

Validation of the Diet Quality Index for Adolescents by comparison with biomarkers, nutrient and food intakes: the HELENA study

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CINDI, Countrywide Integrated Non-communicable Disease Intervention; DIAT, Dietary Intake Assessment Tool; DQI, Diet Quality Index; DQI-A, DQI for Adolescents; FA, fatty acid; FBDG, Food-based dietary guideline; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence.

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Abstract

Food-based dietary guidelines (FBDG) aim to address the nutritional requirements at population level in order to prevent diseases and promote a healthy lifestyle. Diet quality indices can be used to assess the compliance with these FBDG. The present study aimed to investigate whether the newly developed Diet Quality Index for Adolescents (DQI-A) is a good surrogate measure for adherence to FBDG, and whether adherence to these FBDG effectively leads to better nutrient intakes and nutritional biomarkers in adolescents. Participants included 1804 European adolescents who were recruited in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study. Dietary intake was assessed by two, non-consecutive 24 h recalls. A DQI-A score, considering the components' dietary quality, diversity and equilibrium, was calculated. Associations between the DQI-A and food and nutrient intakes and blood concentration biomarkers were investigated using multilevel regression analysis corrected for centre, age and sex. DQI-A scores were associated with food intake in the expected direction: positive associations with nutrient-dense food items, such as fruits and vegetables, and inverse associations with energy-dense and low-nutritious foods. On the nutrient level, the DQI-A was positively related to the intake of water, fibre and most minerals and vitamins. No association was found between the DQI-A and total fat intake. Furthermore, a positive association was observed with 25-hydroxyvitamin D, holo-transcobalamin and *n*-3 fatty acid serum levels. The present study has shown good validity of the DQI-A by confirming the expected associations with food and nutrient intakes and some biomarkers in blood.

Key words: Diet quality index: Adolescents: HELENA study

It is generally accepted that inadequate or excessive nutrient intake can have important health consequences, such as nutritional deficiencies, increased risk of type 2 diabetes, CVD and obesity. Food-based dietary guidelines (FBDG) have been developed to prevent such dietary-related health problems^(1,2). These guidelines are targeted at the general population and contain messages that give an indication of what a person should be eating in terms of foods rather than nutrients. These FBDG can be broad and non-specific, such as 'eat a variety of foods each day' or more targeted such as 'eat five portions of fruits and vegetables a day'. Messages may also specify the type of food, such as 'at least half of the grains consumed should be whole grains', or be meal specific such as 'eat a breakfast every day'^(2,3).

Over the last decades, a number of diet quality indices, measuring adherence to such dietary guidelines, have been developed^(4–7). The advantage of such indices is that they capture the complexity of human diets in a single value, taking into account the interactions between nutrients, food preparation methods and eating patterns^(4,8,9). In general, indices representing overall diet quality showed stronger correlations with health outcomes than individual nutrients or foods⁽⁷⁾.

The majority of existing indices are, however, unsuitable for children and adolescents, because their development is based on dietary recommendations for adults⁽¹⁰⁾. Nevertheless, appropriate indices for children and adolescents have been developed, based on recommendations specific for these age groups, e.g. the Youth Healthy Eating Index⁽¹¹⁾, the Revised Children's Diet Quality Index (DQI)⁽¹²⁾, the DQI for Preschoolers⁽¹³⁾, the Preschoolers Diet-Lifestyle Index⁽¹⁰⁾ and the Healthy Lifestyle-Diet Index⁽¹⁴⁾. However, most of these indices are calculated based on a combination of food and nutrient (e.g. cholesterol, Na) intake. This implies the need for detailed dietary information and use of food composition tables.

The purpose of the present study was to develop a DQI for Adolescents (DQI-A) calculated on food intake data only for assessing adherence to FBDG. Furthermore, the aim of the present study was to investigate whether adherence to FBDG (using the DQI-A as a proxy measure) is associated

with better adherence to nutrient dietary recommendations and a better nutritional biomarker blood profile in European adolescents.

Subjects and methods

Study design

The 'Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) – Cross Sectional Study' is a population-based multi-centre investigation of the nutritional and lifestyle status of adolescents, carried out in ten European cities (Vienna in Austria, Ghent in Belgium, Lille in France, Dortmund in Germany, Athens and Heraklion in Greece, Pécs in Hungary, Rome in Italy, Zaragoza in Spain and Stockholm in Sweden). Data were collected from October 2006 to December 2007. The purpose of the study was to obtain standardised, reliable and comparable data from a random sample of European adolescents on a broad battery of relevant nutrition and health-related parameters like dietary intake, food choices and preferences, serum indicators of lipid metabolism and glucose metabolism, vitamin and mineral status, anthropometry, physical activity, fitness and genetic markers. A detailed description of the HELENA study design and sampling procedure has been published elsewhere^(15–17).

The study population comprised adolescents, aged 12·5–17·5 years. Adolescents were excluded from the database *a posteriori* if they met one of the exclusion criteria, namely age <12·5 or >17·5 years, no measurement of weight and/or height, completion of <75% of the tests, participating simultaneously in another clinical trial or an acute infection during the week prior to the examination⁽¹⁶⁾. The total HELENA population consisted of 3528 eligible adolescents (52·3% females). For the purpose of the present study, adolescents who provided data on two non-consecutive 24 h dietary recalls were included in the analysis, resulting in 2330 subjects. Participants from Heraklion and Pécs were excluded for these analyses, as no nutrient intake information was calculated for these two cities due to logistical problems. Underreporters were excluded for the analyses, as previous reports, using concentration biomarkers and the Triads

method⁽¹⁸⁾, have shown that the validity of food and nutrient intakes compared to 'true' intake was better when excluding underreporters⁽¹⁹⁾. Exclusion of underreporters resulted in a final sample of 1804 adolescents (52.6% females) for statistical analysis. Underreporting was considered when the individual ratio of energy intake divided by the estimated BMR was lower than 0.96⁽²⁰⁾. The group of underreporters consisted of a slightly higher percentage of females (57.8% compared to 52.6% in the plausible reports, $P = 0.036$) and had higher median (minimum, maximum) BMI values (22.5 (14.99, 45.63) kg/m² compared to 20.1 (14.08, 40.77) kg/m² in the plausible reports; $P < 0.001$). No differences in age and DQI-A score were observed.

Blood samples were collected in a randomly selected subset of the total HELENA study population (1089 adolescents), of whom 697 provided two 24 h recalls. Exclusion of underreporters resulted in a final sample of 552 adolescents (52.3% females) with biomarkers in the present study. Characteristics of adolescents for whom no biochemical parameters were obtained were compared to those included in the sub-analysis. No significant differences in age, sex and BMI were observed between these two groups; however, the adolescents in the sub-analysis had higher mean values for the DQI-A compared to the total study population (52.6 (SD 15.6) and 51.3 (SD 16.5)%, respectively, $P = 0.018$).

The study was performed following the ethical guidelines of the Declaration of Helsinki, the Good Clinical Practice rules and the legislation regarding clinical research in human subjects in each of the participating countries. All study participants and their parents provided a signed informed consent form. The protocol was approved by the Human Research Review Committees of the institutions involved⁽²¹⁾.

Dietary intake assessment

Dietary intake was assessed by two non-consecutive 24 h recalls⁽²²⁾, comprising weekdays and weekend-days (except from Fridays and Saturdays), though not necessarily including a week and weekend-day for each individual. The 24 h recalls were collected by use of a computer-based self-administered tool, the HELENA-Dietary Intake Assessment Tool (DIAT). This tool was adapted from a previous version developed and validated for Flemish adolescents⁽²³⁾. This assessment tool is based on six meal occasions (breakfast, morning snacks, lunch, afternoon snacks, evening meal, evening snacks) referring to the previous day. Trained dietitians assisted the adolescents to complete the 24 h recalls when needed. Adolescents selected autonomously all the consumed foods and beverages from a standardised food list in the HELENA-DIAT⁽²⁴⁾. Items not available in the list could be added by the participant at any moment. Consumed foods were translated to nutrients by use of the German Food Code and Nutrient Data Base (Bundeslebensmittelschlüssel, BLS, version II.3.1)⁽²⁵⁾. The Multiple Source Method was used to estimate the usual dietary intake of nutrients and foods^(26,27). This statistical modelling technique takes into account within-person variability and calculates usual intakes corrected for age, sex and study centre.

Diet Quality Index for Adolescents

A previously validated DQI, originally developed for pre-school-aged children⁽¹³⁾, was adapted for use in adolescents to measure their compliance to the Flemish FBDG⁽²⁸⁾. These FBDG put forward three basic principles for a healthy and balanced diet, namely dietary quality, dietary diversity and dietary equilibrium. Furthermore, the daily diet was divided into nine recommended food groups, namely (1) water, (2) bread and cereals, (3) grains and potatoes, (4) vegetables, (5) fruit, (6) milk products (7), cheese, (8) meat, fish, eggs and substitutes and (9) fat and oils. Milk products and cheese were allocated to different food groups because of the important difference in fat content. Meat and fish are considered in the same food group; because of the differences in nutrient content, the FBDG additionally recommended the consumption of fish preferably two times per week. However, as only two 24 h recalls were assessed, the frequency of fish consumption could not be considered separately in the DQI-A calculation. For each of the food groups, a range of recommended daily intakes, specifically for adolescents, was provided by the FBDG. The ranges in these FBDG were based upon the nutrient recommendations of the Belgian Health Council⁽²⁹⁾ and the WHO, combined with data on habitual dietary intake in the Belgian population. These FBDG were very similar to dietary guidelines in other countries and to the CINDI pyramid (Countrywide Integrated Non-communicable Disease Intervention program) developed by the WHO⁽³⁰⁾, making the index applicable for a European population.

The technical aspects of the calculation of the DQI-A are given in Table 1. Parallel to the FBDG, the DQI-A consisted of three components, namely quality, diversity and equilibrium.

Dietary quality expressed whether the adolescent made the optimal food quality choices within a food group and was represented by a 'preference group' (e.g. cereal/brown bread, fresh fruit, fish), an 'intermediate group' (e.g. white bread, minced meat) and a 'low-nutrient, energy-dense group' (e.g. soft drinks, sweet snacks, chicken nuggets). A comprehensive description of the allocation of food items to the different quality groups is given in Table 2.

Dietary diversity expressed the degree of variation in the diet. This diversity component was obtained by giving points ranging from 0 to 9 when at least one serving of food of a recommended food group was consumed.

Dietary equilibrium was calculated from the difference between the adequacy component (which was the percentage of the minimum recommended intake for each of the main food groups, truncated to 1) and the excess component (which was the percentage of intake exceeding the upper level of the recommendation, truncated to 1 if larger than 1 and truncated to 0 when below 0; see Table 1). In the food group of meat, fish, eggs and substitutes, the daily intake of the total group was considered. As such, a too high consumption of meat and fish was penalised in the excess component. However, the fish consumption was granted points in the

Table 1. Overview of the calculation of the Diet Quality Index for Adolescents (DQI-A)*

FBDG		DQI-A components				
FG	Recommended daily intake	DQ	DD	DA	DEx	DE
Recommended foods						
Water	1500–2250 ml	DQ = amount consumed food item (m) × weighting factor Weighting factor: + 1 'preference group' 0 'intermediate group' – 1 'low-nutrient, energy-dense group'	DD = 1 point for each FG if at least one serving is consumed	DA = actual intake FG/ minimum recommended FG Values > 1 were truncated to 1	DEx = (actual intake FG – maximum recommended FG)/maximum recommended FG Values > 1 were truncated to 1; values < 0 were truncated to 0	DE = DA – DEx
Bread and cereal	150–360 g					
Potatoes and grains	210–350 g					
Vegetables	300–450 g					
Fruits	250–375 g					
Milk products	450–600 ml					
Cheese	20–40 g					
Meat, fish and substitutes	75–100 g					
Fat and oils	10–15 g					
Non-recommended foods						
Snacks and candy	< 50 g					
Sugared drinks and fruit juice	< 300 ml					
Score of components		$\Sigma(DQ)/\Sigma m \times 100\%$	$\Sigma(DD)/9 \times 100\%$	$\Sigma(DA)/9 \times 100\%$	$\Sigma(DEx)/11 \times 100\%$	$\Sigma(DE)/11 \times 100\%$
DQI-A score		$(\text{Dietary quality} + \text{dietary diversity} + \text{dietary equilibrium})/3$				

FBDG, food-based dietary guidelines; FG, food groups; DQ, dietary quality; DD, dietary diversity; DA, dietary adequacy; DEx, dietary excess; DE, dietary equilibrium
* Further details on 'preference group', 'intermediate group' and 'low-nutrient, energy-dense group' can be found in Table 2.

Table 2. Classification of food items to the different quality groups within each food group, as advised by the Flemish Food-Based Dietary Guidelines

	Preference group	Intermediate group	Low-nutrient, energy-dense group
Water	Plain water	Non-salted, energy-poor drinks (<20.92 kJ/100 ml)	High-energy drinks (>20.92 kJ/100 ml), bouillons and alcoholic beverages
Bread and cereal	Items rich in fibre and with a low-fat content (e.g. cereal bread)	Items with a low-fibre content and/or added fat (e.g. white bread, raisin bread)	High-fat preparations (e.g. pies, cakes)
Potatoes and grains	Items rich in fibre and with a low-fat content (e.g. boiled potatoes, bulgur, whole-wheat pasta)	Items with a low-fibre content and/or added fat (e.g. white rice, mashed potatoes)	High-fat preparations (e.g. French fries, chips)
Vegetables	Fresh or frozen vegetables without additives	Vegetables prepared with cream/sauces or conserved with added salt	None
Fruits	Fresh or frozen fruits without additives	Conserved and dried fruits	Jam, fruit juices
Milk products	Semi-skimmed or skimmed milk products without added sugar or sweeteners	Sugared and whole-milk products (e.g. milk or soya-based puddings and desserts, flavoured milk)	Creams and desserts (e.g. chocolate mousse)
Cheese	Low-fat cheese (<20 % fat)	Semi-fat and fat cheese	None
Meat, fish and substitutes	Fish products and low-fat meat	Semi-fat and fat meat products (e.g. minced meat) and eggs	Fried meat and snacks (e.g. chicken nuggets)
Fats and oils	Vegetable oils	Margarines and lipids of mixed origins	Butter and animal fats

quality component, as fish is always allocated to the preference group in contrast to semi-fat and fat meat products.

These three components of the DQI-A were presented in percentages. The dietary quality component ranged from -100 to 100%, while dietary diversity and dietary equilibrium ranged from 0 to 100%. To compute the DQI-A, the mean of these components was calculated; as such, the DQI-A ranged from -33 to 100%, with higher scores reflecting a higher diet quality. The score was calculated for each day and a mean of the daily scores was taken as global index score of the individual.

Blood analyses

After an overnight fasting period, venous blood samples were drawn in the morning at school according to a standardised blood collection protocol. Details about the sampling, processing and transportation can be found elsewhere⁽³¹⁾. The studied biomarkers were chosen in view of clinical relevance to evaluate nutritional status (vitamin D, vitamin B₁₂, retinol and TAG) or as a dietary biomarker reflecting true intake (vitamin C, plasma folate, carotenoids, *n*-3 fatty acids (FA) and *trans*-FA). Although plasma vitamin D concentrations are influenced by several factors such as sunlight exposure and adiposity, evidence also showed weak correlations with dietary intakes⁽³²⁾. Strong correlations of dietary intakes of vitamin C and serum ascorbic acid concentrations have been reported mainly when habitual dietary intakes of vitamin C are relatively modest^(33,34). Many factors influence serum folate concentrations and bioavailability of dietary folate; however, intakes correlate moderately with serum concentrations⁽³²⁾. Weak but positive correlations were reported for males and females between dietary vitamin B₁₂ intake and holo-transcobalamin status, being a marker of long-term vitamin B₁₂ status^(35,36). Weak correlations may be linked to the large size of liver vitamin B₁₂ stores. Blood concentrations

of carotenoids appear to be moderately correlated with fruit and vegetable intake^(34,37,38). Plasma retinol concentrations are only responsive to vitamin A intake in individuals with inadequate vitamin A status⁽³⁸⁾. Plasma TAG have been shown to be positively correlated with total fat intake and negatively with fibre intake. Levels may, to some extent, be indicative of the level of dietary fibre intake, but the findings to date are conflicting^(34,39). *n*-3 FA intake is moderately correlated with plasma phospholipid levels, reflecting intake in the short to medium term^(40,41). Correlations between the intake of specific types of *trans*-FA and their levels in blood are generally good; however, correlation between the total sum of *trans*-FA intake and the sum of serum *trans*-FA levels is only weak^(42,43).

Plasma folate was measured by means of an immunoassay using the Immunolite 2000 analyser (DPC Biermann GmbH). Holo-transcobalamin (the biologically active form of vitamin B₁₂) was determined by an automated microparticle enzyme immunoassay with the AxSYM analyser (Abbott Laboratories). Vitamin C, β-carotene and retinol were analysed by HPLC (Sykam) using UV detection (UV-Vis 205, Merck). Serum phospholipid FA composition was determined by capillary GC (GC-2010, FID detection, Shimadzu GmbH) after extraction performed by TLC. Serum TAG were measured enzymatically on the Dimension RxL clinical chemistry system (Dade Behring) using the manufacturer's reagents and instructions. Plasma 25-hydroxyvitamin D (25(OH)D) was analysed by ELISA using a kit (OCTEIA 25-Hydroxy Vitamin D) from Immunodiagnostic System and measured with a Sunrise™ Photometer by TECAN.

Statistical analysis

Statistical analyses were performed using the statistical software PASW for Windows version 18 (SPSS, Inc.).

Descriptive characteristics were summarised by calculating means and standard deviations for continuous variables and percentages for categorical variables. Pearson χ^2 and *t* tests were used to test differences between sexes in categorical and continuous variables, respectively.

Normality was evaluated visually and based on the skewness of the data distributions. Skewness of variables on intake ranged from 0.869 (meat intake) to 9.809 (alcohol intake – due to a high number of non-consumers). As these variables were studied in a large sample size (*n* 1804), it was considered that parametrical tests were allowed without transformations. However, variables with a skewness >3 were transformed (log-transformation and square root transformations were tested) and the multilevel analyses were repeated with the transformed data. For all the variables, except for Na intake, the results before and after transformation were similar. To facilitate the interpretation of the results, it was chosen to display the non-transformed data, except for Na intake which was log-transformed. The skewness of the variables on blood values ranged from –0.210 to 2.017 and the skewness of holo-transcobalamin was 3.110. As these variables were only studied in a sample of 552 adolescents, it was decided to perform a log-transformation of holo-transcobalamin in order to achieve a more normal distribution. Multilevel linear regression analysis with inclusion of a random intercept for study centre was used to examine the relationship between the DQI-A and foods, nutrient intakes or blood biomarkers. Confounders (age and sex) were entered as covariates. The random intercept for centre ranged from 0.19 to 24.42%, with the highest influence of centre observed for oils, butter and animal fats, and milk and yoghurt. Significant differences in mean DQI-A scores were observed between both sexes; however, results of validation were very similar. As such, results were not stratified. To adjust for multiple testing, a Bonferroni correction was applied to lower the significance level (α) taking into account the number of tests (0.05/number of tests). *P* values of 0.0019, 0.0013 and 0.006 were used as thresholds of significance for the associations between DQI-A and foods, nutrients and biomarkers, respectively.

Results

The total study population consisted of 1804 participants (52.6% females) and the mean age was 14.7 (SD 1.2) years. The DQI-A score ranged from –11.1 to 84.1%; the mean DQI-A scores were 49.0 (SD 17.0) and 53.3 (SD 15.9)% for males and females, respectively (*P* < 0.001). No differences were observed in mean DQI-A between adolescents in different BMI classes or between adolescents complying with the recommendation of 60 min physical activity *v.* non-compliers.

Multilevel regression analysis of the DQI-A scores with the usual consumption of different foods is shown in Table 3. A strong positive association between the DQI-A score and water intake (g/d) was observed ($\beta = 19.529$, *P* < 0.0001). In contrast, soft drinks, fruit juices and alcoholic beverages showed a significant negative association with the DQI-A. Furthermore, the DQI-A score and bread/cereals had a positive association, but there was no significant association with

potatoes and grains. Milk and cheese (g/d) were positively associated with the DQI-A score, and animal fat (g/d) and vegetable fat (g/d) showed a small, however, significant positive association with the DQI-A ($\beta = 0.100$, *P* < 0.0001 and $\beta = 0.118$, *P* < 0.0001, respectively). No significant relation was present with meat, fish, eggs and substitutes. All non-recommended (energy-dense and low-nutritious) foods showed a significant negative association with the DQI-A score.

At the level of macronutrients (Table 4), a positive association was observed between the DQI-A and water (g/d) and fibre (g/d) intake, and a negative relationship was found with total energy intake (kJ/d) ($\beta = -2.893$, *P* = 0.0005). The usual intake of polysaccharides (g/d) was positively related to the dietary quality ($\beta = 0.230$, *P* = 0.0004), whilst the intake of mono- and disaccharides (g/d) showed a negative relationship ($\beta = -0.853$ and -0.289 , respectively; both *P* < 0.0001). No significant association was seen between DQI-A and protein intake (g/d) or fat intake (g/d). All investigated minerals (Table 4), except Fe and Cu, were positively associated with the DQI-A score. Furthermore, intake of almost all vitamins, except niacin, vitamin C and vitamin E, showed a significant positive association with the calculated index.

Table 5 describes the results of the multilevel regression analysis between the DQI-A scores and nutritional biomarkers in a subgroup of 552 adolescents. Only for plasma 25(OH)D and holo-transcobalamin, a significant positive association was observed with the index score. The positive association with the *n*-3 FA status ($\mu\text{mol/l}$) was borderline significant ($\beta = 0.376$, *P* = 0.007).

Discussion

Diet quality indices are valuable tools to obtain a global assessment of the dietary quality of a person or population. The present study aimed to investigate whether the developed DQI-A, calculated solely from food items, was an adequate proxy measure for adherence to the FBDG. This was done by comparing the DQI-A scores with the usual intake of different foods, of which some were not included in the calculation. The results showed that DQI-A scores were significantly associated with most food items in the expected direction. Nutrient-dense food items, such as fruits and vegetables, were positively associated, whilst non-recommended foods showed negative associations. No significant relation between the DQI-A score and the usual intake of meat, fish, eggs or their substitutes was found. This was due to the fact that this food group (calculated as the sum of meat, fish and eggs) was often consumed in excess, resulting in a lower score of the dietary equilibrium component. However, it is noteworthy that the overconsumption of this food group is mainly due to an excessive intake of meat products rather than fish or meat substitutes. Fish is only over-consumed in a minority of the adolescents (twenty-six of the 1804 adolescents had a habitual fish consumption of > 100 g/d; range 0–328 g/d). Consumption of meat substitutes ranged from 0 to 143 g/d, with eight adolescents consuming more than 100 g/d. Furthermore, a positive relation was observed between the DQI-A score

Table 3. Association between Diet Quality Index for Adolescents (DQI-A) scores and food intake* (β -Coefficients and 95 % confidence intervals)

	DQI-A (n 1804)		
	β	95 % CI	P
Beverages			
Water (g/d)	19.529	18.298, 20.761	<0.0001
Coffee and tea (g/d)	0.084	-0.243, 0.412	0.6137
Soups/bouillon (g/d)	-0.016	-0.213, 0.182	0.8777
Bread and cereals			
Bread and rolls (g/d)	0.494	0.330, 0.658	<0.0001
Breakfast cereals (g/d)	0.239	0.177, 0.301	<0.0001
Potatoes and grains			
Rice and other grains (g/d)	0.008	-0.110, 0.126	0.8961
Starch roots, potatoes (g/d)	0.063	-0.062, 0.188	0.3247
Pasta (g/d)	0.254	0.094, 0.414	0.0019
Vegetables (g/d)	0.911	0.740, 1.083	<0.0001
Fruits (g/d)	2.181	1.883, 2.479	<0.0001
Milk products			
Milk, yoghurt and milk beverages (g/d)	4.257	3.669, 4.845	<0.0001
Desserts and puddings milk based (g/d)	-0.069	-0.148, 0.010	0.0883
Cheese (g/d)	0.224	0.159, 0.289	<0.0001
Meat/fish/eggs/meat alternatives			
Meat (g/d)	-0.004	-0.202, 0.194	0.9687
Fish products (g/d)	0.074	0.001, 0.146	0.0466
Eggs (g/d)	-0.005	-0.055, 0.044	0.8380
Meat substitutes, nuts and pulses (g/d)	0.124	0.023, 0.224	0.0160
Fat and oil			
Margarine and vegetable oils (g/d)	0.118	0.083, 0.153	<0.0001
Butter and animal fats (g/d)	0.100	0.065, 0.136	<0.0001
Non-recommended foods			
Snacks and candy			
Cakes, pies, biscuits (g/d)	-0.287	-0.409, -0.166	<0.0001
Savoury snacks (g/d)	-0.245	-0.295, -0.195	<0.0001
Sugar, honey, jam, candies, chocolate (g/d)	-0.281	-0.404, -0.158	<0.0001
Sauces and creams (g/d)	-0.143	-0.225, -0.062	0.0006
Drinks			
Carbonated/soft/isotonic drinks (g/d)	-11.456	-12.188, -10.724	<0.0001
Fruit and vegetable juices (g/d)	-3.133	-3.588, -2.677	<0.0001
Alcoholic beverages (g/d)	-0.798	-1.065, -0.531	<0.0001

* Multilevel regression analyses with inclusion of a random intercept for centre and corrected for age and sex as independent variables. Bonferroni correction resulted in level of significance < 0.0019.

and the consumption of fat and oils. A moderate intake of fat and oil is recommended in the Flemish FBDG. Also, a high consumption of vegetable oils is in line with the Mediterranean diet and the FBDG of Greece and Spain⁽³⁰⁾. Moreover, adherence to the Mediterranean diet has been shown to have beneficial effects on cardiovascular risk factors⁽⁴⁴⁾. In the past, it was generally assumed that saturated fats induced a higher risk of CVD; however, this has been questioned lately, as replacement of SFA with refined carbohydrates was suspected to increase the risk of CVD^(45,46). Furthermore, children and adolescents have higher lipid intake needs, which is essential for growth. Besides energy delivery, lipids have an important function as structural components in all tissues, because they are indispensable for cell and plasma membrane synthesis⁽⁴⁷⁾.

Another interesting fact was the inverse relationship between the DQI-A score and energy intake. This suggested that adolescents with large, excessive food intake, and thus more likely to meet minimal intake recommendations, did not necessarily obtain a higher diet quality score. This was in contrast with other DQI validation studies where participants consuming

more food, and thus more total energy, had higher quality scores compared with adolescents who ate less^(11,48).

In the development of the DQI-A, three other variants have been studied, namely, one with inclusion of a measure for meal frequency, one with inclusion of a measure of physical activity and both (data not shown). The DQI-A, as described in the present paper, however, showed the strongest associations with the different food items as well as the largest variance between individuals. Overall, based on these results, it can be concluded that the DQI-A is indeed a good surrogate marker for adherence to FBDG.

The second aim of the present study was to investigate whether adherence to these FBDG, using the DQI-A as a proxy measure, resulted in a better nutritional intake and blood biomarker profile. Indeed, the DQI-A was strongly related to higher intakes of water, fibre and most minerals and vitamins. The high fibre intake was a clear representation of the FBDG to consume sufficient vegetables and fruits, and to choose wholegrain products⁽²⁸⁾. Also, the DQI-A was positively associated with complex carbohydrates, whilst the usual intake of mono- and disaccharides decreased with better

Table 4. Associations between Diet Quality Index for Adolescents (DQI-A) score and usual intake of macro- and micronutrients*

(β -Coefficients and 95% confidence intervals)

	DQI-A (n 1804)		
	β	95% CI	P
Macronutrients			
Energy (kJ/d)	-12.104	-18.886, -5.317	0.0005
Protein (g/d)	0.119	0.043, 0.195	0.0020
Carbohydrates (g/d)	-0.836	-1.072, -0.599	<0.0001
Total fat (g/d)	0.044	-0.034, 0.123	0.2643
Water (g/d)	10.646	8.952, 12.339	<0.0001
Fibre (g/d)	0.107	0.088, 0.125	<0.0001
Carbohydrates			
Monosaccharides (g/d)	-0.853	-939.141, -766.813	<0.0001
Disaccharides (g/d)	-0.289	-405.958, -171.109	<0.0001
Polysaccharides (g/d)	0.230	103.602, 357.275	0.0004
Fats			
SFA (mg/d)	37.695	2.810, 72.580	0.0342
MUFA (mg/d)	32.993	3.639, 62.348	0.0276
PUFA (mg/d)	-17.256	-31.941, -2.570	0.0213
Cholesterol (mg/d)	0.333	-0.017, 0.684	0.0624
Minerals			
Na (mg/d)†	1.003	1.002, 1.005	<0.0001
K (mg/d)	7.687	5.226, 10.147	<0.0001
Cl (mg/d)	8.583	5.531, 11.635	<0.0001
Ca (mg/d)	6.623	5.549, 7.697	<0.0001
Mg (mg/d)	0.923	0.653, 1.192	<0.0001
Fe (μ g/d)	-6.848	-17.827, 4.132	0.2213
Cu (μ g/d)	2.793	0.957, 4.630	0.0029
Zn (μ g/d)	38.728	30.434, 47.023	<0.0001
F (μ g/d)	2.247	1.557, 2.937	<0.0001
I (μ g/d)	0.595	0.500, 0.691	<0.0001
P (mg/d)	5.363	4.233, 6.494	<0.0001
Mn (μ g/d)	11.073	6.669, 15.477	<0.0001
Vitamins			
Thiamine (μ g/d)	1.947	0.848, 3.045	0.0005
Riboflavin (μ g/d)	7.936	6.452, 9.420	<0.0001
Niacin (μ g/d)	17.818	2.495, 33.140	0.0227
Pantothenic acid (μ g/d)	18.807	14.547, 23.066	<0.0001
Pyridoxine (μ g/d)	4.140	2.556, 5.723	<0.0001
Biotin (μ g/d)	0.171	0.133, 0.209	<0.0001
Total folic acid (μ g/d)	0.699	0.511, 0.887	<0.0001
Cobalamin (μ g/d)	0.020	0.013, 0.027	<0.0001
Vitamin C (g/d)	0.169	-0.007, 0.345	0.0601
Retinol equivalents (μ g/d)	5.075	3.713, 6.436	<0.0001
Vitamin D (μ g/d)	0.005	0.002, 0.008	0.0006
Vitamin E (μ g/d)	2.813	-6.578, 12.204	0.5569
Vitamin K (μ g/d)	1.064	0.812, 1.317	<0.0001

* Multilevel regression analysis with inclusion of a random intercept for centre and corrected for age and sex as independent variables. Bonferroni correction resulted in level of significance <0.0013.

† Variable was log-transformed to obtain a normal distribution.

adherence to the guidelines, as would be expected^(28,49). Increased consumption of simple carbohydrate-rich foods has been associated with obesity, type 2 diabetes and the metabolic syndrome^(49,50). The absence of association with vitamin C intake might be attributed to the fact that vitamin C is prevalent in fruit juices, which are considered as non-recommended foods because of their high energy density. As such, high intakes of these items, and thus vitamin C, resulted in a lower DQI-A, whilst a high intake of fruits and vegetables, and thus also vitamin C, tended to increase the DQI-A. Furthermore, the lack of association of Fe and niacin with the adherence to FBDG could be due to the high concentration of these nutrients in meat products, whilst this food group showed no association

with the DQI-A. In the present study population, no association could be found between the DQI-A score and the absolute intake of fat and FA. This might be due to the fact that meat was the largest contributor to fat intake, followed by non-recommended foods and dairy products⁽⁵¹⁾. As such, the 'penalisation' for the excessive intake of meat and non-recommended foods is counteracted by the recommended intake of dairy products. Also, associations might be attenuated due to faults that may arise through linkage with food composition tables. Of course, this finding might also indicate that the present FBDG are well tuned to the micronutrient recommendations, but that the guidelines are not efficient in transferring the recommendations for fats and FA.

Table 5. Associations between the Diet Quality for Adolescents (DQI-A) scores and nutritional biomarkers*

 (β -Coefficients and 95% confidence intervals)

	DQI-A (n 552)		
	β	95% CI	P
Vitamin D (nmol/l)	0.301	0.164, 0.438	<0.0001
Vitamin C (mg/l)	0.125	-0.058, 0.309	0.1807
Plasma folate (nmol/l)	0.051	-0.007, 0.109	0.0826
Holo-transcobalamin (TC-III/B12) (pmol/l)†	1.005	1.002, 1.007	0.0002
β -Carotene (ng/ml)	0.608	-0.445, 1.661	0.2570
Retinol (ng/ml)	0.310	-0.285, 0.905	0.3062
TAG (mg/l)	-0.563	-2.308, 1.181	0.5259
n-3 Fatty acids (μ mol/l)	0.376	0.105, 0.646	0.0066
trans-Fatty acids (μ mol/l)	-0.005	-0.010, -0.001	0.0250

* Multilevel regression analyses with inclusion of a random intercept for centre and corrected for age and sex as independent variables. Bonferroni correction resulted in level of significance <0.006.

† Variable was log-transformed to obtain a normal distribution.

Previously, it has been shown that biomarkers do not always perform better than food intake assessment methods to evaluate true nutrient intake⁽⁵²⁾. Moreover, not all nutrients have well-defined biological markers and many are influenced by other factors than intake. In the present study population, a positive association was found between the DQI-A and levels of 25(OH)D and holo-transcobalamin, representing the bioactive fraction of vitamin B₁₂, and n-3 FA. Amongst others, plasma levels of 25(OH)D are related to a better bone mineralisation⁽⁵³⁾, while deficiency has been linked to the pathogenesis of several disorders, including cancer, hypertension, multiple sclerosis and diabetes⁽⁵⁴⁾. Adequate folate and vitamin B₁₂ levels are essential for good growth and development of the central nervous system in fetal and infant life⁽⁵⁵⁾. Both folate and vitamin B₁₂ are also essential for the synthesis of nucleotide precursors, so if both are deficient, this can result in impaired cell division and anaemia⁽⁵⁶⁾. In addition, deficiencies in folate and vitamin B₁₂ result in high values of homocysteine in blood and tissues, which in turn is associated with organ dysfunction in children that may lead to disease later in life, such as CVD^(16,57). Also, trans-FA levels showed an inverse relation with the DQI-A, which supports the recommendation of discouraging trans-FA intake in the human diet because of their association with an increased cardiovascular risk⁽⁵⁸⁾. This finding was, however, not significant at the corrected level of $P < 0.006$.

The aim of the DQI-A was to obtain a measure for overall dietary quality of an individual. In the present study, statistically significant associations were only found with biomarkers representing long-term dietary intake (25(OH)D and holo-transcobalamin), whilst no statistically significant relationship was seen with biomarkers representing short- to medium-term intake. This might indicate that the DQI-A is a valid measure for long-term dietary habits. Also, the associations with the other biomarkers might be attenuated, as supplement use was not taken into account in the present study.

Several diet quality indices have been associated with specific nutrient intakes and plasma biomarkers. Comparison

is difficult as different statistical approaches have been applied. Similar to the present results, Hann *et al.*⁽⁵⁹⁾ found significant positive associations between the Healthy Eating Index and vitamin C, folate and fibre intake in a sample of adult women. In contrast to the present results, carbohydrates and total energy were also positively associated. Also Newby *et al.*⁽⁶⁰⁾ found significant associations between the DQI-Revised and intakes of several vitamins and minerals. In contrast to the present results, however, total fat and saturated fat were negatively correlated with the index. However, in this index, low total fat intake and low saturated fat intake were incorporated as two of the ten separate components in the calculation.

Both the Healthy Eating Index and the DQI-Revised showed significant correlations with β -carotene serum values (r 0.12–0.42)^(59–61). Furthermore, Weinstein *et al.*⁽⁶¹⁾ found significant, but generally weak, correlations between the Healthy Eating Index and serum vitamin C (r 0.21), serum folate (r 0.15), serum vitamin B₁₂ (r 0.01), serum retinol (r 0.05) and serum TAG (r 0.06) in a large study population ($n > 16\ 000$). Moreover, Neuhauser *et al.*⁽⁶²⁾ could not observe any correlation between the DQI and long-chain n-3 phospholipid FA in a sample of 102 women, whilst Gerber *et al.*⁽⁶³⁾ found a significant association with n-3 erythrocyte FA. Both studies could not observe a relationship with serum β -carotene.

Strengths and limitations

This was the first study to investigate the use and validity of a DQI in a European adolescent population. In the HELENA study, all data were collected according to standardised protocols and strict procedures. Furthermore, in contrast to many other diet quality indices, the DQI-A in the present study was not based on nutrient intakes, avoiding the limitations that coincide with the use of food composition data (such as the use of various tables in different countries with different methods of analysis used, unavailability of food items, loss of dietary information from mixed dishes, etc.).

A possible limitation of the present study is the observed significant differences in sex distribution and BMI between underreporters and non-underreporters. This differential underreporting could most probably attenuate the present results, as generally it would be expected that adolescents with a higher BMI would have a less healthy dietary pattern. Underreporting by these adolescents might, however, result in better DQI-A scores than that in reality. This could attenuate the present results, especially the associations with the biomarkers. Therefore, it was chosen to exclude the underreporters. This decision was supported by a previous evaluation of food and nutrient intake assessment in the HELENA study population⁽¹⁹⁾, showing better correlations with the 'true intake' (calculated with the Triads method⁽¹⁸⁾) after exclusion of underreporters. However, underreporting cannot be fully precluded, as exclusion was only applied for adolescents indicating a negative energy balance. As such, attenuation of results might still be present.

The developed DQI-A score was based on the Flemish FBDG, whilst large variation in dietary habits was observed

in the studied population. These guidelines were selected as the basis of the index score because of the great similarities with the CINDI pyramid developed by the WHO. The Flemish guidelines, however, are more specific concerning quality of different food items and recommended quantities. Compared to dietary guidelines of the other European countries, rather minor differences were found⁽³⁰⁾. The French and Austrian guidelines put greater emphasis on vegetables *v.* cereal intake compared to the Flemish. Furthermore, daily olive oil consumption is a specific recommendation in the Greek and Spanish guidelines. Given the large similarities on the most important aspects of the dietary guidelines, the authors considered it appropriate to apply the Flemish guidelines to a European population.

The DQI-A was calculated based on two self-administered, computer-assisted, non-consecutive 24 h recalls. Following recommendations of the 'European Food Consumption Survey method', 24 h recalls were preferred as these are open-ended questionnaires from which detailed information can be obtained. Furthermore, they are applicable in large populations of different ethnicity, and standardisation is possible by self-administered computer-assisted recall methods with pictures for portion sizes^(22,64). According to Biro *et al.*⁽²²⁾, the 24 h recall method is appropriate to assess both acute and usual intake on the individual level by repeated short-term measurements and modelling.

A limitation of the method used is, however, that only information of 2 d was obtained. Although this allows inclusion of exceptional intakes at the individual level, this effect is neutralised by the large number of observations. The 24 h dietary recall method does not allow quantifying proportions of non-consumers for particular food items, especially for infrequently consumed foods. In order to decrease this influence, nutrient intakes were corrected for within-person variability by applying the Multiple Source Method. Moreover, accuracy of collected data relies on the individual's ability to remember foods and beverages consumed in the past 24 h, and might, therefore, be biased towards underreporting. In this respect, the 24 h dietary recalls were performed through the computer-assisted HELENA-DIAT program⁽²⁴⁾ to standardise the recall procedures as much as possible. Another limitation of the use of 24 h recall interviews is the potential loss of dietary information from mixed dishes, as food ingredients were sometimes counted from mixed dishes.

The same food composition table for conversion of food intake data to estimated nutrient intakes was used for all survey centres. In this way, differences in definitions, analytical methods, units and modes of expression were overcome. In this regard, the German food composition tables (Bundeslebensmittelschlüssel, BLS) were chosen. The BLS is based on German, American, English, Swedish, Danish and Dutch food composition tables, on analytical values of food producing firms, publications and research results of the Federal Research Centres and Universities⁽⁶⁵⁾. The BLS includes about 11 000 raw and cooked foods and recipes, and has been widely used in epidemiological studies⁽⁶⁶⁾.

Conclusion

The present study has shown good validity of the DQI-A by confirming the expected associations with food and nutrient intakes and some biomarkers in blood. However, further investigation is necessary to explore why the present guidelines do not reach their goal of obtaining a more favourable lipid intake with increasing DQI-A scores.

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References

1. Food and Agriculture Organization of the United Nations/World Health Organization (1996) *Preparation and Use of Food-based Dietary Guidelines. Report of a Joint FAO/WHO Consultation*. Nicosia: WHO.
2. Sandstrom B (2001) A framework for food-based dietary guidelines in the European Union. *Public Health Nutr* **4**, 293–305.
3. World Health Organization (2000) *Obesity: Preventing and Managing the Global Epidemic. WHO Technical Report Series* no. 894. Geneva: WHO.
4. Waijers PM, Feskens EJ & Ocke MC (2007) A critical review of predefined diet quality scores. *Br J Nutr* **97**, 219–231.
5. Patterson RE, Haines PS & Popkin BM (1994) Diet quality index: capturing a multidimensional behavior. *J Am Diet Assoc* **94**, 57–64.
6. Kennedy ET, Ohls J, Carlson S, *et al.* (1995) The Healthy Eating Index: design and applications. *J Am Diet Assoc* **95**, 1103–1108.
7. Kant AK (1996) Indexes of overall diet quality: a review. *J Am Diet Assoc* **96**, 785–791.

8. Kant AK (2004) Dietary patterns and health outcomes. *J Am Diet Assoc* **104**, 615–635.
9. Hu FB (2002) Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13**, 3–9.
10. Manios Y, Kourlaba G, Grammatikaki E, *et al.* (2010) Development of a diet-lifestyle quality index for young children and its relation to obesity: the Preschoolers Diet-Lifestyle Index. *Public Health Nutr* **13**, 2000–2009.
11. Feskanich D, Rockett HR & Colditz GA (2004) Modifying the Healthy Eating Index to assess diet quality in children and adolescents. *J Am Diet Assoc* **104**, 1375–1383.
12. Kranz S, Findeis JL & Shrestha SS (2008) Use of the Revised Children's Diet Quality Index to assess preschooler's diet quality, its sociodemographic predictors, and its association with body weight status. *J Pediatr (Rio J)* **84**, 26–34.
13. Huybrechts I, Vereecken C, De Bacquer D, *et al.* (2010) Reproducibility and validity of a diet quality index for children assessed using a FFQ. *Br J Nutr* **104**, 135–144.
14. Manios Y, Kourlaba G, Grammatikaki E, *et al.* (2010) Development of a lifestyle-diet quality index for primary schoolchildren and its relation to insulin resistance: the Healthy Lifestyle-Diet Index. *Eur J Clin Nutr* **64**, 1399–1406.
15. De Henauw S, Gottrand F, De Bourdeaudhuij I, *et al.* (2007) Nutritional status and lifestyles of adolescents from a public health perspective. The HELENA Project – Healthy Lifestyle in Europe by Nutrition in Adolescence. *J Public Health* **15**, 187–197.
16. Moreno LA, González-Gross M, Kersting M, *et al.* (2008) Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutr* **11**, 288–299.
17. Moreno LA, De Henauw S, González-Gross M, *et al.* (2008) Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. *Int J Obes (Lond)* **32**, Suppl. 5, S4–S11.
18. Kaaks RJ (1997) Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. *Am J Clin Nutr* **65**, 1232S–1239S.
19. Vandevijvere S, Geelen A, Gonzalez-Gross M, *et al.* (2012) Evaluation of food and nutrient intake assessment using concentration biomarkers in European adolescents from the HELENA study. *Br J Nutr* **109**, 736–747.
20. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**, 1119–1130.
21. Beghin L, Castera M, Manios Y, *et al.* (2008) Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. *Int J Obes (Lond)* **32**, Suppl. 5, S12–S18.
22. Biro G, Hulshof KF, Ovesen L, *et al.* (2002) Selection of methodology to assess food intake. *Eur J Clin Nutr* **56**, Suppl. 2, S25–S32.
23. Vereecken CA, Covents M, Matthys C, *et al.* (2005) Young adolescents' nutrition assessment on computer (YANA-C). *Eur J Clin Nutr* **59**, 658–667.
24. Vereecken CA, Covents M, Sichert-Hellert W, *et al.* (2008) Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. *Int J Obes (Lond)* **32**, Suppl. 5, S26–S34.
25. Dehne LI, Klemm C, Henseler G, *et al.* (1999) The German Food Code and Nutrient Data Base (BLS II.2). *Eur J Epidemiol* **15**, 355–359.
26. Haubrock J, Hartigg U, Souverein O, *et al.* (2010) An improved statistical tool for estimating usual intake distributions: the Multiple Source Method (MSM). *Arch Public Health* **68**, 14–15.
27. The Multiple Source Method (MSM) (2011) <https://nugo.dife.de/msm> (accessed September 2011).
28. VIG (2006) *De actieve voedingsdrieboek: een praktische voedings- en beweeggids (The Active Food Pyramid: A Practical Guide to Diet and Physical Activity)*. Brussels: Vlaams Instituut voor Gezondheidspromotie (VIG).
29. Belgian Health Council (2009) *Voedingsaanbevelingen voor België. Herziened versie 2009 (Nutritional Recommendations for Belgium. Revised Version 2009)*. Brussels: Belgian Health Council.
30. The European Food Information Council (EUFIC) (2011) <http://www.eufic.org/article/en/page/RARCHIVE/expid/food-based-dietary-guidelines-in-europe/> (accessed September 2011).
31. Gonzalez-Gross M, Breidenassel C, Gomez-Martinez S, *et al.* (2008) Sampling and processing of fresh blood samples within a European multicenter nutritional study: evaluation of biomarker stability during transport and storage. *Int J Obes (Lond)* **32**, Suppl. 5, S66–S75.
32. Jacques PF, Sulsky SI, Sadowski JA, *et al.* (1993) Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am J Clin Nutr* **57**, 182–189.
33. Bates CJ, Rutishauser IH, Black AE, *et al.* (1979) Long-term vitamin status and dietary intake of healthy elderly subjects. 2. Vitamin C. *Br J Nutr* **42**, 43–56.
34. Jenab M, Slimani N, Bictash M, *et al.* (2009) Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum Genet* **125**, 507–525.
35. Bor MV, von Castel-Roberts KM, Kauwell GP, *et al.* (2010) Daily intake of 4 to 7 microg dietary vitamin B-12 is associated with steady concentrations of vitamin B-12-related biomarkers in a healthy young population. *Am J Clin Nutr* **91**, 571–577.
36. Hvas AM, Gravholt CH & Nexø E (2005) Circadian variation of holo-transcobalamin (holo-TC) and related markers. *Clin Chem Lab Med* **43**, 760–764.
37. Rock CL, Swendseid ME, Jacob RA, *et al.* (1992) Plasma carotenoid levels in human subjects fed a low carotenoid diet. *J Nutr* **122**, 96–100.
38. Willett WC, Stampfer MJ, Underwood BA, *et al.* (1983) Vitamins A, E, and carotene: effects of supplementation on their plasma levels. *Am J Clin Nutr* **38**, 559–566.
39. Sonnenberg LM, Quatromoni PA, Gagnon DR, *et al.* (1996) Diet and plasma lipids in women. II. Macronutrients and plasma triglycerides, high-density lipoprotein, and the ratio of total to high-density lipoprotein cholesterol in women: the Framingham nutrition studies. *J Clin Epidemiol* **49**, 665–672.
40. Hodge AM, Simpson JA, Gibson RA, *et al.* (2007) Plasma phospholipid fatty acid composition as a biomarker of habitual dietary fat intake in an ethnically diverse cohort. *Nutr Metab Cardiovasc Dis* **17**, 415–426.
41. Andersen LF, Solvoll K & Drevon CA (1996) Very-long-chain n-3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. *Am J Clin Nutr* **64**, 305–311.
42. Hodson L, Skeaff CM & Fielding BA (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* **47**, 348–380.
43. Baylin A, Kim MK, Donovan-Palmer A, *et al.* (2005) Fasting whole blood as a biomarker of essential fatty acid intake

- in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol* **162**, 373–381.
44. Estruch R, Martinez-Gonzalez MA, Corella D, *et al.* (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* **145**, 1–11.
 45. Mozaffarian D (2011) The great fat debate: taking the focus off of saturated fat. *J Am Diet Assoc* **111**, 665–666.
 46. Willett WC (2011) The great fat debate: total fat and health. *J Am Diet Assoc* **111**, 660–662.
 47. Uauy R, Mize CE & Castillo-Duran C (2000) Fat intake during childhood: metabolic responses and effects on growth. *Am J Clin Nutr* **72**, 1354S–1360S.
 48. Lowik MR, Hulshof KF & Brussaard JH (1999) Food-based dietary guidelines: some assumptions tested for The Netherlands. *Br J Nutr* **81**, Suppl. 2, S143–S149.
 49. WHO (1998) *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation on Obesity Geneva 3–5 June 1997*. Geneva: WHO.
 50. Novac O, Matasaru S, Tataru S, *et al.* (2009) Assessment of complications of excess weight in school-age children and adolescents. *Rev Med Chir Soc Med Nat Iasi* **113**, 740–744.
 51. Vyncke KE, Libuda L, De VT, *et al.* (2012) Dietary fatty acid intake, its food sources and determinants in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Br J Nutr* **108**, 2261–2273.
 52. Kabagambe EK, Baylin A, Allan DA, *et al.* (2001) Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. *Am J Epidemiol* **154**, 1126–1135.
 53. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
 54. Zhang R & Naughton DP (2010) Vitamin D in health and disease: current perspectives. *Nutr J* **9**, 65.
 55. Selhub J & Paul L (2011) Folic acid fortification: why not vitamin B₁₂ also? *Biofactors* **37**, 269–271.
 56. Pepper MR & Black MM (2011) B₁₂ in fetal development. *Semin Cell Dev Biol* **22**, 619–623.
 57. Bjorke Monsen AL & Ueland PM (2003) Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence. *Am J Clin Nutr* **78**, 7–21.
 58. Remig V, Franklin B, Margolis S, *et al.* (2010) *Trans* fats in America: a review of their use, consumption, health implications, and regulation. *J Am Diet Assoc* **110**, 585–592.
 59. Hann CS, Rock CL, King I, *et al.* (2001) Validation of the Healthy Eating Index with use of plasma biomarkers in a clinical sample of women. *Am J Clin Nutr* **74**, 479–486.
 60. Newby PK, Hu FB, Rimm EB, *et al.* (2003) Reproducibility and validity of the Diet Quality Index Revised as assessed by use of a food-frequency questionnaire. *Am J Clin Nutr* **78**, 941–949.
 61. Weinstein SJ, Vogt TM & Gerrior SA (2004) Healthy Eating Index scores are associated with blood nutrient concentrations in the third National Health And Nutrition Examination Survey. *J Am Diet Assoc* **104**, 576–584.
 62. Neuhouser ML, Patterson RE, King IB, *et al.* (2003) Selected nutritional biomarkers predict diet quality. *Public Health Nutr* **6**, 703–709.
 63. Gerber MJ, Scali JD, Michaud A, *et al.* (2000) Profiles of a healthful diet and its relationship to biomarkers in a population sample from Mediterranean southern France. *J Am Diet Assoc* **100**, 1164–1171.
 64. Vereecken C, Dohogne S, Covents M, *et al.* (2010) How accurate are adolescents in portion-size estimation using the computer tool Young Adolescents' Nutrition Assessment on Computer (YANA-C)? *Br J Nutr* **103**, 1844–1850.
 65. Bundeslebensmittelschlüssel (BLS) – German Nutrient Data Base (2011) <http://bls.nvs2.de> (accessed September 2011).
 66. Deharveng G, Charrondiere UR, Slimani N, *et al.* (1999) Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. European Prospective Investigation into Cancer and Nutrition. *Eur J Clin Nutr* **53**, 60–79.