FOOD-BASED DIETARY GUIDELINES: THE RELEVANCE OF NUTRIENT DENSITY AND A HEALTHY DIET SCORE

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EDITORIAL
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Editorial: Food-Based Dietary Guidelines: The Relevance of Nutrient Density and a Healthy Diet Score

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Keywords: nutrient rich food score, dietary muscle health score, bioavailability, drivers of consumption, protein, vitamin B12, vitamin K, dairy

Editorial on the Research Topic

Food-Based Dietary Guidelines: The Relevance of Nutrient Density and a Healthy Diet Score

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Over time the main drivers of eating have shifted from ensuring adequate caloric intake to maintaining optimal health and socializing with appetizing and desirable foods. Sustainable food production is also now considered important. The recent EAT-Lancet Commission (1) suggested a change in the current diet to include lower amounts of animal source foods and a high diversity of plant-based foods, to meet both nutrient requirement and sustainability criteria. The key aim of this Research Topic is to provide a picture of the relevance of different measures of nutritional quality in relation to healthiness of diets such as proposed by the EAT-Lancet with focus on major food- and target groups, whilst taking drivers of food consumption into account.

Nutrients work together, rather than in isolation, and potential beneficial effects of specific nutrients will only appear if intake of other nutrients is optimal. To separate foods that are energy-dense from those that are nutrient-rich, Drewnoski (2) developed a score to assess nutrient density of foods. The nutrient rich food (NRF) score X.Y is based on recommended daily allowances for adult populations with X representing nutrients to encourage and Y nutrients to limit. Within this Research Topic this score was adapted specifically for the European older population, as their proportion is rising and consequently the burden of degenerative diseases (Berendsen et al.) The Elderly NRF score (E-NRF7.3) was composed of nutrients, shown to be of inadequate intake in the aging population, defined as nutrients of public health relevance, and associated with relevant health outcomes. Vegetables, bread, potatoes, and milk and products contributed the most to the new E-NRF7.3 score, which also correlated with a greater adherence to a healthful diet (Berendsen et al.) Moreover, the higher the score the higher the serum folate levels in both Dutch and Polish elderly, the lower the homocysteine (the Netherlands only), and the higher the vitamin B12 levels were (Poland only) (Kramer et al.) The above mentioned articles confirm available literature that the nutrient density score can distinguish nutritious basic food product from unhealthy alternatives, and therewith show validity of the score. However, in the nutrient density score discrimination between healthy and unhealthy foods could still be improved. Recently. Drewnowsky et al., designed an hybrid nutrient density score, which also includes food groups.

He showed that this score would also include the healthfulness of foods not readily captured by a score based on nutrients only (3).

Decreasing consumption of animal foods may increase the risk of certain nutrient deficiencies, such as vitamin B12. An overview of vitamin B12 intake and status in different target groups showed that specifically dairy consumption seemed to be a stronger determinant of vitamin B12 status (Obeid et al.) Cheese is a source of vitamin K_2, a micronutrient with demonstrated positive results on cardiovascular-related outcomes. However, cheese did not add extra to the association between a healthy diet score and incident T2DM or all-cause mortality (Dekker et al.) Nutrient dense animal food products rich in vitamin B12 or vitamin K, are also rich in other nutrients such as calcium, iron, and proteins. A study showed that eliminating dairy foods and replacing them iso-calorically by alternatives with more unsaturated fats led to deficits in some key nutrients and to lower NRF9.3 scores (Rehm and Drewnowski) These papers highlight the fact that the nutrient-based dietary guidance proposed by the EAT-Lancet commission may not account for the complexity of dietary patterns and may have unintended unfavorable nutritional consequences.

In addition to a diversity of single nutrients, a healthy eating pattern should according to Burd et al. include the ingestion of high quality protein, preferably in adequate amounts across all meals throughout the day. Given its role in muscle function and metabolic health, protein recommendations should be adapted to reflect needs throughout life/health-stage, acknowledging food matrix interactions and potential food structure changes resulting from heat treatment, while also ensuring other nutrient adequacies. Schacht et al. therefore developed an older adult-specific dietary muscle health score that is based on associations between food groups and muscle function outcomes. The advantage of this score being it includes nutrient-density, the well-known interactions between nutrients and the bioavailability of these nutrients from the matrix, which is further discussed by Melse-Boonstra.

Reducing the share of ultra-processed foods in the diet has been suggested by Gupta et al. to be a means of improving diets, albeit at a higher cost. They showed that ultra-processed foods tend to be low-cost and represent energy-dense, nutrient poor foods while unprocessed meat, poultry, fish, low fat milk, vegetables, and fruit had lower energy density, higher NRF9.3 nutrient density scores and were considerably more expensive. Drewnowski and Richonnet explored whether the main ingredient in a food could be used as a marker for diet quality. With a focus on snacks, they found that dairy and fruit, when listed as first ingredients, were associated with higher NRF scores. Therefore, the main ingredients in a food have the potential to be used by consumers to quickly differentiate snacks on nutritional value. However, the question remains whether this potentially easy to understand information is sufficient to drive consumers to a NRF choice. Cost also appears to be an important driver of choice, as partly underscored by results of Maillot et al. who found that children from lower-income groups drank less low fat milk and water and more whole milk and caloric sugar sweetened beverages. Another important driver of consumption is food preference. Nutrient poor foods often differ in their taste profiles from NRF by having a sweet, salty, fatty taste profile. Liem and Russell concluded that there is large individual variation in adult consumers' preference for sweet taste, salt taste, and fat taste/texture. This may offer opportunities for other important drivers than taste e.g., smell and texture, to impact food choice and consumption and to improve the nutritional quality of diets.

In conclusion, the present Research Topic provides several examples that both plant- and animal foods are needed to ensure optimal diets in relation to optimal health. The present Research Topic also suggests that the ratio between plant- and animal products needs further research, in which the use of a scientifically valid NRF score might play a key role. Drivers to improve the consumer's intake of NRF are likely to include cost and easy to understand information, in combination with an optimal sensory profile.

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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Characterizing Ultra-Processed Foods by Energy Density, Nutrient Density, and Cost

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Background: The NOVA food classification scheme divides foods into ultra-processed, processed, unprocessed, and culinary ingredients. Ultra-processed foods contribute >60% of energy to diets in the US.

Objective: To characterize ultra-processed foods by energy density, nutrient density, and monetary cost.

Methods: The 384 component foods of Fred Hutch (FHCRC) food frequency questionnaire (FFQ), were assigned to 4 NOVA categories and to 7 USDA MyPyramid food groups. Energy density was kcal/g. Nutrient density was measured using the Nutrient Rich Food index $NRF_{9.3}$. Food prices were collected in local supermarkets from 2004 to 2016. Analyses examined time trends in food prices by NOVA category and by USDA food group.

Results: The ultra-processed classification captured mostly grains (91%), fats and sweets (73%), dairy (71%), and beans, nuts and seeds (70%), but only 36% of meat, poultry and fish, 26% of vegetables, and 20% of fruit. Compared to unprocessed foods, ultra-processed foods had lower nutrient density ($NRF_{9.3}$ per 100 kcal: 21.2 vs. 108.5),higher energy density (mean (SD): 2.2 vs. 1.10 in kcal/g), and lower per calorie cost (0.55 vs. 1.45 in $/100 kcal). Ultra-processed foods did not increase in price as much as unprocessed foods over the 12 year period.

Conclusion: Ultra-processed foods tend to be energy-dense, low-cost, and nutrient-poor. Low energy cost could be one mechanism linking ultra-processed foods with negative health outcomes. Food-based Dietary Guidelines may need to address food processing in relation to economic aspects of food choice.

Keywords: NOVA classification, energy density, $NRF_{9.3}$, monetary cost, ultra-processed foods, unprocessed foods, food frequency questionnaire (FFQ), food-based guidelines

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INTRODUCTION

The NOVA food classification (1) has sought to establish food processing as the primary index of food quality. The four classes of foods were ultra-processed, processed, and unprocessed, as well as culinary ingredients (fat, sugar, salt) (2, 3). The definition of ultra-processed foods has varied over the years (1) and has not always been consistent (4–8). Ultra-processed foods were initially defined as industrial formulations with fats, sugars, and salt added during preparation, alongside

other substances not used in normal cooking. Unprocessed foods were defined as those that were either fresh or that had gone through minimal processing (drying, freezing, pasteurization, or fermentation) mainly to make them safer, accessible and palatable (1). Most studies have contrasted the health impact of industrially engineered multi-ingredient ultra-processed foods with fresh or frozen vegetables and fruit and with unprocessed meat, poultry, and fish.

The level of food processing rather than the foods' nutrient content has thus been suggested as a potential framework for food and nutrition policy (9, 10). Ultra-processed foods, now linked to metabolic syndrome (11, 12), cancer (13), and all-cause mortality (14) are reported to pose a significant threat to human health (2, 15). Analyses of the nationally representative National Health and Nutrition Examination Surveys (NHANES) 2009–2010 suggest that ultra-processed foods accounted for 57.9% of dietary energy and almost 90% of added sugars in diets of US adults (16, 17). Purchase data for >1.2 million products from the 2000–2012 Homescan panel suggested that more than three-fourths of food energy purchased by US households came from highly processed foods and beverages (61%) (17). Similar figures were obtained for Canada (62%) (18) and the UK (63%) (19).

Most of the existing literature on ultra-processed foods, diet quality, and health outcomes has been based on examination of household food purchases (17) or individual diets (16). At this point little is known about the monetary cost of foods by NOVA category (20–22).

This study examined foods assigned into NOVA categories or USDA food groups by energy density, nutrient density, and cost. Energy density was expressed as kcal/100g. Nutrient density was based on the Nutrient Rich Food Index $NRF_{9.3}$ (23). Retail food prices were obtained from local supermarkets Seattle-King County over the period of 12 years (2004–2016). The specific aims of the study were as follows—(a) To examine the quality of ultra-processed foods using a novel nutrient density metric, $NRF_{9.3}$; (b) to examine the relative cost of NOVA categories, (c) to study trends in food prices over 12 years (2004–2016) by for unprocessed, processed and ultra-processed foods.

METHODS

The Fred Hutch (FHCRC) Food frequency questionnaire (FFQ) is a standard dietary data collection tool, widely used in large scale studies such as The Women' Health Initiative and Nurses' Health Study (4, 5).

The FHCRC FFQ was constructed based on 384 component or "recipe" foods that are commonly consumed in the US. The list of these foods was developed based on NHANES and Minnesota Nutrition database. The FFQ list of foods is very specific in terms of whether the item is fresh, frozen, or canned, and which are commercially available or prepared at home. Following those specifics, the lowest retail price at which each item was available were collected. Details of the FFQ methodology have been published (24).

Abbreviations: FFQ, Food frequency questionnaires; NRF, Nutrient Rich Food; FHCRC, Fred Hutchinson Cancer Research Center; NHANES, National Health and Nutrition Examination Survey; RTE, Ready to Eat.

Classifying Foods by Food Groups and Processing

The present novel approach was to apply the NOVA classification scheme to the 384 component foods (**Supplementary file**) in the well-established Fred Hutch food frequency questionnaire (FFQ). First, each food was assigned into one of 7 USDA MyPyramid food groups: dairy; meat, poultry and fish; beans, nuts, and seeds; grains; fruit and juices; vegetables; and fats and sweets (25). Second, each food was also assigned into one of 4 NOVA categories: ultra-processed, processed, unprocessed and culinary ingredients (3). Two researchers applied the NOVA and MyPyramid food group classification independently on 384 FFQ food items. Coefficient correlation was applied to test the inter-rater reliability. There were only 8 items for which discordance was found. Researchers then met to discuss those food items, and came to mutually agreed classification. Following published NOVA classification guidelines, unprocessed foods were defined as fruits, vegetables, grains, or meats that had been subjected to minimal or no processing. These could be fresh, dry, or frozen. Unprocessed foods included fresh meat, milk and plain yogurt, vegetables, eggs, legumes, fish, and other seafood, and unsalted nuts and seeds. Fruit juice was included if freshly squeezed. Tea and coffee were deemed to be unprocessed. Breads were unprocessed if simple and home-made.

Culinary ingredients were sugar, animal fats (butter) and vegetable oils, starches, salt, and vinegar (16). Processed foods were manufactured by adding culinary ingredients (fat, sugar, salt) to wholesome fresh foods. Those foods included cheese, ham, salted, smoked, or canned meat or fish, pickled vegetables, salted or sugared nuts, beer, and wine.

Ultra-processed foods were defined as industrial creations, which contained ingredients not found in home cooking, in addition to fat, sugar, and salt. Ultra-processed foods included commercial breads (refined and whole grain), ready-to-eat breakfast cereals, cakes, sweet snacks, and pizza, French fries, soft drinks (sodas and fruit drinks), ice cream, and frozen meals and soups. In the NOVA scheme, mass-produced whole grain breads, commercial sweetened yogurts, commercial fruit juices, and ready to eat cereals all fell into the ultra-processed category.

According to the NOVA classification, the most desirable foods were those that were fresh and minimally processed and were prepared, seasoned and cooked from scratch during ordinary culinary preparations at home (26).

Developing Food Quality Metrics: Energy Density and $NRF_{9.3}$

Energy density is the ratio of total energy intake over daily weight of total foods consumed (kcal/g) (27). Nutrient Rich Food Index 9.3 ($NRF_{9.3}$) was used as a measure of nutrient density (23). The $NRF_{9.3}$ assigns a nutrient quality score to each food item based on nine qualifying nutrients (protein, fiber, Vitamin A, C, and D, calcium, iron, magnesium, potassium) and three nutrients to limit (saturated fats, added sugar, and sodium) (23).

The final score is the sum of percent daily values for 9 nutrients to encourage minus the sum of percent maximum recommended values for 3 nutrients to limit. All daily values were calculated per 100 kcal capped at 100% for positive nutrients.

$$NRF_{9.3} = \left(\sum_{1-9} (\text{Nutrient/DV}) \times 100\right) - \left(\sum_{1-3} (\text{Nutrient/MRV}) \times 100\right) \quad (1)$$

The Seattle King County Food Prices Database: 2004–2016

Seattle's food prices database 2004–2016 contains lowest retail price for each of the FFQ component foods, collected in 3 large supermarket chains (Safeway, QFC, Albertsons) every 2 years from 2004 to 2016. Standardized data collection protocols, described in past studies, were used (24, 27). Each data collection period was between April and July to account for seasonality. Data were collected during in-store visits and compared to store website prices (Safeway) where available. Temporary promotions, specials, and discounts were excluded.

Shelf and unit prices were corrected for yield, using USDA Handbook 102 (28), to compute food prices per 100 g edible portion. Yield values reflect the edible proportion for each food item after taking losses due to inedible portions or cooking loss into account. Prices per 100 g were then adjusted for energy density for each food item to provide prices per 100 kcal. Price per 100 g of edible portion and price per 100 kcal served as the two primary indicators to study the cost gradient and time trends.

Statistcial Analyses

Descriptive statistics examined the distribution of FHCRC food items by USDA food groups and by NOVA classification. Mean (SD) and median values of energy density and NRF9.3, and mean and median food prices ($/100 g and $/100 kcal) were computed for each group and processing category. ANOVA was applied to compare within group differences. Price trends were analyzed over the 12 years period (2004–2016). For analytical purpose, a list of 371 FFQ component foods were used after excluding 11 outliers (mostly fresh fish, fresh oysters, clams, halibut and crab) and 2 items with missing price data for one or more years. Sensitivity analyses were conducted before and after excluding the outliers. All statistical analyses were conducted using SPSS 22 statistical software and Microsoft Excel (2016).

RESULTS

Figure 1 shows the percent distribution of FFQ component foods by MyPyramid food groups and by 4 food processing categories. 27.5% of the FFQ foods were constituted by grains, followed by meat, poultry and fish (19.4%), vegetables (16.4%), fats and sweets (11.9%), fruits and fruit juices (11.1%), and 6.2% by beans, nuts, and seeds. More than half of the FFQ component foods (57%) fell into the ultra-processed category, with 33% into unprocessed category, and 7% in processed category.

Figure 2A shows the degree of food processing by each food group. Grains were pre-dominantly constituted by ultra-processed foods (91%), followed by fats and sweets (73%), dairy

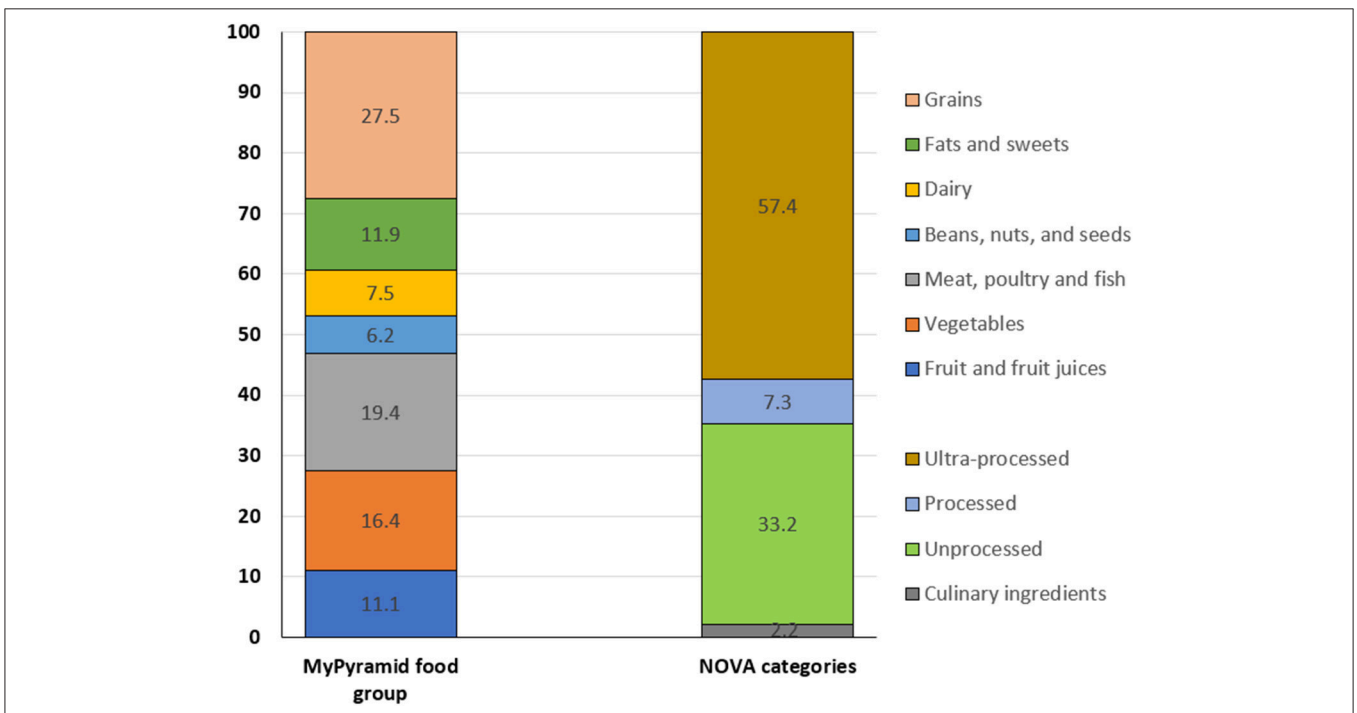

FIGURE 1 | Percent distribution of FFQ component foods by USDA MyPyramid Food groups and NOVA categories.

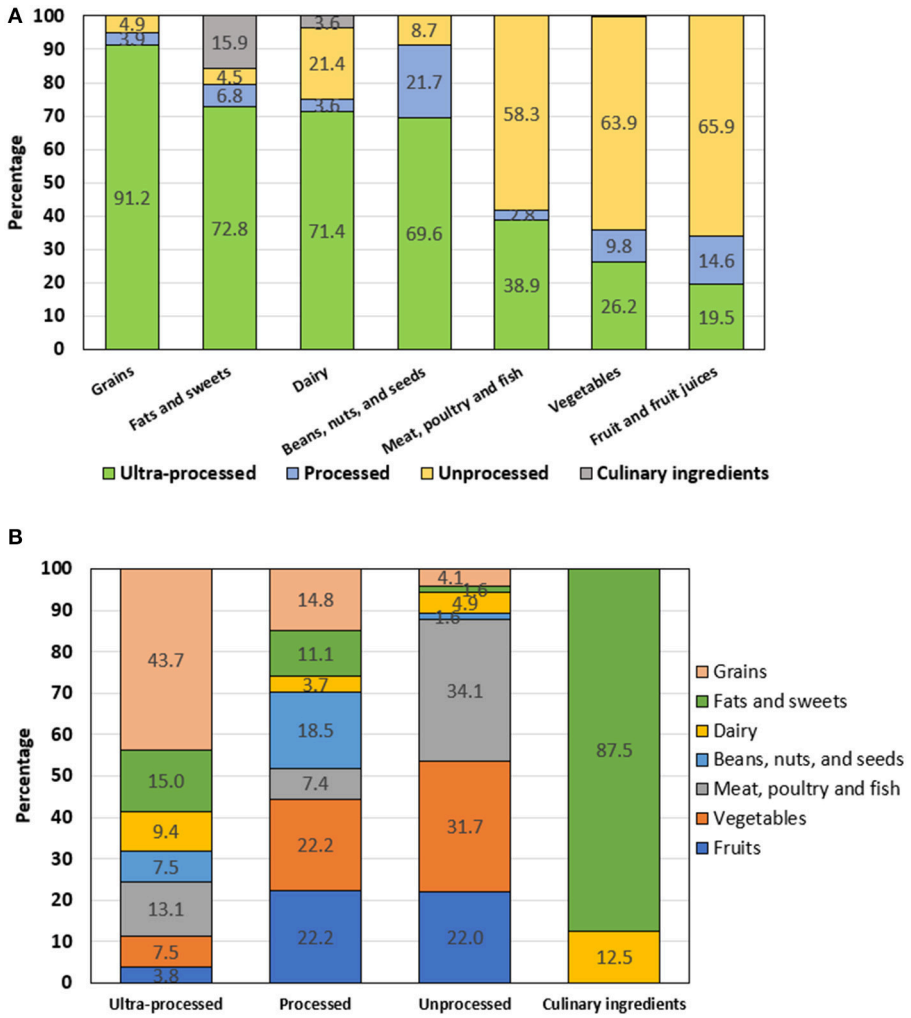

FIGURE 2 | (A) Percent distribution of NOVA categories by MyPyramid food group. **(B)** Percent distribution of MyPyramid food groups by NOVA category.

(71%), and beans, nuts and seeds (70%). Vegetables and fruits contained the lowest proportion of ultra-processed foods (26 and 20%, respectively). In other words, almost 60% of the vegetables, fruits and meat, poultry and fish groups were constituted by unprocessed foods in FHCRC FFQ. **Figure 2B** shows the reverse cross-tabulation, i.e., the distribution of each of 4 food processing categories by food groups. More than 40% of the ultra-processed foods were grains, followed by fats and sweets (15%), and meat, poultry, and fish (13.1%). Unprocessed foods, on the other hand, were mostly fruits and vegetables (54%), followed by meat, poultry, and fish (34%). Culinary ingredients were largely fats and sweets (87.5%).

Table 1 shows the gradient in food groups and food processing categories by two measures of quality (energy density and NRF 9.3 per 100 kcal). Among food groups, fruits, and vegetables had the highest $NRF_{9.3}$ scores [mean (SD): 93.7 (67.3) and 150.0 (117.7), respectively] and the lowest energy density [0.6 (0.6) and 0.7 (0.7) kcal/g, respectively]. Fats and sweets had the highest energy density [2.9 (2.8) kcal/g] but lowest nutrient density [NRF: −1.08 (57.9)]. By degree of food processing, ultra-processed foods had higher energy density [2.3 (1.5) kcal/g] but lower $NRF_{9.3}$ scores [21.2 (52.2)] than did unprocessed foods. Unprocessed foods had highest $NRF_{9.3}$ score [108.5 (100.1)] and lowest energy density [1.10 (0.9) Kcal/g]. ANOVA was applied to compare within group difference. Both energy density and NRF values were statistically significant among both food groups and food processing categories.

If we do tertile of NRF scores, 61% of unprocessed food fall into high NRF score category but only 4% in low NRF score category whereas 50% of ultra-processed food fall in low NRF score category and only 17% in high NRF score category (**Figure 3**). Unprocessed foods undoubtly fall in high NRF category as they have vitamins and mineral, have low energy density and are unprocessed/fresh like meat, fruit, and vegetable. However, some of the ultra-processed foods fall into nutrient rich category.

TABLE 1 | Energy density and nutrient density of 371 FFQ component foods by MyPyramid food group and NOVA category.

		Energy density (Kcal/g)			Nutrient density (NRF 9.3) per 100 kcal		
	N	Mean (SD)	95% CI	Median (IQR)	Mean (SD)	95% CI	Median (IQR)
All items	**371**	**1.96 (1.68)**	**[1.79, 2.13]**	**1.58 (2.22)**	**50.38 (81.95)**	**[41.99, 58.76]**	**21.40 (65.99)**
MYPYRAMID FOOD GROUPS							
Milk and milk products	28	1.66 (1.00)	[1.28, 2.05]	1.41 (1.12)	13.66 (29.67)	[2.16, 25.17]	4.18 (38.75)
Meat, poultry, and fish	72	2.01 (0.79)	[1.82, 2.19]	1.92 (0.86)	29.08 (36.32)	[20.55, 37.61]	23.41 (25.52)
Beans, nuts, and seeds	23	2.04 (1.91)	[1.21, 2.87]	1.13 (1.48)	46.73 (32.69)	[32.59, 60.86]	44.29 (53.26)
Grains	102	2.86 (1.38)	[2.59, 3.12]	2.89 (2.36)	21.39 (47.75)	[12.01, 30.77]	10.48 (25.79)
Vegetables	61	0.68 (0.69)	[0.51, 0.86]	0.37 (0.64)	150.01 (117.74)	[119.86, 180.16]	167.26 (201.07)
Fruits	41	0.66 (0.58)	[0.48, 0.84]	0.48 (0.25)	93.78 (67.30)	[72.26, 115.30]	86.70 (119.74)
Fats and sweets	44	2.93 (2.88)	[2.05, 3.80]	1.85 (4.35)	−1.08 (57.93)	[−18.91, 16.74]	−13.20 (48.10)
NOVA CLASSIFICATION							
Unprocessed	123	1.10 (0.87)	[0.95, 1.26]	0.84 (1.49)	108.50 (100.06)	[90.56, 126.43]	64.19 (144.64)
Processed	27	2.02 (2.03)	[1.22, 2.83]	0.85 (3.15)	37.95 (41.07)	[21.70, 54.19]	18.66 (48.84)
Ultra-processed	213	2.28 (1.54)	[2.07, 2.49]	2.00 (2.38)	21.23 (52.23)	[14.16, 28.30]	8.94 (36.56)
Culinary ingredients	8	6.36 (3.14)	[3.74, 8.99]	8.00 (5.59)	−21.78 (20.33)	[−38.78, −4.78]	−15.28 (40.32)

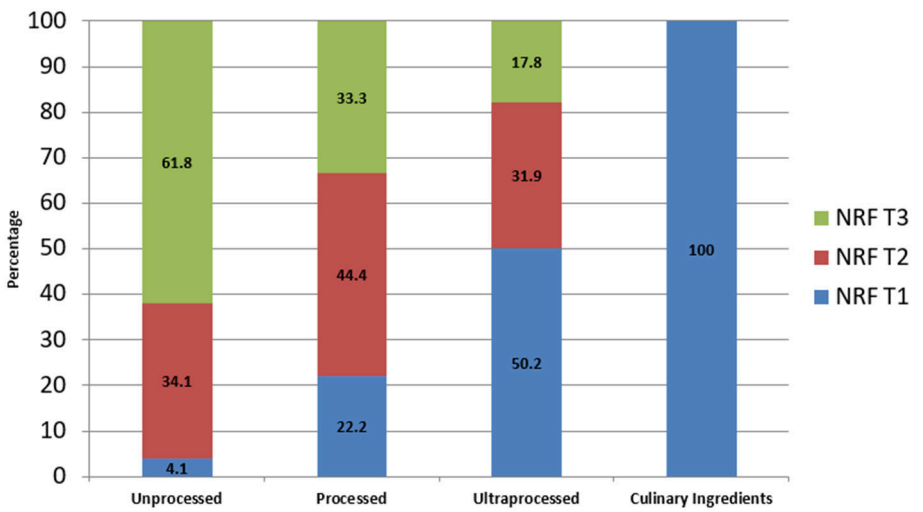

FIGURE 3 | Percent distribution of NOVA categories by tertiles of NRF scores.

Table 2 shows the cost gradient, calculated per 100 kcal and per 100 g, for 371 FFQ component foods. Prices were compared across both food groups and food processing categories using ANOVA test. Vegetables and fruits had the highest cost per 100 kcal [mean (SD): 1.7 (1.4) and 1.3 (1.1), respectively], whereas grains and dairy were the lowest [mean (SD): 0.4 (0.4) and 0.4 (0.3), respectively]. Meat, poultry and fish, and nuts and seeds fell in the middle. By degree of food processing, ultra-processed foods cost \$0.55/100 kcal; processed foods cost \$0.64/100 kcal, and unprocessed foods cost \$1.45/100 kcal. Prices for unprocessed foods were significantly above all other NOVA categories ($p < 0.05$). The lowest cost was for culinary ingredients, mostly fats, oils and sweeteners: \$0.14/100 kcal.

Mean food prices increased by about 37% from 2004 to 2016, as summarized in **Figure 4**. Ultra-processed foods (grains, fats and sweets) rose in price less than did unprocessed foods (fruit, vegetables and fresh meat, poultry and fish). On per calorie basis (\$/100 kcal), price increases were \$0.14 for ultra-processed foods \$0.13 for processed food, and \$0.41 for unprocessed foods, and 0.04\$ for culinary ingredients.

DISCUSSION

This is one of the first few studies to explore nutrient density, energy density, and monetary cost of foods by the degree of processing. Ultra-processed foods were found to be low-cost,

TABLE 2 | Mean 2016 food prices (USD per 100 g and 100 kcal edible portion) by food groups and food processing category.

		Cost per 100 g			Cost per 100 kcal		
	N	$/100 g (SD)	95% CI	Median (IQR)	$/100 kcal (SD)	95% CI	Median (IQR)
All items	**371**	**0.93 (0.76)**	**[0.86, 1.01]**	**0.72 (0.94)**	**0.85 (1.00)**	**[0.75, 0.95]**	**0.49 (0.80)**
MYPYRAMID FOOD GROUPS							
Grains	102	0.94 (0.60)	[0.82, 1.05]	0.89 (0.70)	0.39 (0.39)	[0.32, 0.47]	0.28 (0.30)
Fats and sweets	44	0.67 (0.75)	[0.44, 0.90]	0.44 (0.75)	0.64 (1.18)	[0.28, 1.00]	0.26 (0.47)
Milk and milk products	28	0.58 (0.62)	[0.34, 0.82]	0.37 (0.47)	0.37 (0.31)	[0.24, 0.48]	0.28 (0.24)
Beans, nuts, and seeds	23	0.90 (0.89)	[0.51, 1.28]	0.52 (0.72)	0.62 (0.61)	[0.36, 0.89]	0.39 (0.70)
Meat, poultry, and fish	72	1.58 (0.83)	[1.39, 1.78]	1.55 (1.07)	0.90 (0.59)	[0.76, 1.04]	0.81 (0.60)
Vegetables	61	0.66 (0.57)	[0.51, 0.80]	0.48 (0.43)	1.68 (1.44)	[1.31, 2.05]	1.25 (2.16)
Fruits	41	0.76 (0.60)	[0.57, 0.95]	0.58 (0.70)	1.33 (1.11)	[0.98, 1.68]	0.90 (1.48)
NOVA CLASSIFICATION							
Ultra-processed	213	0.93 (0.78)	[0.82, 1.04]	0.70 (0.88)	0.55 (0.61)	[0.47, 0.64]	0.38 (0.41)
Processed	27	0.71 (0.41)	[0.55, 0.87]	0.66 (0.60)	0.64 (0.48)	[0.45, 0.83]	0.53 (0.73)
Unprocessed	123	1.01 (0.79)	[0.86, 1.15]	0.76 (1.31)	1.45 (1.33)	[1.21, 1.68]	0.99 (1.52)
Culinary ingredients	8	0.73 (0.69)	[0.15, 1.30]	0.42 (0.82)	0.14 (0.12)	[0.04, 0.24]	0.10 (0.19)

energy dense and nutrient poor as compared to unprocessed foods. These findings resonate with past studies suggesting ultra-processed foods as being energy-dense, high in saturated fat, added sugar, and salt and poor sources of protein, dietary fiber, and micronutrients (16, 29).

Utilizing component foods of a well-established FFQ instrument is one way to study the relative cost of different food groups. Separating the foods by NOVA classification and by the USDA food groups yielded some insights. First, as expected, most vegetables and fruits, fresh, frozen, or dried fell into the unprocessed category, as did meat, poultry and fish. Dairy was split into unprocessed (milk), and ultra-processed (commercial yogurt). Second, also as expected, unprocessed meat, poultry, fish, low fat milk, and vegetables and fruit had lower energy density and higher $NRF_{9.3}$ nutrient density scores. Third, the unprocessed foods were more nutrient rich but they were also considerably more expensive.

The ultra-processed NOVA category captured not only fats and sweets (the stated intent) but also most of the commercially prepared breads and cereals, as well as beans, nuts, and seeds. Water, which provides weight but no calories, influences the energy density of foods more than does any macronutrient, including fat (30). As expected, ultra-processed grains, fats and sweets had higher energy density and lower $NRF_{9.3}$ nutrient density scores than did unprocessed foods. Consistent with past observations (16, 17, 31), grains, fats and sweets cost less per calorie than did unprocessed foods.

It would appear that the ultra-processed NOVA designation is a new name for energy-dense grains, fats and sweets. These foods are energy dense, can be nutrient poor, and are distinguished by their low per calorie costs. By contrast, the NOVA unprocessed category successfully captured some of the same food groups that multiple NRF schemes have previously recognized as nutrient-rich.

The NOVA categories were characterized by sharply different food costs. Ultra-processed foods had lower $NRF_{9.3}$ scores than did unprocessed foods but were also much less expensive. Dietary intake studies, applying the NOVA classifications to total diets, will determine how the NOVA classification is linked to sociodemographic determinants of diet choice: minority status, education, and incomes.

While Dietary Guidelines for Americans have emphasized the need to limit energy dense foods, and dietary sugars and fats, most of the US population is not meeting their nutrition goals (32). Having focused on nutrients to limit, the Dietary Guidelines have become more food oriented, specifying amounts of desirable foods and dietary ingredients in healthy food patterns. Food based dietary guidelines can take into account the nature of the food matrix which purely nutrient based calculations are unable to do. Reducing the share of ultra-processed foods in the diet has been suggested as an effective way to improve nutritional quality of diets (26).

The present study had limitations. First, it was based on a market basket of 371 FFQ foods and may not fully capture all the foods consumed by the US population. Second, the pricing was based on the lowest retail price for each item, and the same price was assigned across respondents. However, this is one of the standard widely-used procedures to study diet quality in relation to cost in the literature. The US Department of Agriculture calculates benefits for food assistance by attaching retail prices, similar to the ones here, to dietary intakes data.

Among the strengths of the study were the historical database collected every 2 years since 2004, utilizing the food database that builds the structure for one of the standard dietary data collection tools, and the potential to explore the cost of total diets

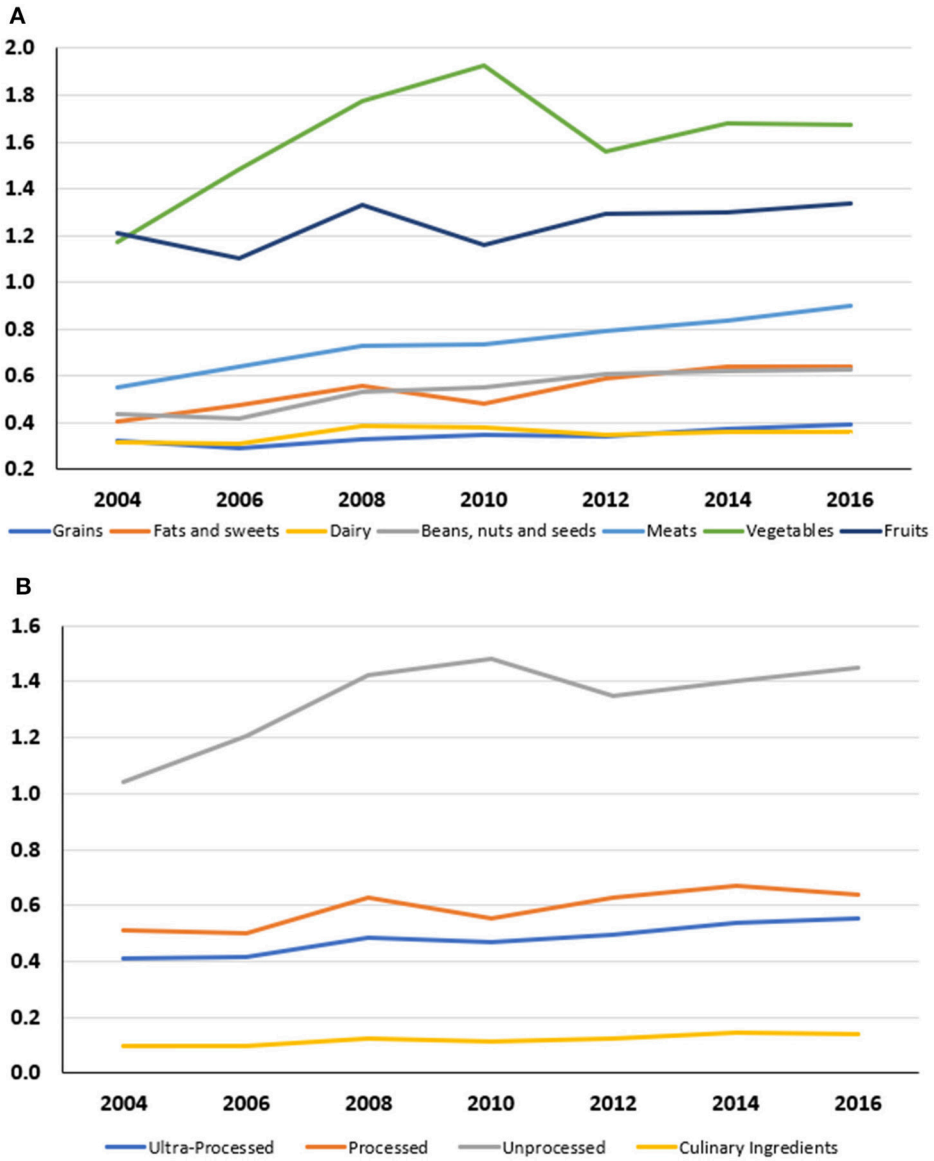

FIGURE 4 | (A) Mean monetary cost in $/100 kcal for FFQ component foods by USDA MyPyramid Food groups (2004–2016). **(B)** Mean monetary cost in $/100 kcal for FFQ component foods by NOVA categories (2004–2016).

featuring fresh vs. ultra-processed foods. The cost component was notably missing from virtually every study published on the topic of the NOVA classification scheme (2, 3, 16, 17, 26, 31, 33–36). Applying NOVA classification to dietary intakes, using standard dietary tools such as FFQ, will help placing processed and ultra-processed foods in the context of total diets.

CONCLUSION

The study has implications for future research and policy efforts. Applying food processing classifications to prospective dietary studies will help clarify the impact of food processing on health outcomes. Food-based Dietary Guidelines focusing on dietary components or foods by degree of processing may be one strategy to make recommendations intuitive for the consumer; developing an overall processing index of the diet might be another.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

AUTHOR CONTRIBUTIONS

SG led data analyses. She assisted in conceptualizing the manuscript, data interpretation, and manuscript writing. TH led data collection and assisted in data analysis. AA led study data collection, assisted in conceptualizing the manuscript, data analyses, data interpretation, and manuscript writing. AD led the study design, data collection, conceptualization of the manuscript, and manuscript writing. All the authors reviewed and approved the submitted version of the manuscript.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Dietary Protein Quantity, Quality, and Exercise Are Key to Healthy Living: A Muscle-Centric Perspective Across the Lifespan

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A healthy eating pattern, regardless of age, should consist of ingesting high quality protein preferably in adequate amounts across all meals throughout the day. Of particular relevance to overall health is the growth, development, and maintenance of skeletal muscle tissue. Skeletal muscle not only contributes to physical strength and performance, but also contributes to efficient macronutrient utilization and storage. Achieving an optimal amount of muscle mass begins early in life with transitions to "steady-state" maintenance as an adult, and then safeguarding against ultimate decline of muscle mass with age, all of which are influenced by physical activity and dietary (e.g., protein) factors. Current protein recommendations, as defined by recommended dietary allowances (RDA) for the US population or the population reference intakes (PRI) in Europe, are set to cover basic needs; however, it is thought that a higher protein intake might be necessary for optimizing muscle mass, especially for adults and individuals with an active lifestyle. It is necessary to balance the accurate assessment of protein quality (e.g., digestible indispensable amino acid score; DIAAS) with methods that provide a physiological correlate (e.g., established measures of protein synthesis, substrate oxidation, lean mass retention, or accrual, etc.) in order to accurately define protein requirements for these physiological outcomes. Moreover, current recommendations need to shift from single nutrient guidelines to whole food based guidelines in order to practically acknowledge food matrix interactions and other required nutrients for potentially optimizing the health effects of food. The aim of this paper is to discuss protein quality and amount that should be consumed with consideration to the presence of non-protein constituents within a food matrix and potential interactions with physical activity to maximize muscle mass throughout life.

Keywords: children, adolescents, aging-old age-seniors, skeletal muscle mass, muscle protein synthesis/breakdown, leucine, anabolic

INTRODUCTION

The development of a healthy eating pattern, or the identification of the best food combinations and amounts to include in the diet, is relevant to support physical performance, weight management, and reduce disease risk. In terms of protein-containing foods, protein quality, and amount are two major considerations within the development of a healthy eating pattern irrespective of age. Food protein quality is traditionally dependent on its amino acid content and the availability of these amino acids in circulation, factors that would influence their metabolism within different body protein pools. Hence, protein quality is often based on protein digestibility ranking methods such as protein digestibility-corrected amino acid score (PDCAAS) or the digestible indispensable amino acid score (DIAAS), as will be highlighted below. The latter method has gained favor by the Food and Agriculture Organization of the United Nations (FAO) after the most recent review of the "best" methods to determine protein quality for human nutrition (1).

Regardless of the method used to measure protein digestibility in human foods (1), it is also important to consider coupling protein digestibility scoring metrics with other relevant human metabolic processes (2), such as the ability to influence protein turnover (i.e., synthesis and degradation) of body proteins. Given the primary role of dietary amino acids are to support protein metabolic demand and cover obligatory protein losses (3), it is perhaps important to consider coupling protein digestibility scoring methods with direct measurements of protein synthesis rates (e.g., within skeletal muscle) and whole body amino acid oxidation rates. For example, amino acids can only be "stored" within functional proteins, which given its size and nutrient sensitivity, positions skeletal muscle protein as a primary reservoir for dietary amino acids (4). Therefore, confirming that ingested protein foods are stimulating postprandial muscle protein synthesis rates without excessive amino acid oxidation rates provides confirmation that the available dietary amino acids in circulation are being used to support this vital tissue.

Protein requirements are set as a minimal need to prevent net nitrogen losses, but arguably are not sufficient to account for all factors contributing to quality of life throughout the lifespan (e.g., exercise habits, aging, hospitalization, or disease) (5). As such, there has been an impetus for a change for better a definition of "optimal" protein intake (5, 6). It has been suggested that greater focus on skeletal muscle is relevant when the goal is to define an optimal requirement of protein intake, especially throughout older adult life (7). The rationale behind this idea is that skeletal muscle represents a large proportion of total body protein in adults, or a large storage depot of energy and dietary amino acids, and contributes ∼25–30% to whole body protein synthesis rates (8). Moreover, muscle has an obvious role in physical performance, but metabolically has important roles in the regulation of glucose disposal (*cf.* insulin resistance), fat oxidation, and energy balance (9). This ostensibly highlights its maintenance as especially pertinent throughout middle and old age. However, the increasing prevalence of metabolic disorders in pediatric populations and the potential for early programming of muscle tissue for later life (10, 11) likely refocuses the issue of optimizing muscle quantity and quality as being essential across the lifespan.

In this review, therefore, we discuss the role of dietary protein quality and quantity in terms of optimizing muscle mass from childhood to old age as a goal toward maintaining metabolic health and physical performance. We also discuss that a transition to a holistic framework within the area of protein nutrition is likely required to truly define optimal protein intakes for muscle. This involves shifting the focus from determining the effect of single nutrients (or the food parts) on metabolic outcomes, in favor of considering how an integrative holistic approach (e.g., exercise habits, eating pattern, and the food matrix in which the protein is consumed) affects the overall protein recommendation and associated muscle metabolic outcomes.

DIETARY PROTEIN QUALITY

It is recommended that a healthy eating pattern consists of ingesting a variety of high quality protein foods to ensure a sufficient supply of amino acids for lean mass (e.g., muscle) maintenance or growth, and overall diet quality (12). In other words, the constituent amino acids of a protein food should match the requirement of the consumer and consist of a variety of protein foods to ensure nutrient density. Indeed, there has been a shift toward plant-based dietary patterns within dietary guidelines to presumably maximize population health benefits and to support environmental sustainability (13). For example, epidemiological findings suggest that minimizing red meat consumption within a dietary pattern is favorable to reduce disease risk (type 2 diabetes, cardiovascular disease, etc) (14, 15). However, these data are challenging to interpret as the comparison diet is often a confounding factor (e.g., different macronutrient compositions, processed vs. unprocessed, or varying fat percentages of meat intake) (16) and/or the lack of control of the physical activity patterns of the participants. It is also relevant to highlight that defining protein intakes based on very general definitions such as "ounce-equivalents" as provided by the USDA's Choose My Plate is discounting the value of protein quality scores and caloric intakes required to meet minimal essential amino acid requirements between animal vs. plant-based foods as indicated elsewhere (17).

The role of dietary protein quality is also perhaps relevant when defining more sustainable diets to meet the nutritional needs of an expanding global population (18). Protecting the planet (i.e., managing greenhouse gas emissions to land and water use) and living sustainably are also important topics in dietary protein quality considerations (19). As such, it is clear that there needs to be a range of methods to evaluate protein quality in order to titrate the claim for food as "high" quality depending on the desired physiological outcome. This also needs to be balanced against the potential environmental impact and importance of maximizing the use of natural resources for production of high quality proteins that provide target amounts of essential amino acids for muscle mass maintenance or growth (19).

DIAAS is the current protein quality ranking method that is recommended by the Food and Agriculture Organization of the United Nations (FAO) (20). The rationale behind this recommendation is that protein digestibility (quality) estimates should be based on true ileal digestibility (i.e., determined at the end of the small intestine where amino acids are absorbed), and ideally performed in humans. Thus, this method aims to determine what amino acid(s) may be limiting in circulation after accounting for digestion and absorption to support whole body protein metabolism. It is not feasible, however, to routinely perform ileal digestibility in humans. Hence, the growing pig model is often used due to the similarly between the digestive tract between pigs and humans, and the willingness of pigs to eat foods within the human diet (21). DIAAS cut-off values have been proposed to provide a basis for protein quality claims, while accounting for the quantity of protein ingested, such as excellent/high (100 or more), good (22–46), and no claim (<75) (20).

Much of the DIAAS work has been done on raw foodstuffs with more recent work focusing on how cooking method impacts food protein quality (47). This is relevant as many of the commonly consumed protein foods within the human diet have experienced heat treatment prior to consumption, which may impact its amino acid content and overall nutritional quality (48). As shown in **Table 1**, it has been established that cooking method (i.e., raw, boiled, grilled, pan-fried, or roasted) of meat affects its structural properties and subsequent DIAAS (47). It generally accepted that cooking (internal temperature of 70°C) increases protein digestibility by denaturing the protein and thus allowing greater bio-accessibility of proteolytic enzymes to its cleavage sites (47, 53). However, it was demonstrated that DIAAS was superior for the raw, boiled, and pan-fried minced beef conditions when compared to roasted or grilled beef in growing pigs (47). Collectively, these data highlight that the food matrix, such as food structure, can be manipulated by heat treatment to modulate protein quality scores. It is important to keep in mind, however, that severe heat treatment, or prolonged storage, can impact the nutritional value of amino acids (e.g., lysine) (54).

Certainly, it is more common to eat mixed meals, as opposed to single nutrients, throughout the day and thus it is relevant to have protein quality scores within the context of mixed foods/ingredients to better inform the various regulatory dietary frameworks (55). The challenge with this food-first approach could be identifying, let alone testing, the myriad of combinations of different food items to assess mixed food/ingredient interactions. However, research has begun to address this challenge through combinations of macronutrient co-ingestion. For example, in terms of protein digestibility, it has been established that the co-ingestion of lipids with protein improves protein digestibility/quality in growing pigs by slowing gastric emptying rates to allow more time for the ingested protein to be exposed to proteolytic enzymes and/or reducing passage rate within the small intestine to allow more time for the amino acids to be absorbed (56).

What is noteworthy, however, is that researchers have developed tools to assess the quality of dietary protein sources for the benefit of supporting whole body and muscle protein remodeling. Specifically, intrinsically labeled food proteins, whereby stable isotope tracers are incorporated into the protein matrix, are more readily applied within a human model to provide an index of protein digestibility and subsequent dietary amino acid availability after food ingestion (57–59). Using a labeled food protein approach, it has been established that macro-nutrient co-ingestion with isolated protein sources modulates postprandial protein derived amino acid availability in circulation, but not the stimulation of postprandial muscle proteins synthesis rates in healthy adults (60, 61). This highlights the potential disconnect between postprandial protein derived amino acid ability in circulation and the subsequent postprandial muscle protein synthetic response that may otherwise be missed without a metabolic tracer that can be tracked from mouth to muscle (60–63). These findings provide support for the notion that protein quality scores need to be coupled with other physiological correlates (e.g., protein turnover) to better define the impact of protein foods from a more "whole-human"

TABLE 1 | Cooking method and its impact on protein quality scores.

	Raw/ Extruded	Boiled	Grilled/ Baked	Pan-fried	Roasted	Source
Surface temp. (°C)		∼80	∼193–225	∼186	∼160	
Valine						
Beef	0.97	0.99	0.80	0.98	0.91	(47)
Pinto beans	0.92	0.95	0.69			(49)
Green peas	0.93	0.98	0.89			(50)
Green lentils	0.80	0.93	0.86			(51)
Isoleucine						
Beef	1.25	1.25	1.11	1.23	1.15	(47)
Pinto beans	1.02	1.23	0.72			(49)
Green peas	1.03	1.16	1.06			(50)
Green lentils	1.11	1.05	0.91			(51)
Leucine						
Beef	1.09	1.11	0.97	1.08	0.99	(47)
Pinto beans	1.13	1.17	0.74			(49)
Green peas	1.00	1.13	1.00			(50)
Green lentils	1.02	1.04	0.83			(51)
Lysine						
Beef	1.28	1.21	1.11	1.11	1.12	(47)
Pinto beans	0.86	1.09	0.66			(49)
Green peas	1.07	1.15	1.10			(50)
Green lentils	1.05	1.04	0.79			(51)
DIAAS						
Beef	97[a]	99[a]	80[c]	98[a]	91[b]	(47)
Pinto beans	0.61	0.7	0.44			(49)
Green peas	0.7	0.67	0.7			(50)
Green lentils	0.53	0.49	0.44			(51)

Beef internal temperature is 71 °C in all conditions. Within a row, values without a common superscript letter differ significantly ($P < 0.001$). Hodgkinson et al. (47) determined DIAAS directly based on true ileal amino acid digestibility using the growing pig model. Nosworthy et al. (49–51) estimated DIAAS based on fecal digestibility using a rat model. Lysine content does not represent reactive lysine and thus the bioavailability of digestible lysine might be overestimated (52). DIAAS, digestible indispensable amino acid scores.

TABLE 2 | Protein recommendations throughout the life span as defined by the recommended dietary allowance (RDA), the population reference intakes (PRI), or muscle-centric meal-based recommendations.

Protein recommendations				
		USA RDA (g/kg)	Europe PRI (g/kg)	Muscle-centric Meal-based (g/kg)
Across the lifespan[a]				
Infants	(0–12 month)	1.50	1.31	?
Young children	(1–3 year)	1.10	1.01[b]	?
Children	(4–13 year)	0.95	0.90[b]	0.30[d]
Adolescents	(14–18 year)	0.85	0.86	0.30[d]
Adults	(19–70 year)	0.80	0.83	0.25
Pregnancy, lactation		1.10	1.07[c]	?
Aging Adult	(>70 years)	0.80	0.83	0.40

Note that the RDA and PRI values are prescribed on a daily basis and obscuring the value of protein distribution and meal frequency as important factors for the stimulation of postprandial muscle protein synthesis rates. Meal-based recommendations should be consumed 4–5 times daily based on normally consumed meal-times (e.g., breakfast, lunch, snack, dinner, evening snack).

[a]Age ranges based on United States Department of Agriculture definition; [b]mean of intake values for ages within given age range; [c]calculated based on European Food Safety Authority absolute recommendation and reference female body weight; [d]based on whole body protein balance data. ? indicates unknown values.

perspective. This in turn will help inform healthy eating patterns and develop effective public health messaging toward the goal of optimizing muscle mass and health (2).

DEFINING OPTIMAL vs. RECOMMENDED PROTEIN INTAKES

Current protein recommendations, as defined by the recommended dietary allowance (RDA) or population reference intakes (PRI), throughout the lifespan are shown in **Table 2**. Protein recommendations are set as the lowest level of protein intake to prevent net nitrogen loss and reduce disease risk in nearly all (97–98%) healthy individuals at energy balance (64). However, these protein recommendation may not be optimal to support the metabolic needs of highly active individuals such as strength (65) and endurance trained populations (66). This is not completely surprising, however, given that protein requirements are designed to prevent protein deficiencies, which is particularly relevant for children and adults in developing countries but less of an issue in more developed nations (67). Therefore, lifestyle and goals of a given population (e.g., athletic performance, muscle growth/maintenance, functional independence, etc.) need to be considered when identifying minimum and optimal protein intakes.

The "best" method to define an optimal protein intake is certainly a matter of debate (68–70), and will depend on the population studied (e.g., children or adults). Stable isotope tracer methods, such as the indicator amino acid oxidation (IAAO) or direct incorporation methods for the determination of muscle protein synthesis, have shown their utility to define protein recommendations across various ages and in relation to an exercise setting (71–74). We believe that studying nutrient requirements in the context of exercise should be a greater consideration as increasing levels of physical activity, including the incorporation of structured exercise regimes, is unquestionably one of the most important lifestyle behaviors for improved health (75), and is arguably our genetic "evolutionary default" as we were born to move. Importantly, exercise also directly affects nutrient utilization and requirement when compared to the sedentary-state. Hence, dietary and exercise guidelines are inherently connected and should be considered together when the goal is to define "optimal" protein intakes for improved health.

Importantly, exercise mode (strength vs. endurance exercise) directly impacts the metabolism of dietary protein at the whole body and muscle levels (**Figure 1**). For example, resistance exercise is inherently anabolic by improving net muscle protein balance (defined as muscle protein synthesis minus breakdown) for up to 2 days (78). Moreover, the performance of resistance exercise results in greater use of dietary amino acids for the stimulation of postprandial muscle protein synthesis rates during the immediate (0–4 h) (71, 79) and prolonged recovery period (∼24 h) (76, 80). In other words, the ingestion of 10 g of essential amino acids (equivalent to ∼25 g of high quality protein) is required to maximize the ingested protein dose-responsiveness of muscle protein synthesis rates in the sedentary-state (81). In the immediately post-exercise period, however, the ingestion of ∼8.6 g of essential amino acids (equivalent to ∼20 g of high quality protein) is required to plateau the postprandial muscle protein synthetic response (71). This implies that resistance exercise enhances the dietary amino acid sensitivity of muscle protein synthesis such that lower protein amounts are required to elicit a robust anabolic effect when compared to the sedentary-state. Similarly, it has been established that skeletal muscle tissue becomes a larger "sink" for dietary amino acids during recovery from resistance exercise as noted by the increased incorporation of dietary phenylalanine into muscle protein when compared to the sedentary-state (82). Finally, regular strength training results in increased whole-body nitrogen retention vs. the untrained-state (83). With these factors in mind, it seems that a greater ratio of circulating amino acids are being retained by the body's largest protein pool (skeletal muscle) in both the fasting and fed-states after resistance exercise. Such findings suggest that regular strength training is a strategy to optimize dietary protein utilization (**Figure 1**).

Interestingly, endurance exercise appears to be on the other end of the spectrum in terms of its impact on dietary protein utilization. Oxidation of endogenous amino acids may only represent a fraction of total energy provision during exercise (∼2–10% depending on carbohydrate availability), but their utilization increases with endurance exercise intensity (84) and duration (85, 86). For example, estimates of leucine oxidation rates during moderate intensity exercise (∼60% of maximal oxygen uptake; VO2max) are ∼8 mg/(kg·h) (87) with rates increasing to ∼10 mg/(kg·h) at higher intensities (∼70% VO2max) (88) in endurance trained athletes. This may translate in a total leucine loss up to ∼1.5 g over 2 h (89). Indeed, regular endurance exercise training blunts the exercise-induced

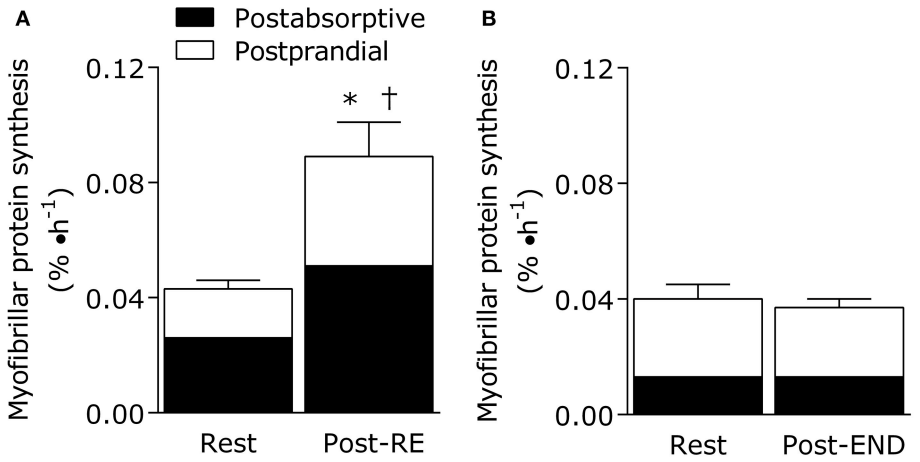

FIGURE 1 | Eating an adequate amount of protein at rest (i.e., in absence of a prior exercise stimulus) generally results in a doubling of the myofibrillar (contractile) protein synthetic response from post-absorptive values in healthy young adults (20–35 years). The fundamentally anabolic nature of resistance exercise results in an interaction between feeding and the exercise stimulus during recovery such that the stimulation of postprandial myofibrillar protein synthesis rates is potentiated when compared to the resting value. This interaction on the stimulation of post-exercise myofibrillar protein synthesis rates is not observed during recovery from endurance exercise (treadmill running at 70% of VO2peak for 1 h). Data adapted from Burd et al. (76) and Abou Sawan et al. (77). *different from post-absorptive value at rest. † different from postprandial value at rest.

stimulation of leucine oxidation rates (90), and it has been shown that 24 h net leucine balance remains unaffected by acute cycling exercise performed twice in a day (∼50% VO2max for 90 min per session) (91). Thus, it could be speculated that there is a dietary protein accommodation occurring, thereby minimizing the extra demand on dietary protein with endurance exercise training (89).

However, our research groups have recently shown that 1 h of treadmill running at 70% VO2peak results in a stimulation of leucine oxidation rates and a net leucine balance that was more negative when compared to the resting-state in athletes (88). What is noteworthy is that net leucine balance remained negative throughout the postprandial period even when providing the athletes a generous amount of high quality protein (18 g whole egg protein) immediately after the acute bout (88). There was also no additive effect of nutrition and endurance exercise on the stimulation of post-exercise muscle protein synthesis rates in these athletes (**Figure 1**) (77), which is a hallmark of the muscle protein synthetic response during recovery from resistance exercise combined with feeding (71). These findings are significant (77, 88) as we provided an amount of protein (∼0.25 g protein/kg per meal) immediately after the acute endurance bout that is commonly recommended to maximize the stimulation of post-exercise muscle protein synthesis rates after resistance exercise (71). Hence, we speculate that endurance exercise places more demand on dietary protein, which is likely intensity and exercise duration dependent, due to the need to compensate for exercise-induced amino acid oxidation losses while also supporting muscle protein remodeling throughout recovery when compared to resistance exercise. These concepts could be supported by recent estimations of an increased daily protein requirement (potentially primarily by the branched chain amino acids that are preferentially oxidized during exercise) to optimize whole body fed-state anabolism in endurance trained athletes during recovery (92, 93). Overall, protein recommendation for physically active adults are likely more nuanced whereby the "optimal" amount of protein to consume needs to take into account exercise mode, intensity, duration, and/or health/performance goals within the recommendation. This notion is consistent with periodized nutrition frameworks for carbohydrates commonly advocated to optimize training prescriptions and adaptations, especially for athletes (94).

Finally, it is also important to recognize that prescribing protein requirements as a single daily value as shown in **Table 2** is likely obscuring the importance of protein distribution and meal frequency to optimize the postprandial muscle protein synthetic response throughout the day (95, 96). In short, dietary guidelines recognize healthy eating patterns for nutrient density and adequacy, but are currently not accounting for meal frequency. For example, it is common for adults, especially Americans, to skew their total protein intakes to dinner with smaller protein portions consumed at breakfast and lunch (96). Contrary to suggestions that there is not a practical maximal anabolic response to a meal protein intake (97), it is clear that muscle protein synthesis (71) and whole body net protein balance (73) have finite capacities to assimilate dietary amino acids. This would ultimately result in more dietary amino acids being irreversibly lost to oxidation as opposed to be used for postprandial muscle protein accretion at dinner when consuming a skewed daily protein distribution (71, 96). Thus, the definition of optimal protein intakes needs to consider meal frequency, and prescribe a recommendation on a meal-by-meal basis to take into account protein distribution as a relevant factor for the stimulation of postprandial muscle protein synthesis rates during the day.

PROTEIN CONSIDERATIONS FOR CHILDREN AND ADOLESCENTS

The development of lean body mass during childhood and adolescence is important for supporting metabolic and skeletal health. Adherence to an active lifestyle is associated with greater lean body and muscle mass across the growth spectrum (98) and, due to the mechanical forces muscle may impose on growing bones, may be an independent predictor of peak bone mass (99, 100). Provided energy intakes are sufficient to support an active lifestyle and the metabolic demand for somatic growth, dietary protein represents arguably the most important macronutrient for the growth and development of lean mass.

General protein requirements are ∼20–60% greater in children and adolescents than the minimum safe intake for adults to account for the metabolic demands of the linear and accelerated, respectively, growth of these young populations (101–103). Currently, the nitrogen balance-derived RDA is set at 0.95 g/kg/d and PRI at 0.90 g/kg/d, and is based largely on data from adult populations with an estimated growth requirement (determined by a factorial method) (102). In contrast, contemporary stable isotope-based methods (i.e., indicator amino acid oxidation) suggest the requirement to maximize whole body protein synthesis (as a metric for offsetting any fasted state protein loss) may be as high as 1.5 g/kg/d (103). However, with protein intakes at ∼15% of energy, these recommended intakes are generally satisfied in the US when total energy intake is sufficient (22). Moreover, consideration for protein quality and individual amino acid requirements in children are unlikely to be an issue when consuming a typical mixed protein diet (i.e., plant and animal-based protein) at the current levels (23). It is important to note that regardless of method (i.e., nitrogen balance vs. IAAO), preliminary research suggests that, similar to adults, protein requirements in active children and adolescents may be (∼50%) elevated, albeit relatively less than similarly active adults (10). This increased daily requirement may be related to a need to offset any exercise-induced losses and/or to support enhanced rates of lean body mass turnover and/or growth (10).

Dietary protein consumption in adults enhances the exercise-induced increase in whole body and skeletal muscle protein synthesis rates (4), the latter of which is generally the targeted outcome to aid in the remodeling and growth of this tissue in adults (24). In contrast to relatively weight stable adults, children experience whole body growth of ∼5 cm height and ∼3 kg body mass per year that may be accelerated 3-fold during the adolescent growth spurt (98). To accommodate for this somatic whole body growth that is enhanced via an active lifestyle (6), it is arguably more relevant to assess the nutritional factors that enhance whole body protein turnover and net protein balance (i.e., surrogate marker of acute "growth") in children and adolescents. Similar to adults, protein consumption after exercise increases whole body net protein balance in children and adolescents in a dose-dependent fashion (25, 26, 73). Perhaps consistent with the requirement to support whole body growth, active children, and adolescents appear to be more "anabolically sensitive" to dietary protein than adults as whole body net protein balance is greater in these young populations at suboptimal (i.e., $<$ ∼0.3 g/kg) meal protein intakes (10). However, similar to adults, whole body net protein balance is saturable with protein ingestion in active children and adolescents (26, 73). For example, whole body leucine oxidation rates (estimate of protein oxidation) plateaus at an intake of ∼34 mg leucine/kg (equivalent of ∼0.34 g/kg of a high quality, leucine-enriched protein) with greater intakes resulting in an expansion of plasma amino acid pool (26), which represents a metabolic profile that could be suggestive of an acute nutrient excess (27). Therefore, available data suggests children and adolescents should target a meal protein intake of ∼0.3 g/kg to maximize whole body net protein balance during the recovery from acute exercise (26, 73), an intake that incidentally has also been shown to maximize post-exercise muscle protein synthesis in adults (71).

The timing and distribution of protein intake throughout the day has been suggested to represent a modifiable factor to optimize dietary protein utilization in adults (95). Similar to adults, children in the United States have been reported to consume a skewed protein distribution with the majority of the daily intake consumed in the evening (28). Whereas, there is some support for consuming a balanced daily protein distribution to enhance protein balance in children (29, 30), this finding is not universal (31). It is possible that the nutrient demands for growth in active children and adolescents render them more sensitive to dietary amino acids and, thus, less influenced by variations in protein distribution. This may be akin to the ability of resistance exercise in adults, the arguable only parallel to "growth" in this population, to increase the sensitivity of muscle protein synthesis to dietary amino acids for up to 24 h (76). Nevertheless, given the anabolic response to bolus protein ingestion is saturable, prudent advice may be to target the repeated ingestion of moderate protein-containing meals to optimize the anabolic efficiency of the daily protein intake. Similar to adults, however, additional research is warranted to identify the anabolic potential of different protein sources independently and within whole food matrices and mixed meals.

PROTEIN CONSIDERATIONS WITH AGE

It is well-established that there is a gradual loss of skeletal muscle mass and function that occurs at a more advanced age, and that this muscle deconditioning is usually coupled to sedentary lifestyle behaviors (32). For example, the age-related loss of skeletal muscle mass is thought to begin at ∼50 years and progress at a rate of ∼0.8% per year (33) whereas the decline in strength, while associated with muscle loss, occurs at a faster rate of ∼2–3% per year (34). Therefore, when an individual reaches 70 years of age, they may have lost ∼16% of their muscle mass and ∼50% of their strength from their younger years.

The age-related decline in overall skeletal muscle mass can be attributed to an imbalance between muscle protein synthesis and breakdown rates that results in a negative muscle protein balance (35). No detectable differences shown to exist in post-absorptive muscle protein synthetic rates between younger and older men (36, 81) and women (37). Hence, the age-related

decline in muscle mass is thought be attributed to the blunting of the postprandial muscle protein synthetic response to protein ingestion when compared to their younger counter-parts (36, 38, 81). The impaired ability of aging muscle to elicit a robust postprandial muscle protein synthetic response to elevated dietary amino acid availability in circulation has been coined "anabolic resistance" (39). Various strategies have been used in an attempt to overcome this age-related anabolic resistance of muscle protein synthesis rate such as increasing the protein density of meals (40, 41), food fortification techniques including extra leucine as an anabolic trigger (42), and food combinations (60, 61). However, what appears to be the most promising, and cost effective, lifestyle strategy to improve the postprandial muscle protein synthetic response to protein ingestion at a more advanced age is regular exercise (82). The final point that has received little attention is the potential sexual dimorphism in the age-related changes in muscle protein synthesis rates in response to protein. There is some indication that aging men and women may respond differently to nutritional stimuli (43, 44), but both sexes are clearly anabolically resistant (43). At this time, however, there is not enough data to clearly define if older women have different protein requirements when compared to older men.

Despite this established anabolic resistance with age, current protein requirements as established by whole body nitrogen balance methods are similar throughout adult life (**Table 2**). When using a muscle-centric approach to protein intake, however, we have observed that the relative quantity of protein to maximize the postprandial muscle protein synthetic response is greater in older when compared to younger men. In particular, we established that older men demonstrated an ingested protein-dose response curve of postprandial muscle protein synthesis rates up to ∼0.40 g/kg per meal, which was nearly doubled when compared to young adults (∼0.24 g/kg per meal) (72). When considering the value of spread distribution pattern of protein intake at each meal time (i.e., breakfast, lunch, dinner, and evening snack) for maximal muscle anabolic potential (45, 96), it seems that protein intakes for older adults is likely higher than the current RDA or PRI of ∼0.8 g/kg/d and nearing values closer to ≥1.2 g/kg/d. These recommendations are supported by whole body tracer estimates using the indicator amino acid oxidation technique of a safe intake of ∼1.25 g/kg/d in older (i.e., >65 years) adults (46). In addition, lean body mass loss over 3 years is lowest in older adults consuming ≥1.2 g/kg/d (104), collectively supporting dietary protein as a modifiable risk factor for age-related lean (and muscle) loss. However, a prospective multi-site randomized control trial with defined protein intakes spanning sufficient to deficient with consideration for habitual activity and functional endpoints (e.g., muscle strength/mass) is ultimately needed to guide best practices in nutritional advice.

HOLISTIC APPROACH FOR BETTER DEFINITIONS OF OPTIMAL PROTEIN INTAKE FOR MUSCLE?

Reductionist approaches have made significant contributions toward the understanding of nutrient-muscle interactions. For example, it has been established that dietary protein derived amino acids, especially the essential amino acids (105), are mainly responsible for the stimulation of postprandial muscle protein synthesis rates. Moreover, the branched chain amino acid, leucine, has received much attention due to its dual role as

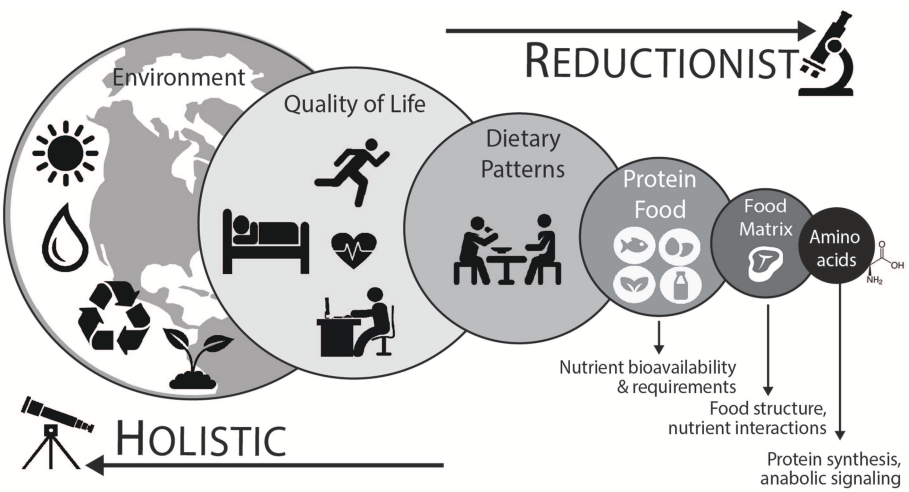

FIGURE 2 | To adequately define optimal protein intakes it is important to consider an integrative holistic approach. This "top-down" approach considers that different levels are additive to the next for the development of dietary advice (110, 111). Dietary patterns (animal based vs. plant based) and their associated protein foods are directly connected. Protein food is more than the sum of its constituent amino acids and the net effect of the food matrix, or food combinations (e.g., complementary protein pairing of plant-based foods), likely has an impact on the stimulation of postprandial muscle protein synthetic responses and overall diet quality. At the highest levels, food sustainability, food waste, and other human choices are important considerations. At the lowest (reductionist) level, amino acids represent the fundamental building blocks of protein and are anabolic agents in themselves (i.e., initiate protein synthesis). Aside from nutrient factors, ample physical activity, including regular structured exercise, is important component of a healthy lifestyle and has a direct impact on protein utilization and the overall nutritional recommendation.

an anabolic signaling molecule (106, 107) as well as a substrate for protein synthesis (108, 109). However, with the general preoccupation of the field studying the individual parts (i.e., isolated proteins and free amino acids) of nutrition in a typical bottom-up fashion, our current approach to understanding human nutrition may be nearing its limits to adequately define the role of protein quality and quantity for muscle mass and health within a complete diet.

As shown in **Figure 2**, a holistic point-of-view considers that protein nutrition follows a hierarchical organization with each level demonstrating a reinforcing factor into the next for the overall protein recommendation (110, 112). Using a top-down approach, which takes into account environmental (e.g., time of year, geographical location, and sustainable agricultural practices), quality of life (e.g., physical activity/exercise habits or injury), dietary pattern (e.g., Western, Mediterranean, or vegetarian), protein foods (e.g., beef or quinoa), net effect of the food matrix (e.g., food structure and nutrient-nutrient interactions), and finally the most basic constituent of protein (i.e., dietary amino acids), will help advance the field of research and perhaps yield the most ecologically valid dietary advice (112, 113).

At the higher levels, it is important to first consider the eating pattern of a population as dietary guidelines consist of eating patterns and their respective food choices to ensure nutrient adequacy and overall diet quality. Dietary eating patterns are often adapted to meet personal preference with common patterns including animal based (e.g., US-style) or plant-based (e.g., vegetarian) eating patterns. Indeed, plant-based diets are often thought to be inferior for the stimulation of postprandial muscle protein synthesis (114). Plant based foods, when viewed in isolation, are lower in leucine, lysine, and methionine by total amino acid content when compared to animal based foods (115). As such, it has been demonstrated that the ingestion of soy protein isolate resulted in a reduced postprandial muscle protein synthetic response when compared to whey protein ingestion in healthy young men (116). However, vegetarian and vegan diets are quite diverse, and generally consist of the ingestion of a variety of plant based foods throughout the day to ensure a more balanced profile of essential amino acids for the stimulation of postprandial muscle protein synthesis rates (117). Direct comparisons, however, are non-existent with regards to the capacity of mixed plant based foods to augment postprandial muscle protein synthesis rates vs. the ingestion of animal based foods.

It is also significant to develop protein recommendations in relation to whole food approaches, which takes into account the amino acid composition of the ingested protein food as well as the associated net effect of the food matrix (118). The food matrix describes the nutrient and non-nutrient components of foods as well as their structure and interactions (113, 119). The food matrix can influence nutrient digestion, absorption, and in terms of protein containing food matrices, the net anabolic action on the stimulation of muscle protein synthesis rates (62, 120–123). Such findings strongly suggest that there are interactions occurring within the food matrix to potentiate the net muscle anabolic effect that is stronger than the individual action of amino acids alone (118). Overall, dietary patterns are composed of foods, food combinations, and their associated food components and nutrients. Certainly, it is relevant to deconstruct dietary patterns, and subsequently understand how the parts of foods (i.e., amino acids) activate anabolic signaling pathways and stimulate the postprandial muscle protein synthetic response to understand the mechanistic basis behind a dietary recommendation. However, it is also important to balance the knowledge gained from studying isolated food components with the interactions occurring between exercise habits, eating patterns, and foods (and their constituent nutrients) when providing dietary advice (**Figure 2**).

CONCLUSION

Identifying the optimal amount and quality of protein foods to consume within a dietary pattern is necessary to provide dietary guidance. We have discussed optimal protein intakes from a muscle-centric point of view given its role in muscle function and metabolic health. There is little uncertainty that there needs to be some level of flexibility when considering what is the "optimal" protein intake to include within a dietary pattern throughout the lifespan. In terms of the protein RDA or PRI, these values represent a minimal target to prevent a protein deficiency within a safety margin, and perhaps are not adequate to support muscle protein remodeling with regular exercise training (6) and/or account for the increased dietary protein amounts required to overcome anabolically resistant aged muscles (7). Moreover, protein quality is also an important consideration of a dietary plan. The DIAAS of a dietary protein may yield more direct information with regards to protein digestibility (2), but there is currently limited DIAAS available based on a wide variety of dietary proteins. Moreover, DIAAS does not consider the impact of exercise training on modulating protein digestibility and the transfer of bioactive food constitutes (118), which will play a role in defining optimal protein quality.

At some point, it is also important to recognize a holistic nutrition framework where there is interplay between environmental considerations, physical activity and exercise patterns, dietary patterns, protein foods, and nutrients (amino acids) that cultivates into the overall dietary advice (**Figure 2**). Likewise, it is essential to keep in mind that there is adaptability for any protein recommendation throughout the life/health-stage, which accounts for health or performance goals, periods of hospitalization, or disease-state. In turn, this will provide a better compass for the definition of "optimal" protein intakes for all ages.

AUTHOR CONTRIBUTIONS

NB, KP, and DM drafted the manuscript. KP, AS, and CM prepared tables and figures. All authors contributed to manuscript revision, read, and approved the submitted version.

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Vitamin B12 Intake From Animal Foods, Biomarkers, and Health Aspects

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The EAT-Lancet commission recently suggested that transformation to healthy diets by 2050 will require a reduction of at least 50% in consumption of foods such as red meat and sugar, and a doubling in the global consumption of fruits, vegetables, nuts, and legumes. A diet rich in plant-based foods and with fewer animal source foods confers both improved health and environmental benefits. Notably, the risk of vitamin B12 deficiency increases when consuming a diet low in animal products. Humans are dependent on animal foods such as dairy products, meat, fish and eggs. Vitamin B12 deficiency is common worldwide, especially in populations with low consumption of animal foods because of low socioeconomic status, ethical reasons, or because of their lifestyle (i.e., vegans). According to the European Food Safety Authoroty, the recommended adequate intake of vitamin B12 is 4.0 μg/d for adults, and vitamin B12 requirements are higher during pregnancy and lactation. Infants and children from deficient mothers and elderly people are at risk for vitamin B12 deficiency. Diagnosis of vitamin B12 deficiency is hampered by low specificity of available biomarkers, and there is no consensus yet regarding the optimal definition of low vitamin B12 status. In general, a combination of at least two biomarkers is recommended. Therefore, this review presents an overview of vitamin B12 biochemistry and its biomarkers. We further summarize current recommendations of vitamin B12 intake, and evidence on the associations of vitamin B12 intake from different nutrient-dense animal foods with vitamin B12 status markers. Finally, potential consequences of low vitamin B12 status on different health outcomes for pregnant women, infants and elderly are presented.

Keywords: vitamin B12 (cobalamin), intake, animal food products, health, infants, pregnancy, elderly

VITAMIN B12

Food Sources of Vitamin B12

Vitamin B12 (cobalamin) is an essential water-soluble micronutrient of microbial origin (1). It is naturally found in animal food products, including meat, poultry, (shell)fish, eggs, milk, and other dairy products (2). Vitamin B12 is generally not present in plant foods, but fortified breakfast cereals are a readily available source of vitamin B12 with high bioavailability (3, 4). Some nutritional yeast products also contain vitamin B12. This paper will only focus on vitamin B12 intake from natural food products, e.g. animal foods. According to recent results of the 2012-2016 Dutch Food Consumption Survey, the contribution of dairy, meat, (shell) fish, supplements, and eggs to total vitamin B12 intake is 38.5, 30, 8.5, 8.4, and 4.6%, respectively[1]. The nutrient dense animal food products rich in vitamin B12 are also rich in other nutrients such as zinc, iron, vitamin D, and proteins. To the best of our knowledge, interactions of vitamin B12 with these nutrients are not fully established, and it is not unlikely that associations of low vitamin B12 with health outcomes may be modified by deficiency of these nutrients or the presence of disorders that affect the digestive system. Notably, vitamin B12 and folate act together within the one-carbon metabolism, and paragraph 4.3.1 will elaborate on a potential role of high folate and low vitamin B12 status in health.

Uptake of Vitamin B12

Vitamin B12 plays an important role in one-carbon metabolism. Dietary vitamin B12 is, once ingested, bound to haptocorin (an animal protein), which carries vitamin B12 to the stomach. In the stomach, HCl and pepsin are released which release vitamin B12 from animal proteins. Free vitamin B12 then binds to haptocorrin in the stomach after which it is transported into the intestine, where vitamin B12 is released by pancreatic enzymes after which vitamin B12 binds to intrinsic factor (IF) (5). Vitamin B12 -IF complex binds to the cubulin receptor in the distal ileum, which takes up vitamin B12 through receptor-mediated endocytosis (5). Once taken up, vitamin B12 is released to the plasma where it is bound to its transport proteins; haptocorrin (HC) and trancobalamin (TC) (6, 7). In the circulation, 20–25% of vitamin B12 is bound to TC (called holo-TC or active B12), which is taken up and used by the cells. The other 75–80% of vitamin B12 is bound to HC, which is stored in the liver (6–8).

Causes of Acquired Vitamin B12 Deficiency

Vitamin B12 deficiency increased with age and is mostly due to malabsorption of the vitamin. In addition, low intake of animal food products—as outlined in chapter 3–and use of certain drugs may also result in vitamin B12 deficiency (**Table 1**). Absorption of vitamin B12 is dependent upon several processes including IF production. If gastric IF production is impaired, like when gastric parietal cells are destructed in case of gastritis or when less gastric parietal cells are strongly reduced in case of a gastric bypass less, this will result in reduced absorption of vitamin B12. Uptake of vitamin B12 takes place in the distal ileum, and in case of an ileal dissection, bacterial overgrowth or intestinal diseases such as Crohn's disease less vitamin B12 can be taken up by the ileal cells resulting in lower intake of vitamin B12. Drugs that regulate secretion of gastric acid production such as proton-pomp inhibitors can also lead to vitamin B12 deficiency due to in impaired release of vitamin B12 from food proteins. In addition, metformin, a drug that is used to lower glucose levels, has been shown to results in lower vitamin B12 levels in serum most likely due to interfering with calcium-related binding of IF-B12 complex to the cubulin receptor (9). Considering drugs the party drug nitrous oxide has gained a lot of attention recently as it has been shown that high intake of nitrous oxide can result in vitamin B12 deficiency due to irreversible oxidation of the cobalt ion of MeCbl and AdoCbl, which makes both coenzymes inactive resulting in increased levels of methylmalonic acid (MMA) and homocysteine (10). Vitamin B12 and active B12 levels are mostly not low in serum in case of nitrous oxide overabuse and functional markers such as MMA and homocysteine should be used for laboratory diagnosis (10).

TABLE 1 | Causes of acquired vitamin B12 deficiency.

Cause	Effect
MALABSORPTION	
Gastric bypass	↓ IF production
Gastrointestinal infection with H. Pylori	↓ IF production
Ileal resection	↓ Absorption of B12-IF
Bacterial overgrowth	↓ Absorption of B12-IF
Intestinal disease (e.g., Crohn)	↓ Absorption of B12-IF
Pernicious anemia	Antibodies against IF or parital cells
Difficulties in chewing foods	Releasing of B12 from food proteins
NUTRITIONAL	
Malnutrition	↓ Vitamin B12 consumption
Vegetarian or vegan diet	↓ Intake of B12 containing animal products
DRUGS	
Proton-pump inhibitors	Defective release of B12 from food
Metformin	↓ Absorption of B12
Nitrous oxide	Inactivation of methionine synthase (in case of NO)

IF, Intrinsic factor; ↓, decreased.

Biochemistry of Vitamin B12

Different derivatives of cobalamin exist of which methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) are the physiological co-enzyme forms. MeCbl is a cofactor in the methionine-synthase dependent remethylation of homocysteine into methionine, which takes place into the cytosol. This remethylation reaction is an important step of the one-carbon metabolism, in which also reduction of folate derivatives takes place, which are important for DNA synthesis. In addition, methionine is an essential amino acid which is involved in formation of the universal methyl donor S-adenosylmethionine. Low dietary intake of vitamin B12 results in elevated homocysteine levels and might affect DNA synthesis and

Abbreviations: IF, intrinsic factor; holoTC, holotranscobalamin; MMA, methylmalonic acid; Hcy, total homocysteine; NTD, neural tube defects.

[1]https://wateetnederland.nl/resultaten/vitamines-en-mineralen/bronnen

DNA methylation. AdoCbl is involved in the l-methylmalonyl-CoA-mutase-dependent conversion of methylmalonyl-CoA into succinyl-CoA, which takes place in the mitochondrium (11). Low dietary intake of vitamin B12 results in accumulation of methylmalonyl-CoA that converts to MMA. Increasing levels of MMA are observed in plasma in case of vitamin B12 deficiency.

Biomarkers of Vitamin B12

Several biomarkers (**Table 2**) exist to evaluate vitamin B12 status in blood. The most used biomarker is total vitamin B12, which measures vitamin B12 bound to both transport proteins (HC and TC), which gives a generally estimation of the vitamin B12 status in the blood (7, 11). In addition, holoTC (active B12), which is the transcobalamin-bound vitamin B12 has been suggested to be an early marker of vitamin B12 status. HoloTC can be used as an initial test to measure vitamin B12 status in blood (12–14). Both total vitamin B12 and holoTC are applied in laboratory diagnostics and functional tests such as homocysteine and MMA are used to confirm diagnosis in case of low-normal vitamin B12 status. No consensus exists about which cut-off values should be applied and which is the best marker or combination of markers to assess vitamin B12 status (15). In general, reference intervals are used or alternative cut-off values are chose based upon sensitivity and specificity (14). However, these cut-off values are not generally applicable, as total vitamin B12 and active vitamin B12 tests are not harmonized, which hampers interpretation difficult for general practitioners.

Consideration

Regarding biomarkers of vitamin B12 there is a need to establish reference intervals of total vitamin B12 in pregnancy as these levels decrease during pregnancy. In addition, better biomarkers are necessary to determin vitamin B12 deficiency as total B12 and active B12 hamper diagnostic specificity.

PRESENT RECOMMENDED DIETARY INTAKE OF VITAMIN B12 AND THEIR LIMITATIONS

Dietary Reference Values for Vitamin B12

Several organizations have followed different approaches to set the dietary reference values for vitamin B12 (**Table 3**). The dietary reference values for adult men and women aged >18 years range between 2 and 4 μg/d depending on the judgments used. Generally, the increased requirements for vitamin B12 in women during pregnancy and lactation have been acknowledged and translated into higher reference values compared with non-pregnant women. No special intake recommendations exist for elderly people, despite the evidence that vitamin B12 malabsorption and deficiency are common in the elderly. Furthermore, the intake recommendations for infants were mainly based on outdated observational studies and on vitamin B12 content in human breastmilk. Measurement of vitamin B12 in breastmilk has been hampered by methodological problems due to the high milk haptocorrin that interferes with most available assays. In general, the European Food Safety Authority (EFSA) panel defined the Adequate Intake of vitamin B12 based on three indicators of vitamin B12 requirements (16):

1- Maintenance of heamatological markers in patients with pernicious anemia in a remission phase (i.e., correcting hemoglobin, mean corpuscular volume, and reticulocyte).
2- Maintenance of the total body stores of vitamin B12 (≈2–3 mg) by adjusting the daily requirements for the daily loss of the vitamin. The absorption efficacy of vitamin B12 from foods is assumed to be 40% and the daily loss is between 2 and 6 μg/d (biliary loss or transfer to the fetus or infant (via the placenta or the breastmilk).
3- Maintenance of normal serum levels of vitamin B12 markers (total vitamin B12, MMA, holoTC, and Hcy).

TABLE 2 | Biomarkers of vitamin B12 status in serum or plasma.

Biomarker	Indication	Interpretation
Total vitamin B12	Global vitamin B12 status	↓ In vitamin B12 deficiency ↑ Myeloid cell proliferation ↓ Pregnancy
Holo-transcobalamin	Vitamin B12-bound to transcobalamin or active B12	↓ In vitamin B12 deficiency ↓ In TC deficiency
Methylmalonic acid	Functional marker of vitamin B12 deficiency	↑ Vitamin B12 deficiency ↑ Renal dysfunction
Homocysteine	Functional marker of vitamin B12 deficiency	↑ Vitamin B12 deficiency ↑ Renal dysfunction ↑ Folate deficiency

↓, decreased; ↑, increased.

Vitamin B12 Intake Necessary for Maintenance of Normal Vitamin B12 Biomarkers

Approximately 1% of a high oral vitamin B12 dose (i.e., derived from supplemental cyanocobalamin) crosses the intestinal barrier into the blood via simple diffusion (17), after saturation of IF (max. 5 μg/meal is absorbed via IF) (18). Vitamin B12 intake shows a dose-response relationship with blood vitamin B12 markers. We focused here on studies on vitamin B12 biomarkers in relation to vitamin B12 intake in the range below 50 μg/d. Studies using therapeutic doses of vitamin B12 are beyond the scope of this review.

In a study among 98 Danish post-menopausal women Bor et al. (19)suggested that an intake of 6 μg/d vitamin B12 (determined from 7-d weighed food records) is sufficient to maintain highest concentrations of vitamin B12 and holoTC, and lowest concentrations of MMA and Hcy with median (25–75th percentiles) of 380 (270–480) pmol/L for vitamin B12, 119 (92–162) pmol/L for holoTC, 0.12 (0.14–0.17) μmol/L for MMA, and 9.8 (8.3–11.4) μmol/L for Hcy compared to intakes lower than 6 μg/d. A similar study in 299 healthy US adults found that mean levels of vitamin B12 and holoTC were highest in the intake range between 4.2 and 7.0 μg/d, while plasma MMA and Hcy reached lowest levels in subjects who achieved an intake of ≥7.0 μg/d (20).

The association between vitamin B12 markers and intake was generally weaker in studies in elderly people (21) compared to those in younger people, in studies considering only dietary

TABLE 3 | Dietary reference values for vitamin B12 (in μg/d) in different age and sex groups as suggested by different organizations.

	EFSA 2015	D-A-CH 2015	NCM 2014	WHO 2004	NL 2003	IOM 1998	SCF 1993	COMA 1991
Infants and children								
Age	7Mo−6y	4–12Mo	6–11Mo	7–12Mo	6–11Mo	7–12Mo	6–11Mo	7–12 Mo
Reference value	1.5 μg/d	0.8 μg/d	0.5 μg/d	0.7 μg/d	0.5 μg/d	0.5 μg/d	0.5 μg/d	0.4 μg/d
Age		1–4 y	1–2 y	1–3 y	1–3 y	1–3 y	1–3 y	1–3 y
Reference value		1.0 μg/d	0.6 μg/d	0.9 μg/d	0.7 μg/d	0.9 μg/d	0.7 μg/d	0.5 μg/d
Age		4–7 y	2–5 y	4–6 y	4–8 y	4–8 y	4–6 y	4–6 y
Reference value		4.5 μg/d	0.8 μg/d	1.2 μg/d	1.3 μg/d	1.2 μg/d	0.9 μg/d	0.8 μg/d
Age	7–10 y	7–10 y	6–9 y	7–9 y	9–13 y	9–13 y	7–10 y	7–10 y
Reference value	2.5 μg/d	1.8 μg/d	1.3 μg/d	1.8 μg/d	2.0 μg/d	1.8 μg/d	1.0 μg/d	1.0 μg/d
Age	11–14 y	10–13 y	10–17 y	10–18 y	10–18 y	14–18 y	11–14 y	11–14 y
Reference value	3.5 μg/d	2.0 μg/d	2.0 μg/d	2.4 μg/d	2.8 μg/d	2.4 μg/d	1.3 μg/d	0.2 μg/d
Age	15–17 y	13–19 y					15–17 y	15–18 y
Reference value	4.0 μg/d	3.0 μg/d					1.4 μg/d	1.5 μg/d
Adults (M+F), > 18 y	4.0 μg/d	3.0 μg/d	2.0 μg/d	2.4 μg/d	2.8 μg/d	2.4 μg/d	1.4 μg/d	1.5 μg/d
Pregnant women	4.5 μg/d	3.5 μg/d	2.0 μg/d	2.6 μg/d	3.2 μg/d	2.6 μg/d	1.6 μg/d	1.5 μg/d
Lactating women	5.0 μg/d	4.0 μg/d	2.6 μg/d	2.8 μg/d	3.8 μg/d	2.8 μg/d	1.9 μg/d	2.0 μg/d

EFSA, European Food Safety Authority; NCM, Nordic Council of Ministers; WHO, World Health Organization; NL, Health Council of the Netherlands; IOM, U.S. Institute of Medicine; SCF, Scientific Committee on Food; COMA, Committee on Medical Aspects of Food Policy.

intake compared to studies on vitamin B12 intake from diet plus supplements, and in studies considering vitamin B12 intake up to 100 μg/d than those using larger doses (21). This could be due to a better absorption of free vitamin B12 from supplements compared to protein-bound vitamin B12 from foods. Van Asselt et al., reported a median vitamin B12 intake (from diet plus supplements) of 6.3 μg/d in elderly Dutch people (mean age 76 years) with normal vitamin B12 markers (vitamin B12 >260 pmol/L and MMA<320 nmol/L) (22). Subjects with mild vitamin B12 deficiency (vitamin B12 <260 pmol/L and MMA>320 nmol/L) had a median intake of 4.9 μg/d, and those with a possible deficiency (either low B12 or elevated MMA) had a median intake of 5.1 μg/d (22). The deficiency in elderly people could be better explained by malabsorption disorders instead of by minor variations in intakes (22). In line with this, associations of plasma concentrations of vitamin B12, MMA, and Hcy with vitamin B12 intake was not present in some studies (23, 24) possibly due to age- and disease-related malabsorption.

A meta-analysis on the association between vitamin B12 intake and biomarkers Dullemeijer et al. estimated that doubling the intake of vitamin B12 is associated with 11.0% (95% CI: 9.4%, 12.5%) higher serum vitamin B12 concentration (21). The association between vitamin B12 intake and biomarkers was stronger in studies conducted in elderly people than in adult populations, which could be related to low baseline concentrations of vitamin B12 in the elderly (21). The slope of the change of plasma vitamin B12 in relation to vitamin B12 intake flattened when vitamin B12 intake was >100 μg/d (21), which could reflect the limited proportional absorption of vitamin B12 from high dose supplements. Compared to plasma vitamin B12, the changes of serum MMA (mean −7%; 95% CI = −10 to −4%) in response to doubling vitamin B12 intake were smaller (21), which could be due to the short observational time of most studies and to the influence of renal function on MMA levels.

The increase in plasma vitamin B12 and the decrease in functional markers appear to depend on population characteristics (mainly age and accompanying diseases), duration of the intervention, starting plasma concentrations of the vitamin, and the administered dose of crystalized cyanocobalamin, even in non-therapeutic ranges. In general, a daily intake of free cyanocobalamin as low as 1.5–2.5 μg provided for approximately 4–6 months may increase plasma vitamin B12 by 50–100 pmol/L.

Consideration

A total intake of vitamin B12 from the diet between 4 and 7 μg/d is associated with normal plasma vitamin B12 and MMA and thus appears to be adequate to maintain body vitamin B12 status in adults. This intake might be insufficient if people have difficulties in chewing foods, releasing the vitamin from its food binding, and/or absorbing it due to disorders as shown in **Table 1** (25, 26). Elderly people with *H-pylori* infection (26), or food-cobalamin malabsorption (25, 27, 28) may be at risk for vitamin B12 deficiency despite sufficient dietary intake. It is unclear if elderly people would generally benefit from higher vitamin B12 intake recommendations.

THE ASSOCIATION OF ANIMAL FOOD PRODUCTS CONTAINING VITAMIN B12 WITH CIRCULATING VITAMIN B12 BIOMARKERS FROM OBSERVATIONAL STUDIES

In addition to supplements or fortified cereals as potential sources of vitamin B12, this paper focusses on vitamin B12

intake from natural food products, e.g., animal foods. In total, 19 observational studies were identified addressing associations of vitamin B12 containing aminal food items with plasma or serum vitamin B12 biomarkers. These studies were performed among infants ($n = 1$ study) (29), children ($n = 5$ studies) (30–35), pregnant women ($n = 1$ study) (36), adults ($n = 7$ studies) (3, 19, 20, 37–45), and elderly ($n = 5$ studies) (22–24, 43, 46–49). The majority of these studies had a cross-sectional design, except for some case-control study conducted among infants (29), children (30), and elderly (24), and a prospective study (3). The observational studies were heterogenous with respect to dietary assessment of animal food items or dietary patterns, usage of different vitamin B12 biomarkers, and statistical analyses, which hampers the direct comparison between studies (**Table 4**). Therefore, this section summarizes main findings from individual studies by different age categories.

Infants and Children

Two case-control studies among infants (29) and children (30) investigated the effects of a macrobiotic dietary regime (no animal foods) on vitamin B12 biomarkers. Plasma vitamin B12 concentrations were significantly lower among macro-biotic fed infants ($n = 47$) as compared to their omnivorous fed controls ($n = 56$) (29). In another study, adolescents who had received a macrobiotic diet until 6 y of age and had then switched a diet containing animal products ($n = 73$) still had significantly lower vitamin B12 concentrations and higher concentrations MMA, but comparable Hcy concentrations, as compared to their age-matched controls who consumed an omnivorous diet from birth onwards. These results suggest that switching from a macrobiotic diet to moderate consumption of animal food products is inadequate to restore vitamin B12 status among children with a low vitamin B12 in early childhood (30). A Swedish study among adolescents [mean (SD) age: 17.5 (1.0) year] compared vitamin B12 intake between 30 vegans (15 males and 15 females) and 30 sex-, age-, and height-matched omnivores. This study revealed significant differences in vitamin B12 intake between vegans and omnivores, with vitamin B12 intakes of 0.0 and 0.1 μg/day for vegan females and males, respectively, and intakes of 5.0 and 5.9 μg/day for omnivorous females and males, respectively (P for differences < 0.001)(50).

In contrast to following a well-defined dietary regime, a Colombian study identified 4 dietary patterns derived from an 28-item FFQ based on principal component analysis. Patterns included diets rich in (1) animal protein (e.g., beef/pork/veal/lamb, chicken/turkey, milk, cheese), (2) cheaper protein (e.g., cow tripe/liver, spleen, chicken giblets), (3) traditional/starch (e.g., rice, potato, plantain), and (4) snacking products (e.g., candy, ice cream, packed fried snacks, soda, fruit punch). Only the pattern rich in animal protein was significantly positively associated with plasma vitamin B12 (P for trend $= 0.003$). This study also studied individual animal food groups, and fully adjusted differences in plasma vitamin B12 for low vs. high consumers were significant only for meat, but not for dairy, fish, cows liver, and eggs (32). In line with these findings, a study conducted in India ($n = 512$) also showed statistically significant positive associations of the meat and fish group with plasma vitamin B12 in fully adjusted models, but not for other animal products (34). Others observed inverse associations between vitamin B12 intake from milk with plasma Hcy, but not for vitamin B12 intake from red meat or cheese (35). One study measured multiple biomarkers for vitamin B12 status. Serum MMA and Hcy concentrations were not correlated with animal food groups, whereas correlation coefficients of serum vitamin B12 and holoTC with dairy intake were 0.16 ($P < 0.05$) and 0.27 ($P < 0.01$), respectively. In addition, intake of liver pate correlated with holoTC ($r = 0.20$, $P < 0.05$). None of the vitamin B12 biomarkers were associated with fish or eggs intakes (33).

When considering different animal products within individual studies among children, differences in vitamin B12 concentrations were most pronounced when comparing high vs. low intake of dairy products, followed by meat and fish intake (32), and dairy products showed stronger correlations with vitamin B12 and holoTC concentrations compared to liver pate, meat and fish (33). In another study, only a combined group of meat and fish was associated with vitamin B12 concentrations, whereas the individual components fish, chicken, eggs, and dairy were not related to plasma vitamin B12 (34).

Pregnancy

Only one study among pregnant women ($n = 1266$) was identified that addressed the association of vitamin B12 intake from dairy, meat, (shell)fish, and eggs with circulating levels of vitamin B12 biomarkers, and presence of vitamin B12 deficiency in week 34–36 of pregnancy. Results showed that vitamin B12 from dairy, meat and fish, but not eggs, independently contributed to plasma concentrations of total vitamin B12, holoTC and MMA, as shown by statistically significant dose-response relationships. Vitamin B12 intake from each of these products groups was also independently associated with a reduced odds of vitamin B12 deficiency (holoTC <35 pmol/L and MMA >0.45 μmol/L). Egg-derived vitamin B12 was negatively associated with holoTC but not associated with other vitamin B12 biomarkers (36).

Adults

Those studies addressing specific animal products revealed that high dairy consumption was associated with significantly lower prevalences of vitamin B12 concentrations <185 pmol/L (3, 42) and 148 pmol/L (3) compared to low dairy consumption(3), significantly higher vitamin B12 concentrations among high milk and cheese consumers compared to low consumers (43), and significantly lower Hcy concentrations with high dairy intake compared to low dairy intake (45). Similarly, those with a fish consumption in the highest quintile had a significantly lower odds of having vitamin B12 deficiency compared to adults who had a fish consumption in the lowest quintile (42), and plasma vitamin B12 concentrations were significantly higher in those consuming high amounts (fourth quartile) compared to low fish consumers (first quartile) (43). In contrast, analyses on meat consumption did not show any relation of meat consumption with vitamin B12 deficiency (3, 42). Moreover, plasma vitamin B12 concentrations (43) and serum Hcy (45) did not differ between high and low meat consumers. Egg consumption was also not related to plasma vitamin B12 status (42, 43, 45). None

TABLE 4 | Main characteristics and results of observational studies addressing the relation between dietary intake and vitamin B12 status biomarkers among different age categories.

Author, year, country, design	Population (*% female)*	Age [range] (years)	Dietary intake	Vitamin B12 status biomarker	Results	Remarks	Final conclusion
INFANTS							
Dagnelie et al. (29), 1989, The Netherlands, Case–control	*N* = 103	10–20 months	Macrobiotic diet (*n* = 47), omnivorous diet (*n* = 56)	vitamin B12 (pmol/L)	Geometric mean ± coefficient of variation in: Marcobiotic group: 149 ± 21.6 Control group: 404 ± 15.6 P for difference < 0.001	None of the macrobiotic fed children had ever received animal produces or vitamin B12 supplements, except for 10 out of 47 infants who had received small amounts of dairy products at some time in their live.	Plasma B12 among macrobiotic fed infants significantly lower than among the control group
CHILDREN							
Van Dusseldorp et al. (30), 1999, The Netherlands, Nested case–control	*N* = 167 (54%) macrobiotic diet (*n* = 73), control (*n* = 94)	12 [9–15]	List of 6 food groups, including intake of cheese, pasteurized milk, buttermilk and yogurt.	Vitamin B12 (pmol/L) MMA (μmol/L) Hcy (μmol/L)	Correlation coefficients (*P*-value) of cobalamin with: Number of years having followed a macrobiotic diet: −0.22 (*P* = 0.06, *n* = 73) Frequency of meat consumption: 0.53 (*P* < 0.0001) Frequency of chicken consumption: 0.47 (*P* < 0.0001) Frequency of dairy consumption: 0.39 (*P* < 0.0001) Geometric mean (±1.96SD) concentrations of Cobalamin in macrobiotic vs. control boys: 213 (107–426) vs. 484 (238–985) pmol/L Cobalamin in macrobiotic vs. control girls: 288 (112–738) vs. 458 (206–1,020) pmol/L MMA in macrobiotic vs. control boys: 0.29 (0.09–0.93) vs. 0.15 (0.06–0.43) μmol/L MMA in macrobiotic vs. control girls: 0.25 (0.09–0.70) vs. 0.17 (0.07–0.40) μmol/L Hcy in macrobiotic vs. control boys: 8.3 (5.2–13.4) vs. 7.0 (4.2–11.7) μmol/L Hcy in macrobiotic vs. control girls: 7.6 (3.8–15.1) vs. 7.2 (3.8–13.7) μmol/L	The group of macrobiotic fed children had received a macrobiotic diet until 6 y of age and had then switched to a lactovegetarian, lacto ovovegetarian, or omnivorous diet (macrobiotic adolescents). The group of macrobiotic fed children switched to a diet containing dairy products (200 g milk or yogurt and 22 g cheese/d (supplying on average 0.95 mg cobalamin/d), and fish, meat, or chicken 2–3 times/wk. In girls, meat consumption contributed more to vitamin B12 status than the consumption of dairy products, whereas in boys these food groups were equally important.	Moderate consumption of animal products after cessation of a macrobiotic diet is insufficient to restore low vitamin B12 status among adolescents
Villamor et al. (32), 2008, Colombia, Cross–sectional	*N* = 972 (49%)	8.7 [5–12]	38 item FFQ obtained by mothers to assess dietary intake among children	Vitamin B12 (pmol/L)	P for trend across quartiles of plasma B12 with: Animal protein pattern: 0.003 Cheap protein pattern: 0.75 Traditional/starch pattern: 0.45 Snacking pattern: 0.84 Adjusted differences (95%CI, P for trend) in B12 concentrations of high vs. low/no intake of: Meat: 24 (1 to 48, 0.04) Dairy: 32 (5 to 95, 0.06) Fish: 17 (−7 to 41, 0.16) Cow liver: 5 (−17 to 28, 0.08) Egg: −25 (−50 to 1, 0.12) Supplement: 9(−8 to 27, 0.31)	PCA derived patterns: Animal protein (beef/ pork/veal/lamb, chicken/turkey, milk, cheese) Cheaper protein (cow tripe/liver, spleen, chicken giblets) traditional/starch (rice, potato, plantain), snacking (candy, ice cream, packed fried snacks, soda, fruit punch). Analyses adjusted for sex, age, frequency of meat, dairy, fish, cow liver, and supplement intake.	Strong dose–dependent positive association between a pattern including frequent consumption of beef, chicken, and dairy products and plasma vitamin B12.

(Continued)

TABLE 4 | Continued

Author, year, country, design	Population (% *female*)	Age [range] (years)	Dietary intake	Vitamin B12 status biomarker	Results	Remarks	Final conclusion
Hay et al. (33), 2011, Norway, Cross–sectional	$N = 155$ (44%)	2 [2–2]	7–day food records. Diary, liver pate, meat (products), fish (products).	Vitamin B12 (pmol/L) and holoTC (pmol/L)	Spearman correlations of Serum Vitamin *B12* with: Dairy products: 0.16 ($P < 0.05$) Serum HoloTC with: Dairy products: 0.27 ($P < 0.01$) Liver pate: 0.20 ($P < 0.05$) P for differences of geometric means by quartiles from food sources: Serum vitamin *B12*: Dairy: $P = 0.197$ Liver pate: $P = 0.212$ Meat & meat products: $P = 0.986$ Fish & fish products: $P = 0.865$ Serum holoTC: Dairy: $P = 0.024$ Liver pate: $P = 0.005$ Meat & meat products: $P = 0.204$ Fish & fish products: $P = 0.680$	Adjusted for sex and energy intake MMA and Hcy were not associated with animal food intake.	In this unfortified toddler population, vitamin B12 status was strongest associated with dairy intake, and with a lesser extend to liver pate'
Christian et al. (34), 2015, India, Cross–sectional	$N = 512$ (52.9%)	9.5 [9–10]	136 item Semi quantitative FFQ, including amongst others minced meat, fish, chicken, mutton, meat and fish, eggs, non–vegetarian, curd foods, milk and dairy	Vitamin B12 (pmol/L)	B (95%CI) T3 vs. T1 of intake with plasma B12: Minced meat: 0.011 (−0.077 to 0.100) Fish: 0.052 (−0.031 to 0.134) Chicken: −0.003 (−0.112 to 0.105) Mutton: 0.101 (−0.007 to 0.209) Meat & fish: 0.126 (0.041 to 0.212) Eggs: −0.031 (−0.122 to 0.061) Non–vegetarian: 0.124 (0.044 to 0.203) Curd (yogurt) foods: −0.075 (−0.157 to 0.007) Milk/dairy: −0.029 (−0.110 to 0.053)	Adjusted for age, sex, BMI, height, SLI score, maternal education, other food groups in the table except traditional fermented foods and raw vegetables, and pregnancy plasma B12 concentrations.	Meat and fish are most important animal derived B12 sources among Indian children
Manioset al. (35), 2017, Greece, Cross–sectional	$N = 600$ (51%)	11 [9–13]	Three 24 h dietary recalls (2 week days, 1 weekend day)	Hcy (μmol/L)	*B* (*p-value*) linear regression of Hcy with *B12* intake from: Milk: −0.120 (0.004) *B* (*p-value*) quadratic regression of Hcy with *B12* intake from: Milk: −0.515 (<0.001)	Adjusted for age, sex, and total vitamin B12 intake from other food sources Vitamin B12 intake from red meat and cheese were not associated with Hcy concentrations.	High vitamin B12 intake from milk was associated with lower Hcy concentrations
PREGNANCY							
Denissen et al. (36), 2019, The Netherlands, Cross sectional	$N = 1{,}266$ (100%)	32.6 ± 3.8	200 item semi–quantitative FFQ, including dairy products (28 items), meat (29 items), (shell)fish (7 items), eggs (1 item)	Vitamin B12 (pmol/L) holoTC (pmol/L) MMA (μmol/L)	%difference (95%CI) Q5 vs. Q1: Dairy–B12: 29 (21 to 37) Dairy–HoloTC: 53 (41 to 66) Dairy–MMA: −21 (−27 to −14) Meat–B12: 15 (8 to 23) Meat–HoloTC: 20 (10 to 30) Meat–MMA: −16 (−23 to −9) Fish–B12: 7 (0.5 to 13) Fish– HoloTC: 15 (7 to 24) Fish– MMA: −15 (−21 to −8) Eggs– B12: 1 (−5 to 7) Eggs– HoloTC: 9 (1 to 17) Eggs– MMA: −5 (−12 to 2)	Multivariable adjusted proportional difference in geometrical means of highest quintile relative to lowest quintile of intake. Values obtained by multiple linear regression analyses adjusted for recruitment group, age, prepregnancy BMI, education, smoking, vitamin B−12 intake from supplements, alcohol use, energy intake, vitamin B−12 intake from mixed dishes, as well as for vitamin B−12 intake from dairy, meat, fish and eggs (except for the food group of interest)	

(Continued)

TABLE 4 | Continued

Author, year, country, design	Population (% *female*)	Age [range] (years)	Dietary intake	Vitamin B12 status biomarker	Results	Remarks	Final conclusion
ADULTS (MEAN AGE POPULATION >18 And ≤64 y)							
Tuckeret al. (3), 2000, United states, Prospective	*N* = 2,999 (52%)	53.6 [26–83]	126 item Semi quantitative FFQ. Vitamin B12 intake was calculated from individual food sources. Food contributions to total vitamin B−12 was calculated, and total vitamin B12 intake was divided into vitamin B12 intake from supplements, breakfast cereals, meat, poultry, fish, dairy sources, and all other foods	vitamin B12 (pmol/L)	Prevalence of *B12* < 185 *vs.* 148 pmol/L for: Dairy upper tertile: 13.4 vs. 6.8 Dairy middle tertile: 21.2 vs. 10.4 Dairy lowest tertile: 24.5 vs. 14.1 P difference T1 and T3<0.001 for both cutoff levels Meat upper tertile: 17.4 vs. 8.5 Meat middle tertile: 20.4 vs. 11.4 Meat lowest tertile: 21.5 vs. 11.4 P difference T1 and T3>0.05 for both cutoff levels	Adjusted for age and sex	Milk appears to protect against lower vitamin B12 concentrations.
					Odds Ratios (95%CI) of having B12 <185 or <148 pmol/L comparing each dietary pattern to high supplement intake group: Meat 57% (*n* = 740): 2.4 (1.7 to 3.4) or 2.0 (1.2 to 3.3) Milk 38%,meat 21%, fish 10% (*n* = 361): 1.6 (1.1 to 2.5) or 1.0 (0.5 to 1.8) Meat 25%, soups 24%, fish 12%, milk 10% (*n* = 592): 2.2 (1.5 to 3.3) or 1.6 (0.9 to 2.6) Fish 35%, meat 21%, other dairy 10% (*n* = 342): 2.1 (1.4 to 3.3) or 1.6 (0.9 to 2.8)	Adjusted for age, sex, total energy intake, total vitamin B12 intake Dietary patterns were derived from cluster analysis.	Participants in all food intake groups were significantly more likely to have B12 concentrations < 185 pmol/L compared to subjects in the supplement group. Only the meat group differed significantly from the supplement group in having vitamin B12 concentrations < 148 pmol/L.
Gao et al. (38), 2003, China, Cross–sectional	*N* = 119 (54%)	42 [35–49]	170 item FFQ from which intake of Fruit and milk, Red meat, and refined cereals were composed	vitamin B12 (pmol/L) Hcy (μmol/L)	OR (95%CI) of having *B12* < 221 pmol/L: Red meat vs. fruit & milk: 2.4 (0.9 to 6.3) Refined cereals vs. fruit & milk: 6.2 (1.9 to 20.8) OR (95%CI) of having Hcy>11 for women and >12 for men: Red meat vs. fruit & milk: 2.6 (0.9 to 7.4) Refined cereals vs. fruit & milk: 5.0 (1.5 to 17.5)	Adjusted for age, sex, total energy intake, BMI, smoking, alcohol use, income and education level.	The pattern high in fruits and milk was associated with a significantly lower risk of having Hcy >11 or 12 μmol/L and of having B12 <221 pmol/L compared to the pattern of refined cereals. Pattern of red meat did not differ in risk of high Hcy or low B12 compared to the fruit and milk pattern.

(Continued)

TABLE 4 | Continued

Author, year, country, design	Population (% *female)*	Age [range] (years)	Dietary intake	Vitamin B12 status biomarker	Results	Remarks	Final conclusion
Koebnick et al. (40), 2005, Germany, Cross–sectional	$N = 187$ (53%)	46 [25–64]	7 day food records including 12 food groups	vitamin B12 (pmol/L) Hcy (μmol/L)	Median (P25, P75) concentrations according to type of raw food consumption: Vitamin *B12* concentrations: Mixed raw food: 175 (142,250) ovo–lacto–vegetarian: 143 (121,176) vegan: 126 (88,182) pmol/L Median (P25, P75) plasma Hcy concentrations: Mixed raw food: 14.7 (11.9,18.3) ovo–lacto–vegetarian: 17.1 (13.1,20.2) Vegan: 18.5 (13.5,28.9) μmol/L	Unadjusted analyses Mixed raw food diet included raw meat and fish	Individuals who consumed a mixed raw food diet had highest vitamin B12 and lowest Hcy concentrations whereas those consuming a stricht vegan diet had lowest vitamin B12 and highest Hcy concentrations.
Hao et al. (42), 2007, China, Cross–sectional	$N = 2,407$ (51%)	49 [35–64]	Semi quantitative FFQ. Animal–based foods were classified in dairy, egg, animal meat and fish.	vitamin $B_{12(}$pmol/L)	Adjusted OR (95%CI, P for trend) for having vitamin B12 deficiency (<185 pmol/L): T3 vs. T1 dairy: 0.5 (0.4 to 0.7, <0.001) Q5 vs. Q1 egg: 0.8 (0.6 to 1.1, 0.196) Q5 vs. Q1 meat: 1.0 (0.7 to 1.4, 0.163) Q5 vs. Q1 fish: 0.4 (0.3 to 0.5, <0.001)	Intake food source divided in tertiles (dairy) or quintiles (egg, animal meat, fish) Adjusted for region, area (urban, rural), gender, age, season	Higher consumption of dairy and fish was associated with a lower likelyhood of having B12 concentrations < 185 pmol/L compared to low or intermediate consumption.
Vogiatzoglou et al. (43), 2009, Norway, Cross–sectional	$N = 3,067$ (55%)	[47–49]	169 item FFQ	Vitamin B12 (pmol/L)	Adjusted mean (95%CI) plasma vitamin B12 concentrations in Q4 vs. Q1 B12 intake from: dairy: 385 (376 to 395) vs. 323 (314 to 332) milk: 388 (379 to 397) vs. 331 (324 to 338) Cheese: 370 (362 to 378) vs. 346 (338 to 335) Meat: 362 (354 to 369) vs. 355 (344 to 366) (shell) fish: 375 (366 to 385) vs. 339 (331 to 346) Eggs: 358 (349 to 366) vs. 356 (350 to 363) P for trend < 0.001, except for meat ($P = 0.189$) and eggs ($P = 0.837$)	Adjusted for sex, energy, use of B vitamin containing supplements, total intake of other food groups	Dairy and fish are significant contributors to plasma vitamin B12. Vitamin B12 appears to be more bioavailable from dairy products than from other animal products.
Yakub et al. (44), 2010, Pakistan, Cross–sectional	$N = 872$ (59%)	32.4 [18–60]	15 item food group frequency questionnaire composing dietary patterns	Vitamin B12 (pmol/L) Hcy (μmol/L)	Adjusted mean plasma vitamin B12 concentrations in Q4 vs. Q1 (P diff) intake from: Prudent diet: 322 vs. 317 (P diff = 0.85) High animal protein diet: 335 vs. 312 (P diff = 0.56) High plant protein diet: 325 vs. 326 (P diff = 0.80) Adjusted mean plasma Hcy in Q4 vs. Q1 (P diff) intake from: Prudent diet: 13.97 vs. 15.78 (P diff = 0.26) High animal protein diet: 18.58 vs. 13.29 (P diff < 0.001) High plant protein diet: 12.50 vs. 18.40 (P diff < 0.001)	Adjusted for age and sex Patterns identified by factor analyses: Prudent pattern: high intake of eggs, fish, uncooked vegetables, juices, and bananas and other fruits. High animal–protein pattern: high intake of meat, chicken, wheat, bananas, and tea with milk. High plant–protein pattern: high intake of cooked vegetables and legumes and a small intake of meat	Patterns high in animal and plant proteins were associated with lower Hcy, but not with vitamin B12 concentrations.
Murakami et al. (45), 2013, Japan, Cross–sectional	$N = 1,050$ (100%)	20 [18–22]	Diet history questionnaire including 17 foods	Hcy (μmol/L)	Adjusted geometric mean (95%CI) Hcy concentrations were significantly lower in Q5 vs. Q1 dairy intake (P for trend 0.02). Hcy did not differ across quintiles of (shell)fish, meats, and egg consumption	Adjusted for survey year, region, municipality level, current smoking, current alcohol drinking, supplement use, physical activity, BMI, energy intake, intakes of other foods.	High consumption of dairy products was associated with lower Hcy concentrations

(Continued)

TABLE 4 | Continued

Author, year, country, design	Population (*% female*)	Age [range] (years)	Dietary intake	Vitamin B12 status biomarker	Results	Remarks	Final conclusion
ELDERLY (MEAN AGE POPULATION >002065 y)							
Kwan et al. (46), 2002, Puerto Rica and Dominicans, Cross–sectional	*N* = 603 (58%) (Hispanic (*n* = 449), non–Hispanic white (*n* = 154))	76.5 [60–93]	Semi qualitative FFQ. Total vitamin B12 intake divided into vitamin B12 intake from supplements, breakfast cereals, dairy sources, eggs, meat, poultry, fish, and all other foods	Vitamin B12 (pmol/L)	Proportions of B12 < 185 pmol/L of vitamin B12 intake from: Hispanics: Dairy: 15.8 (T1), 14.2 (T2), 23.6 (T3), P for diff>0.05 Meat: 15.5 (T1), 15.6 (T2), 22.0 (T3), P for diff>0.05 *Non*–Hispanics whites: Dairy: 9.4 (T1), 10.5 (T2), 20.0 (T3), P for diff>0.05 Meat: 9.8 (T1), 20.6 (T2), 10.0 (T3), P for diff>0.05	Adjusted for age, sex, and energy intake.	Dairy and meat consumption are not significantly related to vitamin B12 status
Lasheras et al. (47), 2003, Spain, Cross–sectional	*N* = 140 (58%)	Men: 73.3 women: 74.2 [60–80]	FFQ for dietary intake, individual foods vegetables, legumes, fruit, cereals, potatoes, fish, meat, eggs, milk and dairy, other foods.	Hcy (μmol/L)	Multiple linear regression with beta (95%CI) for Hcy and intake of: Meat: −0.083 (−0.035 to 0.010) Milk and dairy products: 0.004 (−0.020 to 0.021) Total dietary score: −0.156 (−0.545 to −0.015)	Adjusted for sex, age, and serum creatinine Total diet score based on quartiles of the intakes (grams per day) of main food groups contributing to intake of B–vitamins: i.e., meat, fish, milk, dairy, fruit, and vegetables.	Only the dietary pattern characterized by high intakes of B vitamin–rich foods was associated with lower Hcy concentrations and lower proportion of high Hcy.
Ledikwe et al. (48), 2004, United states, Cross–sectional	*N* = 179 (55%)	76.5 [66–80]	24 h recall, 2 months interval during 10 months, categorized 6 main food sources; High nutrient dense; vegetables, fruit, milk, poultry fish. Low nutrient dense; dairy desserts, meat	Vitamin B12 (pg/mL)	Least Square Mean (95%CI) vitamin B12 (pg/mL): Low–nutrient dense pattern: 455 (406–504) High–nutrient dense pattern: 556 (493–618) P for difference 0.03 Least Square Mean (95%CI) Hcy (μmol/L): Low–nutrient dense pattern: 9.9 (9.1–10.7) High–nutrient dense pattern: 9.9 (8.9–10.8) P for difference 0.981 OR (95%CI) of having B12<350 pg/mL while consuming low nutrient dense pattern group compared to high nutrient dense pattern: 2.15 (0.93–4.96)	Significance tests adjusted for energy intake, age, sex, tobacco use, alcohol use Low–nutrient–dense pattern: higher intake of breads, sweet breads/desserts, dairy desserts, processed meats, eggs, and fats/oils High–nutrient– dense pattern: higher intake of cereals, dark green/yellow vegetables, other vegetables, citrus/ melons/berries, fruit juices, other fruits, milks, poultry, fish, and beans	Consumption of a high–nutrient–dense dietary pattern was associated with higher vitamin B12 concentrations compared to a low nutrient dense dietary pattern. Hcy concentrations did not differ between high and low nutrient dense diets.
Vogiatzoglou et al. (43), 2009, Norway, Cross–sectional	*N* = 2,861	[71–74]	169 item FFQ	Vitamin B12 (pmol/L)	Adjusted mean (95%CI) plasma vitamin B12 concentrations in Q4 vs. Q1 B12 intake from: Dairy: 358 (348,368) vs. 318 (309, 328) Milk: 357 (347,367) vs. 317 (307, 327) Cheese: 343 (332,355) vs. 337 (328, 347) meat & meat products: 342 (332, 354) vs. 340 (332, 349) Fish and shellfish: 359 (350, 369) vs. 321 (311, 330) eggs: 334 (324, 344) vs. 339 (331,347) P for trend < 0.01, except for cheese (*P* = 0.707), meat (*P* = 0.522), and eggs (*P* = 0.677)	Adjusted for sex, energy, use of B vitamin containing supplements, total intake of other food groups	Dairy and fish are significant contributors to plasma vitamin B12. Vitamin B12 appears to be more bioavailable from dairy products than from other animal products.

(Continued)

TABLE 4 | Continued

Author, year, country, design	Population (% female)	Age [range] (years)	Dietary intake	Vitamin B12 status biomarker	Results	Remarks	Final conclusion
Brouwer- Brolsma et al. (49), 2015, The Netherlands, Cross–sectional	$N = 600$ (42%)	72 [>65]	190 item FFQ including meat, fish and shell fish, eggs and dairy products.	Vitamin B12 (pmol/L)	Probability (95%CI) of having serum B12 > 200 pmol/L (T3 vs. T1). B12 intake from: Total vitamin B12 intake: 1.20 (1.06, 1.35) Meat: 1.22 (1.08, 1.37) (Shellfish: 1.16 (1.04, 1.30) Eggs: 1.05 (0.93, 1.18) Dairy: 1.24 (1.10, 1.39)	Adjusted for age, sex, BMI, education, alcohol intake, physical activity, smoking, creatinine, total energy intake, intake of other vitamin B_{12} containing food items.	Higher intakes of dairy, meat, and fish and shellfish were significantly associated with higher vitamin B12 concentrations, with meat and dairy (predominantly milk were the most potent sources)

of the studies investigated the link between animal food products with MMA or holoTC concentrations in adults.

A number of studies described vitamin B12 intake or vitamin B12 biomarkers among omnivores, vegetarians and vegans. All studies consistently observed that vitamin intake was lowest, intermediate and highest among vegans, vegetarians and meat-eaters, respectively (51–55). Similarly, studies also observed lowest, intermediate and highest vitamin B12 concentrations among vegans, vegetarians and meat-eaters, respectively (51, 54–56), or with holoTC concentrations(51). In line with this, prevalences of vitamin B12 deficiency were highest among vegans and lowest among omnivorous (52, 54, 56), although it should be noted that these studies used different criteria to define vitamin B12 deficiency. Other studies addressing dietary patterns in relation to vitamin B12 status have used different approaches to define patterns. Tucker et al derived patterns by cluster analysis. Food groups that contributed to vitamin B12 were entered into the analysis as percentages of total individual vitamin B12 intake. The cluster procedure assigns individuals to predetermined numbers of clusters in a manner that maximizes the difference across groups for the included variables. Factor analyses reveal that 6 patterns led to the clearest separation of vitamin B12 sources, being (1) supplements (61% Supplements, 11% meat), (2) meat (57% meat), (3) milk (38% milk, 21% meat, 10% fish), (4) cereal (37% Cereal, 17% meat, 12% milk, (5) meat and soups (25% Meat, 24% soups, 12% fish, 10% milk), and (6) fish (35% Fish, 21% meat, 10% other dairy). Plasma vitamin B12 concentrations were significantly lower in the meat pattern than in the cereal and milk patterns, despite similar average vitamin B12 intakes in these 3 groups. Subjects in all food intake groups were significantly more likely to have plasma vitamin B-12 concentrations <185 pmol/L compared to subjects in the supplement group, with odds ratios ranging from 1.6 for the milk group to 2.4 for the meat group. For the likelihood of plasma vitamin B-12 concentrations <148 pmol/L, the meat group was the only group that differed significantly from the supplement group [OR (95%CI) = 2.0 (1.2–3.3)] (3). Another study also used factor analyses to identify major dietary patterns. Three patterns were defined as (1) prudent diet (high intake of eggs, fish, uncooked vegetables, juices, bananas, and other fruits), (2) high animal-protein diet (high intake of meat, chicken, wheat, bananas, and tea with milk), and (3) high plant-protein diet (large intake of cooked vegetables and legumes and a small intake of meat). High intakes of the prudent dietary pattern and the plant protein dietary pattern (quartile 4) compared with lowest intake (quartile 1) were associated with a reduced odds of hyperhomocysteinemia (Hcy > 15 μmol/L), with OR (95% CI) of 0.52 (0.30–0.90) and 0.42 (0.25–0.69), respectively. In contrast, a high consumption of the animal-protein diet was positively associated with hyperhomocysteinemia [OR (95% CI) quartile 4 vs. quartile 1= 2.10 (1.22–3.60). Vitamin B12 concentrations did not differ across quartiles of any of the diets (44). Finally, another study investigated if vitamin B12 and Hcy concentrations differed across different degrees of vegetariarism (vegan, ovo-lacto-vegetarian, and mixed raw food diet including raw meat and fish). This study revealed that consumption of

a vegan diet had lowest median vitamin B12 concentrations and highest Hcy concentrations and consumption of a pattern with mixed raw foods had highest vitamin B12 and lowest Hcy concentrations (40).

Elderly

Five observational studies were identified among elderly (average age population >65 y), out of which 4 focussed on specific animal products (43, 46, 47, 49) and two on dietary patterns (47, 48). A study investigating vitamin B12 intake from supplements, breakfast cereals, dairy and meat consumption revealed that only cereal, but not dairy or meat consumption was related to vitamin B12 concentrations and proportions of B12 concentrations <185 pmol/L. In line with this, meat and milk and dairy products were not associated with Hcy concentrations (47). However, other studies showed that high consumption of dairy and fish were accompanied by higher plasma vitamin B12 concentrations compared to low consumption of these food groups(43), and that high intakes (T3 vs. T1) of meat, (shell)fish, and dairy were associated with an increased odds of having vitamin B12 concentrations >200 pmol/L (49).

A dietary pattern characterized by high intakes of foods rich in B-vitamins, such as meat, fish, milk, dairy, fruit, and vegetables, was associated with lower mean Hcy concentrations (47). Another study comparing consumption of high vs. low nutrient-dense dietary pattern revealed higher vitamin B12 concentrations in those consuming a high nutrient dense pattern compared to those consuming a low nutrient dense dietary pattern. Hcy concentrations did not differ between these the high and low nutrient dense patterns. In this study, a high nutrient-dense pattern was defined as higher intake of cereals, dark green/yellow vegetables, other vegetables, citrus/ melons/berries, fruit juices, other fruits, milks, poultry, fish, and beans, whereas a low-nutrient-dense pattern consisted of higher intake of breads, sweet breads/desserts, dairy desserts, processed meats, eggs, and fats/oils (48).

Summary of General Findings and Considerations

Dairy consumption seems to be the strongest determinant of vitamin B12 concentrations. However, when comparing the magnitude of the relation of dairy, meat, fish or egg consumption with vitamin B12 status it is essential to adjust statistical analyses for vitamin B12 intake from other animal food products, which was done in 5 studies (3, 34, 35, 43, 49). In addition, the specific individual food items representing dairy, meat, and fish consumption could not always be derived from the individual studies, which hampers direct comparison between studies. Finally, nutrient-density of different dairy (milk, yogurt, cheese, curd cheese), meat (chicken, pork, veal), and fish (lean vs. fatty) differs considerably. There is a knowledge gap regarding the bioavailability of vitamin B12 from these different animal food products. In addition, associations of different animal product groups with MMA and holoTC remain largely unknown.

VITAMIN B12 INTAKE OR STATUS AND HEALTH OUTCOMES

This section addresses the associations between low vitamin B12 intake or status (defined by abnormal biomarkers) and several health outcomes from epidemiological studies performed in vulnerable population groups.

Maternal Vitamin B12 Status and Pregnancy

Vitamin B12 deficiency can cause megaloblastic anemia/pernicious anemia (57). Women with untreated pernicious anemia have often infertility problems or repeated abortions. When women were diagnosed with vitamin B12 deficiency and had received vitamin B12, pregnancy occurred (58–60).

The metabolisms of vitamin B12 and folate interact. Supplementation of folic acid before pregnancy and in the first pregnancy trimester reduces the risk of neural tube defects (NTDs) in the child. An inverse association has been reported between NTDs risk and vitamin B12 status or polymorphisms in vitamin B12 metabolizing enzymes (61–64). Ray et al. have shown that a low serum holotranscobalamin (<55.3 pmol/L) at 15–22 weeks of gestation was associated with the risk of NTDs in a study that was done in Ontario after the fortification with folic acid (65). There is a general agreement that vitamin B12 concentrations >250 pmol/L in women entering pregnancy is associated with low risk for NTDs compared to when vitamin B12 is below this level (63). These findings may suggest that supplementation with vitamin B12 may reduce the risk for NTD. However, it is unknown if supplementation with both folic acid and vitamin B12 decreases the number of births with a NTD compared to supplementation with folic acid alone.

Maternal vitamin B12 status determines vitamin B12 status of the child at birth and thereafter. Vitamin B12 in neonates at birth is higher than that in plasma of the mother, but it generally declines in the infants after birth. In a nested case-control study, concentrations of vitamin B12, Hcy, and MMA were assessed in healthy pregnant women ($n = 114$) from week 18 of pregnancy through 6 mo postpartum and related to infant cobalamin status at 6 mo, and compared with healthy, never-pregnant women aged 18–40 y controls ($n = 123$). Compared to controls, vitamin B12, Hcy and MMA were lower in pregnant women at 18 w of pregnancy. Vitamin B12 significantly decreased from week 18 to week 36 of pregnancy and increased again by 6 wk postpartum, whereas Hcy and MMA concentrations increased from week 18 of pregnancy to 6 wk postpartum. Infant vitamin B12 concentration at 6 months correlated with maternal vitamin B12 concentration during pregnancy and postpartum (rho = 0.36–0.55, $P < 0.001$). A maternal vitamin B12 concentration <394 pmol/L during week 18 of pregnancy was associated with an increased risk (OR: 4.2; 95% CI: 1.5, 11.5) of infant vitamin B12 deficiency at 6 mo (defined as tHcy ≥6.5 μmol/L) (66). Breastfed infants are at risk for deficiency in this period if their depleted mothers are not taking vitamin B12-containing supplements (67). Most cases of infantile vitamin

B12 deficiency become manifested between 6 and 11 months of age. Neuromuscular and growth or developmental disorders or cerebral atrophy can occur. Symptoms such as irritation, feeding difficulties, stunting, or anemia have been reported in deficient neonates. Vitamin B12 deficiency may leave residual neurological abnormalities (68).

Breastfeeding

Prolonged breastfeeding is related to food insecurity and represent a problem in many parts of the world where the mothers have multiple micronutrient deficiencies. In Indian children (mean age 16 months) from families of low to middle socioeconomic status, prolonged breastfeeding was associated with stunting, anemia, low weight, or wasting (low weight-for-length) in the child (69, 70). A causal role for vitamin B12 deficiency in childhood stunting is possible, but not well-investigated due to possible confounding by intestinal infections, protein deficient diet or multiple nutrient deficiencies that can affect stunting (71–73). Maternal and/or infant vitamin B12 status has been related to infant physical growth (74), anemia, and cognitive and mental function (75). However, it is unknown if requirements for vitamin B12 in pregnant and lactating women should be increased, and if improving maternal or child vitamin B12 status can improve the outcome such as anemia (76) and cognitive development in the child. There are currently some studies ongoing on this topic (77).

Elderly

Several epidemiological studies reported associations between vitamin B12 biomarkers and brain health, cognitive function, or bone health [reviewed in (78)]. In addition, some evidence appears to suggest that lower B12 status is related to increased pro-oxidant and decreased antioxidant status (79). In a 5-y follow up study among dementia-free elderly people, vitamin B12 in the lowest tertile (<308 pmol/L) or holoTC (< 54 pmol/L) was negatively associations with accelerated brain volume loss, compared with those with higher levels (80). No such association was observed for MMA and Hcy with brain volume loss. In contrast, Smith et al., observed an association between high plasma Hcy and MMA and the risk of cognitive impairment in elderly people who were free of dementia at baseline, while higher holoTC and vitamin B12 showed a negative association with the risk of cognitive impairment (81). Most intervention studies to lower tHy have used multivitamins containing folic acid and vitamin B12 among other vitamins, and studies have shown a protective effect of multivitamins containing vitamin B12 on global cognition (82), brain shrinkage (83), or quality of life scores (84).

In a 6-y follow up study in Swedish elderly men (n = 790; age range 70–81 years), lowered holoTC was associated with an increased risk of fracture (hazard risk for the lowest tertile of holoTC= 1.74; 95% CI 1.12–2.69) (85), while Hcy and MMA were not associated with bone mineral density or fracture risk. In a nationally representative cross-sectional study in U.S. women ≥50 years, Baily et al., have reported an association between elevated Hcy and MMA and the risk of lumbar spine osteoporosis, but B12 and MMA were not associated with bone mineral density (86). In an update meta-analysis including RCTs on the association between homocysteine-lowering trials and fracture risk in elderly people (87)., Garcia Lopez et al., found no association between lowering Hcy (using folic acid and vitamin B12) and the risk of fractures (87).

In general, the evidence from homocysteine-lowering trails by B-vitamins on cognition or bone fracture is mixed and there are several negative studies (78). There is some evidence that vitamin B12 supplementation could have positive effects on health in elderly people who are vitamin B12-deficient. Nevertheless, more research in this group of elderly people is still warranted.

Special Considerations

The Health Significance of High Folate and Low Vitamin B12

There is some concern about supplementing high doses of folic acid to women of reproductive age with low vitamin B12 intake. Supplementing folic acid >1 mg/d is common in many parts of the world where low vitamin B12 is endemic, thus causing unbalanced intake and status of the two B-vitamins. Vitamin B12 deficiency is common in pregnant women from many countries such as Colombia (88), Brazil (89), or India (90). Using multivitamin supplements before pregnancy is not common and is related to education and income level (91). Imbalanced levels of folate and vitamin B12 (i.e., high folate and low B12) have raised some concern, although this topic is not well-investigated yet.

Due to low animal source foods, Indian women are a good example of a population with imbalanced folate-to-B12 ratio. A small observational study including Indian women at 36 weeks of gestation and their newborn children within 24 h after birth reported a negative association between folate-to-B12 ratio and birth weight, birth length, and head and chest circumferences (92). It has been reported that neonates born to Indian women with low B12 intake, consuming >1 mg/d of folic acid, and those with a low B12-to-total folate intake ratio are at increased risk of being born small for gestational age (93). Moreover, high red blood cell (RBC)-folate in Indian pregnant women has been related to adiposity in their children (94) and to increased risk of insulin resistance in the children if maternal plasma B12 was also low (94, 95). The risk of gestational diabetes was higher in vitamin B12-deficient Indian women (95), and the risk of persistent diabetes in the deficient women with gestational diabetes was higher in those women with higher folate status (95).

In UK pregnant women, both folate and vitamin B12 status showed inverse associations with maternal BMI (96). Vitamin B12 insufficiency was also associated with insulin resistance in those women (96). Women with a combination of low plasma vitamin B12 (<170 pmol/L) and folate (<10.3 nmol/L) had the highest BMI, while those with high vitamin B12 (>238 pmol/L) and folate status (>18.3 nmol/L) had the lowest BMI (96). Another study in UK pregnant women (beginning of the 3rd trimester) observed a negative association between maternal vitamin B12 and the risk of obesity and gestational diabetes (97). In pregnant women with gestational diabetes, the risk for fetal macrosomia was higher in the highest folate quartile and lowest vitamin B12 quartile (97). In a study among

Spanish women, maternal folate was negatively associated with insulin sensitivity (HOMA-IR test), while low vitamin B12 was associated with insulin resistance (98). Given these close interactions between vitamin B12 and folate, it is not unlikely that other nutrient-nutrient interactions occur, for example with n-3 fatty acids. As such, future studies may also take nutrient-density of specific animal food products or dietary patterns into account.

The Health Significance of High Vitamin B12 Intake and Status

High dietary intake of vitamin B12 has not been shown to be disadvantageous. Supplemental forms of vitamin B12 are considered safe and there is no evidence-based Tolerable Upper Intake Level for vitamin B12. However, elevated plasma concentrations of vitamin B12 (often defined as plasma vitamin B12 levels >600, >800, or >1,000 pmol/L) in individuals not receiving supplemental vitamin B12 have been described in studies in patients with different cancers, liver diseases, or type 2 diabetes (99) that were later attributed to renal dysfunction (100, 101). The clearance of a single dose of radiolabeled vitamin B12 has been shown to be delayed in patients with renal dysfunction (101). Studies conducted in hospital settings have shown that elevated plasma vitamin B12 is associated with elevated plasma levels of liver enzymes and creatinine or albuminurea (102) and several clinical conditions such as chronic kidney disease, diabetes, liver disorders (of any etiology), alcoholism, or malignancies (100, 103, 104).

Elevated plasma vitamin B12 has been shown to predict future cancer (105), cardiovascular mortality in cohort studies (106), and levels above 400 pmol/L may predict short term mortality (within 90 days) irrespective of the cause of death in hospitalized elderly patients (107). A prospective study on 161 patients with different cancers investigated serum vitamin B12 concentrations and the time of death (108). The global median survival time was 45 days (CI 95%: 32–56 days). The highest mortality corresponded to the highest vitamin B12 levels. A significant link was found between elevated vitamin B12 (>600 pmol/l) and the presence of metastasis, a tumor or liver problems (108). In a recent study based on primary care database, the incidence rate ratio for cancer was 4.72 (95% confidence interval: 3.99–5.58) in persons with vitamin B12 >1,000 pmol/L compared to those with a low vitamin B12 levels after multivariate adjustments (109).

Notably, a causal role for vitamin B12 in future diseases or mortality cannot be assumed based on the presence of elevated plasma vitamin B12 levels. High vitamin B12 test results could be due to supplementation (i.e., long storage time), release from damaged tissues, or reduced kidney excretion. In all instances high plasma vitamin B12 levels are likely to be too unspecific to be used as a screening test for existing tumors or to predict future health outcomes. The likelihood of detecting cancer in patients with high vitamin B12 test has not been studied. Considering the high rate of false positive results (with seriously negative impact on patients), there is currently no evidence to initiation of further cancer diagnostic tests in subjects with plasma vitamin B12 levels >600 pmol/L.

Consideration

Studies reporting on the relationship between vitamin B12 intake and health outcomes have limitations due to methodological variations related to quantifying the intake, differences in population characteristics and to the fact that clinical outcomes such as anemia or neuropathy are late manifestations of the deficiency and are not specific for vitamin B12 deficiency.

OVERALL SUMMARY

The current recommendation is to decrease consumption of animal foods and increase consumption of plant foods, as recently suggested by the EAT-Lancet commission (110). However, a major concern of diets low or without animal products is the risk of vitamin B12 deficiency. This review showed that a total intake of vitamin B12 from the diet between 4 and 7 μg/d is associated with normal plasma vitamin B12 and MMA and thus appears to be adequate to maintain body vitamin B12 status in adults. However, this intake might not be sufficient if people have difficulties in chewing foods, releasing the vitamin from its food binding, and/or absorbing it due to intrinsic factor antibodies or medications (25, 26). It is currently unknown if vitamin B12 requirements should be age-specific, since vitamin B12 deficiency is common in elderly.

When considering specific animal food products, dairy consumption seemed to be a stronger determinant of vitamin B12 concentrations than meat, fish and eggs. However, nutritional composition of different dairy (milk, yogurt, cheese, curd cheese), meat (chicken, pork, veal), and fish (lean vs. fatty) differs considerably, and bioavailability of vitamin B12 from these different animal food products together with potential interactions between vitamin B12 and other nutrients from these nutrient-dense animal products are unclear. Therefore, nutrient-density or well-known interactions between nutrients, such as folate and vitamin B12, should also be considered when studying the relations of intake on status or health.

AUTHOR CONTRIBUTIONS

SE, RO, SH, and MV substantial contributions to the conception or design of the work. All authors drafting the work or revising it critically for important intellectual content. All authors provide approval for publication of the content. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. SE, RO, SH, and MV selected extracted relevant papers of this manuscript. SE, RO, and SH wrote the manuscript. SE had primary responsibility for final content. All authors read and approved the final manuscript.

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The Newly Developed Elderly Nutrient-Rich Food Score Is a Useful Tool to Assess Nutrient Density in European Older Adults

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Objective: To develop a nutrient-rich food (NRF) score that captures dietary reference values for older adults and to validate this against a diet index that was specifically designed to assess adherence to dietary guidelines for the older population.

Design: A cross-sectional study within the Dutch National Food Consumption Survey (DNFCS, $n = 735$ men and women aged 70–94 years, enrolled between October 2010 and February 2012) and within the NU-AGE study ($n = 250$ men and women aged 65–79 years, enrolled between April 2012 and March 2013). Dietary intake was assessed by means of two non-consecutive dietary record assisted 24-h recalls and 7-day food records, respectively. Structured questionnaires collected data on lifestyle and socio-economic information. Anthropometrics were measured by trained dieticians or research assistants. We evaluated Elderly NRF (E-NRF) scores against the NU-AGE index, a measure of adherence to European dietary guidelines for the aging population. The E-NRF scores were composed of nutrients that: (1) have been shown to be of inadequate intake in the aging population (>20%), (2) were defined as nutrients of public health relevance, and (3) were associated with relevant health outcomes.

Results: The E-NRF score that best predicted the NU-AGE index included seven nutrients to encourage (protein, dietary fiber, folate, vitamin D, calcium, magnesium, potassium) and three nutrients to limit (saturated fat, sodium and mono- and disaccharides) on a 100-kcal basis, the E-NRF7.3 score (model R^2 0.27 in DNFCS and 0.41 in NU-AGE). Food groups contributing the most to the individual E-NRF7.3 scores were vegetables, bread, potatoes and milk and milk products.

Conclusion: The E-NRF7.3 score is a useful tool for assessing nutrient density of diets within the older population. No index has previously been developed with the aim of evaluating nutrient density of diets and foods specifically capturing dietary reference values for older adults.

Keywords: nutrient density, diet, elderly, nutrient profiling, nutrient-rich food index, diet quality

INTRODUCTION

Changing demographics in Europe result in an increasing proportion of older people; the number of people aged 65 years and over is projected to rise from 97.7 million (19.2%) in 2016 to 151 million (29.1%) in 2080 (1). Longer lives are often accompanied by increased morbidity and suboptimal health. Suboptimal health in people aged 60 years and over comprises 35% of the burden of disease in high-income countries, which is mainly attributable to degenerative diseases, such as cardiovascular disease and cancer (2).

Many degenerative diseases are influenced by inadequate nutrient intakes such as low protein, fiber, and micronutrient intakes on one hand (3–5) and excess intakes of glucose and fat causing low-grade inflammation on the other hand (6, 7). Besides degenerative diseases, low micronutrient intakes among older adults are associated with adverse outcomes such as increased fracture risk for low vitamin D intake (8, 9). Moreover, increasing numbers of nutrient deficiencies within individuals are associated with a higher risk of frailty (10).

Inadequate nutrient intakes are common, as observed in recent studies. A systematic review on macronutrient intakes in community-dwelling elderly from 46 studies showed inadequate protein intakes of 10 and 12% when using an Average Requirement (AR) of 0.66 g/kg bodyweight/day, rising to 27 and 23% in men and women, respectively when applying more recent suggestions of an AR of 0.83 g/kg bodyweight/day (11). Additionally, dietary fiber intakes were below recommendations in most European countries according to a recent review on nutrient intakes from 18 European National Nutrition Surveys (12). A recent systematic review of 37 studies from Western countries including participants aged 65 years and over observed nutrient intake inadequacies that were of public health concern with over 30% reporting an intake below the AR for six nutrients, namely vitamin D, vitamin B1, vitamin B2, calcium, magnesium and selenium (13). Additionally, over 20% showed inadequate intakes for folate, vitamin B6, and vitamins A, C and E (14). This partly matches nutrients identified by EURRECA as the most relevant for public health of elderly people at European level, namely vitamin D, vitamin B12, folate, calcium and iron (15). In the most recently published data on nutrient intakes in the Netherlands including men and women aged 70–94 years of age, 95% of men and 91% of women had inadequate vitamin D intakes, whereas potentially inadequate intakes were observed for vitamin A, B2, B6, folate equivalents, selenium and (for men) vitamin C (16). Another study in the Netherlands (252 men and women aged 65–79 years of age), observed higher percentages of inadequate intakes for vitamin D (98%), selenium (41%) and vitamin B6 (54%), even when taking into account the use of supplements (87, 36, and 20%, respectively) (17). Additionally, omega-3 fatty acids were identified as important nutrients for healthy aging (18). Intakes of sodium, saturated fat and free sugar on the other hand have been reported to be much higher than the current recommendations (12). While energy requirements decrease with age (19), nutrient requirements stabilize or increase, contributing to inadequate nutrient intakes in older adults and necessitating a more nutrient-dense diet (20).

Nutrient density can be expressed by composite indices of nutritional quality. These nutrient density scores reflect the nutrient density of a food or diet in relation to dietary reference values per standard unit (e.g., per 100 gram or 100 kcal). Calculating nutrient content per standard unit, instead of using food groups in dietary indices, means nutrient density can be calculated. This allows for simple calculations, both on the food level as well as the diet level, of how to increase nutrient intake while keeping energy intakes stable. Additionally, nutrient density scores are widely applicable, because they do not rely on specific foods and food groups of which the intake varies between regions. Moreover, nutrient density scores can be used to calculate which foods have maximum nutrient-to-kcal ratios for the lowest price (21). The NRF9.3 score includes nine nutrients to encourage and three nutrients to limit based on nutrients of concern for American adults (22). The NRF9.3 has been validated with the Healthy Eating Index in the USA (23) and with the Dutch Healthy Diet index (DHD) in the Netherlands (24). The NRF9.3 can be used to study associations with health outcomes, such as cardiovascular diseases and mortality (25) and to study the contribution of foods or food groups to the overall nutrient intake (17, 24). However, as the NRF9.3 is based on requirements for American adults it might be of limited use for studying nutrient density within an aging European population as it does not include several important nutrients for older adults, such as vitamin D and folate (17). To our knowledge, there is no nutrient rich food score that specifically captures relevant nutrients for older European adults. Such nutrient density score for older adults could be used to support nutrition and health claims (26), help older people to identify nutrient-rich foods and shape their food purchase decisions by which their diet quality could improve (27).

The aim of the present study was to develop a nutrient-rich food score (Elderly-NRF) that may be used as a tool to distinguish high and low nutrient-dense diets by targeting dietary reference values for the older population. Additionally, we evaluated the E-NRF score against a diet score which was specifically developed to measure adherence to dietary guidelines for older adults (NU-AGE index).

METHODS

Study Design and Population

The E-NRF scores were developed and evaluated within the Dutch National Food Consumption Survey (DNFCS) 2010–2012 (16) and within the NU-AGE study (28).

The DNFCS was conducted in non-institutionalized elderly aged 70–94 years in the Netherlands. In total, 3,138 individuals were invited, of which 2,848 were eligible and 739 agreed to participate. For the present study, 735 participants aged 70–94 years (369 men and 366 women) were included, after excluding participants with unlikely energy intakes (<500 or >3,500 kcal; $n = 4$).

The NU-AGE study is a 1-year, randomized, parallel trial with the aim to investigate whether a newly designed, personally tailored Mediterranean-like dietary pattern, targeting dietary recommendations for people over 65 years of age (NU-AGE diet)

can counteract or slow down the inflammageing process. The study was carried out in five European study centers. For the present study we used baseline data of the Dutch cohort including 252 apparently healthy men and women aged 65–79 years enrolled between April 2012 and March 2013. The rationale and design of this intervention study are described in detail elsewhere (28, 29). Ethical approval was provided by the Wageningen University Medical Ethics Committee (The Netherlands). The trial is registered at clinicaltrials.gov (NCT01754012). For the present study, 250 participants were included, after excluding participants with missing data on supplement use ($n = 1$) and unlikely energy intakes (<500 or >3,500 kcal; $n = 1$).

All study procedures were in accordance with the ethical standards of the Helsinki Declaration. All participants gave written informed consent before participating.

Dietary Intake and Covariate Assessment

Within the DNFCS, dietary intake data were collected by means of two non-consecutive dietary record assisted 24-h dietary recalls from October 2010 to February 2012. Each individual was interviewed twice with an interval of 2–6weeks. The recalls were spread equally over all days of the week and seasons. The two 24-h dietary recalls were conducted during home visits using the computer-directed interview program EPIC-SOFT (30, 31). During these visits the dietary records were checked for incompleteness and for the use of household measures to indicate consumption amounts at home. Consumption data were linked to the 2011 Dutch food composition database (Nederlands voedingsstoffenbestand, NEVO) (32) and averaged over 2 days. Foods were organized into twenty-three food groups by the NEVO classification. A general questionnaire assessed demographics, health and lifestyle factors and dietary supplement use. Highly educated people were defined as having higher vocational education or university. Physically active was defined as a minimum of 30 min of moderately intense activity ≥5 days a week. Weight and height were assessed at the participants home. All intake data collection and anthropometric measurements were carried out by trained dieticians (16).

Within the NU-AGE study, food records on seven consecutive days were used to assess dietary intake. To remind participants to record all foods consumed, a preformatted food record was used including eight meal occasions referring to the current day. In advance, participants had a face-to-face training and received written instructions to keep complete and accurate food records (33, 34). Portion sizes were reported in national household measures, based on pictures or measured in gram or milliliters. During a 1-h interview with a trained dietician/research nutritionist the food record was reviewed and checked for frequently used household measures to ensure an adequate level of detail in describing foods and food preparation methods (34). Consumed foods were coded according to standardized coding procedures. Subsequently, each ingredient or food was translated into nutrients and converted into twenty-three food groups by the Dutch food composition database (NEVO) 2011 (32). Data on supplement use was obtained by means of a self-reported supplement questionnaire and checked by a trained dietician/research nutritionist. Participants completed questionnaires about their health and lifestyle. Education was assessed as years of full-time education (>16 years of education, equivalent to a bachelor degree, was considered as highly educated). Physical activity was assessed by means of the Physical Activity Scale for Elderly (PASE). Anthropometric measurements were done by trained research assistants at the research center.

Development and Calculation of Elderly NRF (E-NRF)

Table 1 gives an overview of the stepwise development of the Elderly-NRF (E-NRF) scores. These scores were based upon a selection of nutrients. All positive models contained protein and dietary fiber since inadequate intakes are common and adequate intakes are associated with disease prevention (11, 12, 35, 36). Furthermore, nutrients were selected if they were both shown to be of inadequate intake (≥20%) as reported in a recent review from ter Borg et al. and if they were defined as nutrients of high public health relevance for elderly by EURRECA (14, 15), resulting in the selection of vitamin D, folate and calcium. Additionally, micronutrients that were associated with a health outcome relevant to elderly according to EURRECA, including magnesium, iron, selenium, iodine, potassium, zinc, and vitamin B6, vitamin B12, vitamin C, vitamin E, and vitamin K (15), were selected and individually added to the models. Lastly, polyunsaturated fatty acids (PUFA) were selected, as they are related to health, according to the latest systematic review on nutrients and aging (18). Eventually, the positive scores included protein, dietary fiber, PUFA and a range of micronutrients, namely vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, folate, calcium, magnesium, potassium, zinc, iodine, iron, copper, and selenium; the negative scores comprised saturated fat, sodium and total mono- and disaccharides.

Population Reference Intakes and Adequate Intakes as set by the European Food Safety Authority (37–50), the Nordic Council of Ministers (51), the Health Council of the Netherlands (52) as well as the labeling reference intake values as set by the European Food Safety Authority (53) were used as Dietary Reference Values (DRV) (**Table 2**). The percentage of DRV for each nutrient was capped at 100% DRV to avoid overvaluing food items that provide very large amounts of a single nutrient, such as fortified foods (22).

The calculation of the E-NRF score comprised several steps, similar to calculating the NRF9.3 (32). First, the scores were calculated for each food item per 100 kcal. Subsequently, these food scores were converted into individual scores by multiplying the amount of energy consumed of each item, in 100-kcal units, by the nutrients to encourage (nutrient-rich; NR) scores and then summing these scores for each subject. Next, the NR index scores were divided by the number of 100-kcal units of the subjects' total energy intake to provide a "weighted average" score. For the nutrients to limit (LIM) score, the same approach was used.

The algorithms used to calculate the E-NRF scores are listed in **Table 3** and are based on sums of nutrients where all nutrients were equally weighted (23). The algorithms which combined positive nutrients and nutrients to limit were based on subtracting the negative from the positive sub score (23).

TABLE 1 | Elderly nutrient-rich food scores; models with nutrients to encourage, nutrients to limit, and full models including both.

E-NRF model	Nutrient-rich components			Nutrients to limit
	Macronutrients	Vitamins	Minerals	
LIM3				Saturated fat, total mono- and disaccharides, Na
E-NR5	Protein, dietary fiber	D, folate	Ca	
E-NR6Zn	Protein, dietary fiber	D, folate	Ca, Zn	
E-NR6I	Protein, dietary fiber	D, folate	Ca, I	
E-NR6VitE	Protein, dietary fiber	D, folate, E	Ca,	
E-NR6VitC	Protein, dietary fiber	D, folate, C	Ca	
E-NR6Se	Protein, dietary fiber	D, folate	Ca, Se	
E-NR6B12	Protein, dietary fiber	D, folate, B12	Ca	
E-NR6B6	Protein, dietary fiber	D, folate, B6	Ca	
E-NR6Fe	Protein, dietary fiber	D, folate	Ca, Fe	
E-NR6Mg	Protein, dietary fiber	D, folate	Ca, Mg	
E-NR6K	Protein, dietary fiber	D, folate	Ca, K	
E-NR7	Protein, dietary fiber	D, folate	Ca, K, Mg	
E-NR8	Protein, dietary fiber, PUFA	D, folate	Ca, K, Mg	
FULL MODELS				
E-NRF5.3	Protein, dietary fiber	D, folate	Ca	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-Zn	Protein, dietary fiber	D, folate	Ca, Zn	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-I	Protein, dietary fiber	D, folate	Ca, I	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-VitE	Protein, dietary fiber	D, folate, E	Ca,	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-VitC	Protein, dietary fiber	D, folate, C	Ca	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-Se	Protein, dietary fiber	D, folate	Ca, Se	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-B12	Protein, dietary fiber	D, folate, B12	Ca	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-B6	Protein, dietary fiber	D, folate, B6	Ca	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-Fe	Protein, dietary fiber	D, folate	Ca, Fe	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-Mg	Protein, dietary fiber	D, folate	Ca, Mg	Saturated fat, total mono- and disaccharides, Na
E-NRF6.3-K	Protein, dietary fiber	D, folate	Ca, K	Saturated fat, total mono- and disaccharides, Na
E-NRF7.3	Protein, dietary fiber	D, folate	Ca, K, Mg	Saturated fat, total mono- and disaccharides, Na
E-NRF8.3	Protein, dietary fiber, PUFA	D, folate	Ca, K, Mg	Saturated fat, total mono- and disaccharides, Na

NR, nutrients to encourage; LIM, nutrients to limit; E-NRF, full elderly nutrient-rich food score.

Moreover, the scores were calculated per 100 kcal, since this led to the highest percentage of variance accounted for in previous validation studies (54). Higher E-NRF scores indicate higher nutrient density per 100 kcal.

NU-AGE Index

The E-NRF scores were evaluated against the NU-AGE index. The NU-AGE index is an a priori dietary index developed by Berendsen et al. (55) The NU-AGE index is meant to reflect adherence to guidance based on DRVs and food based dietary guidelines for elderly individuals from Italy (56), the UK (57), the Netherlands (58–62), Poland (63), and France (64), on the modified MyPyramid for Older Adults (65, 66), and nutrient requirements from the European Community (67), and from the Institute of Medicine (68). These recommendations were jointly integrated into NU-AGE Food Based Dietary Guidelines (**Table 4**), including recommendations on consumption of whole meal bread and wholegrain pasta or rice, fruits, vegetables, legumes, low-fat dairy, low-fat cheese, fish, low-fat meat, and poultry, nuts, eggs, olive oil, fluid and use of a vitamin D supplement, alcohol, salt (sodium), and sweets. The NU-AGE index is a continuous score with 16 components based on adherence to the aforementioned guidelines. For all components a maximum of 10 points can be assigned resulting in a score of 0–160.

Statistical Analyses

All statistical analyses were performed with SPSS version 23.0. General characteristics are expressed as mean ± SD or number (percentage) and differences between men and women were tested with independent *t*-tests or Mann Whitney-*U*-test for continuous variables or chi-square test for categorical variables. Spearman correlation coefficients between all E-NRF scores and the NU-AGE index were calculated. Regression analyses were conducted using the NU-AGE index as the dependent variable and the E-NRF scores as independent variable, testing one E-NRF score at a time. The proportion of explained variance (score R^2 and model R^2) and standardized regression coefficients (STB) were estimated while adjusting for age and sex by using the following equation:

TABLE 2 | Dietary reference values for selected nutrients used in developing the E-NRF7.3.

Nutrient	RDV	References
NUTRIENT-RICH COMPONENTS		
Protein, g[a,b]	112.5 (m), 90 (w)	NNR (51)
Fiber, g	35 (m), 25 (w)	NNR (51)
Vitamin A, μg RE	750 (m), 650 (w)	EFSA (38)
Vitamin C, mg	110 (m), 95 (w)	EFSA (40)
Vitamin E, mg	13 (m), 11 (w)	EFSA (45)
Calcium, mg	1,200	HCNL (52)
Iron, mg	11	EFSA (46)
Magnesium, mg	350 (m), 300 (w)	EFSA (37)
Potassium, mg	3,500	EFSA (48)
Vitamin D, μg	20	HCNL/NNR (51, 52)
Folate, μg DFE	330	EFSA (41)
Vitamin B12, μg	2.8	HCNL (52)
Zinc, mg[c]	11.7 (m), 9.3 (w)	EFSA (42)
Selenium, μg	70	EFSA (43)
Iodine, μg	150	EFSA (44)
Copper, mg	1.6 (m), 1.3 (w)	EFSA (47)
Vitamin B2, mg	1.6	EFSA (50)
PUFA, g[b,d]	22.2 (m), 17.8 (w)	NNR (51)
Vitamin B1, mg[b,e]	1.0 (m), 0.8 (w)	EFSA (49)
Vitamin B6, mg	1.7 (m), 1.6 (w)	EFSA (39)
NUTRIENTS TO LIMIT		
Saturated fat, g	20	EFSA (53)
Sugar, g	90	EFSA (53)
Sodium, mg[f]	2,400	EFSA (53)

Population Reference Intakes and Adequate Intakes as set by the European Food Safety Authority (EFSA) (37–50), the Nordic Council of Ministers (NNR) (51), the Health Council of the Netherlands (HCNL) (52) as well as the labelling Reference Intake values as set by the European Food Safety Authority (53) were used as Reference Daily Values (RDV) m, men; w, women.

[a] *Values equal to18 energy percent.*

[b] *Based on EFSA reference intakes of 2,500 kcal and 2,000 kcal reference intakes for men and women, respectively.*

[c] *EFSA references for mixed diets, containing 600 mg of phytate.*

[d] *Values equal to 8 energy percent.*

[e] *Value equal to 0.4 mg per 1,000 kcal.*

[f] *Value derived from salt reference value using a conversion factor of 2.5.*

NU-AGE index = $\beta_0 + \beta_1$*E-NRF score + β_2*age in years + β_3*gender

Sensitivity analyses were performed to assess the robustness of the results. The regression analyses were conducted separately for men and women, for lower weight and higher weight subjects (median-split BMI $\leq$ 27.1 and $>$ 27.1 kg/m^2 in DNFCS and BMI $\leq$ 26.1 and $>$ 26.1 kg/m^2 in NU-AGE), and across levels of energy intake (median-split $\leq$1,937 kcal vs. $>$1,937 kcal in DNFCS and $\leq$1,844 kcal vs. $>$1,844 kcal in NU-AGE).

The E-NRF score with the highest proportion of explained variance in both the DNFCS and NU-AGE study was used to score all foods. To provide insight into the nutrient density of food groups, mean index scores per 100 kcal on a food-group level were calculated. Additionally, to study the contribution of food groups, taking into account the amount consumed, mean contribution (percent) of food groups to the individual weighted scores were calculated.

TABLE 3 | Algorithms used to calculate the E-NRF index scores.

Model	Algorithm	Comment
NR$n_{100kcal}$	$\sum_i = 1$–n (Nutrient$_i$/RDV$_i$) * 100	Nutrient$_i$ = content of nutrient i in 100-kcal edible portion; RDV$_i$ = recommended daily values for nutrient i
LIM3$_{100kcal}$	$\sum_i = 1$–3 (Nutrient$_i$/MDV$_i$) * 100	Nutrient$_i$ = content of limiting nutrient i in 100-kcal edible portion; MDV$_i$ = recommended daily values for nutrient i
NRF$n.3_{100kcal}$	NRn–LIM3	Difference between sums

NRn, nutrient-rich score consisting of n beneficial nutrients dependent on the evaluated E-NRF score; LIM3, limited nutrient score consisting of three nutrients to limit; NRF, nutrient-rich foods score.

RESULTS

The DNFCS and NU-AGE study populations had a mean age of 77.1 ± 5.2 and 71.0 ± 4.0 years, a BMI of 27.4 ± 3.8 and 26.1 ± 3.6 kg/m^2, consisted of 369 (50%) and 111 (44%) men, respectively and the majority did not smoke (90 and 97%, **Table 5**). Within the DNFCS there was a higher proportion of people with diabetes mellitus (12%) compared to the NU-AGE population (3.6%). Overall, women had higher NU-AGE scores compared to men (64.4 ± 14.2 vs. 60.2 ± 15.0 within DNFCS and 74.2 ± 15.9 vs. 65.1 ± 4.9 within NU-AGE).

Results of the correlation coefficients and linear regression analyses of the evaluated E-NRF scores on the NU-AGE index showed no large differences between the 27 tested E-NRF scores (**Table 6**). The LIM3 was inversely correlated to and least predictive of the NU-AGE index score (STB = −0.27, R^2_{adj} = 0.09 in DNFCS and STB = −0.29, R^2_{adj} = 0.05 in NU-AGE).The E-NRF scores combining nutrients to encourage and nutrients to limit were most predictive of the NU-AGE index. Compared to the E-NRF5.3 score (R^2_{adj} = 0.22 and 0.36 in DNFCS and NU-AGE, respectively), the nutrients that most improved the prediction of the NU-AGE index in both datasets were magnesium and potassium (R^2_{adj} = 0.25 and 0.24 within DNFCS and R^2_{adj} = 0.40 and 0.38 within NU-AGE). Adding PUFA to the E-NRF7.3 score did not substantially improve the prediction (R^2_{adj} = 0.26 in DNFCS and 0.42 in NU-AGE). The E-NRF7.3 score showed best prediction in both datasets with R^2_{adj} = 0.27 and 0.41 in DNFCS and NU-AGE. The correlation coefficient between E-NRF7.3 score and the NU-AGE index was 0.49 in DNFCS and 0.64 in NU-AGE. Actual and predicted E-NRF7.3 scores and NU-AGE index values have been graphically presented in **Figure 1**.

In both study populations, women had a higher mean E-NRF7.3 score compared to men (10.7 ± 6.0 vs. 7.2 ± 5.3 within DNFCS and 12.3 ± 5.1 vs. 8.0 ± 4.6 within NU-AGE). Within the DNFCS, the E-NRF7.3 score predicted the NU-AGE index similarly for men and women (R^2_{adj} = 0.26 and 0.24, respectively). Within NU-AGE, the prediction was stronger

TABLE 4 | Components of the NU-AGE index and their cut-off (maximum score) and threshold (minimum score) values (55).

Component	Servings	Scoring			
		Minimum score (0)	Lower range[b] (1-10)	Maximum score (10)	Upper range[c] (10-1)
Whole meal bread and wholegrain pasta or rice[a]	Bread 4–6 servings/day (140–210 g/day) Pasta/rice 2 × 80 g/week (23 g/day)	Max	1–163 g	163–233 g	233-max
Fruits	2 servings/day (240 g/day)	0 g	0–240 g	≥240 g	
Vegetables	300 g/day	0 g	0–300 g	≥300 g	
Legumes	200 g/week (29 g/day)	0 g	0–29 g	≥29 g	
Low-fat dairy	500 ml/day	0 g	0–500 g	≥500 g	
Low-fat cheese	30 g/day	0 g	0–30 g	≥30 g	
Fish	2 times 125 g/week (36 g/day)	0 g	0–36 g	≥36 g	
Low-fat meat and poultry[a]	4 times 125 g/week (71 g/day)	Max	0–71 g	71–125 g	125-max
Nuts	2 times 20 g/week (6 g/day)	0 g	0–6 g	>6 g	
Eggs	2–4 eggs/week (14–28 g/day)	0 g	0–14 g	>14 g	
Olive oil	20 g/day	0 ml	0–20 ml	≥20 ml	
Fluid	1,500 ml/day	<1,000 ml	1,000–1,500 ml	>1,500 ml	
vitamin D	Use supplement (10 μg/day)	No		Yes	
Alcohol	Max 2 servings/day for men and 1 serving/day for women	>10 g for women >20 g for men		≤10 g for women ≤20 g for men	
Salt[a]	5 g/day (2,000 mg/day sodium)	≥85th	0–1,500 mg	1,500–2,000mg	2,000–85th
Sweets[a]	Limited use	≥85th		0	0–85th

Fruit: maximum 1 glass of fresh fruit juices (120 ml) can be counted as one portion of fruit. Nuts: includes salted and unsalted nuts.

[a]The cut-off value at which a participant would score 0 points was based on the 85 or100th (max) percentile (pct) of the data-specific intake distribution as higher intakes are not necessarily better (100th pct wholegrains (g): 696 DNFCS, 343 NU-AGE; 100th pct meat and poultry (g): 261 DNFCS, 110 NU-AGE; 85th pct sodium (mg); 3141 DNFCS, 2920 NU-AGE; 85th pct sweets (g): 195 DNFCS, 130 NU-AGE).

[b]The range was divided into 10 and then points were given in proportion to the distance from the 0 point cut-off.

*[c]Calculation of points for dietary intake between the upper limit and the standard intake for maximum number of points: 10—(intake—recommendation upper limit) * 10/standard upper limit.*

in women compared to men (R^2_{adj} = 0.43 vs. R^2_{adj} = 0.25, **Supplementary Table 1**).

Within the DNFCS the mean E-NRF7.3 score was lower in subjects with a lower BMI compared to subjects with a higher BMI (mean E-NRF7.3 score 8.4 ± 6.1 vs. 9.6 ± 5.6) whereas the NU-AGE score was comparable between the two groups (62.7 ± 16.3 vs. 62.3 ± 13.3, **Supplementary Table 2**). Within the NU-AGE population the mean E-NRF7.3 score was also comparable between the two BMI groups (10.5 ± 5.8 vs. 10.4 ± 4.8), however, the mean NU-AGE score was substantially higher in those with a lower BMI compared to those with a higher BMI (73.6 ± 17.0 vs. 66.6 ± 14.4). The prediction was consistently higher among subjects with a lower BMI compared to those with a higher BMI within the DNFCS (R^2_{adj} = 0.30 vs. R^2_{adj} = 0.24) and also within the NU-AGE study (R^2_{adj} = 0.45 vs. R^2_{adj} = 0.39).

Food groups that had the highest E-NRF7.3 score on food-item level in both study populations were vegetables, legumes and fish, making up three of the top four in both (**Table 7**). Clinical formulae scored second highest in NU-AGE, whereas in DNFCS miscellaneous foods were the third highest. Food items that had lowest E-NRF7.3 scores were herbs and spices, soups, sugar, sweets and sweet sauces and pastry and biscuits in both populations. With respect to individual E-NRF7.3 scores, taking into account the choice of food items and the amount consumed, vegetables (40 and 33%), bread (35 and 36%), potatoes (24 and 16%) and milk and milk products (18 and 19%) contributed most to individual E-NRF7.3 scores in DNFCS and NU-AGE, respectively (**Figure 2**). However, inter-individual variation was quite high (**Supplementary Table 3**).

DISCUSSION

This study aimed to develop and evaluate a nutrient-rich food score specifically capturing dietary reference values for the older population. We have evaluated multiple combinations of nutrients in relation to an index for a healthful diet specifically for elderly people, the NU-AGE index. The score that best predicted the NU-AGE index in both the DNFCS and NU-AGE study populations included seven nutrients to encourage and 3 nutrients to limit, on a 100-kcal basis, the E-NRF7.3 score, with a model R^2_{adj} of 0.27 in DNFCS and 0.41 in NU-AGE. The E-NRF7.3 score performed well in both men and women and in normal-weight and overweight participants

To our knowledge this is the first study specifically developing a nutrient-rich food score for the aging population. It has previously been discussed that the NRF9.3 could be of limited use within a population of elderly people, as the NRF9.3 is based on recommended daily allowances for a US adult population instead of for an older population (17). As a result, the NRF9.3 does not specifically contain nutrients that are relevant for the

TABLE 5 | Baseline characteristics of the participants of the DNFCS ($n = 735$) and NU-AGE study ($n = 250$).

Characteristic	DNFCS			NU-AGE		
	Total population ($n = 735$)	**Men ($n = 369$)**	**Women ($n = 366$)**	**Total population ($n = 250$)**	**Men ($n = 111$)**	**Women ($n = 139$)**
Age, years	77.1 ± 5.2	76.7 ± 5.0	77.6 ± 5.4*	71.0 ± 4.0	70.9 ± 4.2	71.1 ± 3.9*
BMI, kg/m^2[a]	27.4 ± 3.8	27.2 ± 3.2	27.6 ± 4.3	26.1± 3.6	26.7 ± 3.5	25.5 ± 3.6*
Highly educated[b]	166 (22.6)	104 (28.3)	62 (16.9)*	35 (14.0)	22 (19.8)	13 (9.4)
SMOKING STATUS						
Never	253 (34.4)	55 (14.9)	198 (54.1)*	125 (50)	42 (37.8)	83 (59.7)*
Former	407 (55.4)	269 (72.9)	138 (37.7)	117 (46.8)	65 (58.6)	52 (37.4)
Current	75 (10.2)	45 (12.2)	30 (8.2)	8 (3.2)	4 (3.6)	4 (2.9)
Physically active[c]	576 (78.4)	280 (75.9)	296 (81.1)	137.8 ± 53.3	141.2 ± 54.1	135 ± 52.7
Diabetes mellitus[d]	88 (12.0)	51 (13.9)	37 (10.1)	9 (3.6)	2 (1.8)	7 (5.0)
Hypertension[d]	218 (29.7)	91 (24.7)	127 (34.7)*	83 (33.2)	38 (34.2)	45 (32.4)
Osteoporosis[d]	60 (8.2)	2 (0.5)	58 (15.8)*	26 (10.4)	3 (2.7)	23 (16.5)*
Energy, kcal	1,964 ± 457	2,164 ± 420	1,763 ± 402*	1,901 ± 396	2,086 ± 440	1,754 ± 282*
Sodium, mg	2,318 ± 804	2,563 ± 841	2,070 ± 683*	2,362 ± 634	2,651 ± 690	2,131 ± 474*
Alcohol, g	11.4 ± 16.1	16.0 ± 19.0	6.7 ± 10.6*	12.8 ± 11.9	16.4 ± 13.6	9.9 ± 9.4*
Fat, EN%	34.6 ± 5.8	34.4 ± 5.6	34.8 ± 6.0	34.3 ± 5.1*	33.5 ± 4.5	34.9 ± 5.4*
Carbohydrates, EN%	43.4 ± 6.7	42.7 ± 6.9	44.1 ± 6.5*	42.1± 6	42.3 ± 5.7	42.0 ± 6.2*
Protein, EN%	15.7 ± 3.0	15.4 ± 2.8	15.9 ± 3.1*	16.2 ± 2.4	15.9 ± 2.2	16.4 ± 2.5
NU-AGE index	62.4 ± 14.8	60.2 ± 15.0	64.6 ± 14.2	70.2 ± 16.1	65.1 ± 14.9	74.2 ± 15.9*

Values are presented as mean ± SD and number (%).

BMI, body mass index; EN%, energy percentage; DNFCS, Dutch National Food Consumption Survey.

[a]DNFCS: n = 706 (10 men, 19 women missing).

[b]Highly educated is defined as higher vocational education or university in DNFCS and as >16 years of education (at least Bachelor degree) in NU-AGE.

[c]Physically active is defined as a minimum of 30 minutes of moderately intense activity ≥5 days a week in DNFCS, and as PASE score within NU-AGE (DNFCS: n = 734; NU-AGE: n = 248).

[d]DNFCS: n = 734 (1 man missing).

[*]statistically significant difference between men and women.

older population segment. Therefore, we based our choice of qualifying nutrients on shortfall nutrients, on nutrients that have been shown to be of inadequate intake, and nutrients that were related to relevant health outcomes, specifically within European older populations, resulting in a selection of protein, dietary fiber, vitamin D, folate, calcium, and magnesium as nutrients to encourage and saturated fat, sodium, and total mono- and disaccharides as nutrients to limit.

Of the tested nutrient combinations, the E-NRF7.3 score performed best with an R^2_{adj} of 0.27 in the DNFCS and 0.41 in the NU-AGE study. If there had been a perfect fit between the E-NRF and NU-AGE index, a correlation of $R^2 = 1.0$ would be observed. Participants with a higher E-NRF score can be considered to have a healthier dietary pattern compared to those with a lower E-NRF score. The NRF9.3 has previously been validated against the Healthy Eating Index (HEI) 2005 and the DHD index. The proportion of explained variance of the E-NRF score against the NU-AGE index was slightly lower compared with Fulgoni et al., reporting an R^2_{adj} of 0.45 between the HEI-2005 and the NRF9.3 (23), while it was somewhat higher compared with Sluik et al. who reported an R^2_{adj} of 0.34 between the DHD index and the NRF9.3 (24). Overall, the R^2_{adj} was consistently higher in the NU-AGE study population than in the DNFCS. This might be caused by differences between the study populations. Specifically, the NU-AGE population was younger, had a lower BMI, a lower prevalence of diabetes, and contained fewer smokers compared to the DNFCS. Adding more vitamins and minerals to the model did not necessarily improve the prediction (R^2_{adj} changed from 0.27 for the E-NRF7.3 score to 0.26 for the E-NRF8.3 score in the DNFCS and from 0.41 to 0.42 in the NU-AGE study). Adding some vitamins even lowered the prediction as is visible in the lowest R^2 of 0.16 for E-NRF6.3B6 in DNFCS and of 0.26 for E-NRF6.3B12 score in NU-AGE. This was also demonstrated by the selection of selenium as this mineral has previously been shown to be a shortfall nutrient (14, 17). However, adding this mineral to the E-NRF score resulted in a better R^2_{adj} in neither the DNFCS, nor in the NU-AGE study population. As such, it was decided to select the best model R^2_{adj} with the least nutrients to increase the practical applicability of the E-NRF score.

Within the NU-AGE population, the prediction of the NU-AGE index was higher in women than in men, but not in DNFCS. This could be a result of a higher NU-AGE score and a lower BMI of the women compared to men in NU-AGE. In DNFCS these characteristics did not show large differences.

Moreover, the prediction models were consistently better in the NU-AGE population (R^2_{adj} E-NRF7.3 score = 0.41) compared to the DNFCS (R^2_{adj} E-NRF7.3 score = 0.27). A possible explanation could be the difference in dietary assessment

TABLE 6 | Correlation coefficients and linear regression analyses of the E-NRF scores on the NU-AGE index within DNFCS ($n = 735$) and NU-AGE study ($n = 250$).

Model	DNFCS ($n = 735$)							NU-AGE NL ($n = 250$)						
	Mean	SD	Spearman's ρ	Linear regression with NU-AGE index[a]				Mean	SD	Spearman's ρ	Linear regression with NU-AGE index[a]			
				β	STB	R^2 score	R^2 model				β	STB	R^2 score	R^2 model
LIM3	18.0	2.3	−0.24	−1.76	−0.27	0.05	0.09	17.5	1.9	−0.24	−2.52	−0.29	0.05	0.15
E-NR5	17.5	3.8	0.42	1.72	0.44	0.19	0.19	18.0	3.2	0.59	2.95	0.59	0.34	0.34
E-NR6-Zn	22.5	4.8	0.41	1.36	0.44	0.17	0.17	23.1	4.1	0.57	2.25	0.57	0.30	0.30
E-NR6-I	22.8	4.6	0.41	1.37	0.43	0.18	0.18	23.6	3.8	0.54	2.11	0.50	0.28	0.28
E-NR6-VitE	23.0	4.9	0.42	1.34	0.44	0.18	0.18	23.3	4.4	0.57	2.01	0.55	0.30	0.30
E-NR6-VitC	22.2	5.4	0.44	1.27	0.46	0.20	0.20	22.8	4.9	0.58	1.95	0.59	0.34	0.33
E-NR6-Se	20.7	4.2	0.44	1.63	0.47	0.21	0.21	21.4	3.6	0.56	2.46	0.55	0.32	0.31
E-NR6-B12	25.7	5.8	0.41	0.95	0.37	0.15	0.15	26.1	4.7	0.46	1.41	0.41	0.21	0.22
E-NR6-B6	23.0	5.2	0.36	0.95	0.34	0.12	0.12	22.9	4.2	0.51	1.83	0.48	0.26	0.26
E-NR6-Fe	22.2	4.4	0.44	1.55	0.46	0.20	0.20	23.1	3.7	0.59	2.52	0.58	0.33	0.33
E-NR6-Mg	22.4	4.5	0.46	1.65	0.51	0.23	0.23	23.2	4.0	0.64	2.68	0.67	0.39	0.39
E-NR6-K	22.1	4.4	0.44	1.58	0.47	0.21	0.21	22.7	3.8	0.60	2.59	0.61	0.37	0.36
E-NR7	27.0	5.2	0.47	1.49	0.52	0.24	0.24	28.0	4.7	0.64	2.32	0.67	0.40	0.40
E-NR8	30.7	5.6	0.47	1.42	0.53	0.24	0.25	31.9	5.2	0.65	2.24	0.72	0.42	0.42
E-NRF5.3	−0.5	4.6	0.45	1.48	0.46	0.22	0.22	0.4	3.9	0.61	2.35	0.57	0.36	0.36
E-NRF6.3-Zn	4.5	5.5	0.44	1.26	0.46	0.21	0.21	5.6	4.7	0.60	1.95	0.56	0.33	0.33
E-NRF6.3-I	4.8	5.4	0.44	1.26	0.46	0.22	0.22	6.0	4.5	0.57	1.84	0.52	0.30	0.32
E-NRF6.3-VitE	5.0	5.7	0.44	1.17	0.45	0.21	0.21	5.8	5.0	0.60	1.76	0.55	0.33	0.33
E-NRF6.3-VitC	4.2	6.0	0.48	1.21	0.49	0.24	0.24	5.2	5.3	0.61	1.83	0.60	0.38	0.38
E-NRF6.3-Se	2.7	5.1	0.46	1.40	0.48	0.24	0.24	3.8	4.3	0.59	2.02	0.54	0.33	0.33
E-NRF6.3-B12	7.6	6.4	0.44	1.00	0.43	0.19	0.20	8.5	5.2	0.50	1.41	0.46	0.25	0.26
E-NRF6.3-B6	5.0	6.0	0.39	0.93	0.38	0.15	0.16	5.4	4.9	0.54	1.62	0.49	0.28	0.29
E-NRF6.3-Fe	4.2	5.2	0.46	1.34	0.47	0.23	0.23	5.5	4.4	0.60	2.05	0.56	0.35	0.35
E-NRF6.3-Mg	4.4	5.3	0.47	1.41	0.51	0.25	0.25	5.7	4.7	0.65	2.14	0.62	0.40	0.40
E-NRF6.3-K	4.1	5.2	0.47	1.39	0.49	0.24	0.24	5.2	4.5	0.62	2.12	0.59	0.38	0.38
E-NRF7.3	8.9	5.9	0.49	1.31	0.52	0.27	0.27	10.4	5.3	0.64	1.92	0.63	0.41	0.41
E-NRF8.3	12.7	6.4	0.49	1.20	0.52	0.26	0.26	14.3	5.8	0.66	1.82	0.66	0.42	0.42

E-NRF, Elderly nutrient-rich foods score; E-NR, Elderly nutrient-rich score; LIM, nutrients to limit; STB, standardized regression coefficient.

[a] *Models adjusted for age and sex.*

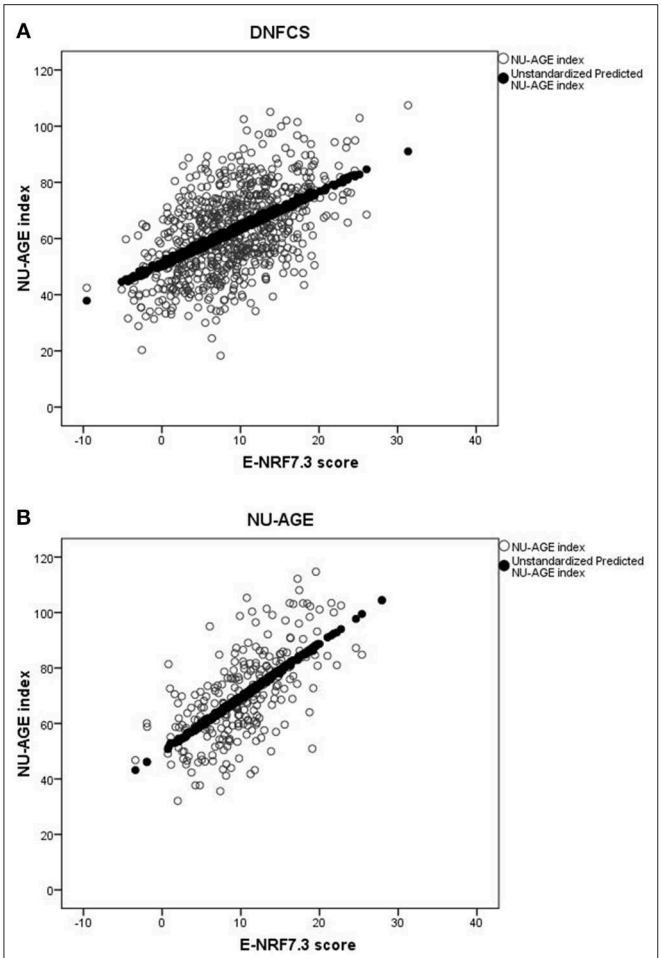

FIGURE 1 | Actual and predicted E-NRF7.3 scores and NU-AGE index values in DNFCS **(A)** and NU-AGE **(B)** populations.

methods, as well as number of days on which dietary intake was assessed. The DNFCS used two-day 24-h recalls, whereas the NU-AGE study used 7-day food records. The two populations also differed in age range (65–79 and 70–94 years of age in the NU-AGE and DNFCS, respectively), which could contribute to differences in reported dietary intake as a result of possible memory complaints, or true differences in dietary intakes as a result of advancing age.

In both study populations, food groups with the highest E-NRF7.3 score were vegetables, legumes and fish. This is partly in line with previous studies in which vegetables, legumes and fruits were observed to have the highest NRF9.3 scores (24, 69). The absence of fruit and presence of fish among the highest scoring food groups in our study compared to NRF9.3 scores in previous studies could be the result of the lack of vitamins present in fruit and the relative importance of protein and vitamin D in fish in the E-NRF7.3 score. An additional difference compared to previous studies using the NRF9.3 is the high E-NRF7.3 score of miscellaneous foods and clinical formulae. Studies using the NRF9.3 used different definitions of food groups or lacked these groups altogether (24, 69) which limits comparability. Considering the various miscellaneous foods such as coconut, cacao, seaweed and the high nutrient density of clinical formulae, which nearly all contain added vitamins and minerals (70) the observed high E-NRF7.3 scores can be explained. The practical relevance of the high E-NRF7.3 scores for these two food groups in our populations is limited as their contributions to the total individual E-NRF7.3 score are very small (<0.5%).

Food groups that had the largest contribution to the individual E-NRF scores within the two study populations were vegetables, bread, potatoes and milk and milk products, similar to the main contributors to the NRF9.3 in another Dutch cohort (24). These food groups are different from the food groups with the highest E-NRF score, as individual weighted E-NRF scores not only depend on the E-NRF score on the food item level, but also on which products are eaten in which amount, as previously discussed by Sluik et al. (24).

While calculating a nutrient-rich food score there are several methodological steps and decisions to be taken which will be discussed below. First of all, in the development of the E-NRF7.3 score, we chose a 100-kcal portion basis. It has previously been shown that the performance of the NRF9.3 on a 100 kcal basis is better than scores based on reference amounts customarily consumed (23) and best reflects the ratio of nutrients to calories, the original definition of nutrient density (22). Additionally, as foods and beverages are consumed in largely varying portions (22), expressing the nutrient density independent of serving size is preferred (21).

Secondly, the decision was made to cap the percentage of nutrients at 100% of the DRV, to prevent high single nutrient contents from producing extremely high scores, as was previously observed by others (21, 24). Moreover, Sluik et al., observed that uncapped scores systematically explained less variance of the DHD index (24).

Thirdly, the E-NRF scores were based on DRVs as set by the European Food Safety Authority, the Nordic Nutrient Recommendations, the Health Council of the Netherlands and the labeling reference intake values as set by the European Food Safety Authority (37–53). There are some differences between these values and the DRVs published by the Institute of Medicine as used for the original NRF9.3. However, it has previously been shown that using American instead of European recommendations did not influence the prediction of the DHD index (24). As we aimed to develop a nutrient-rich food score specifically for the aging European population, we have chosen to use DRV's relevant for this population.

Lastly, selecting nutrients and the way of defining them are important. Data on *total* mono- and disaccharides were available, in contrast to *added* mono- and disaccharides. The latter has been shown to be related to the micronutrient density of the diet (71). However, when Sluik et al. tested both *added* and *total* mono- and disaccharides in the NRF9.3, the model with *total* mono- and disaccharides performed best (24). Furthermore, vitamin K was selected as relevant nutrient as it is associated with relevant health outcomes for the elderly population (15). However, no data on vitamin K intake was available as the NEVO table of 2011 did not contain this nutrient. Future studies could evaluate the effect of including vitamin K in the E-NRF7.3 score.

TABLE 7 | Mean E-NRF index scores per food group, calculated per 100 kcal of foods consumed within DNFCS ($n = 735$) and NU-AGE ($n = 250$).

	DNFCS							NU-AGE						
		LIM3		E-NR7		E-NRF7.3			LIM3		E-NR7		E-NRF7.3	
Food groups	**Number of foods**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Number of foods**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
1 Potatoes	12	9.7	6.4	26.8	9.3	17.1	11.9	20	7.7	6.4	26.3	12.6	18.6	14.8
2 Alcoholic and non-alcoholic beverages	140	23.4	16.5	21.1	37.2	−2.3	32.5	89	21.6	19.2	21.8	42.2	0.2	37.0
3 Bread	83	11.4	3.6	22.4	8.6	11.0	10.1	75	11.5	3.7	21.7	9.4	10.1	10.6
4 Miscellaneous foods	15	26.9	17.0	67.4	66.5	40.5	66.9	19	18.5	19.9	43.4	63.2	24.9	55.0
5 Eggs	2	15.5	0.6	39.2	1.3	23.7	0.7	7	15.8	0.4	31.8	9.4	16.0	9.5
6 Fruits	73	22.6	9.4	34.7	22.8	12.1	26.9	74	22.2	9.4	35.5	22.3	13.3	26.3
7 Pastry and biscuits	126	20.3	5.1	10.3	4.6	−9.9	8.6	91	20.0	5.5	10.2	5.6	−9.8	9.0
8 Cereals and cereal products	41	5.7	5.3	30.5	25.7	24.8	25.0	43	4.0	4.3	25.7	25.1	21.6	24.0
9 Vegetables	142	21.0	22.4	136.2	78.6	115.2	83.3	130	20.9	23.1	130.7	66.3	109.8	72.9
10 Savory bread spreads	6	14.6	8.2	21.9	9.9	7.3	15.4	6	14.6	8.2	21.9	9.9	7.3	15.4
11 Cheese	47	35.8	5.3	30.4	11.9	−5.4	14.1	48	35.4	6.3	29.4	11.8	−6.0	14.5
12 Herbs and spices	17	69.7	39.4	30.5	22.1	−39.2	49.0	11	55.3	44.2	42.3	31.1	−13.0	60.9
13 Milk and milk products	124	24.5	4.8	31.5	19.4	6.9	22.3	102	24.0	4.7	32.3	19.9	8.2	22.8
14 Soy products and vegetarian products	22	29.7	37.0	52.1	29.7	22.4	54.0	23	25.5	32.0	50.6	25.5	25.1	49.3
15 Nuts, seeds and snacks	59	13.2	6.8	18.2	10.8	5.0	15.4	60	14.0	7.5	17.3	10.9	3.3	16.1
16 Legumes	8	9.2	8.9	57.6	6.2	48.4	13.4	8	5.9	9.1	64.2	8.1	58.3	15.2
17 Clinical formulas	9	13.9	7.4	38.4	22.6	24.6	23.9	5	11.5	5.7	70.6	26.9	59.1	24.5
18 Mixed dishes	4	14.4	9.5	16.1	5.9	1.7	7.0	38	16.8	8.4	21.5	13.7	4.7	14.4
19 Soups	20	63.7	30.5	44.2	29.6	−19.5	34.1	21	51.3	30.4	41.0	29.0	−10.3	30.7
20 Sugar, sweets and sweet sauces	77	23.0	6.4	8.6	6.7	−14.4	9.7	67	22.8	6.9	9.2	7.4	−13.6	9.2
21 Fats, oils and savory sauces	145	21.4	17.3	13.3	22.4	−8.0	28.8	88	22.5	20.4	12.8	21.4	−9.7	29.6
22 Fish	39	15.3	10.7	44.8	16.4	29.5	21.9	57	17.2	16.3	42.9	19.4	25.7	27.1
23 Meat, meat products and poultry	136	21.3	13.1	28.2	22.4	6.9	27.6	157	19.7	12.3	26.4	21.0	6.6	26.2

E-NR, Elderly nutrient-rich score; LIM, nutrients to limit; E-NRF, Elderly nutrient-rich foods score.

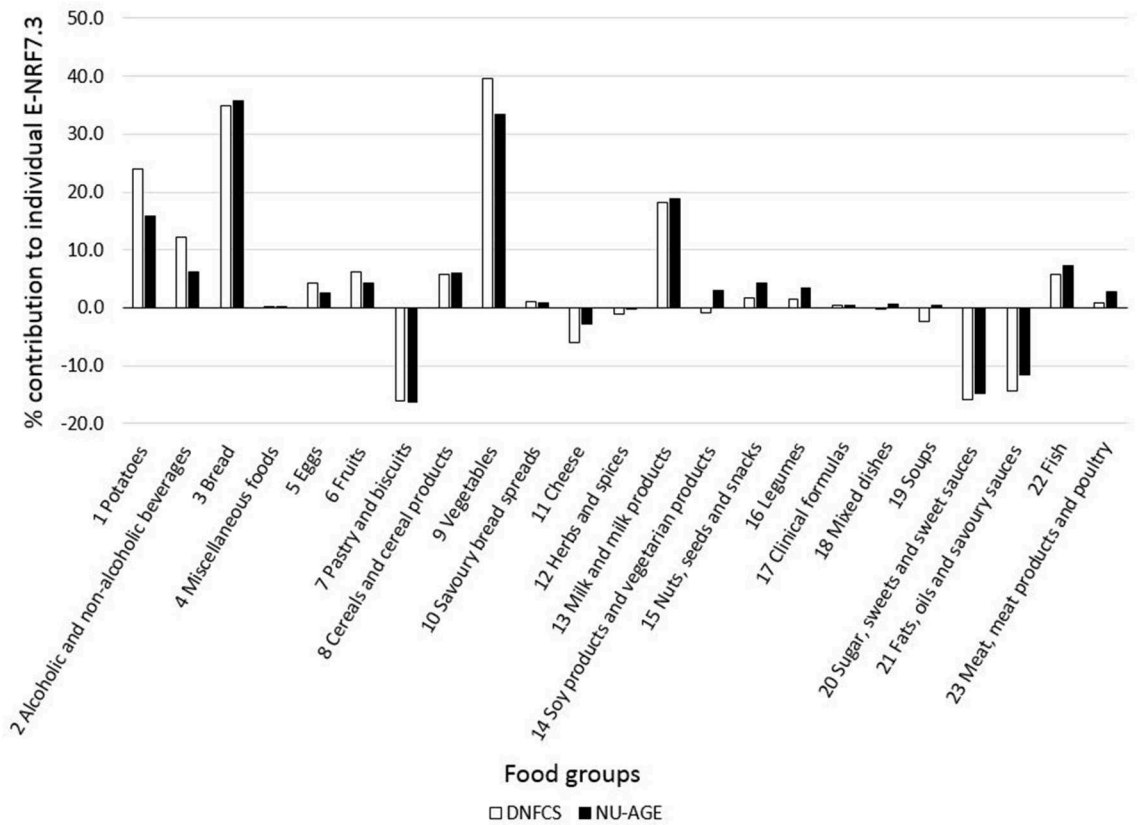

FIGURE 2 | Mean contribution (%) of food groups to individual E-NRF7.3 scores in both DNFCS ($n = 735$) and NU-AGE ($n = 250$).

Our study has some limitations. First, the E-NRF7.3 has been correlated with the NU-AGE index only. The NU-AGE index is the first and only index to measure adherence to guidance based on DRVs and food based dietary guidelines for European elderly individuals (55). The NU-AGE index consists of 16 components, including the use of a vitamin D supplement, resulting in a score ranging from 0 to 160 (55). It has been shown to be able to rank participants from the entire NU-AGE study including over 1,250 older adults according to their adherence to the guidelines (55). Furthermore, higher scores for the NU-AGE index were associated with reduced rates of bone loss at the femoral neck in individuals with osteoporosis (72), an improvement of systolic blood pressure, arterial stiffness (73), global cognition, and episodic memory (74). However, the validity of the E-NRF7.3 score should be confirmed in relation to relevant health outcomes, markers of nutritional status and in other study populations as well. Second, the E-NRF7.3 score does not take into account individual differences in nutrient requirements as personalized nutrition is still a relatively new research area. Once the validity of the E-NRF7.3 score has been studied in more depth, the score could be a useful tool to support nutrition and health claims of foods and to educate older populations to identify nutrient-rich foods for better diet quality. At last, for the E-NRF7.3 score to be used as a tool to address malnutrition, the score could be updated to include multiple risk factors that underlie malnutrition, including physical, social, and medical factors (75).

To conclude, we have developed a nutrient-rich food score specifically targeted at measuring nutrient density of foods and quality of diets from European elderly people. The E-NRF7.3 score was able to rank participants according to their adherence to the NU-AGE index. In future, this newly developed E-NRF7.3 score should be validated against relevant health outcomes for the older population, and more objective markers of dietary intake.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AB and LdG designed the study. CK and AB analyzed the data and interpreted the data and drafted the manuscript. LdG interpreted the data and critically revised the manuscript for important intellectual content.

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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.2019.00119/full#supplementary-material

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Replacing Dairy Fat With Polyunsaturated and Monounsaturated Fatty Acids: A Food-Level Modeling Study of Dietary Nutrient Density and Diet Quality Using the 2013–16 National Health and Nutrition Examination Survey

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Recent dietary guidelines have become more food-based, as opposed to purely nutrient-based. By contrast, assessing the impact of dietary changes on chronic disease risk continues to rely on single-nutrient substitutions. To assess the real-world implications of a nutrient-for-nutrient swap, this study examined dietary nutrient density and healthy diet scores following removal of food sources of dairy fat from diets of 15,260 individuals age ≥4 y in the National Health and Nutrition Examination Survey (NHANES 2013–2016). Those foods were then replaced with foods containing a comparable amount of non-dairy polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). The present food-level substitution model was based on 576 diverse eating patterns of US population subgroups. Diet quality measures were the Nutrient Rich Food (NRF 9.3) Index and the 2015-Healthy Eating Index (HEI-2015). Removing 5% of dietary energy from dairy fat led to lower levels of multiple micronutrients and to lower NRF 9.3 scores. These deficits were not remedied by the modeled replacements. Although swapping dairy fat for foods containing non-dairy MUFA/PUFA did alter the fatty acid ratios, the resulting food patterns were still significantly lower in some key micronutrients. Nutrient-based dietary guidance is prone to ignore the complexity of food patterns and the recommended dietary change may have unintended nutritional consequences.

Keywords: food based dietary guidelines, dietary nutrient density, substitution modeling, healthy diet scores, NRF 9.3, HEI-2015, dairy, dietary fat

INTRODUCTION

Replacing 5% of energy intake from dairy fat with equivalent energy intake from polyunsaturated fatty acids (PUFA) has been linked to a 24% lower risk of cardiovascular disease (CVD) (1). This replacement was accomplished by exchanging different fat sources in regression analyses, substituting one regression coefficient for another (1). The results were taken as evidence in support

of dietary guidelines to replace animal fats, including dairy fat, with PUFA for the prevention of CVD at the population level (1, 2).

The implementation of nutrient-based dietary guidelines has always been problematic (3). In the real world, people eat foods, not nutrients. Foods contain multiple nutrients that cannot be readily swapped for one another. For example, reducing dietary sodium while doubling the intakes of dietary potassium can be a challenge when many frequently eaten foods contain both (4–6).

In practice, a reduction of 5% of energy intake from dairy fat would mean a reduction in the consumption of the major food sources of dairy fat, that is to say milk and milk beverages, yogurt, and cheese, along with many mixed foods containing these ingredients (7–9). To complicate matters, habitual consumption patterns for milk, yogurt, butter, and cheese can vary widely across population subgroups (10, 11). Past studies on sociodemographic profiles of dairy consumers have pointed to sharp differences by gender, age group, income, education, and race/ethnicity (12–14). The type of dairy products and the amounts eaten can also depend on the food pattern chosen by the consumer. The recently issued United States Department of Agriculture (USDA) Healthy Food patterns recommended very different amounts for meat, poultry, fish, milk, and dairy depending on pattern type (15). All those factors are difficult to address in nutrient-based analyses, even after accounting for dietary intakes of fruit, vegetables, coffee, protein, and vegetable fat in multiple regression models (1).

The present aim was to assess the real-world implications of nutrient-driven dietary guidance. The primary outcome was diet quality following total or partial removal of foods containing dairy fat from the diet. The secondary outcome was diet quality following equal weight replacement of dairy fat with foods containing non-dairy PUFA and monounsaturated fat (MUFA). Selected micronutrients and composite measures of dietary nutrient density and a healthy diet score were the measures of diet quality. This food-level modeling was sensitive to the diversity of current dietary habits among multiple population subgroups in the United States.

METHODS

Data Source and Population

Data for this project came from the two most recent cycles of the National Health and Nutrition Examination Survey (NHANES) corresponding to years 2013–2014 and 2015–2016. The NHANES is a nationally-representative diet and health survey of the US population and is the primary source of dietary surveillance data in the US. NHANES dietary intake data are used to inform the US dietary guidelines as well as other federal and state food and nutrition policies.

The present data analyses were based on the first 24 h recall, which is completed in-person by eligible participants. A single dietary recall is sufficient for measuring average intakes for populations, which was the primary goal of the present study (16). A second 24 h recall via telephone is collected as part of NHANES but was not used here. The NHANES dietary recall uses a multi-pass method and measures all foods consumed midnight-to-midnight in the day prior to data collection (17–19). Data were collected on mixed/composite foods such as stews, sandwiches and pasta dishes. The present analyses were based on 15,260 participants aged $\geq$4 y who completed a valid 24 h recall, as defined by National Center for Health Statistics (NCHS) staff.

Identifying Dairy Fat

Foods containing dairy fats cannot be readily identified without combining a number of different data sources. This can be a challenge when identifying mixed foods containing dairy fat as one of many ingredients.

The Food and Nutrient Database for Dietary Studies (FNDDS) includes recipes for each individual food that can be linked to the USDA Standard Reference (SR) nutrient composition database (20) and the Food Patterns Equivalents database (21). This linkage allowed us to identify all dairy fat ingredients, including milk, cream, cheese, yogurt, ice cream, and butter, among many others. We queried the entire database for dairy-fat containing ingredients, allowing us to estimate the amount of dairy fat per 100 gram edible portion for each individual food.

The NHANES dietary data collection flags some consumed food items as combination foods (e.g., milk with cereal, coffee with milk, or a sandwich with cheese). This allowed us to identify any combination food containing dairy fat that was reported as consumed by NHANES participants. This information was then incorporated into the NHANES database that listed all foods consumed by individuals. Summing the amounts of dairy fat consumed from each food by each participant allowed us to estimate dairy fat consumption at the individual level. Summing the amounts of dairy fat over all foods consumed by all participants allowed us to estimate dairy fat consumption at the population level.

Substitution Modeling Approach

All foods/beverages that were available for substitution modeling were identified in the nutrient composition database. Included were all foods consumed by NHANES participants, except those that contained dairy fat or those foods that had more saturated fat than unsaturated fat, in order to not replace food sources of dairy fat with other foods containing saturated fat.

In order to replace dairy fat with an approximately equivalent amount of non-dairy fat (PUFA and MUFA), we developed a multi-step substitution model. Briefly, foods containing dairy fat were replaced with foods containing PUFA and MUFA on a gram-per-gram basis. The replacement foods varied, depending on age, meal type, energy density, and percent energy from fat. We have previously used this approach in substitution modeling of 100% juices and whole fruit (22), ready to eat (RTE) cereals (23) and almonds and other tree nuts (24). Because energy density and percent energy from fat were included in the development of the replacement food, the ensuing substitution was approximately iso-caloric.

All foods were eligible to be included in the replacement model with a few notable exceptions. First, foods that contained dairy fat could not be included, nor could foods that contained more saturated fat than mono- and polyunsaturated fat. It was not possible to exclude foods that contained saturated fat altogether, as many foods that are high in MUFA/PUFA contain

meaningful amounts of saturated fat. Conversely, many foods containing dairy fat also included MUFA/PUFA.

The frequency weighted nutrient profiles of the substitution foods were created based on age, meal type, dietary energy density, and percent of energy from fat. The four age groups were defined as 4–19 y, 20–39 y, 40–64 y, and 65+y. The four meal types were breakfast, snacks (including beverage only eating occasions), lunch/dinner, or dessert. Food energy density (in kcal/100 g) was classified into 6 categories, defined by weighted consumption at <10th percentile [<49.8 kcal/100 g], 10–24th percentile [49.8–70.6], 25–50th percentile [70.7–151.4], 50–74th percentile [151.5–235.5], 75–90th percentile [235.6–308.7] and 90th+ percentile [308.8–682]). Percent energy from fat was likewise classified into 6 categories, defined as: <10th percentile [0.7–16.3%E], 10–24th percentile [16.4–24.8%E], 25–50th percentile [24.9–38.3%], 50–74th percentile [38.4–48.2%], 75–90th percentile [48.3–63.4%], and 90th+ percentile [63.5–97.8%].

Food patterns of the NHANES population sample were thus split by age (4 categories), meal type (4 categories), dietary energy density (6 categories), and percent dietary fat (6 categories) for a total of 576 patterns. All foods consumed by NHANES participants within each of these 576 subgroups were then identified. Food based modeling needs to account for the fact that foods that contain PUFA/MUFA are not eaten in equal amounts by all population subgroups and their consumption can also vary, depending on age, meal type or dietary energy density. The substitution foods were category specific, and their nutrient profiles were weighted moreover by the consumption of each food within that subgroup. This means that the PUFA/MUFA containing foods that were frequently eaten by a given subgroup were weighted more heavily in the substitution model.

To illustrate this very detailed approach, the top-weighted foods in the lunch/dinner meal for the 40–64 y age group in the third sextile of energy density and second sextile of percent energy from fat were white rice made with oil, Spanish rice with added fat, brown rice made with oil, rice pilaf, Lo Mein noodles without meat, beef stew with potatoes and vegetables and gravy, and stewed chicken breast with the skin not eaten, among approximately 240 unique foods. The composite nutrient values per gram for the MUFA/PUFA foods for that group were then weighted based on survey weights and frequency of consumption.

Two substitution models were implemented. Model 1 removed all dairy fat (no limit) and replaced it with the weighted and subgroup-specific substitution foods. Model 2 removed up to 5% of energy from dairy fat for each person and replaced it with subgroup-specific substitution foods. For individuals consuming more than 5% of fat energy from dairy, all foods eaten on that day were randomized and the food contributing >5% of fat energy was replaced up to but not exceeding 5% for that individual. The randomization was necessary since otherwise some meals may systematically be more prone to being replaced (e.g., breakfast), which could bias the results.

Dietary Nutrient Density

Because the models were only approximately iso-caloric, all dietary outcomes were energy-adjusted, with the exception of total energy, which is included in the tables for comparison purposes. The primary outcomes of interest were total fat, saturated fat, polyunsaturated fat, monounsaturated fat, carbohydrates, added sugars, and a number of micronutrients/minerals including calcium, vitamin D, fiber, potassium, vitamin A, and B-vitamins.

Healthy Diet Scores

The Nutrient Rich Foods (NRF) index was the principal measure of diet quality (25, 26). Its development and validation with respect to other measures of diet quality and long term health outcomes have been described in the literature (27–29). The present NRF 9.3 variant applied to total diets was based on nine qualifying nutrients (NR) and three nutrients to limit (LIM), using a new approach to calculate the LIM components to be consistent with other measures of diet quality. Reference daily values (DVs) were based on standards issued by the US Food and Drug Administration (FDA) and the Institute of Medicine (26). The qualifying nutrients and standard reference amounts were as follows: protein (50 g), fiber (28 g), vitamin A (900 RAE), vitamin C (90 mg), vitamin D (20 mcg), calcium (1,300 mg), iron (18 mg), potassium (4,700 mg), and magnesium (420 mg). In the NR calculation, each daily nutrient intake was adjusted for 2,000 kcal and expressed in percentage of DV. Percent DVs for nutrients were truncated at 100, so that an excessively high intake of one nutrient could not compensate for the dietary inadequacy of another. The NR score is then calculated as the sum of the NR components, the minimum score is 0 and the maximum score is 900.

Unlike the NR component, the LIM component that includes added sugars, sodium and saturated fat, are calculated in a manner similar to the HEI-2015 (30). For each nutrient, values above the maximum threshold received a maximum number of limiting points (26%E for added sugars, 16%E for saturated fat and 2,000 mg for sodium); intakes below the minimum threshold received zero limiting points (6%E for added sugars, 8% for saturated fat, and 1,100 mg sodium). Intakes between the minimum and maximum thresholds were calculated on a proportional basis. For example, an individual consuming 12%E from saturated fat would earn 0.5 limiting points. The minimum LIM score is 0 (optimal) and the maximum is 300 (sub-optimal).

The NRF 9.3 was calculated as follows:

$$\text{NRF 9.3} = (\text{NR} - \text{LIM}) \quad (1)$$

The development and validation of the NRF family of nutrient density scores are all well-documented in the literature (28, 29). In recent iterations, vitamin D replaced vitamin E. Fiber, vitamin D, calcium, magnesium, and potassium were all identified in the 2010 Dietary Guidelines for Americans as nutrients of concern (31). The NRF score was adjusted for energy intake, similar to other diet quality scores, including the Healthy Eating Index-2015 (30).

The 2015-Healthy Eating Index (HEI-2015) was used as an additional summary measure of diet quality and specifically measures adherence to the 2015–2020 Dietary Guidelines for Americans. The HEI-2015 is an energy adjusted summary measure of diet quality based on the intake of nine food

groups/nutrients to encourage including total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant protein, and fatty acids ratio (ratio of unsaturated fat to saturated fat), and four food groups/nutrients to discourage, including refined grains, sodium, added sugars and saturated (30). The USDA Food Patterns Equivalents Database (FPED) was used to estimate intakes of each food group component (21). The HEI-2015 is scaled 0 to 100, with higher scores indicating increased adherence to dietary guidelines and vice versa (30, 32).

Analytical Approach

For each dietary outcome of interest, the population mean and corresponding 95% confidence interval (CI) of energy-adjusted intake was calculated. Given the large sample size and statistical precision of the dietary outcomes, even small differences in observed and modeled intakes would be statistically significant. For these reasons, a 10% relative change comparing observed and modeled intakes was used as the threshold for statistical significance and all *p*-values used this threshold. Results are presented for the overall population and also limited to consumers of dairy fat. An alpha-level of 0.05 was used for all analyses. Model implementation and statistical analyses were performed using Stata 13.1 (College Station, TX).

The analysis of publicly available de-identified data is not considered human subjects research by the University of Washington or Albert Einstein College of Medicine.

RESULTS

Table 1 shows population characteristics and mean consumption of energy from dairy fat by sociodemographic variables. Data are for a representative sample of the US population ($n = 15{,}260$) from 2013 to 2016 NHANES cycles. Dairy fat accounted for 5.6% of daily dietary energy on the population-level. Dairy fat consumption did not vary by gender, and tended to decline with age. Dairy fat consumption was not associated with education or income. Non-Hispanic whites consumed most dairy fat (6.1% energy); non-Hispanic Asians consumed the least (3.8%).

TABLE 1 | Sample description and mean consumption of energy from dairy fat overall and by socio-demographic group, United States 2013–2016.

	N	Weighted percent	Mean dairy fat, %
Total	15,260	–	5.6
Age			
4–8 y	1,685	6.7	6.8
9–13 y-	1,658	6.7	6.4
14–19 y	1,853	8.7	5.8
20–39 y	3,433	28.2	5.3
40–59 y	3,380	28.4	5.2
≥60 y	3,251	21.4	5.6
Gender			
Male	7,455	49.1	5.5
Female	7,805	50.9	5.6
Race/ethnicity			
Non-Hispanic white	5,355	62.1	6.1
Hispanic	4,432	16.9	5.4
Non-Hispanic black	3,307	11.9	4.1
Non-Hispanic Asian	1,495	5.4	3.8
Other/mixed race	671	3.7	5.0
Family income to poverty ratio			
<1.3 (lower income)	5,155	25.5	5.5
1.3–1.84	1,842	11.0	5.5
1.85–2.99	2,471	18.2	5.8
≥3.00 (higher income)	4,574	45.4	5.5
Missing	1,218	–	5.4
Education (age ≥ 25 y)			
<High school	2,039	14.3	5.5
High school/equivalent	2,018	21.0	5.5
Some college	2,699	31.6	5.8
≥College	2,458	33.1	5.5

Substitution Effects on Nutrient Intakes

Since dairy fat accounted for about 5% of daily energy at the population level, removing 5% of energy from dairy fat effectively meant removing 65% of dairy products from the diet. **Table 2** shows the observed and modeled dietary intakes for the NHANES sample, including dairy consumers and non-consumers. Model 1 removed all dairy fat; Model 2 removed up to 5% of energy from dairy fat for each individual. In Model 1, dairy fat was reduced to 0% of energy, in Model 2 it was reduced to 1.7%. Saturated fat was significantly reduced in both models.

The removal of dairy fat led to dietary patterns that were significantly lower in energy but were significantly higher in added sugars. There were significant reductions in modeled daily intakes of calcium, vitamin D, vitamin A, riboflavin, and vitamin B12. The same reductions in energy and key nutrients were observed in Model 1 and 2.

Table 3 shows the observed and modeled dietary intakes for dairy fat consumers only ($n = 12{,}831$). following the removal of dairy fat from the diet. Removing dairy fat led to the expected reduction in saturated fat intakes. Calories were significantly lower, as were calcium, vitamin D, vitamin A, riboflavin, and vitamin B12. By contrast, added sugars were higher. The same reductions in energy and some key micronutrients were observed in Model 1 and 2.

Table 4 shows observed and modeled dietary intakes following the swapping of dairy fat for non-dairy fat for the total NHANES sample. Energy intakes were the same, given that the fat swap was approximately iso-caloric. Dairy fat was eliminated; saturated fat declined from 11.4 to 9.2% of energy, and PUFA increased from 8.0 to 9.4% of energy. The swapping of dairy fat for PUFA/MUFA containing foods resulted in modeled food patterns that were significantly lower in calcium, vitamin D, vitamin A, riboflavin, niacin, and vitamin B12. Generally similar results were obtained with Model 1 and 2. While a single 24 h recall cannot be used to estimate the exact proportion of the population meeting or not meeting a specific dietary requirement, the proportion of the population that consumed 1,000 mg/d or more of calcium decreased from

TABLE 2 | Observed and modeled dietary intakes for the total sample following removal of dairy fat, NHANES, 2013–2016.

	Mean (95% Confidence Intervals [CI])		
	Observed	Model 1[a] (remove)	Model 2[b] (remove)
Total population (n = 15,260)			
Macronutrients			
Energy, kcal	2,080 (2,055, 2,105)	1,454 (1,435, 1,474)***	1,580 (1,558, 1,601)***
Protein, %E	15.8 (15.5, 16)	14.2 (13.9, 14.5)	14.7 (14.4, 15)
Total fat, %E	34.7 (34.4, 35)	31.5 (31.2, 31.8)	32.5 (32.1, 32.8)
Saturated fat, %E	11.4 (11.2, 11.5)	8.7 (8.6, 8.9)***	9.6 (9.4, 9.7)***
PUFA, %E	8.0 (7.9, 8.1)	8.3 (8.2, 8.5)	8.2 (8.1, 8.4)
MUFA, %E	12 (11.9, 12.1)	11.4 (11.2, 11.5)	11.6 (11.4, 11.7)
Dairy fat, %E	5.6 (5.4, 5.8)	0 (0, 0)***	1.7 (1.6, 1.9)***
Carbohydrate, %E	48.4 (48, 48.8)	52.3 (51.8, 52.8)	51.1 (50.6, 51.5)
Added sugar, %E	13.4 (13.1, 13.8)	16 (15.5, 16.4)***	15.2 (14.8, 15.6)***
Micronutrients			
Calcium, mg	965 (949, 981)	715 (700, 730)***	784 (772, 796)***
Vitamin D, mcg	4.8 (4.7, 5)	2.7 (2.5, 2.9)***	3.4 (3.2, 3.6)***
Fiber, g	16.6 (16.2, 16.9)	18 (17.6, 18.5)	17.5 (17.1, 17.9)
Potassium, mg	2,547 (2,510, 2,584)	2,618 (2,558, 2,679)	2,577 (2,535, 2,620)
Vitamin A, mcg	637 (618, 656)	490 (465, 516)***	531 (510, 553)***
Thiamin, mg	1.6 (1.6, 1.6)	1.5 (1.5, 1.5)	1.5 (1.5, 1.6)
Riboflavin, mg	2.1 (2.1, 2.1)	1.8 (1.7, 1.9)**	1.9 (1.8, 1.9)*
Niacin, mg	25.1 (24.8, 25.4)	25.8 (25.2, 26.3)	25.6 (25.1, 26)
Vitamin B6, mg	2.1 (2, 2.1)	2.1 (2, 2.1)	2.1 (2, 2.1)
Vitamin B12, mcg	4.8 (4.7, 4.9)	3.6 (3.5, 3.7)***	4 (3.9, 4.1)***
Folic acid mcg	390 (383, 398)	369 (362, 377)	376 (368, 383)

[a]*Model 1 removes all dairy fat. Model 2 removes up to 5% dairy fat for each individual.*
[b]*Asterisks indicate statistical significance comparing whether Modeled changes are a 10% change from observed diets; ***p < 0.001; **0.001 < p < 0.01; *0.01 < p < 0.05.*

TABLE 3 | Observed and modeled dietary intakes for the dairy fat following the removal of dairy fat, NHANES 2013–2016.

	Mean (95% Confidence Intervals [CI])		
	Observed	Model 1[a] (remove)	Model 2[b] (remove)
Consumers of dairy fat only (n = 12,831)			
Macronutrients			
Energy, kcal	2,133 (2,108, 2,157)	1,397 (1,379, 1,416)***	1,545 (1,524, 1,565)***
Protein, %E	15.7 (15.5, 16)	13.9 (13.6, 14.2)**	14.5 (14.2, 14.8)
Total fat, %E	35.1 (34.7, 35.4)	31.3 (30.9, 31.7)**	32.5 (32.1, 32.8)
Saturated fat, %E	11.8 (11.6, 11.9)	8.7 (8.6, 8.8)***	9.7 (9.5, 9.8)***
PUFA, %E	7.9 (7.8, 8)	8.3 (8.2, 8.4)	8.2 (8, 8.3)
MUFA, %E	12.1 (11.9, 12.2)	11.3 (11.2, 11.5)	11.6 (11.4, 11.7)
Dairy fat, %E	6.5 (6.3, 6.7)	0 (0, 0)***	2 (1.9, 2.2)***
Carbohydrate, %E	48.1 (47.7, 48.5)	52.7 (52.2, 53.2)	51.2 (50.7, 51.7)
Added sugar, %E	13.2 (12.9, 13.5)	16.1 (15.7, 16.5)***	15.2 (14.8, 15.7)***
Micronutrients			
Calcium, mg	1,014 (998, 1,030)	720 (702, 737)***	801 (787, 814)***
Vitamin D, mcg	5.1 (5, 5.2)	2.6 (2.4, 2.8)***	3.4 (3.2, 3.6)***
Fiber, g	16.5 (16.2, 16.9)	18.2 (17.8, 18.7)	17.6 (17.2, 18)
Potassium, mg	2,553 (2,517, 2,590)	2,637 (2,568, 2,706)	2,589 (2,543, 2,634)
Vitamin A, mcg	657 (638, 675)	484 (457, 511)***	532 (510, 554)***
Thiamin, mg	1.6 (1.6, 1.6)	1.5 (1.5, 1.5)	1.5 (1.5, 1.6)
Riboflavin, mg	2.2 (2.1, 2.2)	1.8 (1.7, 1.9)***	1.9 (1.9, 1.9)*
Niacin, mg	24.7 (24.4, 25.1)	25.5 (24.9, 26.1)	25.3 (24.8, 25.8)
Vitamin B6, mg	2.1 (2, 2.1)	2.1 (2, 2.1)	2.1 (2, 2.1)
Vitamin B12, mcg	4.9 (4.8, 5)	3.5 (3.4, 3.6)***	4 (3.9, 4.1)***
Folic acid mcg	392 (385, 398)	367 (360, 374)	374 (367, 382)

[a]*Model 1 removes all dairy fat. Model 2 removes up to 5% dairy fat for each individual.*
[b]*Asterisks indicate statistical significance comparing whether Modeled changes are a 10% change from observed diets; ***p < 0.001; **0.001 < p < 0.01; *0.01 < p < 0.05.*

40.6% in observed diets to only 13.8% in Model 1 and 25.1% in Model 2.

Table 5 shows observed and modeled dietary intakes following swapping dairy fat for PUFA/MUFA containing foods for consumers of dairy fat (n = 12,831). Energy intakes were the same, as expected. Dairy fat was eliminated; saturated fat declined from 11.8 to 9.2% of energy, and PUFA increased from 7.9 to 9.6% of energy. The swapping of dairy fat for PUFA/MUFA containing foods resulted in food patterns that were significantly lower in calcium, vitamin D, vitamin A, riboflavin, and vitamin B12. Substantially similar results were obtained with Model 1 and 2. Among dairy fat consumers, the proportion of persons consuming >1,000 mg of calcium dropped from 44.7 to 13.0% in Model 1 and 26.6% in Model 2.

Food-Level Substitution Effects on Healthy Diet Scores

The NRF 9.3 nutrient density score is nutrient based; it contains qualifying nutrients as well as nutrients to limit: saturated fat, added sugar and sodium. **Table 6** shows observed and modeled healthy diet scores following the removal of dairy fat. Shown are mean NRF 9.3 and HEI-2015 overall and component scores and the data are for the total NHANES sample and for dairy fat consumers.

The removal of dairy fat led to lower NRF 9.3 scores; total HEI-2015 scores were not affected. At the HEI sub-score level, a reduction in points from dairy was compensated for by additional points from lower saturated fat and more PUFA. Higher sodium score reflected a lower content of sodium. For dairy consumers there was a small reduction in total protein score.

TABLE 4 | Observed and modeled dietary intakes for the total sample following the replacement of dairy fat with PUFA/MUFA, NHANES, 2013–2016.

	Mean (95% Confidence Intervals [CI])		
	Observed	**Model 1[a] (swap)**	**Model 2[b] (swap)**
Total population (n = 15,260)			
Macronutrients			
Energy, kcal	2,080 (2,055, 2,105)	2,113 (2,087, 2,139)	2,099 (2,073, 2,124)
Protein, %E	15.8 (15.5, 16)	16.1 (15.9, 16.4)	16.0 (15.8, 16.2)
Total fat, %E	34.7 (34.4, 35)	34.7 (34.4, 35)	34.7 (34.4, 35)
Saturated fat, %E	11.4 (11.2, 11.5)	9.2 (9.1, 9.3)***	10.2 (10.1, 10.3)*
PUFA, %E	8.0 (7.9, 8.1)	9.4 (9.3, 9.6)***	8.8 (8.7, 8.9)*
MUFA, %E	12 (11.9, 12.1)	12.8 (12.7, 13)	12.5 (12.4, 12.6)
Dairy fat, %E	5.6 (5.4, 5.8)	0 (0, 0)***	2.6 (2.4, 2.7)***
Carbohydrate, %E	48.4 (48, 48.8)	48.2 (47.8, 48.6)	48.2 (47.9, 48.6)
Added sugar, %E	13.4 (13.1, 13.8)	12.7 (12.4, 13)	13.0 (12.6, 13.3)
Micronutrients			
Calcium, mg	965 (949, 981)	668 (659, 678)***	796 (785, 807)***
Vitamin D, mcg	4.8 (4.7, 5)	3.5 (3.4, 3.7)***	4 (3.9, 4.1)***
Fiber, g	16.6 (16.2, 16.9)	18.2 (17.8, 18.6)	17.6 (17.2, 18)
Potassium, mg	2,547 (2,510, 2,584)	2,599 (2,563, 2,634)	2,575 (2,541, 2,609)
Vitamin A, mcg	637 (618, 656)	564 (547, 580)***	590 (573, 608)***
Thiamin, mg	1.6 (1.6, 1.6)	1.6 (1.6, 1.6)	1.6 (1.6, 1.6)
Riboflavin, mg	2.1 (2.1, 2.1)	1.8 (1.8, 1.8)***	1.9 (1.9, 1.9)
Niacin, mg	25.1 (24.8, 25.4)	27.3 (27, 27.7)**	26.3 (26, 26.7)
Vitamin B6, mg	2.1 (2, 2.1)	2.2 (2.1, 2.2)	2.1 (2.1, 2.1)
Vitamin B12, mcg	4.8 (4.7, 4.9)	4.2 (4.2, 4.3)**	4.4 (4.4, 4.5)
Folic acid mcg	390 (383, 398)	388 (381, 394)	387 (380, 394)

[a] *Model 1 replaces all dairy fat. Model 2 replaces up to 5% dairy fat for each individual.*
[b] *Asterisks indicate statistical significance comparing whether Modeled changes are a 10% change from observed diets; ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$.*

TABLE 5 | Observed and modeled dietary intakes for dairy fat consumers following the replacement of dairy fat with PUFA/MUFA, NHANES, 2013–2016.

	Mean (95% Confidence Intervals [CI])		
	Observed	**Model 1[a] (swap)**	**Model 2[b] (swap)**
Consumers of dairy fat only (n = 12,831)			
Macronutrients			
Energy, kcal	2,133 (2,108, 2,157)	2,172 (2,147, 2,196)	2,155 (2,130, 2,179)
Protein, %E	15.7 (15.5, 16)	16.2 (15.9, 16.4)	16 (15.8, 16.2)
Total fat, %E	35.1 (34.7, 35.4)	35.1 (34.7, 35.4)	35.1 (34.7, 35.4)
Saturated fat, %E	11.8 (11.6, 11.9)	9.2 (9.1, 9.3)***	10.4 (10.3, 10.5)***
PUFA, %E	7.9 (7.8, 8)	9.6 (9.5, 9.7)***	8.8 (8.7, 8.9)***
MUFA, %E	12.1 (11.9, 12.2)	13.1 (12.9, 13.2)	12.6 (12.5, 12.8)
Dairy fat, %E	6.5 (6.3, 6.7)	0 (0, 0)***	3.0 (2.9, 3.2)***
Carbohydrate, %E	48.1 (47.7, 48.5)	47.9 (47.4, 48.3)	47.9 (47.5, 48.3)
Added sugar, %E	13.2 (12.9, 13.5)	12.3 (12, 12.6)	12.6 (12.3, 12.9)
Micronutrients			
Calcium, mg	1,014 (998, 1,030)	666 (656, 676)***	815 (804, 827)***
Vitamin D, mcg	5.1 (5, 5.2)	3.6 (3.4, 3.7)***	4.1 (4, 4.3)***
Fiber, g	16.5 (16.2, 16.9)	18.4 (18.1, 18.8)	17.7 (17.4, 18.1)
Potassium, mg	2,553 (2,517, 2,590)	2,614 (2,580, 2,648)	2,586 (2,553, 2,620)
Vitamin A, mcg	657 (638, 675)	571 (555, 586)***	602 (585, 619)
Thiamin, mg	1.6 (1.6, 1.6)	1.6 (1.6, 1.6)	1.6 (1.6, 1.6)
Riboflavin, mg	2.2 (2.1, 2.2)	1.8 (1.8, 1.8)***	2 (1.9, 2)
Niacin, mg	24.7 (24.4, 25.1)	27.3 (27, 27.7)	26.2 (25.8, 26.5)
Vitamin B6, mg	2.1 (2, 2.1)	2.1 (2.1, 2.1)	2.2 (2.1, 2.2)
Vitamin B12, mcg	4.9 (4.8, 5)	4.2 (4.2, 4.3)***	4.5 (4.4, 4.5)
Folic acid mcg	392 (385, 398)	389 (383, 395)	388 (382, 394)

[a] *Model 1 replaces all dairy fat. Model 2 replaces up to 5% dairy fat for each individual.*
[b] *Asterisks indicate statistical significance comparing whether Modeled changes are a 10% change from observed diets; ***$p < 0.001$.*

Table 7 shows observed and modeled mean NRF 9.3 and HEI-2015 overall and component scores following the replacement of dairy fat with foods not containing dairy fat. Among the total population, the NRF 9.3 and the HEI-2015 scores were not statistically different across observed and modeled diets, though NRF 9.3 scores tended to be lower, and HEI-2015 scores tended to be higher. Across HEI-2015 sub-scores, total vegetables, greens and beans, seafood and plant protein, fatty acid ratio, and saturated fat improved for Model 1 as compared to observed diets. Dairy and sodium worsened. Similar patterns were observed for Model 2, though effects were weaker. When limited to dairy fat consumers the HEI-2015 change for Model 1 was significant, increasing from 50.4 to 55.8 (out of 100). Total vegetables, greens and beans, seafood and plant proteins, fatty acid ratio and saturated fat improved, while sodium and dairy worsened. Whole fruits and total protein foods also increased when limiting the analysis to dairy fat consumers.

DISCUSSION

Replacing dairy fat with other forms of fat, such as a combination of PUFA/MUFA, should not be viewed as a simple exercise in regression modeling. Removing dairy fat from the diet means reducing the consumption of foods where dairy fat is the main source of fat or just an ingredient. In the NHANES sample, a mean reduction in 5% of energy from dairy fat meant removing about 65% of dairy foods from the diet. In the present NHANES 2013–2016 sample, dairy fat provided a mean of 5.6% of dietary energy with some differences by age and race/ethnicity.

TABLE 6 | Observed and modeled mean NRF 9.3 and HEI-2015 overall and component scores following the removal of dairy fat, NHANES 2013–2016.

	Mean (95% Confidence Intervals [CI])		
	Observed	**Model 1[a] (remove)**	**Model 2[b] (remove)**
Total population (n = 15,260)			
NRF 9.3	391 (385, 396)	314 (309, 320)***	337 (332, 343)***
HEI-2015 (100)	50.1 (49.5, 50.8)	49.7 (49, 50.3)	49.8 (49.2, 50.5)
HEI-1: Total fruits (5)	2.1 (2, 2.2)	2.2 (2.1, 2.3)	2.2 (2.1, 2.3)
HEI-2: Whole fruits (5)	2.1 (2, 2.2)	2 (1.9, 2.1)	2.1 (2, 2.2)
HEI-3: Total vegetables (5)	2.9 (2.8, 2.9)	2.8 (2.7, 2.8)	2.8 (2.7, 2.9)
HEI-4: Greens and beans (5)	1.5 (1.4, 1.6)	1.2 (1.1, 1.3)***	1.3 (1.2, 1.3)
HEI-5: Whole grains (10)	2.7 (2.6, 2.8)	2.3 (2.2, 2.4)**	2.4 (2.3, 2.5)
HEI-6: Dairy (10)	5.4 (5.2, 5.5)	2 (1.9, 2.1)***	3 (2.9, 3.1)***
HEI-7: Total protein foods (5)	4.1 (4.1, 4.1)	3.7 (3.6, 3.8)	3.8 (3.8, 3.9)
HEI-8: Seafood and plant protein (5)	2.2 (2.1, 2.3)	2.1 (2, 2.2)	2.1 (2, 2.2)
HEI-9: Fatty acids (10)	4.8 (4.7, 4.9)	6.9 (6.8, 7)***	6.3 (6.2, 6.4)
HEI-10: Refined grains (10)	6 (5.9, 6.1)	6 (5.9, 6.1)	6 (5.9, 6.1)
HEI-11: Sodium (10)	4.3 (4.2, 4.4)	4.9 (4.8, 5)***	4.8 (4.6, 4.9)***
HEI-12: Added sugars (10)	6.5 (6.3, 6.6)	5.9 (5.8, 6)	6 (5.9, 6.1)
HEI-13: Saturated fat (10)	5.7 (5.5, 5.8)	7.7 (7.6, 7.7)***	7.1 (7, 7.2)***
Consumers of dairy fat only (n = 12,831)			
NRF 9.3	402 (396, 407)	313 (307, 318)***	340 (335, 345)***
HEI-2015 (100)	50.4 (49.7, 51)	49.8 (49.2, 50.5)	50 (49.4, 50.7)
HEI-1: Total fruits (5)	2.1 (2.1, 2.2)	2.3 (2.2, 2.4)	2.3 (2.2, 2.3)
HEI-2: Whole fruits (5)	2.2 (2.1, 2.3)	2.1 (2, 2.2)	2.1 (2, 2.2)
HEI-3: Total vegetables (5)	2.9 (2.8, 2.9)	2.7 (2.7, 2.8)	2.8 (2.7, 2.9)
HEI-4: Greens and beans (5)	1.5 (1.4, 1.6)	1.2 (1.1, 1.2)**	1.2 (1.2, 1.3)*
HEI-5: Whole grains (10)	2.8 (2.7, 2.9)	2.4 (2.2, 2.5)**	2.5 (2.4, 2.6)
HEI-6: Dairy (10)	5.9 (5.8, 6.1)	2 (1.9, 2.1)***	3.2 (3, 3.3)***
HEI-7: Total protein foods (5)	4.1 (4, 4.1)	3.6 (3.6, 3.7)*	3.8 (3.7, 3.8)
HEI-8: Seafood and plant protein (5)	2.2 (2.1, 2.3)	2.1 (2, 2.2)	2.1 (2, 2.2)
HEI-9: Fatty acids (10)	4.4 (4.3, 4.5)	6.9 (6.8, 7)***	6.2 (6, 6.3)***
HEI-10: Refined grains (10)	6 (5.9, 6.1)	6.1 (6, 6.2)	6.1 (6, 6.2)
HEI-11: Sodium (10)	4.3 (4.2, 4.4)	5 (4.9, 5.2)***	4.9 (4.7, 5)**
HEI-12: Added sugars (10)	6.5 (6.4, 6.7)	5.9 (5.7, 6)	6 (5.9, 6.1)
HEI-13: Saturated fat (10)	5.3 (5.2, 5.5)	7.7 (7.6, 7.8)***	7 (6.9, 7.1)***

[a]*Model 1 removes all dairy fat. Model 2 removes up to 5% dairy fat for each individual.*
[b]*Asterisks indicate statistical significance comparing whether Modeled changes are a 10% change from observed diets; ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$.*

TABLE 7 | Observed and modeled mean NRF 9.3 and HEI-2015 overall and component scores, following the replacement of dairy fat with PUFA/MUFA, NHANES 2013–2016.

	Mean (95% Confidence Intervals [CI])		
	Observed	**Model 1[a] (swap)**	**Model 2[b] (swap)**
Total population (n = 15,260)			
NRF 9.3	391 (385, 396)	362 (357, 367)	378 (372, 383)
HEI-2015 (100)	50.1 (49.5, 50.8)	54.7 (54.1, 55.3)	52.9 (52.3, 53.5)
HEI-1: Total fruits (5)	2.1 (2, 2.2)	2.3 (2.2, 2.3)	2.2 (2.1, 2.3)
HEI-2: Whole fruits (5)	2.1 (2, 2.2)	2.3 (2.3, 2.4)	2.3 (2.2, 2.4)
HEI-3: Total vegetables (5)	2.9 (2.8, 2.9)	3.3 (3.3, 3.4)***	3.2 (3.1, 3.2)
HEI-4: Greens and beans (5)	1.5 (1.4, 1.6)	2.1 (2, 2.1)***	1.9 (1.8, 2)***
HEI-5: Whole grains (10)	2.7 (2.6, 2.8)	2.8 (2.7, 2.9)	2.8 (2.6, 2.9)
HEI-6: Dairy (10)	5.4 (5.2, 5.5)	2.1 (2, 2.1)***	3.5 (3.4, 3.7)***
HEI-7: Total protein foods (5)	4.1 (4.1, 4.1)	4.5 (4.5, 4.5)*	4.4 (4.4, 4.4)
HEI-8: Seafood and plant protein (5)	2.2 (2.1, 2.3)	3.4 (3.3, 3.4)***	3 (2.9, 3.1)***
HEI-9: Fatty acids (10)	4.8 (4.7, 4.9)	7.9 (7.8, 8)***	6.5 (6.4, 6.6)***
HEI-10: Refined grains (10)	6 (5.9, 6.1)	6.1 (6, 6.2)	6.1 (6, 6.2)
HEI-11: Sodium (10)	4.3 (4.2, 4.4)	3.5 (3.4, 3.6)***	3.7 (3.6, 3.8)***
HEI-12: Added sugars (10)	6.5 (6.3, 6.6)	6.8 (6.7, 6.9)	6.7 (6.5, 6.8)
HEI-13: Saturated fat (10)	5.7 (5.5, 5.8)	7.7 (7.6, 7.8)***	6.7 (6.6, 6.8)***
Consumers of dairy fat only (n = 12,831)			
NRF 9.3	402 (396, 407)	369 (365, 374)	387 (382, 392)
HEI-2015 (100)	50.4 (49.7, 51)	55.8 (55.2, 56.3)*	53.7 (53.1, 54.2)
HEI-1: Total fruits (5)	2.1 (2.1, 2.2)	2.3 (2.3, 2.4)	2.3 (2.2, 2.3)
HEI-2: Whole fruits (5)	2.2 (2.1, 2.3)	2.5 (2.4, 2.6)*	2.3 (2.2, 2.4)
HEI-3: Total vegetables (5)	2.9 (2.8, 2.9)	3.4 (3.4, 3.5)***	3.2 (3.2, 3.3)**
HEI-4: Greens and beans (5)	1.5 (1.4, 1.6)	2.2 (2.2, 2.3)***	2 (1.9, 2.1)***
HEI-5: Whole grains (10)	2.8 (2.7, 2.9)	2.9 (2.8, 3)	2.9 (2.8, 3)
HEI-6: Dairy (10)	5.9 (5.8, 6.1)	2.1 (2, 2.1)***	3.8 (3.7, 3.9)***
HEI-7: Total protein foods (5)	4.1 (4, 4.1)	4.6 (4.5, 4.6)***	4.4 (4.4, 4.5)
HEI-8: Seafood and plant protein (5)	2.2 (2.1, 2.3)	3.6 (3.5, 3.6)***	3.2 (3.1, 3.2)***
HEI-9: Fatty acids (10)	4.4 (4.3, 4.5)	8.1 (8, 8.2)***	6.4 (6.3, 6.5)***
HEI-10: Refined grains (10)	6 (5.9, 6.1)	6.2 (6.1, 6.3)	6.1 (6, 6.2)
HEI-11: Sodium (10)	4.3 (4.2, 4.4)	3.3 (3.2, 3.4)***	3.6 (3.5, 3.8)***
HEI-12: Added sugars (10)	6.5 (6.4, 6.7)	6.9 (6.8, 7)	6.8 (6.7, 6.9)
HEI-13: Saturated fat (10)	5.3 (5.2, 5.5)	7.7 (7.6, 7.8)***	6.6 (6.5, 6.7)***

[a]*Model 1 replaces all dairy fat. Model 2 replaces up to 5% dairy fat for each individual.*
[b]*Asterisks indicate statistical significance comparing whether Modeled changes are a 10% change from observed diets; ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$.*

Consumption patterns for dairy products vary widely across population subgroups. The consumption of fluid milk drops sharply with age; while the consumption of whole- vs. reduced-fat milk tracks socioeconomic status. Milk is more likely to be consumed at breakfast than at dinner. The consumption of dairy products can affect dietary energy density as well as the percent of fat energy in the diet. Realistic food-level substitution modeling needs to take these many social and behavioral considerations into account. Food-level substitutions need to be age-, meal-, and diet-specific.

Food level substitutions can never be perfect. First, many foods that contain dairy fat as an ingredient can also contain MUFA and PUFA. Conversely, many foods that contain MUFA/PUFA also contain meaningful amounts of saturated fat. Our rules were that foods that contained dairy fat could not be included in the substitution model, nor could foods that contained more saturated fat than MUFA+PUFA. Otherwise the substitution modeling was context specific with weighted substitution foods generated for 576 separate population subgroups

This food-level approach contrasts with simpler "fat swapping," that is the swapping of regression coefficients in large scale observational studies (1). Those analyses did not take individual food choices into account but did adjust for age, BMI, energy intake, ethnicity, smoking, physical activity, alcohol consumption, hypertension and hypercholesterolemia. Replacing dairy fat with healthful carbohydrates via the same methods was also shown to reduce CVD risk (1). Nutrient-based dietary guidance is apt to ignore the difficulties inherent in replacing one food group for another, or the unintended nutritional consequences of removing some food groups from the diet altogether. Dairy products are a major source of fatty acids, micronutrients, and bioactive peptides.

The present analyses illustrate the difference between a food-based and a nutrient-based substitution model. The removal of the food sources of dairy fat from the diet led to dietary patterns that were significantly lower in calories and lower in some key micronutrients. Although saturated fat was significantly reduced, calcium and vitamins A, D, and B12 were significantly reduced as well. Diet quality measured using the nutrient-based NRF score was significantly reduced. These results held for partial (5%) or complete removal of dairy fat from the diet and held both for dairy fat consumers and for the whole US population.

The food-level replacement of non-dairy PUFA and MUFA for the missing dairy fat did not remedy the modeled lower intakes of some micronutrients. Measures of diet quality were equivocal. The NRF score showed a reduction in overall dietary nutrient density with the removal of dairy fat and no significant improvement when dairy fat was replaced with non-dairy PUFA and MUFA.

No major improvement in diet quality following PUFA/MUFA replacement was observed using the total HEI-2015 score, though for the dairy fat consumers there was an increase in HEI-2015 scores. This was surprising, given that the HEI-2015 penalizes intakes of saturated fat intake twice; first, as total intake and second, as the ratio of unsaturated to saturated fat. The saturated fat sub-score is very highly correlated with the fatty acid ratio sub-score; ($r = 0.64$) which is one of the strongest bivariate correlations among HEI-2015 components. Replacing saturated fat with unsaturated fat would practically guarantee higher HEI-2015 scores, given that this is how HEI-2015 was constructed. However, no expected improvement in HEI-2015 scores, the federal measure of diet quality, following the removal of dairy fat was observed.

Finally, the observation that modeled nutrient-for-nutrient substitutions were associated with a global reduction in CVD risks (1) seems to contrast with some of the new and emerging evidence for the beneficial impact of dairy fat (33–35). In particular, studies have suggested that dairy foods, particularly fermented dairy products, have neutral or inverse associations with CVD (36, 37). The current view is that overall dietary patterns, as opposed to selected single nutrients are the basis of healthy eating (36). Current analyses are turning to nutrients in the context of the food matrix.

Previous modeling studies did acknowledge the inability to evaluate any potential impact of the food matrix (1). In other words, different foods (butter, hard cheese) had a different impact on plasma biomarkers of CVD, at comparable intakes of total fat and saturated fat. Arguably, the food matrix is itself a simplification. Butter and hard cheese are consumed on different eating occasions and may be accompanied by different foods. The present study addressed the content of dairy fat consumption by taking into account eating occasion and the energy density of the food. Further work on links between diets and CVD risk needs to address food patterns in addition to selected single nutrients.

This study has limitations and strengths that are worth noting. Our results do not represent actual human behavior, which is complex and multi-factorial. We made an effort to build a sophisticated model that accounted for the context of consumption (e.g., meal type) and individual-characteristics (e.g., age), but we could not account for all such variables. However, our modeling strategy took many more factors into account than similar studies and we view this as an improvement. The use of large and nationally representative NHANES datasets ensured a large sample size, making the results highly generalizable and statistically stable. We assessed a number of different dietary outcomes, including individual macronutrients, micronutrients and summary measures of diet quality. A few important diet quality measures were not available, specifically *trans* fatty acids, which are not available in NHANES data. Lastly, a single 24 h recall allows us to examine the change in the population-mean but does not permit us to estimate in an unbiased manner the change in the proportion of individuals meeting or failing to meet a dietary threshold (e.g., calcium adequacy).

CONCLUSION

Food-level modeling that is sensitive to dietary patterns points to some limitations of the theoretical "nutrient swapping" approach. Given that foods contain multiple nutrients, clean replacement of one nutrient for another may be simple in theory but very

complex in real life. The Dietary Guidelines for Americans are increasingly adopting a food based approach with more attention paid to food patterns than to individual nutrients.

DATA AVAILABILITY

The datasets for this study will not be made publicly available because the NHANES databases are publicly available and in the public domain.

AUTHOR CONTRIBUTIONS

CR and AD conceptualized and designed the study. CR developed the databases, carried out the analyses and produced summary tables. AD drafted the initial manuscript. All authors reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Consumption Patterns of Milk and 100% Juice in Relation to Diet Quality and Body Weight Among United States Children: Analyses of NHANES 2011-16 Data

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Background: The American Academy of Pediatrics (AAP) has recommended placing limits on the consumption of milk and 100% juice by children.

Methods: Consumption data for 9,069 children aged 2–19 years came from three cycles of the nationally representative National Health and Nutrition Examination Survey (NHANES 2011-2016). Beverages were classified into 100% juices, milk (whole, reduced fat, and skim), caloric sugar sweetened beverages (SSB), low calorie beverages (LCB), and drinking water. The Healthy Eating Index 2015 and Nutrient Rich Food Index NRF9.3 were two measures of diet quality. Analyses examined consumption patterns for milk and 100% juice in relation to diet quality, AAP recommendations, and BMI z-scores across time and for different age groups.

Results: Intakes of milk and 100% juice declined sharply with age, whereas SSB and water increased. Top quartiles of HEI 2015 and NRF9.3 diet quality scores were associated with higher intakes of water, milk, and 100% juice and with lower intakes of SSB. Lower-income groups drank less skim milk and water and more whole milk and SSB. Only 30% of the children consumed any 100% juice. There was no association between the consumption of milk or 100% juice and BMI z-scores for any age group.

Conclusions: Top quartiles of diet quality were associated with more milk, 100% juice, and water, and less SSB. Higher quality diets were associated with lower compliance with the AAP 100% juice recommendations. Compliance with the AAP 100% juice recommendations was not associated with lower body weights. Attempts to limit the consumption of milk and 100% juice by children might have the unintended consequence of increasing consumption of SSB and may have limited value for obesity prevention.

Keywords: milk, 100% juice, SSB, children, diet quality

INTRODUCTION

The role of milk and 100% fruit juice in the US children's diets continues to be a topic of debate (1–6). To prevent excess weight gain, the American Academy of Pediatrics (AAP) has recommended that children switch to low-fat or non-fat milk after the age of 2 years (7). The AAP has also set limits on the consumption of 100% fruit juices. The suggested amounts for 100% fruit juice were 4–6 oz./d for children aged 4–6 years and up to 8 oz./d for children aged 7–18 years (8). That would limit 100% fruit juice to only one of the recommended 2 to 2 ½ cups of fruit servings per day. For the remainder of the paper 100% juice will be referred to simply as "juice."

Past studies have shown that beverage drinking patterns built around milk and juice were more nutrient-rich than beverage patterns built around sugar-sweetened beverages (SSB) (4). However, only a small minority of children drank mostly milk, juice, or both. The consumption of milk and juice dropped sharply with age, to be replaced by SSB. Efforts are under way to replace SSBs along with juices and milk in the children's diets, with non-caloric and non-nutritive plain drinking water.

Replacing one food or one beverage with another can be problematic when consumption patterns fall along a socioeconomic gradient (9). In past studies, consumption patterns for juice, milk, and even tap water showed very different links to socioeconomic status (SES) (10). For example, higher-SES groups consumed more whole fruit, whereas lower-SES groups drank more fruit juice. Economic issues may have been an underlying factor. A modeling study showed that while eating whole fruit cost more, a combination of whole fruit and juice effectively remedied inadequate fruit consumption, without increasing diet cost (11).

However, social gradients in other beverage choices are not always related to cost. Past analyses of NHANES data showed that higher SES groups drank more low-fat milk, whereas lower SES groups drank more whole milk. Higher SES groups drank more diet soda, whereas lower SES groups drank more regular soda. Higher SES groups also drank more plain water from the tap (12–14).

Obesity prevalence in childhood and in adult life also follows a socio-economic gradient (15). We may therefore expect SES-driven consumption of whole fruit, skim milk, and water to be associated with lower obesity rates. Indeed, reducing added sugars by replacing SSB with plain drinking water is a recognized strategy for obesity prevention (16). However, past studies linking beverage consumption to body weight may not have fully accounted for the potential (and mostly unobserved) SES confounds. Whereas, obesity has been viewed as the direct consequence of eating a specific food or beverage, it can also be viewed as the outcome of eating patterns associated with lower education and lower incomes (17).

This study examined consumption patterns for milk, fruit juice, and water in a representative sample of toddlers, children, and adolescents in the US. The primary goal was to examine the relation between beverage consumption patterns and diet quality measures. A secondary goal was to examine any links between the consumption of milk and juice and body weight by age, within the confines of a cross-sectional study design.

MATERIALS AND METHODS

Dietary intakes data for 9,069 children aged 2–19 years came from the first day of the National Health and Nutrition Examination Survey (2011-2016 NHANES). The NHANES 24-h recall uses a multi-pass method, where respondents reported the types and amounts of all food and beverages consumed in the preceding 24-h, from midnight to midnight. The multi-pass method is conducted by a trained interview using a computerized interface. The recall first identifies a quick list of foods and beverages consumed. The time and occasion for each food item is also obtained. A detailed cycle is then conducted that records the amounts consumed, followed by a final probe for any frequently forgotten foods (beverages, condiments). Day 1 interviews were conducted by trained dietary interviewers in a mobile examination center. For children of 4-5 years, the dietary recall was completed entirely by a proxy respondent (i.e., parent or guardian with knowledge of child's diet). Children aged 4–11 years were the primary respondents, but a proxy respondent was present and able to assist. Children aged 12–19 years were the primary source of dietary recall information, but could be assisted by an adult who had knowledge of their diet.

Participant Characteristics

NHANES participants were stratified by gender and age. The sample was stratified by age into toddlers (2–4 years); young children (5–8 years); older children (9–13 years); and adolescents (14–19 years). These age groups generally correspond to the age groups used by the IOM (18). The cut-points for the family income-to-poverty ratio were: $<$1.3, 1.3–1.849, 1.85–2.99, and $\geq$3.0. Information from the demographic NHANES questionnaires was used to stratify the sample by race/ethnicity, defined as Non-Hispanic White; Non-Hispanic Black, Mexican American, Other Hispanic, Non-Hispanic Asian, and other/mixed race (19). The ethics board review for the NHANES data collection is documented by the National Center for Health Statistics online (20). Analyses of publicly available federal NHANES data are exempt from approvals by Institutional Review Boards.

Beverage Consumption Patterns

Beverages were classified into 6 categories as follows: (1) fruit juices (citrus juices, apple juice, and non-citrus juices) and vegetable juices. (2) Milk and milk beverages, separated into whole, reduced fat, and skim. (3) Other caloric sugar sweetened beverages or SSB ($>$50 kcal/240g). (4) Other non-caloric and low-calorie beverages or LCB ($<$50 kcal/240g). (5) Drinking water, separated into tap and bottled. (6) Baby formula. The 100% fruit juice blends (e.g., apple-cranberry) were included in the juice category but sweetened fruit-based drinks with added sugars were placed among SSBs. All milk analyses were stratified into 3-levels: whole, reduced, and low-fat/skim. Baby formula was included, even though its consumption was low. Very few toddlers$>$ 2 years old consumed breast milk.

Measures of Diet Quality

Energy and nutrient intakes for NHANES participants were calculated using the Food and Nutrient Database for Dietary Studies 2011-2014, customized with the addition of vitamin D and added sugars. This information was supplemented with data from the Food Patterns Equivalents Database (FPED) from the United States Department of Agriculture (USDA) (21).

The HEI-2015 is the latest iteration of the USDA diet quality measurement tool, specifically designed to monitor compliance with the 2015 Dietary Guidelines for Americans (22). The HEI-2015 is a 100-point scale where the adequacy components are fruits (10 points), vegetables (10), grains (10), dairy (10), protein foods (10), and fats (10). The HEI 2015 adequacy component is based around food groups to encourage, with some food categories called out by name (i.e., total vegetables, dark-green and orange vegetables, total fruit, whole fruit, whole grains, total protein foods, protein from seafood and plant sources, the ratio of polyunsaturated and monounsaturated fatty acids to saturated fatty acids, and total dairy). The moderation component listed foods and nutrients to limit that included refined grains, sodium added sugars and saturated fats. Former versions listed SoFAAs, a composite of solid fat, alcohol, and added sugars that served as a summary measure of empty calories.

The Nutrient Rich Foods (NRF) index served as the second measure of dietary nutrient density (23–25). The NRF9.3d variant is an energy-adjusted diet quality score that is based on 9 nutrients to encourage and 3 nutrients to limit. Reference daily values (DVs) were based on the US Food and Drug Administration (FDA) and other standards (26). The qualifying nutrients to encourage and standard reference amounts were as follows: protein (50 g), fiber (28 g), vitamin A (900 RAE), vitamin C (90 mg), vitamin D (20 mcg), calcium (1300 mg), iron (18 mg), potassium (4,700 mg) and magnesium (420 mg). The 3 disqualifying nutrients and maximum recommended values (MRVs) were: added sugar (50 g), saturated fat (20 g) and sodium (2,300 mg). The NRF was calculated as follows.

$$\text{NRF 9.3d} = (\text{NR} - \text{LIM}) \times 100$$

with

$$NR = \sum_{i=1}^{9} \frac{Intake_i/Energy \times 2000}{DV_i}$$

and

$$LIM = \sum_{i=1}^{3} \frac{Intake_i/Energy \times 2000}{MRV_i} - 1$$

where $intake_i$ is the daily intake of each nutrient i, and DVi is the reference daily value for that nutrient. In NR calculation, each daily nutrient intake i was adjusted for 2000 kcal and expressed in percentage of DV. Following past protocol, percent DVs for nutrients were truncated at 100, so that an excessively high intake of one nutrient could not compensate for the dietary inadequacy of another. In LIM, only the share in excess of the recommended amount was considered.

The development and validation of the NRF family of nutrient density indices have been reported in the literature (27). In the present adaptation, vitamin D, a nutrient of public health concern (28), replaced vitamin E. Fiber, vitamin D, calcium, magnesium, and potassium were all identified in the 2015 Dietary Guidelines for Americans as nutrients of concern (28). The NRF score was adjusted for energy intakes, analogous with the recent versions of the USDA Healthy Eating Index, a federal measure of diet quality (22). Both NRF9.3d and HEI 2015 were corrected for dietary energy (1,000 kcal for HEI and 2,000 kcal for NRF).

Plan of Analysis

The sample was stratified by gender, age group, IPR (income-to-poverty ratio) and race/ ethnicity. Diet quality measures—Healthy Eating Index 2015 and the Nutrient Rich Food (NRF) index—were dichotomized to allow for comparisons between high quality diets and diets that needed work (22). Healthy diets were those in the top quartile of HEI 2015 and NRF scores. Weight status was measured using BMI z-scores that were split into 4 classes using clinically meaningful cut-points. We then conducted regression analyses between key indexes of consumption and BMI z-scores, adjusting for covariates.

Analyses of beverage consumption (g/day) were conducted for the entire population and by age group. All analyses accounted for the complex survey design of NHANES data and are representative of the US population. Data analyses were conducted using Stata 13.1 (College Station, TX) and SAS 9.4 (SAS institute, Cary, NC).

RESULTS

Table 1 shows participant characteristics. The sample of children and adolescents was evenly distributed by gender and income-to-poverty ratio (IPR). The sample was 52.1 % Non-Hispanic White; 14.6% Non-Hispanic Black, 15.7% Mexican American, and 8.0% other Hispanic.

Beverages were separated into juice, milk (whole, reduced, skim), SSB, LCB, and water. **Figure 1** shows beverage consumption (in g/d) by age group. Panel 1 (left) shows absolute intakes in g/d; panel 2 (right) shows proportions. The consumption of milk (all types) and juice declined sharply with age. Conversely, the consumption of water, SSB, and LCB increased with age. Absolute intake data presented in **Figure 1** are also available in **Supplemental Table 1**.

Beverage consumption patterns were linked to incomes, as expected. **Figure 2** shows socioeconomic gradient in beverage consumption by 4 IPR strata, separately for each age group. There was a progressive drop in the consumption of whole milk at higher household incomes and a corresponding increase in consumption of reduced fat and skim milk. There was also an income associated drop in SSB consumption and an increase in LCB and water consumption. Consumption of juice was lower at higher IPR but only for young children (2–4y).

Figure 3 shows that beverage consumption patterns varied by race/ethnicity. Non-Hispanic White children drank more water, LCB, and skim milk than other racial/ethnic groups, especially after age 9 years. Non-Hispanic Black children drank more SSB, again especially later in childhood after 9 years.

TABLE 1 | Sample characteristics by age group, demographics, diet quality and BMI z-scores.

	Total *N* = 9069		2–4y *N* = 1729	5–8y *N* = 2133	9–13y *N* = 2501	14–19y *N* = 2706	*p*-value
	n	%	%	%	%	%	
Gender							0.10
Male	4574	51.0	48.3	54.2	50.1	51.0	
Female	4495	49.0	51.7	45.8	49.9	49.0	
Family IPR							0.02
<1.3	2892	24.5	27.1	26.8	23.7	22.3	
1.3-1.849	2280	22.8	23.4	21.3	23.1	23.3	
1.85-2.99	1590	21.1	18.0	19.8	24.6	20.6	
≥3.0	1655	25.8	25.7	26.5	23.7	27.1	
Missing	652	5.7	5.8	5.5	4.8	6.6	
Race/ethnicity							0.58
Non-Hispanic White	2335	52.1	50.7	52.0	51.5	53.4	
Non-Hispanic Black	2345	14.6	15.2	14.5	13.6	15.1	
Mexican American	1925	15.7	15.2	15.9	15.9	15.7	
Other Hispanic	1032	8.0	8.7	8.5	8.6	6.9	
Non-Hispanic Asian	867	4.6	4.5	4.3	4.9	4.8	
Other	565	4.9	5.6	4.9	5.4	4.1	
BMI Z-score cutpoints							<0.001
Underweight	295	3.4	2.9	3.7	3.8	3.0	
Normal	5521	61.8	70.1	64.1	58.4	58.9	
Overweight	1443	15.7	13.6	14.4	17.4	16.1	
Obese	1645	17.6	9.5	17.5	19.9	19.8	
Missing	165	1.6	3.9	0.3	0.5	2.1	
HEI 2015							<0.001
Low (below Q3 = 56.58)	6760	75.0	60.6	74.0	78.7	79.7	
High (above Q3 = 56.58)	2309	25.0	39.4	26.0	21.3	20.3	
NRF9.3							<0.001
Low (below Q3 = 653.38)	6901	75.0	58.4	72.5	77.3	82.7	
High (above Q3 = 653.38)	2168	25.0	41.6	27.5	22.7	17.3	

Data for children and adolescents, United States 2011-2016 NHANES.

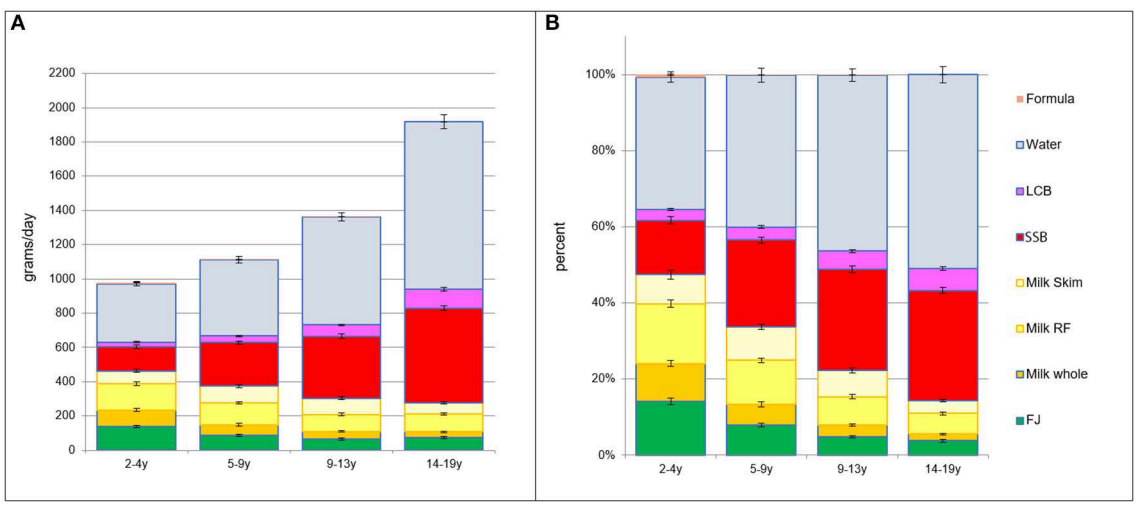

FIGURE 1 | (A) Intakes in grams per day by beverage type and by age group. **(B)** Intakes in percent of daily intake by beverage type and by age group.

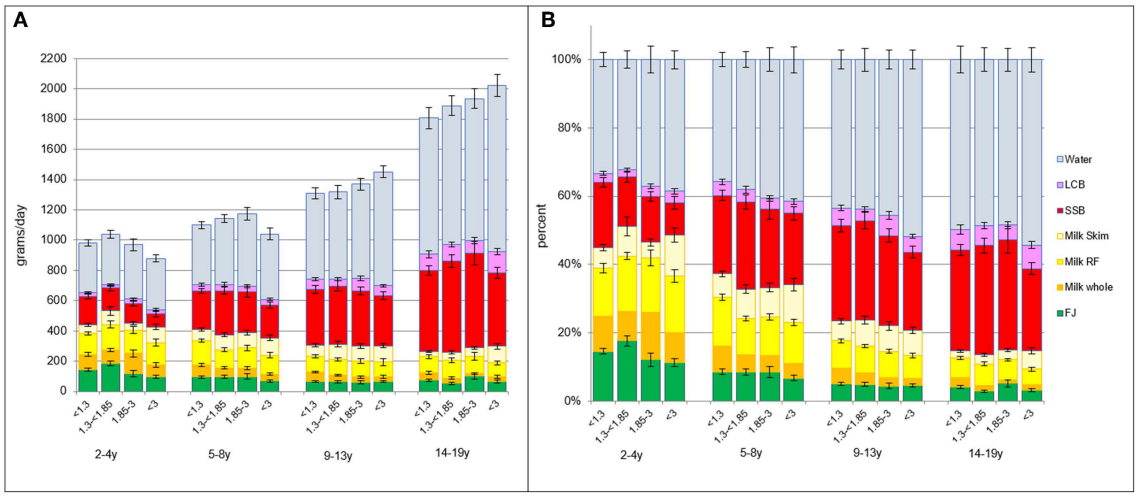

FIGURE 2 | (A) Intakes in grams per day by beverage type and by age group and IPR strata. **(B)** Intakes in percent of daily intake by beverage type and by age group and IPR strata.

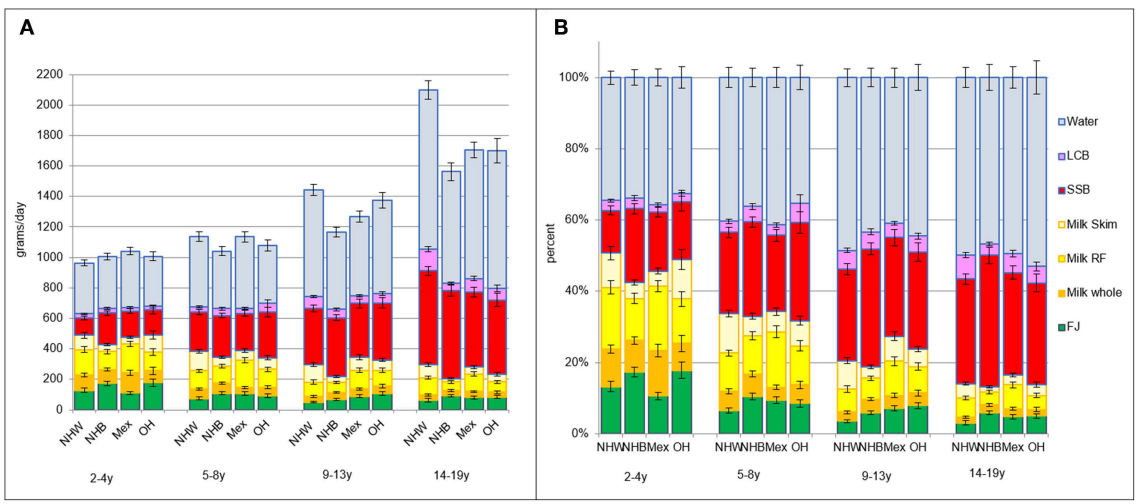

FIGURE 3 | (A) Intakes in grams per day by beverage type and by age group and race/ethnicity. NHW, Non-Hispanic White; NHB, Non-Hispanic Black; Mex, Mexican American; OH, Other Hispanic. **(B)** Intakes in percent of daily intake by beverage type and by age group and race/ethnicity.

Diet Quality and Beverage Consumption

Figure 4 shows beverage consumption patterns that were associated with the top quartile of HEI 2015 scores. Data are shown separately for each age group. The left panels show absolute intakes; right panels show proportions. Diets in the top quartile of HEI 2015 scores had more fruit juice, more reduced-fat and skim milk, and more drinking water. Lower quality diets were associated with higher SSB intakes.

The same pattern was observed using NRF9.3 as the measure of diet quality. **Figure 5** shows beverage patterns associated with lower and higher NRF scores. Data are shown separately for each age group. The left panels show absolute intakes; right panels show proportions. Diets in the top quartile of NRF9.3 scores had more fruit juice, more skim and reduced-fat milk and more water. Lower quality diets were associated with higher SSB intakes.

Beverage Consumption Patterns and Body Weight Status

Table 2 shows beverage consumption patterns as a function of BMI z-scores, separated into 4 classes: underweight, normal, overweight, and obese. There was no relation between fruit juice consumers and body weight status. There was no relation between milk consumption and body weight status. There was no significant relation between water consumption and body weight status.

To test for interactions, additional analyses were conducted with BMI classes as independent variables and fruit juice, milk, and water as dependent variables, adjusting for gender and age group, poverty level, and ethnicity. The age*BMI interaction was also included in the general linear model to test whether any

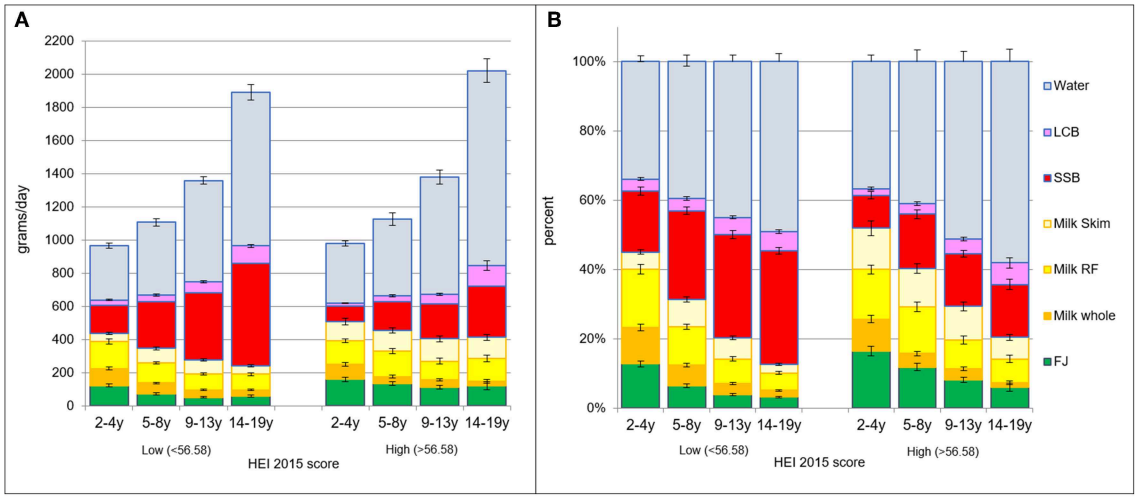

FIGURE 4 | (A) Intakes in grams per day by beverage type and by age group and HEI quartile: lower (<56.58) vs. higher (>56.58). **(B)** Intakes in percent of daily intake by beverage type and by age group and HEI quartile: the lower (<56.58) vs. higher (>56.58).

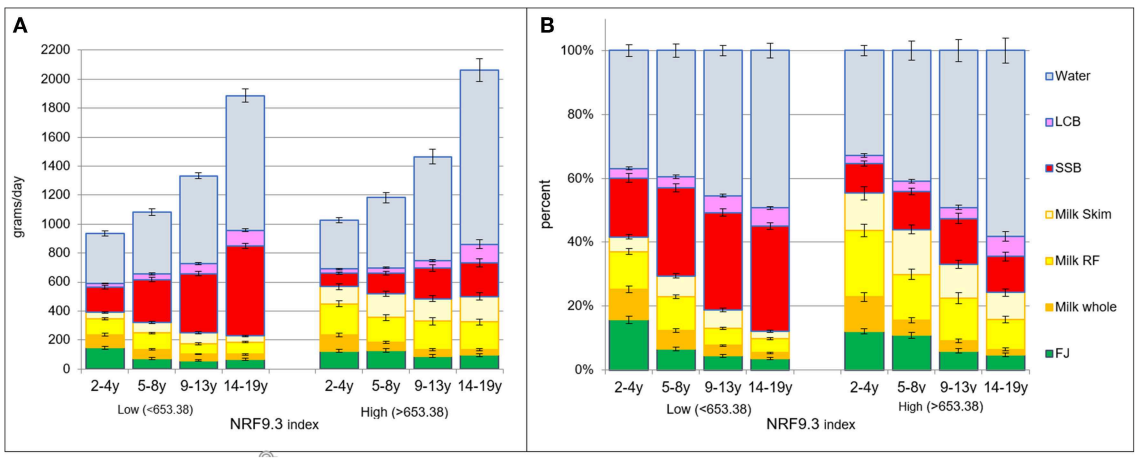

FIGURE 5 | (A) Intakes in grams per day by beverage type and by age group and NRF quartiles: the lower (<653.38) vs. higher (>653.38). **(B)** Intakes in percent of daily intake by beverage type and by age group and NRF quartile: the lower (<653.38) vs. higher (>653.38).

interaction between beverage consumption and BMI classes was age dependent. No significant interactions were observed.

Table 3 shows percent compliance with the AAP recommendations for juice consumption, separately for each age group. Dietary compliance was calculated separately for each age group according to AAP standards. For 1–3 year-olds, the standard was <=2 oz juice per day; for 4–6 year-olds, it was between 4 and 6 ounces per day, and for 7–18 year-olds it was < 8 ounces per day.

The data are split by gender, diet quality and BMI z-scores. First, diets of about 70% of the children were consistent with the AAP recommendations. Compliance rates ranged for 38% to 85% depending on age-specific consumption limits set by AAP. Interestingly, higher quality diets, assessed using two separate diet quality scores, were associated with lower compliance with AAP guidelines for juice. There was no relation between diets consistent with AAP recommendations and the children's body weight status.

DISCUSSION

Based on a representative sample of US toddlers, children, and adolescents aged 2–19 years, the present analyses include the most recent NHANES 2015-16 data. The comparisons made were between milk, juice and drinking water. We have previously identified beverage patterns built around milk and juice as providing an optimum selection of nutrients—from vitamin C to calcium and protein (4). However, few children in the NHANES

TABLE 2 | Average amount (g/d) of fruit juices, milk, and water by BMI class, in the total NHANES sample.

		Fruit juices				Milk				Water			
BMI classes	***N***	**Mean**	**SE**	**Lower limit**	**Upper limit**	**Mean**	**SE**	**Lower limit**	**Upper limit**	**Mean**	**SE**	**Lower limit**	**Upper limit**
Underweight	295	70.30	15.57	38.98	101.62	265.65	36.11	193.01	338.29	536.36	125.47	283.94	788.78
Normal	5521	88.01	4.27	79.42	96.60	259.38	8.66	241.97	276.80	636.51	24.04	588.16	684.87
Overweight	1443	85.70	5.53	74.58	96.81	237.47	8.44	220.49	254.44	667.70	33.92	599.46	735.95
Obese	1645	72.10	6.80	58.41	85.78	235.57	9.08	217.30	253.83	758.78	36.83	684.69	832.87
Missing	165	122.34	32.61	56.72	187.95	212.21	31.68	148.48	275.94	673.41	86.55	499.30	847.52

TABLE 3 | Compliance with American Academy of Pediatrics (AAP) recommendations for fruit juice by gender, diet quality and body weight status.

	Total ***N* = 9069**		**2–4y** ***N* = 1729**	**5–8y** ***N* = 2133**	**9–13y** ***N* = 2501**	**14–19y** ***N* = 2706**
	n	**%**	**%**	**%**	**%**	**%**
GENDER						
Male	4574	68.0	38.1	48.7	83.3	82.8
Female	4495	71.4	44.9	50.0	86.0	85.8
p-value		0.03	0.05	0.66	0.12	0.15
HEI 2015						
Low (below Q3 = 56.58)	6760	73.4	44.0	49.8	88.0	86.7
High (above Q3 = 56.58)	2309	58.4	38.0	47.8	72.1	74.8
p-value		<0.001	0.06	0.55	<0.001	<0.001
NRF9.3						
Low (below Q3 = 653.38)	6901	72.4	39.8	50.4	86.4	85.6
High (above Q3 = 653.38)	2168	61.2	44.2	46.5	78.6	77.9
p-value		<0.001	0.14	0.35	0.008	0.0002
BMI Z-SCORE CUTPOINTS						
Underweight	295	77.5	52.6	70.4	82.7	89.0
Normal	5521	68.1	42.8	47.4	85.7	83.4
Over weight	1443	69.6	36.0	48.0	84.1	83.2
Obese	1645	73.3	35.0	52.9	82.2	86.7
Missing	165	70.2	47.7	51.2	88.9	88.8
p-value		0.02	0.18	0.11	0.64	0.45

Data are shown separately for each age group.

2011-14 dataset drank mostly milk and juice. The vast majority had beverage drinking patterns built around SSB.

In the present study, diets in the top quartile of HEI 2015 and NRF9.3 scores had more milk, more juice, and more water. By contrast, lower quality diets had more SSB.

We also confirm the previously observed age effect—whereas milk and juices sharply decline with age, SSB and water increase. The age-related shifts in beverage choice can have consequences for nutrient density of the diet. Preventing the shift to SSB by continuing to drink milk and juice can confer some nutritional advantage.

Previously observed social gradients in beverage consumption were also confirmed in the present analyses. As expected, children from households with higher SES groups drank more skim and reduced fat milk and more water. By contrast, children from households with lower SES drank more SSB and more whole milk. A similar relationship for race/ethnicity was observed where Non-Hispanic White children drank more water, LCB, and skim milk and NHB children drank more SSB.

Previous studies showed that the consumption of whole fruit vs. fruit juice was income driven. Whereas, higher SES groups consumed more whole fruit, lower SES groups consumed more juice. Those studies also indicated that whole fruit accounted for 66% of total fruit consumption; there was no indication that juice displaced whole fruit in any way (29).

The present analyses showed no association between juice consumption and BMI z-scores, in line with a recent review concluded that there is not enough evidence to support the association of fruit juice intake and weight status or adiposity (2). A recent meta-analysis found that consumption of fruit juice lead to small, not clinically significant weight gain in children aged 1–6 years and no weight gain in older children (7–18 years).

The researchers suggest that the type of fruit juice consumed may account for this age difference; where younger children tend to drink apple juice and older children are more likely to consume orange juice, which may have different effects on cardiometabolic health due to differences in glycemic load (16). Similar analyses were conducted for whole, reduced fat, and skim milks.

Only about 30% of children drank juice on the first day of NHANES data collection. Although beverage drinking patterns built around milk and juice are most nutrient rich, most children drink SSB. Furthermore, both milk and juice consumption drop with age, whereas the consumption of SSB and water increases. Replacing milk and juice with water is one public health goal—however, milk and juice are also being replaced with SSB. Promoting milk and juice consumption into early adolescence may have some nutritional advantages.

The present analyses incorporate one of the few analyses of how compliance with the AAP juice guidelines related to diet quality overall. First, compliance with the AAP guidelines was lower for younger children who drank for juice than older children. Older children drank less juice and were therefore more compliant with the AAP guidelines; however, those children also drank more SSB. Paradoxically, better compliance with AAP guidelines (i.e., less juice) was associated with lower dietary nutrient density scores (i.e., more SSB). Whereas, replacing juice with plain water may be the desirable public health goal, the fact is that both water and SSB consumption increase with age.

Second, there was no association between compliance with AAP recommendations and body weight status. Together these novel results, albeit based on cross-sectional data, suggest that limiting juice intake may not have the intended effect of reducing obesity risk and may be at odds with other measures of diet quality.

The present study had limitations. Most important, the NHANES data were cross sectional, meaning that no causal inferences can be drawn. The present discussion is therefore limited to associations or lack thereof. Dietary intakes data were self-reported, and included proxy report for children 5 years or younger. There are limits to the accuracy of the self-reported data, such that participants are likely not able to report specific details of their intake; i.e., whether a juice was pasteurized or sterilized. Thus, the USDA nutrient composition database only classifies juice into the following categories; freshly squeezed, canned, bottled, frozen reconstituted from concentrate. In addition, self-reported dietary data are always subject to under- and over-reporting. However, the NHANES data are thoroughly examined in several rounds by trained reviewers before being approved for usage, ensuring high quality data (30). Finally, due to the cross-sectional design of the NHANES study, causality cannot be inferred from the data. Despite these limitations, NHANES data are still used as the basis for dietary policies in the US. Nutrient profiling models of nutrient density are nutrient based and may not adequately capture multiple aspects of healthy food patterns. The same limitation applies to the food and nutrient based Healthy Eating Index 2015. Though it is the preferred USDA measure of compliance with the 2015 USDA dietary guidelines, it may not adequately capture food patterns across the social strata.

CONCLUSIONS

Beverage consumption patterns varied with age; older children replaced milk and juice with SSB and to some extent with water. Higher SSB consumption was associated with lower quality diets. Lower compliance with AAP recommendations was associated with higher quality diets. While the current dietary guidance is for children to replace milk and juice with plain water, there is a high likelihood that children will drink palatable SSB instead. Finally, the AAP recommendation to limit juice intake were not associated with weight status, suggesting that compliance with the recommendations may have limited value for obesity prevention.

DATA AVAILABILITY

The datasets for this study will not be made publicly available because Federal data from the NHANES study are already publicly available can be accessed here: https://wwwn.cdc.gov/nchs/nhanes/default.aspx.

ETHICS STATEMENT

The ethics board review for the NHANES data collection is documented by the National Center for Health Statistics online (20). Analyses of publicly available federal NHANES data are exempt from approvals by Institutional Review Boards.

AUTHOR CONTRIBUTIONS

MM, FV, CR, and AD designed the study. CR developed the databases. MM and FV conducted the analyses. AD took the lead on writing the paper, along with CMR. All authors read and approved the final 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.2019.00117/full#supplementary-material

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Development of a Mobility Diet Score (MDS) and Associations With Bone Mineral Density and Muscle Function in Older Adults

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Introduction: Reduced bone mineral density (BMD) and muscle function is associated with increased risk of multiple health related issues. Diet may play a role in sustaining BMD and muscle function throughout old age, but much is still to be learned with regards to which specific food groups and dietary patterns that are important for such outcomes. The aim of the current study was to identify food groups important for both BMD and muscle function.

Methods: A narrative review was performed on studies published on dietary patterns and their association with BMD and muscle function, respectively. Based on these findings, two dietary indices were constructed characterizing food groups associated with BMD and muscle function, respectively. Associations between adherence to these indices and BMD and muscle function were then investigated in a population of older community-dwelling Danes. Food groups found to be associated with both BMD and muscle function in our study population were suggested for inclusion into a common dietary index named the Mobility Diet Score.

Results: In contrast to previous studies, adherence to a dietary index based on foods previously linked to BMD could not be established as important for BMD in our study population of 184 older individuals (53.3% men). We found that adhering to a dietary index characterized by higher intakes of whole grains, dairy products, fish, legumes, nuts, fruit, and vegetables is associated with faster 400 m walking speeds and an increased number of chair stands measured over a 30 s time period. Since no food group could be established as important for both BMD and muscle function in our study population, a Mobility Diet Score could not be established. However, based on our narrative review, the food groups commonly associated with improved BMD and muscle function are similar.

Conclusion: Adherence to a dietary index characterized by high intakes of whole grains, dairy products, fish, legumes, nuts, fruit, and vegetables was not found to be associated with BMD in a group of community-dwelling older Danes. However, our results indicate that the adherence to such foods could be important in sustaining physical function in older individuals.

Keywords: elderly, nutrition, diet, dietary pattern, bone, BMD, muscle, strength

INTRODUCTION

Loss of bone mineral density (BMD) as well as age-related loss of muscle mass and strength (sarcopenia) are potential concerns of older individuals. Both osteoporosis and sarcopenia are associated with adverse health outcomes such as increased risk of falls, bone fractures, hospitalization, disability, poor quality of life and death (1–5). As both bone and muscle deterioration are often correlated in older individuals, the broader term "Osteosarcopenia" has been suggested (6). Furthermore, osteoporosis and sarcopenia are potentially interrelated as osteoporosis is likely to affect physical activity level and thereby muscle maintenance and vice versa. Practicing regular physical activity helps to preserve BMD as well as skeletal muscle mass and strength in older adults (7–9). Moreover, it has been widely demonstrated that diet can positively influence the maintenance of BMD as well as functional capabilities (10–12). However, the specific dietary patterns and food groups that may be important for such outcomes are not yet well-established.

Previously, a diet score that characterizes food groups associated with BMD was developed by de Jonge et al. (13). Based on a narrative review of 15 observational studies investigating associations between different dietary patterns and BMD, eight food groups were identified and included in a BMD-Diet Score. Adherence to this diet score was subsequently found to be associated with BMD in a cohort of +5,000 Dutch older adults (median age 67 years). Other studies have found associations between dietary patterns that are abundant in whole grains, fruits, and vegetables, e.g., dietary patterns inspired by the "Mediterranean" and "healthy Nordic" diets and skeletal muscle mass and strength (14, 15). A recent systematic review of observational studies concluded that the evidence for an association between a range of physical outcomes and adherence to dietary patterns characterized by high intakes of fruit, vegetables, whole grains, fish, lean meat, low-fat dairy, nuts, olive oil and low in refined grains, sweets, and animal products was considered strong and consistent for physical performance, but limited for muscle strength and sarcopenia in older individuals (11). As low BMD can potentially lead to bone fractures, it is an important factor to consider along with muscle strength and functional capabilities as a way to maintain the quality of life and independence of older people. Establishing a dietary index that considers all of these factors is therefore highly relevant. The development of such an index seems increasingly important as the prevalence of both osteoporosis and sarcopenia likely is going to increase significantly in the years to come due to a growing global older population (16).

The aim of the present study was to investigate the food groups most commonly associated with BMD, muscle strength and physical function. Subsequently, we aimed to confirm these associations in a population of older Danish community-dwelling individuals.

METHODS

In order to establish the food groups important for BMD and muscle outcomes, the current study took a two-step methodological approach. Step 1 consisted of a narrative review of the existing literature. Here we identified the food groups that are most strongly associated with BMD and muscle mass, strength and function, respectively (all muscle related outcomes are collectively referred to as "muscle function" in this study). In step 2 we subsequently assessed the potential associations between adherence to the identified food groups and BMD and muscle function, respectively, in a group of older Danish individuals. Food groups confirmed as important for both BMD and muscle function would then be suggested for implementation into a cumulative Mobility Diet Score (MDS) representing a dietary pattern beneficial for both bone and muscle "health".

Study Setup

To investigate associations between the intake of food groups identified in the narrative review and BMD and muscle outcomes, the present study used baseline data from the "Counteracting Age-related Loss of Skeletal Muscle Mass" (CALM) study. The CALM study is a 1 year randomized controlled trial investigating effects of diet and physical activity on functional outcomes such as BMD, muscle size, -strength, and -performance in Danish community-dwelling older adults. The CALM study has been described in detail elsewhere (17).

Study Population

One hundred and eighty four Danish men and women (≥65 years) participating in the CALM study (17) were included in the present study. Only participants with complete baseline dietary registrations were included (184 out of 205).

Assessment of Dietary Intake

The enrolled participants completed 3 days weighed food diaries from Wednesday to Friday. Dietary information was entered into the online dietary registration tool VITAKOST™ (MADLOG ApS, Kolding, Denmark) where average daily consumption of the different food products was calculated. Consumed foods were divided into the respective food groups identified as relevant

in our narrative review for use in later analyses investigating associations with BMD and muscle function.

Assessment of Outcomes

Bone Mineral Density (BMD)

Whole body, femur neck, total femur, and L1-L4 BMD measurements were obtained by dual energy x-ray absorptiometry (DXA) using the enCORE v.16 software [Lunar iDXA; GE Medical Systems, Pewaukee, WI (USA)]. BMD values are presented as g/cm^3.

Muscle Function and Lean Body Mass

30-s chair stands (30 s-cs) A 30 s-cs was used as a measurement of physical function. Participants were instructed to stand up from a seated position with their hands crossed on the chest as many times as possible within a 30 s period. The final number of stands was then registered.

400 m gait time (400 m-gt). A 400 m-gt test was performed by study participants as a measure of walking endurance. Participants were instructed to walk 400 m as fast as possible on a 20-m course marked with two colored cones without personal support or sitting down. Up to 1 min of standing was permitted if the participant felt tired or experienced discomfort, as long as the test was completed within 15 min. Time of completion was then registered.

Hand grip strength (HGS). HGS was used as an indicator of strength and of potential functional limitations. HGS was examined by a handgrip strength dynamometer (DHD-1[SH100]; SAEHAN Corporation, Changwon City, South Korea) using study participant's dominant hand. A minimum of three attempts was performed with at least 30 s rest in between sets. The highest value at the given time point was used. The test was finished when one measurement was lower than the peak value.

Knee extensor MVC. Study participant's maximal isometric thigh strength was measured as an indicator of lower extremity strength. Maximal voluntary contractions (MVC) were performed at a knee angle of 70° flexion (0° = full knee extension) using an isokinetic dynamometer (Kinetic Communicator, model 500-11, Chattanooga, TN, USA). Participants performed three attempts, and the highest attained peak torque was registered.

Lean body mass was measured by DXA. Using the enCORE v.16 software (Lunar iDXA; GE Medical Systems, Pewaukee, WI (USA).

Assessment of Covariates

Personal information such as age and sex were registered at baseline visit to the study center. Body weight in light clothing as well as height without shoes were measured by trained healthcare personal. Physical activity was measured over a 4 day period by an accelerometer-based activity monitor (activPal 3^{TM}, activPal 3c^{TM}, or activPal micro^{TM}; PAL technologies, Glasgow, UK). Calcium, alcohol and total energy intake were calculated based on previously described dietary registrations.

Narrative Literature Review

Two researchers conducted an independent narrative literature review on PubMed searching for articles related to dietary patterns, BMD, osteoporosis and muscle strength and functional outcomes. The search for additional studies related to BMD was performed on articles published between March 2015 [end of search, (13)] and December 2018. The search for studies including muscle outcomes was performed on all articles published in PubMed until December 2018. Search string used in PubMed: ("diet" OR "dietary pattern" OR "diet score" OR "food group" AND "bone" OR "BMD" OR "osteoporosis" OR "muscle" OR, "lean mass," "strength" OR "musculoskeletal" OR "sarcopenia").

The review included studies investigating pre-defined dietary patterns as well as studies investigating exploratively derived dietary patterns. Studies investigating exploratively derived dietary patterns were included only when the contribution of the specific foods was reported as factor loadings. Foods with a factor loading above or below +0.3 and −0.3, respectively, were included in the index. To account for food synergy and complex diet-disease relations (18), studies investigating single nutrients were not included in this search.

Development of the Mobility Diet Score (MDS)

To establish food groups relevant for preservation of both BMD and muscle function, a dietary index including food groups associated with BMD and a dietary index including food groups associated with muscle function were constructed, respectively. Similarities between these two indices were compared and food groups evaluated as relevant for both BMD and muscle function were suggested for inclusion into a common dietary index named the Mobility Diet Score (MDS).

Food Groups Related to BMD (Updating the BMD-Diet Score)

To establish food groups relevant for BMD the "BMD-Diet Score" constructed by de Jonge et al. (13) was updated in the present study. Similar to the original BMD-Diet Score, our updated version (BMD-DS) includes food groups commonly associated with BMD in either positive and negative directions. In the current review update, food groups investigated in relevant studies, but not included by de Jong et al. or published after March 2015 were added to the narrative review results of de Jong et al. (13). In order to establish relevant food groups, the cumulative number of times a food group was associated with BMD in the identified studies was registered. Food groups >25th percentile of this cumulative count were considered as relevant and included in the BMD-DS. A food group was included as a positive contributor to the BMD-DS if >50% of the identified studies reported a positive association with BMD outcomes, and vice versa for negatively associated food groups. Dietary intake expressed in food groups was divided into quartiles and CALM study participants were scored according to adherence. Positively associated food groups contributed positively to the BMD-DS (scoring ranging from 0 to 3 for each food group) and negatively associated food groups contributed negatively to the BMD-DS (scoring ranging from 0 to −3 for each food group).

Food Groups Related to Muscle Function

To identify food groups relevant for muscle function, a similar review approach as the one described above for BMD was performed. A literature search for food groups associated with muscle function and the construction of a Muscle and Functional Diet Score (MF-DS) including food groups relevant for muscle function was conducted. Inclusion of relevant food groups was established by investigating the frequency of which a food group was associated either positively or negatively with muscle function in published studies. Food groups were included into the MF-DS with either a positive or negative contribution if >50% of the identified studies reported an association between the respective food group and any relevant muscle outcome (muscle mass, strength, or function). Scoring was conducted in a similar way as for the BMD-DS with scores ranging from 0 to 3 and 0 to −3 for positively and negatively associated food groups, respectively. Similarly to the de Jonge review, alcohol intake was not included in either the BMD-DS or the MF-DS, but was instead considered a potential confounder (19, 20).

Food groups evaluated to be important in regards to both BMD and muscle function were suggested for inclusion into the MDS.

Statistical Analyses

Descriptive statistics are presented as medians and respective inter quartile ranges (IQR). Tests for associations between index scoring and outcomes related to BMD and muscle function were performed in the CALM study population via multiple linear regression adjusted for available confounders. Model 1 (adjusted for age, sex, and total energy intake), model 2 (adjusted for model 1 + body weight and height), and model 3 (adjusted for model 2 + physical activity level and alcohol intake). As calcium intake potentially explains (some) of the association between diet and BMD, analyses including BMD outcomes were performed with and without adjustment for calcium. Regression coefficients for the investigated associations are presented per SD as well as per quartile of intake (with Q1 as the reference) along with 95% confidence intervals (95% CI). Statistical significance was considered as $p < 0.05$. All analyses were performed in R, version 3.5.1 (21). To identify potential interactions between investigated variables, sex, age and physical activity were included as product terms in the model.

RESULTS

Characteristics and Dietary Intake in the CALM Study Population

General characteristics are presented in **Table 1**. Briefly, the median age of the study population was 70 years, median weight was ≈ 79 and 67 kg for men and women, with a median BMI of 25 and 24 kg/m^2, respectively. Median HGS was ≈ 46 and 27 kg and 400 m-gt ≈ 230 and 245 s, respectively. Median whole body BMD was ≈ 1.28 and 1.0 g/cm^2 for the two sexes and femur total BMD was ≈ 1.1 and 0.9 g/cm^2, respectively.

The median food intake (g/d/10MJ) in the study population is presented in **Table 2**. Large variations were observed for the intake of most foods, exemplified by meat and alcohol intake ranging from total abstainers (intake = 0 g) to intakes >500 and 80 g per day, for the two food groups, respectively. The food intake in this study population is resembling that of the latest national Danish dietary survey of older men and women (22), but with higher intakes of vegetables and coarse grain products.

TABLE 1 | Characteristics of the CALM study population.

Characteristics	Median (IQR)		
	Men ($n = 98$)	Women ($n = 86$)	All ($n = 184$)
Age (year)	69.0 (6.0)	70.0 (5.8)	69.0 (6.0)
Weight (kg)	79.1 (13.7)	66.7 (15.0)	73.7 (16.1)
BMI (kg/m^2)	25.0 (4.2)	24.0 (5.7)	24.7 (5.0)
Waist circumference (cm)	94.0 (13.8)	85.0 (20.0)	92.0 (16.5)
Blood pressure, systolic (mmHg)	144.0 (25.8)	141.5 (21.8)	142.5 (24.5)
Blood pressure, diastolic (mmHg)	85.0 (15.5)	82.0 (11.8)	83.0 (13.3)
400 m walk time (s)	230.0 (37.5)	245.5 (42.0)	240.0 (40.5)
30 s chair stands (stands)	21.0 (7.0)	18 (6.8)	20 (7.0)
Grip strength (kg)	45.9 (16.2)	27.4 (21.1)	36.5 (19.3)
Knee extensor MVC (Nm)	200.9 (64.6)	134.2 (76.3)	163.9 (69.7)
Whole body BMD (g/cm^2)	1.28 (0.24)	1.0 (0.29)	1.17 (0.27)
Femur neck BMD (g/cm^2)	0.95 (0.18)	0.83 (0.19)	0.90 (0.20)
Femur total BMD (g/cm^2)	1.10 (0.24)	0.88 (0.24)	0.96 (0.23)
L1–L4 BMD (g/cm^2)	1.31 (0.26)	1.13 (0.25)	1.24 (0.32)

IQR, inter quartile range (Q3–Q1); BMD, bone mineral density; MVC, maximum voluntary contraction.

Narrative Review and the Identification of Food Groups Associated With BMD and Muscle Function in Previously Published Studies

In total, seven additional papers were identified investigating associations between dietary patterns and BMD (23–29). All seven studies included dietary patterns identified via exploratively-derived methods in the form of either PCA, factor analyses or similar. A short description of the included studies and the investigated dietary patterns are presented in **Tables S1, S2**.

Our search revealed nine studies investigating associations between dietary patterns and outcomes related to muscle function. Three of these studies included dietary patterns exploratively derived via PCA or factor analysis (30–32). The remaining six studies included pre-defined dietary patterns (14, 15, 33–36). A short description of the included studies and the investigated dietary patterns are presented in **Table S3**.

Based on our narrative review, eight food groups were identified as relevant for BMD and included in our updated

TABLE 2 | Median dietary intake in the CALM study population, g/d/10MJ.

Food group	Median (IQR)		
	Men ($n = 98$)	Women ($n = 86$)	All ($n = 184$)
Coarse grains	138.5 (120.3)	136.6 (100.7)	138.5 (102.9)
Refined grains	104.1 (106.7)	60.3 (99.6)	81.3 (116.9)
Meat and meat products	116.2 (130.4)	96.0 (90.7)	104.2 (116.6)
Poultry and poultry products	22.5 (41.3)	21.0 (31.0)	21.8 (36.2)
Fish and seafood	55.9 (81.5)	55.8 (71.6)	55.8 (79.2)
Egg	19.7 (45.6)	26.5 (48.5)	21.4 (48.0)
Fruits	120.6 (208.8)	253.9 (281.9)	182.2 (233.5)
Vegetables	514.2 (479.0)	620.5 (383.8)	574.9 (454.6)
Dairy products	251.6 (301.9)	351.7 (253.0)	308.1 (305.1)
Legumes and nuts	29.2 (22.2)	23.7 (25.7)	26.6 (23.2)
Vegetable oils	4.6 (12.6)	11.3 (14.7)	7.3 (17.0)
Butter and other fats	12.8 (34.4)	8.2 (22.0)	10.4 (30.5)
Confectionery	51.6 (88.3)	60.9 (79.0)	57.0 (84.0)
Coffee and tea	563.9 (465.6)	708.6 (628.6)	618.8 (555.7)
Alcohol	16.0 (22.4)	11.1 (19.0)	13.5 (20.7)

IQR, inter quartile range (Q3–Q1).

version of the BMD-Diet score. These were "grain and cereal products," "red and processed meats," "fruits," "vegetables," "fish and seafood," "confectionery," "dairy products," and "legumes and nuts" (**Figure 1**). Based on our study criteria, red and processed meats and confectionery were the only food groups found to be negatively associated with BMD and hence, the only food groups included in our BMD-DS as negative contributors.

Six food groups were identified as relevant for muscle function and therefore included in the MF-DS. These were "grain and cereal products," "fruits," "vegetables," "fish and seafood," "dairy products," and "legumes and nuts" (**Figure 2**).

The grain and cereal food group were in the present study divided into coarse and refined grain products as primarily whole grain foods were found to be positively associated with the investigated BMD and muscle function and vice versa for refined grain products. Most of the identified studies investigated Mediterranean and Healthy Nordic diets in relation to muscle function (14, 33, 35, 36). Although meat intake is scored negatively in these dietary patterns and should hence contribute negatively to our index, we did not include meat as a negative contributor in the MF-DS as studies investigating meats specifically suggest animal protein to be an important factor in stimulating muscle protein synthesis and preserving skeletal muscle mass (37, 38). Adherence to the two dietary indices (represented by scoring) is presented for the CALM study population in **Table 3**.

Associations Between Adherence to Dietary Indices and BMD and Muscle Function in the CALM Study Population

The investigated associations between the BMD-DS and four different BMD outcomes in the CALM study population are presented in **Table 4**. No statistically significant associations were found between adherence to BMD-DS and the included BMD measures in any of our analyses. Adjusting for calcium intake did not influence any of the investigated associations (results not shown).

The investigated associations between the MF-DS and outcomes related to muscle function are presented in **Table 5**. We found an inverse association between adherence to the MF-DS and 400 m-gt both in the linear analyses (model 3 results: $\beta = -5.4$, 95 CI: -8.6, -0.7) and when comparing Q1 vs. Q3 (model 3 results: $\beta = -11.3$, 95 CI: -22.4, -0.3). Furthermore, we observed a positive association between adherence to the MF-DS and 30 s-cs when comparing Q1 vs. Q2 ($\beta = 2.1$, 95 CI: 0.4, 3.7).

Adherence to MF-DS was positively associated with lean body mass only when comparing Q1 vs. Q3 (model 3 results: $\beta = 1.6$, 95 CI: 0.3, 3.0). This was also seen for adherence to the BMD-DS (model 3 results: Q1 vs. Q2, $\beta = 1.7$, 95 CI: 0.3, 3.0 and Q1 vs. Q3, $\beta = 1.3$, 95 CI: 0.5, 2.9).

As the only difference between the BMD-DS and the MF-DS was the two food groups "red and processed meats" and "confectionery" (included as negative contributors in the BMD-DS, but not in the MF-DS), BMD-DS and MF-DS were interchanged and all linear analyses were performed again. As was the case for BMD-DS, we found no associations between MF-DS and any of the BMD outcomes in model 3 and 400 m-gt was again the only consistent outcome inversely associated with increased adherence (results not shown). No interactions were seen between dependent and independent variables for either sex, age, or physical activity.

Adjusting for total protein intake in our analyses attenuated the associations only very slightly (exemplified by the per SD association between MF-DS adherence and 400 m-gt changing only from $\beta = -4.7$, $p = 0.021$ to $\beta = -4.6$, $p = 0.027$, model 3). Thereby indicating that increased protein intake was not a major explanatory factor in the observed association between MD-DS and 400 m-gt. Adjusting for the dietary groups individually demonstrated that controlling for fruit intake attenuated the association and statistical significance between MF-DS adherence and 400 m-gt ($\beta = -3.2$, $p = 0.18$, model 3). No significant changes were seen when adjusting for other food groups.

DISCUSSION

In the present study, we updated the narrative review and the BMD-Diet Score created by de Jonge et al. (13). The additional studies published since this review resulted in only minor changes to the BMD-Diet Score.

We used the same narrative review approach to produce a similar dietary scoring index (MF-DS) containing the food groups that were most often associated with muscle function in previously published studies. As evident from **Figures 1**, **2**, the two dietary indices were similar. The only difference was that the "red and processed meats" (purposely excluded from the MF-DS) and the "confectionery" food groups were included as negative contributors in the BMD-DS, but not in the MF-DS.

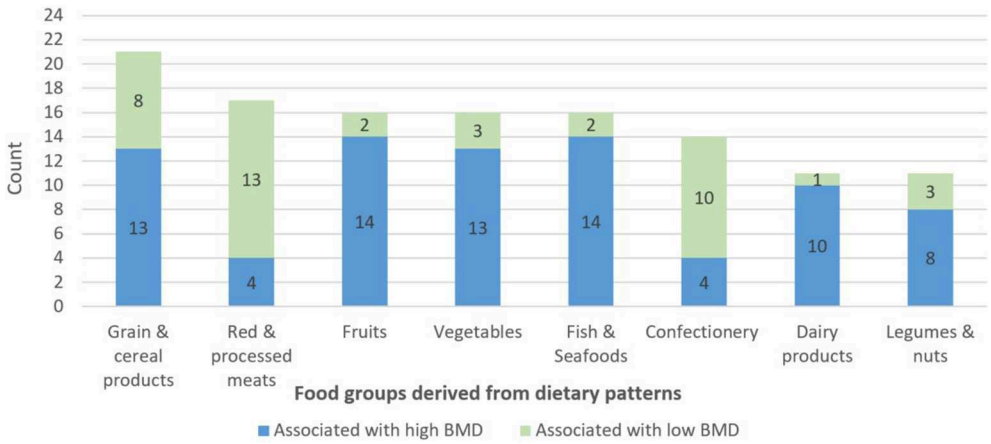

FIGURE 1 | Food groups associated with bone mineral density (BMD). Count equals the number of times a food group was associated with BMD in our narrative review (only food groups with a cumulative count >25th percentile is shown). Update of de Jonge (13) review. Green color indicates that a food group was found to be associated with low BMD in one or several studies in the literature. Blue color indicates that a food group was found to be associated with high BMD.

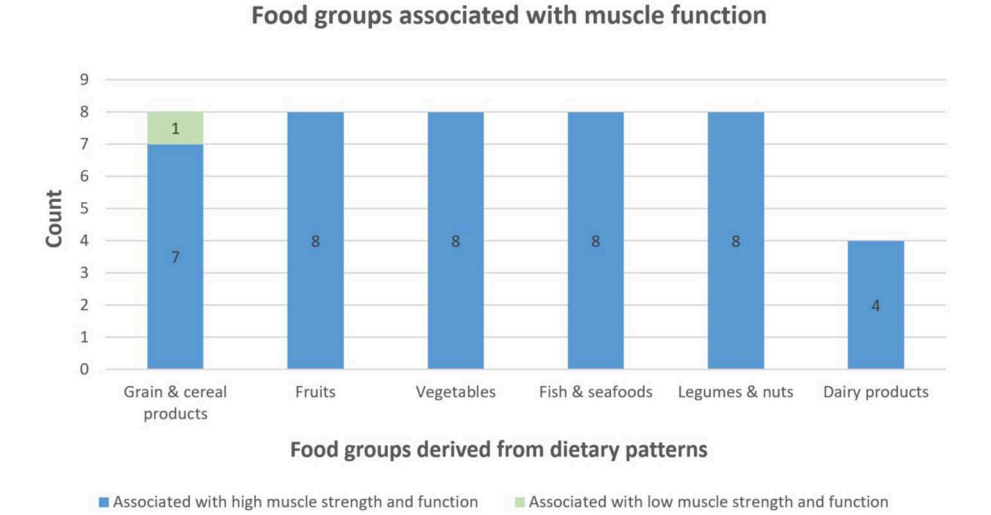

FIGURE 2 | Food groups associated with muscle function. Count equals the number of times a food group was associated with outcomes related to muscle function in our narrative review (only food groups with a cumulative count >25th percentile is shown). Green color indicates that a food group was found to be associated with low BMD in one or several studies in the literature. Blue color indicates that a food group was found to be associated with high BMD.

No differences were seen when interchanging the two indices in any our analyses. Neither adherence to BMD-DS nor MF-DS showed associations with BMD in our study population and adherence to both indices were inversely associated with 400 m-gt and positively associated with 30 s-cs (only in the Q1 vs. Q2 comparisons). No associations were observed with other muscle outcomes. Hence, contrary to expectations, neither of the indices could explain BMD variation in our study population of relatively healthy and active older Danes. Yet, they were equally good at explaining outcomes related to muscle function (mobility).

Since an association between the BMD-DS and BMD was previously found in a Dutch cohort of older adults (13), the current findings may potentially be explained by generally high BMD levels in our study population. It is possible that the intake

TABLE 3 | Scoring according to dietary indices in the CALM study population.

Dietary index	Median (IQR)		
	Men (n = 98)	Women (n = 86)	All (n = 184)
BMD-DS	5 (5)	7 (4)	6 (5)
MF-DS	8 (4)	10 (4)	9 (4)

BMD-DS, Bone Mineral Density-Diet Score; MF-DS, Muscle and Functional-Diet Score; IQR, inter quartile range (Q3–Q1).

of these "bone healthy" foods is more effective in people with lower BMD or that lack of adequate statistical power prevented us from replicating previous findings. The latter consideration may very well be true since the associations observed in the current study are similar to the ones from the de Jonge et al. study (despite the statistical differences). As an example, we observed a per SD β-value of 0.005 (95% CI:−0.014; 0.027) for the association between BMD-DS adherence and femoral neck BMD. For a similarly adjusted model, this association was $\beta = 0.009$ (95% CI: 0.005; 0.012) in the de Jonge et al. study. Alternatively, these foods are not as "bone healthy" as suggested previously. Future studies should investigate associations (or effects) of adherence to similar indices in larger sample sizes preferably with a large variation in baseline BMD measures to establish if baseline BMD status is influencing the "effectiveness" of these foods.

Our results indicate that adhering to dietary indices based on higher intakes of whole grains, dairy products, fish, legumes, nuts, fruit, and vegetables are associated with faster 400 m walking speeds and an increased number of chair stands measured over a 30-s time period. Adherence to a dietary pattern closely resembling that of many dietary recommendations could result in sustained muscle strength and function, partly due to ample amounts of micronutrients, bioactive compounds and quality protein that previously have been associated with reduced rates of age-related muscle loss (11) as well as aging of the brain and nervous system (39, 40), factors that potentially could affect functional capability, particularly in older individuals. To examine the influence of dietary protein on the observed associations between adherence to our indices and functional outcomes, we adjusted for total protein intake in our analyses. This adjustment attenuated the associations only slightly, indicating that increased protein intake was not a major determinant of the investigated outcomes. Adjusting for the dietary groups individually demonstrated that fruit intake attenuated the association and statistical significance for 400 m gait time. This could indicate that this food group could be of relevance in relation to sustaining functional capabilities of older individuals.

As BMD-DS and MF-DS were similarly associated with our outcomes, the two food groups distinguishing the two indices ("red and processed meats" and "confectionery") were evaluated as less important for these outcomes. Previous studies have suggested intake of animal protein to be beneficial in regards to skeletal muscle mass (37, 41) and potentially also prevention of osteoporosis (42, 43). Unfortunately, intake of red and processed meats are strongly associated with increased risk of adverse health outcomes such as colorectal cancers (44). However, we suspect that the intake of red and processed meats is not necessary to sustain muscle mass and function in old age as long as adequate protein intake is reached via other protein rich food sources such as e.g., poultry, dairy products, cereals, legumes, and nuts.

In the current study, adhering to a dietary index containing whole grain, dairy products, fish, legumes, nuts, fruit, and vegetables were not established as important in relation to BMD in our study group of community-dwelling older adults. In contrast, such associations were found in a similarly aged Dutch population by de Jonge et al. The influence of these food groups in relation to BMD needs to be further investigated.

The development of a MDS with established food groups beneficial for both BMD and muscle function was not possible in the current study. Nonetheless, considering the results of our narrative review, it is likely that a large overlap exists between foods that are potentially beneficial for BMD and muscle function. Future studies should investigate whether adherence to a dietary index including these food groups is associated with BMD and muscle function in other populations, or alternatively produce novel dietary indices using data-driven approaches such as reduced rank regression or similar.

The current study is strengthened by the inclusion of several measures related to BMD, muscle strength and functional capability in older individuals. The inclusion of these measures enabled a thorough investigation into potential associations between diet, BMD, and muscle function. A limitation of the current study is its cross-sectional design that entails an inherent risk of reverse causality. Also, we assessed food intake using 3 days weighed dietary records, which may not be sufficient to reach a robust insight into the habitual food intake of our study population. However, study participants not consuming, for instance, dairy or whole grain products within the registration period do likely not consume such foods on a regular basis and would therefore correctly be placed in the "low-intake group" independent of the dietary registration method. A minor change was introduced to our dietary index, compared to the de Jonge review, as intake of beans was too low for any sensible analysis. The food group "legumes and beans" was changed to "legumes and nuts" in order to reach intake levels that enabled meaningful analyses. Nuts and beans share similar properties in relation to, for instance, their content of complex carbohydrates, dietary fibers, vitamins, and minerals. It was therefore the most reasonable substitution feasible. As the CALM study is a randomized intervention trial, information on e.g., social-status, smoking habits, and other potential confounders were not registered. Confounding from these sources can therefore not be excluded. Our study includes a relatively small sample size of 184 well-functioning Danish elderly. This is relevant to consider in terms of our study's statistical power as well as the generalizability of our results. Lastly, it is important to recognize that a narrative review approach is limited by which dietary patterns have previously been investigated in relation to BMD and muscle outcomes. Thus, the current approach does not necessarily answer the question "what is the optimal diet in relation to BMD and muscle function in older adults?" The current approach does nonetheless give an insight into which food groups that are most often associated with BMD and muscle function and therefore could be part of a diet supporting a healthy aging process.

CONCLUSION

In the current study we updated a previously conducted narrative review on food groups associated with BMD and the resulting BMD-Diet Score. Our update did not lead to changes in the originally proposed BMD-Diet Score. Secondly, we conducted a

TABLE 4 | Associations between BMD-Diet Score (BMD-DS) and BMD outcomes in the CALM study population.

	Whole body BMD						**Femur neck BMD**					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Per SD	−0.005	(−0.022, 0.013)	−0.003	(−0.019, 0.013)	0.004	(−0.014, 0.023)	0.006	(−0.014, 0.027)	0.005	(−0.014, 0.024)	0.016	(−0.005, 0.038)
Q1 vs. Q2	−0.004	(−0.052, 0.042)	−0.009	(−0.052, 0.032)	−0.008	(−0.050, 0.034)	0.011	(−0.040, 0.061)	0.004	(−0.044, 0.051)	0.007	(−0.040, 0.055)
Q1 vs. Q3	0.001	(−0.050, 0.052)	0.009	(−0.037, 0.051)	0.013	(−0.034, 0.061)	0.023	(−0.034, 0.080)	0.024	(−0.029, 0.077)	0.033	(−0.022, 0.088)
Q1 vs. Q4	−0.032	(−0.092, 0.026)	−0.008	(0.062, 0.046)	0.002	(−0.057, 0.053)	0.004	(−0.063, 0.071)	0.022	(−0.041, 0.086)	0.034	(−0.031, 0.098)
P for trend	0.621		0.717		0.630		0.528		0.591		0.129	
	Femur total BMD						**L1–L4 BMD**					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Per SD	0.006	(−0.015, 0.027)	0.007	(−0.013, 0.027)	0.017	(−0.006, 0.039)	−0.024	(−0.056, 0.008)	−0.027	(−0.058, 0.004)	−0.007	(−0.042, 0.027)
Q1 vs. Q2	0.016	(−0.037, 0.069)	0.011	(−0.038, 0.062)	0.014	(−0.036, 0.064)	0.038	(−0.044, 0.119)	0.026	(−0.053, 0.104)	0.032	(−0.046, 0.110)
Q1 vs. Q3	0.011	(−0.049, 0.070)	0.015	(−0.041, 0.072)	0.022	(−0.036, 0.080)	−0.067	(−0.158, 0.025)	−0.071	(−0.159, 0.017)	−0.055	(−0.145, 0.035)
Q1 vs. Q4	0.001	(−0.070, 0.070)	0.025	(−0.041, 0.092)	0.034	(−0.034, 0.102)	−0.062	(−0.167, 0.044)	−0.044	(−0.146, 0.059)	−0.021	(−0.125, 0.083)
P for trend	0.590		0.506		0.145		0.145		0.085		0.672	

Coefficients and corresponding 95% CI for the linear modeling of the BMD Diet Score and bone mineral density in the CALM study population. Results are presented per SD and per quartile with Q1 as the reference. Model 1 is adjusted for age, sex and total energy intake. Model 2 is adjusted for model 1 + body weight and height. Model 3 is adjusted for model 2 + physical activity level and alcohol intake. Statistical significance is indicated by bold numbers, $p < 0.05$. Q, Quartiles; SD, Standard Deviation; CI, Confidence Interval; BMD, Bone Mineral Density.

TABLE 5 | Associations between Muscle and Functional Diet Score (MF-DS) and outcomes related to muscle function in the CALM study population.

	Grip strength						**Knee extensor MVC**					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Per SD	0.912	(−0.079, 1.903)	0.892	(−0.08, 1.864)	0.690	(−0.321, 1.701)	2.224	(−2.771, 7.219)	2.392	(−2.491, 7.274)	2.386	(−2.722, 7.494)
Q1 vs. Q2	0.231	(−2.317, 2.778)	0.021	(−2.474, 2.515)	−0.173	(−2.670, 2.324)	11.869	(−0.860, 24.598)	10.925	(−1.547, 23.396)	10.761	(−1.825, 23.347)
Q1 vs. Q3	1.098	(−1.702, 3.899)	1.239	(−1.509, 3.987)	0.661	(−2.175, 3.497)	−1.360	(−15.249, 12.529)	0.031	(−13.597, 13.658)	−0.564	(−14.753, 13.626)
Q1 vs. Q4	−0.705	(−3.950, 2.539)	−0.247	(−3.481, 2.987)	−0.796	(−4.080, 2.489)	−2.677	(−18.765, 13.410)	1.352	(−14.685, 17.388)	1.105	(−15.329, 17.540)
P for trend	0.071		0.072		0.180		0.381		0.335		0.358	
	400 m gait time						**30 s chair stands**					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Per SD	−6.433	**(−10.740, −2.126)**	−5.421	**(−9.308, −1.535)**	−4.682	**(−8.642, −0.722)**	0.4	(−0.316, 1.115)	0.393	(−0.25, 1.035)	0.272	(−0.396, 0.941)
Q1 vs. Q2	−10.762	(−21.642, 0.118)	−10.112	**(−19.973, −0.252)**	−9.355	(−19.062, 0.353)	1.916	**(0.108, 3.723)**	2.170	**(0.559, 3.781)**	2.060	**(0.444, 3.676)**
Q1 vs. Q3	−17.244	**(−29.208, −5.280)**	−14.427	**(−25.289, −3.565)**	−11.34	**(−22.366, −0.313)**	0.427	(−1.560, 2.414)	0.120	(−1.655, 1.894)	−0.208	(−2.044, 1.627)
Q1 vs. Q4	−17.752	**(−31.610, −2.894)**	−10.253	(−23.035, 2.530)	−10.166	(−22.937, 2.604)	1.581	(−0.721, 3.883)	0.650	(−1.439, 2.738)	0.339	(−1.787, 2.464)
P for trend	**0.004**		**0.007**		**0.021**		0.272		0.229		0.423	

Coefficients and corresponding 95% CI for the linear modeling of the Muscle Diet Score and muscle strength and functional outcomes in the CALM study population. Results are presented per SD and per quartile with Q1 as the reference. Model 1 is adjusted for age, sex and total energy intake. Model 2 is adjusted for model 1 + body weight and height. Model 3 is adjusted for model 2 + physical activity level and alcohol intake. Statistical significance is indicated by bold numbers, $p < 0.05$. Q, Quartiles; SD, Standard Deviation; CI, Confidence Interval; MVC, Maximum Voluntary Contraction.

narrative review of food groups associated with muscle strength and functional outcomes and constructed a so-called Muscle and Functional-Diet Score. The food groups included were highly similar for the two dietary scores. Lastly, we investigated associations between adherence to the two dietary scores and outcomes related to BMD and muscle strength, mass, and function in a group of older Danes. Our results showed that adhering to dietary scores based on high intakes of whole grains, dairy products, legumes, nuts, fish, fruit, and vegetables is associated with faster walking speeds and an increased number of chair stands measured over a 30 s time period. We were unable to reproduce earlier findings, which demonstrated that adherence to the BMD-Diet Score was associated with changes in BMD. Future studies should investigate whether adherence to such dietary patterns are associated with BMD and muscle outcomes in other populations of older individuals.

DATA AVAILABILITY

The datasets used and analyzed during the present study are available from the corresponding author on request.

ETHICS STATEMENT

The study was approved by the Danish Regional Ethical Committees of the Capital Region (J-nr. H-4-2013-070) and all participants gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

SS, ML, and IT conceived and designed the study, drafted the manuscript, and read and approved the final version. KM, JB, RB, GH, AS, MH, CS, SA, MJ, SR, and LH drafted the manuscript and read 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/fnut.2019.00114/full#supplementary-material

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The Elderly-Nutrient Rich Food Score Is Associated With Biochemical Markers of Nutritional Status in European Older Adults

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Background: In order to prevent age-related degenerative diseases in the aging population, their diets should be nutrient dense. For this purpose, the Elderly-Nutrient rich food (E-NRF7.3) score has been developed to assess nutrient density of diets by capturing dietary reference values for older adults. To demonstrate its practical importance such score should be validated against markers of nutritional status and health.

Objective: The objective of this study was to examine the association between the E-NRF7.3 score and markers of nutritional status and inflammation.

Design: This study was carried out in a sample of the NU-AGE study including 242 Dutch and 210 Polish men and women, aged 65–79 years. Dietary intake was assessed by means of 7-day food records and structured questionnaires collected data on supplement use, lifestyle, and socio-economic information. Baseline measurements included anthropometrics, physical and cognitive function tests, and a fasting venipuncture. E-NRF7.3 scores were calculated to estimate nutrient density of foods and the diet. Associations between the E-NRF7.3 scores and micronutrient status of vitamin D, folate, vitamin B12, homocysteine, and c-reactive protein (CRP) were examined using linear regression analysis while adjusting for confounders.

Results: Each one unit increase in E-NRF7.3 score was associated with a 2.2% increase in serum folate in Dutch and 1.6% increase in Polish participants in the fully adjusted models (both $p < 0.01$). Each one unit increase in E-NRF7.3 was significantly associated with a 1.5% decrease in homocysteine levels in Dutch participants ($p < 0.01$), whereas, a 0.9% increase in vitamin B12 levels was observed in Polish participants only ($p < 0.01$). Higher E-NRF7.3 scores were not associated with vitamin D or CRP levels. Adjustment for potential confounders did not substantially alter these results.

Discussion: The E-NRF7.3 was developed to reflect dietary intake of relevant nutrients for older adults. Its association with markers of nutritional status could be confirmed for

folate (both populations), vitamin B12 (Poland only), and homocysteine (the Netherlands only). There was no association with vitamin D and CRP. To further demonstrate its validity and practical implication, future studies should include a wider range of nutritional status makers, health outcomes, and inflammation markers.

Keywords: nutrient density, diet quality, micronutrients, status markers, elderly, Europe, health, NU-AGE

INTRODUCTION

The increasing number of older adults and accompanying age-related degenerative diseases necessitate preventive strategies to lower the disease burden. A healthful diet and adequate nutrient intake could be important strategies to prevent degenerative diseases. However, it is known that there is a high prevalence of inadequate intake of beneficial nutrients on the one hand, with high intakes of nutrients with detrimental effects on health on the other hand (1–4). With decreasing energy needs and increasing nutrient needs for some nutrients, diets of elderly should be nutrient dense (5, 6). Nutrient dense diets can be achieved by means of selecting nutrient-dense foods and beverages to meet nutrient goals without exceeding daily energy needs (7).

One frequently studied tool to assess nutrient density of foods and diets is the Nutrient Rich Food (NRF9.3) index, as proposed by Drewnowski and Fulgoni (7). This index has previously been shown to be related to the risk of stroke (8). Recent research, however, has shown that this NRF9.3 might be of limited use specifically studying diets of European older adults, as it lacks relevant nutrients such as vitamin D and folate and uses dietary reference values not targeted to the European older aged population (9, 10).

Therefore, a new nutrient rich food score was developed with the aim to assess nutrient density of diets in European older adults by including dietary reference values that are relevant for the older aged population. The newly developed nutrient rich food score was composed of nutrients that: (1) have been shown to be of inadequate intake in the older aged population (>20%) (11), (2) were defined as nutrients of public health relevance for older adults, and (3) were associated with relevant health outcomes (12). The nutrient rich food score that best predicted adherence to the NU-AGE index, an index assessing adherence to a healthful diet for the aging population (13, 14), was called the E-NRF7.3 score and included protein, dietary fiber, vitamin D, folate, calcium, potassium, magnesium as nutrients to encourage, and saturated fat, total mono- and disaccharides, and sodium as nutrients to limit (15).

While developing the E-NRF7.3 score, previously proposed science-driven rules were followed, namely: (1) the selection of relevant index nutrients and reference amounts, (2) the development of an appropriate algorithm for calculating nutrient density, and (3) the validation of the chosen nutrient profile model against healthy diets (7). However, the E-NRF7.3 score has not been validated yet against markers of nutritional status and health. To demonstrate its practical application, the validity of this score should be studied. Therefore, we aim to assess the validity of the newly developed E-NRF7.3 against markers of nutritional status and inflammation, for the older aged population, in both Northern and Eastern European older adults.

MATERIALS AND METHODS

Study Population

The present study was carried out as part of the NU-AGE project, a dietary intervention study among 1,294 people living in the Netherlands, Poland, Italy, France, and the UK. The NU-AGE study is a 1-year, randomized, parallel trial designed to combat inflammageing by means of a personally tailored Mediterranean-like dietary pattern, targeting dietary recommendations for European people over 65 years of age (NU-AGE diet). The rationale and design of the study have been described previously (13, 16). In short, at baseline and following 1 year intervention, participants completed 7-day food records and structured questionnaires on medical history, current health, and lifestyle factors. Additionally, participants visited the research center for anthropometric measurements, physical performance, and cognitive function tests, and underwent a fasting venipuncture. All participants gave their written informed consent prior to their inclusion in the study. Ethical approval was provided by the Wageningen University Medical Ethics Committee (the Netherlands) and the Bioethics Committee of the Polish National Food and Nutrition Institute (Poland). The trial is registered at clinicaltrials.gov (NCT01754012).

For the present study, we used baseline data of the Dutch and Polish cohort for whom detailed dietary intake data was available consisting of 252 and 259 apparently healthy men and women aged 65–79 years, respectively, who were enrolled between April 2012 and January 2014. Participants who had not completed the 7-day food record ($n = 23$), with an unlikely energy intake of <500 or >3,500 kcal ($n = 7$) and those with missing data on any of the covariates ($n = 40$) were excluded. A total of 242 Dutch and 210 Polish participants were included in the analysis on biochemical markers of nutritional status and inflammation.

Dietary Intake Assessment

Average food and nutrient intake was assessed by means of 7-day structured and pre-formatted food records including eight meal occasions (before breakfast, breakfast, morning snacks, lunch, afternoon snacks, evening meal, evening snacks, night snacks) referring to the current day. Participants had a face-to-face training to complete the food records and received written instructions about the level of detail required to describe foods and amounts consumed, including the name of food, preparation methods, recipes for mixed foods, and portion sizes. Portion sizes were reported in household measures, based on pictures or measured in gram or milliliters. During a 1-h interview at

the participants home (Netherlands) or at the research center (Poland), a trained dietician/research nutritionist reviewed the food record and frequently used household measures were checked to ensure an adequate level of detail in describing foods and food preparation methods. Consumed foods were coded according to standardized coding procedures and translated into nutrients by use of local food composition tables [Nederlands voedingsstoffenbestand, NEVO 2011 (17), in the Netherlands and National Food and Nutrition Institute (18) in Poland].

Calculation of the Elderly-Nutrient Rich Food (E-NRF) Score

The E-NRF7.3 score is based upon a selection of nutrients relevant for older adults (**Table 1**). Nutrients to encourage (NR7) include protein, dietary fiber, folate, vitamin D, calcium, magnesium, and potassium. Nutrients to limit (LIM3) comprise saturated fat, sodium, and total mono- and disaccharides. The development of the E-NRF7.3 score has been described in detail elsewhere (15).

The calculation of the E-NRF7.3 score comprised several steps similar to calculating the NRF9.3 (10, 17). First, the NR7 and LIM3 scores were calculated for each food item per 100 kcal. Subsequently, these food scores were converted into individual scores by multiplying the scores by the individual 7-day average amount of energy consumed of each item, in 100-kcal units, and then summing these scores for each subject. Next, the individual LIM3 scores were subtracted from the NR7 scores, resulting in the unweighted E-NRF7.3 score. Lastly, the E-NRF7.3 scores were divided by the number of 100-kcal units of the subjects' average total daily energy intake to provide a "weighted average" individual E-NRF7.3 score.

TABLE 1 | Dietary reference values for selected nutrients used in calculating the E-NRF7.3.

Nutrient	RDV	References
Nutrient-rich components (NR7)		
Protein, g[a,b]	112.5 (m), 90 (w)	NNR (19)
Fiber, g	35 (m), 25 (w)	NNR (19)
Calcium, mg	1,200	HCNL (20)
Magnesium, mg	350 (m), 300 (w)	EFSA (21)
Potassium, mg	3,500	EFSA (22)
Vitamin D, μg	20	HCNL/NNR (19, 20)
Folate, μg DFE	330	EFSA (23)
Nutrients to limit (LIM3)		
Saturated fat, g	20	EFSA (24)
Sugar, g	90	EFSA (24)
Sodium, mg[c]	2,400	EFSA (24)

Population Reference Intakes and Adequate Intakes as set by the European Food Safety Authority (EFSA) (21–23, 25–35), the Nordic Council of Ministers (NNR) (19), the Health Council of the Netherlands (HCNL) (20) as well as the labeling Reference Intake values as set by the EFSA (24) were used as Reference Daily Values (RDV). m, men; w, women.

[a] *Values equal to 18% EN.*

[b] *Based on EFSA reference intakes of 2,500 and 2,000 kcal reference intakes for men and women, respectively.*

[c] *Value derived from salt reference value using a conversion factor of 2.5.*

The algorithms used to calculate the E-NRF7.3 score are listed in **Table 2** and are based on sums of nutrients where all nutrients were equally weighted (10). The algorithms which combined positive nutrients and nutrients to limit were based on subtracting the negative from the positive sub score (10). Moreover, the scores were calculated per 100 kcal, since this led to the highest percentage of variance accounted for in previous validation studies (36). Higher E-NRF7.3 scores indicate higher nutrient density on a 100 kcal basis.

Biochemical Analysis

Fasting blood samples were obtained by venipuncture in the morning at each of the research centers. Blood samples were stored in a cool storage box with a temperature around 7°C and processed within 3 h after collection.

Concentrations of serum vitamin B12 and folate (chemiluminescence) and plasma homocysteine (enzymatic assay) were measured at the laboratory for biochemical analysis of the Nigrisoli hospital in Bologna, Italy, as described previously (37).

Concentrations of total 25-hydroxyvitamin D (25(OH)D) in all serum samples were measured at the laboratory of the Cork Center for Vitamin D and Nutrition Research, Ireland. 25(OH)D was measured by a modified version of the LCMS/MS method that has been described in detail elsewhere (38).

High sensitivity C-reactive protein (CRP) was quantified via ProcartaPlex^{TM} Immunoassay (Thermo Fisher Scientific) according to the manufacturer's instructions, and with an assay sensitivity of 4.39 pg/mL. Analysis was performed using Luminex 200 instrumentation (Luminex Corporation) in all samples (39) at the gut health Institute of the Quadram Institute Bioscience in Norwich, UK.

Covariates

A standardized general questionnaire was uses to obtain information on smoking status (never, former, current), educational level (years), and medical history (prevalence of diabetes mellitus type II, hypertension, hypercholesterolemia, neurological diseases, osteoporosis, all: yes/no). Physical activity was assessed using the Physical Activity Scale for the

TABLE 2 | Algorithms used to calculate the E-NRF index scores.

Model	Algorithm	Comment
$NR7_{100kcal}$	$\sum_i = 1–7$ $(Nutrient_i/RDV_i)$ * 100	$Nutrient_i$ = content of nutrient i in 100-kcal edible portion; RDV_i = recommended daily values for nutrient i
$LIM3_{100kcal}$	$\sum_i = 1–3$ $(Nutrient_i/MDV_i)$ * 100	$Nutrient_i$ = content of limiting nutrient i in 100-kcal edible portion; MDV_i = maximum daily values for nutrient i
$E\text{-}NRF7.3_{100kcal}$	NR7–LIM3	Difference between sums

NR7, nutrient-rich score consisting of 7 beneficial nutrients: protein, dietary fiber, folate, vitamin D, calcium, magnesium, and potassium; LIM3, limited nutrient score consisting of three nutrients to limit: saturated fat, sodium, total mono-disaccharides; E-NRF, elderly nutrient-rich foods score.

Elderly (PASE) questionnaire (40) and expressed as PASE score. Frailty status (zero being non-frail and one being pre-frail) was assessed with a test described by Fried et al. (41). This test combines measures of unintentional weight loss, handgrip strength, gait speed, self-reported exhaustion, and physical activity. Alcohol intake was assessed by means of food records [virtually no alcohol intake (<0.1 gram of alcohol/day), 0–1 standard glass of alcohol per day (0.1–10 g of alcohol/day) and >1 standard glass of per day (>10 g of alcohol)]. Height was measured with a stadiometer to the nearest 0.1 cm. Weight was measured to the nearest 0.1 kg with a calibrated scale while wearing light clothes. Body mass index (BMI) was calculated as body weight divided by squared body height (kg/m^2). All measures were taken by a trained research assistant.

Statistical Analyses

Participants were divided into tertiles on the basis of individual weighted E-NRF7.3 scores. Baseline characteristics were compared between tertiles of the E-NRF7.3 score using analysis of variance for continuous variables. For categorical variables the X^2 statistic was used, unless expected cell counts were <5 for more than 20% of cells, then Fisher's exact test was used.

Linear regression analyses was used to examine the association between the individual weighted E-NRF7.3 scores and markers of nutritional status and inflammation, while adjusting for age, gender (model 1), education, BMI, smoking status, physical activity, energy intake, and alcohol intake (model 2). Linear regression analyses were both performed with individual weighted E-NRF7.3 score as continuous predictor and as categorical predictor using tertiles. Nutritional status and inflammation marker data were transformed using the natural logarithm when residuals were otherwise skewed and thus violating model assumptions. For analyses using transformed markers, back transformed marginal means and coefficients (e^{β}) are shown for ease of interpretation.

Statistical analyses were carried out using SPSS version 23.0. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

General characteristics of the Dutch and Polish populations are presented in tertiles of individual weighted E-NRF7.3 score in **Table 3**. The mean E-NRF7.3 score was 10.0 ± 5.3 for Dutch and 11.4 ± 7.4 for Polish participants. The Dutch and Polish participants were on average 71.0 ± 4.0 and 71.5 ± 3.8 years old, had a BMI of 25.9 ± 3.6 and 28.1 ± 4.2, completed 12.4 ± 3.6 and 15.2 ± 2.8 years of education and the vast majority did not smoke (96.7 and 93.3%). These characteristics did not differ significantly across tertiles of the E-NRF7.3 score within either country, except for smoking in Dutch participants ($p = 0.03$).

In both countries, participants with higher E-NRF7.3 scores were most likely to be woman (75% in the Netherlands and 81% in Poland, both $p < 0.01$), had lower energy intake ($p < 0.01$), fat intake ($p < 0.01$), and higher protein intake ($p < 0.01$), compared to participants with lower E-NRF7.3 scores. Polish participants with the highest E-NRF7.3 score had a significantly higher carbohydrate intake ($p < 0.01$) and a lower level of physical activity ($p = 0.01$) compared to those with lower E-NRF7.3 scores.

In both populations, folate levels were significantly higher in the group with highest E-NRF7.3 scores [geometric mean (95% CI): 11.1 (9.6–13.0) vs. 7.9 (6.9–9.1), $p < 0.01$ in the Netherlands and 10.4 (9.1–11.8) vs. 7.8 (6.9–8.8), $p < 0.01$ in Poland, after full adjustment, **Table 4**]. Continuously, a one unit increase in E-NRF7.3 score was associated with a predicted 2.2% increase in folate levels in Dutch participants ($e^{\beta} = 1.022$, $p < 0.01$) and 1.6% in Polish participants ($e^{\beta} = 1.016$, $p < 0.01$).

Each 1 unit increase in E-NRF7.3 score was associated with a 0.9% increase in vitamin B12 levels in Polish participants ($e^{\beta} = 1.011$, $p < 0.01$ in the crude model and $e^{\beta} = 1.009$, $p < 0.01$ in the fully adjusted model). In Dutch participants, vitamin B12 levels were not significantly higher with increasing E-NRF7.3 score.

With higher E-NRF7.3 scores homocysteine levels significantly decreased in both populations [geometric mean (95%CI) 9.7 (9.2–10.3) vs. 11.8 (11.2–12.5), $p < 0.01$ in the Netherlands and 10.8 (9.9–11.8) vs. 12.4 (11.4–13.6), $p = 0.07$ in Poland], with a 0.08% decrease in Poland ($e^{\beta} = 0.992$, $p = 0.02$) and 1.6% decrease in the Netherlands ($e^{\beta} = 0.984$, $p < 0.01$) for each unit increase in E-NRF7.3 score in the crude model. When adjusting for potential confounders, the association remained significant in the Dutch population only [geometric mean 10.3 (9.5–11.2) vs. 12.3 (11.4–13.3), $p < 0.01$ and $e^{\beta} = 0.985$, $p < 0.01$).

For vitamin D, a borderline significant positive association was observed in the Polish population across tertiles of E-NRF7.3 score, in all adjustment models [mean value (95%CI) 58.0 (51.7−64.3) in the highest tertile vs. 52.6 (46.8−58.5) in the lowest tertile, $p = 0.06$], but not per one unit increase in E-NRF7.3 score ($\beta = 0.177$, $p = 0.42$ in fully adjusted model).

CRP levels did not differ across tertiles of E-NRF7.3 scores in either the Dutch or Polish population (all $p > 0.10$). Continuously, there was also no association between E-NRF7.3 score and CRP level ($e^{\beta} = 0.984$, $p = 0.26$ in the Netherlands and $e^{\beta} = 0.989$, $p = 0.33$ in Poland after full adjustment).

DISCUSSION

The E-NRF7.3 score was developed with the aim to capture nutrient density of foods and diets of older adults by including nutrients that are of relevance for this population. Although the E-NRF7.3 score was shown to be nicely correlated with greater adherence to a healthful diet for the aging population within a Dutch population, it has not been evaluated in relation to markers of nutritional status and inflammation in other populations. In this cross-sectional study, higher E-NRF7.3 scores were significantly associated with higher folate blood levels in both populations, higher vitamin B12 levels in the Polish population, and with lower homocysteine levels in the Dutch population. These results remained after adjustment for energy intake and various lifestyle and personal factors.

Folate and vitamin B12, as well as other B vitamins are essential for the methylation of homocysteine to methionine (42) and are therefore key players in life maintenance via

TABLE 3 | General characteristics of 242 Dutch and 210 Polish NU-AGE participants across tertiles of the Elderly Nutrient-Rich Food (E-NRF7.3) score.

Variable	Netherlands					Poland				
	Total	T1	T2	T3	*p*	Total	T1	T2	T3	*p*
E-NRF7.3 mean	10.0 ± 5.3	4.5 ± 2.6	9.9 ± 1.2	15.8 ± 3.3		11.4 ± 7.4	3.8 ± 3.0	10.7 ± 2.1	19.8 ± 4.5	
Range	(−3.6–27.2)	(−3.6–7.9)	(7.9–11.9)	(11.9–27.2)		(−6.1–30.5)	(−6.1–7.2)	(7.4–14.1)	(14.2–30.5)	
n	242	81	81	80		210	70	70	70	
Age, years	71.0 ± 4.0	71.0 ± 4.1	70.9 ± 3.8	71.2 ± 4.3	0.85	71.5 ± 3.8	70.9 ± 3.9	71.7 ± 3.9	71.9 ± 3.6	0.26
Women	135 (55.8)	26 (32.1)	49 (60.5)	60 (75.0)	**<0.01**	127 (60.5)	27 (38.6)	43 (61.4)	57 (81.4)	**<0.01**
BMI, kg/m^2	25.9 ± 3.6	26.0 ± 3.3	26.0 (4.0)	25.6 ± 3.3	0.74	28.1 ± 4.2	28.3 ± 3.8	27.2 ± 4.1	28.8 ± 4.4	0.07
Smoking status										
Never	121 (50.0)	30 (37.0)	43 (53.1)	48 (60.0)	**0.03**	104 (49.5)	31 (44.3)	37 (52.9)	36 (51.4)	0.30
Former	113 (46.7)	46 (56.8)	36 (44.4)	31 (38.8)		92 (43.8)	32 (45.7)	30 (42.9)	30 (42.9)	
Current	8 (3.3)	5 (6.2)	2 (2.5)	1 (1.3)		14 (6.7)	7 (10.0)	3 (4.3)	4 (5.7)	
Education, years	12.4 ± 3.6	12.6 ± 3.8	12.4 ± 3.5	12.1 ± 3.6	0.75	15.2 ± 2.8	15.6 ± 2.8	15.2 ± 2.9	14.6 ± 2.7	0.12
Physical activity, PASE score	137.5 ± 53.1	137.9 ± 54.2	136.3 ± 53.8	138.3 ± 52.1	0.97	125.8 ± 55.8	136.1 ± 62.8	131.6 ± 51.6	109.8 ± 49.5	**0.01**
Pre-frail[a]	52 (21.5)	20 (24.7)	14 (17.3)	18 (22.5)	0.50	66 (31.6)	19 (27.1)	21 (30.4)	26 (37.1)	0.43
Diabetes mellitus II	9 (3.7)	3 (3.7)	3 (3.7)	3 (3.7)	1.00	17 (8.1)	7 (10.0)	5 (7.1)	5 (7.1)	0.65
Hypertension	79 (32.6)	28 (34.6)	28 (34.6)	23 (28.8)	0.66	129 (61.4)	45 (64.3)	44 (62.9)	40 (57.1)	0.66
Hypercholesterolemia	61 (25.2)	23 (28.4)	18 (22.2)	20 (25)	0.66	76 (36.2)	20 (28.6)	26 (37.1)	30 (42.9)	0.21
Neurological disease	3 (1.2)	2 (2.5)	0 (0)	1 (1.3)	0.55	3 (1.4)	2 (2.9)	1 (1.4)	0 (0)	0.78
Osteoporosis	25 (10.3)	7 (8.6)	7 (8.6)	11 (13.8)	0.47	42 (20.0)	11 (15.7)	13 (18.6)	18 (25.7)	0.31
Dietary intake										
Energy intake, kcal	1,900 ± 383	1,993 ± 384	1,950 ± 382	1,757 ± 342	**<0.01**	1,844 ± 537	2,049 ± 548	1,813 ± 507	1,669 ± 492	**<0.01**
Carbohydrates, EN%	42.1 ± 6.0	41.7 ± 5.9	42.2 ± 5.9	42.3 ± 6.4	0.79	51.9 ± 7.2	49.6 ± 7.9	52.1 ± 8.6	53.9 ± 6.1	**<0.01**
Fat, EN%	34.4 ± 5.1	36.3 ± 4.4	33.9 ± 4.8	32.8 ± 5.4	**<0.01**	34.1 ± 5.8	36.6 ± 6.3	33.8 ± 5.1	31.9 ± 5.0	**<0.01**
Protein, EN%	16.1 ± 2.4	14.9 ± 1.9	15.8 ± 2.0	17.7 ± 2.3	**<0.01**	17.4 ± 3.0	15.9 ± 2.7	17.0 ± 2.5	19.3 ± 2.8	**<0.01**
Alcohol										
<0.1 g/day	35 (14.5)	12 (14.8)	11 (13.6)	12 (15.0)	0.07	92 (43.8)	26 (37.1)	25 (35.7)	41 (58.6)	0.05
0.1–10 g/day	86 (35.5)	24 (29.6)	24 (29.6)	38 (47.5)		94 (44.8)	34 (48.6)	36 (51.4)	24 (34.3)	
>10 g/day	121 (50.0)	45 (55.6)	46 56.8)	30 (37.5)		24 (11.4)	10 (14.3)	9 (12.9)	5 (7.1)	

Values are expressed as mean ± SD or number (percentage within tertile). Bold values are statistically significant. BMI, body mass index; EN%, energy percent; PASE, physical activity scale for the elderly.

[a] *Poland: n = 209.*

methylation processes and DNA precursors (43). Therefore, high homocysteine levels are the result of low folate and vitamin B12 levels. In turn, high homocysteine levels are associated with increased risk of cardiovascular disease, dementia, stroke, and depression (37, 44–46).

Considering the inclusion of folate equivalents in the E-NRF7.3 score a positive association with serum folate levels can be expected. This is in line with previous analyses of nutrient intakes and blood biomarkers in all five NU-AGE intervention countries by Ostan et al., reporting a significant correlation between folate intake and serum concentrations ($\rho = 0.363$, $p < 0.01$) (37). A study in Italian and British adults reported similar results, where a 100 μg/d increase in dietary folate intake was associated with a 13.8 and 10.5% increase in serum folate levels, respectively (47).

Interestingly, the E-NRF7.3 score showed a significant positive association with serum vitamin B12 levels in Polish participants whereas the index did not include dietary vitamin B12. Although Ostan et al. observed that vitamin B12 intake significantly correlated with serum concentrations ($\rho = 0.151$, $p < 0.01$) (37), Jungert et al. found that vitamin B12 intake was not a predictor of serum vitamin B12 status. In their study, serum folate was the main predictor of serum vitamin B12 in healthy community-dwelling older adults ($\beta = 0.407$, $p < 0.01$) (48), possibly explaining the association found with the E-NRF7.3 score.

While developing the E-NRF7.3, the inclusion of vitamin B12 was considered as it is an important nutrient for older adults and it is related to relevant health outcomes. However, including vitamin B12 to the E-NRF7.3 reduced the validity instead of improving it. Therefore, vitamin B12 was omitted from the E-NRF7.3 (15). This approach is in line with the extensively studied NRF9.3, for which a threshold for the useful number of nutrients exists, after which the ranking of products or prediction of healthy diet index declined (49, 50). We did include serum B12 in the present study as it was hypothesized that a nutrient dense diet based on nutrients included in the E-NRF7.3 is likely to be nutrient dense for other relevant nutrients that are not included in the E-NRF7.3. The Polish data seem to support this hypothesis, however further studies would be useful.

The positive association of the E-NRF7.3 score with vitamin B12 level and the inverse association with homocysteine level were only significant in the Polish and Dutch participants,

TABLE 4 | Association between the Elderly Nutrient-Rich Food (E-NRF7.3) score and markers of nutritional status and inflammation in Dutch and Polish NU-AGE participants.

	Netherlands						Poland					
	T1	T2	T3		Continuous		T1	T2	T3		Continuous	
	mean (95% CI)	mean (95% CI)	mean (95% CI)	*p*	β	*p*	mean (95% CI)	mean (95% CI)	mean (95% CI)	*p*	β	*p*
Folate[a]	*n* = 81	*n* = 81	*n* = 80				*n* = 70	*n* = 68	*n* = 70			
Crude	8.7 (7.8–9.5)	9.9 (9.0–11.0)	12.9 (11.7–14.3)	**<0.01**	1.029	**<0.01**	7.5 (6.8–8.2)	10.1 (9.2–11.2)	10.9 (9.9–12.1)	**<0.01**	1.021	**<0.01**
Model 1	8.9 (8.0–9.8)	9.8 (8.9–10.8)	12.5 (11.3–13.8)	**<0.01**	1.024	**<0.01**	7.7 (7.0–8.5)	9.8 (8.9–10.8)	10.1 (9.1–11.2)	**<0.01**	1.015	**<0.01**
Model 2	7.9 (6.9–9.1)	8.8 (7.6–10.2)	11.1 (9.6 – 13.0)	**<0.01**	1.022	**<0.01**	7.8 (6.9–8.8)	9.9 (8.7–11.2)	10.4 (9.1–11.8)	**<0.01**	1.016	**<0.01**
Vitamin B12[a]	*n* = 81	*n* = 81	*n* = 80				*n* = 70	*n* = 68	*n* = 69			
Crude	368.9 (343.6–396.1)	377.4 (351.5–405.2)	407.1 (379.0–437.3)	0.13	1.008	0.06	315.2 (293.3–338.6)	363.3 (337.8–390.8)	373.4 (347.3–401.4)	**<0.01**	1.011	**<0.01**
Model 1	373.5 (347.2–401.5)	375.0 (349.1–402.8)	400.2 (371.3–431.2)	0.35	1.005	0.24	318.3 (295.9–342.2)	360.0 (334.3–387.5)	364.7 (337.2–394.4)	**<0.01**	1.009	**<0.01**
Model 2	376.5 (340.7–416.3)	378.8 (340.0–421.7)	397.4 (355.0–445.1)	0.56	1.003	0.50	315.5 (289.1–343.9)	349.0 (318.5–382.2)	359.6 (327.2–395.2)	**<0.01**	1.009	**<0.01**
Homocysteine[a]	*n* = 81	*n* = 81	*n* = 80				*n* = 70	*n* = 68	*n* = 70			
Crude	11.8 (11.2–12.5)	10.7 (10.16–11.3)	9.7 (9.2–10.3)	**<0.01**	0.984	**<0.01**	12.4 (11.4–13.6)	11.2 (10.3–12.3)	10.8 (9.9–11.8)	0.07	0.992	**0.02**
model 1	11.7 (11.1–12.4)	10.8 (10.2–11.4)	9.9 (9.3–10.4)	**<0.01**	0.986	**<0.01**	12.2 (11.2–13.4)	11.5 (10.5–12.5)	11.4 (10.4–12.5)	0.45	0.995	0.20
model 2	12.3 (11.4–13.3)	11.4 (10.6–12.4)	10.3 (9.5–11.2)	**<0.01**	0.985	**<0.01**	12.5 (11.3–13.9)	11.9 (10.7–13.3)	11.5 (10.3–12.9)	0.43	0.994	0.12
Vitamin D	*n* = 81	*n* = 81	*n* = 80				*n* = 70	*n* = 69	*n* = 70			
Crude	60.9 (56.9 – 65.0)	61.8 (57.8–65.9)	65.3 (61.3–69.4)	0.28	0.381	0.09	52.7 (47.9–57.4)	60.9 (56.2–65.7)	56.6 (51.9–61.4)	0.06	0.059	0.76
Model 1	61.4 (57.2–65.5)	61.5 (57.5–65.6)	64.8 (60.6–69.1)	0.44	0.314	0.20	52.7 (47.8–57.5)	61.0 (56.1–65.8)	56.7 (51.6–61.8)	0.06	0.033	0.88
Model 2	54.9 (49.3–60.4)	54.6 (48.6–60.5)	58.3 (52.1–64.6)	0.39	0.268	0.28	52.6 (46.8–58.5)	61.0 (54.9–67.1)	58.0 (51.7–64.3)	0.06	0.177	0.42
CRP[a]	*n* = 78	*n* = 79	*n* = 78				*n* = 69	*n* = 63	*n* = 66			
Crude	1,203,357 (952,375–1,520,482)	1,018,196 (807,030–1,284,615)	920,129 (728,219–1,162,614)	0.27	0.979	0.10	801,207 (618,998–1,037,051)	868,473 (662,959–1,137,696)	850,418 (653,219–1,107,149)	0.75	0.999	0.93
Model 1	1,184,700 (932,499–1,505,708)	1,026,843 (811,636–1,299,410)	942,226 (737,113–1,203,436)	0.43	0.982	0.19	814,231 (626,246–1,058,166)	852,561 (647,905–1,122,311)	815,046 (613,549–1,083,055)	0.96	0.995	0.64
Model 2	1,339,759 (971,465–1,846,871)	1,148,538 (816,613–1,616,621)	1,070,889 (748,961–1,531,167)	0.42	0.984	0.26	931,918 (682,155–1,272,178)	1,057,058 (750,284–1,490,238)	914,379 (648,696–1,289,412)	0.70	0.989	0.33

Folate was measured in ng/mL, vitamin B12 was measured in pg/mL, homocysteine as μmol/L, vitamin D as ng/mL, CRP as pg/mL. Bold values are statistically significant.

[a]Natural logarithm used, values are exponentiated values of marginal means (geometric mean), and β (e^{β}).

Model 1: adjusted for age and sex.

Model 2: additionally adjusted for education, BMI, smoking status, physical activity, energy intake, and alcohol intake.

95% CI, 95% confidence interval; CRP, c-reactive protein.

respectively, whereas the non-significant associations did show a similar trend. An explanation for the different findings between the countries could be related to varying ranges of vitamin B12 and homocysteine values within countries. In Dutch participants, the range of vitamin B12 in the highest compared to the lowest E-NRF tertile is around 21, whereas the range for Polish participants is 44. For homocysteine levels the opposite is observed with a wider range in Dutch participants (range of 2) compared to Polish participants (range of 1). A wider range in the study population makes detection of a significant association more likely. This could be a reason that significant associations are only shown for the population with the widest range of the biomarker. Additionally, serum vitamin B12 does not show high sensitivity and specificity, so is limited in its use as a marker (51).

Moreover, although both vitamin B12 and folate levels are considered concentration markers of micronutrient status, several physiological and environmental factors other than diet, such as polymorphisms, and certain drugs, also influence their blood levels (52, 53). For homocysteine, renal function influences levels via clearance (54). For vitamin B12, inflammation of the gastric mucosa can cause reduction in the acid required to cleave vitamin B12 from food protein (55). Since the Polish and Dutch participants were very similar regarding age, sex, disease incidence, and macronutrient intakes, perhaps differences in physiological and environmental factors that have not been measured in these populations, such as kidney function or gastric differences, additionally add to differences in associations of the E-NRF7.3 score with B12 and homocysteine (51).

Previous studies on homocysteine level predictors have not included nutrient density scores, however, indices of the Mediterranean diet have been studied. When developing the E-NRF7.3 score its correlation with the NU-AGE index, a Mediterranean-like dietary pattern (13), was considered. Similar to studies in adults observing a negative association between the MedDietScore and homocysteine levels in adults, our study shows an inverse association between the E-NRF7.3 score and homocysteine levels (56, 57). Folate intake has been shown to be negatively associated with homocysteine levels (58), and folate and folic acid lower homocysteine in people with moderate hyperhomocysteinemia (59). Additionally, low vitamin B2, B6, and B12 levels are associated with increased homocysteine levels (43).

Besides an association of the Mediterranean diet with homocysteine, Chrysohoou et al. found that participants in the highest tertile of the Mediterranean diet score had 20% lower CRP levels (56) compared to participants in the lowest tertile. Similarly, a systematic review on dietary patterns and inflammation markers showed that nearly three-quarters of the studies using dietary indices or scores, and especially using the Mediterranean diet score, found negative associations with CRP levels (60). Other studies reported that close adherence to a Mediterranean diet was related to the inflammation marker fibrinogen, but not to CRP concentrations in community-dwelling older adults. However, "health aware" dietary patterns (low-fat and high-fruit) and high fruit intake were inversely associated with CRP (61).

Although, the E-NRF7.3 score is correlated with the NU-AGE diet, which resembles the Mediterranean diet, the E-NRF7.3 score does not include vitamins such as vitamin C and flavonoids mainly found in fruit. Therefore, the components of the Mediterranean diet that possibly result in the negative association with CRP-levels might not be completely captured in the E-NRF7.3 score.

The E-NRF7.3 score was also not significantly associated with vitamin D serum levels, despite the inclusion of vitamin D in the index. In contrast to folate and vitamin B12 levels, vitamin D is not only derived from oral intake, but additionally synthesized in the skin upon ultraviolet-B light exposure. Even in older adults at relatively northern European latitudes, daily ambivalent ultraviolet-B dose contributes significantly to 25(OH)D levels (62, 63). Moreover, a study by Brouwer-Brolsma et al. in Dutch older community-dwelling adults showed that vitamin D intake from foods, supplements, genetics and education, lifestyle and personal characteristics only explained approximately one-third ($R^2 = 0.35$) of 25(OH) D levels. Similar percentages of 28–33% have been found in by others (64, 65), suggesting that other factors contribute significantly to 25(OH)D variation.

The newly developed E-NRF7.3 score followed specific recommendations as proposed by Drewnowski and Fulgoni (7) by firstly including nutrients that are relevant for the aging population, defined as nutrients that are commonly inadequately consumed by elderly and nutrients that are associated with health outcomes relevant to elderly. Moreover, local nutrient composition databases have been used. Secondly, appropriate reference daily values were used by including the European Food Safety Authority, complemented with reference values more specific to older adults for selected nutrients and labeling reference values for the three nutrients to limit (15). Thirdly, we aimed to keep the algorithm both simple and transparent by adjusting previously developed NRFn and NRFn.3 scores (7, 66). Fourthly, previously the E-NRF7.3 score was validated against the NU-AGE index (15), a measure of adherence to the anti-inflammageing NU-AGE diet (13, 14). The current paper demonstrates its validity against a selection of markers of nutritional status and inflammation.

Strengths of this study include the 7-day food records with a standardized protocol used in both countries. Food records show better association with energy and protein biomarkers than Food Frequency Questionnaires and 24-h recalls (67) and rely less on memory compared to Food Frequency Questionnaires and 24-h recalls since participants record food intake at time of consumption (68, 69). Extensive information on food item level was available for thousands of products per country, as well as a wide range of confounding variables, from diet, physical activity, and anthropometric measurements to alcohol and smoking. An advantage of a nutrient density score is that it does not include foods or food groups that are not consumed as has previously been an issue with dietary indices (70). This allows for use in various regions and countries.

Limitations of this study include differences between national food consumption databases used. For Polish participants sucrose and lactose were used for E-NRF7.3 score calculations, where total mono- and disaccharides were available for Dutch

participants. This could have contributed to higher E-NRF7.3 scores for Polish participants, as the monosaccharides in for example fruits and honey did not contribute to the LIM3 part of the score. However, Streppel et al. found that when using the NRF9.3 index in relation to health outcomes, replacing total sugar with added sugar did not alter the results (8). Therefore, the influence of the different sugars used in calculation on the association with biomarkers is likely to be small. Further differences between the countries could result from variability in estimation of the quantity of nutrients in the same food between food composition databases (37) as well as differences in nutrient densities of similar food items resulting from compulsory margarine fortification with vitamin D (among others) in Poland, compared to only voluntary food fortification in the Netherlands (71).

Although, some dietary patterns and single nutrient intakes have been studied in relation to markers of intake and health outcomes, this is the first study demonstrating an association between a nutrient density score specifically developed to capture relevant nutrients for older adults and markers of nutritional status. In reflection of the current results, the addition of more or other nutrients to the E-NRF7.3 score could be considered as a way to further increase its validity with markers of nutritional status. Future studies should study the association with a wider range of health outcomes relevant to European older adults, and more specific markers of chronic inflammation. Furthermore, to demonstrate the practical applicability of the E-NRF7.3 score, this score should be linked to other determinants of food choice, including food preferences, food costs, food enjoyment, and availability (7).

To conclude, we observed that people with higher E-NRF7.3 scores have significantly higher folate levels, higher vitamin B12 levels (Poland) and lower homocysteine levels (Netherlands). Future studies should be undertaken in which more markers of nutritional status, a wide range of health outcomes and the practical implication of the score can be investigated.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Wageningen University Medical Ethics Committee, the Netherlands Bioethics Committee of the Polish National Food and Nutrition Institute, Poland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS and CF designed the NU-AGE project. AAMB and LG designed the intervention study. LG, AB, and AS were the principal investigators in the Netherlands and Poland. AAMB and ES conducted the dietary intervention in the Netherlands and Poland. AAMB and MS were responsible for the nutrient intake database in the Netherlands and Poland. CK and AAMB analyzed and interpreted the data and drafted the manuscript. LG interpreted the data. LG, AAMB, CK, ES, AB, AS, and CF critically revised the manuscript for important intellectual content.

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REVIEW
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The Influence of Taste Liking on the Consumption of Nutrient Rich and Nutrient Poor Foods

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Repeated consumption of high-energy nutrient poor foods can lead to undesirable health outcomes such as obesity. Taste plays an important role in food choice, and a better understanding of the links between the taste of foods, individual taste preferences, food choices, and intakes will aid in our understanding of why some people might select and consume unhealthy foods. The present review focuses on three main questions: (1) do nutrient poor and nutrient rich foods significantly differ in taste profile? (2) are humans predisposed toward developing a liking or preference for certain taste profiles? (3) how are individual variations in liking of the basic taste qualities related to long term food intake and adverse health outcomes such as obesity? Results indicated that nutrient poor foods were likely to be sweet, salty and fatty mouthfeel, while the taste profiles of nutrient rich foods were diverse. Although humans are born with a universal liking for sweet and aversion for bitter taste, large individual differences exist in liking of all the basic taste qualities. These individual differences partly explain differences in short term intakes of foods varying in taste profiles. However they fail to sufficiently explain long term food choices and negative health outcomes such as obesity. Future studies should focus on how the full sensory profile of food which includes taste, smell and texture interacts with individual characteristics (e.g., taste or health motivations, taste preferences) to affect consumption of nutrient rich and nutrient poor foods.

Keywords: taste, consumer, sensory, food, nutrition

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INTRODUCTION

Overweight and obesity continue to be a major public health challenge in economically developed nations. Approximately 20% of adults in OECD countries are obese which varies from 3.7% in Japan to as high as 27.5% in Australia (1) and 39.8% in the USA (2, 3). In general, obesity is positively correlated with the development of chronic diseases such as diabetes and heart diseases (4, 5), as well as social psychological challenges (6). One of the major contributors to the overweight/obesity epidemic is the food environment and associated dietary choice (7). Dietary patterns that are associated with higher levels of overweight/obesity are often (but not always) characterized by high levels of energy (calories) intake, coupled with low levels of nutrients (7). That is, the foods consumed as part of these dietary patterns are often low in nutrient density.

Nutrient rich foods can be described as "foods which provide substantial amounts of nutrients for relatively few calories, or foods which provide fewer calories than nutrients" (8). Nutrients in this context refer to health promoting nutrients such as protein, fiber, vitamins A, C and E, calcium, magnesium, iron and potassium, and does not refer to non-health promoting nutrients

like saturated fat, sodium, and added sugar. Nutrient density can be reflected by the Nutrient Rich Food (NRF) index which is calculated as the sum of percentage daily values for 9 nutrients that should be consumed in higher quantities (e.g., protein; fiber; vitamins A, C, and E; calcium; magnesium; iron; and potassium) minus the sum of 3 nutrients which should be limited (e.g., added sugar, saturated fat, sodium), with all daily values calculated per 100 kcal and capped at 100% (9). Diets with foods with a high NRF scores (i.e., nutrient rich foods) are positively linked to a higher consumption of foods and nutrients which are encouraged for better health, and a lower energy intake overall (10). In contrast, the over consumption of foods with a low NRF score (i.e., nutrient poor foods) has been associated with weight gain and subsequent negative health outcomes such as diabetes (8, 11). This is not surprising given that, in general, nutrient density (as in its definition) is negatively correlated with energy density (9). In order to increase the consumption of nutrient rich food and to decrease the consumption of nutrient poor foods, it is important to understand what affects the consumption of such foods (12).

A wide range of studies shows that food *liking* is one of the most important driver of food consumption (13–21). Food liking, includes liking of the basic taste qualities [e.g., sweet, sour, bitter, salty, umami and fat (22, 23)]. In this paper we focus on the role of taste liking in affecting choice and consumption of both nutrient rich foods and nutrient poor foods. In order to understand the high levels of consumption of nutrient rich foods and lower levels of consumption of nutrient poor foods, we will address the following questions (1) do nutrient poor and nutrient rich foods significantly differ in taste profile? (2) are humans predisposed toward developing a liking or preference for certain taste profiles? (3) how is individual variation in liking of the basic taste qualities related to long term food intake and adverse health outcomes such as obesity?

To answer these questions we will first discuss the taste profiles of nutrient poor and nutrient rich foods and diets. Next, we will discuss how taste liking develops in humans and how this is related to the consumption of nutrient rich and nutrient poor foods. Lastly, we will propose a series of recommendations, which aim to increase the consumption of nutrient rich foods and decrease the consumption of nutrient poor foods.

TASTE PROFILES OF NUTRIENT RICH AND NUTRIENT POOR FOODS IN THE CURRENT FOODS SUPPLY

Today's modern industrialized food supply is dominated by energy rich, nutrient poor foods (24). These foods are composed of high levels of sugar, saturated fat and sodium and are highly processed and easily accessible due to high volumes, low prices and ease of consumption (e.g., no elaborated preparation required). Data from Australia suggest that the availability of oils for cooking and food production increased by more than 600% from 1961 to 2009 (25). In addition, data from another Australian study showed a large increase in volume and value (around 580%) of imported sweetened products between 1988 and 2010, while exports of similar goods were minimal in comparison (26). Such increase in accessibility of high energy nutrient poor ingredients and foods is associated with the increased consumption of such foods (24, 27). Studies from Western countries suggest that the majority of energy average people in economically developed nations consume now comes from these high (added) sugar, sodium and saturated fat rich foods (12, 28–30).

Sugar (31), fat (32), and sodium (33, 34) significantly impact the taste profile of food (35). Mapping this against the NRF index, it can therefore be hypothesized that individual nutrient poor and nutrient rich foods have different taste profiles. Likewise, it can be hypothesized that healthy diets, which are dominated by nutrient rich foods, have different taste profiles compared to unhealthy diets, which are dominated by nutrient poor foods. These assumptions are explored in the following paragraphs.

Although it makes intuitive sense, there are at least four challenges with the assumed taste-nutrient relationships posited above. Firstly, not all nutrients can easily be sensed. For example, sodium in bread is less accessible to sodium sensing channels on the human tongue, than sodium on the surface of chips (33, 34). Therefore, bread can appear less salty than chips at the same sodium content. Secondly, modern advances have been able to decouple some sensory profiles and nutrient composition. For example, non-nutritive sweeteners provide sweet taste without the calories (36). Therefore, the nutrient content of foods may not match their perceived taste intensity.

A third challenge is that specific taste qualities are dominant in both healthy and unhealthy foods. For example, sweet taste occurs in both nutrient rich (e.g., fruits) and nutrient poor (e.g., sugar sweetened beverages) items. Similarly, bitter taste occurs in both nutrient poor (e.g., alcoholic beverages such as beer and wine) (37) and nutrient rich foods (e.g., cruciferous vegetables such as broccoli and Brussels sprouts (16, 38). The fourth challenge is that there are many taste-taste interactions which potentially disturb the taste-nutrient relationship (39). For example bitter taste suppresses sweet taste (40).

The relationship between the presence of particular nutrients such as sodium or sugar in foods, and their perceived taste intensity is therefore complex. In order to investigate the hypothesis that nutrient poor and nutrient rich food have different taste profiles, firstly foods need to be assessed on their macronutrient composition. Secondly, the same foods need to be sensory profiled by a group of trained human panelists. To this end, several scientists have established taste databases (35, 41–44). These databases contain foods and dishes which are commonly consumed in the country of interest and are assessed on their macro-nutrient profile. By systematically assessing these foods on the presence and intensity of basic taste qualities (e.g., sweet, sour, salty, bitter, and umami) and certain texture properties such as fatty mouthfeel, a food taste database can be established. It is important to note that these databases are mainly focussed on taste, rather than flavor, which is the combination of taste, smell and chemical irritation (45).

To our knowledge the first scientifically peer reviewed published attempt to establish such taste database, came from the Netherlands (43). In this study a small number of frequently consumed foods ($n = 50$) was sensory profiled by 19 minimally trained young consumers. The foods were assessed on the

perceived intensity of sweet, sour, bitter, salty, and umami-taste, by following the Spectrum Method. In this method, panel members are first calibrated on using the same terminology (attributes) for the sensation of interest, by using reference standards and extensive discussions amongst the panel members, facilitated by the panel leader. Next, panel members are calibrated on the intensity of the generated attributes by using reference samples which provide a wide range of intensities of the attribute of interest (46). This methodology to establish a taste database has been repeated with more heavily trained adults (35), in-home panels (41), a higher variety of foods [e.g., 377 food items (35), 237 food items (44), 590 food items (41)], and additional attributes including texture attributes (e.g., hardness, moistness, cohesiveness of mass and fatty mouth feel) (35, 41). These databases provide the means to examine whether nutrient poor and nutrient rich foods can be characterized by particular taste profiles.

NUTRIENT RICH FOODS AND TASTE PROFILE

Despite the potential disparity between taste and nutrient content, as mentioned previously, across a number of studies in different countries it has been shown that taste significantly relates to specific macronutrient content in foods. That is, the sweetness of food is positively correlated with the presence of mono- and disaccharides (35, 42, 43, 47, 48), salty taste is positively correlated with the presence of sodium (35, 42, 43, 47, 48) and to some extent protein (35, 43). Umami taste is positively correlated with the presence of protein (42, 47) and sodium (35). Fat sensation is related to fat content (35, 42, 47). Sour taste is related to the presence of organic acids (47). It is important to note that the association between nutrient content and taste varies amongst foods. Where the association tends to be weaker in more complex foods (35).

Given the high presence of added sugar, sodium and fat in nutrient poor foods and the lower levels of these nutrients in nutrient rich foods, it is to be expected that nutrient rich foods have a lower taste intensity than nutrient poor foods. This is partly confirmed by studies from the Netherlands (44), and France (41). These studies suggest that nutrient rich foods have a rather diverse taste profile. Van Langeveld et al. (44) concluded that staple foods (e.g., bread, potato) and what are generally considered to be nutrient rich foods, such as vegetables and fish, were overrepresented in the neutral taste cluster (e.g., these foods were perceived as having a low taste intensity of all basic taste qualities). However, other nutrient rich foods such as nuts, fruits, meat, and milk were mostly classed as salt/umami/fat, sweet/sour, sweet/fat, respectively. This is in line with data from France whereby fruits were mostly present in the sweet/sour and to some extent bitter clusters. Interestingly, in this French study, vegetables were mostly represented in the salt, umami, sour and bitter clusters which most likely represents the way many consumers in France prepare their vegetables (41).

The taste profile of nutrient poor foods is more consistent than that of nutrient rich foods. In the Netherlands (48) as well as in France (41) is was found that nutrient poor foods mainly have a taste profile which can be summarized as sweet, salty and fatty mouthfeel). Because of the high impact of salt/umami, sweet taste and fat taste on the sensory profile (35) and given the homogeneous taste profile of nutrient poor foods, it can be hypothesized that diets which are high in taste intensity are more likely to be nutrient poor. This indeed seem to be the conclusion from a Dutch study which assessed the taste patterns of different diets, including a diet based on the Dutch dietary guidelines (49). It was concluded that the energy derived from a diet based on the Dutch dietary guidelines mostly comes from neutral/bland tasting foods (49).

INNATE PREFERENCE FOR TASTE PROFILE OF NUTRIENT POOR FOODS

Now that we have more insights in the taste profile of nutrient poor foods, a related question becomes "does the taste profile of nutrient rich and nutrient poor foods affect their consumption"? In this section we specifically focus on sweet, salty and fatty taste as those tastes that are characteristic of nutrient poor foods. In addition, we focus on bitter and sour taste, because nutrient rich foods such as cruciferous vegetables have a bitter note (16) and many fruits have a sour note to them (50). However, it needs to be mentioned that other sensory aspects of food such as smell, which plays an important role in increasing our appetite [see (14) for review], and texture, which plays a role in the amount we consume [see (51) for review], also play a major role in food intake but are not explicitly considered here.

There are several biological underpinnings to the sense of taste. At birth humans are already equipped with a taste apparatus which can distinguish between sweet, sour, and bitter taste as evidenced by distinct facial expressions of newborns when exposed to sweet, sour and bitter tasting substances dissolved in water (52–54). Not only can infants detect these tastants, as judged by facial expression, the sucking responses and to some extent intake of these tastants follow the positive (e.g., sweet taste) or negative (e.g., bitter and to some extent sour taste) facial expressions (52–54).

Most research in the area of taste and infants (and children) has been focussed on sweet taste (55–57). Newborns appear to have a clear preference for sucrose solutions over plain water, as evidenced by relaxed facial expression, increased sucking responses and intake (54). Supposedly liking for sweet taste provides an evolutionary advantage for humans as sweet taste is (in nature) related to energy content, which is needed for growth and development (31). The liking of sweet taste also facilitates the ingestion of breastmilk, which has a sweet taste profile. It has been consistently found that children from a range of countries prefer higher intensities of sweet taste than adults (55) highlighting a clear innate bias toward sweet taste in infants and children.

The perception of salty taste is thought to go through different stages during development [see (58) for review]. Shortly after birth 1 to 4 days old infants seem to be mostly indifferent to salty taste which is likely due to the postnatal maturation of specific central and/or peripheral mechanisms underlying

salt taste perception (59). At ∼4–6 months after birth, infants appear to have a preferential response to salty water over plain water (54, 60) without the need for prior exposure to salty taste. This suggests an innate preferential response to salty taste. Such preference for salty taste is reflected in infants' increased consumption of salty baby cereal over plain baby cereal (61). When infants grow into early childhood (about 3 years of age), they show adult like rejections to salted water, but the liking for salt in culturally accepted salty foods remain high (60). This suggests that liking for salt is food specific and partly learned by exposure.

Whether humans have an innate preference for fat taste remains unclear. Several studies investigated infants' sucking and/or the ingestive response of infants to breast milk (62) or infant formula (63) with varying fat content. These early studies argue against the presence of an innate liking for fat. A more recent study investigated the development of fat liking by following 3 months infants up to the age of 20 months and also concluded that similar to newborns, 3 months old infants were mostly indifferent to the taste of fat emulsions (64). It seems from available evidence, then, that the liking for fat is mostly learned through dietary exposure, a process which may also be affected by the higher energy density of higher fat foods.

Bitter taste is clearly rejected by infants as evidenced by negative facial expressions (e.g., mouth gaping and wrinkling of regions on the forehead). There is little doubt that newborns are able to detect bitter taste (52–54), however they do not seem to diminish intake in response to the sensation of a bitter substance. That is, sucking responses and ingestion in response to a bitter solution are not different from those to water (65, 66). An alternative explanation is that infants dislike plain water. In nature, many toxic substances taste bitter, so it seems to fit a natural survival instinct to reject any foods which are bitter (67).

It is generally thought that strong sour solutions are disliked by newborns, although the facial response to sour solutions (e.g., lip pursing) is remarkably different from the facial response to bitter solutions (e.g., mouth gaping, wrinkling of the regions of the forehead) (52–54, 66). The combination of lip pursing and sucking, seen typically in response to sour tasting substances, may result in compressing the cheeks against the gums, which stimulates salivary flow in the oral cavity. In adults it has been suggested that the increased flow and buffering capacity of saliva neutralizes sour tasting substances (68, 69). Infants' ingestive responses to sour taste do not provide a clear picture indicating a clear rejection. Although a mixture of sucrose and citric acid resulted in lower intake than a sucrose solution in some studies, it does not take into account the suppression of sweet taste by citric acid. Moreover, no difference in the infants' ingestion has been observed in response to water and water with added citric acid (65). In infants close to one and a half years of age it has been shown that about a fifth to one third of these infants have a preference for high concentrations of citric acid in a sugar solution. These infants were more likely to consume fruit than those infants who did not express this preference for high sour tasting sweet solutions (70). Similar observations have been made in children (71–73). This suggests that sour taste preferences directly influence food consumption.

When infants grow into early childhood, the preference for sweet taste (74–76) and the aversion for bitter taste remains high (77, 78). Research conducted in a range of countries suggests that liking for sweet taste is significantly higher in children than adults (55). Liking for sweet seems to coincide with rapid changes of grow during childhood (79), which supports the hypothesis that children's high liking for sweet taste is functional for the rapid stages of growth and development infants and children go through. In addition it has been suggested that children are more sensitive for bitter taste than adults, which might explain why children often reject the slightest note of bitter taste (57, 80) and children's consumption of bitter tasting vegetables can be a struggle (16). When children grow older, new flavors and tastes are added to their repertoire of acceptable foods, mainly through different learning mechanisms, as discussed below.

LEARNED TASTE PREFERENCES

As noted above, humans have innate biological biases that, in the absence of other factors, may predispose them toward liking foods that are sweeter, fattier, and/or saltier and disliking foods that are bitter and possibly sour. However, taste liking arises due to the interacting effects of genetic predispositions and environmental factors. That is, although there are a number of innate taste biases that appear to be common to all humans, they cannot solely explain the wide variation in food/taste liking observed in populations (81, 82). Rather, individual differences in food/taste liking that are observed between individuals reflect interactions between innate biological characteristics and learning processes that occur over time.

Innate biological characteristics include those that are common to all humans such as a liking for sweet taste, as discussed above, along with variations between individuals in these characteristics [e.g., capacity to detect bitter taste (83, 84)]. Individual differences in biological characteristics may influence how individuals respond to the influences within their food environments, and therefore the tastes and foods that are liked or disliked. Evidence in children has shown the important influence of individual genetic differences on liking (85) as well intake of vegetables (86), although this effect seems to diminish in adulthood and older age (80, 87). Furthermore, twin research in adolescents suggest that unique family environmental influences succeed shared environmental influences in young adulthood (unlike in early childhood), highlighting that individuals within similar food environments respond in different ways (88), resulting in taste, and subsequently food, likes and dislikes that are unique to individuals (85, 89)

The ways in which non-shared environmental effects may manifest in unique taste and food liking is through the effects of the social and environmental context of consumption. These effects are also rooted in biologically based predispositions that result in rejection of novel foods (food neophobia), but also greater acceptance of foods with exposure and positive associations: Through the repeated pairing of foods with positive or negative stimuli individuals learn to like and dislike particular tastes and foods. This is why exposure and familiarity are

key mechanisms explaining differences between individuals and populations in taste liking and disliking. Exposure can begin early, with flavors experienced *in utero* and in breast milk influencing liking of flavors/tastes in both shorter and medium terms (90–92). Exposure that is linked to positive affective tones can accelerate the effects of exposure on food liking of particular foods and sensory characteristics (93). In contrast, foods that are presented in ways that elicit negative affect can result in decreased liking of those foods and associated sensory characteristics. Parental use of nutrient poor foods, such as dessert foods, as food rewards, for instance, accelerates children's liking of those foods (94). In contrast, rewarding children for eating nutrient rich foods (e.g., vegetables), or pressuring children to eat them, decreases liking of such foods (95). What determines the nature of individual exposure to a range of tastes and foods, such as use of pressure and reward during parental feeding, is related to a wide range of biological, psychosocial and cultural factors (96, 97).

Also important are learned associations between sensory cues of foods and post-ingestive consequences (98). This works in two important ways: through the formation of food aversions associated with nausea or vomiting (99), and through positive associations between the sensory properties including tastes and flavors of foods that are more filling and satisfying (100). This is one mechanism by which the tastes of more energy dense foods can become liked, and bitter and sour tastes can become liked. However, although this mechanism has, in some instances, been demonstrated in relatively low energy density foods including fruits and vegetables (101) where additional energy is added to increase the overall energy density of the foods, it is more likely to be a mechanism helping to explain liking of foods that are naturally high in energy density, which is a characteristics of many nutrient poor foods.

As noted above, individual differences in biological characteristics can help to explain unique taste and food likes and dislikes. Important individual differences in biological characteristics include variations in taste receptors, which affects sensitivity to the various tastes and taste intensities; notably bitter taste. Greater bitter taste sensitivity has been linked to lower liking of cruciferous vegetables dine, along with greater sensitivity to sweet liking of foods, and lower liking of fatty foods that are strong tasting and sweet tasting (102, 103). However, other biological factors are also associated with taste and food liking, and these also appear to be linked to, and interact with, biological differences in taste acuity. For instance, cognitive approaches to eating such as food neophobia are associated with greater bitter taste sensitivity, as well as reduced exposure to, and liking of vegetables (104). Other differences in individual psychobiological characteristics such as temperament and personality, restraint and disinhibition, and reward circuitry also affect how individuals approach food and eating and, through learning mechanisms, taste and food liking (105).

Finally, it should also be noted that taste liking is not stable within individuals, and can vary with a number of factors including psychophysiological states, across the course of a meal, with hunger levels, mood/emotional state, and eating context and this can affect whether nutrient rich or poor foods are selected and consumed (106).

The interacting influences of unique and common biological factors with the unique characteristics of individual food environments produces a wide range of taste and food likes and dislikes. There are innate predispositions common to all humans that facilitate learned liking and consumption of nutrient poor foods (e.g., flavor-nutrient learning), and retard liking and consumption of some nutrient rich foods (e.g., food neophobia). Further, some individuals, for instance those who have higher sensitivity to bitter taste, are probably more susceptible to developing taste and food likes and dislikes that are consistent with consumption of nutrient poor foods.

RELATIONSHIPS BETWEEN TASTE PERCEPTION AND FOOD CHOICE/LIKING/FLAVOR LIKING (IN DIFFERENT INDIVIDUALS/CONSUMERS)

So far we have discussed how nutrient poor foods have specific taste profiles, how the human biology is designed toward a liking of these typical taste profiles and how humans can learn to like foods associated with these taste profiles. But does individual variation in liking of these taste profiles alone lead to a higher consumption of foods with taste profiles commonly seen in nutrient poor foods? A further question is whether liking for particular taste profiles is related to adverse health outcomes.

SWEET TASTE AND DIETARY INTAKE

Several researchers have sought to find an association between sweet taste sensitivity, perceived intensity, sweet taste liking and intake of sweet tasting foods. A recent systematic review of 17 studies concluded that most studies which were reviewed failed to find an association between sweet taste sensitivity and dietary intake patterns (107). Also the potential relationship between perceived sweet taste intensity and intake is rarely shown (107). If anything there might be a negative association between perceived sweet taste intensity and energy and carbohydrate intake (108, 109), but again this has not consistently been shown.

The strongest and potentially the only association between sweet taste and intake is that of hedonics and intake. In particular, those studies that divided participants in either sweet likers group or sweet dislikers, used a more precise dietary intake tool (e.g., 24 h recall, 4-day weighed food record, 7 day diet record), and had sufficient sample sizes found statistically significant positive relationships between liking for sweet taste and dietary intake (107). That is, those who show a general liking for sweet taste consumed more energy from refined and total sugars (107), which are commonly nutrient poor foods. But as suggested earlier, sweet taste as such does not seem to correlate well with the energy in foods (44). If this holds true one would expect the association between sweet taste liking and obesity to be weak or non-existing. This indeed seems to be the case in that the majority of studies investigating the link between

sweet taste perception/hedonics and obesity failed to find such a relationship (109, 110).

SALT TASTE AND DIETARY INTAKE

The relationship between salt taste liking and intake appears to be malleable. Longitudinal experimental studies in adults suggest that changing the salt content of foods is followed by a change in liking for salt taste as well as perceived intensity of salt taste. That is, a prolonged exposure 5 months (111), 12 months (112) to a low sodium diet resulted in a lower perceived salt intensity and liking for lower salt levels, compared to before subjects went on the low salt diet. Vice versa, when sodium intake increases, preferred levels of sodium in foods increase accordingly (113) [see (114) for review]. These studies suggest that salt taste perception and liking can be modified by changing dietary sodium intake. However, it needs to be noted that dietary sodium reduction in intervention trials are rather severe (24% dietary sodium reduction (111), 21% (112), whether smaller changes in sodium consumption would also result in a change in salt sensitivity and/or liking remains unclear.

When investigating natural variation in salt taste preferences, some studies found a positive association between preference for salty taste and consumption of sodium (115–117), whereas others did not see such correlation (118, 119). Such inconsistent results might be a partly caused by difficulties around the assessment of dietary sodium consumption and the likelihood that sodium intake is influence by more than just taste preferences [see Mattes (120)].

FAT TASTE AND DIETARY INTAKE

Recently fat taste has become of specific interest because of advances in the understanding of the perceptual mechanisms as well the association between fat taste sensitivity and consumption of fat (22, 23, 121, 122). It is important to note that fat taste refers to cellular responses to free fatty acids, rather than the cellular response to the most common form of fat in the diet (123). Observational studies as well as experimental studies have shown that fat taste sensitivity is negatively associated with the (short term) intake of dietary fat (122, 123), potentially this is caused by the influence of fat taste receptors on feelings of satiety. That is, when fat taste receptors (which are present throughout the GI track) are stimulated they trigger the release of various satiety hormones like GLP-1 and CCK (123) which generates a feeling of fullness. Some, but not all, studies found significant associations between fat taste sensitivity and obesity. That is obese people are more likely to be less sensitive to fat taste than lean counter parts (123, 124). The taste of dietary fat is also determined by texture properties. When taking the sensory perception of fat as a whole (e.g., taste and texture properties) some, but not all studies suggest a positive link between liking of dietary fat, fat consumption and obesity (122, 125–129).

Overall it can be concluded that although the taste profile of nutrient poor foods is rather consistent as judged by the basic taste qualities, there is a lack of consistent evidence which suggests that a taste liking of the basic taste qualities is what is driving long term food consumption leading to adverse health outcomes such as obesity. This can be partly due to the variety of methods used to measure liking for the basic taste qualities and they procedures which are followed to estimate dietary intake. In addition it is good to realize that food liking is not only determined by taste, but also by other food related properties such as smell, texture and appearance. Many studies have shown that liking of food as a whole, plays a major role in food consumption (13–21).

DISCUSSION AND RECOMMENDATIONS

The taste profile of energy poor foods are naturally attractive for consumers. Although food liking as a whole (including taste, smell, and appearance) is an important driver of food choice, it is difficult to relate long term food choice of adults to the liking of specific basic taste qualities. This might partly be caused by methodological challenges in which basic taste qualities are often measured in isolation rather than its natural occurrence in foods. In addition taste preferences might play a different role during the life span. That is, children's food choices seem to be stronger related to basic taste preferences, than adults' food choices.

Humans seem to naturally like nutrient poor foods, and this can be reinforced by a range of environmental factors. At the same time segments of consumer can follow a healthy diet. This is not to say that these consumers do not care about how the food tastes, but other factors might make them more resilient to the temptation of taste or prevents consumers to choose the food they like the most. With regards to nutrient rich foods, food liking, perceived health benefits, and price are often seen as trade-offs (130–133). Several studies indicated that when consumer are more focussed on taste than health they, in general, make unhealthier food choices (18, 131, 133). Steering taste focussed consumers, who are less concerned about health, to healthy food choices is difficult and providing more health related information on food packaging [see (134) for review] is unlikely to solve the problem (135–138). In order to attract the attention of taste focussed consumers it has been recommended to emphasize the great taste of healthy products, rather than to fully focus on the health benefits (16). Alternatively, nutrient poor foods can be made less attractive by increasing its pricing (e.g., sugar tax). Such approach has shown to decrease the purchase of those nutrition poor foods which are taxed in modeling studies, experimental studies as well as natural experiments (139, 140). However, it remains unclear how this strategy will benefit long term healthy food choices and whether consumers are not driven to other unhealthy foods which are not taxed (141).

Future studies should focus on strategies which makes it easier for taste focussed consumers to make long term healthy food choices. In addition it needs to be investigated how the full sensory profiles of foods (e.g., taste, smell, and texture), rather than just taste, are associated with food

choices of different segments of the population and how liking for different taste profiles are related to food intake and health outcomes.

In conclusion, the typical taste profile of nutrient poor foods makes them attractive to consumers. The innate liking for sweet and salty taste can make it difficult to move consumers away from nutrient poor foods. However, taste preferences and subsequent food choices can be changed by repeated exposure especially during childhood during which taste preferences play a major role in food choice and consumption. In addition, strategies in which the good taste of nutrient rich foods are emphasized are especially recommended for those consumers who are more taste than health focussed.

AUTHOR CONTRIBUTIONS

DL and CR contributed to the outline of this review and writing of the manuscript.

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Cheese and Healthy Diet: Associations With Incident Cardio-Metabolic Diseases and All-Cause Mortality in the General Population

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Background: Many countries have established Food-Based Dietary Guidelines (FBDG). For some foods, such as cheese, there is no consensus on whether or not to include them in these guidelines. Cheese may, however, be an excellent source of vitamin K2, which is a macronutrient with demonstrated positive results on cardiovascular-related outcomes.

Aim: First, we assessed the role of cheese within the recently developed Lifelines Diet Score (LLDS), a score based on the Dutch FBDG 2015 in relation to incident cardio-metabolic diseases and all-cause mortality. Secondly, we assessed the association of cheese intake with desphospho-uncarboxylated matrix Gla protein (dp-ucMGP), a marker for functional vitamin K2 status, in a subset of the population.

Methods: From the Lifelines cohort study, 122,653 adult participants were included to test the association between de LLDS and health outcomes. In a subset of 1,059 participants aged 60–75 years, dp-ucMGP levels were measured. Dietary intake was assessed using a 110-item Food Frequency Questionnaire. Logistic regression were applied, adjusted for relevant confounders.

Results: Median cheese intake was 23.5 [12.6–40.6] g/day. We found a positive correlation between cheese intake and the LLDS (Spearman's rho = 0.024, $p < 0.001$). The LLDS in quintiles was associated with T2DM [OR (95% CI) Q5 (healthy diet) vs. Q1 (poor diet) = 0.54 (0.43–0.67)] and all-cause mortality [Q5 vs. Q1 = 0.62 (0.50–0.76)]. Inclusion of cheese did not alter these associations. Additionally, we found no significant association of total cheese intake with plasma dp-ucMGP levels.

Conclusion: In this population-based cohort study, the inclusion of cheese in the LLDS did not change the inverse associations with incident cardio-metabolic diseases and all-cause mortality. Furthermore, we found no significant association of total cheese intake with plasma dp-ucMGP. The results suggest that cheese is a neutral food group that fits a healthy diet.

Keywords: nutrition, diet, vitamin K2, cardio-metabolic diseases, all-cause mortality

INTRODUCTION

The consumption of a variety of foods is needed to support growth, provide strength, improve cognitive function, and reduce susceptibility to chronic diseases, illnesses, and infection (1). To help address the nutrition concerns of populations, many countries have established Food-Based Dietary Guidelines (FBDG) (2). These FBDGs are expressed in terms of food and diet rather than in nutrients, to be more easily understood and used by community members, and are created to inform the public about consuming a healthy diet (2). Additionally, substantial evidence indicates that foods and dietary patterns have a stronger influence on chronic disease risk than individual nutrients (3, 4).

In the Netherlands, the Dutch Health Council issued their FBDG in 2015 (3). The guidelines are the result of a systematic and critical evaluation of international peer reviewed literature on relations of foods, dietary patterns, and nutrients with causal risk factors and chronic disease risk. Based on the evidence provided by the Dutch Health Council (3), a food group classification has been made, in which groups were categorized as positive, negative, neutral, or unknown, based on the evidence regarding their health effects (5).

Although cheese is sometimes categorized within the food group "dairy," the composition of cheeses highly differs from dairy products like milk and yogurt, resulting in e.g., differential cardiovascular health effects (6). In the food group classification mentioned above (5), cheese is considered to be a separate food group with unknown health effect. Nevertheless, modest evidence of an association between cheese intake and coronary heart disease and stroke has previously been reported (7). Additionally, among subjects with an impaired glycemic state at baseline, cheese intake was found to be inversely associated with incident diabetes, even after adjusting for BMI and other risk factors (8).

Cheeses are often salted, contributing to high sodium intake (6). Nevertheless, cheese is also nutrient rich, providing a wide range of crucial vitamins (A, B6, B12, D, and K), minerals (calcium, iodine, magnesium, potassium, phosphorus, and zinc), fats, proteins, and other micro constituents (9, 10). Adequate intake of these components is difficult in a diet low in dairy (9). For example, dairy products can provide up to 60% of the recommended daily allowance (RDA) of calcium (10). Additionally, fermented dairy products such as cheese, are an excellent source of fat-soluble vitamins such as vitamin K2 (11–13), vitamin A, and vitamin E (14).

In the Netherlands, cheese is one of the food groups that contribute substantially to the daily food intake (15, 16). In this context it is worthwhile to further study the effect of cheese on health outcomes. The Lifelines cohort, a large general population cohort in the three northern provinces of the Netherlands provides the possibility to assess the role of cheese in a healthy diet in a large representative population. The aim of the present study is two-fold. First, we will assess the possible role of cheese within the recently developed Lifelines Diet Score (LLDS) (5), a score based on the Dutch FBDG 2015. Secondly, we will assess the association between cheese intake and desphospho-uncarboxylated matrix Gla protein (dp-ucMGP), a marker for functional vitamin K2 status (17) in a subset of the population, and is considered a novel risk factor for mortality and CVD (18).

METHODS

Cohort Design and Study Population

The LifeLines cohort study is a prospective population-based cohort study examining the health and health-related behaviors of 167,729 persons living in the North of the Netherlands. The overall design and rationale of the study have been described in detail elsewhere (19, 20). Participants were included in the study between 2006 and 2013, and written informed consent was obtained from all participants. So far, four assessment rounds took place [T1 = baseline, median + IQR of time in months to follow-up rounds: T2 = 13 [12-14], T3 = 24 [23-27], T4 = 44 [35-51]. The Lifelines study is conducted according to the principles of the Declaration of Helsinki and approved by the Medical Ethics Committee of the University Medical Center Groningen, the Netherlands.

Lifelines Population

The Lifelines cohort includes 152,662 adults (age 18–93) and dietary information from food frequency questionnaires (FFQ) was available for 144,095 of them. FFQ data was considered unreliable when the ratio between reported energy intake and basal metabolic rate, calculated with the Schofield equation (21), was below 0.50 or above 2.75, or when energy intake was below 800 kcal/day (males) or 500 kcal/day (females). Fourteen thousand seven hundred and thirty-two participants with unreliable dietary intake data were excluded, leaving 129,363 participants in the study. Furthermore, participants who self-reported to have (had) a stroke, myocardial infarction, heart failure, or diabetes [all types, in addition to self-reporting: fasting glucose $\geq$ 7 mmol/l, HbA1c $\geq$ 6.5 % or medication use (ATC A10A/A10B)] at baseline, were excluded, leaving 122,653 participants in this study (**Figure S1**).

Lifelines Dp-ucMGP Sub-population

Dp-ucMGP measurements were performed in a subset of the LifeLines population consisting of 1,600 subjects, equally distributed over gender and socio-economic status (SES), between 60 and 75 years of age. This subset was selected to investigate vitamin status and subclinical micronutrient deficiency in low vs. high socio-economic status (SES). Since education is more differentiating than income in the Dutch population, classification of SES was based on educational status. Low SES was defined as never been to school or elementary school only, or completed lower vocational or secondary schooling; high SES was defined as completed higher vocational schooling or education. As in the total Lifelines population, participants with missing or unreliable dietary intake data were excluded ($n = 295$) and participants who reported to have (had) a stroke, myocardial infarction, heart failure or diabetes (all types) at baseline ($n = 201$), were excluded. Furthermore, participants reporting to use vitamin K antagonists ($n = 37$) were excluded from this study, leaving 1,059 participants of this sub-cohort in the study.

Data Collection and Measurements

Self-administered questionnaires were used to collect data regarding demographics (education) and lifestyle (smoking, alcohol, physical activity, diet). The validated short questionnaire to assess health-enhancing physical activity (SQUASH) was used to assess physical activity (22). Leisure time and Commuting Physical activity, including sports, at moderate (4.0–6.4 MET) to vigorous (≥6.5 MET) intensity (LC_MVPA) was calculated in minutes per week (22). Anthropometric measurements and blood pressure were measured by well-trained staff. BMI was calculated as weight (kg) divided by height squared (m^2).

Blood samples were collected in fasting state between 8.00 and 10.00 a.m. and subsequently transported to the Central Lifelines Laboratory in the University Medical Center Groningen. Functional vitamin K2 status was assessed by measuring dp-ucMGP in EDTA plasma using a dual-antibody enzyme-linked immunoassay [InaKtif MGP (IDS-iSYS) assay]. The lower limit of quantitation of the InaKtif MGP assay was 300 pmol/L. Serum creatinine (SCr) was measured via an enzymatic assay with colorimetric detection on a Roche Modular chemistry analyzer (Roche, Basel, Switzerland). The creatinine-based CKD-EPI formula was used to obtain the estimated glomerular filtration rate (eGFR) (23). Other laboratory measurements were assessed by commercially available assays on a Roche Modular chemistry analyzer (Roche, Basel, Switzerland).

Dietary Assessment

To assess dietary intake in the LifeLines Cohort, a 110-item semi-quantitative baseline FFQ assessing food intake over the previous month was developed by the Wageningen University using the Dutch FFQTOOL™, in which food items were selected based on the Dutch National Food Consumption Survey of 1997/1998 (24). Energy and macronutrient intake was estimated from the FFQ data by using the Dutch food composition database of 2011 (25). Alcohol consumers were defined as those participants who consumed at least one alcoholic beverage in the past month.

Cheese intake was assessed with three main questions, asking for habitual consumption of cheese on bread, bread-rolls or crackers, with hot meals and as snack. Additionally, it was asked what type of cheese was most frequently chosen (low fat cheese (20/30% fat), regular high fat cheese (40/48% fat), cream cheese or foreign cheeses (e.g., brie or blue cheese). From these data, daily cheese intake in g/day was calculated.

The Lifelines Diet Score

The Lifelines Diet Score (LLDS) was calculated as a measure of relative diet quality. The development of this food-based diet score has been described in detail elsewhere (5). In short, the LLDS is based on the scientific evidence underlying the 2015 Dutch Dietary Guidelines, and ranks the relative intake of nine food groups with proven positive health effects (vegetables, fruit, whole grain products, legumes and nuts, fish, oils and soft margarines, unsweetened dairy, coffee, and tea) and three food groups with proven negative health effects (red and processed meat, butter and hard margarines and sugar-sweetened beverages) (3). For each of the food groups, quintiles of consumption in grams/1,000 kcal are determined and awarded zero to four points, with four points being awarded to the highest quintile of consumption for positive food groups, and to the lowest quintile for negative food groups. The sum of the 12 component scores resulted in a LLDS score ranging from 0 to 48. The LLDS scores were then categorized into quintiles, with quintile 1 including 20% of participants with the lowest diet quality and quintile 5 including 20% of participants with the highest diet quality. As previously shown, the LLDS is higher in women and positively associated with age category and educational level. For men, mean LLDS ranged from 19.5 (SD = 5.30) in males aged below 40 with low educational level, to 25.9 (SD = 5.50) in highly educated males aged 60 or higher. For women, this range is 20.8 (SD = 5.74) to 29.1 (SD = 5.61) (5).

To investigate the role of cheese in the LLDS, cheese was included in the LLDS as an additional, 13th group. In line with the methods used for other food groups of the LLDS, quintiles were made for cheese intake in grams per 1,000 kcal. Higher scores were either awarded to the quintile of highest intake (when cheese was considered a positive food group), or to the quintile of lowest intake (when cheese was considered a negative food group).

In collaboration with the National Institute for Public Health and the Environment (RIVM), the Netherlands Nutrition Center has calculated various diets that comply with the Dutch dietary guidelines and with the Dietary Reference Values (26). Based on the saturated fat and sodium dietary reference values, a maximum for cheeses of 40 g/day has been set to fit a healthy diet Therefore, a third approach to score cheese intake was used, in which intake up until 40 g was scored as positive, and intake above 40 g as negative. This resulted in four variants of the LLDS to be investigated in statistical analyses.

Clinical End Points

In the present study, we examined associations of LLDS (with or without cheese) with incidence of cardio-metabolic diseases [i.e., stroke, myocardial infarction, heart failure, and type 2

diabetes (T2DM)] and all-cause mortality. Incident cases of stroke, myocardial infarction and heart failure were based on self-reported questionnaires that were issued in the three follow-up assessment rounds. Incident T2DM was defined as self-reported T2DM according to the questionnaires in the three follow-up rounds, or a fasting glucose ≥7.0 mmol/L or HbA1c ≥ 6.5 mmol/mol during the last follow-up assessment when blood samples were collected. Data on prescribed medication was not available during follow-up. Data on mortality were obtained from the municipal register.

Statistical Analyses

Continuous data as medians with interquartile ranges (IQR) because of the non-normal distribution of many of the variables involved. Discrete and categorical data are presented as frequencies (%). Logistic regression was applied to investigate the association of cheese intake in quintiles (based on intake in g/1,000 kcal) and incident stroke, myocardial infarction, heart failure, T2DM and all-cause mortality. To evaluate the effect of inclusion of cheese in the LLDS on the association of the LLDS with clinical end points, logistic regression was applied on the four defined LLDS (with or without cheese) and these same five health outcomes. For cardio-metabolic health outcomes, only participants with complete follow-up were included in the analyses since it was unknown whether participants who dropped-out became an incident case or not. For all-cause mortality this does not apply, since this outcome does not rely on self-reporting.

Furthermore, in the subset as described above, we assessed whether cheese intake was associated with dp-ucMGP, a marker for functional vitamin K2 status and a novel risk factor for mortality and CVD (18). The association was investigated using logistic regression analyses with dp-ucMGP dichotomized into ≤300 vs. >300 pmol/L as the dependent variable.

All regression analyses were adjusted for relevant confounders including energy intake, education level, age, gender, smoking status, alcohol intake, leisure time, and commuting moderate-vigorous physical activity and BMI. Analyses with quintiles of cheese intake were additionally adjusted for the regular LLDS, and with respect to the dp-ucMGP analysis were additionally adjusted for eGFR. Furthermore, it was tested whether gender was an effect modifier in all regression analyses involving cheese consumption by including the interaction-term of gender and cheese intake in quintiles.

RESULTS

Baseline Characteristics

In the present study, we included 122,653 subjects [median age 44 (IQR 35–51) years, 40.8% male] of the adult Lifelines cohort and 1,059 [aged 64 (62–68) years, 48.8% male] of the subset with available dp-ucMGP data. Baseline characteristics of the total study population and for the subpopulation with available dp-ucMGP measurements are presented in **Table 1**. The median cheese intake was 23.5 g/day in the total study population and 28.8 g/day in the subset population. The mean LLDS was 24 and 27 in the total and subset study population, respectively. We found a significant correlation between daily cheese intake (in g/day) and the LLDS (Spearman's rho = 0.024, $p < 0.001$).

TABLE 1 | Baseline characteristics of the total study population and subset with available dp-ucMGP measurements.

	Total study population	Subset with available dp-ucMGP
	(*N* = 122,653)	(*n* = 1,059)
Demographics		
Male gender (n, %)	50,101 [40.8]	513 [48.4]
Age (years)	44 [35–51]	64 [62–68]
Education		
Low (%)	2.1%	40.2%
Middle (%)	66.6%	-
High (%)	31.3%	59.8%
Smoking status		
Never (%)	47.1%	34.8%
Former (%)	31.9%	54.0%
Current (%)	21.0%	11.2%
BMI [kg/m^2]	25.3 [23.0–28.0]	25.8 [23.7–28.4]
Laboratory parameters		
dp-ucMGP > 300 pmol/L (n, %)	N.A.	243 [22.9%]
Total cholesterol-HDL ratio	3.38 [2.75–4.22]	3.5 [2.9–4.3]
LDL cholesterol	3.2 [2.6–3.8]	3.6 [3.0–4.2]
Triglycerides [mmol/L]	0.96 [0.7–1.36]	1.0 [0.8–1.4]
Serum creatinine [μmol/L]	72 [64–81]	74 [65–85]
eGFR [mL/min/1.73 m^2]	98 [87–108]	84 [75–91]
Nutrition		
Lifelines Diet Score [0–48]	24 [20–28]	27 [23–31]
Energy intake [kcal/day]	1985 [1636–2412]	1862 [1554–2206]
Cheese intake		
Total cheese [g/day]	23.5 [12.6–40.6]	28.8 [17.5–44.2]
High fat cheese [g/day]	16.1 [5.9–32.0]	20.9 [7.8–39.6]
40+ cheese/cheese spread [g/day]	0 [0–10.7]	0.9 [0–14.2]
48+ cheese/cheese spread [g/day]	0 [0–6.3]	0 [0–7.1]
Cheese with hot meal [g/day]	1.1 [0–3.0]	0.9 [0–2.8]
Cheese as snack [g/day]	2.7 [0–4.6]	2.7 [0.7–5.9]
Cream cheese or foreign cheeses [g/day]	0 [0–0]	0 [0–0]
Low fat cheese		
20+/30+ cheese/cheese spread [g/day]	0 [0–8.9]	0 [0–12.3]

Cheese and Incident Stroke, Myocardial Infarction, Heart Failure, T2DM, and All-Cause Mortality

After a median follow-up time of 3.7 [IQR 2.9–4.3] years for cardio-metabolic diseases, 306 (0.38%) incident cases of stroke, 325 (0.40%) incident cases of myocardial infarction, 859 (1.06%) incident cases of heart failure, and 1,099 (1.36%) incident cases of T2DM occurred. After a median follow-up time of 7 years [IQR 6–8] for all-cause mortality, 1,305 subjects (1.06%) had died.

TABLE 2 | Associations of quintiles of cheese intake (based on intake in g/1,000 kcal) with cardio-metabolic diseases ($N = 78,774$) and all-cause mortality ($N = 119,435$).

Outcome	Quintiles of Cheese intake					
	Q1 (Reference)	Q2 [OR (95% CI)]	Q3 [OR (95% CI)]	Q4 [OR (95% CI)]	Q5 [OR (95% CI)]	*P-value*
Stroke–w/o LLDS	N.A.	1.37 (0.90–2.07)	1.20 (0.79–1.82)	1.28 (0.85–1.93)	1.07 (0.70–1.62)	0.495
Stroke–with LLDS	N.A.	1.38 (0.91–2.08)	1.20 (0.79–1.83)	1.29 (0.86–1.94)	1.08 (0.71–1.63)	0.486
Myocardial infarction–w/o LLDS	N.A.	0.75 (0.50–1.12)	0.84 (0.57–1.23)	0.98 (0.67–1.41)	1.09 (0.76–1.56)	0.267
Myocardial infarction–with LLDS	N.A.	0.76 (0.50–1.13)	0.85 (0.58–1.25)	0.99 (0.68–1.43)	1.10 (0.77–1.58)	0.273
Heart failure–w/o LLDS	N.A.	1.07 (0.82–1.40)	1.34 (1.04–1.72)	1.25 (0.97–1.60)	1.26 (0.99–1.62)	0.115
Heart failure–with LLDS	N.A.	1.07 (0.82–1.40)	1.34 (1.04–1.72)	1.25 (0.97–1.61)	1.27 (0.99–1.63)	0.109
T2DM–w/o LLDS	N.A.	1.13 (0.91–1.40)	1.02 (0.83–1.27)	1.12 (0.91–1.38)	1.08 (0.88–1.33)	0.717
T2DM–with LLDS	N.A.	1.16 (0.94–1.43)	1.05 (0.85–1.31)	1.15 (0.94–1.42)	1.12 (0.91–1.38)	0.595
All-cause mortality–w/o LLDS	N.A.	0.96 (0.78–1.18)	0.87 (0.71–1.07)	1.03 (0.85–1.25)	0.94 (0.78–1.14)	0.425
All-cause mortality–with LLDS	N.A.	0.98 (0.80–1.20)	0.90 (0.73–1.10)	1.06 (0.87–1.28)	0.96 (0.79–1.16)	0.472

Q1 (low cheese intake) was used as reference quintile.
Models are adjusted for: energy intake, education level, age, gender, smoking status, alcohol intake, moderate-vigorous physical activity and BMI, and additionally for diet quality (LLDS).

The association of cheese intake with incident cardio-metabolic diseases is depicted in **Table 2** and **Figure 1** (upper panel). Cheese intake was not associated with incident cardio-metabolic diseases, adjusted for relevant confounders [OR (95%CI) for Q5 (healthy diet) vs. Q1 (poor diet) = 1.08 (0.71 – 1.63) for stroke, 1.10 (0.77–1.58) for myocardial infarction, 1.26 (0.99–1.62) for heart failure and 1.12 (0.91–1.38) for T2DM]. In addition, the associations of the LLDS with incident cardio-metabolic diseases in which cheese is not included in the score (regular LLDS), as a negative or positive food group, or using a 40 g/day cut-off to define cheese as a positive or negative food group are also shown in **Figure 1**. In multivariable analysis, higher scores on the regular LLDS were associated with a modest, borderline significant, decrease in the risk for myocardial infarction [OR (95% CI) Q5 vs. Q1 = 0.69 (0.47–1.02), $P = 0.056$], and a substantial and significant decrease in risk for T2DM [0.54 (0.43–0.67), $P < 0.001$] and all-cause mortality [0.62 (0.50–0.76), $P < 0.001$]. The inclusion of cheese either as a positive or negative food group in the LLDS resulted in an attenuation of the borderline association of the LLDS and myocardial infarction, while the inverse associations with T2DM and all-cause mortality remained statistically significant, independent on how cheese was introduced in the score. Inclusion of cheese defined by the 40 g/day cut-off did not affect the associations with cardio-metabolic diseases.

The association of cheese and the different LLDS with all-cause mortality is depicted in **Figure 2.** Adjusted for relevant confounders, cheese was not associated with all-cause mortality [0.94 (0.78–1.14)]. The inclusion of cheese in the LLDS, either as a positive or negative food group, or when applying the 40 g/day cut-off, did not result in a different association with all-cause mortality compared to the regular LLDS. All types of LLDS were significantly inversely associated with all-cause mortality. None of the associations between cheese intake and health were modified by gender.

Cheese Intake and Dp-ucMGP

The association between cheese intake and dp-ucMGP was assessed in a subset of 1,059 Lifelines participants. A total of 816 participants (77.1%) had a plasma dp-ucMGP level under the detection level of 300 pmol/L. We found no significant association between cheese intake in g/day and plasma dp-ucMGP levels (Spearman's rho = 0.032, $p = 0.304$). However, when investigating the association in 243 participants with dp-ucMGP levels above the detection level of 300 pmol/L, a non-significant but negative association was found (Spearman's rho = −0.029, $p = 0.656$). The association between cheese intake, presented for quintiles of cheese intake, and plasma dp-ucMGP levels is depicted in **Table 3**. We found no significant trend across these quintiles (p for trend = 0.193). Also in confounder adjusted logistic regression, no significant association between cheese intake in quintiles and having a dp-ucMGP above 300 pmol/L was found ($p = 0.093$) (**Table 4**). Furthermore, the association was not modified by gender.

DISCUSSION

In this study in the general population, the LLDS was significantly associated with a lower risk of T2DM and all-cause mortality. Inclusion of cheese intake in the LLDS did not alter the associations compared to the regular LLDS. In an elderly subset

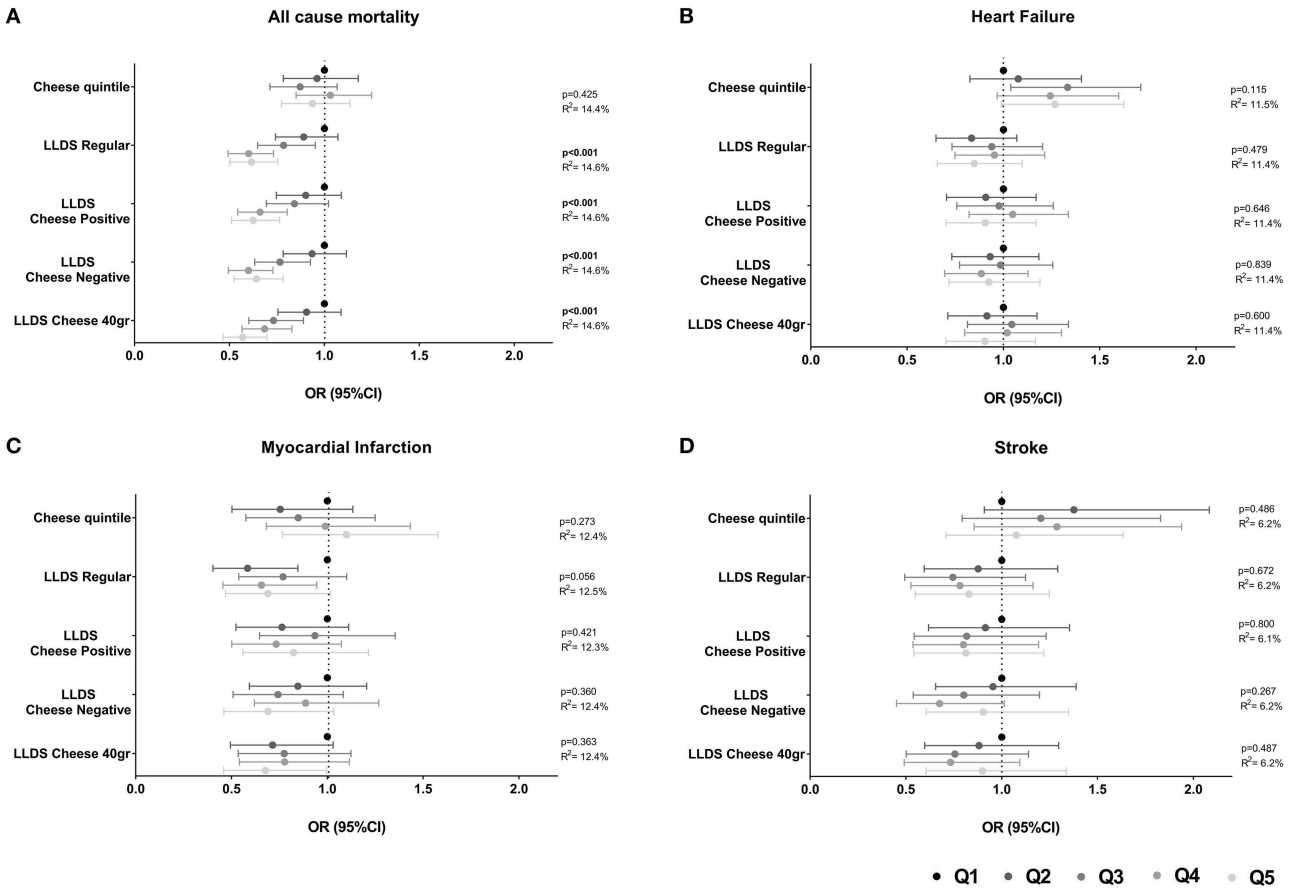

FIGURE 1 | (A–D) Associations of quintiles of cheese intake (based on intake in g/1,000 kcal) with cardio-metabolic diseases ($N = 78,774$). Low cheese intake or Q1 of the LLDS as reference. Adjusted for: energy intake, diet quality (LLDS), education level, age, gender, smoking status, alcohol intake, moderate-vigorous physical activity, and BMI.

of the population we investigated whether cheese intake was associated with plasma dp-ucMGP level. We found no significant association of total cheese intake with plasma dp-ucMGP levels.

Our findings are in line with current literature. There is no scientific consensus on the health effects of cheese (27–29). In the present study the absence of an association with health outcomes may be the result of the exclusion of high risk individuals, but may also be explained by the observation that cheese was consumed by those who had a relatively healthy diet (30). However, the association between cheese and health outcomes did not differ in models with and without adjustment of diet quality (LLDS).

The absence of an significant association of cheese with health outcomes may be the result of cheese being a relative "balanced" food with both healthy and less healthy components. While cheese is one of the major sources of saturated fat, which potentially increases plasma levels of low-density lipoprotein-cholesterol (LDL-C) (31), it has also a high content of calcium, which binds with fatty acids in the intestine to form insoluble soap leading to reduced absorption of fat, promoting a higher excretion of fecal fat (32). The both potential positive and negative effects of cheese consumption have been previously been observed with respect to blood lipids (33–35), and may well-explain the largely unchanged associations of the LLDS with and without cheese with clinical end points in the present study.

In the current study, for the regular LLDS we found no association with cardiovascular diseases. This may be due to the relatively low incidence—and hence low discriminative power-in this population with an average age at baseline of 44 years, but also be the result of the exclusively self-reported data that defined these health outcomes. This, in contrast to T2DM and all-cause mortality for which laboratory parameters or municipal register data was available.

Since it has been shown that cheese is the richest source of long-chain menaquinones (vitamin K2) in the Western diet (36), it would be expected that higher cheese intake is associated with lower dp-ucMGP levels. We could, however, not detect such an association in the total sample of participants with measured dp-ucMGP. The absence of this association may be related to the high lower limit of quantification of the dp-ucMGP assay (i.e., 300 pmol/L), as in the small subgroup of participants above this lower limit, an inverse association between cheese intake and

dp-ucMGP was found, although not significant. Additionally, it should be noted, however, that actual vitamin K2 content varies considerably and relies on the type of cheese, the time of ripening, the fat content and the region where the cheeses are produced (12). Since the vitamin K2 content varies substantially between different types of cheese, total cheese intake may not be an accurate marker for actual vitamin K2 intake. Given the unavailability of more specific and detailed data on cheese intake (i.e., exact type of cheese and time of ripening) in this study population, we were not able the assess the association of intake of different types of cheese with dp-ucMGP levels or adjust for intake of different types of cheese in analyses. Furthermore, although it has been suggested that vitamin K2 is more effective in activating extra-hepatic vitamin K-dependent proteins than vitamin K1 (16), vitamin K1 also contributes to vitamin K2 status (37). In the present study, intake of vitamin K1 (mainly from green vegetables) may also have contributed to dp-ucMGP levels.

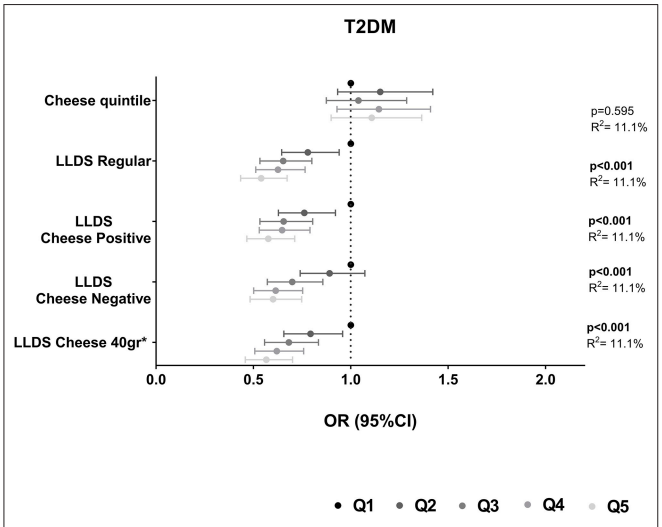

FIGURE 2 | Associations of quintiles of cheese intake (based on intake in g/1,000 kcal) with all-cause mortality (N = 119,435). Low cheese intake or Q1 of the LLDS as reference. Adjusted for: energy intake, diet quality (LLDS), education level, age, gender, smoking status, alcohol intake, moderate-vigorous physical activity, and BMI.

Some limitations of the present study need to be addressed. First, dietary intake was based on self-reported data, and are subject to recall bias. Second, given the lower limit of quantitation of the dp-ucMGP assay (i.e., 300 pmol/L), this assay may not be sensitive enough to detect effects of cheese intake in the LifeLines study population in which 77% of the subjects had dp-ucMGP levels <300 pmol/L. In addition, the cross-sectional design may not have been the most appropriate for testing the association of cheese intake and plasma dp-ucMGP levels, as it is not possible to fully adjust and account for factors affecting plasma dp-ucMGP levels. Intervention studies may be more appropriate to investigate the effect of cheese intake on dp-ucMGP levels. For example, Dalmeijer et al. demonstrated that plasma dp-ucMGP concentrations decreased significantly and dose-dependently after 12 weeks of menaquinone supplementation (38).

Major strengths of the present study are the large sample size, prospective design, long-term follow-up and the availability of data on many potential confounding factors. Furthermore, median daily cheese intake in the dp-ucMGP sub-cohort (28.8 g/day) is highly comparable to the estimated intake in the adult Dutch population (29.0 g/day). As expected, the evidence based LLDS shows clear dose-response associations with health outcomes like T2DM and all-cause mortality, which adds to the validity of the score as a measure of relative diet quality. The

TABLE 3 | Cheese intake (median [range]) and number of participants with dp-ucMGP >300 pmol/L across quintiles of daily cheese intake.

	Quintiles of cheese intake					
	Q1 (N = 140)	**Q2 (N = 169)**	**Q3 (N = 230)**	**Q4 (N = 260)**	**Q5 (N = 260)**	$P_{linear\ trend}$
Cheese intake in g/day [median (range)]	6 (0–10)	16 (10-19)	24 (19-29)	39 (29-44)	64 (45–161)	
Dp-ucMGP > 300 pmol/L [n (%)]	23 (16.4%)	44 (26%)	49 (21.3%)	64 (24.6%)	63 (24.2%)	0.193

TABLE 4 | Logistic regression analysis to investigate the association of cheese intake quintile (g/day) and the odds of having a dp-ucMGP > 300 pmol/L.

	Quintiles of Cheese intake					
	Q1 (Reference)	**Q2 [OR (95% CI)]**	**Q3 [OR (95% CI)]**	**Q4 [OR (95% CI)]**	**Q5 [OR (95% CI)]**	***P-value***
Model 1	N.A.	1.92 (1.08–3.40)	1.60 (0.91–2.81)	2.00 (1.15–3.47)	2.11 (1.20–3.73)	0.093
Model 2	N.A.	1.92 (1.08–3.40)	1.60 (0.91–2.82)	2.00 (1.15–3.48)	2.11 (1.20–3.74)	0.093

N = 1,048.

Models 1: Adjusted for energy intake, education level, age, gender, smoking status, alcohol intake, moderate-vigorous physical activity, and BMI. Models 2: adjusted for model 1 + diet quality (LLDS).

absence of a clear (dose-response) association of the LLDS with stroke, myocardial infarction and heart failure may be related to a lower data quality for these cardiovascular health outcomes. In contrary to T2DM and al-cause mortality, the identification of incident cases for these three cardiovascular outcomes relied solely on self-reporting.

In conclusion, in this population based cohort study, the intake of cheese was not associated with cardio-metabolic health outcomes or all-cause mortality and we found no significant association of total cheese intake with plasma dp-ucMGP. Furthermore, the inclusion of cheese in the LLDS did not change the inverse associations with T2DM and all-cause mortality compared to the LLDS without cheese. Therefore, the results suggest that cheese is a neutral food that fits a healthy diet.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available. Data is available by contacting the lifelines research office. Please see www.lifelines.nl.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Lifelines study is conducted according to the principles of the Declaration of Helsinki and approved by the Medical Ethics Committee of the University Medical Center Groningen, the Netherlands. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LD, PV, IR, GN, and EH contributed to the conception and design of the study. LD, PV, and IR organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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The funder, the Dutch Dairy Association, had no involvement with the study design, data collection and analysis, decision to publish, or preparation 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.2019.00185/full#supplementary-material

Figure S1 | Flow-chart of included participants.

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Dairy and Fruit Listed as Main Ingredients Improve NRF8.3 Nutrient Density Scores of Children's Snacks

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Background: The US Food and Drug Administration has modified its regulations on nutrient content claims by considering healthy dietary ingredients as well as nutrients.

Objective: To assess the relation between dairy and fruit as main ingredients in children's snacks and the Nutrient Rich Food (NRF8.3) nutrient density score.

Methods: Commonly consumed children's snacks in the United States, Canada, France, and the United Kingdom (n = 261) were assigned into USDA What We Eat in America (WWEI) categories. Nutrient composition data came from industry websites, open-source government databases (USDA Standard Reference SR28; CIQUAL), and back-of-pack food labels. Nutrient density was calculated using the Nutrient Rich Food Index NRF8.3. Snacks with dairy or fruit as the first listed ingredient (n = 115) were compared to those that listed neither (n = 146). Snacks that contained fruits-vegetables-nuts (FVN) (n = 88) were compared to those that did not (n = 173).

Results: NRF8.3 scores were higher for snacks listing dairy or fruit as main ingredients. Dairy or fruit when listed as the first ingredient were associated with higher percent daily values of protein, fiber, calcium, vitamin A, vitamin C, and vitamin D, lower saturated fat content and a 30-point increment in NRF8.3 scores. The presence of FVN was associated with a 22-point increment in NRF8.3 scores.

Conclusion: The correspondence between back-of-pack food ingredients and the nutrient based NRF8.3 scores suggests that ingredients can also be used to communicate the nutritional value of foods to the consumer. Dairy and fruit, when listed as first ingredients, were an important component of the NRF8.3 nutrient density score.

Keywords: nutrient profiling, nutrient-rich food index, ingredient list, dairy, fruit, snacks, WWEIA, food-based dietary guidelines

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INTRODUCTION

Methods to assess nutrient density of foods, based on their nutrient composition, have become known as nutrient profiling (NP) (1–6). Foods contain a variety of beneficial nutrients that may include protein, fiber, and a variety of required vitamins and minerals but they can also include excessive amounts of saturated fat, sugar, and salt (7). Designed to capture each food's overall nutritional value (2, 7). NP models aim to separate foods that are energy-dense from those that are nutrient-rich. Most NP models strive to include the beneficial nutrients to encourage and to limit excessive fat, sugar, and salt (4–7).

The development of NP models in the US and in the European Union (EU] has been guided by the regulatory environment (7–9). With some exceptions (6, 10), regulatory decisions on what foods qualify as "healthy" have been for the most part nutrient-based. The US Food and Drug Administration (FDA) standards required "healthy" foods to contain adequate amounts of protein, fiber, vitamin A, vitamin C, calcium, and iron (qualifying nutrients), without exceeding the limits for fat, saturated fat, cholesterol, and sodium (disqualifying nutrients) (1, 11). The initial NRF9.3 NP model in the US was accordingly based on protein, fiber, vitamin A, vitamin C, vitamin E, calcium, iron, potassium, and magnesium. It was recently modified to include vitamin D (1, 12). Nutrients to limit were saturated fat, added sugar, and sodium (8, 9).

In 2015, the food company KIND LLC submitted a citizen petition requesting that the FDA revisit its definition of a "healthy" food (13). This was in response to an FDA Warning Letter, which requested the company remove the word "healthy" from its packaging (14). KIND bars with nuts failed to meet the implicit nutrient content claim of "healthy" because they contained >1 g of saturated fats per Reference Amount Customarily Consumed (RACC) and because >15% of energy came from saturated fats. The KIND petition argued that purely nutrient-based standards were no longer supported by science and that the inclusion of healthy ingredients was more important than was the overall saturated fat content. The KIND petition asked the FDA to allow nutrient content claims on those products that contained meaningful amounts of health promoting foods, defined as vegetables, whole fruits, whole grains, legumes, and nuts. By May 2016, FDA reversed its earlier decision and allowed KIND to use the term "healthy" on its products.

This policy shift from healthy nutrients to healthy food ingredients was in line with current research on healthy food patterns in the US (15, 16). While the US Dietary Guidelines for Americans 2015–2020 have continued to stress the importance of excess saturated fat, sugar and salt, the focus of dietary advice has shifted toward the overall quality of habitual food patterns (17). Healthy food patterns are described as those with more low-fat dairy, more fruits and vegetables, and more whole grains, legumes, and nuts (17). Consistent with the US advice, the French National Plan for Nutrition and Health (PNNS) has recommended five servings of fruits and vegetables and three servings of milk and dairy per day (18).

The likely recognition of healthy food ingredients by US federal and other agencies opens the door to new hybrid NP approaches to NP modeling (1). While milk and fruit are viewed as desirable components of children's snacks, few studies have quantified the relation between ingredients listed on back-of-pack and algorithm-based nutrient density scores (19). The present approach was to screen a selection of commonly consumed children's snacks in the US, Canada, France, and the UK using a version of the Nutrient Rich Foods (NRF8.3) model. The contribution of milk and fruit listed as first ingredients on the back-of-pack ingredient label to NRF8.3 scores was examined as was the contribution to NRF8.3 scores of fruit, vegetables, and nuts.

MATERIALS AND METHODS

Nutrient Composition of Snacks

Popular children's snacks ($n = 261$) in the US ($n = 65$), Canada ($n = 60$), France ($n = 65$), and the UK ($n = 71$) were identified through marketing research data in respective countries. The data were informed by sales figures from consumer research conducted by Nielsen (France, Canada, US), Ipsos (France, Canada), BCG (US), and Kantar (UK) and made available to MOM Materne Mont Blanc Group. The nutrient composition of snacks and the lists of ingredients were obtained from two main sources: (1) Back-of-pack nutrition fact panels and lists of ingredients, available from product packaging or from company websites; (2) government agency nutrient composition databases, some of them with data for branded products. The databases included the branded US Department of Agriculture Standard reference database SR28, now supplemented with a list ingredients for each product and the French nutrient composition database CIQUAL maintained by the French agency for food, environmental, and occupational health safety (ANSES), the French equivalent of the FDA. Nutrients missing from the nutrition facts panels or from company websites were imputed from nearest matches in the SR-28 for US and Canada and from the CIQUAL databases for France and the UK. Data for added sugar in US and Canada came from the USDA; data for added sugar in France came from a customized version of CIQUAL used in previous studies.

The 261 snacks were categorized by major categories and subcategories used in analyses of the What We Eat in America (WWEIA) data by the US Dietary Guidelines Advisory Committee 2015–2020 (US DGAC) (20). The major DGAC categories are dairy, protein foods, mixed dishes, grains, snacks and sweets, fruit, vegetables, beverages, and condiments (20). Not surprisingly, the typical children's snacks did not include vegetables or legumes, meat, poultry or fish, or eggs, mixed dishes, or condiments. Rather, most of the best-selling snacks came from such categories as sweet grains, candy and other sweets, dairy products (milk, yogurt, sugar sweetened beverages and 100% juices, fruits snacks, nuts, and seeds.

The Nutrient Rich (NRF8.3) Model

The public-domain algorithm for the Nutrient Rich Food Index (NRFn.3) is given by NRn—LIM = NRFn.3, where n represents a variable number of nutrients to encourage and LIM represents 3 nutrients to limit (1–3). In published studies, the number of qualifying nutrients to encourage has varied from 6 (NRF 6.3) to 15 (NRF 15.3) (8, 9). The LIM score was always based on the same 3 nutrients of public health concern: saturated fat, added sugar, and sodium (8, 9). The basis of calculation was 100 kcal. The NRF score can be applied to foods, composite meals, and to total diets (12).

The present NR8 subscore was the sum of percentage daily values (%DVs) for 8 qualifying nutrients: protein, fiber, vitamins A, C, and D, calcium, iron, and potassium. Vitamin D replaced vitamin E and magnesium was omitted. The %DVs for qualifying nutrients were capped at 100%. The negative LIM sub-score was the sum of %MRVs (Maximum Recommended Values) for 3

disqualifying nutrients: saturated fat, added sugar, and sodium. Percent MRVs were also calculated for total sugar (21). Total sugars include those naturally present in fruit and/or dairy.

Nutrient density calculations are generally based on nutrient standards per reference amount of food, whether 100 kcal, 100 g, or serving size (11, 22, 23). Nutrient standards for the US and the European Union are close but not exactly the same. For ease of transnational comparisons, a single set of US-based standards was applied to snacks from all markets to assure uniformity in NP modeling.

The 8 nutrients to encourage and standard reference amounts were as follows: protein (50 g), fiber (28 g), vitamin A (800 μg), vitamin C (80 mg), vitamin D (15 μg), calcium (1,000 mg), iron (18 mg), and potassium (4,700 mg). The three nutrients to limit and maximum recommended values (MRVs) were: added sugar (50 g), saturated fat (20 g), and sodium (2,400 mg). The NRF8.3 was calculated as follows:

$$NR8 = \sum_{i=1}^{8} \frac{\frac{Content_i}{DVi} \times 100}{Energy\ density}$$

and

$$LIM = \sum_{i=1}^{3} \frac{\frac{Content_i}{MRV_i} \times 100}{Energy\ density}$$

where content i is the food's content of each nutrient i, and DVi is the reference daily value (DV) for that nutrient. In NR calculation, each nutrient i is expressed in percentage of DV per 100 kcal (rather than per gram or serving). Percent DVs for nutrients to encourage were truncated at 100, so that an excessively high content of a single nutrient would not lead to excessive NRF scores.

The Back-of-Pack Ingredient List

The USDA SR28 branded nutrient composition database, developed in collaboration with ILSI North America, includes both nutrients and ingredients (24). Ingredients are also provided on the back-of-pack label alongside the nutrition facts panel and can be obtained from company websites.

While no specific amounts are provided on the ingredients list, ingredients are typically listed in the order of importance. In the US, the section 101.4, Title 21 of the Code of Federal Regulation (CFR) (25) specifies how ingredients must be designated. The current rule of the US Food and Drug Administration is that the ingredients on a product label must be listed in order of predominance, with the ingredients used in the greatest amount first, followed in descending order by those in smaller amounts.

The number of back-of-pack ingredients can vary widely, depending on local regulatory requirements and the manufacturer's own clean label policy. For example, in the US, GoGoSqueez lists "apple, apple puree concentrate, lemon juice concentrate" whereas Haagen Dazs ice cream lists "cream, skim milk, egg yolks, vanilla extract." KIND granola bar lists "oats, tapioca syrup, semi-sweet chocolate (unsweetened chocolate, cane sugar, cocoa butter, vanilla extract), honey, canola oil, brown rice, brown rice flour, cane sugar, sorghum, sea salt, quinoa, vanilla extract, vitamin E." The ingredients of Oreo chocolate sandwich cookies are given as "sugar, unbleached enriched flour (wheat flour, niacin, reduced iron, thiamine mononitrate [vitamin B1], riboflavin [vitamin B2], folic acid), high oleic canola and/or palm and/or canola oil, cocoa (processed with alkali), high fructose corn syrup, leavening (baking soda and/or calcium phosphate), cornstarch, salt, soy lecithin, vanillin—an artificial flavor, chocolate."

The more recent European Commission notice 2017/C393/05 has taken this a step further by providing guidelines on the quantitative declaration of ingredients (QUID) used in the manufacture of preprocessed foods (26). QUID is required when the ingredient (or category of ingredients) is included in the name of the food and is expressed as numerical percentage by weight. At this time, the QUID requirement covers products containing fruit or dairy (e.g., strawberry yogurt) and can be found on food labels in the EU. For example, strawberry flavored yogurt from Danone lists "whole milk (59.5%), skim milk powder, sugar (8.2%), red fruit (blackberry, strawberry, raspberry 5%), raspberry (5%), strawberry (5%).

The present analyses used two sets of criteria to examine the contribution of dairy or fruit to nutrient density. The presence of dairy was counted only if milk, yogurt, or cheese were listed as the first ingredient. Milk chocolate or milk powder, skim milk powder, or modified milk solids occurring further down the ingredient list did not qualify. The presence of fruit was counted only if fruit was listed as the first ingredient. Second place following water was allowed, but not after sugar or another ingredient Ingredients lists for e.g., orange juice from concentrate often take the form of: "water, oranges." While fruit purees were included, cakes with fruit jam, fruit pectins or fruit flavors did not qualify. Following FDA position on added sugars, concentrated fruit juices (used as sweeteners in processed foods) did not count and neither did dairy components such as dehydrated milk solids.

Second, the products were classified by the presence of fruit, vegetables, or nuts (FVN), a more common approach, already used in some NP models (10). The FVN scoring system developed for the Food Standards Agency—Office of Communications (FSA-Ofcom) in the UK was initially based on recipes provided by the manufacturers (10). However, these procedures were recently modified by Public Health England (27). In the absence of recipes, we looked for the occurrence of FVN on the ingredient list. Peanuts and seeds were included in the FVN category. Vegetables were not a frequent ingredient of popular children's snacks. Starchy vegetables (potatoes, corn) in the form of chips did not qualify for inclusion in the vegetable category.

Plan of Analysis

Nutrient content of snacks was expressed as % Daily Value (%DV) for nutrients to encourage and as % Maximum Recommended Value (%MRV) for nutrients to limit. Energy density (kcal/100 g), the NRF9.3 nutrient density scores and the LIM subscores were calculated for snacks aggregated by WWEIA categories. Snacks that contained fruit or dairy were compared to those that did not on both nutrients and nutrient density scores. Snacks that contained FVN were compared to those that did not.

TABLE 1 | Energy Density (ED) in kcal/100 g, LIM subscore, and NRF8.3 scores for snacks aggregated to WWEIA categories.

	WWEIA food group	*N*	Energy density kcal/100 g		LIM subscore		NRF8.3 nutrient density score		WWEIA codes
			Mean	SD	Mean	SD	Mean	SD	
1	Low fat milk, plain and flavored	6	59	22	16.09	6.16	37.67	24.48	1004, 1006, 1204, 1206
2	Cheese	13	293	114	22.95	10.31	14.38	15.99	1602
3	Yogurt, whole/reduced	21	96	26	23.38	8.10	25.49	29.30	1802
4	Yogurt, low fat/non-fat	18	80	18	18.20	8.26	30.25	18.59	1804
5	Nuts and seeds	16	504	97	14.28	9.89	2.72	13.99	2804
6	Muffins, biscuits	2	428	29	13.82	4.75	13.57	31.59	4402
7	Potato chips	6	534	22	8.60	2.97	1.98	9.60	5002
8	Corn chips	4	508	24	7.08	3.82	−1.93	3.94	5004, 5008
9	Popcorn	2	541	21	11.41	1.64	−0.62	7.24	5006
10	Crackers	10	463	36	11.59	3.84	0.66	8.11	5202, 5204
11	Cereal bars	15	390	35	11.49	6.72	22.06	21.03	5402
12	Nutrition bars	2	378	40	13.81	13.88	42.01	2.61	5404
13	Cakes, cookies, brownies	27	438	55	22.42	7.86	−9.53	11.46	5502, 5504, 5506
14	Candy, chocolate	19	517	34	32.02	8.16	−21.79	7.63	5702
15	Candy, fruit	27	331	68	12.67	13.83	22.38	44.44	5704
16	Ice cream	8	209	80	31.72	5.19	−11.79	11.39	5802
17	Pudding/dairy dessert	16	140	47	30.22	8.69	−2.62	17.09	5804
18	Ices	2	108	35	35.75	2.62	26.85	58.33	5806
19	Apples, bananas, berries	18	79	71	2.69	7.24	48.88	45.87	6002, 6004, 6010
20	Fruit, dried	2	316	23	0.59	0.29	19.58	2.76	6016
21	Fruit salad	7	70	8	10.08	15.86	40.62	36.78	6018
22	Citrus, apple, and other juices	7	48	2	0.33	0.26	67.55	24.11	7002, 7004, 7006
23	Sugar sweetened sodas	5	59	55	44.61	17.25	−43.98	16.94	7202
24	Fruit based soft drinks	8	40	10	30.46	18.51	−1.90	38.57	7204
	Total:	261	277	185	18.63	13.40	12.26	33.87	

Tests for significant differences between means were based on one-way ANOVAs.

RESULTS

Energy density (kcal/100 g), the LIM subscores, and the NRF 8.3 scores for WWEIA categories are shown in **Table 1**. WWEIA identification codes and the number of items per category are provided as well. As expected, the most popular children's snacks in 4 countries were mostly cakes, cookies, brownies ($N = 27$), fruit candies ($N = 27$), chocolate candies ($N = 19$), sweet grains, and flavored yogurts. Mean energy density of children's snacks ranged from <50 kcal/100 g for whole fruit and fruit based soft drinks to more than 500 kcal/100 g for energy-dense chocolate, chips, and nuts. Among snacks with energy density <200 kcal/100 g were milk and yogurts, unsweetened fruit, 100% fruit juices, and other sweetened beverages. Among snacks with energy density of >200 kcal/100 g were fruit candy and snacks with added sugar, sweet grains, savory snacks, candy bars, and cheeses.

Figure 1 is a scatterplot of the relation between energy density (kcal/100 g) and nutrient density (NRF8.3) scores per 100 kcal for snack categories. Individual snacks were aggregated to WWEIA categories and the size of the circle denotes the number of items in each category. There was a clear separation between energy-dense snacks and snacks of lower energy density, which included unsweetened fruit and low-fat milk and yogurt. Chocolate candy, cakes, and crackers had relatively low NRF8.3 scores. Ice cream and pudding (dairy desserts) had lower energy density but also low NRF8.3 score, because of added sugar and saturated fat. Higher NRF8.3 scores were awarded to energy dense nutrition bars, cereal bars, dried fruit, cheese, and nuts and seeds.

Energy density (kcal/100 g) and LIM scores (saturated fat, added sugar and sodium) are correlated but not the same (8, 9). **Figure 2** is a scatterplot of the relation between the LIM subscore and the nutrient density (NRF8) subscore, with snacks aggregated to WWEIA categories. There was an inverse relation between LIM subscores and NRF8.3 nutrient density score. The continuum of nutrient densities ran from sugar sweetened soft drinks to 100% citrus juice. Snacks based on milk, yogurt, and fruit had higher NRF scores as compared to candy, cakes and desserts.

Figure 3 is a scatterplot of the relation between energy density of individual snacks and their nutrient density (NRF8.3). The lowest nutrient density scores were for Coca-Cola; jellies and

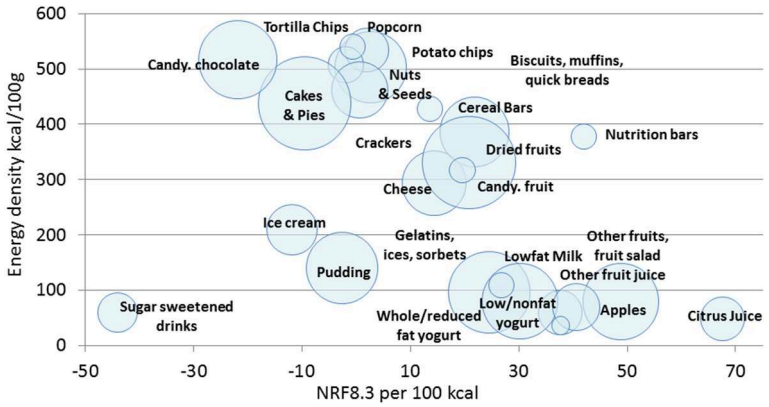

FIGURE 1 | A scatterplot of NRF8.3 nutrient density per 100 kcal (x axis) and energy density kcal/100 g (y axis) of children's snacks the US, Canada, France and UK ($n = 261$). Snacks are aggregated by WWEIA food categories and labeled.

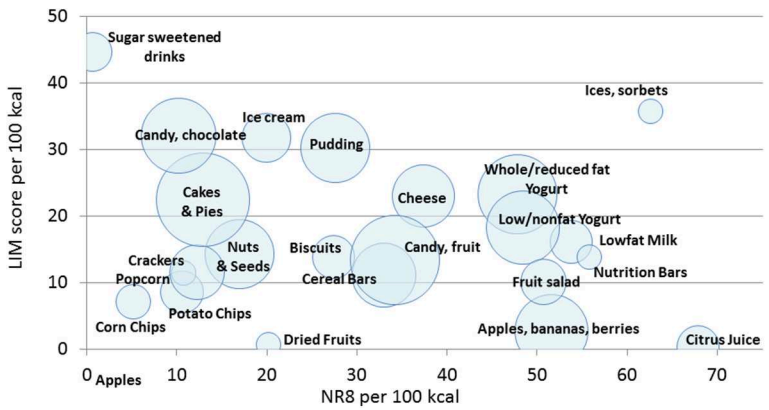

FIGURE 2 | A scatterplot of NR8 nutrient density subscore per 100 kcal (x axis) and LIM scores (y axis) of children's snacks the US, Canada, France and UK ($n = 261$). Snacks are aggregated by WWEIA food categories and labeled.

lollipops, chocolate candy, fruit drinks with added sugar and cookies and cake. The highest scores were for low-fat milk and low-fat plain yogurts, whole fruit, orange juice, Greek yogurt, and strawberries.

Again, energy density and LIM scores were not the same. Lower- energy density foods had variable LIM scores, depending on added sugar content. **Figure 4** shows that the highest LIM and lowest nutrient density NR8 subscores were for a sugar sweetened beverage followed by other sweetened foods. By contrast, low-fat milk and low-fat yogurt scored higher as did apples, bananas and berries and citrus juice. Children's snacks based on unsweetened fruit, plain yogurt, and low-fat milk ranked relatively high on the nutrient density continuum.

The Contribution of Dairy and Fruit as Main Ingredients to NRF8.3 Scores

The relation between dairy or fruit, listed as first ingredients on back-of-pack and the NRF8.3 score is shown in **Table 2**. Also shown are percent daily values (%DV) for NRF8.3 components and the NR8 and LIM subscores. When milk, yogurt, cheese or fruit were listed as the first ingredient, the snacks were higher in protein, fiber, vitamin A, vitamin C, vitamin D, calcium, iron, and potassium. Total sugar was higher because of naturally occurring sugar in milk and fruit; there was no difference in saturated fat, added sugar, or sodium. As might be expected, higher protein, vitamin A, vitamin D, calcium, and potassium came from dairy, whereas higher values for fiber and vitamin C were due to fruit.

Those snacks that listed dairy or fruit as the first ingredient had higher NRF8.3 scores compared to those that did not. Energy density was significantly lower (126 kcal/100 g compared to 395 kcal/100 g). The NR8 score was higher and the LIM subscore was lower. The NRF8.3 score was higher by an average of 30 points ($p < 0.001$).

Those snacks that listed FVN also had higher NRF8.3 scores compared to those that did not. Fewer children's snacks contained vegetables and so the category size was reduced. As expected, listed FVN content was associated with higher %DV values for fiber, vitamin C and potassium (but not protein). Saturated fat was reduced, total sugar was increased (fruit) and there was a reduction in added sugar and sodium. Energy density was significantly lower (213 kcal/100 g compared to 309

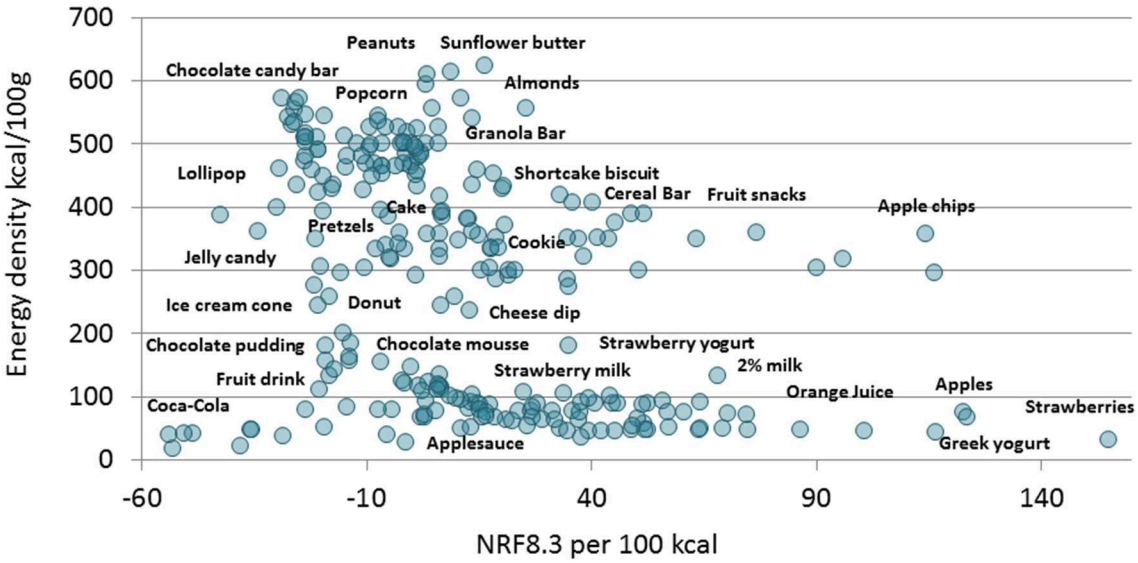

FIGURE 3 | A scatterplot of NRF8.3 nutrient density per 100 kcal (x axis) and energy density kcal/100 g (y axis) of children's snacks the US, Canada, France and UK ($n = 261$). Snacks are shown individually and partly labeled.

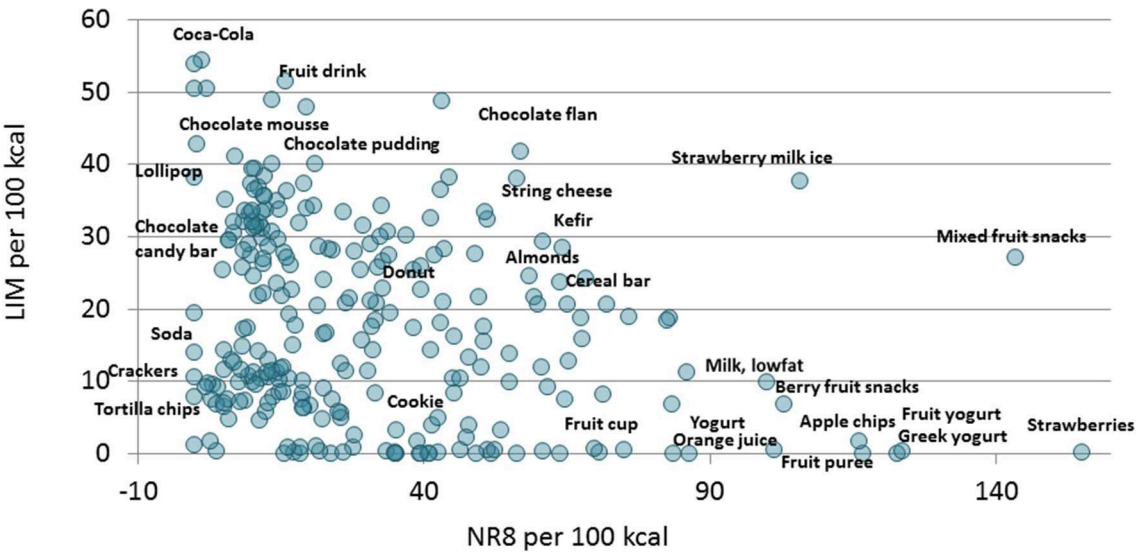

FIGURE 4 | A scatterplot of NR8 nutrient density subscore per 100 kcal (x axis) and LIM scores (y axis) of children's snacks the US, Canada, France and UK ($n = 261$). Snacks are shown individually and partly labeled.

kcal/100 g). The NR8 score was higher and the LIM subscore was lower. The NRF8.3 score was higher by an average of 22 points ($p < 0001$).

DISCUSSION

The present analyses were based on branded open-source nutrient composition databases for commonly consumed children's snacks in the United States, Canada, France and the United Kingdom, as identified by marketing research. Energy density and nutrient density measures were obtained. Energy density of children's snacks has been used in the past to distinguish between healthier and less healthy snacks (28). The present snacks varied greatly by energy density per 100 g. Energy-dense snacks (>200 kcal/100 g) were chocolate, nuts, chips, cakes, cookies and brownies and other sweetened grains. Lower energy density snacks (with energy density <200 kcal/100 g) were fruits and juices, milks, and plain and flavored yogurts.

Energy density per 100 g is a measure of policy interest. In January 2014, Mexico passed an 8% tax on non-essential foods—that is snacks—with energy density ≥275 kcal/100 g. The tax

TABLE 2 | Nutrient composition %DV 100 kcal of children's snacks by the first ingredient listed on the ingredient label.

	Fruit or Dairy $n = 115$	Neither $n = 146$		Fruit vegetables nuts $n = 88$	No. fruit vegetables nuts $n = 173$	
	Mean (SEM)	Mean (SEM)	*P*	Mean (SEM)	Mean (SEM)	*P*
%Daily Value/100 Kcal						
Protein	6.13 (0.46)	2.71 (0.18)	<0.001	4.03 (0.43)	4.31 (0.31)	0.587
Fiber	3.63 (0.58)	2.39 (0.28)	<0.05	5.04 (0.60)	1.86 (0.30)	<0.001
Vitamin A	5.48 (0.80)	1.15 (0.43)	<0.001	2.81 (0.74)	3.17 (0.56)	0.697
Vitamin C	12.14 (2.30)	6.16 (1.50)	<0.05	15.98 (2.90)	5.13 (1.27)	<0.001
Vitamin D	3.03 (0.48)	0.31 (0.11)	<0.001	1.66 (0.42)	1.35 (0.26)	0.544
Calcium	10.40 (1.08)	2.44 (0.35)	<0.000	5.30 (1.26)	6.27 (0.57)	0.419
Iron	1.47 (0.33)	3.08 (0.32)	<0.001	2.16 (0.44)	2.49 (0.28)	0.522
Potassium	3.35 (0.22)	0.99 (0.10)	<0.001	2.96 (0.26)	1.55 (0.14)	<0.001
%Maximum Recommended Value/100 Kcal						
Saturated fat	5.29 (0.64)	6.00 (0.47)	0.365	3.42 (0.47)	6.84 (0.51)	<0.001
Total sugar	16.28 (0.76)	10.62 (0.65)	<0.001	16.89 (0.94)	11.19 (0.58)	<0.001
Added sugar	9.25 (0.92)	11.66 (1.10)	0.105	8.34 (1.06)	11.67 (0.96)	<0.05
Sodium	2.06 (0.21)	2.53 (0.18)	0.095	1.21 (0.13)	2.89 (0.19)	<0.001
ED (kcal/100 g)	126 (9)	395 (12)	<0.001	213 (20)	309 (13)	<0.001
NR8 subscore	45.65 (2.46)	19.12 (1.80)	<0.001	40.00 (3.30)	26.16 (1.82)	<0.001
LIM subscore	16.60 (1.20)	20.19 (1.11)	<0.05	12.97 (1.35)	21.49 (0.97)	<0.001
NRF8.3 score	29.05 (3.01)	−1.07 (2.25)	<0.001	26.99 (3.93)	4.68 (2.14)	<0.001

was imposed on salty snacks, chips, cakes, pastries, and frozen desserts; and a 1 peso/liter (∼10%) tax on sugar-sweetened beverages. Basing the tax on energy density rather than on healthy ingredients or nutrient content meant that nuts and cereal bars were taxed whereas ice cream was not. By contrast, some past evaluations of nutrient density of snacks, some based on the NRF nutrient density score (29), have focused more on the snacks' nutritional value rather than on their energy density alone. When it comes to snacks, dietary advice has focused on promoting snacks of lower energy density and higher nutrition value (29–31).

It should be noted that some energy-dense snacks were relatively nutrient rich (32); for example, nuts and fortified cereals both had high NRF8.3 scores. By contrast, some low energy-density beverages were nutrient-poor (soda and fruit drinks). As shown above, the LIM score was distinct from energy density since it penalized added sugar in low energy density beverages. In the present dataset, about 60% of the snacks contained added sugar. Although the mean %DV for added sugar was 10%, sugar sweetened beverages and sugar candy were essentially all sugar, providing 100% of energy from added sugar.

Examining the contribution of first-listed ingredients to NRF8.3 scores is a novel component of this paper. This approach follows on the Code of Federal Regulations in the US and the more recent 2017 notice by the European Commission. In the US, the ingredients on a product label must be listed in the order of predominance, with the ingredients listed in the greatest amount listed first. The European Commission has imposed quantitative requirements for key ingredients to be listed as numerical percentage by weight. The implementation of these requirements will open the door to a new generation of NP models that make use of the ingredient list. In the present analyses, dairy or fruit listed as the first ingredient were associated with a 30-point increase in the NRF8.3 nutrient density score. The presence of FVN produced comparable results but the differences were less sharp.

While most NP models are based on nutrients only, some do award additional points to selected food groups, notably FVN. For example, the FSA-Ofcom guidelines (10) and the Australian Health Star Rating System (33) award extra points to foods containing fruit, vegetables, and nuts. The French Nutri-Score (34), directly derived from FSA-Ofcom, has awarded extra points to foods containing fruit, vegetables, legumes, nuts or rapeseed, and to olive or nut oils.

However, the relation between the foods' content of FVN and the nutrient profiling algorithm has always seemed arbitrary. The algorithm used to assess the amounts of fruits, vegetables, or nuts in complex foods for the FSA-Ofcom score was particularly complex (10). The FVN content of foods was then rated along a wholly arbitrary range of 5 points, based on the percent content of FVN per 100 g of food product. Only products that contained >80% FVN were awarded 5 points; products that contained <40% FVN got 0 points, those that contained >40% got 1 point and those that contained >60% got 2 points. The nutrient profiling technical guidance published in 2011 by the Obesity Team at the UK Department of Health runs to 18 detailed pages (10). It is a summary of a more extensive document that is no longer available online (35).

The principal consideration was that only intact fruit and vegetables (including those that were cooked and dried) and those that were minimally processed (peeled, sliced, tinned,

frozen, 100% juices, and purees) could be included when calculating the FSA-Ofcom score. More detailed instructions on how to calculate the FVN content of foods for an updated UK nutrient profiling model were recently published by Public Health England (27). Neither document was meant to assist the shopper. Rather, the PHE guidelines had been produced to assist food manufacturers, retailers and advertisers to correctly calculate nutrient profiling scores for their products (10, 27). That required manufacturers to provide the weight of each ingredient in a product, which would then allow an exact calculation of the fruit, vegetable, and nut content in a food. The present approach was to base calculations on the ingredient listed first, consistent with the US and the EU guidelines. Following the FDA practice, concentrated fruit juice sugars, powders, or leathers were not counted as fruit in the present study. The nut category included peanuts as well as tree nuts.

There are other studies suggesting that the back of pack ingredient list is a potential additional tool for nutrition education. Lacking detailed information on the weight of FVN in food products, the Environmental Working Group in Washington DC (36) developed a separate algorithm that used the order of the ingredient's listing as a proxy for the percentage of fruit, vegetable, or nut in the product. The nutrient content of FVN ingredients was then compared to the foods' content of carbohydrates, sugar or fat in a ratio-based metric (36). The present approach was to quantify the contribution of dairy or fruit listed as first ingredients to the previously established NRF8.3 score. On average, snacks with dairy or fruit as first ingredients scored 30 points higher. Arguably, the inclusion of dairy is more relevant to the rating of children's snacks than are vegetables, which were barely represented in the present market-driven database.

As the dietary guidance is shifting from nutrients to whole foods and food ingredients, NP models need to follow suit. The 2015–2020 DGA defined healthy dietary patterns as composed of a variety of vegetables from all of the subgroups; fruits; grains, at least half of which are whole grains; fat-free or low-fat dairy; a variety of protein foods; and oils (1). Hybrid NP models that combine both nutrients and selected dietary ingredients are more closely aligned with dietary guidance that is increasingly targeted at foods and food groups, rather than isolated nutrients. Some hybrid scores have attempted to include whole grains, plant proteins, seafood, or healthy oils, consistent with the DGAs. However, some of those food groups seem to be underrepresented in children's snacks—hence the resent focus on dairy and fruit, in comparison to a modified FVN approach.

Children's snacks are not often viewed as particularly nutritious and many fall into the category of processed or "ultra-processed foods" (37–39). Whereas, NP models were initially intended to guide consumer behaviors at the point of sale, the NP methodology is increasingly being used by food companies for product screening and reformulation. Those voluntary efforts are monitored by the Access to Nutrition Index (ATNI), a global initiative to evaluate the world's largest food and beverage manufacturers on their policies and practices, including the nutritional value of their product portfolios (40).

CONCLUSIONS

Dairy or fruit, when listed as first ingredients, made an important contribution to the NRF8.3 nutrient density score. The correspondence between nutrient based NP models and the back-of-pack food ingredients suggests that ingredients can also be used to communicate the nutritional value of foods to the consumer. Data from the ingredient list, increasingly available in electronic format, can also be used to construct a new generation of hybrid NP scores for potential use in the (re) formulation and optimization of food products.

DATA AVAILABILITY STATEMENT

The US Department of Agriculture Branded Food Products database is available at FoodData Central: https://fdc.nal.usda.gov/. The French food composition table CIQUAL is available at https://ciqual.anses.fr/. Additional data came from manufacturers' websites and food labels.

AUTHOR CONTRIBUTIONS

AD and CR conceptualized the study and reviewed and approved the manuscript. CR generated the list of snacks from multiple markets and provided nutrient composition data. AD conducted nutrient profiling analyses and took the lead on writing the manuscript. The views expressed in this work are those of the authors and do not necessarily reflect the position or policy of MOM Materne Mont Blanc Group.

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REVIEW
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Bioavailability of Micronutrients From Nutrient-Dense Whole Foods: Zooming in on Dairy, Vegetables, and Fruits

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In order to fully exploit the nutrient density concept, thorough understanding of the biological activity of single nutrients in their interaction with other nutrients and food components from whole foods is important. This review provides a narrative overview of recent insights into nutrient bioavailability from complex foods in humans, highlighting synergistic and antagonistic processes among food components for two different food groups, i.e., dairy, and vegetables and fruits. For dairy, bioavailability of vitamins A, B2, B12 and K, calcium, phosphorous, magnesium, zinc and iodine are discussed, whereas bioavailability of pro-vitamin A, folate, vitamin C and K, potassium, calcium, magnesium and iron are discussed for vegetables and fruits. Although the bioavailability of some nutrients is fairly well-understood, for other nutrients the scientific understanding of uptake, absorption, and bioavailability in humans is still at a nascent stage. Understanding the absorption and bioavailability of nutrients from whole foods in interaction with food components that influence these processes will help to come to individual diet scores that better reflect absorbable nutrient intake in epidemiologic studies that relate dietary intake to health outcomes. Moreover, such knowledge may help in the design of foods, meals, and diets that aid in the supply of bioavailable nutrients to specific target groups.

Keywords: bioavailability, vitamins, minerals, dairy, fruits, vegetables

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INTRODUCTION

Historically, the nutritional sciences are built on the study of single nutrients or food components in relation to health outcomes. Although this has been a useful concept when it comes to specific deficiency diseases, the picture became blurry when studying the role of nutrition in complex diseases. The notion that the sum of the parts does not necessarily explain the result of the whole has prompted a shift in focus from single nutrients to whole foods, meals, and dietary patterns. Studying the biological activity of single nutrients in their interaction with other nutrients and food components from whole foods, especially during their stay in the gastro-intestinal tract, helps to better understand the underlying positive and adverse health effects of whole foods, meals, and dietary patterns.

Bioavailability of nutrients from whole foods is a research area that has received ample attention in the past decades, although studies in humans are still limited. Over time,

some consensus has been reached on the definition of bioavailability, which is the fraction of an ingested nutrient that becomes available for use and storage in the body (1). In this definition, bioavailability goes beyond mere absorption from the gut and also includes the use and storage (retention) in body tissue. The study of absorption and bioavailability of nutrients from foods in humans requires sophisticated methods that take into account endogenous nutrient losses through the enterohepatic circulation as well as incorporation of nutrients into storage tissue. Use of isotopes, both radio-isotopes and stable isotopes, have greatly improved accuracy and precision of *in vivo* nutrient bioavailability studies, either as a single nutrient or as part of a food, meal, or dietary pattern (2). Although *in vitro* methods are much cheaper and faster than *in vivo* methods, allowing for large numbers and experimental conditions, translation of findings to full body human conditions is still cumbersome (3, 4). Therefore, this review uses only bioavailability data from *in vivo* studies in humans, although the underlying mechanisms of interactions between food components are mostly based on *in vitro* or animal studies.

The main objective of this review is to provide an overview of insights into nutrient bioavailability from complex foods in humans, thereby highlighting the current state of knowledge on synergistic and antagonistic processes among food components. Two different food groups are put in the spotlight for this purpose, i.e., dairy, and fruits and vegetables. Both of these food groups contain a myriad of nutrients, for some of which the bioavailability is now well-understood, whereas others still require further study. Both food groups also contain many bioactive components and have a complex matrix, which affect the kinetics of nutrient release, absorption, and bioavailability. Understanding of these processes will help to better predict the true nutrient value of foods and to incorporate this information into diet scores in the future.

MILK AND DAIRY FOODS

Dairy refers to food products that have milk—mostly cow's milk—as their main ingredient such as buttermilk, yogurt, cheese, and all closely related products. Dairy is characterized by a relatively high amount of protein and fat, and can therefore make an important contribution to calorie intake unless low-fat alternatives are consumed. Intake of dairy varies greatly between and within world regions, with an estimated average intake of milk (excluding other dairy products) of ∼200–240 g per day in Western Europe and North America, ∼130–300 g per day in Latin America, ∼100–200 g per day in Africa, and 20–150 g in Asia[1]. Despite controversy around the healthfulness of dairy with respect to non-communicable disease risk, scientific evidence consistently points toward either beneficial or neutral effects (5–10). In addition, positive effects on bone mineral density have been found, as well as reduced fracture risk in some populations (9, 11). Such beneficial effects have been attributed to calcium as well as to various other nutrients and bioactive components present in milk (11).

In industrialized countries, dairy stands out as source of calcium, but it also contributes for 20–40% to the intake of vitamins A, B2, B12, and K as well as of phosphorous, magnesium, zinc, and iodine (12–23)[2]. In the next sections, the absorption and bioavailability of these nutrients from milk and dairy foods will be described, with special attention for calcium.

DAIRY AS A SOURCE OF CALCIUM

Dairy is by far the most important source of calcium in the human diet and it has therefore been studied most extensively among the nutrients derived from dairy. Bovine milk contains an average of 120 mg calcium per 100 mL. In Europe and North America, ∼75% of dietary calcium is derived from milk and dairy products, with an additional 15% from vegetables and fruits, 5% from mineral water and the rest from other foods (24, 25). Approximately 40% of calcium from dairy sources is absorbed under normal circumstances, with higher absorption in children and lower absorption in elderly (26, 27). In the body, 99% of calcium is present in the skeleton. The efficiency of calcium storage in bone tissue is mainly determined by physiological factors (e.g., related to growth, pregnancy, and lactation), and is regulated by several hormones, such as PTH, calcitonin, calcitriol, and estrogens. Excessive absorbed calcium is excreted in urine, feces, and sweat. Adults are generally in negative calcium balance after their peak bone mass (∼35 y) and loose ∼10 mg of calcium each day, although in post-menopausal women the daily loss may be 40 mg per day or more (28). Bioavailability of calcium is determined by absorption in the small intestine on the one hand and by incorporation into bone tissue on the other hand. Both of these processes can be influenced by dietary factors. The bioavailability of calcium may therefore be defined as the fraction of dietary calcium that is absorbed by the intestine and is used for bone mineralization.

Intestinal absorption of calcium mostly happens by passive diffusion, whereas active transport at low and moderate calcium intake is under regulation of vitamin D (24). Fortification of milk with vitamin D2 was shown to enhance calcium absorption (29). A number of milk and dairy components have been found to aid in the passive absorption of calcium (and also of other divalent cations), such as phosphopeptides, casein and whey proteins, lactose and phosphorous (**Table 1**). Phosphopeptides, which are products of the enzymatic hydrolysis of casein, sequester calcium thereby protecting it from precipitation by anions like phosphates in the intestine (30–32). The same is true for alpha lactalbumin and beta lactoglobulin, both whey proteins (24), and for amino acids such as L-lysine and L-arginine (30). The calcium bound to these amino acids, peptides, and proteins is readily released during digestion by bringing it slowly into solution, which is an important prerequisite for passive diffusion.

Lactose also seems to enhance calcium absorption, although the mechanism has long been unclear (42). The most likely explanation is that, like other sugars, lactose widens the

[1]https://www.globaldietarydatabase.org/our-data/data-visualizations/dietary-data-region

[2]https://wateetnederland.nl/resultaten/vitamines-en-mineralen/bronnen

TABLE 1 | Synergistic and antagonistic effects of dairy food components on calcium bioavailability.

Dairy food component	Synergism/antagonism	References
Vitamin D	↑ active absorption of calcium at low and moderate intake	(24)
Casein and phosphopeptides	↑ passive diffusion by binding and slow release of calcium into solution in the chymus	(30–32)
Whey proteins alpha lactalbumin, beta lactoglobulin	↑ passive diffusion by binding and slow release of calcium into solution in the chymus	(24)
Amino acids (L-lysine, L-arginine)	↑ passive diffusion by binding and slow release of calcium into solution in the chymus	(30)
Lactose	↑ passive diffusion by widening the paracellular spaces in the enteric cell lining	(33)
Phosphorous	↓ absorption by binding into undigestible complexes ↑ re-absorption of calcium in the distal part of the nephron ↑ uptake of absorbed calcium into bone	(24)
Sulfur-containing proteins	↓ calcium balance by inducing hypercalciuria	(34–40)
Fat	↔ formation of insoluble soaps with calcium, but these are dissociated at the low pH of the stomach	(41)

↑ Indicates an increase; ↓ indicates a decrease; ↔ indicates no effect.

paracellular spaces in the enteric cell lining, thereby enhancing passive diffusion (33). However, studies have shown that this mostly happens at relatively high doses of lactose (43, 44), whereas in the amounts present in milk and dairy it is less likely to contribute much to absorption (45, 46). When lactose is hydrolyzed, such as in yogurt, or absent, such as in cheese, calcium absorption does not seem to be affected (47). Nevertheless, lactose seems to be important for calcium absorption in case of high calcium intake in combination with poor solubility, such as seen in babies and the elderly (48, 49). It may be that, similar to galactic-oligosaccharides, lactose functions as a prebiotic and stimulates calcium absorption in the cecum and colon by enhancing the growth of bifidobacteria and thereby maintaining low pH (50, 51). This hypothesis is supported by evidence from lactose-deficient patients, who do not seem to have compromised calcium absorption (52).

Apart from factors that enhance calcium absorption, several dairy components can inhibit the uptake of calcium (**Table 1**). Protein, especially sulfur-containing protein, has been shown to lead to a negative calcium balance through increased urinary calcium excretion (24). Nevertheless, the conclusion of a working group that more recently reviewed the current evidence linking dietary protein intake with bone health was that, although a high protein diet—either of animal or vegetable origin—is associated with increased urinary calcium excretion, this is more likely due to higher intestinal calcium absorption than to bone resorption (53, 54). Milk fats can form insoluble soaps with calcium; however, these are dissociated at the low pH of the stomach and therefore do not affect calcium bioavailability negatively (41). This explains that calcium from cheese is readily available for absorption despite the high content of saturated long chain fatty acids (24, 55).

Phosphorous plays a dual role in calcium absorption, by, on the one hand, binding calcium and inhibiting its absorption in the small intestine resulting in increased fecal excretion of calcium, and, on the other hand, after being absorbed, by increasing the reabsorption of calcium in the distal part of the nephron or by enhancing the uptake of absorbed calcium into bone (24, 54). The inhibitory properties of phosphorous on intestinal calcium absorption may partly be countered by phosphorylation of lactose, thereby keeping calcium in solution (24). However, this hypothesis has not yet been confirmed (41, 55). Also, the high phosphorous content of milk may counter the hypercalciuria induced by protein (54, 56). The recommended dietary intake ratio for calcium (mg) to phosphorous (mg) ranges from 1:1 to 1.5:1, with ratios <0.5 being associated with decreases in bone mineral density (57). Moreover, excessive intake of phosphorous has been shown to induce the secretion of fibroblast growth factor 23 (FGF-23) from bone, thereby decreasing the formation of 1,25-dihydroxyvitamin D3 and decreasing intestinal calcium absorption (57, 58). Cow's milk provides calcium and phosphorus in a reasonable balanced ratio of ∼1.2:1.

Overall, although intestinal absorption of calcium from milk and dairy is very similar compared to other sources such as calcium salts, vegetables, or mineral water, its net effect on calcium retention is generally higher (59) with little difference between the various dairy products (milk, acidified milk, yogurt, skim milk, cream cheese, hard cheeses) (24). Diets including dairy products can therefore be considered as the most optimal calcium-dense option to prevent adverse health effects related to a negative calcium balance.

DAIRY AS A SOURCE OF OTHER NUTRIENTS

Vitamins

Vitamin A

The content of vitamin A in dairy ranges between 15 and 50 μg in 100 mL of milk to over 300 μg per 100 g of full-fat cheese (**Table 2**). Vitamin A occurs in dairy products predominantly as retinyl palmitate (60), but small amounts of ß-carotene can also occur. Little is known about the bioavailability of vitamin A from milk and other dairy products, but one study reports that ∼15% of vitamin A from milk is absorbed and this appeared not to be different for fortified milk (60). Moreover, it did not appear to be different for full fat or skim milk, despite the fact that absorption of fat-soluble vitamins is generally regarded to depend on the fat content of a meal for solubilization and stimulation of biliary secretion and for the formation of micelles (60).

TABLE 2 | Bioavailability of vitamins and minerals from milk and dairy foods in humans.

Nutrient	PRI/AI	Content (per 100 g)		Bioavailability (%)	Enhancing factors	Inhibiting factors
Vitamin A	650 μg/d	Milk Yogurt Soft cheese Cheese	15 μg 31 μg 200–300 μg >300 μg	15%	Dietary fat	
Vitamin B2	1.6 mg/d	Milk Yogurt Soft cheese Cheese	0.18 mg 0.16 mg 0.30–0.60 mg 0.28 mg	67%	Non-covalent binding to protein	Covalent binding
Vitamin B12	4 μg/d	Milk Yogurt Soft cheese Cheese	0.45 μg 0.25 μg 1.1 μg 2.0 μg	65%	Binding to transcobalamin or casein	Binding to haptocorrin
Vitamin K-2	70 μg/d	Milk Yogurt Soft cheese Cheese	0.7–1.4 μg 0.1–3.3 μg 2.0–2.5 μg 68 μg	Unknown	Dietary fat Fermentation products Long-chain menaquinones (MK_{7-9})	Medium-chain menaquinones (MK_4)
Calcium	950 mg/d	Milk Yogurt Soft cheese Cheese	120 mg 125–150 mg 400–600 mg 800 mg	40%	Binding to casein and whey peptides Lactose, amino acids Vitamin D (fortification)	Phosphorous Sulfur-containing proteins
Phosphorous	550 mg/d	Milk Yogurt Soft cheese Cheese	100 mg 100–120 mg 300–500 mg >500 mg	Unknown	Binding to casein and whey peptides Binding to phospholipids	Complexing with unbound calcium
Magnesium	300 mg/d	Milk Yogurt Soft cheese Cheese	10 mg 13 mg 15–25 mg >30 mg	24–75%	Binding to casein and whey peptides Lactose	High dosing
Zinc	7.5 mg/d	Milk Yogurt Soft cheese Cheese	0.4 mg 0.4–0.6 mg 2–3 mg 3.4 mg	25–30%	Mild acidic conditions Whey and casein peptides Low molecular ligands (amino acids, organic acids)	
Iodine	150 μg/d	Milk Yogurt Soft cheese Cheese	15 μg 15 μg 20–40 μg 21 μg	90%	Inorganic, unbound	

PRI, Population Reference Intake; AI, Adequate Intake for adult females. Source: EFSA: https://www.efsa.europa.eu/sites/default/files/assets/DRV_Summary_tables_jan_17.pdf.

Vitamin B2

With 0.18 mg of riboflavin per 100 mL of milk and 0.28 mg per 100 g of cheese, dairy forms an important source of this water soluble vitamin (**Table 2**). In dairy, riboflavin is mostly bound non-covalently to protein, predominantly as flavin adenine dinucleotide (FAD) and to a lesser extent as flavin mononucleotide (FMN). Milk also contains free riboflavin bound to specific binding proteins (21). Hydrolysis of FAD and FMN to riboflavin by phosphatases in the small intestine is a prerequisite for its carrier-mediated absorption (21). Riboflavin has been reported to be readily bioavailable from milk at ∼67% (61).

Vitamin B12

Milk contains ∼0.40–0.45 μg of vitamin B12 per 100 mL, whereas cheese can contain up to 2 μg per 100 g (**Table 2**). The major derivatives of vitamin B12 in bovine milk are hydroxycobalamin, adenosylcobalamin, and methylcobalamin, and it is mostly bound to the proteins haptocorrin, transcobalamin and casein depending on the cow breed (62, 63). Vitamin B12 bound to transcobalamin appeared to be better released *in vitro*, whereas this was cumbersome when bound to haptocorrin (mainly present in buffalo milk) and this may have implications for *in vivo* bioavailability (63). A study in healthy adults > 60 y old, however, revealed that ∼65% of vitamin B12 from milk was absorbed (64), whereas, in comparison, absorption of vitamin B12 from animal foods is generally 50% or lower and even <5% for synthetic supplements (62, 65). Nevertheless, a study comparing cyano-B12 from a supplement with hydroxo-B12 from whey powder improved vitamin B12 status similarly (66).

Vitamin K-2

Menaquinones are primarily synthesized by bacteria, and therefore fermented dairy products such as yogurt and cheese are good sources of this vitamin (67). Milk contains ∼0.7–1.4 μg of menaquinones per 100 mL, whereas full-fat hard cheese can contain up to 68 μg per 100 g (**Table 2**). Intake of long-chain menaquinones (MK_n) in particular has been associated with decreased risk of cardiovascular disease (68, 69), in contrast

to phylloquinone (vitamin K-1) derived from plant-based foods. The menaquinone content of dairy products was assessed to be highest in fermented cheeses and to be positively related to fat content (70, 71). Vitamin K is a fat soluble vitamin, and as such is absorbed through the lipid pathway. The bioavailability of menaquinones from dairy sources has not been studied in humans to date, with the exception of a study showing that MK_7-fortified yogurt resulted in slightly higher plasma concentrations as compared to MK_7 from a soft-gel capsule (72). The length of the isoprene chain strongly determines absorption and metabolism of menaquinones in the sense that MK_{7-9} are better absorbed than MK_4 and have a longer half-life than vitamin K-1 (67, 73).

Minerals and Trace Elements

Phosphorous

With a content of ∼100 mg of phosphorous per 100 mL milk and >500 mg per 100 g cheese, dairy is an important source of phosphorous in the diet (**Table 2**). Although data are still scarce, it is assumed that phosphorous derived from animal foods is more bioavailable than phosphorous derived from plant-based foods, as revealed by balance studies relating phosphorous intake from dietary sources to urinary excretion (74, 75). This may be explained by the binding of phosphorous to digestible compounds in animal foods, such as proteins and phospholipids. However, phosphorous also easily forms indigestible complexes in the gastro-intestinal tract (e.g., with calcium), and its bioavailability from dairy sources may strongly depend on interaction with other meal components (74). No studies have been done to date to directly measure the bioavailability of phosphorous from dietary sources in humans.

Zinc

With a concentration of 0.4 mg of zinc per 100 mL milk forms an important source of zinc (**Table 2**). Zinc is predominantly present in the protein fraction in milk, specifically in casein micelles, but is easily released under mild acidic conditions (76). Approximately 25–30% of zinc is absorbed from milk (77, 78). Apart from whey and casein peptides, also other low molecular ligands and chelators that can bind Zn, such as amino acids (histidine, methionine) and organic acids (citric, malic and lactic acid), may promote zinc absorption (79).

Magnesium

Milk contains ∼10 mg of magnesium per 100 mL, but can be triple that in cheese (**Table 2**). Absorption of magnesium from milk was found to be strongly dose-dependent, with ∼75% absorption reported from a serving of milk containing 46 mg of magnesium (80). With intake of magnesium at physiological doses, absorption seems to be predominantly due to a saturable mechanism and at higher amounts mainly by simple diffusion (80). As for other divalent metals, i.e., calcium, iron and zinc, peptides from casein or whey can bind magnesium, which may promote absorption (81). Also, lactose appeared to promote absorption of magnesium from milk in rats (81, 82), but this was not confirmed in humans (83). As for calcium, unabsorbed lactose may act as a prebiotic and stimulate magnesium uptake in the large intestine, but this needs further investigation (84, 85).

Iodine

Iodine content of milk can vary substantially with a reported range of 3.3–53.4 μg per 100 mL, depending on the way of farming, iodine intake of dairy cows, use of iodine-containing udder cleansers, season, and processing (86, 87). Iodine in milk is predominantly (>80%) present as inorganic iodide, and in line with this iodine bioavailability from milk is high (∼90%) (88).

VEGETABLES AND FRUITS

Vegetables and fruits form a widely diverse food group that contains a broad range of essential nutrients. Vegetables and fruits are generally low in fat and proteins and therefore contribute relatively little to energy intake. Ample consumption of vegetables and fruits is promoted worldwide. Such recommendations are based on studies consistently showing that higher intake of vegetables and fruits is negatively associated with all-cause mortality and mortality from cardiovascular disease and cancer (89, 90). Close to 75% of the world population consumes less than the recommended 400 g of vegetables and fruits on a daily basis (91). Low consumption of vegetables and fruits is estimated to contribute 1.8% to the total global burden of disease, primarily through cardiovascular diseases and cancer (92).

So far, studies have failed to attribute the healthful effects of vegetables and fruits to any of its isolated components. Therefore, health benefits from vegetable and fruit consumption are rather to be explained as the resultant of additive and synergistic effects of its components (63–66). They are a particular rich source of pro-vitamin A carotenoids, vitamin C, folate, vitamin K-1, potassium, calcium, magnesium, iron, and several other trace elements (14, 93)[2].

Non-nutritive bioactive compounds are also present in multitude, comprising of phenolics, carotenoids, and glucosinolates. Although these bioactive compounds are regarded as non-essential for human survival, they may exert health effects such as reduced risk of non-communicable and degenerative diseases (71–76). Delivery of fiber, both digestible and indigestible, is another important nutritional aspect of vegetables and fruits. It has an important impact on satiety, gastrointestinal processing, metabolic parameters, and microbiota composition. It constitutes a group of heterogeneous polymers such as non-starch polysaccharides, cellulose, resistant starch, inulin, lignins, chitins, pectin, beta-glucans, and oligosaccharides. Dietary fiber may stimulate intestinal fermentation, thereby altering the production of microbial phenolic metabolites and enhancing mineral absorption (94, 95). However, dietary fiber can also negatively affect the absorption of nutrients because of gel formation, increased viscosity, or binding and entrapment (96–98). Other compounds present in vegetables and fruits may have negative consequences for human nutrition and health, such as alkaloids, oxalates, phytic acid, lectins, trypsin and protease

TABLE 3 | Bioavailability of vitamins and minerals from vegetables and fruits in humans.

Nutrient	PRI/AI	Content (per 100 g)		Bioavailability (%)	Enhancing factors	Inhibiting factors
(Pro)-vitamin A	650 μg/d	Carrot Kale Mango Orange	694 μg 335 μg 26 μg 8 μg	0–36%	Lipid droplets Dietary fat	Entrapment in cell matrix/ structures Crystallization Dietary fiber
Folate	330 μg/d	Spinach Broccoli Orange Banana	130 μg 77 μg 33 μg 9 μg	60–98%	5-methyl tetrahydrofolate vitamer	Presence of polyglutamate chain
Vitamin C	95 mg/d	Kale Broccoli Orange Kiwi	100 mg 47 mg 51 mg 79 mg	80–90%	Vitamin E	Flavonoids
Vitamin K	70 μg/d	Kale Spinach Kiwi	623 μg 394 μg 11 μg	5%	Fermentation products	Entrapment in cell matrix/ structures
Potassium	3,500 mg/d	Spinach Kale Banana Kiwi	539 mg 400 mg 374 mg 312 mg	60–85%		Food matrix of unprocessed vegetables and fruits
Calcium	950 mg/d	Kale Spinach Kiwi Orange	180 mg 105 mg 30 mg 23 mg	20–40%		Phytate Oxalate
Magnesium	300 mg/d	Spinach Kale Banana Kiwi	55 mg 34 mg 28 mg 14 mg	25–35%	Proteins Medium chain triglycerides Indigestible carbohydrates	Phytate Oxalate Cellulose Lignin Pectin
Iron	11 mg/d	Spinach Kale Broccoli Kiwi	2 mg 1 mg 0.6 mg 0.5 mg	∼12%	Vitamin C Lactic fermentation	Entrapment in cell matrix and structures Phytic acid

PRI, Population Reference Intake; AI, Adequate Intake for adult females. Source: EFSA: https://www.efsa.europa.eu/sites/default/files/assets/DRV_Summary_tables_jan_17.pdf.

inhibitors, tannins, and cyanogens. Anti-nutrients can be removed or inactivated by various food processing procedures, such as fermentation, germination, boiling, leaching, and extraction (99).

VEGETABLES AND FRUIT AS SOURCES OF NUTRIENTS

Vitamins

Pro-Vitamin a Carotenoids

β-carotene, α-carotene and β-cryptoxanthin are the most common dietary carotenoids that can be converted to vitamin A (retinol) through central cleavage by β-carotene monooxygenase (bco1). B-carotene has the highest affinity to the cleavage enzyme, and, based on its chemical structure, can provide twice as much retinol as compared to the other two carotenoids. Therefore, and also because it is more abundant in the diet, β-carotene has received the most attention in vitamin A research. Liberation of β-carotene from the fruit or vegetable matrix is one of the main limiting steps in its bioavailability (100, 101). Green leafy vegetables, such as spinach and kale, are rich in β-carotene (**Table 3**), but only around 5–10% of the total content is bioavailable. In contrast, β-carotene from fruits show higher bioavailability despite their relatively lower β-carotene content (102, 103). This is explained by the digestibility of the particular plant compartment where the β-carotene is stored. Notably, green leafy vegetables store β-carotene in chloroplasts, which is not easily digestible for humans, whereas mangoes, for instance, store β-carotene in chromoplasts from which it is more readily available. Moreover, β-carotene in its crystallized form, as found in carrots, is not easily absorbed, in contrast to β-carotene present in lipid droplets as found in papaya (102, 103). The amount (μg) of β-carotene required to form 1 μg of retinol is referred to as conversion factor; this is estimated as 2.1–3.8 μg of β-carotene when it is provided as a supplement dissolved in oil (**Table 4**). Conversion factors for β-carotene from a wide variety of vegetables and fruits have been comprehensively summarized (104). In contrast to the earlier retinol equivalents (RE) which assumed that intake of 6 μg of β-carotene would yield 1 μg of retinol, current insights have shown that the bio-conversion efficiency is much lower for an average western diet. Therefore, new retinol activity equivalents (RAE) for β-carotene have been set at 12:1 (105). Conversion efficiency of α-carotene and β-cryptoxanthin have hardly been studied, although lately there is renewed interest in the latter (106, 107). Fat content of the diet

TABLE 4 | Reported conversion factors and bioefficacy for β-carotene from vegetables and fruits.

Food	Conversion factor[a]	Bioefficacy (%)[b]
Synthetic β-carotene in oil	2.1:1–3.8:1	26–48
Fruits	12:1	8.3
Tubers	2.8:1–13.4:1	7.5–36
Cooked vegetables	10:1–28:1	1–3.5
Raw vegetables	13:1–77:1	0.01–7.7

[a] *Amount (μg) of ingested β-carotene required from the food source to form 1 μg of retinol.* [b] *Proportion of ingested β-carotene that is absorbed and converted to retinol. Based on: Van Loo-Bouwman et al., Br J Nutr 2014 (104).*

is the most important enhancer of carotenoid absorption (108–110), whereas fiber present in the diet can reduce absorption efficiency (96).

Folate

Green leafy vegetables and citrus fruits are important dietary sources of folate (**Table 3**). In vegetables and fruits, folate is mostly present in its polyglutamated form. Before absorption, enzymatic cleavage of this glutamate chain by folylpoly γ-glutamyl carboxypeptidase (FGCP) is necessary. It has been shown that, as compared to supplemental folic acid, which is a monoglutamate, polyglutamated folate has a bioavailability of ∼70% (111, 112). Others have shown that 5-methyl-tetrahydrofolate is the best bioavailable natural form of the vitamin (113). Folate bioavailability ranges between 60 and 98% from a diet high in vegetables and fruits (91). Whereas, the food matrix, dietary fiber, and low pH may inhibit folate bioavailability, zinc enhances FGCP activity and therefore would promote folate absorption (114). Dietary folate equivalents (DFE) have been defined as 1.7 μg of dietary folate to deliver 1 μg of folate to the body circulation (115).

Vitamin C

Certain fruits, such as kiwi and orange, but also many vegetables are rich sources of vitamin C (**Table 3**). Unlike some other vitamins, vitamin C derived from vegetables and fruits largely shows similar bioavailability as compared to synthetic vitamin C at 80–90% in human studies (116–118). Nevertheless, entrapment of vitamin C in the food matrix, premature degradation or inhibition by other food components may decrease its bioavailability. Vitamin C interacts with vitamin E by reducing tocopheroxyl radicals; vice versa, vitamin E might preserve vitamin C *in vivo* (119). Although it is uncertain if flavonoids can affect vitamin C absorption *in vivo*, several *in vitro* studies showed that flavonoids inhibit the absorption of vitamin C (120–122).

Vitamin K

Dark green leafy vegetables and herbs such as kale, parsley, spinach, and green cabbage (**Table 3**) are rich in phylloquinone (vitamin K1), whereas among the fruits kiwi and avocado by exception contain reasonable amounts as well (123, 124). Menaquinones (vitamin K2) are generally not found in vegetables and fruits, but an exception to this is fermented vegetables such as sauerkraut (124). Data on the bioavailability of phylloquinone from dietary sources are scarce, but some studies show <5% bioavailability from dark green leafy vegetables, while addition of fat or oils improves bioavailability markedly (124–126). Low bioavailability can be explained by binding of phylloquinone to the membranes of plant chloroplasts (127).

Minerals

Potassium

Consumption of vegetables and fruits contributes importantly to potassium intake, especially from dark green leafy vegetables and certain fruits such as banana and kiwi (**Table 3**). High intake of potassium has consistently been associated with reduced blood pressure and risk for hypertension (128, 129). Potassium is almost completely absorbed from dietary sources, although matrix effects may hinder potassium absorption from unprocessed vegetables and fruits to some extent. Estimates of bioavailability range between 60 and 85% from such sources (130, 131). Little is known about factors that promote or inhibit the absorption of potassium from individual dietary sources (132).

Calcium

Especially dark green leafy vegetables such as kale and spinach contribute to dietary calcium intake (**Table 3**). Studies have shown that calcium absorption from various vegetables is either inferior or comparable to calcium absorption from milk with bioavailability estimates ranging between 20 and 40% (133–135), although *Brassica* sp. vegetables showed slightly higher absorption (136). Phytate and oxalate content determine the efficiency of calcium absorption from vegetables. Phytic acid, or inositol polyphosphate, as well as oxalate, or ethanedioate, form insoluble and non-digestible complexes with divalent cations such as Fe^{2+}, Zn^{2+}, Ca^{2+}, and Mg^{2+}, which limits the bioavailability of these minerals. Oxalate is the conjugate base of oxalic acid, which is present in high amounts in certain vegetables such as spinach, cabbage, broccoli, brussels sprouts, beetroot, and rhubarb.

Magnesium

Magnesium can be derived in moderate amounts from fruits and vegetables (**Table 3**). Magnesium from dark green leafy vegetables was shown to have a bioavailability of 25–35% (137). Magnesium is assumed to be absorbed as the ion rather than as in the form of a complex (138). The absorption of magnesium is inhibited by oxalate (139). As explained for calcium above, oxalic acid can form indigestible complexes with divalent cations at physiological pH. It has been shown before that addition of oxalate-rich vegetables to the diet resulted in negative zinc and magnesium balances. Spinach, an oxalate rich vegetable, indeed showed lower magnesium bioavailability as compared to kale, a vegetable low in oxalate (137). Other known dietary based inhibitors of magnesium absorption are phytic acid, cellulose, lignin, and possibly pectin, whereas proteins, medium chain triglycerides, and indigestible carbohydrates are among the enhancers (139).

Iron

Green leafy vegetables are rich in iron (**Table 3**), but the bioavailability of iron is relatively low—around 12% (140). The low bioavailability is attributed to the indigestibility of cellular components such as chloroplasts and mitochondria where iron is stored (141). Vitamin C is well-known to aid non-heme iron bioavailability, either by enhancing iron solubility or by acting as a co-factor in the reduction of iron from the ferric to the ferrous form by duodenal cytochrome B (142, 143). Fytic acid is a strong inhibitor of iron absorption (144), whereas the inhibiting properties of oxalate are less clear. One study showed that oxalic acid did not reduce iron absorption from kale (145). A study in human volunteers showed that lactic fermentation of vegetables doubled iron absorption, which was explained by the acidic conditions that promote the presence of ferric iron, which is more stable in the gastrointestinal tract (146).

CONCLUSION

Both milk as well as vegetables and fruits are nutrient-dense foods that provide a myriad of nutrients which impact human metabolism and health. Bioavailability is an important explanatory step between the food source and potential health effects of its food components. Much of the health benefits of foods may be explained by additive, antagonistic and synergistic processes at the level of uptake and absorption of nutrients. As has become clear from this review, bioavailability values from whole foods have been established in humans for some nutrients, but are still lacking or need confirmation for others. Translation of this information to individual diet scores will require detailed dietary intake information, preferably at the meal level, while taking information on bioavailability of nutrients from separate foods as well as food-to-food interactions into account. This is all the more complex, since bioavailability estimates are currently already incorporated into dietary reference intakes at the population (group) level to a certain extent. Furthermore, host-related factors, e.g., nutrient status, disease state and genetics, also play an important role in nutrient uptake and bioavailability at the individual level and are often unknown. Nevertheless, accounting for nutrient bioavailability based on food intake pattern may result in better estimates of true individual absorbable nutrient intake in relation to health outcomes. Moreover, such knowledge may help in the design of foods, meals and diets that aid in the supply of nutrients to specific target groups.

AUTHOR CONTRIBUTIONS

AM-B conducted the literature review and wrote the manuscript.

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