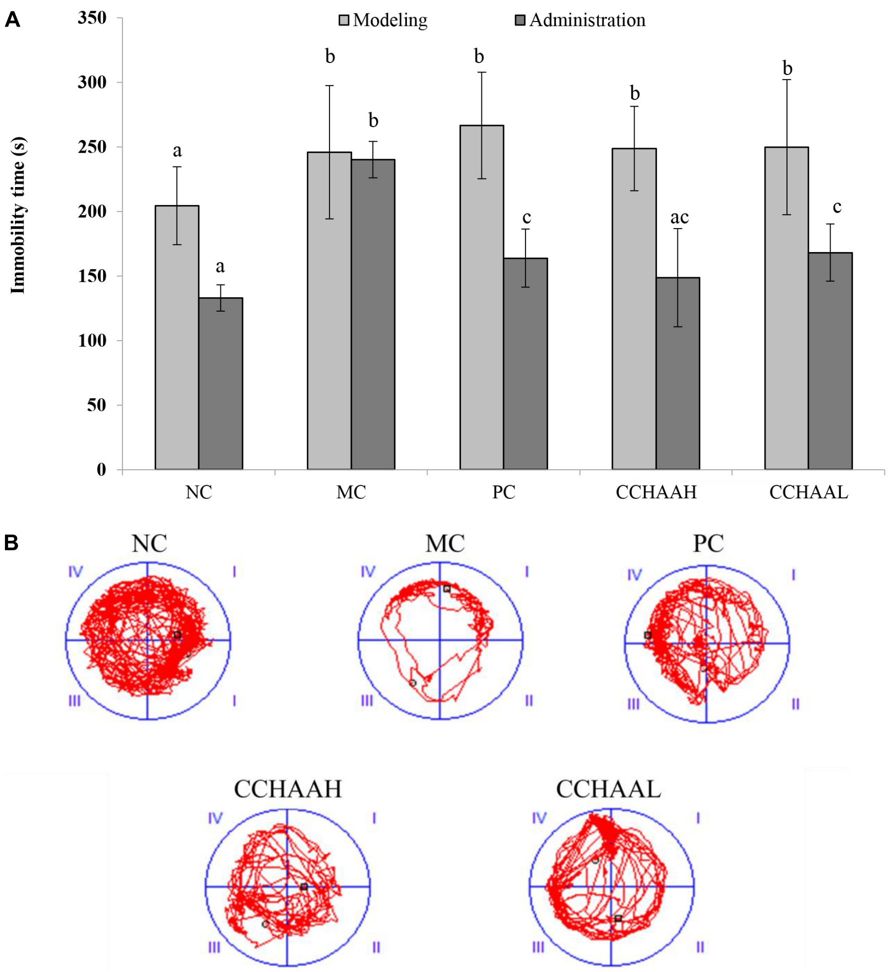


FIGURE 3
(A) The effects of CCHAA formula on the behaviors of mice in the forced swimming test. Different letters indicate significant differences ($P < 0.05$). **(B)** The total movement tracked in FST: The black dots indicate where the mice started the test, with the black boxes showing where the mice stopped after the 10-min test, and the red lines were the recorded motion track/routes of mice.

that the hypothalamus releases corticotropin-releasing hormone (CRH); then, CRH stimulates the anterior pituitary to release ACTH; further, ACTH stimulates the adrenal gland to secrete CORT, which in turn has a negative feedback effect on CRH and ACTH, thereby affecting the functional state of the HPA axis (54). Studies have shown that plasma CRH and ACTH levels were significantly increased in patients with anxiety and depression (55). CORT, as the main plasma corticosteroid (75–90%), was essential for anxiety-depressive disorder and the body's anti-stress response (including activation of tryptophan-2,3-dioxygenase, and immune system responses) (56, 57). Studies have also shown that chronic exposure to high levels of glucocorticoids reduced the expression of glucocorticoid receptors (presented in the hippocampus and provided negative feedback to the hypothalamus to prevent further release of glucocorticoids) (52, 58) and impaired the negative feedback regulation mechanism of the HPA axis (52, 59).

The result of the effect on plasma CRH and ACTH levels in anxiety-depressed mice are listed in **Table 2**. CRH (33.26 pg/mL) and ACTH (37.58 pg/mL) in NC mice were at normal levels, while The CRH and ACTH levels in MC group were significantly higher ($p < 0.05$) than those in the NC group. After 4 weeks oral gavage administration with CCHAA, the levels of plasma CRH and ACTH in the mice were significantly lower ($p < 0.05$) than those in MC group. According to the previous studies (27, 28, 41), CCHAA have the effect on improving the levels of plasma CRH and ACTH in anxiety/depression mice.

The effects on neurotransmitter secretion in the brain tissue

After the 4-week treatment of this study, the NC mice had normal 5-HT, DA, and GABA level in the brain tissue (**Table 3**).

TABLE 2 The effects on the secretion of plasma hypothalamic-pituitary-adrenal (HPA) axis (n = 12).

Groups	CRH (pg/mL)	ACTH (pg/mL)
NC	33.26 ± 1.85^{a}	37.58 ± 2.90^{a}
MC	60.29 ± 6.52^{b}	91.82 ± 10.60^{b}
PC	45.85 ± 1.18^{c}	59.84 ± 5.92^{c}
CCHAAH	41.70 ± 2.02^{c}	57.46 ± 5.31^{c}
CCHAAL	42.56 ± 2.84^{c}	64.31 ± 1.78^{c}

Different letters in the same column of data indicate significant differences between groups ($p < 0.05$).

TABLE 3 The effects on neurotransmitter secretion in the brain tissue (n = 12).

Groups	5-HT (ng/mL)	DA (ng/mL)	GABA (μmol/L)
NC	391.53 ± 55.30^{a}	7.03 ± 0.51^{ac}	7.39 ± 0.41^{a}
MC	285.79 ± 26.00^{b}	5.43 ± 1.32^{b}	4.54 ± 1.10^{b}
PC	360.43 ± 14.29^{ac}	8.11 ± 0.23^{c}	6.81 ± 1.28^{a}
CCHAAH	325.50 ± 18.67^{c}	8.57 ± 0.63^{c}	7.19 ± 1.66^{a}
CCHAAL	332.68 ± 76.28^{c}	6.40 ± 1.20^{b}	7.19 ± 1.13^{a}

Different letters in the same column of data indicate significant differences between groups ($p < 0.05$).

The level of 5-HT, DA and GABA in the MC group were significantly lower ($p < 0.05$) than those in the NC group, which indicated that chronic restraint stress combined with subcutaneous injection of CORT on the back could induce a significant decrease ($p < 0.05$) in the levels of 5-HT, DA, and GABA in the brain tissue. The levels of 5-HT and GABA in the brain tissue of PC group (fluoxetine hydrochloride treatment group) and CCHAA (casein hydrolysate: GABA) mice were significantly higher than ($p < 0.05$) in the MC group. Compared with mice in MC group, DA level in brain tissue of PC and CCHAAH-treated mice were significantly increased ($p < 0.05$). There was no significant different ($p > 0.05$) between MC and CCHAAL group, and CCHAAL was slightly higher than MC mice.

The effects on the histopathology of hippocampal CA3 region

The hippocampus is a high-level regulatory center of the subcutaneous center of emotional management (59). Long-term CORT exposure will atrophy and reduce the number of vertebral cells in the hippocampal CA3 region of patients with anxiety and depression, resulting in structural and functional damage to the hippocampus (60–62). Hence, the histopathological observation of the mouse hippocampus was performed, and the results of H&E staining of the hippocampal CA3 region were illustrated in **Figure 4**. It can be seen that the hippocampal structure of the NC mice was clear, the pyramidal cells were closely arranged, and the tissue cell shape (in oval shape) was regular. Compared with the NC mice, the number of pyramidal cells in CA3 area of MC mice was significantly reduced, cytoplasmic vacuolation, nuclear pyknosis, hyperchromatic cytoplasm, cell membrane shrinkage, irregular cell morphology, and blurred cell boundaries. After 4-week of treatment with fluoxetine hydrochloride and CCHAA, compared with the MC group, the number of cells in the CA3 region of hippocampus was significantly increased ($p < 0.05$), neatly arranged, the cell morphology was basically normal, and the outline of the hippocampus was clear. Despite the presence of pyramidal cell lesions in the CA3 area, the histopathological sections of the hippocampal CA3 region of CCHAA-treated mice were more similar to those of NC mice. In conclusion, fluoxetine hydrochloride and CCHAA have the effect on improving the pathological changes in the CA3 region of hippocampus in mice with anxiety/depression.

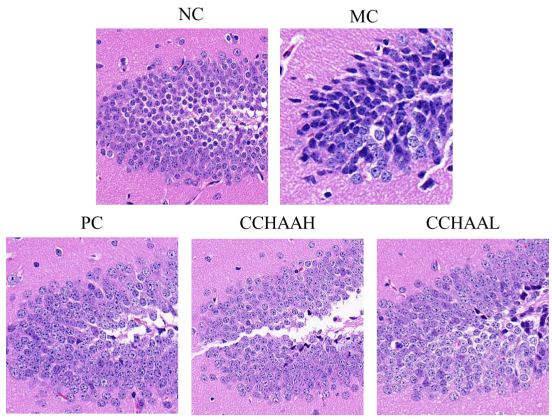


FIGURE 4
The histopathological alterations in the hippocampal CA3 regions caused by CCHAA.

Here, we have shown that CCHAA formulation has an improving effect on anxiety/depressive function in mice. Specifically, the effects of CCHAA formula on anxiety-depression-like behaviors including weight change, elevated plus maze test, open-field test, and forced swimming test in anxiety/depressed mice, the effects on plasma HPA axis secretion, the effects on neurotransmitter secretion in brain tissue, and the effects on histopathology in hippocampal CA3 region, were studied, respectively. Fluoxetine, a widely used new antidepressant drug (63), was used as a positive control in the experiment. It can be found from these experimental results that the CCHAA (H and L) formulations and the PC groups effectively reduced anxiety/depression-like behaviors in mice subjected to chronic stress. In addition, the effects of CCHAA (H and L) formula groups were very similar to that of PC group, which indicates that CCHAA (H and L) formulas could achieve the same degree of anti-anxiety/depression effect as fluoxetine.

It was found in our previous work that Trp oligopeptides (EW and WPH) could improve anxiety/depression function and four possible mechanisms by which Trp oligopeptides enhanced 5-HT synthesis and anti-anxiety/depression effects were proposed (27, 28). Accordingly, we can fully guess that the possible mechanism of Trp oligopeptide enhancing 5-HT synthesis and anti-anxiety/depression effects may be very similar to that of the CCHAA (H and L) formulations (27).

Conclusion

This study aimed to investigate the improvement of the anxiety/depression function by the compound of casein hydrolysate and γ-aminobutyric acid (GABA) (casein hydrolysate: GABA = 4:1; CCHAA) on mice induced by chronic restraint stress-corticosterone injection. The anxiety/depression-like behaviors, GABA and 5-HT synthesis, histopathological changes in the hippocampus CA3 region which is related to anxiety/depression in mice, was further studied. Animal experiments revealed that oral gavage administration of CCHAA significantly reversed the anxiety/depression-like behaviors. Compared to the model control group, body weights were increased after treatment with CCHAA groups [1.5, 0.75 mg/(g·d)]. As a diagnostic index of anxiety and depression, we assessed GABA and 5-HT levels in response to CCHAA ingestion. The GABA and 5-HT levels were increasingly enhanced by the CCHAA diet. In addition, histopathological changes in the hippocampus CA3 region of the anxious/depressed mice were also alleviated after the treatment with the CCHAA. Thus, the casein hydrolysate and GABA formula diets may induce beneficial effects on the mice with anxiety/depression. According to the demonstrated effects of the CCHAA formula in reversing the anxiety/depression-like behaviors, regulating the HPA axis, alleviating the histopathological changes in the hippocampus CA3 region of the anxious/depressed mice, the CCHAA formula might be an effective therapeutic product to help treat anxiety/depression. Moreover, it is particularly important that it also provides a new direction and guiding significance for our team to further development of new drugs for the treatment of anxiety/depression.

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/s.

Ethics statement

The animal study was reviewed and approved by the Committee for the Care and Use of Laboratory Animals in the South China Agricultural University [IACUC No. 2018D047].

Author contributions

LC, CC, and WL conceived the idea. LC and QT conducted the statistical analyses. LC and XZ drafted the manuscript. XZ and QT carried out experiments. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

Author QT and WL were employed by Infinitus (China) Company Ltd. Author CC was employed by Guangdong Weiwei Biotechnology Co., 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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Awareness of nutrition and health knowledge and its influencing factors among Wuhan residents

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Background: Nutrition and health knowledge play a crucial role in promoting healthy dietary behaviors, and have been found to be related to sociodemographic characteristics. However, the existing evidence is limited and inconsistent. We aimed to evaluate the awareness level of nutrition and health knowledge and its influencing factors among Wuhan residents, and to provide scientific basis for carrying out targeted nutrition education programmes.

Methods: By stratified random sampling, residents aged 18–64 in Wuhan were selected for self-administered questionnaire survey. We adopted the structured questionnaire to investigate respondents' sociodemographic characteristics, nutrition and health knowledge, and the way to acquire knowledge. Among them, nutrition and health knowledge includes the following four parts: dietary guidelines recommendations, food and nutrients, nutrition and disease prevention, and nutrition skills. Chi-square tests were used to analyze the associations between total awareness rate and sociodemographic characteristics. Multiple linear regression models were used to analyze the influencing factors of nutrition and health awareness.

Results: A total of 33,436 valid questionnaires were obtained, with a response rate of 97.8%. The total awareness rate was 20.4%, with the highest in nutrition and disease prevention (72.7%) and the lowest in nutrition skills (46.3%). Responders aged 35–44 (23.3%), females (22.8%), educational workers (24.8%), obtaining a master's degree or above (34.1%), living in downtown area (23.1%), and without a history of chronic disease (24.6%) were more likely to have higher awareness rates (all $p < 0.001$). The multiple linear regression models showed that age, gender, education level, occupation, residential address, and the history of chronic disease were the potential factors affecting individual nutrition awareness.

Conclusion: The total awareness rate of nutrition and health knowledge among Wuhan residents was not optimistic. Besides, our findings suggested that sociodemographic characteristics are closely related to nutrition awareness, which may provide important clues for carried out nutrition education campaigns.

KEYWORDS

nutrition awareness, influencing factors, sociodemographic characteristics, health literacy, survey

Introduction

With the rapid economic development and the improvement of living standards, significant changes have taken place in the lifestyle and dietary patterns of Chinese people. Meanwhile, the prevalence of nutrition-related chronic diseases such as obesity, hypertension and diabetes has increased year by year, causing huge burden on individuals and society (1, 2). Current evidence strongly suggests that dietary and nutritional status plays a critical role in the development of chronic diseases (3). However, date from the latest China Health and Nutrition Survey (1, 2, 4) revealed a series of nutrition-related problems in Chinese population: (a) the level of nutrition and health knowledge among Chinese residents varies greatly, and most people maintain unreasonable dietary habits; (b) unreasonable diet structure and unbalanced nutrition intake are common; and (c) more seriously, the dietary pattern of Chinese people is gradually shifting from a plant-based diet to a high-energy, high-fat and high-protein dietary pattern. The reasons for the above problems may be partly attributed to lower nutrition literacy, incorrect nutrition concepts and lack of relevant nutrition skills (5, 6). According to the knowledge, attitude and practice (KAP) model, acquiring knowledge is a key first step to promoting belief and changing undesirable behaviors (7). Therefore, given the potential health hazards caused by the above problems, there is an urgent need to find out the shortcomings of people's nutrition and health knowledge, and further improve people's nutrition and health literacy.

It is well accepted that a higher level of nutrition knowledge contributes to promoting healthier dietary behaviors, while a lower level of nutrition knowledge is closely related to poor eating habits, unbalanced dietary patterns (5, 6), and a higher risk of nutrition-related chronic diseases (8), since those who have a lower level of nutrition knowledge but perceive themselves as healthy consumers have no incentive to change their poor behavior (2, 9). Specifically, a randomized controlled trial conducted in sub-Saharan African compared the effects of supplementary nutrition education and dietary counseling on nutrition knowledge and dietary behavior among Malawian pregnant women, and found that the nutrition awareness and dietary behavior of the intervention group receiving nutrition and dietary guidance were significantly improved compared with the control group (10). Similarly, Bottcher et al. (11) pointed out that college students who received formal nutrition education were more likely to adhere to the Mediterranean diet. Moreover, a growing body of literature shows that there is a significant positive correlation between nutrition knowledge and favorable dietary-related behaviors, such as healthy weight loss (12), purchasing intention of nutritious claims (13), consuming more green vegetables (14), grains and dairy products (15). However, other studies did not find any associations between nutrition knowledge and healthier food choices (16) and healthy effects (17), suggesting that nutrition knowledge may be a necessary but insufficient factor to change people's behavior (18).

Individual sociodemographic characteristics, including age, education level, economic status, and residential area, are key factors affecting their nutrition and health knowledge (19, 20). However, due to the discrepancies in the study population, survey design and analysis methods, some available results on the relationship between sociodemographic characteristics and nutrition and health knowledge remain controversial (7, 21, 22). For instance, a cross-sectional study conducted in Nanjing, China found female students have a higher level of nutrition knowledge than male students (7). Whereas, Xu et al. (21) did not observe any relationship between nutrition knowledge and gender. With respect to the relationship between nutrition knowledge and age, the National Health and Nutrition Examination Survey 2005–2006 reported a negative linear correlation (22), while other studies did not observe any association (23, 24). Despite the contribution of nutrition and health knowledge to the change of dietary behavior is intricate and affected by various sociodemographic and environmental factors, a better understanding of the relationship between nutrition and health knowledge and its possible influencing factors is urgently needed.

To date, however, there are few large-scale studies on nutrition and health knowledge of the Chinese population and its possible socio-demographic factors. Thus, we aimed to acquire the level of nutrition and health knowledge of Wuhan residents and to explore its influencing factors. Our findings will help to provide scientific basis for carrying out targeted nutrition education programmes.

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Materials and methods

Study design and participants

The China Nutrition and Health Knowledge Survey is an ongoing cross-sectional study launched in April 2021 in 31 provinces, cities and autonomous regions in China. The survey aims to explore the awareness level and its influencing factors of nutrition and health knowledge among Chinese adult residents and providing a scientific basis for the policy decision on nutrition intervention. Wuhan, a representative city in central China, conducted part of the survey from April 2021 to October 2021, and the permanent residents aged 18–64 from Wuhan were included in the analysis. Based on the results from the sixth national population census in China (25), we adopted the method of stratified cluster sampling, and selected participants according to the proportion of half male and half female, and the proportion of 20, 20, 20, 30, and 10% for 18–24, 25–34, 45–54, 35–44 and 55–64 years, respectively. The respondents were selected from 15 monitoring sites (3–10 communities were selected from each monitoring site). According to the population size of each monitoring site, 1–15 communities were selected for each monitoring site, and it is planned to survey 330 residents in each community, who were representative in terms of gender, age and regional distribution. After excluding people who refused to participate in the project, 33,436 of the 34,190 respondents were included in the analysis, with a response rate of 97.8%. The actual distribution of each monitoring site is shown in Supplementary Table S1. All respondents provided their written informed consent before conducting the survey. This study was approved by the Ethics Review Committee of the Wuhan Center for Disease Control and Prevention (approval number: WHCDCIRB-K-2021033).

Questionnaire

The questionnaire was designed by the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, and formed after expert review, pre-survey, reliability and validity test. We adopted the structured questionnaire to investigate respondents' sociodemographic characteristics (age, gender, educational level, occupation, residential address, and history of chronic disease), nutrition and health knowledge, and the way to acquire knowledge.

The nutrition and health knowledge consisted of 32 items, including 22 single choice questions and 10 multiple choice questions, involving the following four parts: (a) dietary guidelines recommendations (items 1–18); (b) food and nutrients (items 19–22); (c) nutrition and disease prevention (items 23–26); and (d) nutrition skills (items 27–32). For single choice questions, a score of 1.5 (items 1–8, 19, 23, 27, 28) or 2.0 (items 9–13, 24, 29–32) was assigned to the correct answer, and 0 was assigned to the wrong or "don't know" answers. For multiple choice questions with 5 options (one option is the "don't know" answer, and at least two options are the correct answer), a score of 0 was assigned to the "don't know" answer, and 1.5 was assigned to each correct answer. Besides, if the wrong answer was not selected, 1.5 points will be further added, with a total score of 6.0 (except for item 18). The specific scoring criteria was shown in Supplementary Table S2. Further, the scores of all items were added up to calculate individual awareness levels of nutrition and health knowledge, ranging from 0 to 100. A total score of 75.0 and above was defined as "awareness", otherwise defined as "unawareness".

For multiple choice questions, we defined both choosing the correct answer and not choosing the wrong answer as knowing this option. To assess the responders' awareness of each multiple choice questions, we also calculated the proportion of knowing 0, 1, 2, 3 and 4 options.

Definition of analysis indicators as follows:

Awareness rate of each item (single choice question):

$$\frac{\textit{the number of responders who selected the correct answer}}{\textit{total number of responders who completed the survey}} \times 100\%$$

Awareness rate of each item (multiple choice questions):

$$\frac{\begin{array}{c}\textit{the number of responders who were aware of 3 or more}\\ \textit{options in each item}\end{array}}{\textit{total number of responders who completed the survey}} \times 100\%$$

Awareness rate in each part:

$$\frac{\begin{array}{c}\sum \textit{the number of responders who were aware}\\ \textit{of each item}\end{array}}{\begin{array}{c}\textit{total number of responders who completed the survey}\\ \times \textit{total number of items in each part}\end{array}} \times 100\%$$

The total awareness rate:

$$\frac{\textit{the number of responders with a total score of 75 or above}}{\textit{total number of responders who completed the survey}} \times 100\%$$

Covariates

Age was divided into five groups ($\leq$ 24, 25–34, 35–44, 45–54, and 55–64 years). Gender was divided into two categories (male and female). Education level was allocated into six categories (primary school diploma or below, junior school diploma, high school diploma, junior college diploma, bachelor's degree, and master's degree or above). The occupation was allocated into five categories (medical workers, catering service workers, other health-related workers, educational workers, and others). The residential address included the downtown area and the remote area. A history of chronic diseases (including hypertension, diabetes, stroke, coronary heart disease, dyslipidemia, or other chronic diseases) was divided into three categories (Yes/No/Don't know).

Quality control

The questionnaire was revised after review by an expert group, preliminary investigation and reliability and validity test. Prior to the online survey, the investigators at each monitoring site must receive professional training and assessment. During the survey, all investigators carried out the investigation strictly in accordance with the unified survey manuals, and the participants should be informed of the purpose and filling method prior to answering the questionnaire. After completing a questionnaire, an on-site inspection is conducted immediately. Additionally, after the investigation of all monitoring sites is completed, the quality control personnel of the Centers for Disease Control and Prevention will conduct a telephone return visit to the respondents according to the specially designed review questionnaire. If there are 3 or more unqualified questionnaires at one monitoring site, the on-site investigation was considered unqualified and should be re-investigated.

Statistical analysis

The sociodemographic characteristics of respondents were all set as categorical variables and expressed as frequencies (percentages). Chi-square tests were used to analyze the associations between the total awareness rate of nutrition and health knowledge and sociodemographic characteristics. The awareness level of nutrition and health knowledge was a continuous variable with skewed distribution, presented as median (lower and upper quartile) and the differences between the two groups were compared using the Mann-Whitney U test. Multiple linear regression models were used to analyze the influencing factors of nutrition and health awareness. All statistical analyses were performed using SPSS version 25.0 (IBM Corporation). A two-tailed value of $p < 0.05$ was considered indicative of statistical significance.

Results

Sociodemographic characteristics of respondents

The average age of 33,436 respondents was (37.76 ± 12.29) years. 9430 (28.2%) were aged 35–44 years, and 7393 (22.1%) were aged 25–34 years. Males accounted for 47.0% and females for 53.0%. In terms of education level, 6.5% of respondents had a master's degree and 28.1% had a bachelor's degree or above. For the remainder, 23.7, 23.5, 15.6, and 2.7% of respondents had junior college, high school, junior school, and primary school diplomas or below respectively. In terms of occupation, respondents working in medical institutions, catering industry, other health-related industries, educational institutions and other work units accounted for 11.2, 4.0, 1.9, 8.5, and 74.4%, respectively. More than half of the responders (51.5%) were resident in the downtown city while the rest (48.5%) were resident in the far city. Additionally, respondents with chronic history accounted for 20.5% (Table 1).

TABLE 1 Sociodemographic characteristics of responders.

Sociodemographic characteristics	Frequency (n)	Percentage (%)
Age (years)		
18–24	6,444	19.3
25–34	7,393	22.1
35–44	9,430	28.2
45–54	6,521	19.5
55–64	3,648	10.9
Gender		
Male	15,724	47.0
Female	17,712	53.0
Education level		
Primary school diploma or below	893	2.7
Junior school diploma	5,206	15.6
High school diploma	7,845	23.5
Junior college diploma	7,916	23.7
Bachelor's degree	9,391	28.1
Master's degree or above	2,185	6.5
Occupation		
Medical workers	3,760	11.2
Catering service workers	1,342	4.0
Other health–related workers	624	1.9
Educational workers	2,848	8.5
Others	24,862	74.4
Residential address		
Downtown area	16,201	48.5
Remote area	17,235	51.5
History of chronic disease		
No	14,715	44.0
Yes	6,848	20.5
Don't know	11,873	35.5

Awareness rate of nutrition and health knowledge among respondents

Among the four parts of nutrition and health knowledge, the part of nutrition and disease prevention knowledge had the highest awareness rate of 72.7%, followed by the knowledge of core recommendations of dietary guidelines (59.6%), food and nutrients (49.4%), and nutrition skills (46.3%). The top three items for the awareness rate in single choice questions were: Q12 (84.3%), Q23 (82.6%), and Q27 (80.6%). The bottom three single choice questions were Q29 (21.5%), Q10 (21.6%) and Q11 (28.7%). The top three items for the awareness rate in multiple choice questions were: Q18 (80.9%), Q17 (78.3%), and Q14 (71.0%). The bottom three items for the awareness rate in multiple choice questions were: Q21 (21.0%), Q22 (35.2%), and Q15 (56.8%) (Table 2). Besides, for nutrition and health knowledge multiple choice questions, we calculated the proportion of knowing 0, 1, 2, 3, and 4 options, respectively. The percentage of responders who got all the answers correct ranged from 2.4 to 57.9% (Supplementary Table S3).

TABLE 2 Awareness rate of nutrition and health knowledge of respondents.

The items of nutrition and health knowledge	Awareness rate (*n*, %)
Core recommendations of dietary guidelines	358,806 (59.6)
Q1. Recommendations on vegetable intake in the Dietary Guidelines for Chinese Residents (2016)	25,897 (77.5)
Q2. Recommendations on fruit intake in the Dietary Guidelines for Chinese Residents (2016)	20,897 (62.5)
Q3. Recommendations on dairy products intake in the Dietary Guidelines for Chinese Residents (2016)	13,335 (39.9)
Q4. Recommendations on soybean and its products intake in the Dietary Guidelines for Chinese Residents (2016)	9,719 (29.1)
Q5. Recommendations on meat intake in the Dietary Guidelines for Chinese Residents (2016)	22,981 (68.7)
Q6. Recommendations on egg intake in the Dietary Guidelines for Chinese Residents (2016)	24,001 (71.8)
Q7. Recommendations on processed meat intake in the Dietary Guidelines for Chinese Residents (2016)	22,462 (67.2)
Q8. Recommendations on sweet foods or beverage intake in the Dietary Guidelines for Chinese Residents (2016)	25,303 (75.7)
Q9. How much salt is recommended for healthy adults every day?	19,644 (58.8)
Q10. How much added sugar is recommended for healthy adults every day?	7,227 (21.6)
Q11. How much cooking oil is recommended for healthy adults every day?	9,591 (28.7)
Q12. Which of the following is more nutritious for lunch?	28,173 (84.3)
Q13. If an adult's body mass index is 26.1 kg/m^2, what is his/her weight classification?	11,312 (33.8)
Q14. Which of the following statements about vegetables and fruits are true? *	23,754 (71.0)
Q15. Which of the following statements about Dietary Guidelines for Chinese Residents are true? *	18,986 (56.8)
Q16. Which of the following can help maintain a healthy weight? *	22,273 (66.6)
Q17. Which of the following are the correct explanations for saving food? *	26,204 (78.3)
Q18. Which of the following are the correct explanations for dietary hygiene? *	27,047 (80.9)
Food and nutrients	66,088 (49.4)
Q19. Which food is best for supplementing calcium?	24,570 (73.5)
Q20. Compared with refined staple foods, what are the nutritional values of coarse cereals? *	22,723 (68.0)
Q21. Which of the following foods is rich in iron and is easily absorbed by the body? *	7,020 (21.0)
Q22. Which foods below can supplement vitamin A? *	11,775 (35.2)
Nutrition and disease prevention	97,200 (72.7)
Q23. Which food contains more cooking oil and salt?	27,623 (82.6)
Q24. Which food is most beneficial to prevent dyslipidemia and cardiovascular disease?	26,108 (78.1)
Q25. Which of the following statements about salt/sugared beverages and chronic disease are true? *	19,735 (59.0)
Q26. Which of the following statements about foods and chronic disease are true? *	23,734 (71.0)
Nutrition skills	92,969 (46.3)
Q27. Read the food labels below. Which product contains more protein?	26,939 (80.6)
Q28. Read the food labels below. Which product belong to dairy products?	21,291 (63.7)
Q29. How much does a handful of vegetables weigh?	7,192 (21.5)
Q30. How much does a palm-sized piece of lean meat weigh?	10,061 (30.1)
Q31. How much does an ordinary egg weigh?	16,632 (49.7)
Q32. How much does a fist-sized steamed bread weigh?	10,854 (32.5)

*Multiple choice questions with 5 options (one option is the "don't know" answer, and at least two options are the correct answer). We defined both choosing the correct answer and not choosing the wrong answer as knowing this option. Responders are defined as knowing an item if they are aware of 3 or more options for this item.

Analysis of the influencing factors of nutrition and health knowledge

There were 6,825 responders whose total score of nutrition and health knowledge was 75 or above, with a total awareness rate of 20.4% (6,825/33,436). Across the age groups, the awareness rate of responders aged 35–44 years was the highest (23.3%), while responders aged 18–24 years had the lowest awareness rate (17.6%). A significant association was found between age and the nutrition awareness rate ($\chi^2 = 90.519$, $p < 0.001$). A higher proportion of the female respondents (22.8%) were aware of nutrition and health knowledge compared with the male respondents (17.7%) ($\chi^2 = 129.495$, $p < 0.001$). The results also showed that there was a significant correlation between the awareness rate of nutrition and health knowledge and education level ($\chi^2 = 715.809$, $p < 0.001$), occupation ($\chi^2 = 115.568$, $p < 0.001$), residential address ($\chi^2 = 140.783$, $p < 0.001$), and a history of chronic disease ($\chi^2 = 338.625$, $p < 0.001$) (Table 3).

Socio-demographic factors influencing the awareness level of nutrition and health knowledge were presented in Table 4. The final multivariable model showed that compared with responders aged 18–24 years, the awareness level was higher in the older group ($\beta = 3.939$, $p < 0.001$). Females were more aware of nutrition and health knowledge than males ($\beta = 2.780$, $p < 0.001$). Responders with higher education levels were more likely to have higher awareness levels ($\beta = 15.259$, $p < 0.001$). Compared with medical workers, catering service workers ($\beta = -1.355$, $p = 0.001$) and other health-related workers ($\beta = -4.727$, $p < 0.001$) had lower awareness levels, while educational workers ($\beta = 2.059$, $p < 0.001$) and other workers ($\beta = 2.303$, $p < 0.001$) had higher awareness levels. The awareness level of residents in a remote area was lower than that of downtown residents ($\beta = -0.691$, $p < 0.001$). Besides, compared with those without a history of chronic disease, those with chronic disease and those who did not know whether they had a chronic disease had a lower awareness level (Table 4).

Discussion

Based on the China Nutrition and Health Knowledge Survey, we conducted a large-scale cross-sectional study to comprehensively assess the nutrition and health knowledge level of Wuhan residents, and to examine how awareness levels varied across sociodemographic characteristics. Our results showed that the total awareness rate of nutrition and health knowledge of Wuhan residents was not optimistic (20.4%), especially in nutrition skills and food and nutrients aspects. Besides, we found individual sociodemographic characteristics, including age, gender, education level, occupation, residential address, and the history of chronic disease, were significantly associated with their awareness level of nutrition and health knowledge, suggesting that divergent demographic characteristics should be considered in carrying out various forms of nutrition education and promotional activities.

The awareness rate of nutrition and health knowledge in this study was similar to the results of the survey among adults in 9 provinces in China (21.1%), but much lower than that of adult residents in 3 municipalities (Beijing, Shanghai and Chongqing; 45.0%) (26), Suzhou (52.3%) (27) and Zhengzhou (40.5%) (28). Compared with foreign countries, the awareness rate of Wuhan residents is also lower than that of adults in the three central cities and rural areas of Italy (46.0%) (29) and the adults aged≥ 35 years in Central and Southern Italy (30). These discrepancies could be partially attributed to differences in study participants (race, economic status, age composition, etc.), questionnaire composition, and definition of nutrition awareness. For one-third of the items, nevertheless, about half of the respondents could not answer correctly. These misconceptions may further lead to inaccurate nutritional skills and undesirable dietary behaviors (7, 31). As Dickson-Spillmann et al. (32) indicated, for instance, participants with a lower level of nutrition knowledge consumed fewer fruits and vegetables, but more sausages than those with higher nutrition knowledge. Among the four parts of nutrition and health knowledge, we found respondents had the lowest overall awareness in nutrition skills (46.3%), especially when it came to estimating food weight (items Q29–Q32), with awareness rates ranging from 21.5 to 49.7%. These results suggested that respondents do not have a good grasp of the skills of estimating food portions, which may naturally lead to overconsumption or underconsumption, resulting in a massive gap between recommended intake and actual intake. Due to the complex interaction between nutritional information processing, motivational factors and behavior change, there is a significant gap between perceived knowledge and practical dietary intake (33, 34). Despite this, it is widely acknowledged that popularizing nutrition knowledge, including but not limited to nutritional skills, is essential.

In terms of food and nutrition, the awareness rate of each item ranged from 21.0 to 73.5%. The lower awareness rate of items 21 and 22 highlights the fact that the nutritional value of food is not present in many responders' minds and is not factored into their daily food choices. Therefore, more efforts are needed to increase people's awareness of food nutrition in order to facilitate people to choose food more rationally.

Given that awareness of dietary guidelines is a key factor in whether people are likely to adopt the recommended behavior, adherence to dietary guidelines may be particularly valuable in promoting rational dietary intake and reducing the risk of nutrition-related chronic disease (5, 35, 36), more than half of the items in this study are related to the core recommendations of Dietary Guidelines for Chinese Residents. The overall awareness rate of core recommendations of dietary guidelines in this study was 59.6%. However, the awareness rate of the recommended intake of added sugar

TABLE 3 Correlation of respondents' sociodemographic characteristics and the awareness rate of nutrition and health knowledge.

Basic characteristics	Awareness rate (*n*, %)	χ^2	*p*-value
Age (years)		90.519	<0.001
18–24	1,133 (17.6)		
25–34	1,511 (20.4)		
35–44	2,193 (23.3)		
45–54	1,327 (20.3)		
55–64	661 (18.1)		
Gender		129.495	<0.001
Male	2,791 (17.7)		
Female	4,034 (22.8)		
Education level		715.809	<0.001
Primary school diploma or below	96 (10.8)		
Junior school diploma	703 (13.5)		
High school diploma	1,292 (16.5)		
Junior college diploma	1,548 (19.6)		
Bachelor's degree	2,440 (26.0)		
Master's degree or above	746 (34.1)		
Occupation		115.568	<0.001
Medical workers	835 (22.2)		
Catering service workers	169 (12.6)		
Other health-related workers	79 (12.7)		
Educational workers	707 (24.8)		
Others	5,035 (20.3)		
Residential address		140.783	<0.001
Downtown area	3,744 (23.1)		
Remote area	3,081 (17.9)		
History of chronic disease		338.625	<0.001
No	3,618 (24.6)		
Yes	1,373 (20.0)		
Don't know	1,834 (15.4)		

(Q10), cooking oil (Q11), and the classification of body mass index (Q13) was lower than or close to 30.0%, indicating that responders were insensitive to number-based dietary recommendations, which may result in excessive intake of added sugar and cooking oil, and even increase the risk of diabetes and obesity (37, 38). In addition, we found that, compared with the awareness about the recommended intake of vegetables (Q1), eggs (Q6), meats (Q5 and Q7), and fruits (Q2), responders had a lower awareness about the recommended intake of soybean and its products (Q4) and dairy products (Q3), which tend to be the most overlooked daily foods. In this regard, it is suggested to emphasize the recommended intake of soybean and its products and dairy products, as well as the adverse effects caused by their deficiencies when carrying out the education about Dietary Guidelines for Chinese Residents. In respect of nutrition and disease prevention, the responders demonstrated higher awareness (72.7%), suggesting that although the respondents had a clear comprehension of diet related to possible chronic diseases, they could not regulate their daily diet well. Consistently, Bullen et al. (39) revealed that those who acquired factual knowledge did not make good use of their knowledge to change their unhealthy eating behavior. Generally speaking, although nutrition awareness is not always associated with a distinct dietary behavior, we still need to persist in popularizing people's nutrition and health knowledge from multiple perspectives. Specifically, in the future, it is suggested to disseminate rich and easy to accept nutrition and health knowledge to the population from multiple perspectives such as promoting dietary guidelines and nutrition skills training through various forms, for example, dietary training, expert consultation and thematic discussion.

A considerable amount of literature has been published on the relationship between sociodemographic characteristics and nutrition knowledge (40, 41). Similar to the findings in

TABLE 4 Factors influencing the awareness level of nutrition and health knowledge*.

Variables	Awareness level [M (P25, P75)]	Univariable model		Multivariable model	
		β (95% CI)	p-value	β (95% CI)	p-value
Age (years)					
18–24	64.5 (53.5, 72.0)	ref.		ref.	
25–34	66.0 (55.0, 73.5)	1.262 (0.799, 1.724)	<0.001	1.432 (0.989, 1.875)	<0.001
35–44	66.5 (55.0, 74.0)	1.780 (1.342, 2.219)	<0.001	2.937 (2.509, 3.365)	<0.001
45–54	65.5 (55.0, 73.5)	1.337 (0.861, 1.814)	<0.001	4.339 (3.848, 4.831)	<0.001
55–64	64.0 (53.0, 72.5)	−0.215 (−0.778, 0.347)	0.453	3.939 (3.344, 4.535)	<0.001
Gender					
Male	64.0 (52.5, 72.0)	ref.		ref.	
Female	67.0 (56.5, 74.0)	2.871 (2.575, 3.167)	<0.001	2.780 (2.496, 3.064)	<0.001
Education level					
Primary school diploma or below	54.5 (42.0, 67.5)	ref.		ref.	
Junior school diploma	61.5 (49.5, 70.0)	0.028 (−0.001, 0.056)	0.057	5.336 (4.398, 6.273)	<0.001
High school diploma	64.0 (53.0, 71.5)	0.057 (0.030, 0.085)	<0.001	8.202 (7.280, 9.123)	<0.001
Junior college diploma	65.0 (55.0, 73.0)	0.088 (0.060, 0.116)	<0.001	10.012 (9.075, 10.948)	<0.001
Bachelor's degree	68.0 (59.0, 75.0)	0.152 (0.125, 0.180)	<0.001	13.024 (12.083, 13.965)	<0.001
Master's degree or above	71.5 (63.5, 77.0)	0.234 (0.203, 0.265)	<0.001	15.259 (14.188, 16.330)	<0.001
Occupation					
Medical workers	65.0 (50.5, 74.0)	ref.		ref.	
Catering service workers	61.0 (47.5, 69.0)	−4.251 (−5.109, −3.392)	<0.001	−1.355 (−2.186, −0.524)	0.001
Other health-related workers	58.5 (43.6, 70.5)	−5.854 (−7.023, −4.685)	<0.001	−4.727 (−5.842, −3.611)	<0.001
Educational workers	68.0 (59.5, 74.5)	3.424 (2.763, 4.086)	<0.001	2.059 (1.411, 2.707)	<0.001
Others	65.5 (55.0, 73.0)	1.029 (0.571, 1.487)	<0.001	2.303 (1.860, 2.745)	<0.001
Residential address					
Downtown area	67.0 (56.5, 74.0)	ref.		ref.	
Remote area	64.0 (53.0, 72.0)	−2.256 (−2.552, −1.959)	<0.001	−0.691 (−0.984, −0.399)	<0.001
History of chronic disease					
No	67.5 (58.5, 74.5)	ref.		ref.	
Yes	64.5 (52.0, 73.0)	−3.745 (−4.137, −3.354)	<0.001	−3.666 (−4.062, −3.270)	<0.001
Don't know	63.0 (50.5, 71.5)	−5.287 (−5.617, −4.956)	<0.001	−5.143 (−5.462, −4.824)	<0.001

*Values are linear regression coefficients and 95% CIs. Only variables with statistically significant in univariate model were included in multivariate analysis.

other studies (23, 42), we found females tended to have higher nutrition awareness than males, which might be related to the fact that Chinese women mostly assume the roles of housewives and thus master more nutrition-related knowledge than men. Previous studies on the relationship between age and nutrition awareness have been equivocal (22, 23, 42). Compared to responders aged 18–24 years, we found the older tended to be more nutritionally aware, among which the middle-aged and elderly showed the highest nutrition awareness. Similarly, Glanz et al. (42) showed that the elderly were more nutritionally conscious than younger people. Plausible explanations for these results might be that older people have greater access to nutrition education activities. Conversely, the National Health and Nutrition Examination Survey 2005–2006 reported that the younger were more likely to have a higher nutrition awareness rate than the older (22), while Girois et al. (23) did not report any associations between age and nutrition awareness. However, divergent measures of nutrition awareness used in these comparisons should be taken into consideration. Several previous studies pointed out that individual nutrition level was positively correlated with their education level (17, 41), which was consistent with our findings. Moreover, we found medical workers and educational workers tended to be more aware of nutrition knowledge than other professions. The possible explanation for this result might be that the higher the level of education, the greater the learning capacity and the more opportunities for nutrition information. Besides, our study indicated that responders without the chronic disease had higher awareness levels, which may be related to their more attention to health care and nutrition information. In turn, those who do not know whether they have chronic diseases may also be indifferent to nutrition and health knowledge (43). Last but not least, our findings highlight the need to strengthen nutrition education for responders living in the remote area, as their access to nutrition information and basic public health services is significantly lower than that of responders living in the downtown area. The above findings of this study suggested that nutrition and health knowledge level varies across populations with various demographic characteristics. In the future nutrition campaign, we should give full play to the role of professional teams such as medical personnel, nutritionists and educators, and go deep into communities, rural areas, schools and other places to provide targeted dietary guidance for those younger, less educated, living in remote areas and with weak nutrition awareness.

Our study has several strengths. Firstly, the large sample size and representative study population came from 15 monitoring sites in Wuhan, which provided strong evidence for examining the nutrition awareness level of Wuhan residents. Secondly, we used validated questionnaires to evaluate participants' awareness of nutrition and health knowledge from various aspects. The results of our study can provide a reference for future nutrition questionnaire design. Finally, we have adopted strict quality control before, during and after the investigation to minimize potential deviations.

Our study also has some potential limitations. Firstly, since this survey uses a self-administered questionnaire, there might be some cases where responders filled in the questionnaire carelessly, however, three-level quality control measures were taken to reduce the information bias as much as possible. Secondly, although the participants in this study were representative of the Wuhan population in terms of age, gender, and residential area, we could not rule out the potential sampling bias. Thirdly, various definitions of nutrition awareness may contribute to different results, therefore, the results of interpolation and extrapolation need to be treated with caution. Last but not least, different scoring criteria and questionnaire design, such as the representative questions selected in each part may contribute to different results. However, in order to acquire the shortcoming of residents' nutrition and health knowledge in a large scale, the questions designed in each part should not only be closely related to nutrition and health, but also easy to understandable and representative. Additionally, the questionnaire used in this study were determined by expert review, pre-survey, reliability and validity test, which can ensure the design quality of the questionnaire.

Conclusion

In conclusion, in this large-scale cross-sectional study, the total awareness rate of nutrition and health knowledge among Wuhan residents was far from reaching the national requirements, especially in terms of nutrition skills. Besides, we found evidence that sociodemographic characteristics were associated with nutrition and health knowledge. Further nutrition education campaign is needed to target certain subgroups of the population or specific nutrition concerns.

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/s.

Author contributions

YW, SW, MS, LH, and XW designed the research. LH and XW supervised the study conduct. YW, SW, MS, XW, HL, SG, and LT conducted the research. SW analyzed the data and wrote the manuscript. YW and MS coedited, revised, and finally reviewed the manuscript critically for important intellectual content. All the authors read and approved the final version of the manuscript.

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Conflict of interest

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.987755/full#supplementary-material

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Vitamin D insufficiency is high in Malaysia: A systematic review and meta-analysis of studies on vitamin D status in Malaysia

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Purpose: To estimate the vitamin D status of participants residing in Malaysia.

Methods: PubMed, Scopus, Web of Science, and MyJurnal were searched up to June 2022 without language restrictions. Studies that reported the 25-hydroxyvitamin D [25(OH)D] concentrations and defined their cut-off for deficiency or insufficiency from healthy participants residing in Malaysia were included. The random effects model was used to pool vitamin D status using established cut-offs of <30, <50, and <75 nmol/L according to age group.

Results: From 299 studies screened, 32 studies were included in the meta-analysis. The pooled proportion for <30 nmol/L was 21% (95% CI 9–36, *n* = 2,438 from 10 studies), while the pooled proportion <50 nmol/L was 64% (95% CI 56–72, *n* = 13,977 from 30 studies), and <75 nmol/L was 85% (95% CI 61–100, *n* = 1,376 from five studies). Heterogeneity was high (I^2 ranged from 98–99%). Higher proportions of vitamin D insufficiency (defined as <50 nmol/L) were found in participants living in the urban areas (compared to rural areas), in females (compared to males), and in Malays and Malaysian Indians (compared to Malaysian Chinese) ethnicities.

Conclusion: More than half of Malaysians have insufficient vitamin D levels, despite being a country that is close to the equator. We strongly urge prompt public health measures to improve the vitamin D status in Malaysia.

Systematic review registration: [https://www.crd.york.ac.uk/prospero/], identifier [CRD42021260259].

KEYWORDS

Malaysia, vitamin D, 25-hydroxyvitamin D, prevalence, cholecalciferol

Introduction

Vitamin D is well-recognized as a hormone that plays an essential role in maintaining adequate serum calcium and phosphate concentrations and bone health. The importance of vitamin D in maintaining a sound immune system has also been well-discussed (1). Following the coronavirus disease-2019 (COVID-19) pandemic, there has been a renewed interest in understanding the role of vitamin D in managing COVID-19 infection (2). Several systematic reviews have demonstrated that vitamin D levels are associated with the severity of COVID-19 conditions (3–11). This is echoed by several clinical trials showing positive outcomes of supplementing vitamin D in COVID-19 infection, especially in deficient patients (8, 12–15). Although the exact benefit of vitamin D in COVID-19 management remains debatable (16–19), the importance of maintaining adequate vitamin D levels for general health is not disputed.

There is no consensus on the optimal vitamin D concentration in the body [measured as 25-hydroxyvitamin D; 25(OH)D]. It is generally agreed that concentrations <30 nmol/L (or 12 ng/ml) should be avoided in all age groups (20), but the definition of deficiency and insufficiency varies between guidelines. The Endocrine Society Clinical Practice Guidelines 2011 defines vitamin D deficiency as 25(OH)D concentrations <50 nmol/L (or <20 ng/ml), while 50–75 nmol/L (or 20–30 ng/ml) is defined as insufficient (21). The Endocrine Society further recommends levels above 75 nmol/L to reduce the risk of infectious diseases and to obtain other non-calcemic benefits of vitamin D. On the other hand, the Institute of Medicine (IOM) defines vitamin D deficiency as <30 nmol/L, while 30–50 nmol/L is insufficient (22). The IOM have used bone health as the main basis for their recommendations. Misra et al. (23), define vitamin D deficiency as ≤37.5 nmol/L and insufficiency as 37.5–50 nmol/L (23). Although there is no consensus on the terminology, a cut-off of <50 nmol/L is commonly used between guidelines. At this concentration, there are compensatory mechanisms activated to maintain calcium homeostasis, which will affect bone and muscle health (24).

Malaysia is a Southeast Asian country close to the equator, with latitudes ranging from 1.2°N to 6.8°N. Malaysia consists of two regions: Peninsular Malaysia, which houses approximately 80% of the Malaysian population, and Malaysian Borneo. It has an equatorial climate with relatively stable temperatures all year that are hot, sunny, and humid with an average daily temperature that ranges between 21 and 32°C. Seasonal climate variability is closely tied to the drier Southwest Monsoon, which occurs from April to September, and the wetter Northeast Monsoon, from October to March (25). In theory, during periods of heavy rainfall, more of the population may be confined indoors, limiting their sun exposure during the day. However, in a recent study, Aris et al. (26) reported that while there was a significant decrease in vitamin D concentrations during the monsoon season for some subgroup of individuals, there was no significant difference in the vitamin D status between monsoon and non-monsoon season (26). Approximately 69.8% of the 29.9 million Malaysian population is Malay and Bumiputera, 22.4% is Chinese (i.e., Malaysian Chinese), 6.8% is Indian (i.e., Malaysian Indian), and the remainder is other ethnicities (27). Malaysian Chinese generally have fair skin tone, whereas, Malays' skin tone may vary from light to tanned, and most Malaysian-Indians have darker skin tone (28). As vitamin D is synthesized in the skin following sunlight exposure and is highly correlated with skin color, the Malay and Malaysian Indian populations are at higher risk of lower vitamin D status.

A recent review article has highlighted several studies have investigated the vitamin D status of subpopulations in Malaysia (29). Most of the results have indicated that vitamin D status is low in Malaysia. However, the prevalence of vitamin D status has not been pooled across studies. Therefore, the primary aim of this research is to combine the data from studies that have been conducted on the vitamin D status in populations living in Malaysia.

Methods

This systematic review was conducted following the PRISMA guidelines (37) and was registered in PROSPERO (CRD42021260259).

Identifying published studies

We searched through PubMed, Scopus, Web of Science, and MyJurnal (a Malaysian Journal database) from the inception of each database until June 2022. A systematic search was conducted to identify all studies that reported vitamin D status in Malaysian residents. The search terms included (Vitamin D OR its synonyms) AND (Malaysia OR its synonyms). The full record of the search strategy is presented in **Supplementary material 1**. We did not use any language or other search limits. Key review publications were also identified and searched for further relevant studies. In addition, the reference lists from the identified studies were also examined for potentially relevant studies.

Inclusion and exclusion criteria

For a study to be included in the systematic review, there were two inclusion criteria: (i) the study measured serum 25(OH)D levels in healthy Malaysians, and (ii) it was non-interventional study and conducted either as a cross-sectional,

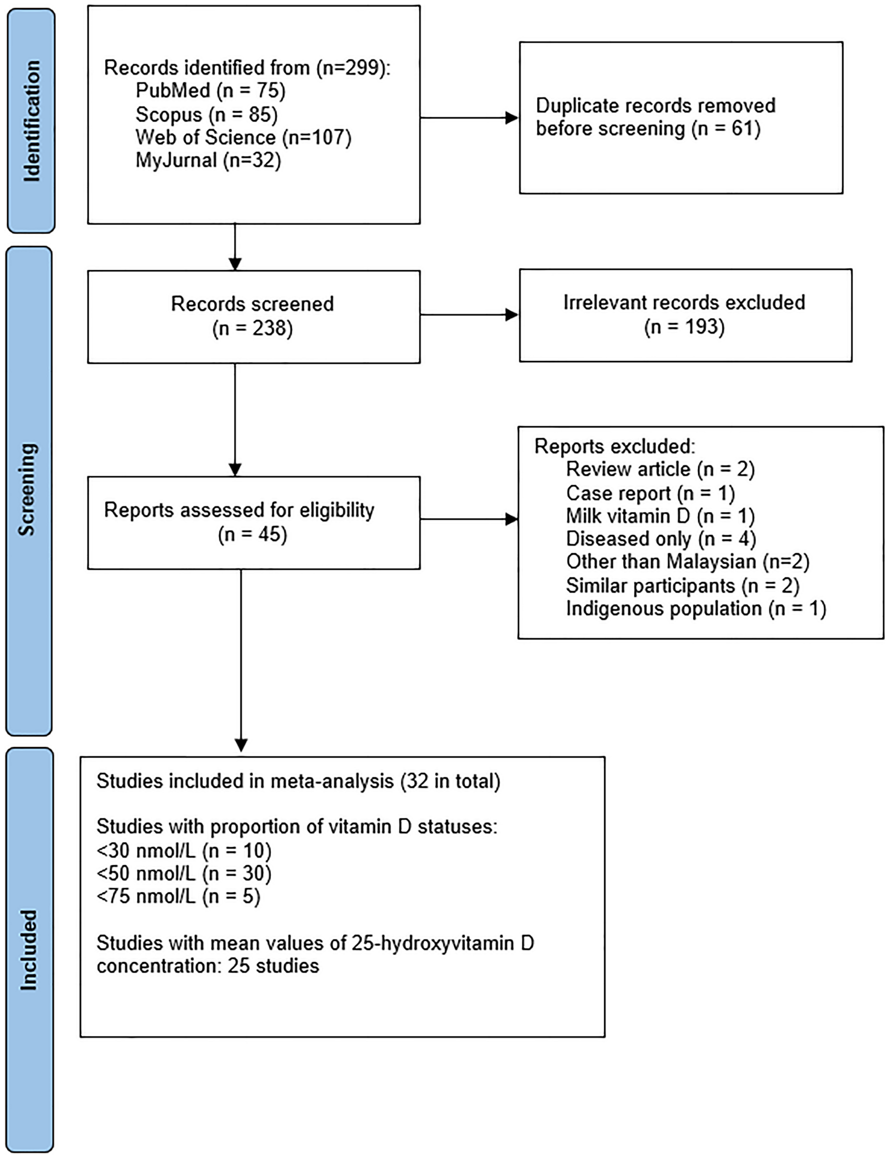

FIGURE 1
Flow-chart of study selection.

case-control, or longitudinal study design. For longitudinal studies, baseline vitamin D levels must be provided. Studies were excluded if they were: (i) case reports or case series, or (ii) meeting abstracts or unpublished materials, (iii) articles that were not written in English, (iv) review articles and meta-analyses.

The meta-analysis considered only data from healthy population subgroups for case-control studies. We excluded studies where the lifestyle differed significantly from Malaysia's general population from the meta-analysis. If a subsequent research included data from a previous cohort of individuals, the study with the larger sample size would be included in the meta-analysis.

Three investigators (NAJ, SMS, NAMT) developed the search strategy. Then, SMS and NAMT performed the database search and independently screened the retrieved articles based on the titles and abstracts. All four authors assessed the full-text articles and selected studies based on the inclusion and exclusion criteria. Any disagreements in the study selection were resolved by consensus. All data were extracted from the selected studies using a standardized extraction form, including information on the study location, data collection period, population demographics (ethnicity, sex, age), assay method, vitamin D status (including cut-off definitions), and vitamin D concentrations.

Critical appraisal of studies included

After considering various quality assessment tools for prevalence studies (38), the Joanna Briggs Institute Prevalence Critical Appraisal Tool, 2014 (39, 40) was used for quality assessment. Three investigators (EMH, NAJ, and SMS) critically appraised and rated the studies included in the meta-analysis using the tool. The tool consists of nine questions with four standard answer options (*yes/no/unclear/not applicable*). Each rater rated independently; however, discussions were carried out to ensure that discrepancies were discussed and agreed upon through consensus.

Statistical analysis

For the overall data, we performed a meta-analysis using three predetermined vitamin D cut-off values (<30, <50, and <75 nmol/L) sub-grouped by age category and a meta-analysis of mean 25(OH)D levels. The random-effects model was used to pool the proportion and mean values given an *a priori* assumption of heterogeneity between prevalence studies (41). If the mean and standard deviation were not provided, they were estimated from the median, interquartile range, range, and sample size, assuming that the data were not significantly skewed (42). For all meta-analyses of specific subgroup proportions (e.g., gender, ethnicity), a cut-off of <50 nmol/L was used, in view that this cut-off is present in almost all of the studies. We examined the presence of heterogeneity using the Q statistic with $P < 0.05$ indicating significant heterogeneity exists. The heterogeneity between studies was then quantified using the I^2 statistics with values of <25, <50, and <75% indicating low, moderate, and high, and the $\chi 2$ test with $P < 0.05$ to denote significance (43). All analyses were performed using MetaXL, version 5.3 (EpiGear International, Sunrise Beach, QLD, Australia). A sensitivity analysis is, by default, reported by MetaXL by excluding one study at a time and recalculating the pooled effect sizes and the associated heterogeneity statistics. The funnel plot was not used as it is inaccurate to assess publication bias for prevalence studies (44). The overall mean vitamin D levels and the proportion of vitamin D deficiency/insufficiency were also summarized by study location.

Results

Literature search results and characteristics of the eligible studies

From the 299 articles identified, 45 full-text articles were assessed, and 32 studies were included in the systematic review and meta-analysis (see **Figure 1**). **Table 1** shows the characteristics of the studies included in the systematic review. Two studies were published before 2010 (45, 46). Twenty-five studies were mainly from participants in Malaysia's capital city (Kuala Lumpur and its surrounding areas). Four studies were excluded as they only presented data on specific patients (47–50) and did not include healthy controls. One study on indigenous people (51) was excluded because their lifestyles differed significantly from the rest of the population. Two studies were excluded from the meta-analysis because it has part of the same individuals from a previous research (52, 53). The summary the of study quality assessment is presented in **Supplementary material 2**. The included studies measured 25(OH)D levels using immunoassays, except for four studies that used chromatographic methods (54–57).

Meta-analyses

For the <30 nmol/L cut-off, ten studies with a total of 2,438 participants were included. The pooled proportion for this cut-off was 21% (95% CI 9–36%, see **Figure 2**). Pregnant women had a higher proportion of participants with 25(OH)D levels <30 nmol/L (n = 613, 40%).

All studies eligible for the meta-analysis, except for two studies (58, 59), had a <50 nmol/L cut-off. The pooled proportion for <50 nmol/L from three studies with data from 13,977 individuals was 64.5% (95% CI 56.1–72.5, see **Figure 3**). Again, pregnant women represent the highest proportion with vitamin D levels of <50 nmol/L. The pooled proportion for each subgroup of participants was above 50% except for one study (60). Mat et al. (60) conducted a study on adults above 55 years old, and 203 out of the 1,011 participants were taking vitamin D supplements, which could explain the lower proportion of individuals with levels <50 nmol/L.

Only five studies with 1,376 participants reported a cut-off of <75 nmol/L, and the pooled proportion was 85% (95% CI 61–100, see **Figure 4**). All pooled proportions, including most subgroups, were considered high, indicating substantial heterogeneity (>99%).

The pooled mean (95% CI) according to the location of the study is reported in **Figure 5**. Not surprisingly, the urban population had a higher proportion of vitamin D insufficiency (66.8%, 95% CI 57.8–75.3, n = 10,893) compared to the rural population (45.6%, 95% CI 21–71.1, n = 3,487), respectively (see **Supplementary materials 3, 4**). Chua et al. (51) conducted a study on the indigenous population, considered a significant outlier and thus excluded from the meta-analysis. However, Chua et al. reported that only 1.4% (out of 555 indigenous people) had 25(OH)D levels <50 nmol/L, and 26.3% had levels <75 nmol/L.

Vitamin D insufficiency is also more common in females (76%, 95% CI 65–86, n = 6,264, see **Supplementary material 5**) compared to males (46%, 95% CI 25.6–67%, n = 3,664,

TABLE 1 Characteristics of studies included in the systematic review.

References	State	Period data was collected	Total sample size (n), population	n, Ethnicity	Male (%)	Vitamin D measurement method	Number of participants, vitamin D classification [25 (OH)D concentrations are in nmol/L]
Rahman et al. (45)	Kuala Lumpur	Not reported	274, postmenopausal women	101, Malay 173, Chinese	0	Radio-immunoassay	2, <25 deficient 93, 25–50 insufficient 179, 50–100 hypovitaminosis
Green et al. (46)	Kuala Lumpur	January and March 2005	378, non-pregnant women	133 Malay 123 Chinese 122 Indian	0	Radio-immunoassay	113, <17.5 deficient 227, <50 insufficient
Khor et al. (81)	Kuala Lumpur	2008	402, from five primary school children	51.2% Malay 42.8% Chinese 6% Indian and others	44.8	CLIA	142, <37.5 deficient 149, <37.5–50 insufficient
Moy and Bulgiba (71)	Kuala Lumpur	May to July 2010	380, Malay employees	100% Malay	41.6	CLIA	258, <50 insufficient
Hawa et al. (82)	Kelantan	Not reported	150, (51 pre-menopausal and 99 post-menopausal)	100% Malay	0	ELISA	1, <25 deficient 124, 25–49.8 insufficient 25, 50–100 hypovitaminosis
Nurbazlin et al. (83)	Kuala Lumpur and Negeri Sembilan	Aug 2010 to July 2011	400, women (293 urban, 107 rural)	267, Malay 67, Chinese 66, Indian	0	ECLIA	48, <30 deficient 74, 30–50 insufficient 278, ≥50 sufficient
Poh et al. (84)	Six regions in Malaysia (northern, central, southern, east coast, Sabah & Sarawak)	May 2010 to October 2011	2,936, children 4–12 years old	Not reported	49.7	CLIA	1,395, <50 insufficient
Chin et al. (85)	Klang Valley	September 2009 to September 2011	383, men	Malay (39.2%), Chinese (60.8%)	100	ELISA	2, <30 deficient 85, 30–50 insufficient
Jan Mohamed et al. (56)	Kelantan	April 2010 to December 2012	102, pregnant women	100% Malay	0	HPLC	4, <25 severe deficient 57, 25–50 mild deficient 35, 50–75 insufficient
Al-Sadat et al. (86)	Perak, Selangor and Kuala Lumpur	March 2012 to May 2012	1,361 school students	Malay 1091 (80.2) Chinese 105 (7.7) Indian 105 (7.7) Others 41 (3)	38.6	ECLIA	20, <12.5 severe deficient 1,053, 12.5–37.5 deficient 187, 37.5–50 insufficient
Shafinaz and Moy (87)	Kuala Lumpur	February to May 2013	858 teachers from 30 schools	76.9% Malay 16.4% Chinese 8.3% Indian 0.4% Others	9.1	ECLIA	578, <50 deficient
Bukhary et al. (75)	Selangor	January to April 2014	396, pregnant women	77.5% Malay 11.2% Chinese 8.8% Indian 2.5% Others	0	ECLIA	174, <24.99 184, 25–49.99 deficient 33, 50–74.99
Lee et al. (55)	Kuala Lumpur	August 2013 to August 2015	575 women completed 37 weeks of pregnancy (term)	73.4% Malay 18.4% Chinese 4.9% Indian 3.3% others	0	UPLC	412, <50 deficient 121, 50–75 insufficient
Moy et al. (88)	Kuala Lumpur	March to October 2013	770, female teachers	76.6% Malay 15% Chinese 8.4% Indian	0	ECLIA	557, <50 deficient

(Continued)

TABLE 1 (Continued)

References	State	Period data was collected	Total sample size (*n*), population	*n*, Ethnicity	Male (%)	Vitamin D measurement method	Number of participants, vitamin D classification [25 (OH)D concentrations are in nmol/L]
Rahmadhani et al. (70)	Kuala Lumpur	January 2012 to July 2012.	941 boys and girls from 23 schools	75% Malay 13% Chinese 10% Indian 2% Others	28	ECLIA	305, <37.5 deficient 166, 37.5–50 insufficient
Ralph et al. (54)	Kota Kinabalu, Sabah	Ethics approved in 2010/1	92, controls for cases (tuberculosis)	Not reported	35.8	LCMS	23, <50 deficient
Ariffin et al. (78)	Kuala Lumpur	March to August 2017	57, pregnant women	86% Malay 14% non-Malay	0	ELISA	15, <25 severe deficient 37, 25-49.9 mild deficient
Mat et al. (60)	Kuala Lumpur	November 2013 to October 2015	1,011, elderly	31% Malay 36.3% Chinese 32.7 Indian	43	CLIA	409, <50 deficient
Quah et al. (89)	Selangor, Perak and Kuala Lumpur	1 April 2014 to 30 June 2014	1,016 students (14–15-year-olds) from 15 urban and rural schools	Not reported	38	CLIA	338, ≤50 678, >50
Jamil et al. (74)	Kuala Lumpur	March to July 2019	147, Malay office workers	100% Malay	0	Enzymatic immunoassay	133, <50 insufficient
Lee et al. (90)	Johor Bahru	1st December 2016 and 31st May 2017	65, controls for cases (atopic dermatitis)	67.7% Malay 16.9% Chinese 5% Indian 5% Others	52.3	ECLIA	19, <50 deficient 22, 50–75 insufficient
Abd Aziz et al. (91)	Kuala Lumpur	Not stated, ethics approved in 2018	60, women	87% Malay 7% Chinese 5% Indian	0	ELISA	6, <30 deficiency 34, 30–50 insufficiency
Arumugam et al. (92)	Kuala Lumpur	June 2014 to February 2015	38, controls for cases (atopic dermatitis)	Not reported	36.8	ECLIA	1, <30 deficient 22, 30–50 insufficient
Ismail et al. (93)	Kuala Lumpur	Not reported	78, pregnant women	77% Malay 15.4% Chinese 5.1% Indian 2.5% Others	0	ECLIA	27, <30 deficient 36, 30–50 inadequate
Leiu et al. (94)	Kuala Lumpur and Selangor.	Not reported	214, post-menopausal Chinese women	100% Chinese	0	CLIA	71, <30 deficient 106, 30–50 insufficient
Ismail et al. (95)	Kelantan	August 2017 to October 2017	126, healthy volunteers	100% Malay	43.7	CLIA	92, <30 deficient 12, 30–50 insufficient
Woon et al. (58)	Kuala Lumpur and Selangor	November 2016 and January 2018	535, late pregnancy	Not reported	0	CLIA	227, <30 deficient
Aris et al. (26)	Kelantan	May to June 2012	119, indoor workers, 119, outdoor workers	100% Malay	31 (indoor)93.3 (outdoor)	ECLIA	64, <50 deficient 46, 50–75 insufficient
Hussain and Elnajeh (59)	Kelantan	Not stated, ethics approved in 2012	361, adolescents from 10, schools	85% Malay 15% Chinese	37.1	ECLIA	59, <30 deficiency
Chee et al. (96)	Kuala Lumpur	August 2017 to August 2019	243, 9- to 11-year-olds	90.5% Malay 9.5% Indian	52.3	LC–MS/MS	46, <30 deficient 123, <50 inadequate
Mustapa Kamal Basha et al. (57)	Kuala Lumpur	November 2017 to March 2019	179, longitudinal study of pregnant women	78% Malay 13.6% Chinese 6.8% Indian 1.7% others	0	HPLC	161, <50 deficient
Razip et al. (97)	Selangor	Not reported	50, controls for adult diabetes patients aged 30 to 65	90% Malay 2% Chinese 8% Indian	56	HPLC	41, <50 deficient 8, 50–200 Optimal 1, >200 Toxic

HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescent immunoassay.

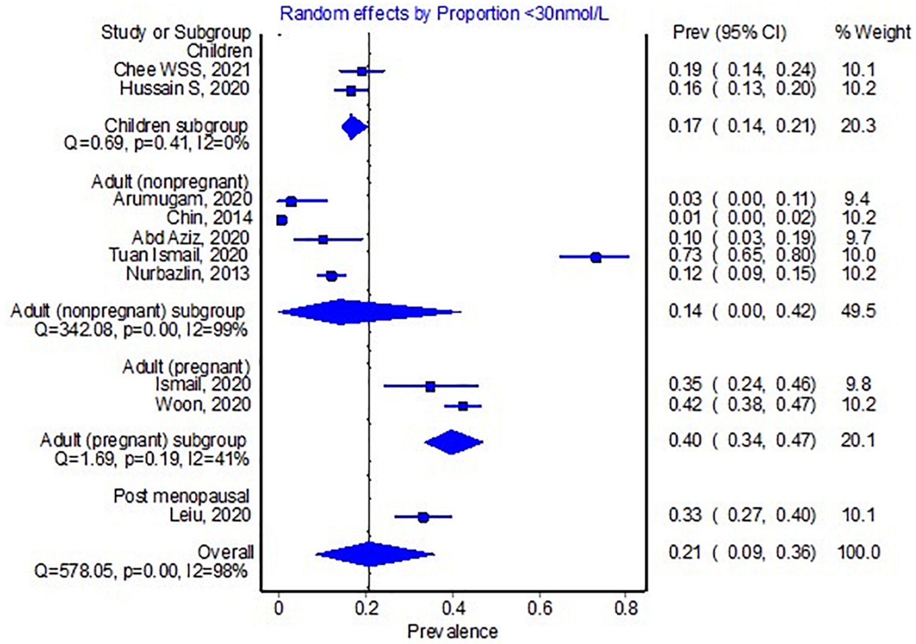

FIGURE 2
Pooled proportion of the <30 nmol/L 25-hydroxyvitamin D cut-off.

see **Supplementary material 6**). In terms of ethnicity, Malays (77%, 95% CI 65–87; n = 4,928, see **Supplementary material 7**) and Indians (77%, 95% CI 57–92; n = 768, see **Supplementary material 8**) had higher proportions of vitamin D insufficiency than the Chinese population (34.5%, 95%CI 17–54; n = 1,353, **Supplementary material 9**). One study examined the relationship between monsoon seasonality and vitamin D status and found no significant association (26).

Discussion

This study found that vitamin D deficiency and insufficiency, as defined by three cut-offs, is common in Malaysia. Precisely, over half of the population is estimated to have 25(OH)D levels <50 nmol/L (see **Figure 3**). Only two studies (45, 46) were conducted prior to 2010, and the majority were studies within the past 5 years, indicating that the data would reflect the current situation. However, all of the studies reported data collected before the COVID-19 pandemic. COVID-19 pandemic-related confinement has been shown to worsen vitamin D status in several studies outside of Malaysia (61, 62), however it is unclear if the same impact will be observed in Malaysia. We found that individuals living in the urban areas have higher prevalence of vitamin D insufficiency compared to rural areas. This is not surprising considering individuals living in rural areas tend to spend more time outdoor and hence receive higher doses of UVB radiation compared to individuals living in urban areas. Vitamin D deficiency was still detected in the indigenous population, whose lifestyle would involve more outdoor activities and sun exposure. However, the proportion is much lower than the general Malaysian population (51). It should be noted that there was substantial heterogeneity between the studies, which could partly be explained by age group, living in urban areas, gender, and ethnicity. Pregnant women had a much higher risk of vitamin D deficiency than all other age groups for all cut-offs. A longitudinal study followed 179 pregnant women from early pregnancy to birth found that at early pregnancy, 89% had levels <50 nmol/L, and this rose to 96.1% at birth (57). Although 11.1% took vitamin D supplements, none of the women had sufficient vitamin D status at birth.

Our study indicates that vitamin D deficiency/insufficiency in Malaysia can be considered very high compared to other regions worldwide. **Table 2** is adapted and updated from a commentary on reviews or systematic reviews of vitamin D status for a country or continent by Bouillon (24). Vitamin D levels < 30 nmol/L in Malaysia are approximately equal to the African continent, but the proportion of levels <50 nmol/L is much higher. However, the prevalence for both cut-offs is comparable to estimates from Mainland China (36). It is also interesting to note that Malaysian Chinese have a much lower proportion of vitamin D insufficiency (35%, <50 nmol/L) compared to Chinese from Mainland China. We are unaware of any studies exploring the difference between Chinese in different international geographical regions and their vitamin D status.

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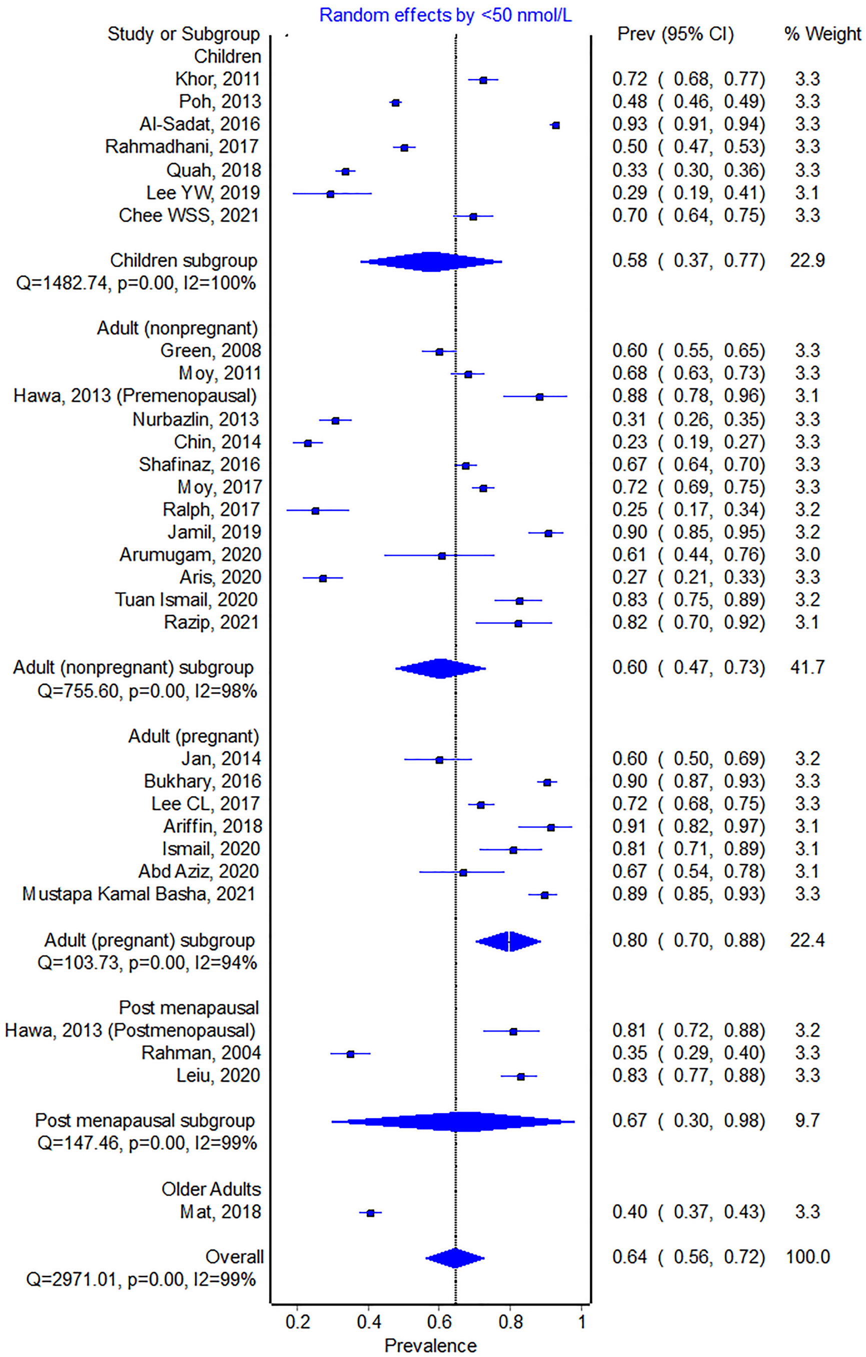

FIGURE 3
Pooled proportion of the <50 nmol/L 25-hydroxyvitamin D cut-off.

The prevalence of vitamin D deficiency could be associated to the prevalence of several non-communicable diseases in Malaysia. It has been shown that obese individuals have lower vitamin D levels compared to non-obese subjects (63) due to the reduced bioavailability of the fat-soluble vitamin D (64). According to Malaysia's National Health and Morbidity Survey 2019, approximately half of Malaysians were above the ideal body weight, with 30.4% overweight and 19.7% obese (65). Vitamin D deficiency has also been linked to the onset of insulin resistance and diabetes mellitus (66). The prevalence of diabetes mellitus in Malaysia is increasing at an alarming rate, with the overall prevalence exceeding 18% in 2019 (67). In addition, the prevalence of raised blood glucose in individuals with unknown diabetes is even higher at 43.4% (95% CI 37.37–49.65) among people aged 65–69 years, which can be attributed to a high intake of sugary beverages (65). In mechanistic animal studies, chronic

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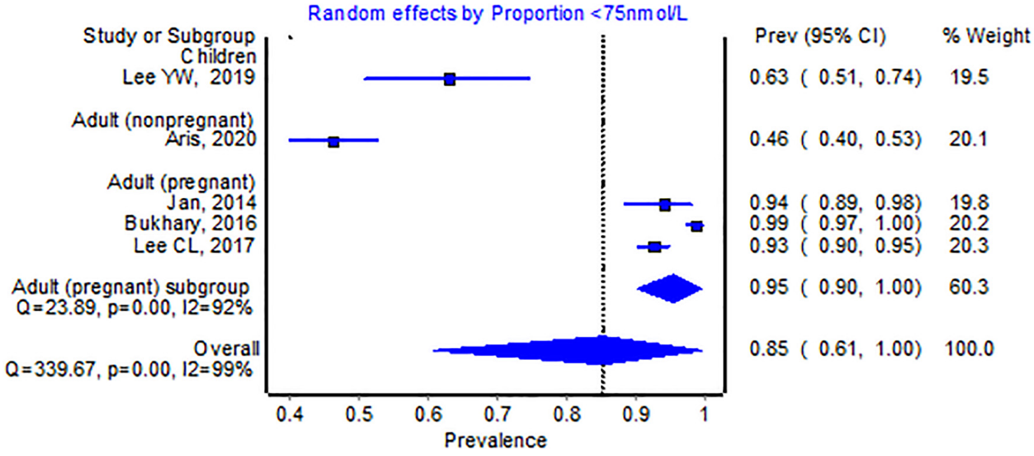

FIGURE 4
Pooled proportion of the <75 nmol/L 25-hydroxyvitamin D cut-off.

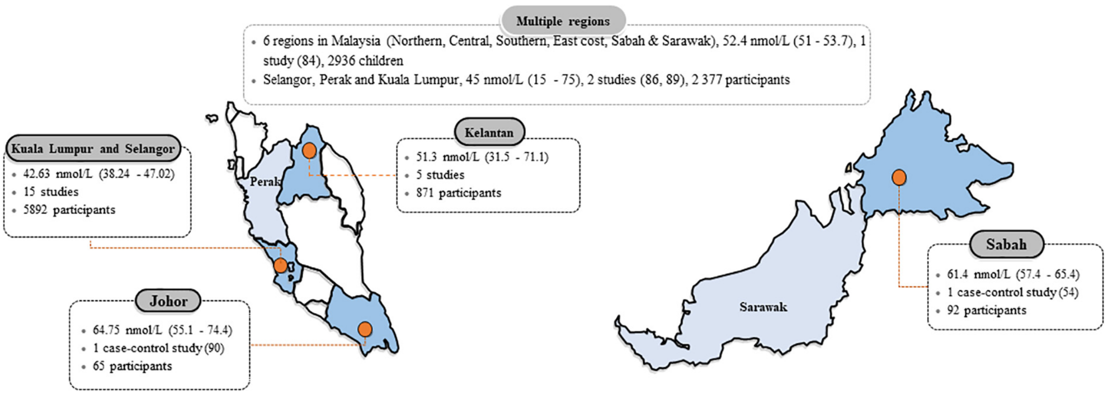

FIGURE 5
Mean 25-hydroxyvitamin D [25(OH)D] concentrations in Malaysia according to states. Data are the mean (95% confidence interval) of 25(OH)D concentrations reported in studies done in each state. The 25(OH)D mean values were pooled if there were more than one study from a form and were computed only from studies that stated the mean (SD), or median and interquartile range or range.

TABLE 2 Vitamin D deficiency by geographical area and 25-hydroxyvitamin D cut-offs, updated from Bouillon (24).

	Geographical area	Prevalence, %	
		Cut-off of <25 or <30 nmol/L	Cut-off of <50 nmol/L
Hilger et al. (30)	Global	7%	37%
Herrick et al. (31)	USA	5%	18%
Cashman et al. (32)	EU countries (adults)	13%	40%
Arabi et al. (33)	Iran and Jordan	50%	90%
Durazo-Arvizu et al. (34)	Ghana and Seychelles	<1%	<7%
Mogire et al. (35)	African continent	18%	34%
Liu et al. (36)	Mainland China	20.7% (Adults) 23.0% (Adolescents)	63.2% (Adults) 46.8% (Adolescents)
This study	Malaysia	21%	64%

Cut-offs refer to serum concentrations of 25-hydroxyvitamin D used to define vitamin D deficiency.

consumption of high fructose diets can reduce circulating 1,25-$(OH)_2D_3$ (68, 69). In this study, only two articles explored the association between vitamin D status and obesity in our study, but the results were conflicting (70, 71). This prompts for more studies exploring the association of vitamin D and metabolic syndrome in Malaysia.

Dietary vitamin D can be obtained by consuming food that naturally contains vitamin D, fortified food, or supplements. However, limited food choices naturally contain vitamin D in the Malaysian diet (72). Furthermore, vitamin D fortification in Malaysia is voluntary by the manufacturers, with only a few fortifying milks for children and adults (72). The Malaysian Adult Nutrition survey reported that the highest consumer of full cream milk is amongst adults aged 50–59, with only 24% reported daily consumption, while only 15% of individuals aged 18 and 19 reported daily consumption (73). In this meta-analysis, only approximately 10–20% of the participants take vitamin D supplements (57, 60, 74, 75), although the frequency and dosage of supplementation are unclear.

Without significant dietary sources, Malaysians are left to sunlight exposure to achieve adequate vitamin D levels. However, there are numerous reasons why Malaysians tend to avoid sun exposure. Some of the barriers to sun exposure identified amongst Malay women with low vitamin D status include misunderstanding about vitamin D, health concerns toward sun exposure, including effects on skin color and surface, weather (hot and rainy), and religious/cultural clothing practices (76). Additionally, there are limited studies investigating vitamin D synthesis following sun exposure in Malaysia (77). Due to the scarcity of studies, it is unclear exactly how long and how much skin surface is required to be exposed to the sun to obtain adequate vitamin D levels in Malaysia. Different ethnicities further complicate recommendations with different skin tones and different cultural/religious clothing. Hence, more research on sun exposure in Malaysia to meet adequate vitamin D levels is required to provide more explicit guidelines.

There are several notable limitations in this study. Most studies do not report vitamin D supplementation, although some include this information (78). However, vitamin D supplementation is not a common practice among the Malaysian population, and therefore it is unlikely to affect our results significantly. Secondly, 25(OH)D levels were measured using different chromatographic and immunoassays. This is a common issue when comparing studies that involve measurements of vitamin D plasma levels as there is no worldwide standardization (79, 80). Nevertheless, in Mogire's (35) study, different vitamin D assays accounted for only 5% of the heterogeneity and had no significant impact on the overall 25(OH)D mean concentrations estimate. Thirdly, most of the studies included in the meta-analysis used convenient sampling, which is prone to bias. Finally, although we tried to be inclusive by having a broad inclusion and minimal exclusion criteria, there were limited data for many states in Malaysia.

Conclusion

Malaysia has a high prevalence of vitamin D deficiency and insufficiency, with more than half of the population estimated to have levels <50 nmol/L. Vitamin D deficiency is more prevalent in Malay and Malaysian Indian ethnic groups than in Malaysian Chinese. The female gender has lower vitamin D levels compared to the male. We strongly recommend immediate public health measures such as the refinement of nutritional guidelines, development of government policies, and awareness campaigns to improve vitamin D status in Malaysia.

Data availability statement

The requests to access the datasets analysed in this study should be directed to SM, shamin@ukm.edu.my.

Author contributions

SM, NJ, and NM conducted the literature search. EH, NJ, and SM critically appraised the included studies. SM performed the data analysis and EH and NJ checked it. SM wrote the first draft of the manuscript. All authors contributed to the study's conception and design and data extraction, commented on previous versions of the manuscript, read, and approved the final manuscript.

Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1050745/full#supplementary-material

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Systematic review on fiscal policy interventions in nutrition

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Introduction: Both the World Health Organization and the Lancet Series on Adolescent nutrition recommend that governments adopt fiscal policies to combat diet-related non-communicable diseases (NCDs). However, rigorous, systematic evidence regarding the effects of these interventions is lacking.

Methods: We synthesize the available evidence regarding the impacts of taxes and subsidies that directly affect consumer prices on availability and accessibility of foods and beverages, purchasing behavior, diet quality, health and well-being outcomes as well as considerations for implementation, sustainability and equity.

Results: Our initial search returned 2,113 de-duplicated studies, and ultimately 24 impact evaluations and two systematic reviews met final eligibility criteria and represented unique evaluations. Our meta-analysis of these studies suggests that taxes may decrease purchases of taxed beverages (SMD = −0.14 [95% CI: −0.29 to −0.07], n = 15). Results should be interpreted cautiously due to considerable heterogeneity ($Q\,(14) = 335.19$, $p = 0.01$, $\hat{\tau}^2 = 0.03$, $I^2 = 95.82\%$).

Discussion: The evidence base is too limited to draw conclusions about the effects of taxes on beverages and calorie-dense foods on purchases, or on the effects of subsidies on purchasing or diet quality. Overall, the evidence base is inconclusive on whether fiscal policies can meaningfully influence the availability and accessibility of foods and beverages, diet quality, and health outcomes. Policymakers implementing fiscal policies should consider information campaigns on health benefits and health risks associated with certain food and beverage consumption. For taxes, exposure to health information may amplify signaling effects of taxes and reduce avoidance behaviors, such as cross-border shopping. Future evaluations should diversify data sources to better understand impacts on diet and health outcomes.

KEYWORDS
fiscal policies, nutrition, sugar-sweetened beverage consumption, taxes, subsidies

Introduction

Malnutrition in all its forms, including undernutrition (wasting, stunting, and underweight), overweight and obesity, affects at least 2.6 billion people worldwide (1). In 2021, non-communicable diseases (NCDs) accounted for over 70 percent of deaths globally, led by cardiovascular disease (17.9 million people), cancers (9.3 million), respiratory diseases (4.1 million), and diabetes (1.5 million) (2). Both the WHO and the Lancet Series on Adolescent nutrition recommend that governments adopt fiscal policies, such as taxes and subsidies, to combat diet-attributed NCD risk. The goal of such policies is to either discourage the consumption of calorie-dense beverages and foods or encourage diverse diets that include fruit, vegetables, legumes, nuts and whole grains (3–5). Taxes on non-alcoholic sugar-sweetened beverages (SSBs) have been implemented in over 50 countries, generally in the form of per-unit excise taxes (e.g., a juice in Mexico is taxed at one peso per liter) or ad-valorum excise taxes [e.g., an energy drink in Saudi Arabia is taxed at 50 percent of pre-tax price; (6)]. The effects of these taxes on changes in price, including pass-through rates from distributors to consumers, is well documented. However, the effects of taxes on consumption, diet, and health outcomes remains unclear (7). Subsidies are implemented in nearly every country in the world, and previous reviews have synthesized the effects of monetary subsidies on food purchases and consumption in field experiments (8) and in modeling studies (9). To our knowledge, this is the first attempt to synthesize the empirical evidence base on the impacts of subsidies in which the government pays a *portion* of the price of a good on diet and health outcomes.[1] Since they affect people globally, we need to know if taxes and subsidies meaningfully improve diet, health, and well-being.

To address this gap and support evidence-informed decision-making, we conducted a systematic review of the effects of fiscal policies linked to food and beverages on the availability of and access to diverse diets. This systematic review challenges and verifies the hypothesis of the international community that these interventions improve diet, health, and well-being. Researchers and implementers can use this work to better understand how to structure and implement taxes or subsidies to facilitate behavioral change among consumers and industry.

1 We define subsidies as interventions in which the government pays a *portion* of the price of a good. The evidence base on interventions which provide food, cash, or vouchers as subsidies is extensive. While these programs can all be conceptualized as reducing costs and increasing accessibility of food, the behavioral responses of consumers to the various delivery mechanisms are likely to be different. Therefore, we do not consider food vouchers, in-kind food provision, and cash transfers in our definition of subsidies.

Methodology

This systematic review is based on topically relevant studies identified by the Food Systems and Nutrition Evidence Gap Map and a systematic literature search of key academic databases (10). We assessed literature for quality and summarized it visually and in a narrative format. The review followed the rigorous Campbell Collaboration and Cochrane approaches to systematic reviewing (11, 12).

Expected theory of change

The theory of change is that policymakers implement taxes and subsidies on foods and beverages to influence the availability and accessibility of foods and beverages. When the price of taxed goods increases, we expect consumers to change their purchasing behavior by decreasing their consumption of taxed foods. When the price of subsidized goods decreases, we expect that consumers will change their purchasing behavior by increasing their consumption of subsidized foods. Taxes generate revenue for the implementor, which, if invested in health or nutrition initiatives, may contribute to changes in consumption in the population. Changes in consumption may generate financial incentives for manufacturers to reformulate or modify production of target foods. Changes in diet attributable to fiscal policies will promote consumers' diet quality, anthropometrics, health and well-being outcomes (**Figure 1**).

Objectives and research questions

The objective of this work is to synthesize the available evidence regarding the impacts of tax and subsidy interventions on availability and accessibility of foods and beverages, purchasing behavior, diet quality, anthropometrics, health and well-being outcomes. We also identify considerations for implementation, sustainability and equity. We specified the following research questions (RQ) *a priori:*

> RQ1: What are the effects of fiscal policies on food and beverages on the availability and access to diverse diets?

> RQ2: Are there unintended consequences of these actions, such as food substitutions or regressive effects?

> RQ3: What policy design features moderate impact? For example, do effects vary by the specific approach taken, food targeted, socio-economic status, or context, including the joint implementation of fiscal policies with other initiatives?

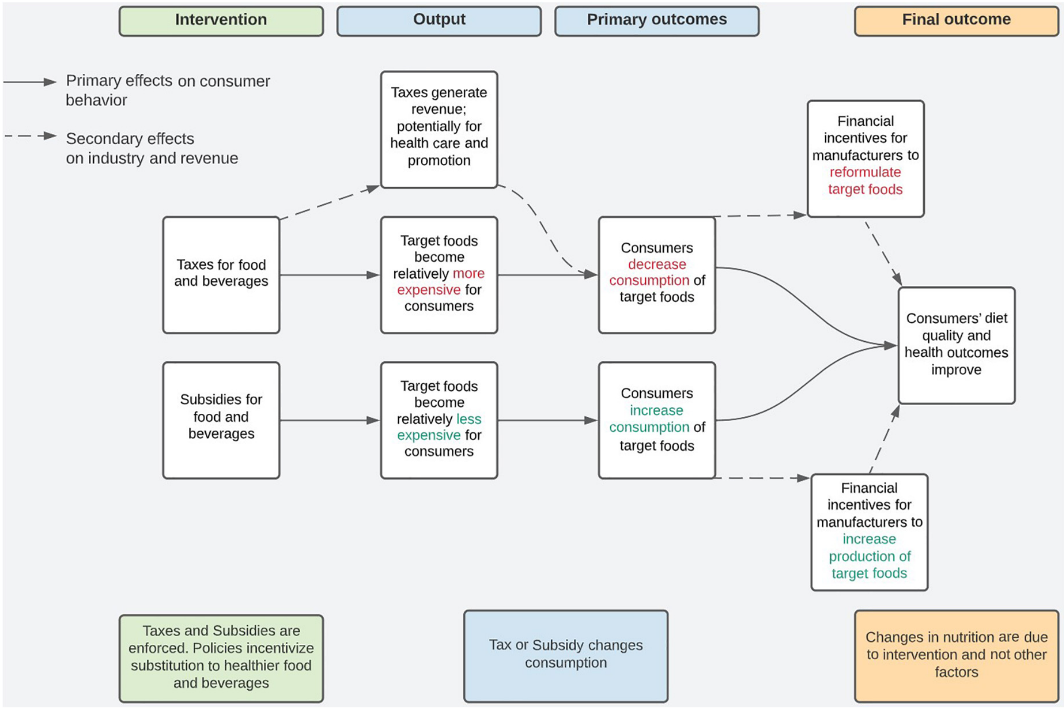

FIGURE 1
Fiscal policy theory of change, World Health Organization (4).

> RQ4: What evaluation design strategies are used? What relationships and data sources are key to allowing for evaluation?

> RQ5: What are common implementation challenges, sustainability issues, and implications for practitioners in both high-income countries (HICs) and low- and middle-income countries (L&MICs)?

We conduct meta-analysis to synthesize effects (RQ1) and unintended consequences (RQ2) related to substitutions. We conducted qualitative thematic analysis to further probe unintended consequences (RQ2), consider policy design features (RQ3), identify study design strategies (RQ4), and develop implications for implementation, sustainability and equity (RQ5).

Criteria for including and excluding studies in the review

Types of study participants

There were no limitations on study participants' country of origin, gender, ethnicity, age, or other demographic trait (**Table 1**).

Types of interventions

We considered two types of fiscal policies, implemented by governments, for this systematic review:

1. Taxes that increase prices of high-sugar foods and non-alcoholic beverages, such as SSBs, to discourage consumption.
2. Subsidies to decrease prices of targeted foods and beverages, such as fruits, vegetables and pulses, to encourage consumption and diversify nutrient uptake (5).

We considered studies of taxes that directly increase the consumer price of calorie-dense foods and beverages that are high in sugar, fat or salt through the government charging an additional fee to manufacturers, stores, or the consumers. Taxes on producers, processors, or other downstream actors in the value chain were not considered (**Table 1**).

We define subsidies as interventions in which the government pays a *portion* of the price of a good. Studies must explicitly mention that they evaluated subsidies. Often, authors refer to interventions which provide food, cash, or vouchers as subsidies. While these programs can all be conceptualized as reducing costs and increasing accessibility of food, the behavioral responses of consumers to the various delivery mechanisms are likely to be different. Therefore, we did not include these interventions. Subsidies can be implemented

TABLE 1 Description of participations, intervention, comparison, outcomes, study designs.

Criteria	Included	Excluded
Participants	Individuals in L&MICs and HICs	Niche populations, such as astronauts, people in the military, professional athletes, etc.
Intervention	Taxes for of calorie-dense foods and beverages that are high in sugar, fat or salt (e.g., sugar-sweetened)	In-kind food provision (e.g., free school meal handouts) or fully subsidized products Food vouchers or cash transfers/cashbacks/food stamps even if they are referred to as subsidies [e.g., the Supplemental Nutritional Assistance Program (SNAP) in the United States]. These interventions do not explicitly decrease the market price of targeted foods and beverages Fiscal policies that are not subsidies or taxes, such as price ceilings Reduction on import taxes for vegetables and fruits.
	Consumer subsidies for nutritious foods (e.g., fruits, vegetables, legumes, pulses, and fortified wheat) and beverages (e.g., fortified milk for pregnant mothers)	General consumption behavior change interventions Agricultural input subsidies Consumer subsidies not directly aimed at supporting a diverse, nutritious diet (e.g., for corn, rice, wheat, salt, wine grapes unless fortified with micronutrients) Price changes, which would not be considered an intervention by itself Lab in the field or field experiments such as virtual supermarkets
Comparison	Business as usual, including pipeline and waitlist controls An alternate intervention	If there is no counterfactual
Outcome(s)	Availability and accessibility of targeted foods and beverages Purchasing behavior/patterns	Affordability of diverse, nutritious foods and beverages, including post-tax or post-subsidy changes in price
	Diet quality and adequacy Anthropometrics Health, including diseases associated with nutrition (e.g., diabetes or heart disease) Well-being (e.g., psychological measures and the acceptability of diet)	Outcomes unrelated to nutrition, such as tax revenue, public finance
Study designs	Experimental and quasi-experimental impact evaluations Systematic reviews of experimental and quasi-experimental impact evaluations Ex-post cost evidence	Qualitative impact evaluations Descriptive or observational studies that do not assess effectiveness Modeling studies* Ex-ante cost evidence

*We excluded studies that use observed data to predict outcomes for both treatment and control groups. However, in some cases, these models are employed for evaluating taxes and subsidies due to the nature of implementation and data availability.

alongside other governmental programs, such as subsidizing a portion of the cost of a school meal which students are expected to pay. We considered subsidies that targeted foods such as fruits, vegetables, pulses, legumes, and fortified grains. We excluded unfortified staple crops, wine grapes, and salt (Table 1).

Types of outcome measures

We considered outcomes related to availability and accessibility of foods and beverages, such as food assets and production; purchasing behavior, such as sale of foods or frequency of purchases; diet quality and adequacy, such as composite diet scores or dietary diversity; anthropometrics, such as body mass index; health, such as incidence of non-communicable diseases; and well-being, such as measures of anxiety related to food insecurity. *A priori*, we specified preferred outcomes and alternate outcomes for synthesis (Table 2). We preferred composite measures over disaggregated ones. Additional information on indicators that we considered for each

TABLE 2 Included outcomes and indicators extracted for evidence synthesis.

Outcome	Indicators*
Availability and accessibility of foods and beverages	Preferred outcomes: food assets, production (community gardens), and stores
	Other measures: distance and accessibility to markets, were considered if these are not available
Purchasing behavior	Preferred outcome: sales of food in monetary units
	Secondary outcome: frequency or change of purchase of foods
Diet quality and adequacy	Preferred outcomes: composite diet scores such as the nutrient rich food index
	Secondary outcome: dietary diversity and other food variety measures
	Tertiary outcome: intake of specific foods
Anthropometrics	Preferred outcomes: body mass index, weight for length, length for age, and weight for age
	Other measures, such as mid-upper arm circumference (MUAC) and ponderal index, were considered if these are not available
Health	Incidence of diseases *directly* tied to nutrition, especially with regard to nutrition-related non-communicable diseases (NCDs), including diabetes, anemia, metabolic syndrome, and cardiovascular disease. Indirect diseases, such as cancer, will not be considered.
Well-being	Preferred outcome: perceived well-being
	Secondary outcome: anxiety, often regarding food security

*Indicators are listed by preference based on *a priori* specification. Such *a priori* specification reduces bias by preventing subjective reporting of outcomes by the team conducting the systematic review. Most indicators were ultimately not found in the studies.

outcome are specified in the protocol (**Supplementary material 1**).

Types of comparators

We considered alternate intervention or business as usual comparators, including pipeline and waitlist controls, as valid comparators. Studies with no valid counterfactual were excluded.

Types of study design

We considered experimental and quasi-experimental studies for inclusion in the meta-analysis, including:

- Randomized controlled trial
- Regression discontinuity design
- Controlled before-and-after studies, including
    - Propensity-weighted multiple regression
    - Instrumental variable
    - Fixed-effects models
    - Difference-in-differences (and any mathematical equivalents)
    - Matching techniques
- Interrupted time series

We also included ex-post cost-effectiveness analyses and systematic reviews that include a quantitative or narrative synthesis.

Date, language, and form of publication

We included studies published after 2000 and written in English.

Search strategy

An information specialist developed the search string with subject-matter input by the research team. The team verified the sensitivity of the search strings by ensuring that search results included the eligible studies from the Food Systems and Nutrition Evidence Gap Map. Ultimately, one of these studies was not identified through the final search because it was published in a relatively less well-known journal that is not indexed in major databases, the Latin American Economic Review journal. Due to resource constraints, we limited the number of databases searched. Search terms are provided in **Supplementary material 1**.

Electronic searches of bibliographic databases and library catalogs

The information specialist searched the following twelve databases:

- CAB Abstract (EBSCO)
- Agricola (EBSCO)
- Medline (EBSCO)
- Academic Search Complete (EBSCO)
- PsycInfo (EBSCO)
- Africa-Wide (EBSCO)
- CINAHL (EBSCO)
- Scopus
- Embase (Ovid)
- CAB Global Health (Ovid)
- Cochrane Library (this contains 2 databases—Trials Register and the SR database).

Other searches

In addition to the search of academic databases, the team searched for additional, relevant studies that had been previously identified from the search by Moore et al. (10) and its recent update. These studies may have been excluded because they considered participants from high-income countries or used ineligible study designs. The following studies were added to the search:

- Studies from the original map excluded using the code 'High income country' on title and abstract or full text, with the term 'tax*' or 'subsid*' on title or abstract.
- Studies from the original map included on title and abstract that have the term 'tax' or 'subsid*' in title or abstract.
- Studies from the EGM update (as of 21/01/2022) with the code 'Exclude- High income country' on title and abstract or full text that have the term 'subsid*' or 'tax*' on title or abstract.
- Studies from the EGM update (as of 19/01/2022) with the code 'FSN marker TA screening—FSN relevant' with subsid* or tax* on title or abstract.

Selection of studies

Screening

For title and abstract screening, the team developed a machine learning classifier in EPPI Reviewer. Two research associates screened studies with a prioritization score of 0.3 (30 percent likelihood of inclusion) or higher independently at title and abstract. One research assistant screened records with a prioritization score of 0.2–0.29. Research associates did not screen records with a probability of inclusion below 20 percent. Two research associates then screened all records included at the title and abstract stage at full text. The research team subsequently trained research assistants on the screening protocols and instructed them to apply exclusion codes in a hierarchical order for consistency in coding. Research assistants discussed differences in inclusion decisions, consulting with the research lead if disagreements could not be reconciled.

Data extraction and coding procedures

Once included impact evaluations and systematic reviews were identified, the team conducted an initial round of data extraction to determine the methods, interventions, and outcomes used. Because many studies considered the same intervention and outcomes within the same population, they did not represent unique evaluations; so, they could not all be included in the final analysis. We used the following, hierarchical criteria to select a single study for each intervention-outcome-population combination for inclusion in the meta-analysis: (1) the most biologically relevant outcomes, (2) the most rigorous analytical method, or (3) the longest time frame (**Supplementary Appendix Tables 1**, **2**). The ranking criteria were only employed when selecting among outcomes that fell within the same category outlined in **Table 2**. Effect estimates from systematic reviews were not considered in meta-analysis as all the policies considered within the systematic reviews were already reflected in the studies included in this review.

Using 3ie's repository coding protocols, we modified data extraction templates typically used for systematic reviews (**Supplementary material 2**). For analyzed (**Supplementary Appendix Table 1**) and linked studies (**Supplementary Appendix Table 2**), we extracted bibliographic and geographic information, equity considerations, standardized methods, project-specific interventions and outcomes, population of interest (disaggregated by gender and age, where possible), barriers and facilitators to implementation, sustainability, cost, and other considerations for practitioners. For analyzed studies, we also extracted effect sizes relevant to the theory of change (**Figure 1**). If a single study reported several different analyses on the same outcome (e.g., presented an adjusted and unadjusted model), we selected the model preferred by the authors for extraction. If the authors did not clearly state a preferred model, we extracted data from the model with the most control variables. Two independent reviewers completed data extraction, except in the case of two systematic reviews and linked publications which were extracted by one person each.

Critical appraisal

Two independent reviewers appraised all analyzed quantitative impact evaluations and systematic reviews of impact evaluations using a critical appraisal tool based on their study design (**Supplementary material 3**).

Analytical approach for quantitative and qualitative data

To reply to RQ1, we selected studies with sufficient data for meta-analysis. We chose the appropriate formulae for effect size calculations in reference to, and dependent upon, the data provided in included studies. We conducted random effects meta-analyses when we identified two or more studies that measured similar underlying concepts, such as purchasing behavior or consumption. We assessed heterogeneity by calculating the Q statistic, I^2, and τ^2 to provide an estimate of the amount of variability in the distribution of the true effect sizes (13). We explored heterogeneity using moderator analyses if the data allowed. Moderators considered included taxes vs. subsidies, food targeted and socioeconomic status (SES). There were not enough studies in L&MICs to conduct moderator analysis by country income level. We also tested for the presence of publication bias if at least ten studies were included in the analysis.

There was insufficient data to answer the remaining research questions quantitatively, so we conducted qualitative, thematic analysis on quantitatively analyzed and linked studies. While reviewing data extracted from each study, one coder identified common topics, ideas and conclusions across studies. She created themes around these common ideas and grouped extracted information accordingly. For example, if an author mentioned 'tax avoidance' as a barrier, the coder created a theme 'Barriers—Tax avoidance' and subsequently grouped similar information from other studies under this theme. Once the qualitative information was organized by theme, the coder prioritized findings by frequency and relevance to quantitative findings. Five reviewers from the research team validated findings from the thematic analysis.

Data presentation

We provide a narrative summary of the papers identified. This includes an overall description of the available literature and a general synthesis of findings. We summarize key information from each study, including intervention type, study design, country, outcomes, measurement type, effect sizes and confidence rating. Then, we present results from meta-analyses and their associated forest plots in the findings section. We also present qualitative information in a section on theories of change, unintended effects and implementation considerations to provide actionable insights for policy design.

Results

Search results and characteristics of the included studies

Our initial search returned 6,585 studies, of which 2,113 remained after de-duplication (**Figure 2**). After title and abstract screening and full text retrieval, 422 impact evaluations and 32 systematic reviews remained for full text review. Ultimately, 49 impact evaluations and two systematic reviews met eligibility criteria (n = 51). Half of these did not represent unique evaluations as they considered the same tax and outcome. For example, three studies evaluated the impact of a SSB tax on purchases of taxed beverages in Berkley, CA, United States. Therefore, 24 impact evaluations and two systematic reviews met eligibility criteria, considered unique intervention-outcome-population combinations and were included in the quantitative analyses. Studies included the meta-analysis are presented in **Supplementary Appendix Table 1**, and linked studies are listed with qualitative information in **Supplementary Appendix Table 2**. Qualitative information from analyzed studies are presented in **Supplementary Appendix Table 3**.

Impact evaluations

Most of the 24 impact evaluations were in HICs, primarily in the United States (n = 8) and Europe (n = 8) (**Figure 3**). Six studies took place in L&MICs contexts, which included Mexico (n = 3) and India (n = 3).

We included 20 studies that evaluated taxes in the main analysis. Most of the tax studies evaluated taxes targeting SSBs alone (n = 13), but four taxes targeted SSBs and high-sugar foods, and two targeted carbonated or aerated beverages. Eleven countries, states or cities implemented excise taxes on beverages using a per-unit tax, such as the Public Health Product Tax in Hungary that taxes soft drinks at HUF 200 per liter (14) or the Oakland Sugar-Sweetened Beverage Distribution Tax in CA, United States that taxes SSBs at USD 0.01 per ounce (15). Four countries implemented ad-valorum excise taxes on beverages or foods, such as the Excise Tax Implementing Regulations that included a 50 percent excise tax on SSBs in the Kingdom of Saudi Arabia (16) or Mexico's 8 percent tax on solid foods with high caloric density (17). Two studies evaluated 'tiered-taxes' implemented in Catalonia and Portugal that had higher tax rates for high- and low-sugar beverages. While most taxes targeted any SSB, including soda and juice (n = 18), two policies exclusively taxed carbonated beverages, two policies imposed additional taxes on caffeine content, and one policy taxed artificially sweetened beverages. Most of the tax studies measured the impact of taxes on purchases of taxed or untaxed beverages.[2] A few also considered outcomes related to purchases of high-sugar foods (n = 4) or diet quality (n = 2). Two studies exclusively measured the impact of taxes on diet quality or health without focusing on purchasing outcomes.

Four included studies evaluated subsidies: two targeted fruits and vegetables, one targeted fortified wheat and one targeted pulses. All four studies measured the impact of subsidies on diet quality or health outcomes (**Table 3**).

The evidence was overwhelmingly quasi-experimental (n = 23), and one impact evaluation used a randomized design. Quasi-experimental studies used difference-in-differences (n = 10), interrupted time series (n = 9), synthetic control (n = 2), instrumental variables (n = 1) and both synthetic control and difference-in-differences for two interventions evaluated separately (n = 1). Nearly all (n = 17) quasi-experimental studies relied on consumer purchase data from global bases such as Kantar World Data, Nielsen and Euromonitor.

Systematic reviews

After the initial search and title and abstract screening, 32 systematic reviews were screened at full text. Common exclusion reasons for systematic reviews were evaluation methods (many

2 Untaxed beverages varied by policy. Commonly, study authors include untaxed beverages such as water or diet versions of soda. Most study authors do not report differential effects of taxes on substitute or complementary untaxed beverages.

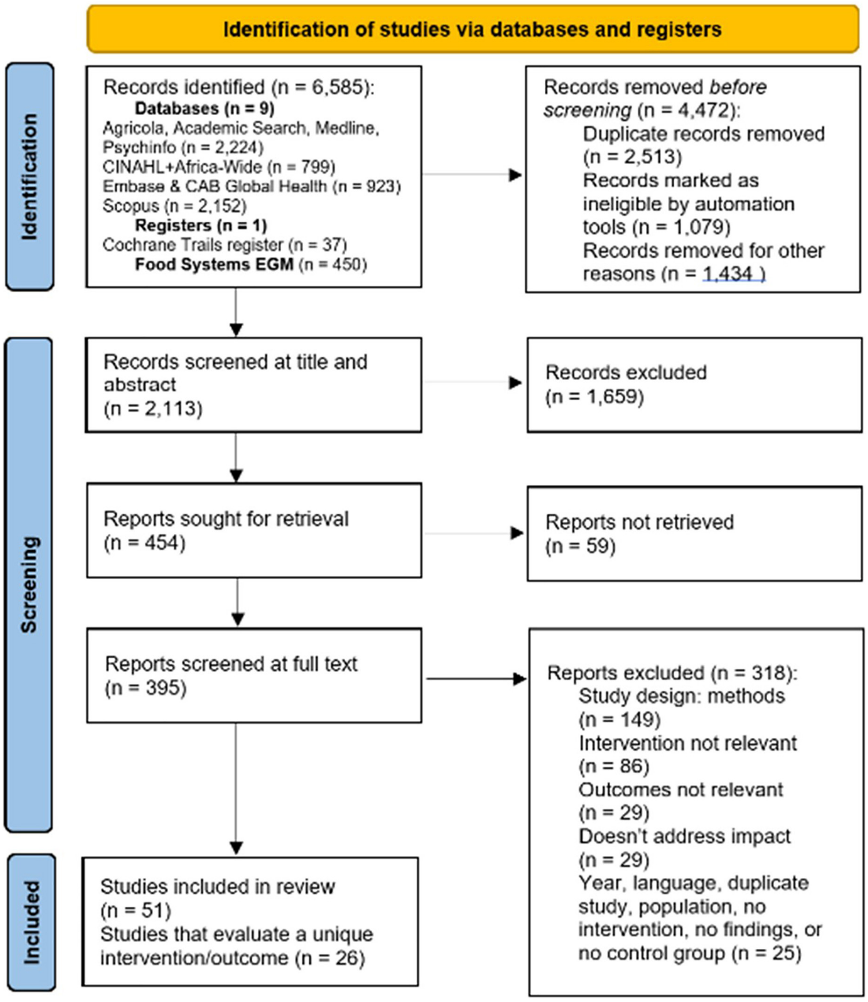

FIGURE 2
PRISMA diagram, 3ie (2022).

of the included studies within these SRs did not meet the eligibility criteria), and interventions not relevant to the scope of this review. Ultimately, two systematic reviews met the inclusion criteria. One review included one study from Hungary, and the other conducted a quantitative meta-analysis of evidence from the United States, Chile, France, Mexico, and Spain. Both included reviews synthesized the impacts of taxation. One searched for taxes on sugar and sugar added foods but found only one evaluation of a tax on foods high in sugar, salt and caffeine, including SSBs. The other study reviewed the effectiveness of taxes on SSBs.

Risk of bias in impact evaluations and systematic reviews

Overall, the quality of the impact evaluations is low; we assessed all evaluations to have some concerns or high risk of bias for at least two criteria (Table 4). Common quality concerns were related to confounding and reporting bias. Both systematic reviews were assessed with high confidence (Table 5). There were minimal concerns with the causal chain used in the review to analyze studies and the type of evidence incorporated to inform the analysis and reporting.

Effects of fiscal policies

We present the meta-analysis results for taxes and subsidies separately in Tables 6, 7, with additional results in Supplementary material 4. Results which consider only one or two studies should be interpreted with caution.

Effects of taxes

Taxes on sugar-sweetened beverages have no overall effect on purchasing of beverages

The meta-analysis suggests taxes on SSBs have no overall effect on beverage purchases ($\widehat{\mu} =$

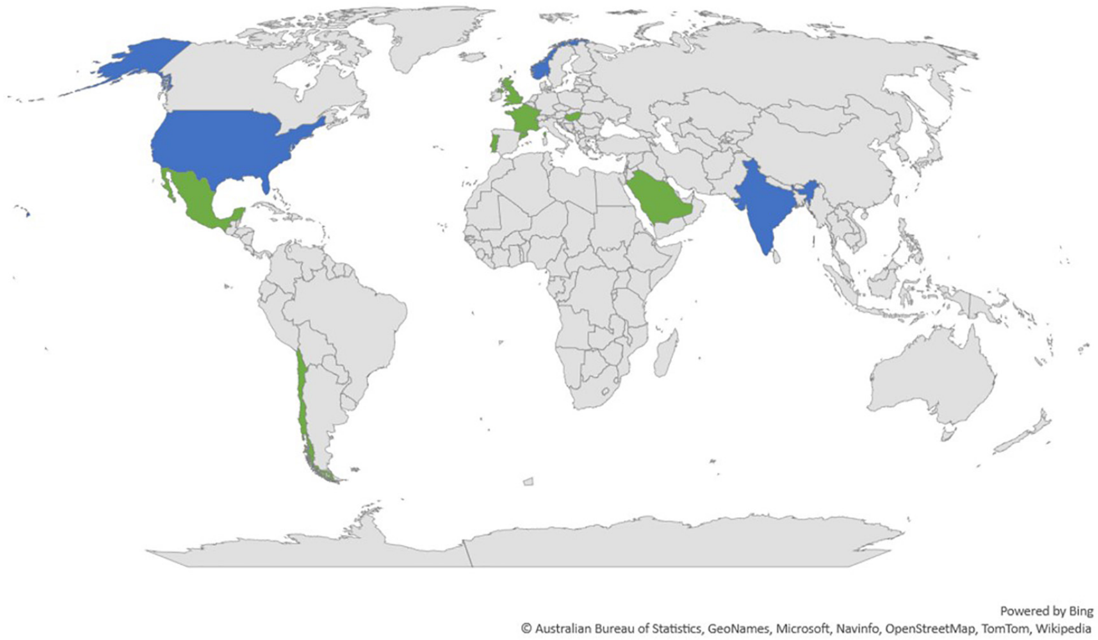

FIGURE 3
Map of analyzed studies, 3ie (2022).

TABLE 3 Summary of included studies.

Intervention group	Number of studies	Implementation countr(ies)	Outcomes evaluated	Indicators evaluated
Taxes on SSBs, ASBs or carbonated beverages (14–16, 20, 21, 24, 26, 29–31, 34–40, 42)	*N* = 18	Barbados. United States, Spain, Chile, France, Portugal, Saudi Arabia, United Kingdom	All purchases	Calories purchased in beverages; calories purchased in high-sugar foods; sugar purchased in beverages; sugar purchased in high-sugar foods; volume of purchased beverages
			Taxed purchases	Calories purchased in taxed beverages; volume of beverage purchases
			Untaxed purchases	Calories purchased in untaxed beverages; grams of sugar purchased in beverages; volume of purchased beverages
			Diet quality	Consumption of grams of added sugar; ratio of post to pre-tax prevalence of regular consumption of taxed beverage
Taxes on SSBs and high-sugar foods (17, 32, 33, 41)	*N* = 4	Mexico, Hungary, Norway	All purchases	Calories purchased in beverages; volume of purchased beverages
			Taxed purchases	Calories purchased in taxed beverages; volume of beverage purchases
			Untaxed purchases	Calories purchased in untaxed beverages; volume of purchased beverages
			Health	Outpatient visits for dental carries
Subsidies for staples (pulses, fortified wheat) (22, 25)	*N* = 2	India	Subsidized purchases	Purchases of pulses
			Diet quality	Daily household intake of protein
			Health	hemoglobin levels
Subsidies for fruits and vegetables (18, 28)	*N* = 2	Norway, United States	Diet quality	Intake of fruits (excluding fruit juices) and vegetables (excluding potatoes); servings of fruit in previous week

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TABLE 4 Risk of bias of analyzed impact evaluations.

Study name	Review criteria: Randomized control trial, difference-in-difference, and instrumental variable designs								
	Assignment mechanism	Unit of analysis	Selection bias	Confounding	Deviations from intended interventions	Performance bias	Outcome measurement bias	Reporting bias	Overall risk of bias:
Øvrum and Bere (18)	3	3	3	3	8	2	8	3	High
Alsukait et al. (16)	N/a	N/a	3	4	2	1	1	8	High
Cawley et al. (15)	N/a	N/a	1	2	2	8	8	1	Some concerns
Cawley et al. (19)	N/a	N/a	1	3	2	1	1	1	High
Gonçalves and Pereira dos Santos (20)	N/a	N/a	2	3	2	1	1	1	High
Rojas and Wang (21)	N/a	N/a	8	3	8	1	1	1	High
Chakrabarti et al. (22)	N/a	N/a	8	4	8	1	1	1	High
Bleich et al. (23)	N/a	N/a	3	8	8	2	1	1	High
Royo-Bordonada et al. (24)	N/a	N/a	2	3	8	2	2	4	High
Chakrabarti et al. (25)	N/a	N/a	2	3	3	1	1	1	High
Powell et al. (26)	N/a	N/a	2	4	8	1	1	3	High
Colchero et al. (27)	N/a	N/a	8	3	3	1	2	2	High
Howard and Prakash (28)	N/a	N/a	8	8	1	2	2	8	Some concerns
	Review criteria: Interrupted time series and fixed effect designs								
	Assignment mechanism	Unit of analysis	Selection bias	Confounding	Deviations from intended interventions	Bias due to missing outcome data	Outcome measurement bias	Reporting bias	Overall risk of bias:
Alvarado et al. (29)	N/a	4	N/a	0	1	0	0	1	Some concerns
Silver et al. (30)	N/a	1	N/a	1	2	0	0	2	High
Law et al. (31)	N/a	4	N/a	1	1	0	0	0	Some concerns
Hernández-F et al. (32)	N/a	4	N/a	1	0	0	0	1	Some concerns
Øvrebø et al. (33)	N/a	4	N/a	1	0	0	0	1	Some concerns
Pell et al. (34)	N/a	4	N/a	1	0	1	0	1	Some concerns
Aguilar Esteva et al. (17)	N/a	4	N/a	1	0	0	0	1	Some concerns
Powell and Leider (35)	N/a	4	N/a	1	1	0	0	2	High
Nakamura et al. (36)	N/a	4	N/a	1	1	0	0	1	Some concerns

TABLE 5 Risk of bias of systematic reviews.

Review criteria	Teng et al. (37)	Pfinder et al. (38)*
Methods used to identify, include and critically appraise studies	High confidence	High confidence
Methods used to analyze the findings relative to the primary question addressed in the review	High confidence	High confidence
Overall reliability of the review	High confidence	High confidence

*Pfinder et al. (38) only includes one evaluation on the Hungary tax, which was evaluated in one analyzed study (14).

$-0.07 (95\% CI: -0.25 \text{ to } 0.11); p = 0.42$; **Figure 4**). We included five effect estimates from four unique studies. Studies considered calories purchased in beverages (17), sugar purchased in beverages (36), and volume of beverages purchased (14, 30).

There was significant heterogeneity in results ($Q(4) = 22.17$, $p = 0.001$, $\hat{\tau}^2 = 0.02$, $I^2 = 81.96\%$). However, results did not differ between studies that considered volume and those that considered other outcomes (−0.14 [95% CI: −0.51 to 0.24], $p = 0.48$). There was no variation in results among studies that used synthetic control methods or interrupted time series and computationally similar methods (0.40 [95% CI: −0.31 to 1.11], $p = 0.27$), or between studies scored as high risk of bias and some risk of bias (−0.37 [95% CI: −0.99 to 0.25]; $p = 0.25$).

We examined the studentized residuals and found that two studies (17, 36) had values larger than 2.58 and may be potential outliers in the context of this model. Results did not change meaningfully when (36) was dropped from analysis ($\hat{\mu}$ =0.01 [95% CI: −0.05 to 0.04]; $p = 0.76$), but became significant when (17) was removed from analysis $\hat{\mu} = -0.20 [95\% CI: -0.28 \text{ to } -0.13]; p = 0.001$).

Taxes on beverages may reduce purchases of taxed beverages

The evidence from twelve studies (with 15 independent effect estimates) suggests taxes on SSBs reduced consumers purchasing such beverages ($\hat{\mu} = -0.18 [95\% CI: -0.29 \text{ to } -0.07]; p = 0.001$; **Figure 5**). We examined the studentized residuals and found that one study (26) had a value larger than 2.94 and may be a potential outlier in the context of this model. Our results remain significant when we removed this effect from the analysis ($\hat{\mu} = -0.07$ $[95\% CI: -0.12 \text{ to } 0.03]; p = 0.001$). Neither the rank correlation nor the regression test indicated any funnel plot asymmetry ($p = 0.38$; $p = 0.29$, **Supplementary material 5**, **Figure 1**), indicating that publication bias was not present.

Powell and Leider (35), which evaluated a tax on sugar-sweetened and artificially sweetened beverages in Cook County, IL, United States, report that, after the implementation of the tax, purchases of taxed beverages reduced immediately ($g = -1.30$ [95% CI: −1.65 to −0.96]) but there was no change in trend in purchase patterns ($g = -0.08$ [95% CI: −0.41 to 0.25]). When the county government repealed the tax, consumption increased ($g = 1.09$ [95% CI: 0.53 to 1.65]) but, once again, there was no change in trend in consumption patterns ($g = 0.22$ [95% CI: −0.32 to 0.77]). Law et al. (31), which evaluated changes in take-home aerated soft drink purchases after implementation of India's Goods and Services Tax (GST), consider linear and quadratic trend changes in the sale of taxes, aerated beverages. They find no change in either coefficient ($g = 0.83$ [95% CI: −0.15 to 1.8] and $g = -0.77$ [95% CI: 1.74 to 0.2], respectively). We could not consider these results within the main analysis because study authors report multiple measures within the same regression.

Taxes on sugar-sweetened beverages have no effect on purchases of untaxed beverages

Based on evidence from 11 studies, our meta-analysis suggests there is no effect of taxes on SSBs on the purchasing of untaxed beverages ($\hat{\mu} = 0.02 (95\% CI: -0.06 \text{ to } 0.02); p = 0.33$; **Figure 6**). Studies considered calories purchased (17), grams of sugar purchased (26), volume purchased (15, 20, 29, 30, 36, 39–42). There was moderate heterogeneity in results ($Q(10) = 19.61, p = 0.03$, $\hat{\tau}^2 = 0.00$, $I^2 = 49.01\%$). However, impacts were generally, consistently null or very small. There was no difference in impacts between those that considered volume of purchases and those that considered other outcomes (−0.03 [95% CI: −0.12 to 0.06], $p = 0.46$). Results were not different between studies that used difference-in-difference approaches when compared to those that used either synthetic control, interrupted time series, or computationally similar approaches (0.02 [95% CI: −0.06 to 0.11], $p = 0.59$). Similarly, results were not different among studies we assessed as high risk of bias and the other studies (0.03 [95% CI: −0.04 to 0.11]; $p = 0.40$).

We examined the studentized residuals and found that none of the studies had a value larger than ±2.84 and hence there was no indication of outliers in the context of this model. Neither the rank correlation nor the regression test indicated any funnel plot asymmetry ($p = 0.45$ and $p = 0.08$, respectively, **Supplementary material 5**, **Figure 2**), indicating that there was no publication bias present.

Powell and Leider (35) find a tax on sugar-sweetened and artificially sweetened beverages had no effect on purchases of untaxed beverages, nor do they find an effect of repealing the tax. However, Pell et al. (34) find that the *announcement* of England's tax on SSBs increased purchases of untaxed beverages ($g = 0.76$ [95% CI: 0.49 to 1.03]). We did not consider these results within the main analysis because study

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authors evaluated the *announcement* of the tax, rather than an implemented fiscal policy.

The evidence is too limited to draw conclusions about effects of taxes on sugar-sweetened beverages on the purchasing of untaxed high-sugar foods

Two studies reported on the impacts of standalone SSB taxes on sugary food consumption and find conflicting results. Bleich et al. (39) find that there is no change in the total calories purchased from high-sugar-foods ($g = 0.02$ [95% CI: −0.06 to 0.10]) in Philadelphia, PA, United States. However, Powell et al. (26) find an increase in the amount of sugar sold in sweets ($g = 0.22$ [95% CI: 0.13 to 0.31]) in Seattle, WA, United States. When we consider these effects jointly, we find no effect on the purchasing of sugary foods ($\widehat{\mu} = 0.12$ [95% CI: −0.08 to 0.32]; $p = 0.23$, **Supplementary material 5**, **Figure 3**). However, because the evidence base is limited and heterogeneous, we cannot make a definitive conclusion.

The evidence base is too limited to draw conclusion about effects of taxes on sugar-sweetened beverages have on evaluated measures of diet quality

Two studies reported on the impacts of SSB on measures of diet quality, each reporting two effects. Cawley et al. (15) find that the consumption of added sugar did not change in adults ($g = -0.09$ [95% CI: −0.30 to 0.12]) or children ($g = 0.01$ [95% CI: −021 to 0.23]) across several cities in the United States. Royo-Bordonada et al. (24) find no change in the consumption of taxed ($g = 0.45$ [95% CI: 0.36 to 0.54]) or untaxed ($g = 0.61$ [95% CI: 0.52 to 0.70]) beverages in Catalonia, Spain. When the change in taxed beverage and sugar consumption of adults were pooled, we find no overall effect ($\widehat{\mu} = 0.19$ [95% CI: −0.34 to 0.72]; $p = 0.48$; **Supplementary material 5**, **Figure 4**). However, the evidence is too limited to make a definitive conclusion.

The evidence base is too limited to draw conclusions about effects of taxes on sugar-sweetened beverages and high-sugar foods on the frequency of dental visits

Mexico's tax on SSBs and energy dense foods resulted in an immediate increase in outpatient visits for dental carries ($g = 0.66$ [95% CI: 0.34 to 0.98]) but an overall decrease in the trend in the frequency of these visits ($g = -0.81$ [95% CI: −1.13 to −0.49]; (32)). Because these results were based on a single study, they should be interpreted with caution.

The evidence base is too limited to draw conclusions about effects of taxes on sugar-sweetened beverages and high-sugar foods on the purchasing of taxed foods

There were no effects of taxes on high-sugar foods and SSBs in Mexico or Norway. In Mexico, Aguilar Esteva et al. (17) find no effect on total calories purchased ($g = -0.04$ [95% CI: −0.09 to 0.00]). In Norway, Øvrebø et al. (33) find no change in the sale of candy ($g = 0.01$ [95% CI: −0.06 to 0.07]). When considered jointly, these results suggest no change in the purchasing of sugary foods ($\widehat{\mu} = -0.02$ [95% CI: −0.07 to 0.02]; $p = 0.34$; **Supplementary material 5**, **Figure 5**). However, the evidence is very limited and should be interpreted with caution.

Sub-group analysis of effects of taxes

Although two studies interrogate the impacts of high and low tax levels on purchasing patterns, the evidence base is too limited to make definitive conclusions. Gonçalves and Pereira dos Santos (20) find no change in purchases of taxed beverages as a result of high- ($g = -0.01$ [95% CI: −0.04 to 0.02]) or middle-tier ($g = 0.00$ [95% CI: −0.03 to 0.03]) taxes in Portugal. The report a small reduction in purchases of low-tax beverages ($g = -0.03$ [95% CI: −0.05 to −0.01]). However, Nakamura et al. (36) find that a high-tier tax resulted in a reduction in purchases of high-tax beverages ($g = -0.21$ [95% CI: −0.28 to −0.13]) and no change in purchases of low-tax beverages ($g = 0.02$ [95% CI: −0.06 to 0.09]) in Chile. No middle-tier tax was imposed in Chile. When pooled, there was no effect on purchases of the high-tax beverages ($\widehat{\mu} = -0.11\,[95\%\mathrm{CI}: -0.30 \text{ to } 0.09]$; $p = 0.27$) or low-tax beverages ($\widehat{\mu} = -0.02\,[95\%\mathrm{CI}: -0.05 \text{ to } 0.02]$; $p = 0.30$; **Figure 7**). A third study, Pell et al. (34) evaluated the *announcement* of England's tiered tax on SSBs, which also had no effect on purchases of high-sugar beverages ($g = 0.21$ [95% *CI:*−0.06 to 0.47]) but decreased the purchases of low-sugar beverages ($g = -0.51$ [95% CI: −0.78 to −0.24]). However, we did not consider these results within the main analysis because study authors evaluated the *announcement* of the tax, rather than an implemented fiscal policy. Given the conflicting findings from the limited evidence base, the effects of these taxes are unclear.

Similarly, the evidence is too limited to draw any conclusions about differential effects across socioeconomic status (SES) groups. Two studies assessed the effects of SSB taxes on consumption of taxed and untaxed beverages by socioeconomic status. Colchero et al. (41) found that, in Mexico, the tax resulted in a no change in SSB purchases among individuals in the highest socioeconomic levels ($g = -0.03$ [95% CI: −0.11 to 0.04]). However, purchases decreased slightly in the lowest ($g = -0.15$ [95% CI: −0.26 to −0.05]) and middle ($g = -0.09$ [95% CI: −0.15 to −0.03]) socioeconomic levels. Nakamura et al. (36) find that consumption of high-tax beverages decreased among middle ($g = -0.15$ [95% CI: −0.28 to −0.03]) and higher ($g = -0.29$ [95% CI: −0.40 to −0.17]) socioeconomic levels but did not change among the lowest socioeconomic class ($g = -0.01$ [95% CI: −0.13 to 0.11]). There was no change in consumption of low-tax beverages in any socioeconomic class. When considered jointly, these two studies show that there was no change in the purchases of untaxed beverages among

TABLE 6 Results from meta-analysis considering the effects of taxes and subsidies.

Outcomes	# of included effects (total number of beneficiaries)	Overall effect size [95% CI]	Estimated percentile change compared to control group [95% CI]	Heterogeneity of overall effect: Q, I^2	Range of effects
Impacts of taxes on purchases of any beverage					
Total beverage purchases	5 (9,812)	−0.07 [−0.25; 0.11]	−2.8% [−9.9%; 4.4%]	22.17,** 81.96%	−0.21 to 0.40
Taxed beverage purchases	15 (86,971)	−0.18* [−0.29, −0.07]	−7.1% [−11.4%; 2.8%]	335.19,** 95.82%	−2.51 to 0.91
High-tax beverage purchases	2 (20,835)	−0.11 [−0.30; 0.09]	−4.4% [−11.8%; 3.6%]	23.51,** 95.7%	−0.21 to −0.01
Low-tax beverage purchases	2 (33,598)	−0.02 [−0.05; 0.02]	−0.8% [−2%; 0.08%]	1.35, 25.8%	−0.03 to 0.02
Untaxed beverage purchases	11 (34,977)	−0.02 [−0.06; 0.02]	−0.8% [−2.4%; 0.8%]	19.61,* 49.01%	−0.09 to 0.62
Untaxed beverage purchases among high SES	2 (3,474)	−0.08 [−0.25; 0.09]	−3.2% [−9.9%; 3.6%]	5.78,* 82.71%	−0.17 to 0.00
Untaxed beverage purchases among middle SES	2 (4,848)	−0.03 [−0.17; 0.11]	−1.2% [−6.7%; 4.4%]	3.91,* 74.40%	−0.09 to 0.05
Untaxed beverage purchases among low SES	2 (2,495)	−0.29 [−0.72; 0.14]	−11.4% [−26.4%; 5.6%]	29.30,** 96.59%	−0.51 to −0.07
Taxed, high-sugar food purchases	2 (10,819)	−0.02 [−0.07; 0.02]	−0.8% [−2.8%; 0.8%]	1.57, 36.13%	−0.04 to 0.01
Untaxed, high-sugar food purchases	2 (4,423)	0.12 [−0.08; 0.32]	4.8% [−3.2%; 12.6%]	11.06,** 90.96%	0.02 to 0.22
Impacts of taxes on diet					
Diet quality	2 (2,270)	0.19 [−0.34; 0.72]	7.5% [−13.3%; 26.4%]	21.58,** 95.4%	−0.09 to 0.45
Impacts of subsidies					
Purchases of pulses	4 (450,998)	0.02 [0.01; 0.03]	0.8% [0.4%; 1.2%]	3.04, 1.6%	0.01 to 0.02
Diet quality	3 (119,039)	0.06 [−0.01; 0.14]	2.4% [−0.4; 5.6%]	12.79,* 84.37%	0.01 to 0.22
Hemoglobin levels	2 (4,676)	−0.005 [−0.06; 0.05]	0.0% [−2.4%; 2%]	0.05, 0.0%	−0.01 to −0

*$p \leq 0.05$; **$p \leq 0.01$.

high ($\mu = -0.08$ [95%CI : -0.25 to 0.09] ; $p = 0.35$), middle ($\mu = -0.03$ [95%CI : -0.17 to 0.11] ; $p = 0.67$), or low ($\mu = -0.29$ [95%CI : -0.72 to 0.14]; $p = 0.19$; Figure 8). However, given the limited and heterogeneous evidence base, the variation in effects of taxes across socioeconomic classes remains uncertain.

Effects of subsidies

Chakrabarti et al. (22) find that a subsidy for pulses in India increased purchases of pulses ($\mu = 0.02$ [95% CI: 0.01 to 0.03]; $p < 0.001$; Supplementary material 5, Figure 6A). Effects were too small to be meaningful; however, they were generally consistent across the four states considered (Himachal Pradesh, Punjab, Andhra Pradesh, and Tamil Nadu, $Q(3) = 3.05$, $p = 0.38$, $\widehat{\tau}^2 = 0.00$, $I^2 = 1.60\%$). Because these results are based on a single study, they should be interpreted with caution.

Only one study considered the impacts of subsidies to support a diverse diet on health. Chakrabarti et al. (25) consider the impacts of a subsidy for iron-fortified wheat on hemoglobin levels in Punjab ($g = -0.01$ [95% CI: -0.11 to 0.09]) and Tamil Nadu ($g = -0.00$ [95% CI: -0.07 to 0.07]), India. When considered jointly, the subsidies have no effect on hemoglobin levels (($\widehat{\mu} = -0.005$ [95% CI: -0.06 to 0.05]; $p = 0.88$; Supplementary material 5, Figure 6B). However, since these results also come from a single study, they are also inconclusive.

Subsidies that incentivize diversifying diet with vegetables, fruits, or pulses have no effect on diet quality ($\widehat{\mu} = 0.06$ [95% CI: -0.01 to 0.14]; $p = 0.10$; Supplementary material 5, Figure 6C) in the included studies. We included three effect estimates, all from unique studies in this meta-analysis. The specific outcomes reported are daily household intake of protein [g/day; (22)], intake of fruits (excluding fruit juices) and vegetables (excluding potatoes) consumed on a typical day (18); and servings of fruit in the previous week (28). According to the Q-test, there was significant heterogeneity in the true outcomes ($Q(2) = 12.79$, $p = 0.01$, $\widehat{\tau}^2 = 0.00$, $I^2 = 84.37\%$). We examined the studentized residuals and found that study (18) had a value larger than ± 2.39 and may be a potential outlier in the context of this model. When it was removed, the estimate becomes statistically, but not practically, significant ($\widehat{\mu} = 0.01$ [95% CI: 0.002 to 0.02]; $p = 0.03$) According to the Cook's distances, none of the studies could be considered to be overly influential. Given the limited number of studies, this finding needs to be interpreted with caution.

TABLE 7 Effect estimates from included studies.

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Purchases of any beverage							
Nakamura et al. (36)	2018	Chile	Fixed effects	Grams sugar sales from all soft drinks*	−0.21 [−0.29; −0.14]	2,836 households	1 observation per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Gram sugar sales from all soft drinks (high SES)	−0.27 [−0.39; −0.16]	1,138 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Gram sugar sales from all soft drinks (medium SES)	−0.18 [−0.31; −0.06]	963 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Gram sugar sales from all soft drinks (low SES)	−0.11 [−0.23; 0.01]	1,120 households	60 observations per household
Aguilar Esteva et al. (17)	2019	Mexico	Regression discontinuity design	Total calories contained in all purchased products*	−0.01 [−0.06; 0.04]	6,935 households	104 observations per household
Silver et al. (30)	2017	Berkley	Interrupted time series	% change in volume of taxed or untaxed beverages sold per transaction relative to counterfactual developed based on pre-intervention trends*	0.14 [−1.02; 1.3]	9 stores	1,128 observations per store
Kruz et al. (14)	2020	Hungary	Synthetic control	SSB sales in milliliters*	0.20 [−0.73; 1.13]	16 stores	15 observations per store
Kruz et al. (14)	2020	France	Synthetic control	SSB sales in milliliters*	0.40 [−0.53; 1.33]	16 stores	15 observations per store
Powell et al. (26)	2021	Seattle	Difference-in-difference	Ratios of incidence rate ratios (RIRRs) showing the percentage change in grams of sugar sold in Seattle compared with Portland—from standalone sugar	0.12 [−0.31; 0.55]	81 brands	2 observations per brand
Purchasing of taxed beverages							
Alsukait et al. (16)	2020	Saudi Arabia	Difference-in-difference	Carbonated drinks' annual volume sales (liters per capita)*	−2.51 [−4.06; −0.95]	7 years	1 observation per year
Powell et al. (26)	2021	Seattle	Difference-in-difference	Ratios of incidence rate ratios (RIRRs) showing the percentage change in grams of sugar sold in Seattle compared with Portland—from taxed beverages*	−1.01 [−1.13; −0.90]	1326 brands	2 observations per brand
Powell and Leider (35)	2020	Cook County	Interrupted time series	Taxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in level after tax	−1.30 [−1.65; −0.96]	138 weeks	1 observation per week

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TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Powell and Leider (35)	2020	Cook County	Interrupted time series	Taxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in slope after tax	−0.08 [−0.41; 0.25]	138 weeks	1 observation per week
Powell and Leider (35)	2020	Cook County	Interrupted time series	Taxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in level after tax repeal	1.09 [0.53; 1.65]	51 weeks	1 observation per week
Powell and Leider (35)	2020	Cook County	Interrupted time series	Taxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in slope after tax repeal	0.22 [−0.32; 0.77]	51 weeks	1 observation per week
Puig-Codina et al. (42)	2020	Catalonia	Synthetic control	Liters cola purchased per person per month*	−0.67 [−1.59; 0.24]	17 regions	78 observations per region
Alvarado et al. (29)	2019	Barbados	Interrupted time series	Weekly sales of SSBs in mL/capita*	−0.19 [−0.47; 0.08]	200 weeks	1 observation per week
Cawley et al. (15)	2020	Philadelphia	Difference-in-difference	Taxed beverages monthly purchases (in ounces)*	−0.17 [−0.27; −0.06]	1447 households	12 observations per household
Cawley et al. (40)	2020	Oakland	Difference-in-difference	Volume purchased of taxed beverages in ounces*	−0.07 [−0.18; 0.03]	1360 individuals	2 observations per individual
Bleich et al. (39)	2021	Philadelphia	Difference-in-difference	Purchased fluid ounces of taxed beverages*	−0.16 [−0.24; −0.08]	2369 purchases	2 observations per purchase
Kruz et al. (14)	2020	Hungary	Synthetic control	SSB sales in milliliters*	0.91 [−0.05; 1.86]	16 regions	15 observations per region
Kruz et al. (14)	2020	France	Synthetic control	SSB sales in milliliters*	−0.11 [−1.03; 0.82]	16 regions	15 observations per region
Silver et al. (30)	2017	Berkeley	Interrupted time series	% change in volume of taxed beverages sold per transaction relative to counterfactual developed based on pre-intervention trends*	−0.08 [−1.24; 1.08]	9 stores	1128 observations per store
Aguilar Esteva et al. (17)	2019	Mexico	Regression discontinuity design	Total calories contained in all purchased taxed drinks*	−0.05 [−0.10; −0.00]	6935 households	104 observations per household
Øvrebø et al. (33)	2020	Norway	Interrupted time series	Exponentiated (Log) of sale of liters of soda*	0.01 [−0.06; 0.07]	3884 stores	50 observations per store
Rojas and Wang (21)	2021	Seattle	Difference-in-difference	Log volume purchased of taxed SSBs*	−0.02 [−0.04; −0.01]	61139 brands in a region	36 observations for brands in a region
Rojas and Wang (21)	2021	Berkley	Difference-in-difference	Log volume purchased of taxed SSBs*	−0.10 [−0.17; −0.02]	2548 brands in a region	24 observations for brands in a region

(Continued)

TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Colchero et al. (41)	2016	Mexico	Difference-in-difference and Fixed effects	Log volume purchased (mL/capita/day) of taxed beverages*	−0.07 [−0.13; −0.02]	5698 households	36 observations per household
Colchero et al. (43)	2016	Mexico	Difference-in-difference and Fixed effects	Log of volume purchased (mL/capita/day) of taxed beverages (high SES)	−0.03 [−0.11; 0.04]	2686 households	27 observations per household
Colchero et al. (41)	2016	Mexico	Difference-in-difference and Fixed effects	Log of volume purchased (mL/capita/day) of taxed beverages (middle SES)	−0.09 [−0.15; −0.03]	3885 households	27 observations per household
Colchero et al. (43)	2016	Mexico	Difference-in-difference and Fixed effects	Log of volume purchased (mL/capita/day) of taxed beverages (low SES)	−0.15 [−0.26; −0.05]	1375 households	27 observations per household
Law et al. (31)	2021	India	Interrupted time series	Year-on-year growth rate change trend in % volume aerated drinks sold—change in linear time coefficient	0.83 [−0.15; 1.80]	15 regions	51 observations per region
Law et al. (31)	2021	India	Interrupted time series	Year-on-year growth rate change in % volume aerated drinks sold—change in quadratic time coefficient	−0.77 [−1.74; 0.20]	15 regions	51 observations per region
Law et al. (31)	2021	India	Interrupted time series	Year-on-year growth rate change in % volume aerated drinks sold - change in linear time coefficient in high income states	0.19 [−0.77; 1.14]	15 regions	51 observations per region
Law et al. (31)	2021	India	Interrupted time series	Year-on-year growth rate change in % volume aerated drinks sold - change in quadratic time coefficient in high income states	−0.13 [−1.09; 0.82]	15 regions	51 observations per region
Law et al. (31)	2021	India	Interrupted time series	Year-on-year growth rate change in % volume aerated drinks sold - change in linear time coefficient in low income states	0.79 [−0.18; 1.76]	15 regions	51 observations per region
Law et al. (31)	2021	India	Interrupted time series	Year-on-year growth rate change in % volume aerated drinks sold—change in quadratic time coefficient in low income states	−0.75 [−1.73; 0.22]	15 regions	51 observations per region
Purchases of high and medium tax beverages							
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases high tax soft drinks*	−0.21 [−0.28; −0.13]	2836 households	60 observations per household

(Continued)

TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of high tax soft drinks (low SES)	−0.01 [−0.13; 0.11]	1120 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of high tax soft drinks (middle SES)	−0.15 [−0.28; −0.03]	963 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of high tax soft drinks (high SES)	−0.29 [−0.40; −0.17]	1138 households	60 observations per household
Gonçalves and Pereira dos Santos (20)	2020	Portugal	Difference-in-difference and Fixed effects	ln (Liters sold of High Sugar products)*	−0.01 [−0.04; 0.02]	17999 stores	36 observations per store
Gonçalves and Pereira dos Santos (20)	2020	Portugal	Difference-in-difference	ln (Liters sold of Medium Sugar products)	0.00 [−0.03; 0.03]	15772 stores	36 observations per store
Pell et al. (34)	2020	United Kingdom	Interrupted time series	Absolute change (ml/g) purchases of high tax beverages per household	0.21 [−0.06; 0.47]	212 weeks	1 observation per week
Purchases of low tax beverages							
Gonçalves and Pereira dos Santos (20)	2020	Portugal	Difference-in-difference	ln (Quantity of liters sold of Low Sugar products)*	−0.03 [−0.05; −0.01]	30762 households	36 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of low tax soft drinks*	0.02 [−0.06; 0.09]	2836 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of low tax soft drinks (low SES)	−0.10 [−0.22; 0.02]	1120 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of low tax soft drinks (middle SES)	0.11 [−0.02; 0.23]	963 households	60 observations per household
Nakamura et al. (36)	2018	Chile	Fixed effects	Log ml purchases of low tax soft drinks among (high SES)	0.05 [−0.70; 0.16]	1138 households	60 observations per household
Pell et al. (34)	2020	England	Interrupted time series	Absolute change (ml/g) purchases of low tax beverages per household	−0.51 [−0.78; −0.24]	212 weeks	1 observation per week
Purchasing of untaxed food and beverages							
Cawley et al. (15)	2020	Philadelphia	Difference-in-difference	Untaxed beverages monthly purchases (in ounces)*	−0.09 [−0.19; 0.02]	1447 households	12 observations per household
Cawley et al. (40)	2020	Oakland	Difference-in-difference	Volume purchased of untaxed beverages in ounces*	0.03 [−0.07; 0.14]	1363 individuals	2 observations per individual
Nakamura et al. (36)	2018	Chile	Fixed effects	Log of per capita volume of no-tax soft drink purchased by the household*	−0.07 [−0.15; 0.00]	2836 households	60 observations per household

(Continued)

TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Bleich et al. (39)	2021	Philadelphia	Difference-in-difference	Purchased fluid ounces of nontaxed beverages*	−0.01 [−0.09; 0.07]	2369 purchases	2 observations per purchase
Powell and Leider (35)	2021	Seattle	Difference-in-difference	Percent grams sugar sold from untaxed beverages relative to comparator*	0.00 [−0.25; 0.25]	239 brands	2 observations per brand
Powell and Leider (35)	2020	Cook County	Interrupted time series	Untaxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in level after tax	0.27 [−0.07; 0.60]	138 weeks	1 observation per week
Powell and Leider (35)	2020	Cook County	Interrupted time series	Untaxed Beverage Volume Sold in Cook County, Illinois, Relative to St Louis County and City, Missouri—change in slope after tax	−0.21 [−0.54; 0.12]	138 weeks	1 observation per week
Powell and Leider (35)	2020	Cook County	Interrupted time series	Untaxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in level after tax repeal	−0.08 [−0.62; 0.46]	51 weeks	1 observation per week
Powell and Leider (35)	2020	Cook County	Interrupted time series	Untaxed beverage volume sold in Cook County, Illinois, relative to St Louis County and City, Missouri—change in slope after tax repeal	0.36 [−0.18; 0.91]	51 weeks	1 observation per week
Gonçalves and Pereira dos Santos (20)	2020	Portugal	Difference-in-difference	ln (Liters sold of zero sugar products)*	0.01 [−0.03; 0.04]	13864 stores	36 observations per store
Aguilar Esteva et al. (17)	2019	Mexico	Regression discontinuity design	Calories purchased in untaxed drinks*	0.01 [−0.04; 0.06]	6935 households	104 observations per household
Aguilar Esteva et al. (17)	2019	Mexico	Regression discontinuity design	Calories purchased from untaxed food	0.02 [−0.03; 0.07]	6935 households	104 observations per household
Colchero et al. (43)	2016	Mexico	Difference-in-difference and Fixed effects	Volume purchased (mL/capita/day) of untaxed beverages*	−0.07 [−0.13, −0.02]	5698 households	36 observations per household
Silver et al. (30)	2017	Berkeley	Interrupted time series	% change in volume of nontaxed beverages sold per transaction relative to counterfactual developed based on pre-intervention trends*	0.29 [−0.88; 1.45]	9 stores	1128 observations per store

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TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Alvarado et al. (29)	2019	Barbados	Interrupted time series	Absolute difference in beverages that are not water or SSBs purchased ml/capita in the final week*	0.30 [0.03; 0.58]	200 weeks	1 observation per week
Puig-Codina et al. (42)	2020	Catalonia	Synthetic control	Liters diet cola purchased per person per month*	0.62 [−0.30; 1.53]	17 regions	78 observations per region
Pell et al. (34)	2020	United Kingdom	Interrupted time series	Absolute change (ml/g) purchases of untaxed beverages per household	0.76 [0.49; 1.03]	212 weeks	1 observation per week
Chakrabarti et al. (22)	2016	India	Difference-in-difference	Month household consumption (purchases) of all pulses (kg/household/month)	0.02 [0.01; 0.03]	112750 households	2 observations per household
Chakrabarti et al. (22)	2016	India	Difference-in-difference	Month household consumption (purchases) of all pulses (kg/household/month) in Himachal Pradesh	0.02 [0.01; 0.03]	112750 households	2 observations per household
Chakrabarti et al. (22)	2016	India	Difference-in-difference	Month household consumption (purchases) of all pulses (kg/household/month) in Punjab	0.01 [0.00, 0.02]	112750 households	2 observations per household
Chakrabarti et al. (22)	2016	India	Difference-in-difference	Month household consumption (purchases) of all pulses (kg/household/month) in Andhra Pradesh	0.02 [0.01, 0.03]	112750 households	2 observations per household
Chakrabarti et al. (22)	2016	India	Difference-in-difference	Month household consumption (purchases) of all pulses (kg/household/month) in Tamil Nadu	0.02 [0.01; 0.04]	112750 households	2 observations per household
Purchase of untaxed beverages among high SES households							
Nakamura et al. (36)	2018	Chile	Fixed effects	Log of per capita volume of untaxed soft drink purchased by the household*	−0.17 [−0.29; −0.06]	1138 households	60 observations per household
Colchero et al. (41)	2016	Mexico	Difference-in-difference and Fixed effects	Volume purchased (mL/capita/day) of untaxed beverages*	0.00 [−0.08; 0.08]	2336 households	27 observations per household
Purchase of untaxed beverages among middle SES households							
Nakamura et al. (36)	2018	Chile	Fixed effects	Log of per capita volume of untaxed soft drink purchased by the household*	0.05 [−0.07; 0.18]	963 households	60 observations per household
Colchero et al. (43)	2016	Mexico	Difference-in-difference and Fixed effects	Volume purchased (mL/capita/day) of untaxed beverages*	−0.09 [−0.015, −0.03]	3885 households	27 observations per household

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TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
Purchase of untaxed beverages among low SES households							
Nakamura et al. (36)	2018	Chile	Fixed effects	Log of per capita volume of untaxed soft drink purchased by the household*	−0.51 [−0.63; −0.39]	1120 households	60 observations per household
Colchero et al. (41)	2016	Mexico	Difference-in-difference and Fixed effects	Volume purchased (mL/capita/day) of untaxed beverages*	−0.07 [−0.18; 0.04]	1375 households	27 observations per household
Purchase of untaxed, high-sugar food							
Bleich et al. (39)	2021	Philadelphia	Difference-in-difference	Total calories from high-sugar food purchases*	0.02 [−0.06; 0.10]	2369 purchases	2 observations per purchase
Powell et al. (26)	2021	Seattle	Difference-in-difference	Percent grams sugar sold from sweets relative to comparator*	0.22 [0.13; 0.31]	2054 brands	2 observations per brand
Purchase of taxed, high-sugar food							
Aguilar Esteva et al. (17)	2019	Mexico	Regression discontinuity design	Total calories contained in all purchased taxed food*	−0.04 [−0.09; 0.00]	6935 households	104 observations per household
Øvrebø et al. (33)	2020	Norway	Interrupted time series	Exponentiated log of sale of candy sold (kg)*	0.01 [−0.06; 0.07]	3884 stores	50 observations per store
Diet quality							
Cawley et al. (15)	2020	Oakland	Difference-in-difference	Consumption of grams of added sugar in adults*	−0.09 [−0.30; 0.12]	341 individuals	2 observations per individual
Cawley et al. (40)	2020	Oakland	Difference-in-difference	Consumption of grams of added sugar in children	0.01 [−0.21; 0.23]	318 individuals	2 observations per individual
Royo-Bordonada et al. (24)	2019	Catalonia	Difference-in-difference	Ratio of post to pre-tax prevalence of regular taxed beverages (soft drinks, fruit drinks, energy drinks)*	0.45 [0.36; 0.54]	1929 individuals	2 observations per individual
Royo-Bordonada et al. (24)	2019	Catalonia	Difference-in-difference	Ratio of post to pre-tax prevalence of regular consumption of untaxed beverage	0.61 [0.52; 0.70]	1929 individuals	2 observations per individual
Chakrabarti et al. (22)	2016	India	Difference-in-difference	Daily household intake of proteins (gm/day)*	0.01 [0.00; 0.02]	112750 households	2 observations per household
Howard and Prakash (28)	2011	United States	Instrumental variable	Servings of fruit in previous week*	0.03 [−0.03; 0.08]	5140 individuals	1 observation per individual
Øvrum and Bere (18)	2013	Norway	Randomized control trial	Portions of fruits or vegetables consumed per day *	0.22 [0.11; 0.34]	1149 individuals	1 observation per individual
Health outcomes							
Chakrabarti et al. (25)	2019	Punjab	Difference-in-difference	Hemoglobin*	−0.01 [−0.11; 0.09]	1587 individuals	2 observations per individual

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TABLE 7 (Continued)

First author	Year	Region(s)	Evaluation or synthesis method	Outcome	Standardized effect estimate (confidence interval)	Independent units	Number of repeated measures
		Tamil Nadu		Hemoglobin*	−0.00 [−0.07; 0.07]	3089 individuals	2 observations per individual
Hernández-F et al. (32)	2021	Mexico	Interrupted time series	Intercept change in outpatient visits related to dental caries	0.66 [0.34; 0.98]	156 months	1 observation per month
Hernández-F et al. (32)	2021	Mexico	Interrupted time series	Slope change in outpatient visits related to dental caries	−0.81 [−1.13; −0.49]	156 months	1 observation per month
Other outcomes							
Bleich et al. (39)	2021	United States	Difference-in-difference	Total calories from sweetened beverage and high-sugar food purchases	−0.09 [−0.17; −0.01]	2369 purchases	2 observations per purchase

*Indicates estimates included in meta-analysis.

Design and implementation considerations

We summarize qualitative findings for both analyzed and linked studies in the following sections.

How are taxes on sugar-sweetened beverages, high sugar foods or aerated beverages 'supposed' to work?

Despite the call to implement these taxes because of their effects on health and wellbeing, few authors include health outcomes, such as obesity and diabetes, in their theories of change (17, 20, 21, 30, 42). Instead, most of the hypothesized theories of change focus on consumption. Authors argue that taxes on SSBs, sugary foods and aerated beverages will increase prices of taxed goods and decrease their consumption, but do not link these primary outcomes to health and wellbeing. In general, we observed three theories for how changes in price may affect consumption of SSBs or high-sugar foods: (1) signaling effects of adverse health risks, (2) price elasticity and substitution effects, or (3) product reformulation.

The 'signaling effect' mechanism suggests that the publicizing and adoption of taxes with associated price increases of taxed foods and beverages communicates to consumers that these products are inferior to untaxed products. Awareness of health risks associated with their consumption may influence consumers to reduce purchases (14, 15, 24, 34, 35, 44, 45). Two studies reported that signaling effects from taxes were amplified by information campaigns that may have increased awareness of the health risks associated with calorie-dense foods and beverages that are high in sugar, fat or salt (35, 46). Pell et al. (34) also suggest signaling effects and found that the *announcement* of England's tax on SSBs increased purchases of untaxed beverages before the policy went into effect.

Several authors observe theorized 'price elasticity effects' and conclude that raising the prices of taxed goods discourages purchases (14–16, 21, 29, 30, 33, 40–42). Others argue that consumers may purchase untaxed goods instead, substituting their purchases of taxed goods for untaxed goods (16, 47–50). Alsukait et al. (16) suggest decreased SSB consumption may increase the sale and consumption of bottled water; Taillie et al. (50) anticipate substitution effects from taxed to untaxed goods; Edmondson et al. (47) hypothesize that decreased consumption of taxed beverages will increase sales of juice or milk; and Leider and Powell (49) argue that taxes will increase in sales of other snacks, untaxed beverages or alcohol. Although many authors anticipated changes in purchases of untaxed goods, we find no change in purchases of untaxed beverages (**Figure 6**).

The final theorized mechanism from taxes to nutritious diets is through manufacturer product reformulation to reduce sugar (20, 34, 51). Both Portugal and the United Kingdom

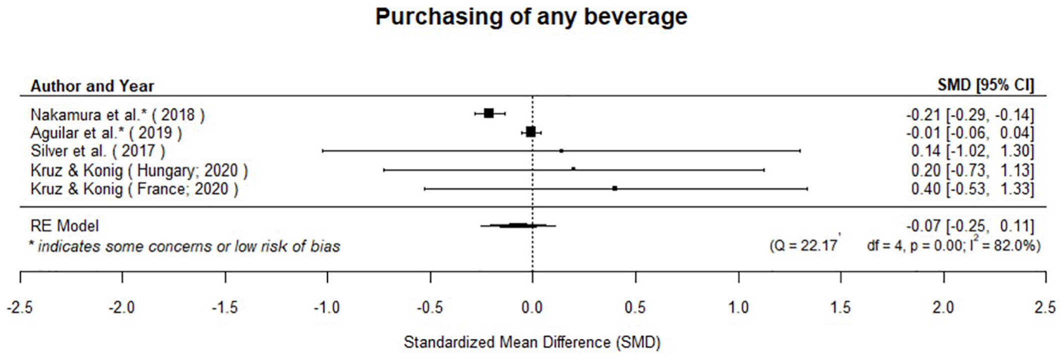

FIGURE 4
Taxes on SSBs have no overall effect on purchasing of beverages.

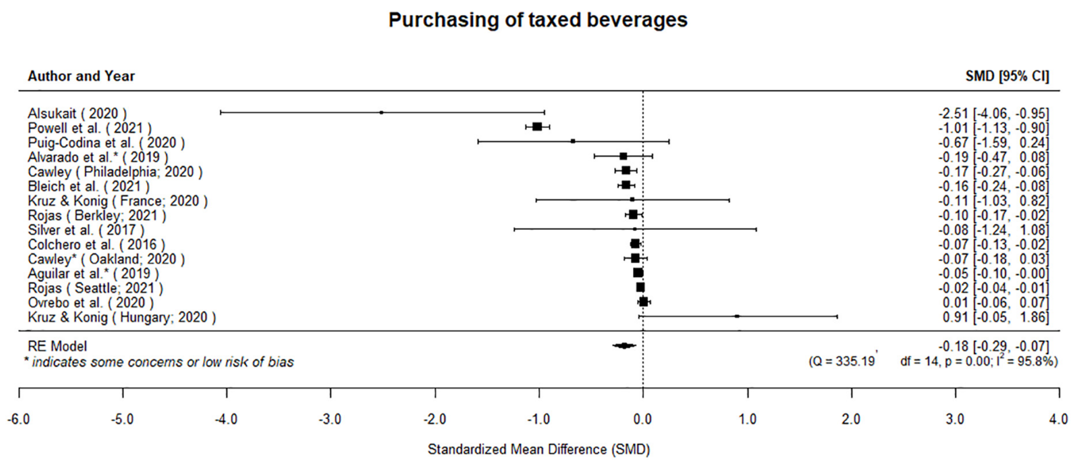

FIGURE 5
Taxes on beverages may reduce purchases of taxed beverages.

implemented tiered taxes that levied higher taxes for high-sugar products relative to low-sugar products. Gonçalves and Pereira dos Santos (20) and Pell et al. (52) observed that SSB manufacturers in both countries reformulated products to reduce added sugars to pay a lower tax rate. Industry reformulation was also observed in Mexico, which had a progressive tax (51). In contexts where volume of purchases of SSBs or high-sugar foods did not change, product reformulation may still have achieved changes in consumption of free sugars.

How are subsidies supposed to work?

Unlike proposed mechanisms of action for taxes, studies considering subsidies generally posit an effect on diet quality and health. Studies of subsidies for nutritious foods suggest the main mechanism of action is that they reduce prices, causing an increase in the purchases and consumption of foods, leading to improved nutrition outcomes (18, 22, 25, 28). For example, Chakrabarti et al. (25) argue that subsidies for iron-fortified wheat flour can reduce incidence of anemia in pregnant women. Chakrabarti et al. (22) posit that subsidies for pulses can improve nutritional outcomes for below-poverty-line families. While there was limited evidence, the studies considering relevant outcomes found that subsidizing pules increased household purchases and daily protein intake. However, the limited evidence does not suggest that subsidies for iron-fortified wheat flour impact anemia.

Do fiscal policies to encourage diverse diets have (unintended) regressive effects?

Consumers in lower socioeconomic classes may be more responsive to changes in prices than those in who are better off because changes in price are likely to represent larger relative changes in their total expenditures. This reasoning posits that fiscal policies may have variable impacts across socioeconomic levels. However, with few studies investigating impact across socioeconomic level and heterogeneous impacts, variation in impacts by socioeconomic level is uncertain. There were relatively larger decreases in consumption of taxed products

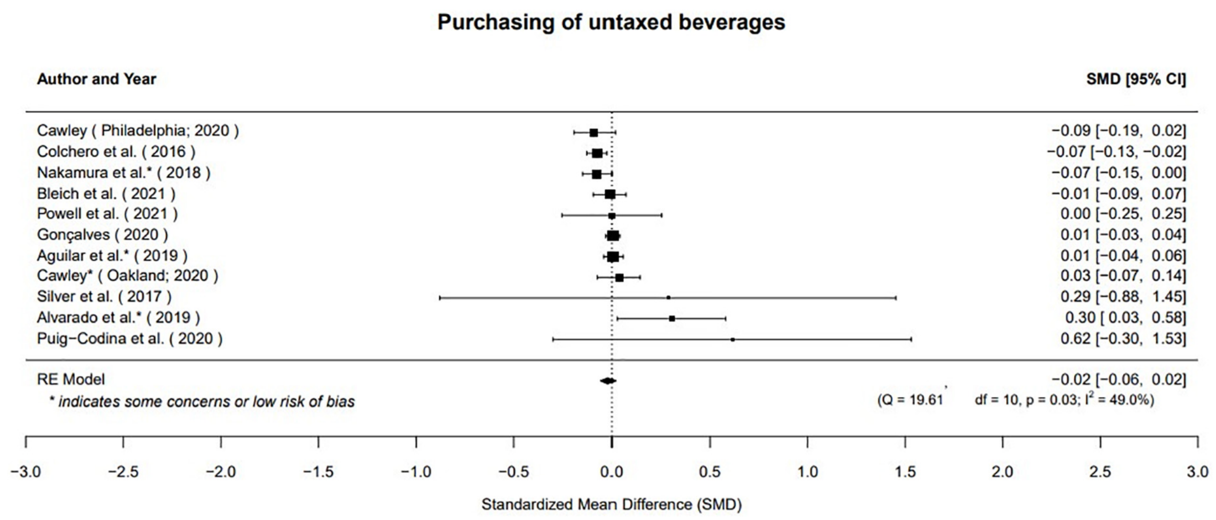

FIGURE 6
Taxes on SSBs beverages have no effect on purchases of untaxed beverages.

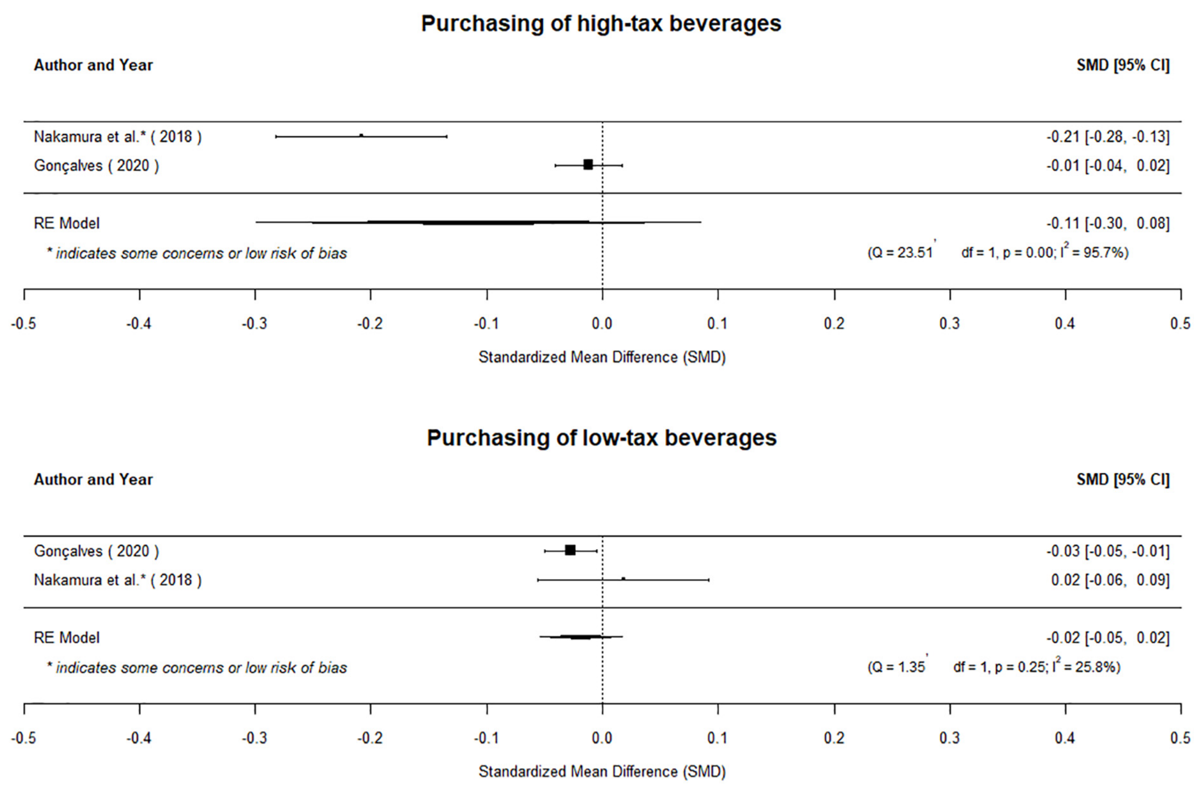

FIGURE 7
The evidence base is too limited to draw conclusions about the effects of high-tax and low-tax beverages on purchases.

among low-income households relative to high-income and middle-income households in Mexico and Philadelphia (39, 41). Nakamura et al. (36) report that SSB taxes decreased consumption among high- and middle-income households, but did not detect any change in consumption among the lowest socioeconomic class in Chile. Fichera et al. (53) did not observe any impact by socioeconomic status. Most studies did not have the income data necessary to conduct differential analysis by

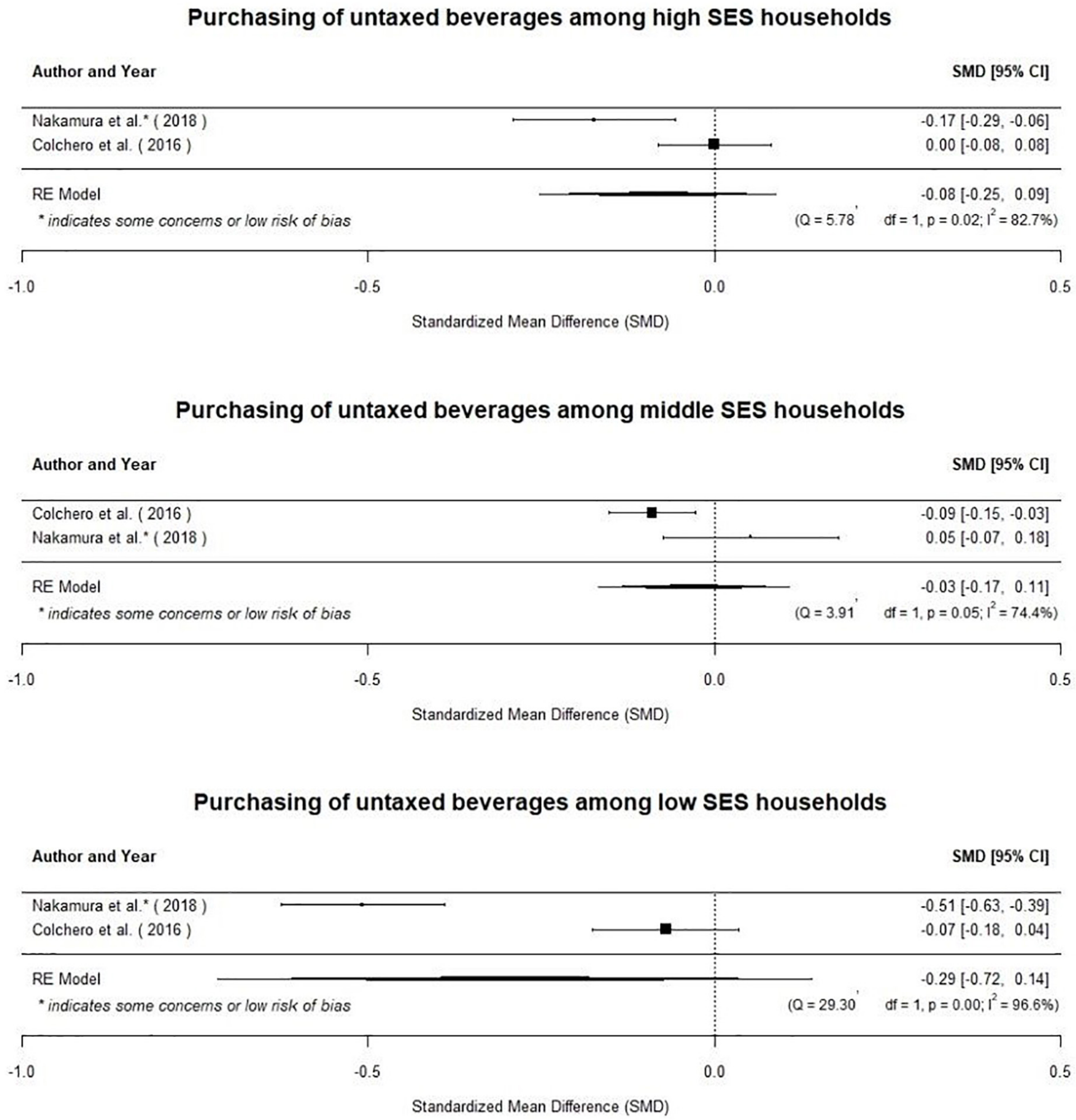

FIGURE 8
The evidence base is too limited to draw conclusions about the effects of taxes by socio-economic status.

income status. Given the variability of results, it is not possible to determine if the hypothesized variation in impacts across socioeconomic classes occurs.

Only a handful of studies reported differential analysis for other relevant groups. Bleich et al. (39) reported findings for education completed; Cawley et al. (15) and Teng et al. (37) reported findings for both adults and children; Chakrabarti et al. (25) reported findings for women at high-risk for anemia; Nakamura et al. (36) reported findings by weight.

Fiscal policies had slightly more positive effect sizes in HICs relative to L&MICs. In HICs, ten out of 20 studies report impact on consumer purchases in response to taxes. In L&MICs, two studies out of six report impact on consumer purchases in response to a tax or subsidy. There were not enough studies in L&MICs to conduct moderator analysis by country income level.

Facilitators of fiscal policies

Based on thematic analysis of all 49 impact evaluations of eligible interventions, we identified key factors that may facilitate the implementation and effectiveness of fiscal policies. Positive effects of fiscal policies were facilitated by (1) information to increase awareness on adverse health effects of SSBs or high-sugar foods and health benefits of subsidized foods, (2) large geographic coverage, and (3) the potential for revenue generation.

Information and media campaigns delivered in conjunction with the rollout of fiscal policies may facilitate beneficial effects on consumption and diet

Media campaigns may have increased awareness of adverse health effects of consumption of SSBs or high-sugar foods and health benefits of consumption of subsidized foods (21, 25, 33, 35, 40, 51). A pro-SSB tax campaign, "Berkeley vs. Big Soda," focused on adverse health outcomes and the "inappropriate behavior in the SSB industry" (46). The campaign's success was attributed to endorsements from a wide range of supporters, early stage coalition building, and prominent features by community representatives.

Better information may also amplify signaling effects and facilitate policy implementation. Chakrabarti et al. (25) report that new guidelines on safe levels of iron, folic acid and vitamin B12 published in 2016 may have encouraged two state governments in India to implement fortified wheat subsidies through an existing food subsidy program, the Public Distribution System (PDS). On the other hand, Law et al. (31) suggest that the focus of India's tax on revenue generation rather than health may have led to the null effects.

However, pro-tax campaigns are susceptible to retaliation from manufacturers. Powell and Leider (35) found that powerful manufacturer-backed anti-tax campaigns influenced the quick repeal of the Cook County, Illinois SSB tax just 4 months post-implementation. In June 2018, California state lawmakers passed a bill to prevent any municipality from passing a beverage or food tax for the next 12 years (54). Implementers should anticipate engaging with manufactures of taxed products.

Taxes covering larger geographic areas may facilitate compliance

Several studies report that a larger geographic coverage makes it more difficult for consumers to avoid SSB or high-sugar food taxes by cross-border shopping (15, 21, 23, 39, 55–57). Rojas and Wang (21) observed that implementing the tax in Berkley, CA, United States resulted in smaller changes in consumption relative to taxes implemented in larger geographic areas such as Seattle, WA, United States. Taxes that cover larger geographic areas limit opportunities for shopping in nearby, tax free locations, facilitating compliance and changes in consumer purchasing behaviors away from foods and beverages that are high in sugar, fat or salt.

Highlighting the potential for tax revenue may facilitate implementation by local governments

When framed by their revenue-generating potential, taxes experienced greater buy-in from implementing governments at local [Cook County, IL as observed by Powell and Leider (35)], state [Catalonia, Spain as observed by Puig-Codina et al. (42)] and national [Portugal, as observed by Gonçalves and dos Santos (20) and Norway, as observed by Øvrebø et al. (33)] levels of governance. While taxes on candy and SSBs in Norway were mainly implemented to create revenues, health benefits were later emphasized by the government (33).

Barriers to effects of fiscal policies

We identified several barriers to the implementation and effectiveness of fiscal policies including (1) tax avoidance through cross-border shopping, (2) vendor non-compliance, and (3) low awareness of health risks associated with SSBs, high-sugar foods or aerated beverages. Subsidy evaluations did not report any barriers to implementation.

Opportunities for cross-border shopping may limit compliance and provide a barrier to changing consumer habits toward nutritious diets

Cross-border shopping or tax avoidance is a barrier to compliance with the taxes on SSBs or high-sugar foods (15, 21, 23, 39, 40, 56, 57). For instance, Cawley et al. (15) describe how residents of Oakland, CA, United States avoid paying taxes by traveling to stores outside of the city to purchase SSBs at lower prices. Oakland is a medium-sized city, and authors suggest that cross-border shopping may be harder to deter in smaller tax locales. To mitigate cross-border shopping in Saudi Arabia, neighboring countries simultaneously implemented taxes on high-sugar foods or beverages (16). Tax avoidance by cross-border shopping likely weakens the effects of taxes by providing access to foods at cheaper prices in un-taxed locales and reducing the need to change behavior and substitute with more nutritious options.

Resistance from industry can present a barrier to both implementation and effects

Resistance from industry presented a barrier to implementation through stores failing to comply with details of new tax regimes. For example, stores in smaller localities did not always pass increases in price of taxed products on to their customers (21, 53, 58). These stores and SSB brands were concerned about competition from cross border shopping. Some stores preferred to incur the cost of the tax to avoid losing customers to stores outside the tax jurisdiction. Stores also did not always explicitly communicate the price change to customers. Powell and Leider (35) observes that while the Cook County, IL, United States tax ordinance required stores to display shelf prices inclusive of the total price with tax, retailers may not have complied. If retailers did not comply, consumers would not have known about the price change before check-out. In Chile, Caro et al. (58) found stores were not willing to incur the additional 'menu' costs required to physically change shelf prices to include the new tax. In this way vendor non-compliance could prevent the tax from affecting the prices of high-sugar foods or beverages within tax locales.

A few studies observed negative responses to taxes from producers (35, 41). Colchero et al. (41) report an increase in targeted advertising for SSB products in Mexico after implementation of the tax that may have influenced SSB purchases and consumption. This way of countering the effect of taxes on consumers may have presented a barrier to behavior change.

Low awareness of health risks associated with sugar-sweetened beverages and aerated beverages may present a barrier to behavior change

Low consumer awareness of the health risks associated with SSBs and high-sugar foods may have contributed to small changes in consumption post-implementation of taxes. Gonçalves and Pereira dos Santos (20) observed stockpiling of SSBs in the quarter before the tax was implemented and high rates of cross-border shopping. They conclude that SSB taxes in Portugal may not have produced the desired signaling effect to reduce consumption of high-sugar and medium-sugar products. In India, Law et al. (31) report that consumers may not have perceived the aerated beverage taxes as signaling health risks because the tax was rolled out as a part of general Goods and Services Taxes (GST), and not specifically as a health-related policy. Low awareness of health risks could prevent the tax from influencing consumption of foods and beverages that are high in sugar, fat or salt, reducing impact on diet and health outcomes.

Cost and sustainability information

None of the included studies conducted cost analysis. However, tax policies have the potential to be cost effective and sustainable because they generate revenue. Three studies report increases in revenue because of these taxes (20, 42, 55). While studies do not include information on how tax revenue was allocated within city, state or national budgets, there is potential for revenue to be invested in nutrition and health programming. Revenue from SSB taxes can be used to fund other health promoting activities, such as the Seattle SSB tax which supports nutrition, child health and education initiatives (59). The justification of these taxes as a revenue generating mechanisms should be considered separately from their justification as a public health tool. However, the signaling effect of these taxes may be diminished if revenue generation rather than health is seen as the goal.

Though some taxes, such as the Cook County, IL, United States taxes on SSBs (35) and the high-sugar food and beverage taxes in Norway (33) and Denmark (60) were repealed, nearly all taxes and subsidies were still implemented as of April 2022 (6). The costs of subsidy interventions were not reported.

Discussion

We identified 49 impact evaluations related to fiscal policies to support a diverse diet. These represented 24 unique intervention-outcome-population combinations due to repeated evaluation of the same taxes. Most studies took place in high-income countries (n = 18). We assessed all 24 analyzed impact evaluations as having some concern or high risk of bias for at least two criteria. We assessed the two SRs with 'high' confidence, but Pfinder et al. (38) only included one evaluation. Common quality concerns were related to confounding and reporting bias. We did not observe publication bias. Taxes on SSBs reduced purchases of taxed beverages (**Figure 5**). The results were inconclusive on whether fiscal policies can meaningfully influence the availability and accessibility of targeted foods and beverages, diet quality, health and well-being outcomes. Although these policies are largely supported due to their assumed effect on diet and health, only seven studies evaluated diet or health outcomes. These outcomes are inherently more challenging to measure relative to purchasing outcomes. Positive effects of fiscal policies were facilitated by information to increase awareness on adverse health effects of SSBs or high-sugar foods and health benefits of subsidized foods, large geographic coverage, and the potential for revenue generation. Tax avoidance through cross-border shopping and vendor non-compliance may have prevented some taxes from achieving desired effects. In the following sections we summarize the effects of taxes, the effects of subsidies, and limitations in the evidence base.

Effects of taxes on beverages and foods

Taxes on SSBs reduced purchases of taxed beverages with a standardized mean difference of -0.18 ([95%CI :-0.29 to -0.07], n = 15, **Figure 5**). Taxes had no impact on the overall volume of purchases of any beverage or the substitution of taxed beverages with untaxed ('healthier') beverages. Much of the reason for the apparent inconsistency in a reduction in taxed beverage purchases but no change in overall purchases is that there are different studies considering these different outcomes. The evidence base is too limited to determine if taxed beverages were substituted with untaxed substitute or complementary purchases. The available evidence is also too limited to determine if taxes on high-sugar foods and beverages impact consumption of taxed foods. Furthermore, it was not possible to make conclusions about the effects of these taxes on diet or health.

We observe mixed evidence for signaling effects of taxes. In many studies, consumers were affected by information or media campaigns. Without additional data on consumer choice, such as qualitative data on food decision-making or metrics of diet quality, we do not know if the effects were signaled by changes in prices of taxed goods or exposure to health information. The taxes may not influence consumers to substitute or purchase more nutritious untaxed beverages instead of taxed beverages or food.

Effects of subsidies

With only four studies evaluating subsidy interventions, we do not have sufficient evidence to comment on their potential for impacting health and nutrition outcomes. However, these studies generally report null or small impacts on the selected diet quality and health measures evaluated.

Limitations

This systematic review approach is limited by heterogeneity in pooled analyses, which may be explained in part by variation in outcomes used by study authors (e.g., volumes, grams of sugar, etc.). We conduct sensitivity analyses to determine if results varied by outcomes for purchases of taxed products and purchases of untaxed products and there were no differences. For diet and health outcome categories, we have presented effects individually in addition to presenting the combined analyses. The combined analyses are intended to provide additional information on the effects of these policies.

Gaps in the evidence base

Most included studies (n = 19) took place in HIC contexts, and only four studies evaluate subsidy interventions. Just two studies exclusively measured the impact of taxes on diet quality or health without focusing on purchasing outcomes, and only one study measured both purchasing and diet quality outcomes. Very few studies included sub-group analysis by socioeconomic status. Gibson et al. (48) explained that few stores in low-income neighborhoods participated in the study, and that common data sources do not include demographic information on consumers. Zhong et al. (62) observed that using purchase data limits generalizability of findings to relevant subpopulations, such as soda drinkers and low-income consumers. Because there are so few evaluations, there is insufficient evidence to be able to determine if key features of the taxes themselves, such as the size of the tax, the type of tax, or targeted beverages of foods, may affect the results.

High risk of bias

Nearly all studies used quasi-experimental designs, as the roll out of a fiscal policy would be challenging to evaluate using experimental methods. The most common evaluation designs were difference-in-differences (n = 11) and interrupted time series (n = 9) that relied on large panel or time series data. We assessed all studies in the evidence base to have concerns of bias, most often related to confounding, independence or contamination from other events or programs occurring simultaneously. High risks for confounding were common in difference-in-difference studies because authors did not include relevant time-varying characteristics in their model specifications (16, 20, 21, 26, 40). This may have been due to limited availability of data of seasonal consumption trends, socio-demographic and economic characteristics of purchasers, store characteristics and others. Nearly all difference-in-difference studies tested parallel trends and verified the core assumption of the method. For interrupted time series studies, there was limited discussion of independence of interventions from other factors which may have confounded the impact of the policies, such as information campaigns (34), or large overhauls of the whole tax system (31).

Reverse causation is not addressed

The theory of change justifying the adoption of fiscal policies assumes that changes in price influence purchasing behavior (**Figure 1**). However, given that nearly all included evaluations occur in democratic countries, it is possible that changes in perception of targeted foods precipitated adoption of a fiscal policy to influence consumption, for example pre-tax media campaigns to advocate implementing SSB taxes (35). While authors did not often explicitly mention potential for reverse causality in-text, we caution that constituents may have elected to tax or subsidize themselves in some contexts. A key determinant of implementing a fiscal policy could be changes in constituents' attitudes toward calorie-dense foods and beverages that are high in sugar, fat or salt. As such, interrupted-time series, which assume that trends would have remained the same without the intervention and was commonly used in the included evaluations, may not be an appropriate evaluation approach for these policies.

Diet quality and health are not evaluated

These policies are largely supported due to their assumed effect on diet and health. However, only seven studies evaluated diet or health outcomes, likely because these outcomes are inherently more challenging to measure relative to purchasing outcomes. Nearly all studies (n = 17) used datasets that track consumer point-of-sale purchases at select stores such as Nielsen scanner data, Euromonitor, and Kantar World Panel. A strength of this method was that point-of-sale data avoided common measurement errors. Data was collected independent of the intervention and primary outcomes were assessed objectively. However, sales data do not directly measure changes in calories consumed, diet and health outcomes (17, 26, 51, 58, 61). These datasets rarely include household demographic information or even all the products subject to the tax. For example, Zhong et al. (62) reported that Euromonitor data did not have data on all the products included in the Philadelphia, PA, United States taxes. While point-of-sale data sources can illuminate changes in sales of products, purchases do not provide information on food and beverage intake, diet quality, or consumption patterns within households and neighborhoods. To elucidate the impact of fiscal policies on diet and health outcomes, Hernández-F et al. (32) used data from the Mexican government to connect

Mexico's SSB and sugary food taxes to dental health outcomes. Future research can similarly use population health data to investigate the causal links among fiscal policies, nutrition, and health outcomes.

Data sources not suitable to quantify impacts on selected outcomes

In many studies, the data sources used did not have key information needed to quantify impacts and adequately respond to their research questions. Authors report that they did not have requisite purchase data to measure changes in cross-border shopping (15, 35, 56) or purchases or volume sold in non-store venues such as restaurants, kiosks, or vending machines (17, 26, 41). Others could not measure intake of home-cooked foods, or perishable fresh foods such as fruits and meat and lacked household panel data (20, 43). Limited purchase data also prevented a few authors from testing for substitution effects. For instance, Falbe et al. (46) did not have data on purchases of non-SSBs, including diet soda, and could not analyze beverage substitution. Gibson et al. (48) attributed their inability to measure substitution effects to unavailability of data at the individual consumption level. Colchero et al. (43) report that they were not able to account for factors that may impact SSB sales independent of the tax in Mexico, such as temperature or advertising. Missing data on these purchases and consumption trends prevented authors from quantifying changes in their target outcomes.

Implications for policy and practice

- The evidence base is too limited to make firm conclusions as to whether fiscal policies such as taxes and subsidies improve diet and health outcomes. Considering the widespread adoption of such policies, and the significant costs of subsidies in particular, it would be prudent to integrate rigorous research with the implementation of such policies. Only two studies considered the impacts of these taxes on diet quality.
    - The evidence suggests that beverage taxes reduce purchases of taxed beverages, but there is considerable heterogeneity in results.
    - The few studies evaluating subsidies suggest no or very small effects, but the evidence base is too small to draw firm conclusions.
- Taxes do not appear to generate substitution from calorie-dense beverages to relatively more nutritious beverages.
- Tax policymakers should consider conducting needs assessment to better understand health knowledge in their population. If appropriate, they may incorporate health information campaigns to amplify signaling effects of the taxes. This may improve adherence to the tax and reduce avoidance behaviors, such as cross-border shopping.
- Tax policies can trigger reformulation processes that can contribute to an anti-obesogenic food environment and a food systems transformation.
- Tax policies pay for themselves by generating revenue and may be more sustainable than other nutrition interventions. Revenue from taxes can be allocated toward nutrition and health programming.
- Integrating subsidies into existing food support systems may facilitate greater access among low-income populations.
- Because subsidies may require significant financial investment, additional research is needed to justify their implementation.
- Fiscal policies can be more rigorously evaluated if governments share routine monitoring data with researchers.

Implications for research

- Anticipate limitations in data sources by developing rigorous evaluation design strategies that account for common sources of bias, such as confounding and independence.
    - Synthetic control analysis may be appropriate in these settings where there is a single intervention unit and non-intervention sites are likely to be fundamentally different than intervention sites (42, 14).
- Evaluations should use all available data and diversify data sources to better understand the impacts of SSB and high-sugar food taxes on diet and health outcomes.
    - Consider collecting new data or leveraging large scale data sources such those available through DHIS2, DQQ and the FAO (63–65). When using existing data sources, be sure to acknowledge any known limitations in their sampling procedures and data quality.
    - Consider partnering with government to access nutrition and health information.
- Prioritize evaluations in L&MIC contexts and evaluations of subsidies in all contexts, especially those focusing on nutritious foods, such as fruits, vegetables and pulses (2).
- To the extent practical, include equity aspects, such as subgroup analysis by socioeconomic status, body-mass index or pre-existing health conditions that may correlate with consumption of taxed or untaxed foods and beverages.
- Theory-based evaluations should prioritize measuring the effects of these interventions on health and wellbeing, rather than purchasing behavior.

- Mixed-methods evaluations could elucidate how consumers respond to these fiscal policies.
- Consider investigating variation in impacts based on the size and type of the tax or subsidy.
- From the perspective of policymakers, cost-evidence is needed to justify the use of subsidies, but may not be needed for the implementation of taxes, which generate revenue.
- Long-term outcomes should also be investigated as some consumer behaviors, such as purchases of diet cola, may change over time.

Data availability statement

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

Author contributions

CL and SS led the conception and design of the study and the meta-analysis. DA and TK led the search and screening, data extraction, and risk of bias analysis. MB contributed to the quantitative data analysis. VB served as research coordinator. JH performed the qualitative data analysis, served as publication manager, and wrote the first draft. All authors substantively contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.967494/full#supplementary-material

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Food-based dietary guidelines for children and adolescents

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Objective: This study aimed at reviewing food-based dietary guidelines (FBDGs) with content targeted at children and adolescents to present their main characteristics, thus enabling comparisons among countries.

Design: We conducted a search of the FBDGs available on the Food and Agriculture Organization (FAO) website, followed by a scoping review with a gray literature search to find FBDGs for children or adolescents non-listed on the FAO's website. Data extraction included the year of publication, language, and guidelines for the target group.

Results: From FAO website searches, 109 documents were found, and 17 of them could not be translated. The Scoping review search conducted in 5,190 articles, and none led to new guidelines, nor from the gray literature. Regarding the 92 FBDGs explored, 41 were specific for infants under 24 months old, children, and/or adolescents, and 51 were for the general population with information for the studied group. Twelve percent of the general FBDG and 35% of the specific ones have food icons. All of the guidelines were published after 2001. Latin America and the Caribbean were the regions that presented more specific FBDGs and the majority of countries with guidelines for fruits and vegetables. The information about fat (15 countries) and sugar (26 countries) consumption reduction is frequent. Reduction of sodium intake appears to be in the majority of guidelines after 2015. Food hygiene guidelines are recurrent in Latin American documents. NOVA classification was adopted in five countries and 21 countries approach recommendations for mealtimes. Both exclusive and continued breastfeeding guidance and healthy complementary feeding orientation are present in over 50% of the specific FBDG for infants and children under 24 months old.

Conclusion: Food-based dietary guidelines are diverse due to both the nutritional and political aspects of each region. Latin America stands out for its orientations for the studied group. Further studies should measure the possible impacts and comprehension of FBDGs.

KEYWORDS

adolescent, child, health promotion, dietary recommendations, scoping review

Introduction

Food-based dietary guidelines (FBDGs) are a practical tool for building a more conscious diet based on healthy and sustainable habits since they provide advice on foods, food groups, and dietary patterns to promote overall health, foster healthy eating habits and lifestyles and prevent chronic diseases (1, 2). They can effectively assist the general population, health professionals, and policymakers in different areas, such as nutrition in public health, agriculture, and nutrition education (2). However, the ways in which these guidelines are presented are diverse, varying between countries and according to the stages of life they are targeted (3, 4).

Specific FBDGs for each stage of life are necessary due to the individualities they present. A healthy eating pattern for each stage of life can demand different habits, which should lead to different dietary guidelines (5). The 1st years of life, especially from 0 to 2 years old, are considered an important window of opportunity as it comprises the formation of eating behavior that will be maintained in adulthood (6). In addition, adolescence has particular characteristics, involving elements of biological growth and major social role transitions, that affect eating patterns in this stage of life (7).

Horta et al. have already conducted a review of FBDGs aimed at children and adolescents (8); however, it is outdated, since it was conducted in 2010. Recently, two systematic reviews compared FBDGs for adults (9, 10), but no other studies focusing on children and adolescents were found.

Thus, this study aimed at reviewing the FBDGs with content targeted at children and adolescents around the world, providing information to policymakers on their main characteristics, in order to enable and assist improvements in the country's tool and comparison among countries.

Methods

This study was divided into two parts. The first one corresponds to a search conducted on the Food and Agriculture Organization (FAO) website (11): https://www.fao.org/nutrition/education/food-based-dietary-guidelines,—in order to explore the FBDG repository available. This website was launched in 2014 and it has been continually updated. It collates information about FBDG from many countries, including the official name of the national guidelines; publication year; a description of the process and stakeholders involved in its development; the intended audience; a brief description of the food guide; and its key messages. Links to downloadable documents are available too, which were the object of this first part of our study. All FBDGs available were considered, independent of the version, as a complete FBDG, folder, or food guide. "Food guide" and FBDG were considered synonymous. The information was sought not only in materials specifically for children and adolescents but also in those directed to the general population.

Two reviewers (1R and 2R) were responsible for the extraction of FBDG with information for the intended group on FAO's website. In case there was no information for children and adolescents, the material was excluded. For documents whose existence was indicated in FAO's website, but were not available, when there was an indication of the existence of a more recent version of the material in the website, and/or when an earlier FBDG was from previous knowledge of 1R and 2R, a search in official government pages of the respective countries was done. This phase lasted from February 2021 up to March 2021 and it was updated in July 2022 up to August 2022. Guidelines in English, Spanish, Portuguese, French, German, Italian, Chinese, Korean, and Japanese were translated and their information was extracted.

A table used for the extraction of FBDG found on FAO's website was designed based on previous studies (8–10). Information registered of each material found was: whether it was specific or not for children and/or adolescents, country of origin, region (Latin America and the Caribbean, Europe, Asia and the Pacific, North America, Africa, and Near East), language, year of publication, the age group for which the information was intended (infants and children under 24 months old, named group 1; preschoolers and school-age children between 25 months old and 9 years old, named group 2; and adolescents from 10 to 19 years old, named group 3), intended audience (e.g., general population and health professionals), disposition of information in the general FGDBs (e.g., in a specific chapter or annex), and the content directed to the age group. Regarding the content, variables registered were about the presence of a food icon, food groups and/or portion recommendations, recommendations for mealtimes/commensality (e.g., encourages involving the child in preparing meals or eating with the child), and other relevant recommendations, such as fruits and vegetable guidelines, NOVA food classification system, hygiene guidelines, healthy complementary feeding guidelines, recommendation to avoid sugary foods and sweets, fats related information (to limit consumption or about the adequate sources), water ingest, sodium/salt limitation, exclusive breastfeeding, and continued breastfeeding.

NOVA classifies foods into four groups, according to the extent and purpose of the industrial processing they undergo: (1) minimally processed foods and unprocessed food (no addition of salt, sugar, oils or fats, or other food substances to the original food); (2) processed culinary ingredients (like oils and fats, sugar, and salt); (3) processed foods (industrial products made by adding culinary ingredients to the first groups of foods, to increase their durability or enhancing their sensory qualities);

TABLE 1 Search strategy for PubMed database.

Database	Search strategy
PubMed	Infant OR Infants OR Baby OR Babies OR "Preschool child" OR "Preschool children" OR Newborn OR Newborns OR "Young child" OR "Young children" OR Child OR Children OR Kid OR Kids OR Toddler OR Toddlers OR "School child" OR "School children" OR Adolescent OR Adolescents OR teenager OR teenagers OR Teen OR Teens OR Adolescence OR Infante OR Infantes OR Niño OR Niños OR Chico OR Chicos OR Bebé OR Escolares OR "Recién nacido" OR "Recién nacidos" OR Adolescente OR Adolescentes OR Criança OR Crianças OR Bebê OR Bebês OR "Pré escolar" OR "Pré escolares" OR "Recém nascido" OR "Recém nascidos" OR Escolar OR Escolares OR Adolescência. AND "Food based dietary guidelines" OR "Food based dietary guideline" OR FBDG OR "Food guide" OR "Food guides" OR "Nutrition Guidelines" OR "Nutrition Guideline" OR "Nutrition policy" OR "Nutrition policies" OR "Nutritional requirement" OR "Nutritional requirements" OR "Nutritional education" OR "Guías alimentarias" OR "Orientación nutricional" OR "Educación nutricional" OR "Necesidades nutricionales" OR "Política nutricional" OR "Necessidades nutricionais" OR "Educação nutricional."

and (4) ultra-processed foods (formulations of ingredients, most of exclusive industrial use, which result from a series of industrial processes; products are usually highly profitable, convenient, and hyper-palatable) (12).

The second part of the study corresponds to an additional search in databases following the guidelines of a scoping review in order to check FBDGs for children or adolescents not found on the FAO's website. A scoping review seems to be a more effective way to identify the types of available evidence in a given field (13). This scoping review was written according to the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews) checklist (14), and the protocol registration is available online under DOI registration, 10.17605/OSF.IO/J5Z6R (15).

A scoping review aiming to identify scientific articles that mentioned FBDGs non-listed in FAO's website for children or adolescents (groups 1, 2, and/or 3) was conducted in March 2021 by two reviewers (1R and 2R). We searched for articles that mentioned guidelines for children and adolescents or the general population with information for children and adolescents, but articles that mentioned FBDGs already identified on FAO's page were not included. Searches were conducted in the following databases: Lilacs, Scielo, PubMed, and Web of Science. This search was complemented by gray literature from Google Scholar (first 100 results). The primary search strategy adopted for the PubMed database (Table 1) was adapted for the other ones. Articles could be in English, Spanish, or Portuguese, without a limit of the year of publication. The risk of bias was not performed since the aim of the study was not to evaluate the included articles. For the entire selection process, the app Rayyan was used (16). If there was a new FGDB identified, the data extraction would be the same as described before.

The materials will be described in a narrative form and with data organized in tables, with no intention of qualifying them.

Results

As presented in Figure 1, from the FAO's page extraction, 274 documents were found, of which 109 had information for children and adolescents. Of them, 17 could not be translated. Regarding the 92 FBDGs explored, 41 (17–57) were specific for children and/or adolescents, and 51 (58–108) were directed to the general population with information targeted at children and/or adolescents.

As shown in Figure 2, the search for the systematic review resulted in a total of 5,190 articles, of which 700 were duplicates. After the analysis by 1R and 2R, 21 articles were fully read. Neither of the documents were used, nor were the ones found in gray literature, since they mentioned FBDGs for children or adolescents already listed on FAO's website.

Overview of included material

Ninety-two documents found were from a total of 59 countries, accordingly to the regions listed on FAO's website: 72% of the listed countries in Latin America and the Caribbean (25–44, 81–95), 67% of the five countries in the Near East (76–80), 61% of 18 in Asia and the Pacific (17–24, 66–75), 35% in Europe (45–58, 96–102), all nine countries in Africa (59–65, 105–108), and all two countries in North America (103, 104).

Most of the FBDG documents found were written in English (51%) (17–24, 39, 40, 48, 60–81, 83, 89, 93, 96–98, 100–108), followed by Spanish (31%) (25, 26, 28–38, 41–44, 57, 82, 85–88, 90–92, 94, 95), French (45–47, 49, 50, 59), and German (51–56), representing 7% each, and the remaining documents (4%) were written in Portuguese (27, 84, 99) and Italian (58). Regarding the region of origin, 38% of the documents were from Latin America and the Caribbean (25–44, 81–95), 23% from Europe (45–58, 96–102), 19% from Asia and the Pacific (17–24, 66–75), 12% from Africa (59–65, 105–108), 6% from the Near East (76–80), and 2% from North America (103, 104).

About a third (30%) (27, 32, 33, 41, 42, 45–48, 50–56, 58, 82, 86, 89, 92, 97, 103–108) were dated from the past 5 years (2018–2022), 40% (17–26, 28–31, 39, 40, 43, 44, 49, 59, 60, 64–70, 76, 78, 79, 81, 84, 90, 91, 94, 101, 102) were from 2013 to

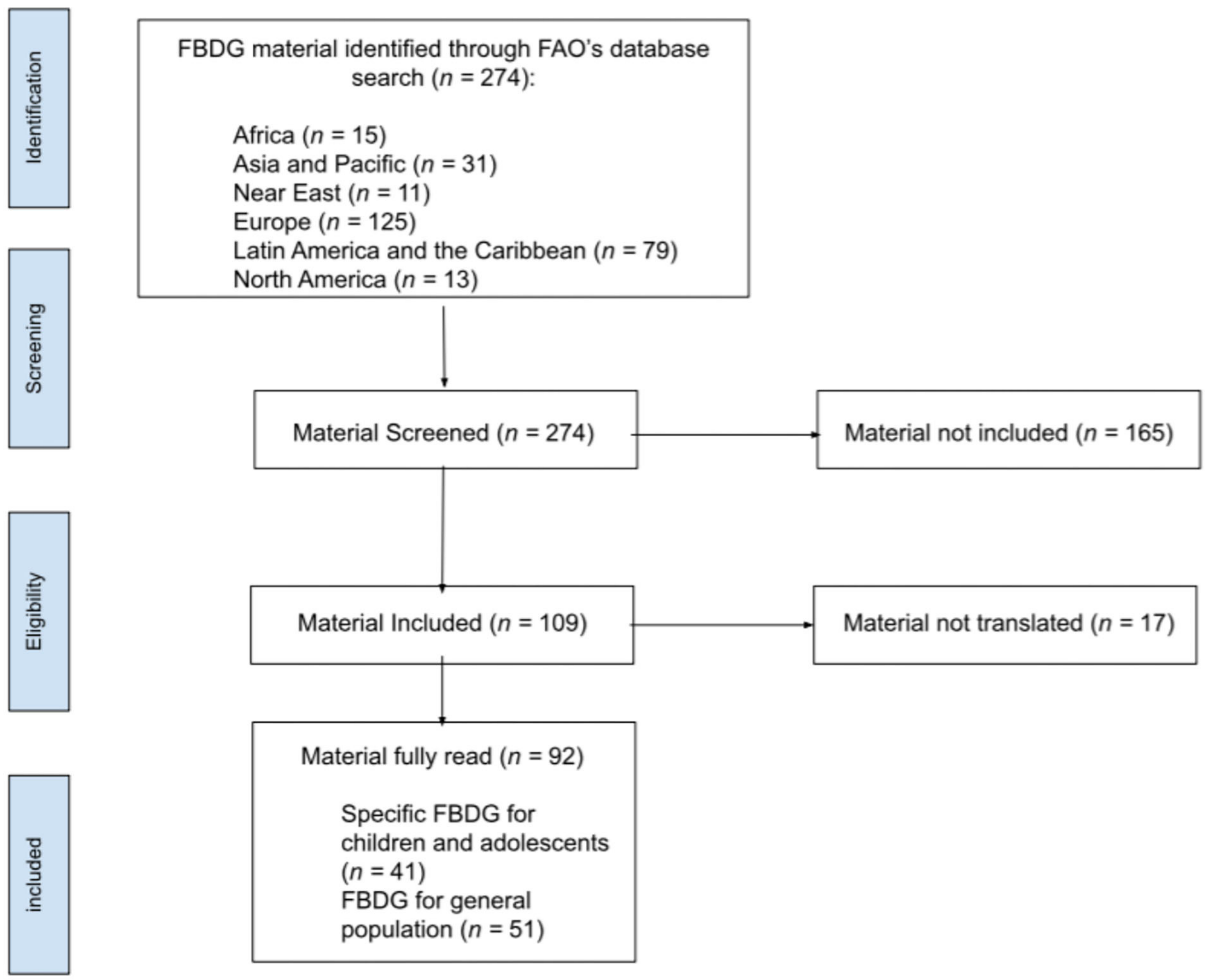

FIGURE 1
Flow diagram representing the selection process of material from FAO's database.

2017 and 19% (34–37, 71–74, 77, 80, 85, 87, 88, 93, 95, 96) were published between 2008 and 2012. The minority (11%) (38, 57, 61–63, 75, 83, 98–100) are dated before 2008, and none were published before 2000. The distribution of those FBDGs around the world is shown in Figure 3.

FBDG for the general population with information for children and adolescents

Forty-seven countries with general FBDG with specific information about the studied groups around the world were found: 15 in Latin America and the Caribbean (81–95), two in North America (103, 104), eight in Europe (58, 96–102), nine in Asia and the Pacific (66–75), nine in Africa (59–65, 105–108), and four in the Near East (76–80). Of those, only 12% (58, 69, 70, 85, 97, 102) have food icons directed at children and adolescents. Their main characteristics are summarized in Supplementary Table 1.

The intended age group most frequently addressed in the general FBDG corresponds to group 1 (13%) (63, 72, 74, 81, 83, 89, 108), followed by group 2 (4%) (61, 94). None presented guidelines exclusively for group 3. As for those materials which comprehend more than one group, the majority contains recommendations for groups 1, 2, and 3 (40%) (58, 60, 62, 64, 69, 71, 77–79, 86, 90, 93, 95–101, 104), 33% for groups 2 and 3 (59, 67, 68, 73, 75, 76, 80, 82, 84, 88, 91, 92, 102, 105–107), and 10% for 1 and 2 (65, 66, 70, 85, 87). About the intended audience, most of the FBDG (54%) were targeted at the general population (58, 59, 61–64, 67–71, 73, 75, 77, 80, 81, 83, 87–89, 91, 94, 95, 99, 100, 102, 108), 28% were directed to professionals of a certain field, mainly health (65, 66, 72, 76, 85, 86, 90, 92, 96–98, 101, 103, 104), and 18% were directed for both the general

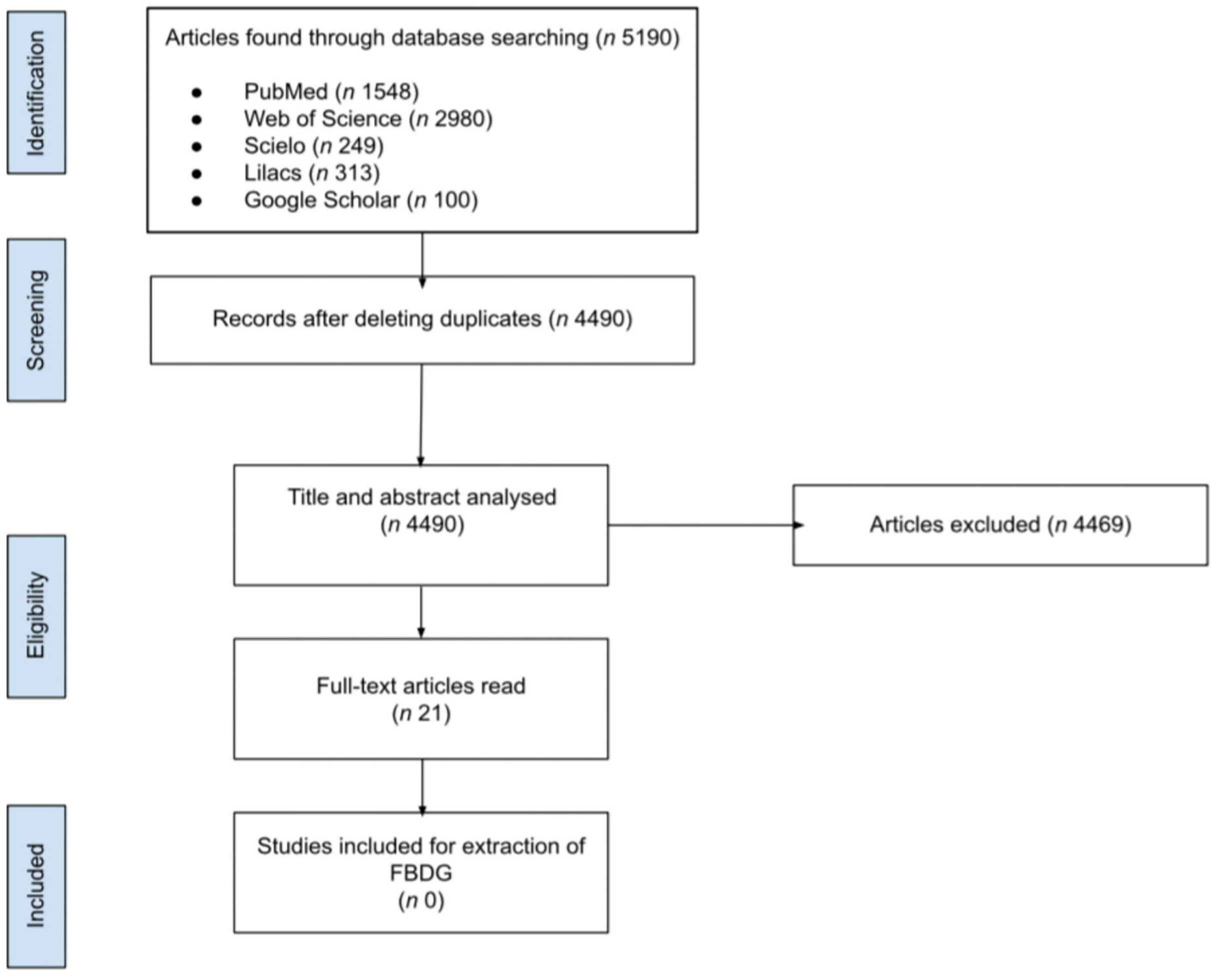

FIGURE 2
Flow diagram representing the selection process of articles for part 2 (systematic review) of the present study.

population and professionals (60, 74, 78, 79, 82, 84, 93, 105–107) (Supplementary Table 1).

Most of the documents (57%) (58–62, 64–66, 71, 73–75, 77, 80, 87, 88, 90, 91, 95–98, 100–102, 104–107) presented food groups directed for the target population of this study. Nineteen percent (60–62, 65, 69, 90, 95–98) have recommendations for mealtimes, directed at children and adolescents (Supplementary Table 1).

Specific FBDG for children and adolescents

Seventeen countries with specific FBDG for the studied group were found: 59% in Latin America and the Caribbean (25–44), 23% in Europe (45–57), and 18% in Asia and the Pacific (17–24). None was found in North America, Africa, and the Near East. From those 17 countries, 35% (23–26, 32, 33, 45–56) have specific icons for children and adolescents. The characteristics of those FBDGs for children and adolescents are summarized in Supplementary Table 2.

About half of the specific FBDG were aimed at group 1 (24%) (27, 34–38, 41–44), followed by group 2 (10%) (28–31) and group 3 (5%) (39, 40), and documents intended for more than one age group: 39% (17–22, 45–47, 51–57) corresponds to groups 1, 2, and 3, 12% (32, 33, 48–50) to groups 1 and 2, and 10% (23–26) to groups 2 and 3. Sixty-four percent (17–22, 25, 26, 28–31, 34–37, 41–50) were organized in guidelines and 32% (23–26, 28–31, 38–42) did not present recommendations for mealtimes. From the studied material that presents food groups, 85% (17–22, 27–38, 41–57) present at least four food groups: cereals or similar, animal protein sources, milk and dairy, and fruits and vegetables (Supplementary Table 2).

FIGURE 3
Distribution of FBDG with content targeted at children and adolescents around the world.

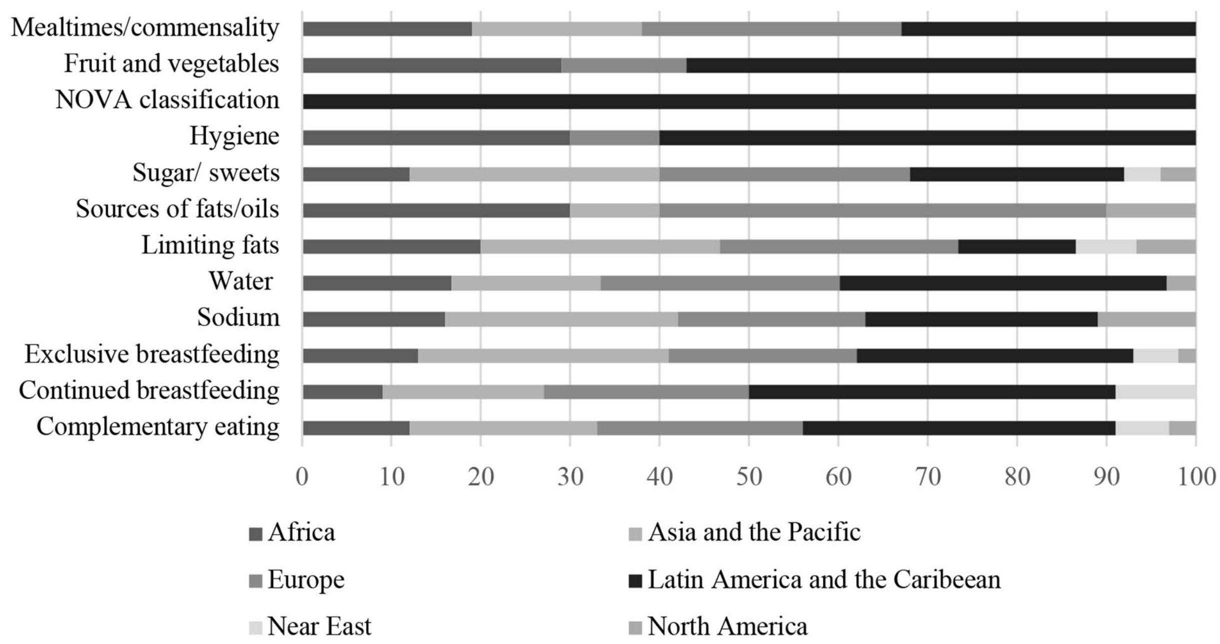

FIGURE 4
Percentage distribution of FGDBs with specific nutritional recommendations according to world regions.

Figures 4, 5 show the distribution of relevant specific recommendations found in the FBDGs, according to world regions and year of publication, respectively.

Figure 6 shows the distribution of important specific recommendations found in the FBDGs according to the type of document (general or specific for the studied age groups) and

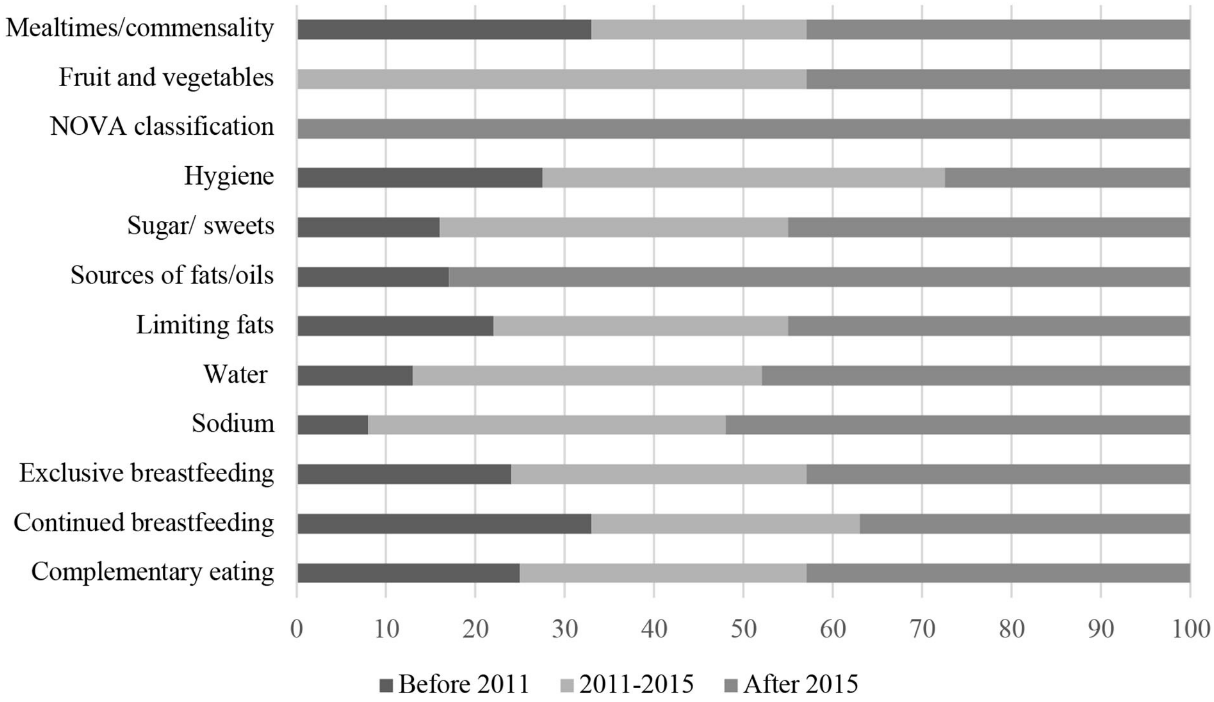

FIGURE 5
Percentage distribution of FGDBs with specific nutritional recommendations according to the year of publication.

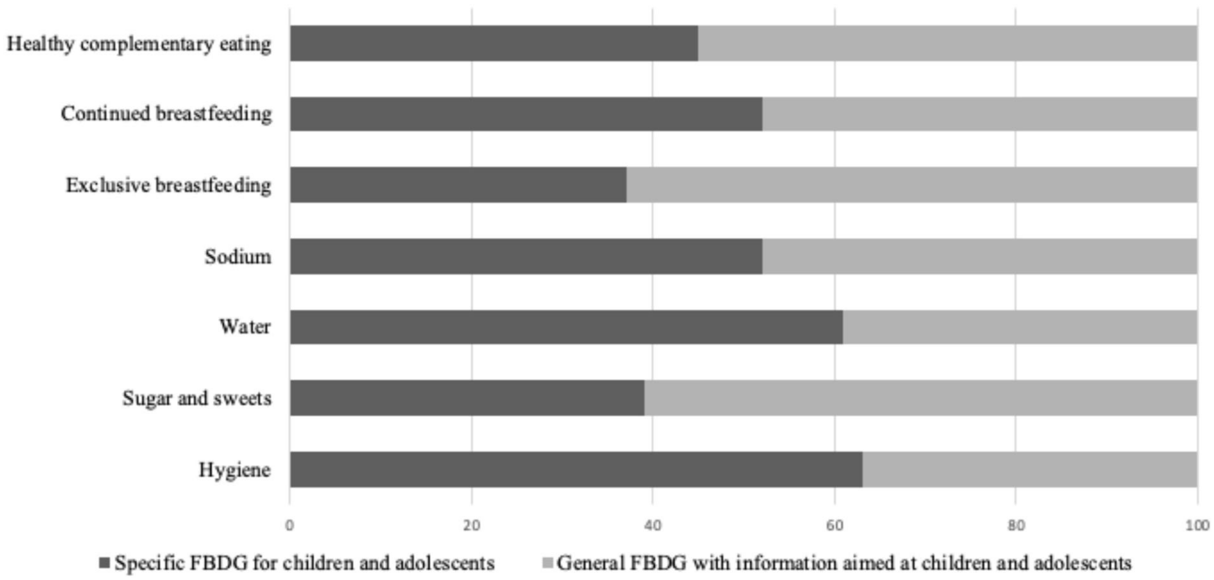

FIGURE 6
Percentage distribution of FBDGs with specific nutritional recommendations according to the type of document.

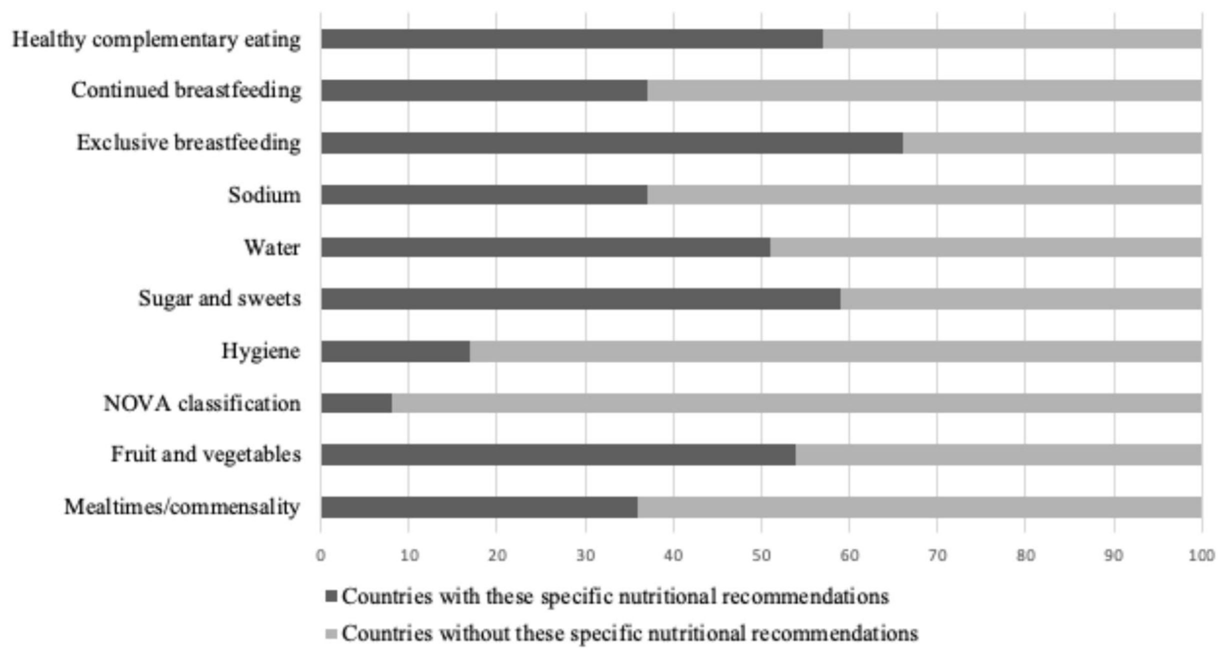

FIGURE 7
Percentage distribution of countries with specific nutritional recommendations in their FBDGs.

Figure 7 presents, in percentage, the number of countries with these specific recommendations in their FBDGs.

Recommendations for mealtimes and commensality

It was found 21 out of the 59 countries included guidance for eating habits in mealtimes, such as eating with the family, without using cellphones or television, in a calm environment (17, 21, 27, 32, 34, 37, 43, 45, 50, 53, 60–62, 65, 69, 90, 96–98). From these 21, 19 FBDG included guidance for eating habits in mealtimes for children and/or adolescents. There was no significant variation between specific and general documents on this topic (Supplementary Table 2).

Fruit and vegetables

Of the 27 countries, whose national FBDG had dietary guidelines aimed at children and adolescents, seven have an exclusive guideline for fruit and/or vegetable consumption (25, 26, 28–30, 49, 60, 65, 90), 67% of those documents were specific FBDG (25, 26, 28–30, 49). Also, 32 countries out of the 59 analyzed in this study bring some guidance about fruit consumption on the FBDG (17, 19–22, 25, 26, 28–31, 35, 48, 49, 57–60, 62, 64–67, 69, 71, 74, 75, 77, 80, 87, 90, 91, 96, 104–107), 43% were specific documents for children and adolescents (17, 19–22, 25, 26, 28–31, 35, 48, 49, 56, 57), and 26 encourage or guide vegetable consumption for the studied group (17, 20, 22–31, 35, 48, 57–60, 62, 64–66, 69, 71, 74, 75, 77, 90, 91, 96, 105–107), 45% were in specific FBDG for the target population of this study (17, 20, 22–31, 35, 48, 57).

NOVA classification

Five (8%) out of the 59 countries showed the NOVA food classification system to gather the foods in groups in the FBDG, according to the processing level (27, 84, 86, 87, 92, 94). These countries are all from Latin America: Brazil, Ecuador, El Salvador, Peru, and Uruguay. Of the six FBDGs, which included the NOVA classification, most of them (83%) were general FBDGs (83, 85, 86, 91, 93).

Sugar and sweets

Among the 35 countries in which FBDG includes sugar and sweets guidance for children and adolescents, 26 (74%) showed guidance to reduce or avoid its consumption (17–22, 27, 29, 38–41, 49, 52, 58, 60, 64, 65, 67, 70, 71, 74, 75, 80, 85, 96–98, 101, 104–107). Of the 33 FBDG that provided this information, about a third (39%) were specific documents for the studied group (17–22, 27, 29, 38–41, 49, 52).

Besides the recommendations for reducing or avoiding sugar consumption, 11 countries (32%) (25, 26, 32–35, 41–44, 58, 69, 71, 90, 91, 100, 101) brought guidance on the number of portions or grams of sugar per day, accordingly to age. As for the minimum age to be introduced to sweets, 20% (20–22, 41, 42, 48, 49, 58, 74, 97, 100) recommended 12 months old, 17% (17–19, 27, 60, 96, 101, 104), 24 months old, and 1 (65) country advised to not offer sugar to children under 3 years old. Some of the materials also correlated high sugar consumption with chronic diseases, such as diabetes, corresponding to 6% (86, 105–107) of the 35 countries. One country (45, 49) presented the recommendation to introduce sugar for 8 months old children.

Approximately, 15% (64, 67, 97, 104–107) of the countries recommended sugar intake below 10% of total daily calories, of those two (64, 105–107) countries limit sugar intake to 5% of daily.

More than half (65%) (17, 25, 28, 29, 31, 34, 35, 41, 42, 48, 49, 57, 60, 64, 65, 69, 71, 74, 75, 90, 91, 96, 97, 100, 101, 104–107) of the countries presented some information about sugar and sweets intake addressed for age group 2 (preschool-age and school-age children), 53% (18–20, 27, 41–45, 48, 49, 60, 64, 65, 71, 74, 96, 97, 100, 101, 104–107) for age group 1 (infants and children under 2 years old), and 44% (17, 26, 30, 31, 34, 35, 39, 40, 60, 75, 90, 91, 96–98, 100, 101, 104) for age group 3 (Adolescents).

Fats

From the 33 countries whose FBDG included fat and oils consumption guidance (17–22, 25–32, 34, 35, 37, 38, 43, 45, 49, 56–58, 60, 62, 64–66, 71, 74, 75, 78, 79, 88, 90, 91, 95–98, 100, 101, 104), 10 (30%) provided guidance on the best sources (22, 27, 45, 49, 60, 62, 64, 96, 97, 104), 15 (45.5%) highlighted the limit of fat consumption (17, 19, 21, 22, 49, 56, 60, 64, 65, 69, 75, 80, 85, 88, 98, 101, 104), and 6 (18.2%) guided good sources and limit the consumption of oils and fats (22, 49, 56, 60, 64, 104). Among the 44 FBDGs that provided fat and oils consumption guidance, 55% were specific documents for the studied group (17–22, 25–32, 34, 35, 37, 38, 43, 45, 49, 56, 57, 101).

Sodium

Among the 22 countries that the FBDG provides guidance on sodium consumption for the age group studied, 19 focus on limiting its consumption (17–22, 25–27, 33, 41, 45, 56, 58, 60, 64, 65, 71, 74, 75, 84, 95, 97, 103, 104). There were 25 FBDGs that included guidance for sodium consumption for children and/or adolescents, 52% of them were specific FBDGs for the studied group (17–22, 25–27, 33, 41, 45, 56).

About a third (36%) (58, 60, 64, 96, 97, 101, 103, 104) of these countries brought recommendations per age, limiting sodium intake and 23% (20–22, 32, 33, 41, 42, 58, 60) discourages its intake for children under 12 months old. Five countries (17, 60, 65, 74, 104) provide orientation in their FBDG about avoiding salty snacks and foods rich in sodium and one country (94) recommended avoiding having salt on the table at mealtimes.

As for the guidance, about half (45%) (26, 58, 60, 64, 74, 75, 96, 97, 101, 104) of the countries presented information addressed for age group 2 (preschool-age and school-age children), 45% (26, 58, 60, 64, 74, 75, 96, 97, 101, 103, 104) for age group 3 (Adolescents) and 36% (20–22, 27, 32, 33, 58, 60, 97, 101, 104) for age group 1 (infants and children under 2 years old).

Exclusive breastfeeding

It was found that 39 countries had exclusive breastfeeding until 6 months old guidance in their FBDGs (18–20, 27, 31, 33, 34, 36, 38, 41–43, 45, 49, 51, 52, 58, 60, 62–74, 78, 80, 81, 89, 90, 95–98, 100, 104, 108). Among the 43 FBDG that provided this information, 37% were specific documents for the studied group (18–20, 27, 31, 33, 34, 36, 38, 41–43, 45, 49, 51, 52). The majority (70%) (27, 34, 36, 38, 41–43) of specific FBDG aimed at group 1 presented guidance about exclusive breastfeeding.

Continued breastfeeding

Twenty-two countries have also continued breastfeeding orientation in their FBDG (18, 19, 27, 33, 34, 36–38, 41–43, 51–53, 58, 62, 64, 68, 72, 74, 78, 80, 90, 95–99). Of the 27 FBDGs that provided this information, 52% were specific documents for the studied group (18, 19, 27, 33, 34, 36–38, 41–43, 51–53). About 80% (27, 34, 36–38, 41–43) of specific FBDG directed to group 1 have this recommendation.

Healthy complementary eating

Thirty-four countries had FBDG that addressed guidance on healthy complementary eating (18–20, 27, 31, 33, 34, 37, 38, 41, 43, 45, 48, 51–53, 57, 58, 60, 62, 64–67, 69, 71, 72, 74, 78, 80, 88–90, 95–98, 100, 105). There were 38 documents that included guidance for healthy complementary eating, 45% of them were specific FBDG for the studied group (18–20, 27, 31, 33–35, 38, 41, 43, 45, 48, 51–53, 57). More than half (60%) (27, 34, 37, 38, 41, 43) of the materials specific to group 1 presented guidance on this subject.

Discussion

This article intends to fill a gap about the main characteristics of the current general and specific FBDGs around the world that bring guidance aimed at children and adolescents. A previous study (8) identified 17 specific FBDGs for children and adolescents around the world, so the growing elaboration of this type of material can be seen, given that the present study identified 41 FBDGs aimed specifically at this age group. This can be explained by the relevance that the food habits in childhood and adolescence have in dietary patterns through adulthood and in the child's development (6, 109, 110).

It can also be noticed that the regions that had the highest growth of countries with specific FBDGs since 2011 (8) were Latin America and the Caribbean, with 10 new specific FBDGs since 2011 (25–44). That growth is in line with the context of the double burden of malnutrition that Latin America and the Caribbean countries are experiencing. In this way, the focus of nutrition policies shifts from an undernourished population to a developing country with overweight and obese population (111, 112).

Even though there was an increase in the number of specific FBDGs aimed at children and adolescents around the world, it is not yet widespread in many countries, which is explicit in this study as most of the documents found were aimed at the general population. This shows a limitation to adapt the contents of the guidelines in order to make them understandable and motivational to the target population (113). Also, specific documents are important as each life stage has its singularities, such as nutritional needs and interests (114).

Age groups most cited in general FBDGs are schoolers and preschoolers. This is justified as most countries that have specific documents have those types of guides only aimed at children under 2 years old (20, 27, 32, 34–38, 41–45, 48, 51–53). Thus, it is clear that groups 2 and 3 are neglected, as they also need specific nutritional recommendations and the number of specific materials for each of them is fewer (18, 19, 21–26, 28–31, 33, 39, 40, 46, 47, 54–56).

When analyzing the results obtained by the study, it is possible to notice the great differences in the way of presenting nutritional guidance for the studied group, both in specific and general FBDGs. This information can be exposed in the documents through nutritional guidelines, icons, didactic design, food groups, and others. Those differences can be explained by the singularities of each region, the prevalence of nutritional inadequacies, and food culture, e.g., China's food icon being an abacus (69, 115).

Food icons or graphic representations are a tool for nutritional education, as they can represent quantities and even the frequency of consumption recommended for each food group in a succinct way (115). There are some differences between the shapes of food icons found. There is often a relation between the countries' cultures and the shapes chosen, as it seems to evoke the cultural food choices and some cultural food elements suggest cooking, besides the proportion between food groups (116). It is important to remember the relevance of culture in food choices since food practices such as cooking and having meals can be part of identity (117).

However, literature has suggested limitations when it comes to FBDGs' graphic representations, such as Food Pyramids and Plates, due to the attempt to summarize all choices consumers need to make in order to maintain healthy eating habits (118). Another important aspect is that the interpretation of such elements can demand subjective comprehension and the possible struggle of the population on comprehending abstract concepts (119).

About food groups, most of the specific FBDG presented a meat/animal source protein. When it comes to recommendations, developing countries with high undernutrition prevalence recommend red meat consumption in order to prevent anemia, especially among younger groups (9), such as Guatemala's FBDG (38). Others recommend altering between protein sources, which is an important recommendation and seems to be related to environmental sustainability concerns, such as Panama's document (41), Brazil's (27), China's (69), Belgium's (46, 47), and Australia's materials (17, 19), in which the recommendations were to consume other protein sources, such as lean meats and poultry, besides vegetable protein sources as legumes and nuts.

Another feature explored related to diet quality is commensality. Eating in the company of the family has proved to have a protective effect against obesity in children and adolescents, besides supporting healthy eating habits. This practice elevates the consumption of fruits and vegetables and several micronutrient intakes. It also decreases the risk of being overweight and obese in adolescents (120). Therefore, it is valuable that the FBDG present recommendations to endorse family meals as recommendations for mealtimes, both general and specific FBDG (17–22, 27, 36, 37, 45–56, 60, 65, 90, 95, 96).

Asia and the Pacific are the regions with most countries with FBDG that brought guidance on fruit (29%) (17, 19–22, 66, 67, 71, 74, 75) and vegetable (32%) (17, 20, 22–24, 66, 69, 71, 74, 75) consumption, which might demonstrate concern with the population's eating habits such as the growth rates of ultra-processed food consumption (121).

It is observed that Latin America is in a food transition, characterized by lower consumption of fruits and vegetables and with a high or growing participation of ultra-processed products in diet (108). This can justify why most countries with specific guidelines for fruit and/or vegetable consumption were in Latin America and the Caribbean (25, 26, 28–30, 90). It is also related to the nutritional transition presented in these countries, in which there are growing rates of overweight, obesity, and chronic diseases associated with weight gain (111). All of these FBDGs have been published after 2011, 43% after 2015, which is related to the fact that both food and nutritional transitions in Latin America are still considered recent processes.

All FBDG which use the NOVA food classification system were from Latin American countries (39, 40, 53, 54, 68, 70), which can be explained by the development of this tool being made in Brazil, by researchers of the Center for Epidemiological Research in Nutrition and Health of the University of São Paulo (12). Two recent systematic reviews (122, 123) have highlighted an association between high ultra-processed food intake and a variety of adverse health outcomes for adults, such as overweight, obesity, and different non-communicable chronic diseases, including cancer, hypertension, diabetes, and dyslipidemia. Among children and adolescents (123), the outcomes of high ultra-processed food consumption include cardio-metabolic risks and asthma; thus, there is already a body of evidence supporting the incorporation of the NOVA classification in dietary guidelines as a scientific concept to evaluate the "healthiness" of foods, including those directed to children and adolescents. Knowledge about the processing level of food is needed in order to design effective nutritional guidance to prevent chronic diseases and to promote adequate food production and distribution systems (111).

In developing countries, it can be noticed that food-related illness such as diarrhea has an expressive role in children's mortality rates (124). Also, a study showed that there is a substantial correlation between the Human Development Index (HDI) of a country and diarrhea-associated deaths among children (125). Thus, developing countries with lower HDI, such as those in Latin America (25, 26, 34, 37, 41, 43, 90) and Africa (61, 64, 65), represent almost all the countries with hygiene guidelines in the FBDG, to guide the population and prevent those diseases. Also, this explains the lack of a hygiene guideline in FBDGs in regions with developed countries with higher HDI, such as those from North America and Europe.

As most of the materials analyzed that brought orientations on topics such as hygiene and water consumption were specific FBDGs (17–31, 34, 38–41, 43, 45, 47, 49, 51, 52, 56, 57), it is also evident that specific documents can include more about topics indirectly related to food consumption. It is possible because there is space to embrace those topics in specific documents as it provides guidelines for a narrower target audience than general FBDGs.

By 1999, the United States Department of Agriculture (USDA) determined a limitation in the consumption of fats for children, which might explain no FBDG with such guidance before 2001. Also, there is an evident limitation of the FBDGs in relation to the consumption guidelines for the groups of fats and sugars, as this often occurs through recommendations of "moderate use" or "minimum quantity," which can lead to different interpretations of the amount that should or can be consumed (113).

The World Health Organization (WHO) recommendations for sugar intake are up to 5% of daily calorie intake (126), which was present in 6% (64, 105–107) of the countries that advised about sugar and sweets consumption for children. Dietary patterns rich in sugar can lead to oral caries, diabetes, and other non-communicable diseases (NCDs) (126); therefore, guidance on high-sugar foods and beverages should be present in FBDG (127).

Furthermore, the rising prevalence of obesity and non-communicable diseases in childhood and adolescence is concerning. According to WHO (127), some strategies to prevent obesity are related to limiting the consumption of foods and beverages high in fat, sugar, and salt by infants and young children. Guidance to avoid those foods and regulations on the marketing and sale of beverages and snacks of that category are measures that can be adopted by the government to prevent excess weight gain among children.

Guidance on sodium consumption is recent, which can be noticed as most of the FBDG analyzed with this orientation have been published after 2015 (21, 22, 27, 33, 41, 45, 56, 58, 60, 64, 97, 103, 104). This characteristic can be associated with the greater accumulation of scientific evidence about the harmful effects of excessive sodium consumption and the increasing salt or sodium consumption data among the studied age group (128). Guidance on sodium consumption for children and adolescents is necessary because of the growing pace of pathogenic processes of chronic diseases in these stages of life (129). Research demonstrates that 80% of Brazilian adolescents consume above the upper level of sodium and almost 10% of them have hypertension (128). Sodium consumption has an impact not only in the economic sphere, in relation to diseases associated with excessive consumption, but it is also related to premature death (130).

Yet, reducing salt intake is related to increasing population health, by preventing outcomes such as cardiovascular diseases, besides being a low-cost measure. On this path, it is essential to maintain the population's awareness of the necessity to reduce salt consumption, such as by not having salt shakers on the table at mealtimes, and avoiding high in sodium snacks and foods (131).

Among the 39 countries that presented exclusive breastfeeding guidance, 17 did not mention continued breastfeeding orientation in their FBDGs (20, 31, 45, 49, 61, 64, 66–68, 70–72, 74, 82, 90, 100, 104, 108), recommended by the WHO for the practice to be continued for up to 2 years old or longer (132). As known in the literature, breast milk contains all nutrients to promote the healthy growth and development of infants (133). Besides, a study has shown a negative correlation between breast milk intake with the consumption of ultra-processed food and sweetened beverages, being evident in this impact on childhood obesity and NCDs (134). It seems this type of orientation could reinforce the practice among breast feeders and health professionals; it is, therefore, suggested that updates in the FBDG take this into account.

The study has some limitations regarding the translation of some documents, which were left out such as those in Russian, Khemer, and Hebrew. Also, there might be FBDG not mentioned in the FAO; however, a systematic review was carried out to maximize the possibility of identifying them, but no additional material was found.

Conclusion

The present study summarized different countries' official recommendations for children and adolescents in order to compare and acknowledge the available content in this field. It was possible to notice the materials' diversity, due to both the nutritional and political aspects of each region. In this context, Latin America stands out for its orientations for the studied group. The relevance of understanding the tendencies around the world is to be aware of possible gaps, without putting aside the specificities of each population. This review did not aim to measure the possible impacts and comprehension of FBDGs, nor other subjective evaluations of the materials, which can be explored by further studies.

Author contributions

JC and MM: parts 1 and 2 of the study, writing, data extraction, and analysis. GM: data extraction, writing review, and advising/orientation. LS: writing review. NT: design of the study, writing review, and advising/orientation. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1033580/full#supplementary-material

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Poor adherence to iron-folic acid supplementation and associated factors among pregnant women who had at least four antenatal care in Ethiopia. A community-based cross-sectional study

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Background: In developing countries, including Ethiopia, maternal mortality is a major public health concern. The Ethiopian Demographic Health Survey (EDHS) reported that the maternal mortality ratio (MMR) was 420 per 100,000 live births in 2016. Iron-folic acid supplementation (IFAS) is a key intervention to reduce these deaths. Therefore, this study aimed to assess the magnitude of poor adherence to IFAS and associated factors among pregnant women who had at least four antenatal care in Ethiopia.

Methods: Secondary data analysis was used using 2016 Ethiopian Demographic and Health Survey (EDHS). We analyzed the data using Stata version 14. To identify factors associated with poor adherence to IFAS, a multilevel mixed-effect logistic regression model was fitted. Variables with a $p < 0.05$ in the multilevel mixed-effect logistic regression model were declared as significant factors associated with poor adherence to IFAS.

Result: The magnitude of poor adherence to IFAS was 82.87% (95% CI: 80.96–84.65). Women education; primary [adjusted odds ratio (AOR) = 0.48; 95% CI: 0.31–0.75] and secondary (AOR = 0.52; 95% CI: 0.29–0.96), husband education; primary (AOR = 0.56; 95% CI: 0.36–0.86) and secondary (AOR = 0.51; 95% CI: 0.29–0.95), and community media exposure (AOR = 0.47; 95% CI: 0.27–0.79) were significantly associated with poor adherence to IFAS.

Conclusion: In the current study, more than eight out of ten pregnant women who had at least four antenatal care had poor adherence to IFAS. Thus, promoting maternal and husband education and establishing community media with a priority on iron-folic acid supplementation and health-related programs are essential strategies to reduce poor adherence to IFAS.

KEYWORDS

adherence, associated factors, iron-folic acid, EDHS, Ethiopia

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Introduction

The nutritional status of the mother during pregnancy has a significant impact on the health, development, and wellbeing of the child (1). Anemia is associated with maternal and infant morbidity and mortality during pregnancy since iron stores decrease and iron requirements increase (2). Anemia affects 38.2% of pregnant mothers worldwide, with Africa contributing 44.6%. Iron deficiency contributes to half of all anemia cases (3). Furthermore, more than one-fifth of maternal mortality in Sub-Saharan Africa (SSA) is indirectly attributable to anemia (4).

Iron deficiency is the major cause of anemia globally, particularly in SSA (5). Both the mother and the fetus can suffer from iron deficiency anemia during pregnancy (6). Preterm delivery, spontaneous abortion, low birth weight, and fetal distress are associated with anemia (6, 7).

Globally, anemia reduction is the second nutritional goal for 2025 and is a key component of achieving women's and children's health. The goal is to reduce anemia in women of reproductive age by 50% (8). Iron-folic acid supplementation (IFAS) is one of the most important interventions to reduce anemia among pregnant women (3, 8). A daily supplement containing 60 mg of elemental iron with 400 micrograms of folic acid is recommended for pregnant women by the World Health Organization (WHO) for 6 months (9). IFAS should be continued for three months postpartum in areas with high anemia prevalence (9). Furthermore, Ethiopia's national guideline for preventing and controlling micronutrient deficiency recommends taking IFAS daily for 6 months during pregnancy and 3 months after delivery (10). The Ethiopian national nutrition program (NNP II) also set a key target to increase the number of women receiving iron-folic acid supplements for more than 90 days during pregnancy to 50% by 2024 and 90% by 2029 (11, 12).

The poor adherence to IFAS varies across different settings, for instance, in urban and rural areas of Tigray, Ethiopia was 62.8 and 71.1%, respectively (13), Northeast, Ethiopia 52.4% (14), Shire refugee camps, Northern Ethiopia 35.3 (15), Tikur Anbessa Specialized Hospital, Ethiopia 36.4% (16), systematic review and meta-analysis in Ethiopia 58.6% (17), Tanzania 79.7% (18), and SSA 71.3% (19). Some of the identified factors that can affect poor adherence to IFAS include the age of the women, previous anemia, educational status, wealth index, knowledge about anemia, receiving counseling about nutrition, and husband support (13, 15, 17, 19–21).

The magnitude of anemia has persistently increased despite many efforts to fight it over the past three decades, including free provision of IFAS of mothers and the promotion of ANC. This can be supported by the evidence of EDHS, which found that the magnitude of anemia among reproductive-age women increased from 17% in 2011 to 24% in 2016. Only 5% of reproductive age women adhere to IFAS during their most recent pregnancy (22). Furthermore, various studies have been conducted in Ethiopia about IFAS (6, 7, 23–25). However, the majority of these focused on the effects of IFAS in newborns and pregnant women and only a limited number of studies assessed the adherence to IFAS and associated factors among pregnant women in Ethiopia. Nevertheless, there was no adequate literature that answered the reason why pregnant women who attended the recommended ANC had poor adherence to IFAS in the country. Therefore, this study aimed to determine the magnitude of poor IFAS and tried to explore factors determining adherence to IFAS among pregnant women who had at least four ANC.

Methods

Study design, setting, and sampling procedure

This study used data from the most recent Ethiopia Demographic and Health Surveys (EDHS). EDHS is a nationally representative household survey conducted every 5 years in low- and middle-income countries (26). Using the women's recode (IR file) data set, we extracted independent and dependent variables. The dataset is freely available for download at: https://dhsprogram.com/data/available-datasets.cfm.

The EDHS employs two-stage stratified sampling technique. Which makes the data nationally representatives (7). A total weighted sample of 1,606 pregnant women within 5 years before the survey who had at least four antenatal care (ANC) were included in the study (Figure 1).

Study variables

Outcome variable

The outcome variable for this study was poor adherence to iron-folic acid supplementation. It was defined as not using iron-folic acid supplementation for ≥90 days during the pregnancy of the most recent birth. This was measured in the DHS data by the number of days when iron supplements (tablets) were taken as part of antenatal care. The poor adherence of <90 days threshold was chosen in accordance with previous studies (19, 27, 28).

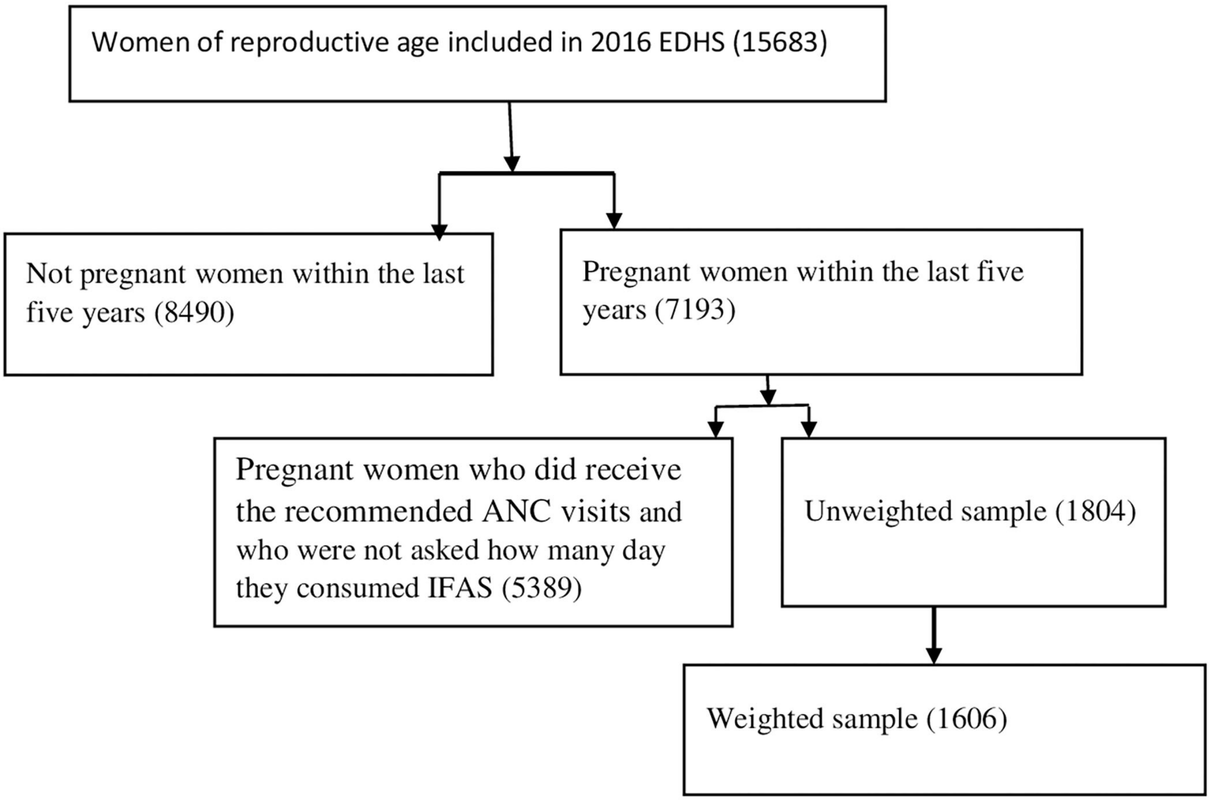

FIGURE 1
Data extraction procedures and sample size.

Independent variables

In this study, individual and community-level factors that are associated with poor adherence to IFAS were considered. Individual level factors considered in the analysis were age (15–24, 25–34, and 35–49), women education (no formal education, primary education, and secondary and above), husband education (no formal education, primary education, and secondary and above), occupation of the respondents (employed, non-employed), wealth index (poor, middle, and rich), nutrition counseling (yes, no), and religion (Orthodox, Muslim, protestant, and catholic). Regarding media exposure (yes, no), we coded yes if the women read newspaper, listened radio, or watched television for at least once a week, and no for otherwise (7).

Of the community level variables, region (small peripheral, large central, and metropolitan) and place of residences (rural, urban) were directly accessed from DHS data sets. However, community media exposure (low, high) and community-level education (low, high) were constructed by aggregating individual-level characteristics at the cluster level. They were categorized as high or low based on the distribution of the proportion values computed for each community after checking the distribution by using the histogram. The aggregate variable was not normally distributed, and the median value was used as a cut-off point for the categorization (29, 30).

Statistical analysis

Data analysis was performed using Stata version 14. Before data analysis, all frequency distributions were weighted (v005/1000000) to ensure that the DHS sample was representative and to obtain accurate estimates and standard errors. In order to account for the hierarchical nature of the DHS data, a multi-level logistic regression analysis was conducted. First, bivariable multilevel logistic regression analysis was conducted and those variables with a $p < 0.2$ were considered for multivariable analysis.

After selecting variables for multivariable analysis, four models were fitted; null model (Model 0) which shows the variations in the poor adherence to IFAS in the absence of any independent variables. Model I adjusted for the individual-level variables, Model II adjusted for the community level variables and Model III adjusted for both individual and community level variables. Simultaneously, Model fitness was done using the deviance since these models were nested (20). In addition, Variance inflation factors (VIFs) were

TABLE 1 Individual and community level factors associated with poor adherence to IFAS in Ethiopia.

Variables	Weighted frequency	Percentage (%)
Women age		
15–24	364	22.69
25–34	895	55.70
35–49	347	21.61
Women education		
No formal education	777	48.31
Primary education	517	32.21
Secondary education and above	313	19.49
Women occupation		
Employed	790	49.19
Unemployed	816	50.81
Husband education		
No formal education	538	36.06
Primary education	557	37.39
Secondary education and above	396	26.55
Wealth index		
Poor	481	29.96
Middle	276	17.17
Rich	849	52.87
Media exposure		
Yes	816	50.81
No	790	49.19
Religion		
Orthodox	873	54.37
Catholic	28	1.76
Muslim	309	19.25
Protestant	396	24.62
Region		
Small peripheral	57	3.56
Large central	1,415	88.07
Metropolitan	135	8.38
Resident		
Rural	1,180	73.49
Urban	426	26.51
Community media exposure		
Lower	983	61.2
Higher	623	38.80
Community-women education		
Lower	930	57.90
Higher	676	42.10

used to test for multicollinearity. Each independent variable had a VIF < 5, with a mean VIF of 1.84, indicating no significant multicollinearity. AORs were presented with a 95% confidence interval.

Results

Individual and community-level factors

A total weighted sample of 1,606 pregnant women were included in this analysis. The median age of the study participants was 28 years (IQR: 24–33) and 73.49% of the women were rural dwellers. Nearly half (48.31%) of the pregnant women had no formal education. Of the study participants, 49.19% were employed and 50.81 % had media exposure. The majority (88.07%) of the pregnant women were from large central regions. More than half (52.87%) of the pregnant women were from households with rich wealth quantiles (Table 1).

Magnitude of poor adherence to IFAS

The magnitude of poor adherence to IFAS in Ethiopia was 82.87% (95% CI: 80.96–84.65).

Factors associated with poor adherence to IFAS

The poor adherence to IFAS varied significantly across clusters. In the baseline model without an independent variable, 26.23 % of the variance in poor adherence to IFAS was explained by the variation in characteristics between clusters (ICC = 0.2623). In model 3, which included both individual and community level factors, the between-cluster variation, was reduced to 24.43%. Accordingly, the variance in IFAS adherence could be explained by differences in clusters. Model 3, which incorporated both individual and community-level factors, exhibited the best goodness of fit for predicting poor adherence to IFAS. The final model was selected because it has the lowest (1,250.90) deviance as compared with the other models (Table 2).

In the multilevel multivariable logistic regression model, both the individual and community level factors were fitted simultaneously. Thus, women education, husband education, and community media exposure were statistically associated with poor adherence to IFAS at 95% confidence level.

This study showed that pregnant women attended primary and secondary education and above were 52% (AOR = 0.48; 95% CI: 0.31–0.75) and 48% (AOR = 0.52; 95% CI: 0.29–0.96) less likely to have poor adherence to IFAS compared to

TABLE 2 Model comparison and random effect analysis result in Ethiopia.

Parameters	Null model	Model I	Model II	Model III
Variance	1.21	1.17	1.10	1.06
ICC (%)	26.23	25.06	26.16	24.43
PCV (%)	Ref	3.31	9.10	12.40
Log-likelihood	−696.30	−631.96	−687.75	−625.45
Deviance	1,392.60	1,263.92	1,375.50	1,250.90

women with no formal education, respectively. Women with husband education attended primary and secondary education and above were 44% (AOR = 0.56; 95% CI: 0.36–0.86) and 49% (AOR = 0.51; 95% CI: 0.29–0.95) less likely to have poor adherence to IFAS compared to their counterparts, respectively. The odds of poor adherence to IFAS in the community with high media exposure were 53% (AOR = 0.47; 95% CI: 0.27–0.79) low compared to their counterparts (Table 3).

Discussion

The aim of this study was to determine poor adherence to IFAS and identify associated factors among pregnant women who had at least four ANC. This study found that 82.87% (95% CI: 80.96–84.65) of pregnant women who had at least four ANC had poor adherence to IFAS. The result of the study found that women's education, husband's education, and community-level media exposure were identified as the factors associated with poor adherence to IFAS.

The current study is lower than a study conducted in Ethiopia 87.6% (7). The possible explanation might be the difference in the study population. The current study exclusively included pregnant women who received the recommended ANC, whereas the previous study included pregnant women who were asked how many days they consumed IFA tablets regardless of the number of ANC visits (7). Women who received the recommended ANC visits had better knowledge about anemia compared to those who did not receive the recommended ANC visits (31). Previous studies have documented that ANC visits and knowledge of anemia have a negative relationship with poor adherence to IFAS (7, 23–25, 32). However, this finding is higher than that of studies conducted in Ethiopia (23–25, 33–35), Tanzania 79.7% (18), and sub-Saharan African countries 71.3% (19). The possible explanation could be because of the difference in the study setting, the quality of service delivery in the health institutions, socio-demographic differences, and women's awareness of the importance of IFAS during pregnancy. For example, the previous studies done in Ethiopia were small-scale surveys compared to the EDHS survey, which was a national representative survey and included developing regions. Regarding socio-demographic variation, a previous study done in SSA reported that only 34.4% of the pregnant women had no formal education, which was lower than that of the current study (48.3%). Previous research has shown that women's education has a negative relationship with poor adherence to IFAS (21, 24, 36). Furthermore, the reason could also be the difference in access to health institutions and the availability of IFA in the nearby health facilities (7).

Pregnant women with primary education and secondary education and above were decreases poor adherence to IFAS by 52 and 48% as compared to those with no formal education, respectively. This result is in line with previous studies done in Ethiopia (24, 36), and Indonesia (37). Educated women are better informed about iron deficiency anemia and therapy, supplement benefits, and pregnancy in general. In addition, education may enhance awareness of micronutrient deficiency and ways to overcome it (7, 38).

Pregnant women with husband education who had formal education were less likely to have poor adherence to iron supplementation than those pregnant women with husbands who had no formal education. The finding is consistent with other study done in Ethiopia (39). This might be due to mothers with husband who were educated were autonomous on utilization of iron supplementation without the consent of their husbands.

Community media exposure is also negatively affected with poor adherence to IFAS. This study was supported by studies conducted in Ethiopia (40), and Asia (41). This is because different maternal and child health services including the importance of iron-folic acid supplementation during pregnancy are frequently given to the community through mass media. Therefore, pregnant women who are exposed to community media would have better understanding of the advantages of IFAS during pregnancy compared to those who do not have community media exposure (40).

Strengths and limitations

This study used nationally representative data sets, which were collected with a standardized and validated data collection tools. A multilevel analysis was used in this study to account for the hierarchical nature of the data. The cross-sectional nature of the survey does not show the causal relationship

TABLE 3 Multivariable multilevel logistic regression model results of poor adherence to IFAS in Ethiopia.

Variables	Null model	Model I (AOR, 95%CI)	Model II (AOR, 95%CI)	Model III (AOR, 95%CI)
Age of the respondent				
15–24		1		1
25–34		1.28 (1.0.85, 1.92)		1.33 (0.87, 2.02)
35–49		0.90 (0.53, 1.52)		0.92 (0.55, 1.56)
Women education				
No formal education		1		**1**
Primary education		0.52 (0.33, 0.78)		**0.48 (0.31, 0.75)***
Secondary education and above		0.53 (0.30, 0.93)		**0.52 (0.29, 0.96)***
Women occupation				
Employed		0.91 (0.65, 1.28)		0.94 (0.66, 1.32)
Unemployed		1		1
Husband education				
No formal education		1		**1**
Primary education		0.57 (0.37, 0.87)		**0.56 (0.36, 0.86)***
Secondary education and above		0.46 (0.27, 0.81)		**0.51 (0.29, 0.95)***
Wealth index				
Poor		1		1
Middle		1.05 (0.63, 1.76)		1.06 (0.63, 1.77)
Riche		1.49 (0.94, 2.35)		1.67 (0.89, 2.05)
Nutrition counseling				
Yes		0.86 (0.57, 1.31)		0.88 (0.58, 1.33)
No		1		1
Religion				
Orthodox		1		1
Muslim		1.18 (0.73, 1.91)		1.26 (0.78, 2.07)
Protestant		1.14 (0.69, 1.92)		0.96 (0.56, 1.61)
Catholic		1.5 (0.26, 8.79)		1.32 (0.22, 7.70)
Region				
Small peripheral			1	1
Large central			1.25 (0.56, 2.81)	1.33 (0.56, 3.13)
Metropolitan			0.82 (0.31, 2.17)	0.83 (0.30, 2.29)
Resident				
Rural			0.81 (0.43, 1.51)	0.87 (0.45, 1.72)
Urban			1	1
Community media exposure				
Lower			1	**1**
Higher			0.44 (0.26, 0.73)	**0.47 (0.27, 0.79)***
Community-women education				
Lower			0.98 (0.91, 1.07)	1
Higher			1.10 (0.68, 1.76)	1.63 (0.95, 2.72)

AOR, Adjusted Odds Ratio; PNC, Postnatal care; Model 1: adjusted for individual-level factors, Model 2: adjusted for community-level factors, Model 3: adjusted for both individual and community-level factors (full model), and *Statistically significant at $p < 0.05$ in the full model.
Bold value indicate statistical significant.

between outcome and independent variables. Furthermore, due to the use of secondary data, essential factors like knowledge of anemia, and socio-cultural factors were not available in the EDHS data set, hence, they were not included in the analyzed.

Conclusion

In the current study, more than eight out of ten pregnant women who had at least four antenatal care had poor adherence to iron-folic supplementation. Thus, promoting maternal and husband education and establishing community media with a priority on iron-folic acid supplementation and health-related programs are essential strategies to reduce poor adherence to iron-folic acid supplementation.

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 review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements.

Author contributions

DA conceived the idea, extract the data, data analysis, and draft the manuscript. WD, TB, and DB participant in the data analysis, interpretation, and revising of the manuscript. The final manuscript has been read and approved by all authors.

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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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The indirect effects of food insecurity on obesogenic environments

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Introduction: The Centers for Disease Control and Prevention (CDC) estimates 39.8% of United States (US) residents have obesity. This study examined obesity-related factors at the county-level to determine the indirect effects on physical inactivity, insufficient sleep duration, income inequality, food insecurity, on obesity rates.

Methodology: Using the 2018 Robert Wood Johnson Foundation (RWJF) County Health Rankings data set, a multiple regression analysis was conducted to measure the percentage of the obesity rate explained by physical inactivity, insufficient sleep duration, food insecurity, and income inequality *via* geographically weighted county means. RWJF combines US federal and state datasets to produce a composite dataset comprised of information primarily from adults over the age of 18 from the 3,143 counties found within US borders. The aggregate county-level data serves as the unit of measure ($N = 3{,}143$). The indirect relationships (the product of two direct relationships) between obesity-related variables and obesity were measured and illustrated through a path analysis model.

Results: This study found the combination of independent variables explained 53% of the obesity rates in the US, $R^2 = 0.53$, $p < 0.001$, two-tailed. This study also found that food insecurity has both a direct and indirect effect on obesity, physical inactivity, and insufficient sleep duration. Physical inactivity has a direct effect on obesity and insufficient sleep duration, along with an indirect effect on obesity. Insufficient sleep duration has a direct effect on obesity.

Conclusion: This analysis found that food insecurity indirectly impacts an obesogenic environment and drives county-level BMI averages. The dataset used for analysis predates the COVID-19 pandemic but presents the effect of food insecurity during a normative year. The findings, though interesting, provide an opportunity for future research.

KEYWORDS
food insecurity, obesity, physical inactivity, insufficient sleep, health inequities, nutrition

Introduction

Since the 1960's, obesity rates in the United States (US) have tripled from 13% in the mid-twentieth century (1) to a national high of 39.8% based on the Centers for Disease Control and Prevention (CDC) National Health Statistics Center data brief (2). It is estimated up to one-third of the world's population (∼1.46 billion) is obese (3) while two-thirds of Americans are either overweight or obese (4). The medical costs of obesity in the US were estimated to be $149.4 billion in 2014 which included the economic effect of direct costs, disability, and premature mortality (5).

Lower income adults are more likely to become obese due to working long hours, exercising less, suffering from insufficient sleep, and consuming fewer fruits and vegetables than their higher income counterparts (6). The relationship between income, physical inactivity, obesity, and poor diet (as a result of food insecurity) is not only present in minority-majority urban communities; it is found in majority white rural populations, as well. Low income minority populations are more likely to live near unhealthy food retailers increasing the possibility of consuming a poor diet. Understanding the food environment may provide avenues to reducing obesity in these areas. High levels of obesity were validated in areas with limited access to full-service supermarkets in food deserts (7). Food swamps, areas with high concentrations of junk food retailers, and food deserts appear in communities with higher levels of income inequality and obesity (8). Quick service restaurants and discount retail outlets in "food deserts", those economically depressed communities without access to a supermarket or healthy food outlet, serve as food outlets in lower income communities (9). The current literature involving the effect of food insecurity on obesity does not consider its effect on a combination of obesity-related community variables. Either food insecurity does or does not affect obesity directly but its effect is negligible in the studies examined for this analysis. This study primarily seeks to determine the direct and indirect effects food insecurity has on a community of variables commonly found in obesogenic environments.

CDC recognizes that the current community-level obesity reduction strategies should include additional variables and approaches to begin reducing the US obesity rate (10). RWJF has also recognized that broadening the social system variables contributing to obesogenic environments in research has the potential to more effectively develop community-level strategies to combat a variety of health disparities, including obesity. An obesogenic environment has been described as the "the sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations (11)."

Physical inactivity (12–14), insufficient sleep duration (15), food insecurity (16, 17), and income inequality (18, 19) have each been associated with obesity in the literature. Based on an ecological model of obesity, this study will employ multiple regression and path analysis to examine these obesity-related factors in individuals at the county-level to determine the effects of physical inactivity, insufficient sleep duration, income inequality, and food insecurity on US obesity rates, based on available data from the 2018 County Health Rankings and Roadmaps dataset. The primary focus of this study is to determine the direct and indirect effects of food insecurity on obesity rates. In addition, the results of the path analysis will reveal the estimated direct, indirect (the product of two direct effects), and synergistic effects of food insecurity and the additional independent variables on obesity county-level obesity rates. Further, existing efforts and suggestions to blunt factors found within the community environment that can create an obesogenic environment will be discussed.

Research questions

1. To what extent does the combination of insufficient sleep duration, physical inactivity, food insecurity, and income inequality influence obesity rates in the US?
2. In an obesogenic environment that includes insufficient sleep duration, physical inactivity, and levels of income inequality, what are the direct and indirect effects (the product of two direct effects) of food insecurity on obesity rates?

Methodology

Participants

This study employed the Robert Wood Johnson Foundation (RWJF) County Health Rankings and Roadmaps data set for the 2018 calendar year. Rather than surveying smaller participant populations through a primary data survey instrument, the RWJF County Health Rankings and Roadmaps data set is comprised of current data from more than 20 large federal and state cross-sectional surveys to provide geographically-weighted county averages offering an opportunity to measure the relationships among obesity and obesity-related aggravators on a national level (20). Obesity-related aggravators reviewed include physical inactivity, food insecurity, income inequality, and insufficient sleep duration, since these aggravators exist in nearly every county in the US. While the relationship between insufficient sleep and obesity is the subject of more current research, a noteworthy amount of US obesity research has focused on physical inactivity and obesity amongst the working poor. Food insecurity has been associated with obesity and the remaining independent variables separately but a cursory review of the literature did not reveal any prior research that included this combination of variables or considered the indirect effects on obesity created by the coexistence of these variables at the county-level. The unit of analysis for this study was 3,143 US counties, which is every county found within US borders. Per their terms of use, the data is available to use for personal, informational or non-commercial purposes (20).

Variables

Adult obesity data are the age-adjusted, county-level average derived from the CDC Diabetes Interactive Atlas. BMI calculated from the height and weight proportions reported by respondents 20 years of age or older (20, 21). BMI for less populated counties reflects a several year average for the weighted measure. Physical inactivity data are the county-level average of adults 20 years of age or older reporting no leisure-time physical activity during CDC Diabetes Interactive Atlas interviews (20, 22). Insufficient sleep is the 2016 Behavioral Risk Factor Surveillance System (BRFFS) self-reported county-level average of adults 18 years of age or older reporting less than seven hours of sleep per night (20, 23). Food insecurity is the county-level percent average of respondents 13 years of age or older who reported not having a reliable source of food during the last year. The information was originally compiled by Map the Meal Gap with 2015 data from the Census Population Survey (CPS), American Community Survey (ACS), and the Bureau of Labor Statistics (BLS) and made available at the county-level (20, 24). Income inequality data represents the self-reported household income averages between 2012–2016 collected by the US Census Bureau to produce the ACS 5-year estimates (20, 25).

Research design

The publicly-available secondary data set was downloaded over the internet from the RWJF County Health Rankings and Roadmaps website then stored as a Microsoft Excel spreadsheet on a password-protected personal computer. The variables included in this study were copied from the original Excel file to a second study-specific Excel spreadsheet for analysis. The reduced Excel spreadsheet was then exported to SPSS for data analysis *via* correlation analysis and multiple regression. The RWJF data set was then entered into SPSS AMOS to produce a path analysis model.

Data reliability/quality assurance

The reliability of the primary data used is one of the concerns when estimating values for relatively small areas like counties. Users should be aware that reliability can vary by place and by measure. An easy estimate of reliability is the error margin for a measure. Larger error margins suggest lower reliability (20). To forestall the issue of reliability, the measures used in this study had a CI of 95%. "Although the reliability of some Couthy Health Rankings' measures varies, when multiple measures are used to capture an underlying concept, reliability improves" (20). The food insecurity variable is comprised of multiple measures to improve reliability.

Data analysis

The variables were first reviewed *via* correlation analysis to determine significant relationships and preclude any concerns regarding multicollinearity. The variables exhibiting significant correlation with obesity were placed in the multiple regression matrix to determine the collective effect of physical inactivity, food insecurity, insufficient sleep, and income inequality on county-level obesity rates. In addition, the assumptions of multiple regression were validated. Path analysis can measure the R^2 found in multiple regression but does not contain the ability to examine whether the assumptions of regression, linearity, multivariate normality, homoskedasticity, no multicollinearity, and an independence of errors, were met. The RWJF data set was then imported into SPSS AMOS to illustrate both the direct effects among the independent and dependent variables and their indirect effects on obesity relationships to provide a window into future research. Path analysis is an extension of multiple regression, which utilizes the standardized beta weights found in a multiple regression output (26). Path analysis is measured through direct, indirect, and cumulative effects the independent variables have on obesity. Path analysis modeling allows the reader to quantify and envision the indirect effects of each variable on obesity. The visualization of relationships may provide avenues of further research into the indirect effects of food insecurity.

Results

The first research question seeks to examine the influence among the combination of physical inactivity, insufficient sleep duration, food insecurity, and income inequality on the variance in obesity rates in the US. The correlation analysis reveals that physical inactivity, insufficient sleep, food insecurity, and income inequality were all significantly correlated with obesity and each other ($p < 0.001$). When physical inactivity [r (3,143) = 0.71, $p < 0.001$, two-tailed], insufficient sleep duration [r (3,143) = 0.46, $p < 0.001$, two-tailed], food insecurity [r (3,143) = 0.40, $p < 0.001$, two-tailed], and income inequality [r (3,143) = 0.15, $p < 0.001$, two-tailed] increase, obesity rates rise. Following the correlation examination, a multiple regression model (Table 1) was examined to resolve the first research question and to establish whether these variables produced a good fit for path analysis modeling.

The regression model predicting obesity rates from physical inactivity, insufficient sleep duration, food insecurity, and income inequality was significant, F (4, 3,139) = 884.90, $p <$ 0.001, two-tailed. Specifically, 53% of the variance in obesity was explained by model; when adjusted for sample size and number of predictors (independent variables), the amount of variance remained unchanged at 53%. For every percent increase in physical inactivity, food insecurity, insufficient sleep duration, and increases obesity rate by 0.53, 0.13, 0.17%, respectively. Every percent increase in income inequality reduces obesity by 0.07%.

To satisfy the remaining research question, a path analysis model (Figure 1) was created to illustrate and explain the direct (Table 2) and indirect (Table 3) effects the independent variables have on obesity rates. Table 2 displays the direct effects for each variable. This model has a good fit, NFI = 0.91, IFI = 1.0, and CFI = 1.0. Figure 1 indicates food insecurity has a direct effect on obesity (β = 0.11), a direct effect on physical inactivity (β = 0.44), and a direct effect on insufficient sleep duration (β = 0.36). Physical inactivity has a direct effect on obesity (β = 0.61) and a direct effect on insufficient sleep duration (β = 0.28). Insufficient sleep duration has a direct effect on obesity (β = 0.15). Income inequality has a direct effect on obesity (β = −0.11), a direct effect on insufficient sleep (β = 0.18), and a direct effect on food insecurity (β = 0.55).

Figure 1 displays only direct effects. Indirect effects are calculated by multiplying two direct effects. Table 3 indicates food insecurity has an indirect effect on obesity (β = 0.29), an indirect effect on physical inactivity (β = −0.02), and an indirect effect on insufficient sleep duration (β = 0.22). Physical inactivity has an indirect effect on obesity (β = 0.04). The variable with the most outsized indirect influence on obesity in this obesogenic environment is food insecurity.

Physical inactivity remains a primary predictor of obesity within this environment of variables but the indirect effects of physical inactivity on obesity were negligible resulting in little change in the cumulative effect of physical inactivity on obesity. Food insecurity has a small direct effect on obesity (β = 0.11) in this environment but the inclusion of indirect effects (β = 0.29) advances the influence of food insecurity to a medium-sized cumulative effect (β = 0.40) on obesity.

TABLE 1 Regression analysis summary for variables predicting obesity.

Variable	B	$^{+}$SEB	β
Physical inactivity	0.53	0.01	0.61**
Food insecurity	0.13	0.02	0.11**
Insufficient sleep	0.17	0.02	0.15**
Income inequality	−0.07	0.00	−0.11**

N = 3143. B is the unstandardized coefficient.
$^{+}$Standard Error of B.
β is the standardized coefficient.
$^{**}p < 0.001$.

Discussion

This study found that food insecurity not only directly affects community obesity rates; it indirectly affects them, as well. This

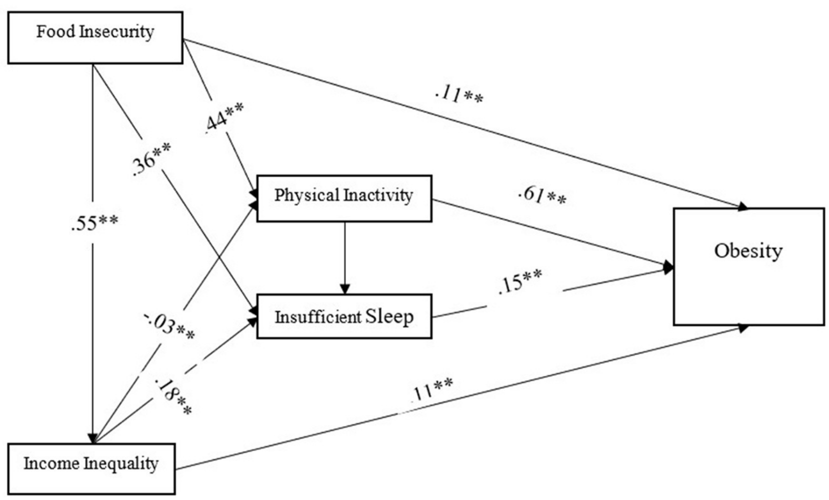

FIGURE 1
Path analysis model. N = 3143. $^{**}p < 0.001$, two-tailed.

TABLE 2 Path analysis direct effects.

Variable	Physical inactivity	Insufficient sleep	Food insecurity	Income inequality	Obesity
Physical inactivity	–	–	–	–	–
Insufficient sleep duration	0.28**	–	–	–	–
Food insecurity	0.44**	0.36**	–	–	–
Income inequality	−0.03**	0.18**	0.55**	–	–
Obesity	0.61**	0.15**	0.11**	−0.11**	–

** $p < 0.001$, two-tailed.

TABLE 3 Path analysis indirect effects.

Variable	Physical inactivity	Insufficient sleep	Food insecurity	Income inequality	Obesity
Physical inactivity	–	–	–	–	–
Insufficient sleep duration	–	–	–	–	–
Food insecurity	−0.02**	0.22**	–	–	–
Income inequality	–	0.01**	–	–	–
Obesity	0.04**	–	0.29**	0.01**	–

** $p < 0.001$, two-tailed.

study differs from the current food security literature by focusing on the indirect effect food insecurity has on common, obesity-related variables. A cursory review of the current literature did not reveal articles that appear to have connected these four phenomena to show that there appears to be causal variation. The studies reviewed for this analysis focused primarily on the impact food insecurity, as a consequence of location, has on obesity rates. Low-income households, those without adequate transportation, those in rural areas, those in food deserts, and those living in areas populated by dollar stores are often food insecure and obese (27). Additionally, rural infrastructure often does not support mass grocery deliveries, furthering limiting access to healthy foods (28). When measuring the effect of discount retailers on obesity in Kings County Washington, census tracts measures of obesity ranged from 5% in higher income communities to 30% in low-income communities. The lower-income areas included disproportionate saturation of discount retailers compared with full-service supermarkets in communities with higher mean income (16). Food environment alone, however, is not the only indicator of both the obese and food insecure. High levels obesity were validated in areas with limited access to full-service supermarkets (food deserts) but higher income neighborhood residents have higher overweight status (29). The presence of overweight suburbanites in higher income communities would suggest eating behaviors are more responsible for weight status than simply plotting supermarkets numbers and locations.

None of the studies reviewed, however, considered the indirect effect food insecurity has on other modifiable health behaviors in an obesogenic environment. Poverty seems to drive the relationships between food insecurity and both physical inactivity and sleep duration. The food insecure often rely on a combination of sources to satisfy their appetite, regardless of nutritive content. An inadequate nutrient balance can affect their ability to engage in regular physical activity and has been associated with sleep deprivation in adolescents (16).

Implications for providers

Providers could begin to address food insecurity experienced by their patients by including specific questions regarding food security in patient intake forms, including it in a patient's history, and discuss, when applicable, during an appointment. Providing patients with budget-friendly resources, nutrition education, and support could begin to reduce the effects of food insecurity. Offering courses in budgeting, food preparation and storage, and menu planning would allow patients of limited means the knowledge essential to feel more food secure. Smartphone applications, text messaging, and internet resources can also promote nutrition education, food preparation education, and provide resources to improve nutrition in the income insecure, as well.

As an example of the resources providers could share, the Health Bucks program promoted in New York City provided nutrition education through smartphone applications, online, through live cooking demonstrations, and was available through participating food vendors throughout the city. The Health Bucks program, supported by the Healthy Food Initiative, provided a $2 match for every $5 worth of SNAP benefits used to purchase produce from neighborhood farmer's markets (20). The program provided supporting educational materials at small food vendor locations in neighborhoods considered "food deserts" (30).

Implications for county-level public health governance

There are a variety of opportunities for county-level Public Health governance to improve food insecurity and physical activity in communities with higher levels of obesity and income inequality. Some of these options include: regulating nutritional content on restaurant menus, requiring small vendors carry a limited amount of fresh or frozen fruits and vegetables, and offering tax incentives to the owners of vacant lots to allow transient, seasonal use, venues for community gardens and farmer's markets. County or state initiatives have the opportunity to improve mass transit or ride share initiatives to reduce food insecurity in low income communities with low levels of car ownership (31). Ride share initiatives would allow low-income community members the ability to travel to full-service supermarkets. In addition, blighted vacant lots could be seized by local governments to be repurposed for community gardens, farmers markets, and school gardens. Cities which seize vacant lots for delinquent taxation have the opportunity to encourage community members to engage in collective gardening to reduce food insecurity while increasing physical activity (32).

In addition, county-level governance should encourage the participation of local schools and businesses to develop multi-component obesity remission programs. Similarly, The SHAPE UP program conducted in Somerville, MA presents another multi-component program to improve nutritional education, promote physical activity, and reduce obesity by pairing local schoolchildren with their parents to reduce the child's BMI. By involving the parent in a program designed to reduce their children's BMI, the parent would also experience a reduced BMI. In fact, parental BMI was reduced by.411 points overall (33). Through this program, education regarding food selection was printed on the menus of local restaurants, nutrition was promoted through local mass media and social media, and feedback regarding the child's progress was sent home from school with the day's healthy recipe and nutrition education of the day. This nutritional education is particularly necessary in low-income communities. Community members with less than a college degree who earn <\$15,000 per year are more likely to misinterpret the nutritional value of a variety of foods or fully understand the effects of a diet replete with processed foods (34). Though these educational efforts are valuable, they should be accompanied by policy changes to improve the opportunities to reduce obesity by increasing physical activity, reducing food insecurity, and reducing income inequality at the community level.

Limitations

This study is the product of a variety of national surveys joined at the geographic (county) level. The national surveys are the product of self-reported data and could have been improved by the use of activity diaries to catalog daily physical activity time and sleep duration. The RWJF dataset averages BMI data from several years and several sources to improve reliability but could have been improved by using objective measures of BMI exclusively rather than including self-reported height and weight. There are a variety of factors not included in this analysis which could explain the remaining 47% of the variance in obesity rates including: status and perceptions of the built environment, increased screen-time activities, perceptions and objective measures of neighborhood crime, access to exercise, insurance access and affordability, access to medical facilities, health and basic literacy, access to transportation, housing, and access to fresh food.

Conclusions

This analysis found that food insecurity directly and indirectly impacts an obesogenic environment and may influence county-level BMI averages. The dataset used for analysis predates the COVID-19 pandemic but presents the effect of food insecurity during a normative year. The findings, though interesting, provide an opportunity for future research. Enhanced unemployment benefits during the COVID-19 pandemic reduced food insecurity by 30% and demonstrated a 42% decline in eating less (35). A comparison between the current findings and data retrieved during 2020 and/or 2021 could provide support for the indirect relationship between food insecurity and obesity.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.countyhealthrankings.org/explore-health-rankings/rankings-data-documentation/national-data-documentation-2010-2019.

Ethics statement

The studies involving human participants were reviewed and approved by Indiana State University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

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

Conflict of interest

The author declares 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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Nutrient combinations exhibit universal antianxiety, antioxidant, neuro-protecting, and memory-improving activities

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Anxiety disorders are the most common mental disorders and, without proper treatment, may lead to severe conditions: e.g., somatic disorders or permanent damage to central nervous system. Although there are drugs in clinical trials, this study focuses on exploring the efficacy of nutrients in treating these diseases. We built different zebrafish models and screened several nutrient combinations for their antianxiety, antioxidant, neuro-protecting, and memory-improving activities. Our results showed that the combinations of nutrients (e.g., Walnut Peptides + Theanine at 14.2 + 33.3 μg/ml) have similar or better activities than the positive control drugs. In addition, we discovered that the effects of the nutrients in the above four aspects were universal and highly related. This study is noteworthy as it suggested that nutrients could be healthier and greener drug alternatives and provide similar or better universal treatments for anxiety and related conditions.

KEYWORDS
nutrients, zebrafish, antianxiety, antioxidant, neuroprotection, memory-improving

1. Introduction

Anxiety disorders are the most common mental disorders that generally begin before or during early adulthood. Major characteristics of anxiety disorder include excessive fear and anxiety about threats or their prevention. In this condition, the brain circuits that respond to danger are dysfunctional. Anxiety disorders are influenced by genetic, environmental factors and epigenetic interactions. It is also common for anxiety disorders to be comorbid with other somatic disorders and mental disorders (e.g., depression) (1). Unlike the state of anxiety, anxiety disorders impair individuals' daily life performance, resulting in high costs of public health care worldwide (2). According to the ICD-10 classification, there are eight types of anxiety disorders. Among them, specific (isolated) phobias are the most common anxiety disorders, with a 12-month prevalence of 10.3%, followed by social anxiety disorder (SAD, also called social phobia; 2.7%) and general anxiety disorder (GAD; 2.2%) (3, 4). While there continues to be extensive research on treating other mental disorders (e.g., depression and schizophrenia) (5), new treatments for anxiety disorders are still needed.

In addition, anxiety disorders are known to be associated with brain damage (6). Effective brain-protective strategies are thus needed during the disease's progression. Up to now, no specific drug is available to remission the symptoms of these diseases. Nevertheless, nutritional samples supplemented with food extracts were reported to have such effects recently (7). In addition, studies have shown that nutrients can often outperform drugs in achieving health goals as they do without any side effects, with which prescription drugs are often riddled (8). Recently, the use of nutrients such as tryptophan, vitamin B6, B12, folic acid, and glutamic acid has drawn much attention in the research of anti-depression and antianxiety (7, 9). Despite this, identifying more nutrients for their antianxiety and neuroprotective activities is still needed and requires low-cost and high-throughput screening techniques (9).

In this study, we built anxiety, oxidative stress (OS), nervous system injury (NSI), and Alzheimer's disease (AD) zebrafish models. With these models, we investigated several nutrients and combinations for their antianxiety, antioxidant, neuro-protecting and memory-improving capabilities. Our results demonstrated that using nutrient combinations (e.g., Walnut Peptides + Theanine at 14.2 + 33.3 μg/ml) as healthier and greener drug alternatives could provide similar or better universal treatments for anxiety and related conditions.

2. Materials and methods

2.1. Experimental model animals

In this study, wild-type AB strain zebrafish were bred in natural pairs. Zebrafish aged four days post-fertilization (dpf) were chosen for antianxiety activities and memory-improving [responsiveness and acetylcholinesterase (AChE) production] evaluation. Zebrafish aged one dpf were used to evaluate the samples' protective abilities to the central nervous system (CNS). Translucence melanin allele of Albino-type zebrafish bred in natural pairs aged three dpf were used to evaluate the antioxidant efficacy.

All zebrafish were raised in 28°C fish farming water (water quality: 200 mg of instant sea salt per 1 L reverse osmosis water, conductivity: 450–550 μS/cm; pH: 6.5–8.5; hardness: 50–100 mg/L $CaCO_3$) as culture, license number of experimental animals used: SYXK (Zhejiang) 2022-0004. The feeding practice was carried out following the requirements of the international AAALAC certification (certification number: 001458).

2.2. Model establishment and grouping

The anxiety zebrafish model was established by adding 1-(3-chlorophenyl) piperazine hydrochloride (mCPP, 20.0 μg/mL, 1 h incubation at 28°C and washed off) (10, 11). After that, in the positive drug group, Selegiline was added (20.0 μg/ml, 24 h incubation at 28°C). The OS model was established by adding menadione (2.25 μM, 22 h incubation at 28°C) to the culture. In the positive drug group, N-Acetyl-L-cysteine (NAC) was also added (62.5 μg/ml, 22 h incubation at 28°C). The zebrafish NSI model was established by adding Mycophenolate mofetil (0.4 μM, 48 h incubation at 28°C) to the culture. In the positive drug group, Glutathione was also added (615 μg/ml, 48 h incubation at 28°C). The zebrafish AD model was established by adding aluminum chloride (140 μM, 24 h incubation at 28°C) to the culture. Donepezil hydrochloride was also added to the positive drug group (3.33 μg/ml, 24 h incubation at 28°C). The nutrients in each test were incubated under the same condition as their respective positive drug group. The control (blank control, culture-only) was set up simultaneously with the other groups.

2.3. Sample information

Four positive drugs were used in this experiment: Selegiline, NAC, Glutathione, and Donepezil hydrochloride are commercially available synthetic chemicals. The nutrients, including Stachyose, Lactic acid bacteria-*Lactobacillus Plantarum* (PS128), Walnut Peptide (WP), Desert Cistanche (DC), and Theanine (Th) were all plant extracts. Casein Peptides (CP) were originated from milk proteins. All the nutrients are commercially available. Their combinations were nutrient samples with determined concentrations. The names and concentrations of the positive drugs, individual nutrients and combinations tested were summarized in **Table 1**. Their doses in zebrafish and the estimated doses in humans were also listed.

2.4. Zebrafish behavioral test

In this test, four dpf wild-type AB zebrafish were randomly selected and raised in the six-well plates with 30 fish per well (for

TABLE 1 The names, origins and doses of the samples tested.

Group	Name	Origin	Human dose (estimated)	Zebrafish dose (μg/ml)
Positive drugs	Selegiline	Chemical substance (Orion Corporation, Finland)	N.A.	20
	N-acetyl-L-cysteine (NAC)	Chemical substance (Shanghai Aladdin Biochemical Technology Co., Ltd)	N.A.	62.5
	Glutathione	Chemical substance (Sigma)	N.A.	615
	Donepezil hydrochloride	Chemical substance (Eisai China Inc.)	N.A.	3.33
Nutrients	Stachyose	The plant extract, a galactose derivative of sucrose (Xi'an APP-Chem (Tech) Co., Ltd)	3 g/day	500
	Lactic acid bacteria-*Lactobacillus Plantarum* (PS 128)	Traditional fermented mustard products of Taiwan (Asian Probiotics and Prebiotics corporation)	1.8×10^{12} CFU/g	1,000
	Walnut peptides (WP)	The plant extract, bioactive peptide extracted from the protein of walnut residues (Sinphar group Co.)	0.75 g/day	125
	Desert cistanche (DC)	The plant extract, tonic traditional Chinese medicine (Sinphar group Co.)	0.75 g/day	125
	Casein peptides (CP)	Milk (Guangdong Huapeptides Biotechnology Co., Ltd.)	0.6 g/day	100
	Theanine (Th)	The natural amino acid in green tea (Shanghai Novanat Co., Ltd)	400 mg/day	66.6
Combinations	Stachyose + PS128	N.A.	0.5 g/day + 9×10^{11} CFU/g	83.3 + 500
			1.5 g/day + 9×10^{11} CFU/g	250 + 500
			3 g/day + 9×10^{11} CFU/g	500 + 500
	WP + DC	N.A.	0.375 + 0.375 g/day	62.5 + 62.5
			0.1875 + 0.562 g/day	31.2 + 93.7
			0.562 + 0.1875 g/day	93.7 + 31.2
	WP + CP	N.A.	0.085 + 0.15 g/day	14.2 + 25
			0.17 + 0.3 g/day	28.4 + 50
			0.34 + 0.6 g/day	56.7 + 100
	WP + Th	N.A.	85 + 200 mg/day	14.2 + 33.3
			170 + 400 mg/day	28.4 + 66.6

N.A, not applicable, CFU, colony-forming unit.

all groups). After treatments, all zebrafish were transferred into a 96-well plate (one tail/well) and 200 μl of culture solution was added to each well. A behavioral analyzer was then used to detect the total moving distance of zebrafish for further analysis.

2.5. Cortisol production measurement

In this test, the wild-type AB strain zebrafish of four dpf were randomly selected and raised in the six-well plate, with 30 zebrafish per well (for all groups). After treatments, the cortisol concentration of zebrafish (three replicates per treatment) was measured using the enzyme-linked immunosorbent assay (ELISA kit, **Supplementary Table 1**: Instruments and consumables). The data were then collected and analyzed.

2.6. Antioxidant effect evaluation

The translucent albino zebrafish with three dpf melanin allele mutations were randomly selected and raised in six-well plates. Thirty zebrafish were kept in each well (for all groups). After treatments, zebrafish were transferred into 24-well plates and stained with specific reactive oxygen species (ROS) fluorescent dye. After staining, 10 zebrafish were randomly selected from each test group and placed under a fluorescence microscope for imaging. Nis-elements D 3.20

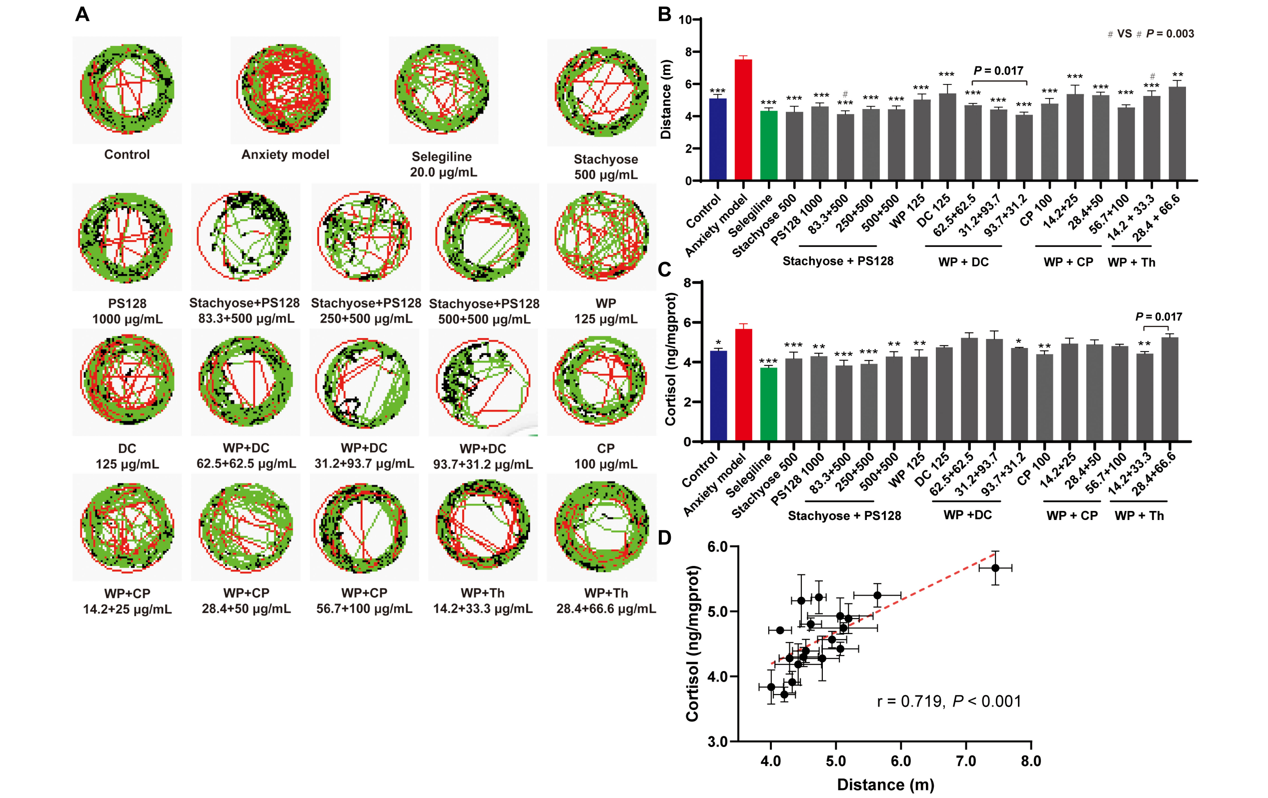


FIGURE 1
Antianxiety effects of nutrient combinations. **(A)** Representative trace map of the zebrafish behavioral tests (n = 10). The moving speed <4, 4–20, and >20 mm/s were marked in green, black, and red lines, respectively. **(B)** Moving distance of zebrafish in each group (n = 10). **(C)** Cortisol production level of zebrafish in each group (zebrafish in three wells were combined and measured as one sample, n = 3). **(D)** The cortisol production level of zebrafish in each group was plotted against the distance measured in the behavioral test. n = 3. Data in panels **(B–D)** were presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 compared with anxiety model. In panel **(B)**, Stachyose + PS128 (83.3 + 500 μg/ml) **(#)** vs. WP + Th (14.2 + 33.3 μg/ml) (#), P = 0.003. Data were collected from three biological replicates.

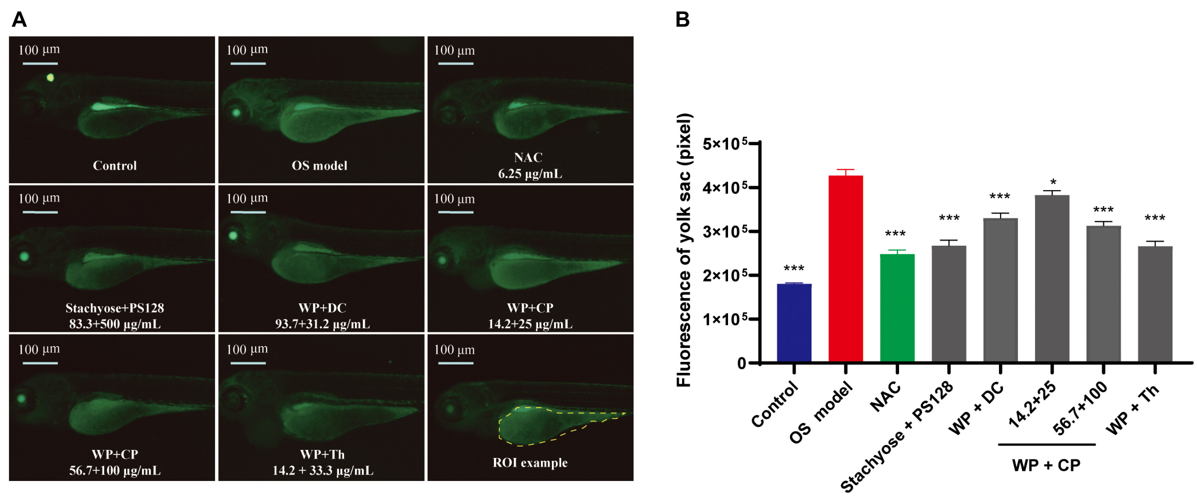


FIGURE 2
Antioxidant effects of nutrient combinations. **(A)** The representative images of yolk sac fluorescence in each group. **(B)** The fluorescence intensity of the yolk sac in each group (n = 10). Data were presented as mean ± SEM. *P < 0.05, ***P < 0.001 compared with OS model. Data were collected from three biological replicates.

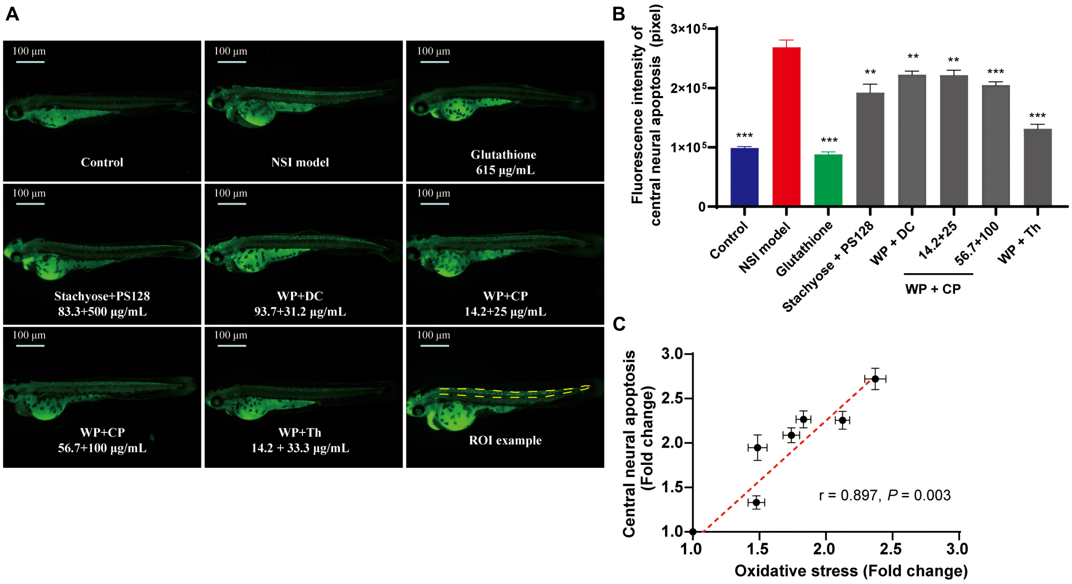


FIGURE 3

Central neuroprotective effect of nutrient combinations. **(A)** The representative fluorescence images of central neural apoptosis in zebrafish. **(B)** The quantification of fluorescence intensity of central neural apoptosis in each group. n = 10, $^{**}P$ < 0.01, $^{***}P$ < 0.001. **(C)** The oxidative stress (from Figure 2B, value was normalized to the control) was plotted against the central neural apoptosis level (normalized to the control), (r = 0.897, P = 0.003). Data in panels **(B,C)** were presented as mean ± SEM. $^{**}P$ < 0.01, $^{***}P$ < 0.001 compared with NSI model. Data were collected from three biological replicates.

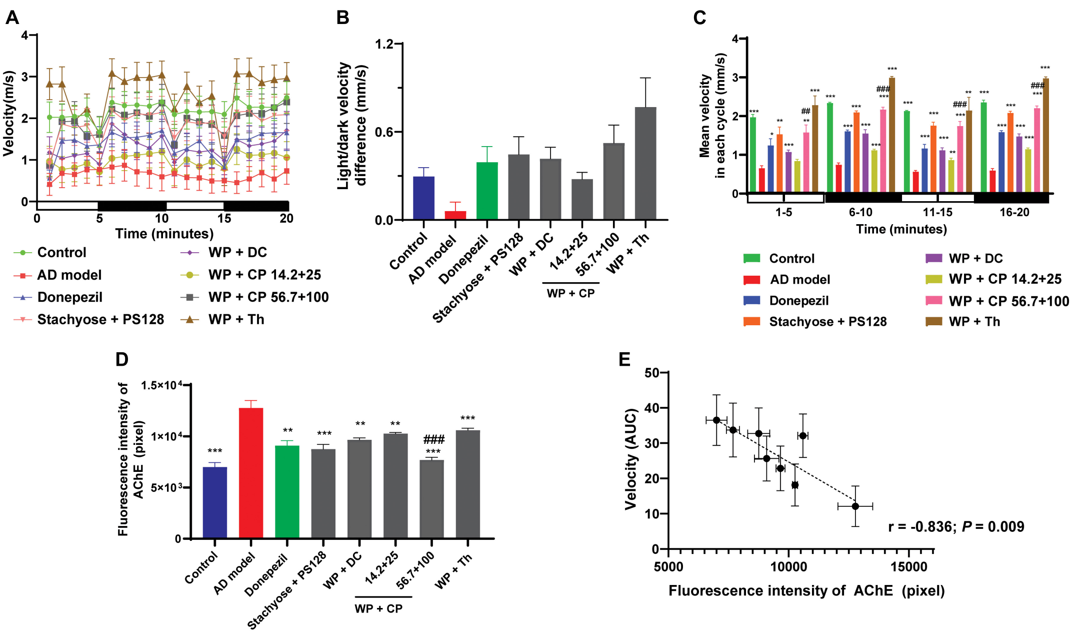
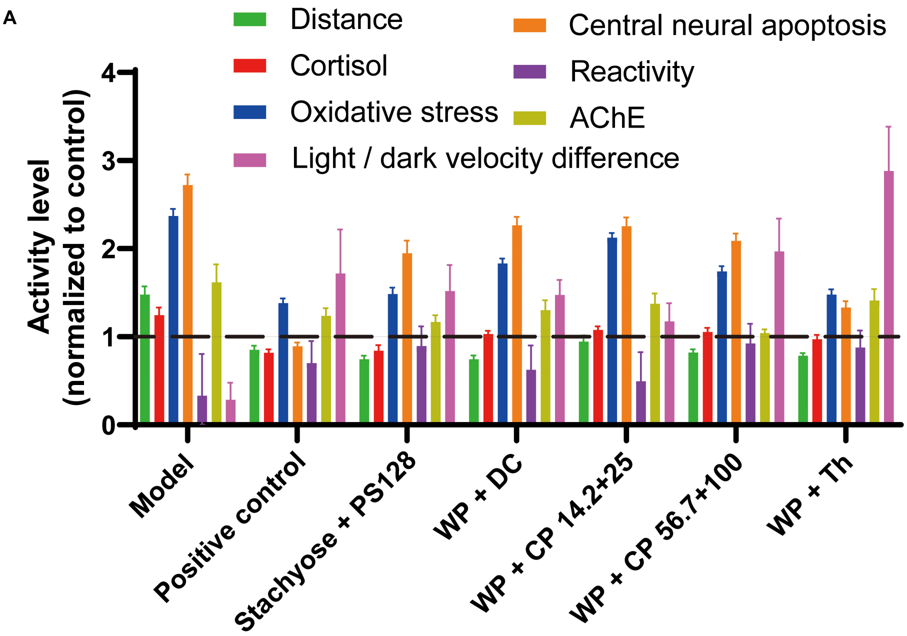


FIGURE 4

Memory-improving effects of nutrients. **(A)** The lines represented the average moving speed of zebrafish per minute under light and in dark environments for 20 min (n = 12). **(B)** The difference in velocity in light (0–5 and 10–15 min) and dark (5–10 and 15–20 min) was measured (n = 12). **(C)** The histogram of zebrafish's average movement velocity in each cycle (5 min per cycle, n = 12). **(D)** The fluorescence intensity of AChE in each group (n = 6). **(E)** The intensity of AChE was plotted against the AUC of the mean velocity from panel **(A)** (n = 6, r = −0.836, P = 0.009). Data in panels **(B,C)** were presented as mean ± SEM. $^{*}P$ < 0.05, $^{**}P$ < 0.01, $^{***}P$ < 0.001 compared with AD model; $^{\#\#}P$ < 0.001, $^{\#\#\#}P$ < 0.001 compared with WP + CP (14.2 + 25 μg/ml). Data were collected from three biological replicates.

A



Model includes: Anxiety model, OS model, NSI model and AD model.

B

r & *P*	Distance	Cortisol	Oxidative stress	Central neural apoptosis	Reactivity	AChE	Light / dark velocity
Distance		r = 0.743; *P* = 0.035	r = 0.529; *P* = 0.178	r = 0.402; *P* = 0.323	r = -0.641; *P* = 0.087	r = 0.558; *P* = 0.151	r = -0.758; *P* = 0.030
Cortisol			r = 0.731; *P* = 0.039	r = 0.712; *P* = 0.047	r = -0.601; *P* = 0.115	r= 0.516; *P* = 0.191	r = -0.529; *P* = 0.178
Oxidative stress				r = 0.897; *P* = 0.003	r = -0.867; *P* = 0.005	r = 0.743; *P* = 0.035	r = -0.420; *P* = 0.301
Central neural apoptosis					r = -0.648; *P* = 0.083	r = 0.510; *P* = 0.197	r = -0.454; *P* = 0.258
Reactivity						r = -0.833; *P* = 0.010	r = 0.580; *P* = 0.132
AChE							r = -0.224; *P* = 0.595
Light / dark velocity							

FIGURE 5
The effects of nutrient combinations were universal and related. **(A)** Results of all the effects from the test groups were consolidated and presented in bar chat. The four models, including anxiety, OS, NSI and AD, were shown as "Model" for short. Data were normalized to the control (value of which was normalized to 1) of each test and presented as mean ± SEM. **(B)** The effects tested from test groups were plotted against each other. The r-value and *P*-value of each plot are shown in panel **(B)**.

advanced image processing software was used for analysis. The fluorescence intensity at the zebrafish yolk sac was measured for further analysis.

2.7. CNS protective effect evaluation

Zebrafish of the wild-type AB strain of one dpf were randomly selected and raised in six-well plates with 30 zebrafish per well (for all groups). After treatments, zebrafish were stained with acridine orange (AO, Supplementary Table 1: Instruments and consumables). After staining, 10 zebrafish were randomly selected from each group and placed under a fluorescence microscope for imaging. Images were processed by Nis-elements D 3.20 advanced image processing software. After that, the fluorescence intensity in the zebrafish CNS was measured.

2.8. Memory improvement

2.8.1. Responsiveness

The wild-type AB strain of four dpf zebrafish was randomly selected and raised in 6-well plates, 30 zebrafish per well (for all groups). After treatments, 10 zebrafish in each group were randomly selected and studied. The behavioral analyzer measured the zebrafish's average moving speed per minute under light and in dark environments for further analysis.

2.8.2. AChE measurement

Zebrafish of the wild-type AB strain of four dpf were randomly selected and raised in six-well plates with 30 zebrafish per well (for all groups). After treatments, an AChE assay kit was used to analyze zebrafish by ELISA. The AChE-fluorescence value was measured for further analysis.

2.9. Statistical analysis

Statistical analysis was conducted using SPSS v.20 software (IBM Ltd., UK). Data following normal distributions were represented as means ± standard errors of the mean (SEM). Differences among groups were analyzed by one-way ANOVA, followed by the *post hoc* Tukey Dunnett's multiple comparison test. In the non-parametric test, the Kruskal–Wallis single-factor ANOVA test (K samples) was used. Comparative analysis of the two groups: independent t-tests and non-parametric tests were used for normal distributions. Mann–Whitney tests were used for non-normal distributions. The correlations between each effect were analyzed statistically by Pearson's correlation. The area under curve (AUC) of velocity in 20 min was used for correlations between reactivity and other effects. When putting all the indexes together in one graph, we normalized every index to control to compare the full impact of each combination. P values less than 0.05 were considered significant in all analyses. Data were collected from at least three biological replicates.

3. Results

3.1. The nutrient combinations exhibited antianxiety effects

We used mCPP to increase zebrafish's anxiety-like behaviors and stimulate the production of cortisol (10, 12). The zebrafish's movement (representative trace maps in **Figure 1A** and **Supplementary Figure 1A**) reflected the zebrafish's anxiety level (13). From the results in **Figure 1B** and **Supplementary 1B**, the distance in the model group was significantly longer than the control ($P < 0.001$), indicating that the zebrafish anxiety model was effectively established. As expected, zebrafish exhibited a shorter distance in the Selegiline (an irreversible monoamine oxidase (MAO)-B inhibitor that increases 5-HT levels) (14) group than in the model or the control. Based on the preliminary screening (**Supplementary Figure 1B**), we selected several nutrients and their combinations for further tests.

Figure 1B revealed that all the individual nutrients and combinations exhibited shorter distances than the model group, indicating their antianxiety effects. Among them, Stachyose at 500 μg/mL was the most effective nutrient, with comparable activity to the positive drug. For the combinations, firstly, we noticed that when the ratio of WP + DC was adjusted based on weight (1:1, 1:3, and 3:1), their effects varied and, at 93.7 + 31.2 μg/ml (this concentration was selected for downstream tests) showed the highest capability which is comparable to the positive drug. Secondly, the effects of the combinations were dose-specific but not dose-dependent in all groups. For instance, in Stachyose + PS128 group and WP + Th group, the increase of both components' concentrations did not promote their antianxiety capabilities. Thus, in Stachyose + PS128 and WP + Th groups we selected the lowest concentrations (83.3 + 500 and 14.2 + 33.3 μg/ml, Stachyose + PS128 is more effective, $P = 0.003$, **Figure 1B**) for downstream tests. Nevertheless, the concentration increases in the combination of WP + CP promoted the antianxiety effect, showing a preliminary dose dependence.

The production of cortisol in each group was also measured to show the zebrafish's anxiety level (15). As expected, most tested nutrients and combinations showed lower cortisol levels than the model group, with Stachyose + PS128 (83.3 + 500 μg/ml) being the most effective combination (**Figure 1C**). Surprisingly, specific concentrations of WP + DC and WP + CP combinations did not inhibit the production of cortisol, which was not consistent with the zebrafish's moving distance. Despite this, a positive correlation ($r = 0.719$, $P < 0.001$) was still observed between the distance and the cortisol level (**Figure 1D**), showing that the antianxiety effects assessments were reliable.

3.2. The nutrient combinations were effective in antioxidation

It was reported that biological processes such as oxidative stress are partly affected by mood states (16). Therefore, we continued to investigate the antioxidant effects of the nutrients. Menadione was used to induce oxidative stress, which triggered green fluorescence expression in the yolk sac of zebrafish (**Figure 2A**). The increase in fluorescence intensity represents a decrease in antioxidant function (17). The intensity (**Figure 2B**) was observed to be much higher in the model than in the control, indicating that the model establishment was successful. All the combinations showed antioxidant effects. Although none of them could recover the model to the level of the control, Stachyose + PS128 and WP + Th combinations showed similar effects as the positive control drug NAC, a glutathione precursor, which modulates glutamatergic transmission and targets oxidative pathways (18). Their antioxidant effects were also affected by concentrations: in WP + CP, the higher concentration at 56.7 + 100 μg/ml was more effective than the lower concentration, indicating a preliminary dose-dependence (**Figure 2B**).

3.3. The nutrient combinations showed central neuroprotective effects

It was also reported that there are links between anxiety, oxidative stress and neuron damage (19, 20). Thus, we continued to investigate the central neuroprotective effect of nutrients. Mycophenolate mofetil was used to induce NSI in zebrafish, which triggered green fluorescence (**Figure 3A**) in apoptotic cells at CNS (21). Firstly, in the model group, the fluorescence intensity (**Figure 3B**) was almost three times higher than the control, showing the model establishment was successful. Secondly, the combination of WP + Th showed the strongest activity in reducing central neural apoptosis, close to the positive drug Glutathione, whose depletion is a common feature of apoptotic cell death (22). Unlike the antianxiety tests, the other combinations (WP + DC, WP + CP, and Stachyose + PS128) exhibited a similar but much lower protective effect than WP + Th. A preliminary dose-dependent effect was observed in WP + CP and further tests with more concentrations are needed. Noteworthily, when we plotted the antioxidant effect of the nutrients against their central neural apoptosis level, a good positive correlation was established ($r = 0.897$, $P = 0.003$, **Figure 3C**), showing that both effects from the nutrients were highly related.

3.4. The nutrient combinations improved zebrafish's memory

Apart from the above tests, we further investigated the memory-improving abilities of the nutrients, as anxiety was reported to cause memory damage (23). During model establishment, aluminum chloride was used to build zebrafish's AD model: Excessive intake of aluminum chloride causes toxic effects on the nervous system, resulting in abnormal zebrafish behavior (24). It also destroys the cholinergic nerve function, increases the production of amyloid, reduces learning ability and causes memory loss (24, 25). Donepezil hydrochloride, an AChE inhibitor, was used as the positive drug in this test. The nutrients' memory-improving abilities were evaluated by measuring the velocity of zebrafish using the response capability test (26). From the graph shown in **Figure 4A**, the mean velocity of the model group was the lowest of all, demonstrating the effectiveness of the modeling. The results showed that the nutrients improved the mean velocity, which was slower and less stable under light (0–5 and 10–15 min) than in the dark (5–10 and 15–20 min). WP + CP significantly improved zebrafish's response capability, with a preliminary concentration-dependent effect. It is noteworthy that WP + Th showed the most substantial capability in improving velocity, even recovered to a higher level than the control.

Furthermore, zebrafish with nervous system damage were reported to have slower swimming speed; But after treatment, their swimming speed was recovered with the stimulation of light-dark alternation (27). Consistent with this report, the difference in velocity between light and dark was much higher in nutrient combinations (e.g., WP + Th) than in the model (**Figure 4B**). The mean velocity was further broken down into cycles of 5 min in **Figure 4C**. The patterns of the results in each period were similar, further proved that the effects of nutrients in the shorter period are consistent. Another crucial memory-improving index was the production of AChE (28), the intensity of which (**Figure 4D**) confirmed the effectiveness of all the combinations, with WP + CP at a higher concentration being the most effective. When we plotted the AChE intensity against the AUC of the mean velocity of all groups, a good negative correlation was observed ($r = -0.836$, $P = 0.009$, **Figure 4E**), indicating the memory-improving assessments were reliable.

3.5. The effects of nutrient combinations were universal and highly-related

With all these data in hand, we further analyzed the relationships between different effects of the nutrient combinations. The results of all effects were consolidated in **Figure 5A**, from which we observed that the pattern of the effects from test groups are similar and most of the nutrients improved their respective models. Among them, WP + Th was almost the most promising in all tests. When the value of individual effect was plotted against each other, good correlations (17 out of 21 have absolute r value larger than 0.5, representative plots were shown in **Supplementary Figure 2**) were observed (**Figure 5B**), suggesting the effects of the nutrient combinations were universal and highly related.

4. Discussion

In this study, we used different zebrafish models to identify nutrient combinations as potential drug alternatives for treating anxiety and related conditions. In the antianxiety test, Stachyose + PS128 (83.3 + 500 μg/ml) exhibited similar capability (**Figures 1B, C**) as the positive drug Selegiline. Stachyose is a soluble carbohydrate that works as a probiotic that reduces the negative impacts on antibiotic-destroyed microbiota (29) and improves long-term potentiation impairment caused by stress through the gut-brain axis (30). PS128 improved stress, anxiety and insomnia in humans (31). It could serve as a therapeutic adjuvant for treating major depressive disorder (32). These effects might be related to ameliorated inflammation and oxidative stress through microbiota modulation and related metabolites (33). In preliminary screening, PS128 at 500 μg/ml showed an antianxiety effect (**Supplementary Figure 1**), and Stachyose is reported to be an absorption enhancer (34).

Therefore, we speculate that PS128 is the more effective component while Stachyose plays an auxiliary role.

In the downstream antioxidant, neuro-protecting and memory-improving tests, three positive drugs rescued their respective models via different mechanisms of action. Our study showed that WP + Th, with a relatively lower concentration of 14.2 + 33.3 μg/ml, was the most effective in the above three tests, with similar or better activities than these drugs. WP has been reported to promote sleep quality by regulating neurotransmitters in the brain tissue in mice (35). It could also enhance memory, cognition and improve sleep quality in clinical trials (36). Th, a non-protein amino acid abundantly present in tea leaves, has a similar structure as glutamate and glutamine that has been shown to improve anxiety, sleep and cognitive function, as well as reduce oxidative stress (37). The mechanism might be associated with its cerebro-protective effect, the neuroprotective effect and its glutamine carrier for inhibiting the combination of extracellular glutamine with neurons (38) or regulating the gamma-aminobutyric acid (GABA) receptor (39). We thus speculate that WP + Th might have a synergistic regulatory effect on neurotransmitters in the brain. Furthermore, in the aluminum chloride-induced AD model, the WP + Th group showed the best memory-improving abilities, even better than the control (**Figures 5A–C**), indicating WP + Th may have a potential effect on improving memory in other animals or even in the human context, which implies a great commercial value in the memory-improving food market.

In this study, we found a significantly high correlation between distance and light/dark velocity (**Figure 5B**), which was in line with the previous research (40). Oxidative stress is the essential pathophysiological process in hypoxia and aging. It causes glial activation, neurodegeneration, neurotransmitter/receptor dysregulation and induces CNS disorders (depression, anxiety, and dementia) (41). In our study, positive correlations were also found between oxidative stress and central neural apoptosis, as well as oxidative stress and AChE level, indicating that oxidative stress, central neural apoptosis and AChE production were reciprocal causation in the anxiety process. Noteworthily, not in many studies were these four effects studied together, let alone analyzing the correlations between them. In the following studies, we will further investigate the relationships between these four effects and the causation.

Zebrafish are widely used in CNS-related studies as they have human-like CNS: similar main structure of the brain, identical physiology and neurochemistry, etc. (42). Studies also compared the zebrafish and mouse models of anxiety. They also proved the predictive power of zebrafish for behavioral research (43). Since mammals are more genetically and physiologically similar to humans (44), further animal studies using rodents and large animals are also needed prior to human trials.

As most animal behavioral tests are subjective and unstable (45), using different behavioral tests to evaluate and recognize emotions is recommended. However, due to time constraints, only two behavioral tests were used in this study to assess anxiety and memory loss. We couldn't perform more cognitive tests like the novel dark diving test, the T-maze test, or the object recognition test. In the following rodents experiment, we would select more behavioral tests to evaluate the nutrients and combinations on anxiety, recognition and memory.

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

XJ, YZ, and QZ designed the experiments. BZ, CT, YH, JD, and XB performed the experiment. HF and ZiZ analyzed the data. ZhZ, JH, HL, and YL contributed to the reagents, nutrients, and nutrient combinations. BZ, YZ, and QZ prepared the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

XJ, HF, ZiZ, JH, HL, JD, YL, and XB are employed by Inner Mongolia Yili Industrial Group Co., Ltd. CT and QZ are employed by Sichuan SAFE Pharmaceutical Technology Co., Ltd. ZhZ and YZ are employed by Sichuan Kangcheng Biotech Co., Inc.

The remaining authors declare that the research was conducted without any commercial or financial relationships 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.2022.996692/full#supplementary-material

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A multilevel analysis of factors associated with vitamin A supplementation among children aged 6–35 months in Ethiopia

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Background: Vitamin A deficiency is among the leading preventable causes of childhood morbidity and mortality that might be attributable to the low uptake of vitamin A supplementation (VAS). Factors contributing to its low utilization are not researched at the national level and with the appropriate model. Therefore, this study aimed at identifying the magnitude and the individual- and community-level factors associated with vitamin A supplementation among children aged 6–35 months in Ethiopia.

Methods: We have used the Ethiopian mini demographic and health survey data, which was conducted from 21 March to 28 June 2019. A weighted sum of 2,362 mothers having children aged 6–35 was extracted. Considering the hierarchical nature of the data, we fitted the multilevel multivariable logistic regression model. Adjusted odds ratio (AOR) with a 95% confidence interval (CI) was reported and variables with a p-value of <0.05 were declared to be significantly associated factors.

Results: In this study, 43.4% (95% CI: 41.4–45.4%) of children have taken the VAS. Moreover, the 12–23 age of the child (AOR = 2.64; 95% CI: 1.88–3.72), 30–34 age of the mother (AOR = 3.34; 95% CI: 1.21–9.20), middle household wealth status (AOR = 1.75; 95% CI: 1.06–2.90), and four and above antenatal care (AOR = 2.90; 95% CI: 1.90–4.43) are the individual-level factors associated with VAS whereas being from Amhara (AOR = 2.20; 95% CI: 1.29–3.76) and Tigray (AOR = 2.16; 95% CI: 1.17–3.98) regions is a community-level factor significantly associated with the uptake of VAS.

Conclusion: Overall, a low proportion of children have taken the VAS in Ethiopia. The higher age of the child and mother, full antenatal care, and improved wealth status positively influence VAS. Moreover, a child from the Tigray or Amhara regions was more likely to get VAS. Therefore, an intervention has to be designed to address the VAS uptake among young mothers, and working to improve the wealth status of the household would be helpful. Moreover, the advocacy of antenatal care and minimizing the regional disparity through encouraging the uptake in the rest of the regions would help increase the national-level uptake of VAS.

KEYWORDS

vitamin A supplementation, Ethiopia, multilevel analysis, childhood illness, vitamin A deficiency (VAD)

Background

Vitamin A deficiency (VAD), the leading preventable contributor to childhood blindness, diarrhea, and measles infection (1, 2), is a disorder that occurs when our body is unable to satisfy the physiological needs due to the inadequate body storage of vitamin A (3, 4).

Even though VAD is decreasing worldwide, its prevalence in Sub-Saharan Africa (SSA; 48%) and South Asia (44%) is alarmingly high in children (5). In 2013, nearly 94,500 and 11,200 deaths from childhood diarrhea and measles infection were attributable to VAD (5). The incidence of deaths was also skewed to the developing countries. For instance, SSA and South Asia account 95% of deaths due to VAD (5). In Ethiopia, 37.7% of children had deficient clinical serum vitamin A levels (6).

Hence, one of the strategies for treating VAD is vitamin A supplementation (VAS) during a childhood period (7, 8). According to the World Health Organization (WHO) recommendation, a 100,000 international unit (IU) dose of vitamin A supplementation should be given for 6–11 months and 200,000 IU for 12–59 months aged children every 4–6 months who are living in affected areas (9). Moreover, the regular uptake of the recommended dose of vitamin A supplementation decreases childhood mortality by 12—28% (10, 11).

However, the coverage of VAS is not in line with its public health importance. In 2020, there was a 60% unmet demand for VAS worldwide (12). Moreover, the VAS coverage in SSA was 65% in 2018 (13) and it ranges from 40.8% in Guinea to 88.4% in Senegal (14).

In Ethiopia, VAS is given to children aged 6–59 two times a year as part of the expanded program of immunization (EPI) for the last two decades (15, 16). The country implemented a variety of strategies such as routine health extension programs, enhanced outreach strategies, and community health day modalities to address the unmet demand for VAS (17). The routine VAS has saved 167,563 to 376,030 child lives between the years 2005 and 2019 (18). However, more than half (55%) of the eligible children were not supplemented in 2016 (19) and 43% of children were in demand of VAS in 2019 (20). It is below the 95% country's target of VAS in 2020 (21). On the other hand, the country has planned to strengthen and scale up VAS to children by 2025 (22).

According to different studies, different factors were associated with the uptake of VAS. Among them, sex and age of the child, mother's age, maternal occupation, maternal education, households' wealth status, possessing a television, residence, region, knowledge, information provider, antenatal care, place of delivery, and postnatal check-up were the determinants of the uptake of VAS (14, 23–30).

Despite the presence of evidence on identifying the factors contributing to the uptake of VAS in Ethiopia, the available studies were not identified factors at the national level (26) and the national-level studies were not conducted with the appropriate model that considers the hierarchical nature of the national survey. Hence, in the multilevel model (random effect model), unlike the fixed effects model (the conventional logistic analysis), the effects of group-level predictors will not be confounded and the effects of different level (individual and community level) variables can be estimated. The methodological issues of multilevel analysis are depicted elsewhere (31). In addition, some of the available evidence is not up-to-date to explore the individual- and community-level factors for the uptake of VAS (29, 30).

Therefore, this study aimed at identifying the factors associated with the uptake of VAS in Ethiopia using the most recent Ethiopian demographic and health survey conducted in 2019 and employing the multilevel analysis to account for the hierarchical nature of the survey. Thus, the finding of this study will help to make evidence-based decisions by having updated information on the country's national-level VAS and its determinants.

Methods and materials

Study settings

Ethiopia is the second most populous country in Africa, with a total population of near 122 million according to the 2022 United Nations data (32). Based on the World Bank report, Ethiopia is one of the world's poorest countries, with a per capita income of US$944 in 2021 (33). Currently, a three-tier healthcare delivery system is being implemented in Ethiopia. Primary-level health care includes health posts, health centers, and primary hospitals, secondary-level health care delivered by the general hospitals, and tertiary-level health care carried by specialized hospitals (34).

Data source, sampling procedure, and population

The 2019 Ethiopian Mini Demographic and Health Survey (EMDHS) was the data source for this study. The cross-sectional survey was conducted from 21 March to 28 June 2019, using a complete list of 149,093 enumeration areas (EAs) as a sampling frame. The survey was conducted by a two-stage stratified sampling. That is each region was stratified into urban and rural areas to yield 21 sampling strata. First, a total of 93 urban EAs and 212 rural EAs were chosen with a probability proportionate to EA size. Then, in the second stage of selection, a fixed number of 30 households per cluster were selected with an equal probability of systematic selection from the newly created household listing, after a household listing operation was carried out in all selected EAs from January to April 2019 (the resulting lists of households served as a sampling frame for the selection of households in the second stage) (20). In this study, we used the birth record dataset (BR data). From a total of 2,403 mothers having children aged 6–35 months, 2,362 weighted samples were included for analysis. Sample weighting was conducted using the "*Svy*" command.

Abbreviations: ANC, antenatal care; AOR, adjusted odds ratio; CI, confidence interval; EAs, enumeration areas; EMDHS, Ethiopia Mini Demographic and Health Survey; ICC, Intraclass Correlation Coefficient; PCV, Proportional Change in Variance; SNNPR, Southern Nation Nationalities and Peoples Region; SSA, Sub-Saharan Africa; VAD, vitamin A deficiency; VAS, vitamin A supplementation; WHO, World Health Organization.

Study variables

The outcome variable of this study was vitamin A supplementation among children aged 6–35 in the last 6 months (*yes/no*). The independent variables were individual-level variables such as the age of the child, age of the mother, sex of the child, sex of household head, religion, marital status, mother's education, husband's education, wealth status, working status, birth order, parity, possession of radio, possession of a television, number of under-five children in the household, place of delivery of the child, mothers having ANC, and mothers having postnatal care. The community-level variables were residence and region. A region in this study was classified into five Oromia, Amhara, Southern Nation Nationalities and Peoples Region (SNNPR), Tigray, and others. The 'others' region contains Addis Ababa, Somalia, Afar, Dire Dawa, Benishangul, and Gambella due to their low number of eligible participants for this study. The questionnaire of the survey was pretested and 2 days of training were given to the data collectors and supervisors before the onset of actual data collection.

Data management and analysis

Stata version 14 was used for data management (extraction, re-coding, and categorization) and statistical analysis (identifying the factors contributing to VAS). Considering the hierarchical nature of the data, a multilevel multivariable logistic analysis was employed for assessing the factors associated with VAS. While conducting multilevel analysis, four models were fitted, namely, the null model (a model without explanatory variables), model I (a model with individual-level explanatory variables only), model II (a model with community-level variables only), and model III (a model with both individual- and community-level variables). Both bivariable and multivariable multilevel analyses were used. Variables with a p-value of <0.20, in the bivariable analysis, were eligible for the multivariable analysis. In the multivariable analysis, an adjusted odds ratio (AOR) with a 95% confidence interval (CI) was reported and variables with a p-value of <0.05 were declared to be a significantly associated factor. For assessing the cluster-level variability of VAS, we have employed the random effect analysis calculating the Intraclass Correlation Coefficient (ICC) and Proportional Change in Variance (PCV). Finally, model fitness was checked using Deviance.

Ethical considerations

We have used the secondary data from the EDHS, which was conducted under the Declaration of Helsinki. Through online request, we accessed the dataset from the DHS website (https://dhsprogram.com) and personal identifiers were not available on the dataset. Since we have used publicly accessible data, ethical approval was not needed.

Results

Background characteristics of the respondents

In this study, 2,362 mothers having a child aged 6–35 months were included. The mean (± standard deviation) age of the mothers and children was 28.13 ± 6.42 years and 19.16 ± 8.29 months, respectively. Among eligible children, only 43.4% (95% CI: 41.4–45.4%) have taken the VAS. The majority 391 (47.07%) of the children who have taken the supplementation were aged between 24 and 35 months and 279 (54.55%) of the children who have supplemented with vitamin A were from the Amhara region (Table 1).

Maternity-related characteristics of the respondents

In this study, 236 (44.05%) of children whose mother is nulliparous have taken the vitamin A supplementation, whereas 529 (52.26%) of children whose mothers had four and above antenatal care have taken the supplementation. A total of 1,194 (59.01%) children whose mothers have not had a postnatal check-up within the last 2 months have not taken the VAS (Table 2).

Multilevel logistic regression analysis

In this study, the ICC of the null model indicated that 25.4% of the variability of VAS is attributable to the differences between the clusters. Moreover, 27.7% of the variability of VAS was explained by both individual- and community-level variables. Furthermore, model III was the best-fitted model since it had the lowest deviance.

In a multilevel binary logistic regression, the age of the mother, age of the child, wealth index, the household that has television, type of place of residence, region, the number of ANC follow-ups, place of delivery, having postnatal checkups within 2 months, the number of under-five children in the household, type of residence, and region were associated with VAS with a p-value of <0.2. In the multivariable logistic analysis model, the age of the mother, the age of the child, wealth index, ANC follow-up, and region were significantly associated with VAS with a p-value of <0.05.

A child whose mother aged 30–34, 35–39, and 40–49 had 3.34 (AOR = 3.34; 95% CI: 1.21–9.20), 3.06 (AOR = 3.06; 95% CI: 1.00–9.34), and 2.62 (AOR = 2.62; 95% CI: 1.02–6.70) times higher odds of getting VAS than a child whose mother's age was between 15 and 19, respectively. Similarly, a child with the age range of 12–23 and 24–35 months had 2.64 (AOR = 2.64; 95% CI: 1.88–3.72) and 2.20 (AOR = 2.20; 95% CI: 1.37–3.56) times higher odds of VAS than a child with an age range of 6–11 months. Moreover, a child from a middlingly wealthy family was 1.75 (AOR = 1.75; 95% CI: 1.06–2.90) times more likely to get a VAS as compared to a child from the poorest family. In addition, having three ANC follow-ups and four

TABLE 1 Background characteristics of the respondents having children aged 6–35 months in Ethiopia ($N = 2{,}362$).

Variables	VAS non-uptake		VAS uptake	
	Frequency	Percent	Frequency	Percent
Sex of household head				
Male	1,165	56.59	894	43.41
Female	180	59.36	123	40.64
Age of the mother (years)				
15–19	109	71.17	42	28.83
20–24	305	60.49	199	39.51
25–29	455	59.02	316	40.98
30–34	237	50.56	232	49.44
35–39	158	52.06	142	47.94
40–49	91	51.12	87	48.88
Sex of the child				
Male	713	57.07	536	42.93
Female	932	56.80	481	43.20
Age of the child (months)				
6–11	355	70.58	148	29.42
12–23	551	53.53	478	46.47
24–35	439	52.93	391	47.07
Highest educational level				
No education	694	59.91	464	40.09
Primary	500	55.71	398	44.29
Secondary and higher	151	49.37	155	50.63
Current marital status				
Unmarried	66	48.09	71	51.91
Married	1,279	57.49	946	42.51
Religion				
Orthodox	406	48.31	435	51.69
Muslim	480	60.57	313	39.43
Protestant	420	61.63	261	38.37
Other*	39	82.43	8	17.57
Wealth index				
Poorest	321	65.13	172	34.87
Poorer	320	62.95	188	37.05
Middle	212	45.99	249	54.01
Richer	244	59.94	163	40.06
Richest	249	50.35	245	49.65
Household has television				
No	1,165	58.79	817	41.21
Yes	180	47.35	200	52.65

(Continued)

TABLE 1 (Continued)

Variables	VAS non-uptake		VAS uptake	
	Frequency	Percent	Frequency	Percent
Household has radio				
No	996	56.71	761	43.29
Yes	349	57.61	256	42.39
Type of place of residence				
Urban	334	52.33	305	47.67
Rural	1,011	58.66	712	41.34
Region				
Oromia	531	58.28	380	41.72
Amhara	233	45.45	279	54.55
SNNPR	313	66.10	161	33.90
Tigray	65	41.24	92	58.76
Others**	204	65.95	105	34.05

*Catholic and traditional.
**Addis Ababa, Somalia, Afar, Dire Dawa, Benishangul, Gambella, and Harari.

and above ANC follow-up increases the odds of VAS by 1.91 (AOR = 1.91; 95% CI: 1.05–3.45) and 2.90 (AOR = 2.90; 95% CI: 1.90–4.43) times as compared to no ANC follow-up. On the other hand, a child residing in Amhara and Tigray would increase the odds of VAS by 2.20 (AOR = 2.20; 95% CI: 1.29–3.76) and 2.16 (AOR = 2.16; 95% CI: 1.17–3.98) times as compared to other regions (Afar, Somalia, Dire Dawa, Addis Ababa, Harari, Benishangul, and Gambella; Table 3).

Discussion

This study was designed to identify the individual- and community-level factors, which affect the uptake of VAS in Ethiopia. Maternal and child age, wealth index, ANC follow-up, and region were among the factors, which affect the VAS of the child significantly.

In this study, 43.4% (41.4%–45.4%) of children have taken VAS, which is in line with another similar study conducted in Ethiopia which showed that the VAS in Ethiopia was 44.4% (35). But the finding of this study is higher than another study conducted that indicated that the uptake of vitamin A-rich food in Ethiopia was 38.99% (36). This might be used as an indicator that food-enriched uptake of vitamin A is falling behind the supplementation. Hence, action has to be taken to enhance the uptake of vitamin A-rich food hand in hand with supplementation. The trend on VAS has not shown a significant change from the 45% of VAS in 2016 (19). However, it is lower than the 59.4% coverage of VAS in SSA countries (25) and the 65% average proportion of VAS for lower-income countries (37). The difference might be due to the later studies including children of 6–59 months whereas this study included children of 6–35 months. On the one hand, this finding also underlines that Ethiopian progress is distant even from its peer SSA and low-income countries.

TABLE 2 Maternity-related characteristics of the respondents having children aged 6–35 months in Ethiopia ($N = 2,362$).

Variables	VAS non-uptake		VAS uptake	
	Frequency	Percent	Frequency	Percent
Parity				
Null parity	230	55.95	236	44.05
Multiparity	611	57.58	450	42.42
Grand multiparity	434	56.75	331	43.25
Number of ANC follow-up				
No visit	423	70.18	180	29.82
One visit	53	63.02	31	36.98
Two visits	116	64.77	63	35.23
Three visits	270	55.79	214	44.21
Four and above visits	483	47.74	529	52.26
Place of delivery				
Home	703	62.25	427	37.75
Health facility	641	52.08	590	47.92
Having postnatal check-up within 2 months				
No	1,194	59.01	829	40.99
Yes	151	44.59	188	55.41
Number of children 5 and under in the household				
0–1	614	54.15	520	45.85
2–3	712	59.22	491	40.78
4–5	19	73.28	6	26.72

Moreover, this study identified important individual- and community-level factors. Accordingly, the older maternal and child age was significantly associated with the fewer odds of VAS. A child whose mother was aged 30–34, 35–39, and 40–49 had 3.34, 3.06, and 2.62 times higher odds of getting VAS than a child whose mother's age was between 15 and 19, respectively. The finding has supported another study conducted in Ethiopia and elsewhere in SSA (14, 38). The variation could be associated with a lack of awareness and paying less attention among young mothers (30, 39). This might become a huge loss when we consider that teenage pregnancy is common in Ethiopia (40). Therefore, health education programs on the importance of VAS shall target younger mothers to increase the uptake of VAS. Similarly, a child with the age range of 12–23 and 24–35 months had 2.64 and 2.20 times higher odds of getting a VAS than a child in the age range of 6–11 months. This might be related to the culture of Ethiopia where after delivery a mother exposes herself late during the postpartum period. A study conducted somewhere showed a different association (14, 39). Nevertheless, the health extension workers and the women development armies should pay attention to the good uptake of VAS among lower-aged children. Moreover, a child from a middle-

TABLE 3 Multilevel multivariable logistic regression output for factors associated with vitamin A supplementation in Ethiopia.

Variables	Null model	Model I AOR (95% CI)	Model II AOR (95% CI)	Model III AOR (95% CI)
Age of the mother (years)				
15–19		1		1
20–24		2.06 (0.85–4.99)		2.03 (0.84–4.97)
25–29		2.42 (0.85–5.91)		2.22 (0.84–5.88)
30–34		3.46 (1.27–9.46)*		3.34 (1.21–9.20)*
35–39		3.13 (1.03–9.46)*		3.06 (1.00–9.34)*
40–49		2.72 (1.06–6.95)*		2.62 (1.02–6.70)*
Age of the child (months)				
6–11		1		1
12–23		2.65 (1.89–3.71)**		2.64 (1.88–3.72)***
24–35		2.19 (1.36–3.52)*		2.20 (1.37–3.56)**
Wealth index				
Poorest		1		1
Poorer		0.91 (0.62–1.33)		0.88 (0.60–1.30)
Middle		1.80 (1.10–2.97)*		1.75 (1.06–2.90)*
Richer		0.91 (0.51–1.60)		0.88 (0.49–1.58)
Richest		0.79 (0.33–1.88)		0.69 (0.27–1.77)
The household has a television				
No		1		1
Yes		1.20 (0.55–2.61)		1.14 (0.53–2.49)
Number of under 5 children in the household				
0–1		1		1
2–3		0.83 (0.61–1.12)		0.85 (0.63–1.15)
4–5		0.52 (0.06–4.86)		0.59 (0.07–5.10)
Place of delivery				
Home		1		1
Health facility		1.22 (0.75–1.95)		1.20 (0.74–1.94)
Number of ANC follow-up				
No visit		1		1
One visit		1.60 (0.73–3.47)		1.48 (0.68–3.22)

(Continued)

TABLE 3 (Continued)

Variables	Null model	Model I AOR (95% CI)	Model II AOR (95% CI)	Model III AOR (95% CI)
Two visits		1.34 (0.72–2.49)		1.27 (0.68–2.37)
Three visits		2.00 (1.10–3.62)*		1.91 (1.05–3.45)*
Four and above visits		3.09 (2.03–4.68)***		2.90 (1.90–4.43)***
Having postnatal check-up within 2 months				
No		1		1
Yes		1.35 (0.87–2.10)		1.37 (0.88–2.13)
Type of place of residence				
Urban			1	1
Rural			0.58 (0.38–0.88)*	0.68 (0.35–1.33)
Region				
Oromia			1.65 (0.96–2.83)	1.36 (0.75–2.47)
Amhara			2.96 (1.78–4.93)*	2.20 (1.29–3.76)**
SNNPR			1.03 (0.67–1.58)	0.84 (0.52–1.36)
Tigray			3.21 (1.84–5.62)*	2.16 (1.17–3.98)*
Others$			1	1

*p < 0.05. **p < 0.01. ***p < 0.001. $Addis Ababa, Somalia, Afar, Dire Dawa, Benishangul, and Gambella.

wealth family had 1.75 times more likely to get a VAS as compared to a child from the poorest family. This is similar to the other study conducted in Ethiopia (35). Even though VAS is given in Ethiopia free of charge, associated indirect costs might be hindering the poorest households from getting the supplementation.

Consistent with other studies conducted in Ethiopia (30, 35), the odds of having three and four and above ANC follow-up increases the odds of VAS by 1.91 and 2.90 compared with no ANC follow-up. This is supported by antenatal contact, which is usually the entry point for a mother for the subsequent maternal–child health service utilization including the VAS, which leads to a high level of uptake. This is because, during antenatal care, the health professional is usually aware and consults the pregnant woman on the care of the child and herself. Therefore, we also underline that inclusive maternal and child health service awareness creation including the VAS should be strengthened during antenatal contact.

Furthermore, a child who was in Amhara and Tigray would increase the odds of VAS by 2.20 and 2.16 times as compared to other regions (Afar, Somalia, Dire Dawa, Addis Ababa, Harari, Benishangul, and Gambella). The socio-demographic and cultural variation between regions might be contributed to the uptake variation in the regions. A spatial analysis conducted in Ethiopia also indicated the need for immediate interventions in Afar, Somali, and some pocket areas in Addis Ababa for the uptake of vitamin A (30). Moreover, interventions such as promoting VAS in other regions should be considered to achieve the national target of VAS.

Even though the study used the national-level data and fitted the appropriate model for the nature of the data (multilevel model), the survey dataset has limitations on having observations for children aged 37–59 months for VAS. Therefore, factors affecting VAS in the above age group were not able to be identified in this research. In addition, due to the use of EMDHS, the absence of important facility and behavioral variables, such as distance of household from the health facility, knowledge of maternal and husband toward the importance of VAS, and household information about the schedule of VAS, might limit the findings of this study.

Conclusion

Overall, the proportion of VAS among children in Ethiopia is low. The higher age of the child and mother, full antenatal care, and improved wealth status was positively associated with VAS. Moreover, a child from other than Tigray and Amhara regions was less likely to recieve VAS. Therefore, the intervention has to be designed to improve the uptake of VAS among young mothers as most of the country's population is young and teenage pregnancy is common in our country special attention should be paid by the health extension workers and women's health development army to improve the initiate VAS to children in the postpartum period as soon as possible. Working to improve the wealth status of the household would also have a spillover effect on the uptake of VAS. Moreover, the advocacy of antenatal care and minimizing the regional disparity through encouraging the uptake in the rest of the regions would help increase the national-level uptake of VAS.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://dhsprogram.com.

Author contributions

TA, TS, GL, MF, and MA developed the concept, reviewed the literature, carried out the statistical analysis, interpret and discussed the results, and drafted the manuscript. All authors reviewed the drafted manuscript and approved the submission of 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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