Measuring nutritional knowledge using Item Response Theory and its validity in European adolescents

Thanise Sabrina Souza Santos[1,*], Cristina Julian[2], Dalton Francisco de Andrade[3], Betzabeth Slater Villar[1], Raffaela Piccinelli[4], Marcela González-Gross[5], Fréderic Gottrand[6], Odysseas Androutsos[7], Mathilde Kersting[8], Nathalie Michels[9], Inge Huybrechts[10], Kurt Widhalm[11], Dénes Molnár[12], Ascensión Marcos[13], Manuel J Castillo-Garzón[14] and Luis A Moreno[2] on behalf of the HELENA Study Group†
[1]Public Health Faculty, University of São Paulo, Av. Dr Arnaldo 715, Cerqueira César, São Paulo – SP, 01246-904, Brazil: [2]Growth, Exercise, NUtrition and Development (GENUD) Research Group, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria de Aragón (IIS Aragón) and Centro de Investigación Biomédica en Red de Fisiopatología de la Nutrición y la Obesidad (CIBEROBN), Zaragoza, Spain: [3]Department of Informatics and Statistics, Federal University of Santa Catarina, Florianópolis, SC, Brazil: [4]Council for Agricultural Research and Economics, Research Centre for Food and Nutrition, Rome, Italy: [5]Department of Health and Human Performance, Universidad Politécnica de Madrid, Centro de Investigación Biomédica en Red de Fisiopatología de la Nutrición y la Obesidad (CIBEROBN), Madrid, Spain: [6]Department of Pediatrics, Regional Hospital Center, University of Lille, Lille, France: [7]Department of Nutrition and Dietetics, School of Health Sciences and Education, Harokopio University, Athens, Greece: [8]Research Department of Child Nutrition, Pediatric University Clinic, Bochum, Germany: [9]Department of Public Health, Ghent University, Ghent, Belgium: [10]Nutritional Epidemiology Group (NEP), International Agency for Research on Cancer, Lyon, France: [11]Division of Nutrition and Metabolism, Department of Pediatrics, Medical University of Vienna, Vienna, Austria: [12]Department of Pediatrics, University of Pécs Medical School, Pécs, Hungary: [13]Institute of Food Science, Technology and Nutrition, Spanish National Research Council (ICTAN-CSIC), Madrid, Spain: [14]Department of Physiology, Medicine School, University of Granada, Granada, Spain

Abstract

Objective: To analyse the Nutritional Knowledge Test (NKT) using Item Response Theory (ITR) analysis and to assess the construct validity of the Nutritional Knowledge Scale (NKTS) and its associations with adolescent food group consumption and nutritional biomarkers.
Design: Cross-sectional study.
Setting: Multicentre investigation conducted in ten European cities.
Participants: Adolescents aged 12·5–17·5 years (n 3215) who completed over 75 % of the NKT.
Results: Factor analysis indicated that the NKT can be analysed with a one-dimensional model. Eleven out of twenty-three items from the NKT presented adequate parameters and were selected to be included in the NKTS. Nutrition knowledge was positively associated with consumption of fruits, cereals, dairy products, pulses, meat and eggs, and fish, as well as with blood concentrations of vitamin C, β-carotene, n-3 fatty acids, holo-transcobalamin, cobalamin and folate; nutrition knowledge was negatively associated with intake of olives and avocado, alcohol and savoury snacks.
Conclusions: The NKTS assessed nutritional knowledge adequately and it is proposed as a new tool to investigate this subject in future studies.

Unhealthy diet contributes to nutrition- and obesity-related diseases, such as heart disease, cancer and type 2 diabetes[1]. One strategy to improve overall health and dietary habits is to increase knowledge about nutrition. Adolescence is a period in life during which nutritional education can help adolescents establish healthy eating habits and maintain them throughout life[2,3].

---

† Members of the HELENA Study Group are listed in the Appendix.

Corresponding author: Email thanisesouza@gmail.com

Nutritional knowledge is usually evaluated in healthy adolescents[3–5] with classical test theory[2], based on the sum of correct answers from a true/false or multiple-choice questionnaire[3–5]. The Nutritional Knowledge Test (NKT) was used in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, a cross-sectional and multicentre investigation. The HELENA study explored the level of nutritional knowledge among European adolescents and its potential determinants based on the percentage of correct answers[3].

The classical test approach has some limitations. The characteristics of each item may vary across different populations. Thus, the validation of the questionnaire should be analysed again[2,6]. Item Response Theory (IRT) analysis provides information on the discrimination and difficulty of each item across different levels of the latent trait. The information of items remains constant even if the analyses are performed in other samples. In this regard, IRT analysis allows selection of items with a better distinction of the latent trait and acknowledges the level of the construct required by each item. Moreover, IRT scores are not only estimated from the sum of the correct answers but calculated based on a probabilistic model. Besides, IRT scores are also independent of the considered set of items[6].

Therefore, IRT analysis may refine the measurement provided by the NKT, selecting items that better discriminate adolescent nutritional knowledge while characterizing the ability inherent on each level. The IRT scores calculated could also improve study of the relationship between nutritional knowledge and adolescent food intakes and biomarkers. Thus, the objectives of the present study were: (i) to analyse the NKT based on ITR analysis; and (ii) to assess the construct validity of the Nutritional Knowledge Scale (NKTS) and its associations with adolescent food group consumption and nutritional biomarkers. To address these objectives, nutritional knowledge was considered the latent trait on the IRT analysis encompassing energy metabolism, nutrient contents, oral health, food knowledge and nutritional terms.

Methods

Study design and participants

The HELENA study is a cross-sectional, multicentre investigation conducted in ten European cities: Vienna (Austria), Ghent (Belgium), Lille (France), Dortmund (Germany), Athens and Heraklion (Greece), Pécs (Hungary), Rome (Italy), Zaragoza (Spain) and Stockholm (Sweden)[7]. Between October 2006 and December 2007, the HELENA study was carried out in a random cluster sample of 3528 European adolescents between 12·5 and 17·49 years old and stratified by geographical location, age and socio-economic level. To guarantee this stratification, school and class of adolescents by age group were

considered. Overall, ten schools and fifteen to twenty classes (350–400 students) were selected in each city. The eligibility of each class within the selected schools was based on at least 70 % of students agreeing to participate in the HELENA study. Blood samples were then obtained by randomly selecting one-third of the adolescents (*n* 1089) from the total sample[7].

Exclusions from the HELENA study were performed *a posteriori* when: adolescents presented simultaneous participation in another clinical trial; were <12·5 or ≥17·5 years old; and/or had an acute infection less than one week before inclusion in the study. To process the IRT analyses, a total of 222 adolescents who did not complete more than 75 % of the NKT on the first run of the test were excluded. In addition, ninety-one adolescents providing 100 % of the answers as either right or wrong were also excluded from the current analyses because of the non-variability of the latent trait, which could affect the development of the NKTS. In total, 313 adolescents did not meet the inclusion criteria for the current analyses. Therefore, the final sample comprised 3215 adolescents.

However, a different sample size was considered in the analyses. Those adolescents who had no information on dietary assessment and under-reported energy intake using the Goldberg *et al.* approach[8,9] were excluded. This approach defines the minimum reasonable value of energy consumption that is compatible with energy balance, considering the level of physical activity[8,9]. In total, 487 adolescents under-reported energy intake. Moreover, for the mixed-model linear regression analyses, adolescents had to provide complete information on the corresponding confounding factors (age, gender, socio-economic level, maternal education level, centre and BMI). The final sample on the mixed model-linear regression analyses with consumption of food groups and biomarkers as the outcome variables included 1623 adolescents. The final sample for the mixed-model linear regression analyses with biomarkers as the outcome variable included 609 adolescents.

In each of the participating countries, the corresponding institution approved the HELENA study protocol and all participants and both parents or guardians provided their written consent[7,10]. Each participant was identified anonymously using a specific coding system which was included on the questionnaires and blood samples[10].

The study design is further explained in Fig. 1.

Sociodemographic characteristics

Socio-economic status was evaluated with a self-reported questionnaire that collects data on living conditions, family structure, employment status of parents, parental occupation and educational level[11]. The Family Affluence Scale (FAS) index was calculated based on this questionnaire and was used as an indicator of adolescents' material affluence (reflecting the number of bedrooms,
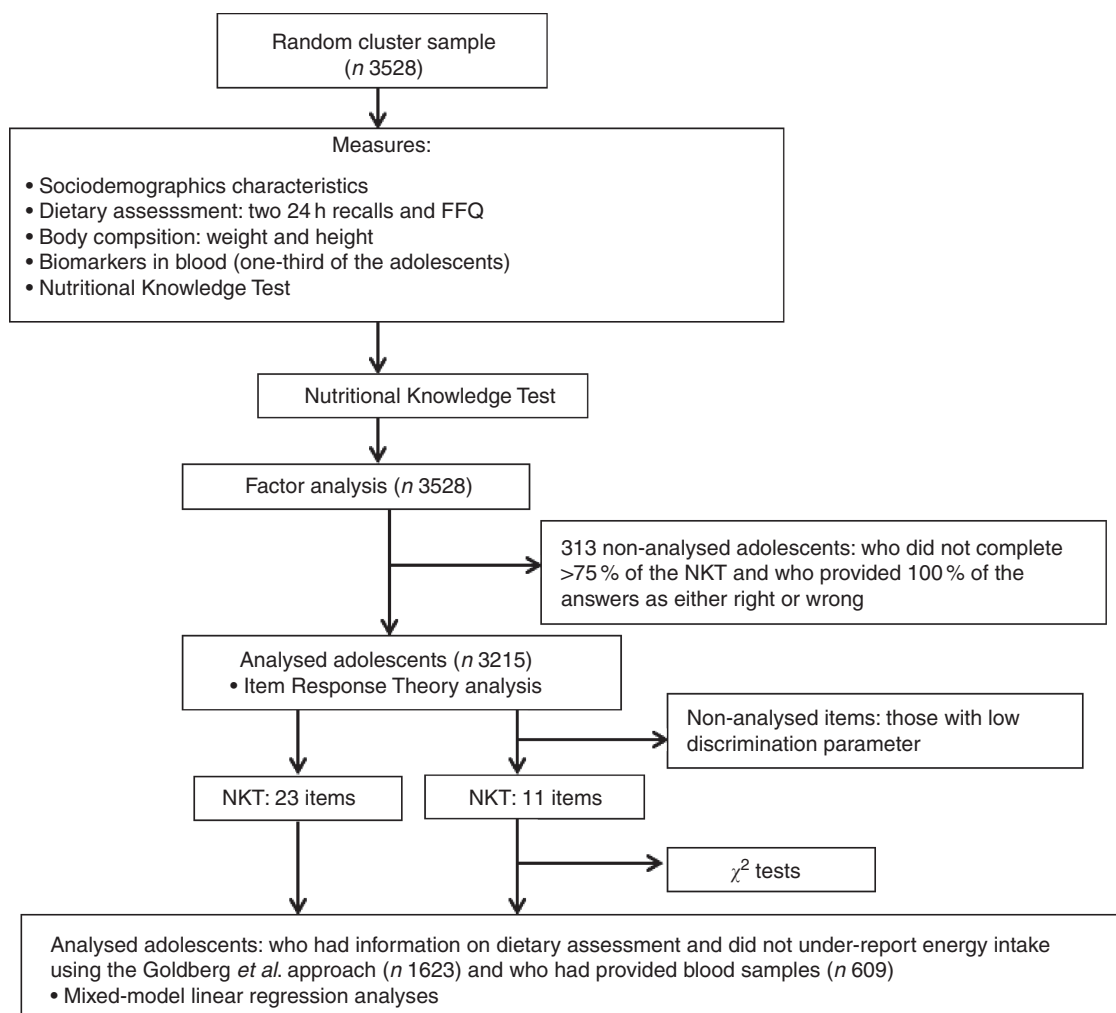
Fig. 1 Study design flowchart (NKT, Nutritional Knowledge Test; NKTS, Nutritional Knowledge Scale)

cars and computers possessed by the family and Internet access in their home). The scale ranged from 0 to 8 and was dichotomized into 'low familial wealth' (0–4) and 'high familial wealth' (5–8). Maternal education level was categorized into 'primary education', 'lower secondary education', 'higher secondary education' and 'university degree'[12].

Dietary assessment

Participants' diet was considered to assess the construct validity of the NKTS and its associations with adolescent food intakes. In the HELENA study, dietary intake data were collected using the validated HELENA-DIAT software by two non-consecutive computerized 24 h recalls, including weekdays and Sundays. The second assessment was performed within two weeks after the first one[13]. Adolescents autonomously selected all the foods and beverages consumed from a food list that included six 'meal occasions'. Some questions were presented to respondents to help them remember what they ate the day before. Some slides were presented for each food selected

detailing the portion sizes[13,14]. In addition, a self-administered FFQ was applied. This questionnaire included fifteen items and seven weekly response categories ranging from 'never' to 'more than once a day, every day'[13]. Both dietary assessment methods were administered during school time with the assistance of trained nutritionists.

Data from 24 h recalls regarding foods and beverages were expressed as grams and millilitres per day, respectively, and organized into food groups. Furthermore, to remove the effect of day-to-day variability and random error in the 24 h recalls, the individual usual food intake from these recalls was estimated using the multiple source method. This statistical modelling technique considers information from the FFQ as a covariate of the two 24 h recalls[15].

In the present study, the foods groups that emerged from the 24 h recalls were categorized into fifteen food groups according to their similarity in nutritional content and health-related characteristics. These categories are: (i) vegetables (excluding potatoes); (ii) fruits; (iii) vegetables (excluding potatoes) and fruits; (iv) sweets (including carbonated/soft/isotonic drinks, cakes, biscuits, chocolate

and other sugar products); (v) cereals (including potatoes); (vi) nuts and seeds; (vii) vegetable oils; (viii) olives and avocado; (ix) alcohol; (x) dairy products (including milk and yoghurt); (xi) pulses; (xii) meats and eggs; (xiii) fish; (xiv) savoury snacks (including chips, salty biscuits, crackers, and popcorn); and (xv) water.

Anthropometric measurements

Weight was measured with an electronic scale (model SECA 861) with 0·1 kg precision. Height was measured with participants barefoot and head in the Frankfort plane using a telescopic height-measuring instrument (model SECA 225) with 0·1 cm precision and range from 70 to 200 cm. Both measurements were taken at the same time by trained staff[16].

BMI (kg/m^2) was calculated as weight (in kilograms) divided by the square of height (in metres). The values of BMI were categorized into 'thinness', 'normal weight', 'overweight' and 'obesity' considering the reference values for BMI in adults[17,18].

Biochemical analyses

Blood biomarker concentrations were considered to assess the construct validity of the NKTS and its associations with adolescent nutrition. In the HELENA study, the blood collection was performed after a 10 h overnight fast following a standardized protocol and up to two weeks apart from the NKT. A handling and transportation system was developed to guarantee the quality assurance and stability of blood samples[19].

Out of all the biomarkers analysed in the HELENA study, vitamin C, β-carotene, *n*-3 fatty acids, cobalamin and folate presented strong correlations with nutrient intakes and were included in the present analyses. Blood concentration of vitamin C, β-carotene and *n*-3 fatty acids presented a strong correlation with fruits, vegetables and *n*-3 fatty acids, respectively[13]. *Trans*-fatty acids were also considered due to their association with nutrition- and obesity-related diseases[20]. Folate (plasma and erythrocyte) and cobalamin were measured by a competitive immunoassay (Immulite 2000; DPC Biermann GmbH, Bad Nauheim, Germany). β-Carotene and vitamin C were analysed by HPLC (Sykam, Gilching, Germany) using UV detection (UV-Vi 205; Merck, Darmstadt, Germany). Fatty acid concentrations were determined by capillary GC (model 3900) after extraction performed by thin-layer chromatography. The total amount of fatty acids was obtained from the percentage area by integrating the area under the peak and dividing it by the total area for all fatty acids. The labels *n*-3 and *trans* include all these types of fatty acids identified during blood analyses. Holo-transcobalamin was measured by a microparticle enzyme immunoassay (Active B$_{12}$; Axis-Shield Limited, Dundee, UK) with the use of an AxSYM analyser (Abbott Diagnostics, Abbott Park, IL, USA)[13,19].

To measure plasma folate, cobalamin and vitamin C (previously stabilized with metaphosphoric acid) the blood sample was collected in heparinized tubes, transported on dry ice and centrifuged within 30 min. The serum sample for fatty acid determination was also centrifuged within 30 min, stored at −80°C and transported on dry ice as soon as possible. The serum sample for β-carotene was stabilized with a synthetic antioxidant. For erythrocyte folate analysis, the whole blood was diluted in prepared ascorbic acid and incubated in the dark before storing it at −80°C. To measure holo-transcobalamin, blood was collected in evacuated tubes without anti-coagulant and then the aliquot was stored at −80°C until its transport to the laboratory[13,19].

Nutritional knowledge assessment

Nutritional knowledge was evaluated with the NKT, a validated questionnaire designed by a nutritional psychologist[21]. The NKT contains twenty-three multiple-choice items encompassing concepts related to energy intake and metabolism, nutrient contents, sweeteners and oral health, food knowledge, and special terms and definitions. This test was designed for those who did not receive any nutritional education programme in the past. Each item offers four possible answers, including a 'don't know' category, but only one answer category is correct. Furthermore, the NKT comprises common misconceptions as well as easy items in order to motivate individuals to answer the test. In the HELENA study, the NKT was completed by students during class with the supervision of the research team[3,21,22].

Statistical analyses

The dimensionality of the NKT was analysed by factor analysis with estimation using principal component analysis and Varimax orthogonal rotation. The use of a one-dimensional model was acceptable if the first principal component explained 20 % or more of the total variance[23].

The biserial coefficient was calculated for each item to indicate the correlation behind the correct answer to items and the level of nutritional knowledge. Items with a negative biserial coefficient were excluded from the analyses[24].

A one-dimensional logistic model of the three parameters was used to calculate the probability of a person to correctly answer an item, with a given nutritional knowledge level. This model is represented by the following equation[24]:

$$P\left(U_{ij} = 1 \middle| \theta_j\right) = c_i + (1 - c_i) \frac{1}{1 + e^{-Da_i(\theta_j - b_i)}},$$

where θ_j is the IRT score of a person j; $P(U_{ij} = 1|\theta_j)$ is the probability of a person j with an ability θ_j to correctly answer an item i; D is a scale constant factor and equal to

1·7; c_i is the guessing parameter of item i; a_i is the discrimination parameter of item i; and b_i is the difficulty parameter of item i.

The discrimination parameter a_i indicates the quality of an item to discriminate people with different levels of nutritional knowledge. Items with higher values on this parameter present better discrimination. The difficulty parameter b_i identifies the point on the scale at which the probability to answer correctly is higher than or equal to $(1+c_i)/2^{(24)}$. Finally, the guessing parameter c_i indicates the probability that someone gives the correct answer by chance, with value equal to or above 0 and equal to or below 1 $(0 \leq c_i \leq 1)^{(25)}$.

All parameters (a_i, b_i and c_i) were estimated by marginal maximum likelihood with a maximum number of twenty quadrature points, fifty EM cycles, ten Newton interactions and convergence criterion equal to 0·01. These parameters estimates were analysed with the corresponding SE (see online supplementary material, Supplemental Table 1). Items with discrimination parameter a_i value below or equal to 0·70 were considered as having less discrimination and were excluded from further analyses. The estimation of the parameters mentioned above was repeated only for items with adequate discrimination. To control the lack of identification of the model, b_i and IRT scores were estimated on a scale with mean equal to 0 and SD equal to 1 by the expected *a posteriori* method[25]. Empirical reliability, a precision measure analogous to Cronbach's α, was calculated to analyse the reliability of the NKTS. Besides the empirical reliability, the test information curve was analysed to identify the accuracy of the measurement along the NKTS.

The probability of the correct answer for each item was calculated across IRT scores. The item was located at the level of the correct answer probability when it was higher than or equal to $(1+c_i)/2$. Some levels were grouped into the same range of nutritional knowledge according to the technical similarity between items. This step was performed by two nutritionists (T.S.S.S. and C.J.) using Microsoft® Excel version 2010.

To analyse the associations between IRT scores and demographic, socio-economic and health characteristics, Kolmogorov–Smirnov tests were carried out to test the normality in the distribution of the studied variables and guide further analyses. The χ^2 test was performed to evaluate differences between demographic, socio-economic and BMI categories by NKTS levels. Mixed-model linear regression analyses were performed to assess the construct validity of NKTS and its associations with nutritional knowledge, food group consumption and biomarkers. The IRT scores were included as the independent variable. To explore these associations, analyses were carried out separately for the NKTS (considering only items with adequate IRT parameters) and for all items in the original NKT (calculating all IRT scores although there were items with inadequate IRT parameters). Food groups and biomarker

variables were included separately in the model with the outcome variable, and centre was included as the random intercept. Age, gender, maternal education, FAS index, BMI and energy intake were entered as covariates in the model. Statistical significance was set at $P < 0.05$.

Analyses were performed using the statistical software package Stata version 14[26] and the software BILOG-MG version 3[27].

Results

As suggested by the factor analysis, the first principal component explained 46 % of the total variance (see online supplementary material, Supplemental Fig. 1). None of the items presented a negative biserial coefficient (Supplemental Table 1). Twelve items presented low discrimination of nutritional knowledge and were excluded from further analyses. Biserial coefficient and IRT parameters (a_i, b_i and c_i) of the remaining items are described in Table 1.

The estimation of the IRT parameters was completed with eleven EM cycles and three Newton interactions. Along the nutritional knowledge continuum, the item 'A breakfast merely consisting of bread, jam and butter does not contain enough…?' presented the lowest discrimination parameter ($a_i = 0.88$). On the other hand, the item 'The ingredients list found on food items may contain a number of different terms for sugar. Which row lists three terms for special types of sugar?' had the highest discrimination parameter ($a_i = 1.65$).

The item 'Marcel has been playing with a ball all afternoon. During this time Kevin has been sitting at home watching television. Which of the following statements is most applicable?' had the lowest difficulty parameter ($b_i = -2.13$), suggesting that energy expenditure is a relatively easy concept for the adolescents. The item 'Which row lists three terms for energy-free sweeteners?' presented the highest difficulty parameter ($b_i = 1.50$), suggesting that advanced nutritional terms were the most difficult ones to be identified by the HELENA adolescents.

All items presented low values of the guessing parameter c_i, with values ranging from 0·01 to 0·09. In the studied sample, NKTS scores varied from –0·83 to 0·83. NKTS showed a good empirical reliability (0·69) and presented more accurate information between IRT scores, ranging from –1·0 to 1·0 (Fig. 2).

Results from the mixed-model linear regression analysis with food group consumption as the outcome variables and NKTS scores as the independent variable are shown in Table 2. Nutritional knowledge was positively associated with consumption of fruits, cereals, dairy products, pulses, meats and eggs, and fish, and was negatively associated with intake of olives and avocado, alcohol and savoury snacks.

Table 1 Biserial coefficient and discrimination, difficulty and guessing parameters, with their SE, from the Item Response Theory analysis of the Nutritional Knowledge Scale

Item	Correct answer	Biserial	a_i†	SE	b_i‡	SE	c_i§	SE
Marcel has been playing with a ball all afternoon. During this time Kevin has been sitting at home watching television. Which of the following statements is most applicable?	Marcel burns more energy than Kevin	0·44	1·22	0·09	−2·13	0·13	0·02	0·03
Which row lists three dishes that have all been prepared using very little fat?	Boiled egg, boiled potatoes, steamed fish	0·37	0·91	0·06	−1·13	0·09	0·02	0·02
Which substance is good for your teeth?	Fluoride	0·39	0·91	0·07	−0·66	0·10	0·02	0·03
Bread, cake, pasta, potatoes and rice contain mainly…?	Carbohydrates	0·45	1·17	0·13	−0·19	0·16	0·09	0·07
Dietitians use the American term 'junk food' to describe certain foods. What do they mean by this?	Foods that contain a lot of energy but are of very little nutritional value	0·41	0·98	0·07	−0·18	0·07	0·02	0·02
What effect does the fibre contained in our food have on the human body?	It stimulates the process of digestion	0·51	1·32	0·09	0·02	0·06	0·02	0·02
The ingredients list found on food items may contain a number of different terms for sugar. Which row lists three terms for special types of sugar?	Dextrose, fructose, maltose	0·56	1·65	0·11	0·19	0·04	0·01	0·01
A breakfast merely consisting of bread, jam and butter does not contain enough…?	Protein	0·38	0·88	0·08	0·35	0·12	0·03	0·04
Which row lists three foods that contain a lot of vitamin C?	Peppers, cabbage, citrus fruit	0·40	0·98	0·10	0·48	0·11	0·04	0·04
What is the other commonly used term for energy?	Joule	0·41	1·08	0·11	0·96	0·08	0·03	0·03
Which row lists three terms for energy-free sweeteners?	Aspartame, saccharin, cyclamate	0·45	1·28	0·14	1·50	0·08	0·01	0·01

†Discrimination parameter.
‡Difficulty parameter.
§Guessing parameter.
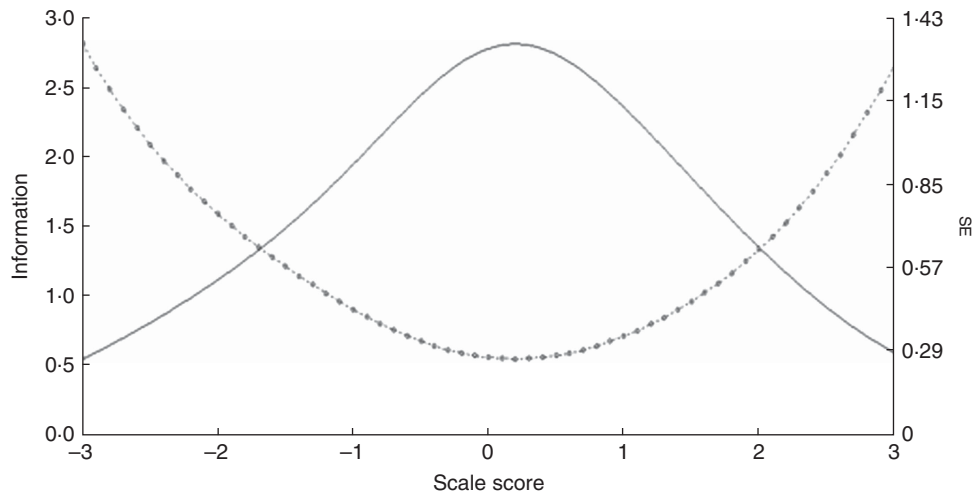
Fig. 2 Nutritional Knowledge Scale information curve (*n* 3215): ——, test information curve; --●--, standard error curve

Results from the mixed-model linear regression analysis with the concentration of biomarkers as the outcome variables and NKTS scores as the independent variable are presented in Table 3. Nutritional knowledge was positively associated with blood concentrations of vitamin C, β-carotene, *n*-3 fatty acids, holo-transcobalamin, cobalamin and folate. Additionally, both mixed-model linear regression analyses presented similar results when IRT scores were calculated for all items of the original NKT that presented low discrimination of nutritional knowledge (see online supplementary material, Supplemental Tables 2 and 3) and when the number of cigarettes consumed was included as a covariate (data not shown).

NKTS scores were categorized into three different levels to facilitate their interpretation: basic, adequate and advanced. The basic level (IRT scores below −0·5) included knowledge about energy expenditure and ingredients from recipes. This level comprised the items: 'Marcel has been playing with a ball all afternoon. During this time Kevin has been sitting at home watching television. Which of the following statements is most applicable?' and 'Which row lists three dishes that have all been prepared using very little fat?' The adequate level (IRT scores from −0·5 to 1·0) included knowledge about nutrients from foods and their role in health. This level presented the following items: 'Which substance is good for your teeth?'; 'Bread,

Table 2 Mixed-model analyses between intakes of food groups (g/d) and Item Response Theory scores from the Nutritional Knowledge Scale, adjusted for age, gender, maternal education, Family Affluence Scale index and energy intake, among European adolescents aged 12·5–17·5 years (n 1623), HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study

Food group	β†	95 % CI	P value
Vegetables	−33·03	−78·88, 12·81	0·16
Fruits	109·75	30·53, 188·96	0·01*
Fruits and vegetables	83·58	−9·17, 176·33	0·08
Sweets	−98·80	−338·44, 140·83	0·42
Cereals	114·42	51·77, 177·06	<0·001*
Nuts and seeds	−4·25	−11·41, 2·91	0·24
Olives and avocado	−6·19	−11·60, −0·79	0·02*
Vegetable oils	−2·21	−9·10, 4·67	0·53
Alcohol	−160·41	−237·06, −83·77	<0·001*
Dairy products	401·06	233·18, 568·93	<0·001*
Pulses	32·29	9·88, 54·69	<0·01*
Water	293·82	−122·91, 710·55	0·17
Fish	39·91	21·46, 58·36	<0·001*
Meats and eggs	150·28	96·12, 204·45	<0·001*
Savoury snacks	−14·85	−27·40, −2·30	0·02*

Centre was used as the random intercept.
*Significant P values.
†Fixed-effects estimates.

Table 3 Mixed-model analyses between concentrations of biomarkers and Item Response Theory scores from the Nutritional Knowledge Scale, adjusted for age, gender, maternal education, Family Affluence Scale index, BMI and energy intake, among European adolescents aged 12·5–17·5 years (n 609), HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study

Biomarker	β†	95 % CI	P value
Vitamin C (mg/l)	10·90	5·68, 16·12	<0·001*
β-Carotene (ng/ml)	476·05	179·56, 772·54	0·002*
n-3 Fatty acids (%)	5·09	2·89, 7·29	<0·001*
n-3 Fatty acids (µmol/l)	155·21	71·34, 239·08	<0·001*
Holo-transcobalamin (pmol/l)	66·22	11·23, 121·22	0·02*
Cobalamin (pmol/l)	539·86	335·23, 744·49	<0·001*
Plasma folate (nmol/l)	42·24	25·37, 59·11	<0·001*
Erythrocyte folate (nmol/l)	1253·45	648·63, 1858·27	<0·001*
Trans-fatty acids (µmol/l)	1·20	−0·01, 2·41	0·05

Centre was used as the random intercept.
*Significant P values.
†Fixed-effects estimates.

cake, pasta, potatoes and rice contain mainly…?'; 'Diet-itians use the American term "junk food" to describe certain foods. What do they mean by this?'; 'What effects does the fibre contained in our food have on the human body?'; 'The ingredients list found on food items may contain a number of different terms for sugar. Which row lists three terms for special types of sugar?'; 'A breakfast merely consisting of bread, jam and butter does not contain enough…?'; and 'Which row lists three foods that contain a lot of vitamin C?' The advanced level (IRT scores above or equal to 1·0) included knowledge about basic and advanced nutritional terms. This last level contained the items: 'What is the other commonly used term for energy?' and 'Which row lists three terms for energy-free sweeteners?' Therefore, a person who shows a nutritional knowledge level below −0·5 would have knowledge on energy expenditure and ingredients from recipes. On the other hand, a person with a nutritional knowledge level above or equal to 1 would present advanced nutritional

knowledge, such as knowledge of energy expenditure, nutrients from foods and their role in health, ingredients from recipes and advanced nutritional terms.

The demographic and socio-economic characteristics of participants are described in Table 4. Girls, older adolescents, those whose mothers had a university education and those with a high FAS index presented higher nutritional knowledge.

Discussion

When evaluating if the NKT assessed nutritional knowledge, factor analysis indicated that the first principal component explained 46 % of the total variance. Therefore, an one-dimensional logistic model of three parameters was applied to develop the NKTS[23,24].

The original NKT calculated only the percentage of correct answers[3]; in this way, adolescents with different

Table 4 Demographic and socio-economic characteristics, by Nutritional Knowledge Scale level, of European adolescents aged 12·5–17·5 years (*n* 3215), HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study

			Nutritional Knowledge Scale level						
	Total sample		Basic (*n* 376; 11·7 %)		Adequate (*n* 1912; 59·5 %)		Advanced (*n* 927; 28·8 %)		
	n	%	*n*	%	*n*	%	*n*	%	*P* value†
Age (years)									<0·001*
12·5–13·99	1022	31·8	161	42·8	656	34·3	205	22·1	
14–14·99	795	24·7	99	26·3	482	25·2	214	23·1	
15–15·99	783	24·4	65	17·3	457	23·9	261	28·2	
16–17·49	615	19·1	51	13·6	317	16·6	247	26·6	
Gender									0·04*
Male	1526	47·5	183	48·7	936	49·0	407	43·9	
Female	1689	52·5	193	51·3	976	51·0	520	56·1	
FAS index (*n* 3044)									<0·001*
Low	1397	45·9	198	57·7	852	47·1	347	38·8	
High	1647	54·1	145	42·3	955	52·9	547	61·2	
Maternal education (*n* 3072)									<0·001*
Primary	252	8·2	43	12·5	168	9·2	41	4·6	
Lower secondary	819	26·7	104	30·2	533	29·0	182	20·4	
Higher secondary	966	31·4	100	29·1	568	31·0	298	33·4	
University degree	1035	33·7	97	28·2	566	30·8	372	41·7	
Centre									<0·001*
Athens	272	8·5	80	21·3	149	7·8	43	4·6	
Dortmund	422	13·1	55	14·6	290	15·2	77	8·3	
Ghent	322	10·0	28	7·4	196	10·3	98	10·6	
Heraklion	245	7·6	59	15·7	154	8·1	32	3·5	
Lille	243	7·6	35	9·3	189	9·9	19	2·0	
Pécs	376	11·7	29	7·7	199	10·4	148)	16·0	
Rome	293	9·1	21	5·6	195	10·2	77	8·3	
Stockholm	292	9·1	36	9·6	173	9·0	83	9·0	
Vienna	383	11·9	24	6·4	174	9·1	185	20·0	
Zaragoza	367	11·4	9	2·4	193	10·1	165	17·8	
BMI									0·01*
Thinness	193	6·0	28	7·4	109	5·7	56	6·0	
Normal weight	2280	70·9	248	66·0	1346	70·4	686	74·0	
Overweight	561	17·4	67	17·8	347	18·1	147	15·9	
Obesity	181	5·6	33	8·8	110	5·8	38	4·1	

FAS, Family Affluence Scale.
*Significant *P* values.
†Likelihood ratio *P* value.

nutritional knowledge levels could get the same score[28]. The IRT analysis complements the classical approach as it calculates the scores and the pattern of correct answers by comparing the difficulty of each item[29].

Twelve items from the original NKT presented low discrimination of nutritional knowledge and thus were excluded. The NKTS provides a high-quality tool to assess nutritional knowledge, as it includes items with the best discrimination of nutritional knowledge. In addition, results from the mixed-model linear regression analyses presented similar associations between food group consumption and nutritional biomarkers in two different approaches: when considering the NKTS (IRT scores calculated from eleven items) or the original NKT (IRT scores calculated from all twenty-three items). One of the benefits of using a lower number of items is the accuracy of participants' responses, as large periods for questionnaire completion tend to increase fatigue and boredom[30]. Therefore, the reduction in the number of items on the questionnaire may improve the quality of answers and

decrease the time of completion. This aspect is important for epidemiology researchers working with large sample sizes and assessing information in a short period of time.

Besides this refinement of the NKT, by selecting the items that best discriminated nutritional knowledge, all items maintained on the NKTS presented an adequate guessing parameter. If an item presents a high value concerning the guessing parameter, it should be excluded from the final questionnaire because guessing is influenced by a high probability of correct answers[24]. Therefore, adequate guessing parameters may confirm the precision of the NKTS. In this regard, the IRT analyses improved the quality of nutritional knowledge assessment.

In addition to the reduced number of items and greater accuracy of the measurement, the current analysis confirmed the construct validity of the NKTS. The mixed-model linear regression analysis confirmed that nutritional knowledge is positively associated with the consumption of healthy foods and an appropriate profile of nutritional biomarkers. The NKTS scores were positively associated

with intakes of fruits, cereals, dairy products, pulses, meat and eggs, and fish, as well as with blood concentrations of vitamin C, β-carotene, n-3 fatty acids, holo-transcobalamin, cobalamin and folate; on the other hand, nutritional knowledge was negatively associated with consumption of olives and avocado, alcohol and savoury snacks. A recent study on Japanese children and their guardians also observed a positive association between nutritional knowledge and consumption of healthy foods[31]. Among adolescents, some studies have demonstrated that nutritional knowledge is related to a higher overall variety of food[32,33], higher serum retinol and lower intakes of total fat and added sugars[33]. Finally, considering the challenges to obtain accurate food intakes, the positive associations between NKTS scores and nutritional biomarkers improved the results from the mixed-model linear regression analysis with food groups' consumption as the outcome variable. Despite the non-expected negative association with intakes of olives and avocado ($P < 0.05$), our results highlight the importance of acquiring nutritional knowledge as a strategy to improve dietary habits among adolescents.

After calculating IRT scores, the next step was to describe the nutritional knowledge in each NKTS level. Based on this description, it may be possible to better assess what adolescents know about nutrition and with which items they may have more difficulty[29]. The basic level includes knowledge about energy expenditure and ingredients from recipes. Individuals who are classified on this level would correctly answer to the item with the lowest difficulty level: 'Marcel has been playing with a ball all afternoon. During this time Kevin has been sitting at home watching television. Which of the following statements is most applicable?' ($b_i = -2.13$). The item 'Which row lists three terms for energy-free sweeteners?' presented the highest difficulty parameter ($b_i = 1.50$), suggesting that advanced nutritional terms were the most difficult to be identified by adolescents of the HELENA study. This item would probably be answered correctly by those adolescents on the advanced level and with NKTS scores higher than 1·50. Therefore, the identification of the NKTS levels in the studied individuals may improve the strategic direction of scientific interventions and policy making.

This description makes it possible to determine the proportion of individuals on each NKT level. In this regard, adolescents from Heraklion and Lille presented the lowest NKTS scores. In agreement with a previous study that evaluated the NKT[3], the scores from the NKTS significantly increased in adolescents who were older in age, female and whose mothers had more education. Moreover, the positive associations between NKTS scores and socio-economic level are consistent with previous research. A population-based study indicated a linear association between nutritional knowledge and socio-economic level in urban and rural regions in

Iraq[34]. Finally, in the current study, NKTS scores were negatively associated with BMI among thin and obese adolescents. However, the relationship between nutritional knowledge and nutritional status is not well known in the literature. The previous study evaluating the NKT[3] and other studies have demonstrated no significant associations in adolescents and adults[35,36]. However, it is important to mention that these studies used classical test theory and, therefore, their results may not be accurate.

The test information curve indicated that the NKTS is more precise within medium scores. For this reason, it is necessary to include new items measuring other levels of knowledge. In future studies, the item parameters could be maintained and new items included in the NKTS. This could be possible using computerized adaptive testing which allows calculating more precise estimates and creating a flexible questionnaire making it less burdensome for respondents[37].

The current analysis aimed to evaluate the NKT based on the ITR analysis and assess the construct validity of the developed NKTS and its associations with food consumption and nutritional biomarkers in adolescents. The factor analysis confirmed that the NKT assessed nutritional knowledge. However, if we were evaluating nutritional knowledge in other populations, the classical approach would not be applicable by using the original NKT. The IRT analysis complemented the classical test approach, identifying those items with better discrimination and the location of each item on the continuum. Additionally, IRT analysis added the characterization of each nutritional knowledge level. Results from the mixed-model linear regression analysis confirmed the validity of the NKTS, endorsing its use in epidemiological studies. Furthermore, due to the invariance in the estimation of the IRT parameters, the NKTS can be applied to other populations such as adolescents and adults from different countries[28]. When analysing NKTS scores in other populations it will be necessary that participants answer some or all of the eleven items. Moreover, to calculate these scores, it is important to consider the IRT parameters calculated in the current study. In addition, IRT scores can be applied to further studies in continuous or categorical forms.

Limitations and strengths
One limitation of the present study concerns the cross-sectional design which does not allow pointing out causal inferences. Besides, the missing values reduced the sample size for the IRT analysis, but it was large enough to estimate IRT parameters and detect associations between IRT scores and both food intakes and nutritional biomarkers. The HELENA study did not assess information on supplement intake that may affect the results from the mixed-model linear regression analyses with the

concentration of biomarkers as the dependent variable. The dietary assessment was based on self-reporting which may affect the precision of the information, although the HELENA-DIAT software includes some questions to help adolescents remember what they ate the day before. However, the study has some strengths; the stratified nature of the sampling increased the precision of the data that may differ across strata. The standard procedures used to collect all measurements throughout the cities and the analyses were controlled for several confounding factors. Furthermore, the development of the NKTS was based on statistical analyses that solve the limitations of the classical test approach previously described. In this regard, the use of the NKTS may generate new and promising research partnerships, and after its standardization, researchers can apply this new method to assess nutritional knowledge in different studies and populations.

Conclusions

The IRT analysis selected those items from the NKT which presented better nutritional knowledge discrimination. The NKTS provides a more precise assessment of the latent trait with a short and high-quality pool of items. The mixed-model linear regression analysis confirmed the validity of the NKTS, indicating positive associations between the NKTS and healthy food consumption as well as nutritional biomarkers. The NKTS is proposed as a new instrument to assess nutritional knowledge in Europe and other parts of the world.

Acknowledgements

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1368980018003269

References

1. Robertson A, Tirado C, Lobstein T *et al.* (editors) (2004) *Food and Health in Europe: A New Basis for Action. WHO Regional Publications, European Series* no. 96. Copenhagen: WHO Regional Office for Europe.
2. Parmenter K & Wardle J (2000) Evaluation and design of nutritional knowledge measures. *J Nutr Educ* **32**, 269–277.
3. Sichert-Hellert W, Beghin L, De Henauw S *et al.* (2011) Nutritional knowledge in European adolescents: results from the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. *Public Health Nutr* **14**, 2083–2091.
4. Dickson-Spillmann M, Siegrist M & Keller C (2011) Development and validation of a short, consumer-oriented nutrition knowledge questionnaire. *Appetite* **56**, 617–620.
5. Whati LH, Senekal M, Steyn NP *et al.* (2005) Development of a reliable and valid nutritional knowledge questionnaire for urban South African adolescents. *Nutrition* **21**, 76–85.
6. Nguyen TH, Han HR, Kim MT *et al.* (2014) An introduction to Item Response Theory for patient-reported outcome measurement. *Patient* **7**, 23–35.
7. Moreno LA, De Henauw S, Gonzalez-Gross M *et al.* (2008) Design and implementation of the Healthy Lifestyle in Europe by Nutritional in Adolescence Cross-Sectional Study. *Int J Obes (Lond)* **32**, Suppl. 5, S4–S11.
8. Goldberg GR, Black AF, Jebb SA *et al.* (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45**, 569–581.
9. Black AF (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.
10. Beghin L, Castera M, Manios Y *et al.* (2009) 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.
11. Iliescu C, Beghin L, Maes L *et al.* (2008) Socioeconomic questionnaire and clinical assessment in the HELENA Cross-Sectional Study: methodology. *Int J Obes (Lond)* **32**, Suppl. 5, S19–S25.
12. Julian C, Mouratidou T, Vicente-Rodrigues G *et al.* (2017) Dietary sources and sociodemographic and lifestyle factors affecting vitamin D and calcium intakes in European adolescents: the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study. *Public Health Nutr* **20**, 1593–1601.
13. Vandevijvere S, Geelen A, Gonzalez-Gross M *et al.* (2013) Evaluation of food and nutrient intake assessment using concentration biomarkers in European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr* **109**, 736–747.
14. Vereecken CA, Covents M, Sichert-Hellert W *et al.* (2008) Development and evaluation of a self-administered computerized 24h dietary recall method for adolescents in Europe. *Int J Obes (Lond)* **32**, Suppl. 5, S26–S34.
15. Currie C (editor) (1998) *Health Behavior in School-aged Children: A WHO Cross-National Survey (HBC) Research Protocol for the 1997/1998 Survey.* Edinburgh: University of Edinburgh, Research Unit in Health and Behavioural Change.
16. Nagy E, Vicente-Rodriguez G, Manios Y *et al.* (2008) Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. *Int J Obes (Lond)* **32**, Suppl. 5, S58–S65.

17. Cole TJ, Flegal KM, Nicholls D et al. (2007) Body mass index cut offs to define thinness in children and adolescents: international survey. BMJ 335, 194–202.

18. Cole TJ, Bellizzi MC, Flegal KM et al. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320, 1240–1243.

19. González-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.

20. Wang Q, Inamura F, Lemaitre RN et al. (2014) Plasma phospholipid trans-fatty acids levels, cardiovascular diseases, and total mortality: the Cardiovascular Health Study. J Am Heart Assoc 3, e000914.

21. Diehl JM (1999) Ernährungswissen von Kindern und Jugendlichen. Verbraucherdienst 44, 282–287.

22. Kersting M, Sichert-Hellert W, Vereecken CA et al. (2008) Food and nutrient intake, nutritional knowledge and diet-related attitudes in European adolescents. Int J Obes (Lond) 32, Suppl. 5, S35–S41.

23. Reckase MD (1979) Unifactor latent trait models applied to multifactor tests: results and implications. J Educ Behav Stat 4, 207–230.

24. Andrade DF, Tavares HR & Valle RC (2000) Teoria de Resposta ao Item: Conceitos e Aplicações. In 4º Simpósio Nacional de Probabilidade e Estatística – SINAPE 2000, Caxambu, Minas Gerais, Brazil. São Paulo: Associação Brasileira de Estatística.

25. Chernyshenko O, Stark S, Chan K et al. (2001) Fitting Item Response Theory models to two personality inventories: issues and insights. Multivariate Behav Res 36, 523–562.

26. StataCorp (2015) Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.

27. Zimowski MF, Muraki E, Mislevy RJ et al. (2003) BILOG-MG 3. Chicago, IL: Scientific Software.

28. Wilson M, Allen DD & Li JC (2006) Improving measurement in health education and health behavior research using item response modeling: comparison with the classical test theory approach. Health Educ Res 21, Suppl. 1, i19–i32.

29. Primi R (2004) Advances in scale interpretation with the application of Item Response Theory. Aval Psicol 3, 53–58.

30. Burns KEA, Duffett M, Kho ME et al. (2008) A guide for the design and conduct of self-administered surveys of clinicians. CMAJ 179, 245–252.

31. Asakura K, Todoriki K & Sasaki S (2017) Relationship between nutrition knowledge and dietary intake among primary school children in Japan: combined effect of children's and their guardians' knowledge. J Epidemiol 27, 483–491.

32. Augustine LF, Vazir S, Rao SF et al. (2012) Psychometric validation of a knowledge questionnaire on micronutrients among adolescents and its relationship to micronutrient status of 15–19-year-old adolescent boys, Hyderabad, India. Public Health Nutr 15, 1182–1189.

33. Oldewage-Theron W, Egal A & Moroka T (2014) Nutrition knowledge and dietary intake of adolescents in Cofimvaba, Eastern Cape, South Africa. Ecol Food Nutr 54, 138–156.

34. Heshmat R, Salehi F, Qorbani M et al. (2016) Economic inequality in nutritional knowledge, attitude and practice of Iranian households: the NUTRI-KAP study. Med J Islam Repub Iran 30, 915–922.

35. Brien GO & Davies M (2005) Nutrition knowledge and body mass index. Health Educ Res 22, 571–575.

36. Meseri R, Mermer G, Ergin I et al. (2015) Evaluation of obesity prevalence and nutritional knowledge in adolescents in semi urban area of Turkey. Prog Nutr 17, 58–67.

37. Cella D, Riley W, Stone A et al. (2010) The patient-reported outcomes measurement information system (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008. J Clin Epidemiol 63, 1179–1194.

Appendix

The HELENA Study Group