Title: Socioeconomic factors associated with folate and vitamin B_{12} intakes and related biomarkers concentrations in European adolescents: The HELENA Study

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Abbreviations:

SEF: Socioeconomic Factors

NDNS: National Diet and Nutrition Survey

HELENA-CSS: Healthy Lifestyle in Europe by Nutrition in Adolescence- Cross-Sectional Study

ANCOVA: One-way analysis of covariance

RBC-folate: Red Blood Cell folate

FAS: Family Affluence Scale

PC: Personal Computer

ISCO: International Standard Classification of Occupations

BMI: Body Mass Index

YANA-C: Young Adolescents' Nutrition Assessment software

MSM: Multiple Source Method

EDTA: Ethylene Diamine Tetraacetic Acid

HoloTC: Holotranscobalamin

CV: Coefficient of Variation

SPSS: Statistical Package for Social Sciences

SE: Standard Error

CI: Confidence Intervals

EAR: Estimated Average Requirement

Abstract

Socioeconomic factors (SEF) may influence dietary quality and vitamin intakes, so this study aims to examine associations between socioeconomic factors and folate and vitamin B_{12} intakes, and related biomarkers in the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. Vitamins intakes were obtained from two 24h-recalls in 2,253 participants (47% males). Vitamin B biomarkers were assessed in a subsample of 977 participants (46% males). SEF were assessed by questionnaire. One-way analysis of covariance (ANCOVA) and linear regression analysis were applied. Mean intakes of folate were 211.19 and 177.18 μg/d; and for vitamin B_{12}, 5.98 and 4.54 μg/d, for males and females, respectively. In males, levels of plasma folate, RBC-folate, serum B_{12}, and holoTC, were 18.74, 807.19, 330.64, and 63.04 nmol/L; and for females: 19.13, 770.16, 377.9, and 65.63 nmol/L, correspondingly. Lower folate intakes were associated with several SEF, including maternal and paternal education in both sexes. Regarding folate biomarkers, only lower plasma folate were associated with single/shared-care in males, and with lower paternal occupation in females. Lower vitamin B_{12} intakes were associated with almost all the studied SEF, except with paternal occupation in both sexes. Considering vitamin B_{12} biomarkers, only in females, lower plasma vitamin B_{12} was associated with lower maternal education and occupation; and lower holoTC, with lower maternal education and lower paternal occupation. From the set of socioeconomic determinants studied in a sample of European adolescents,

maternal education and paternal occupation were more consistently associated with folate and vitamin B_{12} intakes and biomarkers concentrations.

Keywords: Socioeconomic factors, folic acid, vitamin B_{12}, cross-sectional, adolescent,

1. Introduction

Adequate micronutrient status is essential during critical periods of rapid growth and development such as adolescence [1]. Irregular patterns in meals, and the increased consumption of unhealthy products in this period may lead to nutritional deficits, especially when considering micronutrients [2]. Folate [3] and vitamin B_{12} [4] deficiencies during childhood and adolescence are not uncommon, at least at subclinical level [5]. These vitamins ,contribute to healthy growth and development due to their important role in cell formation [6].

Socioeconomic factors (SEF) are known to influence dietary quality [7] and vitamin intakes [8]. The reported associations between diet quality and health, found in epidemiologic studies, may have been mediated by unreported SEF. Available literature addressing such issues is limited. For instance, the results of the National Diet and Nutrition Survey of 4-18 years old in the UK [8], indicated that participants in lower socioeconomic positions (measured as social class of the head of the household, receipt of benefits by the young person's household, household income and family composition) tended to have lower micronutrient intakes and corresponding biomarkers concentrations. In a sample of Swedish adolescents participating in The Nord-Trondelag Health Study [9], a positive association was suggested between folate intakes but not with folate biomarkers concentrations and maternal education and paternal income.

The most appropriate SEF in epidemiological studies is subject to debate and often the final choice reflects data availability and study resources [10]. For instance, social factors like household composition and migration background [11] have demonstrated to be strongly linked with eating behaviour [12]. Economic factors like education, occupation, and income [7] are also considered to be related to health outcomes due to its direct influence on lifestyle behaviours [13].

Differences between SEF regarding health-related issues, highlight the need of using parallel social and economic factors to obtain an in-depth picture of the influence of social inequalities on dietary habits in order to develop effective strategies for health promotion.

These challenged proxies are mediated by the reporting bias in dietary assessment. For instance, it is well-established that higher position socioeconomic groups are used to over-reported healthy foods in dietary surveys [14]. Therefore, serum vitamin analyses may be essential to better understand the interrelations between SEF and micronutrient adequacy [15, 16].

There is a lack of large studies addressing micronutrient status both in terms of intakes and plasma concentrations, in relation to SEF among adolescents in Europe. The present study aims to examine socioeconomic factors as determinant of intakes and statuses, which contributes to better understand the DISH model for food, nutrition and health research [17] which is sum up in figure 1, adapted from Romana Novakovic´s thesis [18]. In this case, the relationships are based on folate and vitamin B_{12} intakes and their related blood concentrations in a large sample of European adolescents aged 12.5 to 17.5 years.

2. Methods and materials

2.1. Subjects, recruitment and study design

The Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study, (HELENA-CSS), is a multi-centre study of lifestyle and nutrition among adolescents, from 10 European cities from nine countries; Athens and Heraklion (Greece), Dortmund (Germany), Ghent (Belgium), Lille (France), Pecs (Hungary), Rome (Italy), Stockholm (Sweden), Vienna (Austria), and Zaragoza (Spain). The mean participation rate for adolescents in our study was 67%, which can be considered acceptable for such a demanding epidemiological study [19]. Inclusion criteria were; being 12.5-17.5 years old, not participating simultaneously in another clinical trial and being free of any acute infection occurring < 1 week before inclusion [20]. A total of 3,528 adolescents (47% males) were recruited between October 2006 and December 2007. Data from Heraklion and Pecs were not included in the dietary intake analysis (7% of the total sample) because no nutrient intake was calculated for these two cities. The final sample available for the dietary analysis included 2,253 adolescents (46% males) with complete data on two non-consecutive 24-hour recalls and SEF of interest. A random sub-sample of 977 adolescents (46% boys) were included in the blood parameters analysis related to vitamin status (data from Heraklion and Pecs were available in this instance). More details on the sampling procedures, pilot study and reliability of the data have been published elsewhere [20]. Informed consent was obtained from all participants and their parents, and the protocol was approved by the Human Research Review Committees of the corresponding centres (cities) [21].

2.2. Assessment of SEF

Information on SEF was obtained via a self-administered questionnaire completed by the adolescents. The questionnaire addressed a wide range of social factors including family affluence, paternal and maternal education and occupation levels, migration background and household composition. As some questions related to SES questionnaire, produced difficulties, these questions were reformulated or deleted. The SES questionnaire was then modified, corrected and finally approved by all partners involved in the HELENA [22]. This methodology used to asses SEF had been previously demonstrated to be accurate proxy for parental socioeconomic status [23]. A modified version of the Family Affluence Scale (FAS) developed by Currie et al. [24] was used. The FAS [25] included questions on i) bedroom availability, ii) number of family cars, iii) number of PCs at home, and iv) internet availability. Thereafter, each possible answer was given a numerical value and a final score was created for each individual ranging from 0 to 8. Scores thereafter were grouped into three levels: low (from 0 to 2), medium (from 3 to 5) and high (from 6 to 8) [26].

Parental education (both paternal and maternal) was assessed using four levels (elementary, lower secondary, higher secondary or tertiary education). In terms of parental occupation, reported information was classified into one of the twelve classification categories based on the International Standard Classification of Occupations (ISCO) from 1988 [27], and, subsequently grouped into four respective categories: i) unemployed, ii) low occupational level, iii) medium occupational level, and iv) high occupation level. Mean intakes and biomarkers levels were presented into these four categories. However, both parental education and occupation were categorized into two categories (low and high grouping the lowest and the highest categories together) to make more interpretable the found associations.

Information on parental migration background was also obtained by asking: i) if both parents were born abroad, ii) if only one parent was born abroad, and iii) if both parents were born in

the country where the study was performed. Household composition included: i) those living in single-parent families (either lone parent household or 'shared-care' between parents) and ii) those living with both parents (parents and/or step parents) [28]. The coding of the SEF indicated by ascending numbering denoted gradual higher socioeconomic position, i.e., (i) low position, (ii) medium-low position, (iii) medium-high position, and (iv) high position. Subsequent comparisons considered the high position category as the reference group.

2.3. Weight and height

Following standard protocol procedures, weight was measured in underwear and without shoes with an electronic scale (Type SECA 861) to the nearest 0.05 kg, and height was measured barefoot in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm. Body mass index (BMI) was calculated using the Quetelet formula and used as a covariate.

2.4. Assessment of folate and vitamin B_{12} intakes

The dietary intake analysis included 1,029 males and 1,224 females from 8 centres. Dietary intakes were assessed using the HELENA-DIAT self-administered, computerized 24-hour recall based on the Young Adolescents' Nutrition Assessment software (YANA-C) adapted for European adolescents [29]. The HELENA-DIAT is based on six 'meal occasions' (breakfast, morning snacks, lunch, afternoon snacks, evening meal, and evening snacks). The adolescents, supported by trained staff including dieticians, completed the 24-hour recalls at school-time, twice within a two-week timeframe [29]. Data were linked to the German Food

Code and Nutrient Data Base (BLS -Bundeslebensmittelschlüssel-, version II.3.1, 2005), containing approximately 12,000 coded foods, menus and menu components with up to 158 nutrient data points available for each product. Data from each country were linked to the database to ensure standardization of available measures. If a food item was missing in the German food composition table, then calculations were made via recipes or a local food composition table for the specific country [29]. The Multiple Source Method (MSM) [30] is a new statistical method for calculating usual dietary intake based on two or more dietary assessment methods such as 24-h dietary recalls, and it may include habitual use or non-use of a food as a covariate in the model, as well as a parameter for identifying consumers and non-consumers. The result is a method which removes the effect of day-to-day within-person variability and random error in the recalls. The software is hosted on a website established at the DIFE and can be accessed at https://nugo.dife.de/msm.

2.5. Assessment of folate and vitamin B_{12} biomarkers concentrations

On the day of the first 24 h-recall, a blood sample was taken [31]. At school setting and following an overnight fast, 30 ml of blood was drawn early in the morning according to a standardized blood collection protocol. More details on sample transport and quality assurance can be found elsewhere [32].

For the measurement of plasma folate and serum cobalamin, heparinised tubes were collected, placed immediately on ice, and centrifuged within 30 min (3,500 g for 15 min). The supernatant fluid was transported at a stable temperature of 4-7°C to the central laboratory at the University of Bonn (Institut fuer Ernährungs und Lebensmittelschaften -IEL-, Germany) and stored at -80°C until assayed. After measuring the hematocrit in situ, ethylene diamine

tetraacetic acid (EDTA) whole blood was used for the RBC-folate analysis. EDTA whole blood was diluted 1:5 with freshly prepared 0.1% ascorbic acid for cell lysis and incubated for 60 min in the dark before storage at -80°C. Plasma and RBC-folate and serum cobalamin were measured by means of a competitive immunoassay using the Immunolite 2000 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany) (CV for plasma folate = 5.4%, RBC folate = 10.7%, Cobalamin = 5.0%). [32]. Serum for measuring HoloTC were obtained by centrifuging blood collected in evacuated tubes without anticoagulant at 3,500 g for 15 min within 1 hour. Once send to the laboratory, the sera were aliquoted and stored at -80°C until transport in dry ice to the biochemical lab at the Universidad Politécnica de Madrid for analysis (Laboratory number 242 of the Laboratory Network of the Region of Madrid). Holotranscobalamin (HoloTC) was measured by microparticle enzyme immunoassay (Active B_{12} Axis-Shield Ltd, Dundee, Scotland, UK) with the use of AxSym (Abbot Diagnostics, Abbott Park, IL, USA) (CV = 5.1%) [33] .

2.6. Statistical analysis

The Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used to analyse the data. All statistical tests and corresponding *p* values were two-sided, and $p<0.05$ was considered statistically significant. Statistical analysis was stratified by gender. Descriptive data are presented as means and standard deviations (SE), and confidence intervals 95% (CI).

This study is a city-based sample, striving for representativeness on the level of these cities. Statistically, the group is considered homogeneous without the variability caused by the center.

For this study, we used the reference values for vitamin intakes based on the American EAR (Estimated Average Requirement [34]), and for the biomarkers, those previously used in another publication based on the HELENA study [35]. Variables were log-transformed to improve their normality in distribution.

Initially, a generalized linear model with the inclusion of a random intercept for study centre was used to examine the relationship between related intakes and biomarkers concentrations and SEF. Age, BMI, and adjusted energy intakes (kcal) were entered as covariates, based in other similar studies like, for instance the one from Galobardes. et al in 2001 [36]. Due to the observed low variance component associated with study centre (<5%) the authors proceeded with models that did not include random effects for centre. Therefore, the relationship between folate and vitamin B_{12} intakes and biomarkers concentrations (dependent variables) and SEF (independent variables) was examined using multiple linear regression analysis. Age, BMI, adjusted energy intakes (kcal), and the set of socioeconomic factors were included in the model as covariates to establish the associations between SEF and vitamins, both intakes and biomarkers, independently. Values are presented as adjusted β values (estimated unstandardized regression coefficient) and 95% confidence intervals (CI). Additionally, differences in folate and vitamin B_{12} mean intakes and respective biomarkers concentrations according to SEF were analysed by one-way analysis of covariance (ANCOVA), adjusted for age, body mass index (BMI) and MSM adjusted energy intakes (kcal). Bonferroni corrections were used for post hoc multiple comparisons test and the p for trend was provided based on the F test. This information is provided in supplemental material.

3. Results

Subjects included in the dietary analysis were significantly older from those not included, and also they had lower BMI and lower energy (kcal) consumption. The ratio males/females were significantly higher in subjects not included (p<0.05). In addition they differ significantly in terms of all SEF variables addressed (p<0.05) except for parental migration background. However, the associations found in the sample including those excluded adolescents were the same as without them. The biomarkers concentrations analysis included 457 males and 520 females from all 10 centres. The characteristics of the subjects included in the biomarker analysis did not differ significantly from those not included. Table 1 presents the adolescents' characteristics by SEF categories, stratified by gender correspondingly to those in the dietary and the biomarker groups.

3.1. Folate intakes and relates biomarkers between different SEF groups

Mean intakes of folate were 211.19 for males (3 % meet the American EAR) and 177.18 for females (2 % meet the American EAR). In males, levels of plasma folate, and RBC-folate, were 18.74, 807.19 nmol/L; and for females: 19.13, 770.16 nmol/L, accordingly. Among males, 18.4 % and 2 % do not meet plasma folate and RBC-folate concentration recommendations. These percentages for females, are 17.7 % and 2 %, respectively. Adolescents in categories denoting higher socioeconomic positions had significantly higher intakes and biomarkers levels of folate compared to those in the rest of the socioeconomic positions (table 1 supplemental material). More specifically, in males, folate intakes were significantly different for household composition (p <0.05), and paternal education (medium-low vs. high position groups at p <0.05 level). In females, folate intakes were significantly

different for paternal education (medium-low vs. medium-high, and medium-low vs high respectively position groups, at p <0.05 level) (table 1 supplemental material).
Regarding biomarkers, for plasma folate concentrations, significant differences were observed in males by household composition (p <0.05), indicating that males living with both parents had higher plasma folate values (19.03 nmol/L) in comparison to those living in single-parent families (15.78 nmol/L), (p<0.05). In females, no significant differences were observed. Maternal education was the only SEF for which significant differences were found for RBC-folate concentrations both in males and females. In males, significant differences were observed between the low and high position groups, and in females, between low and medium-high position groups (p <0.05) (table 1 supplemental material).

3.2. Vitamin B_{12} intakes and relates biomarkers between different SEF groups

Vitamin B_{12} intakes were 5.98 in males and 4.54 µg/d in females (91% and 98 % meet the American EAR, respectively). Serum vitamin B_{12} were 330.64 and 377.9 nmol/L; and holoTC were 63.04 and 65.63 nmol/L, for males and females, respectively. Among males, 3.7 % and 9 % do not meet serum vitamin B_{12} and holoTC concentration recommendations. These percentages for females, are 6.3 % and 8.5 %, respectively. Adolescents in categories denoting higher socioeconomic positions had significantly higher intakes and biomarkers levels of vitamin B_{12} compared to those in the rest of the socioeconomic positions (table 2 supplemental material). In males, vitamin B_{12} intakes were significantly different according to FAS (low vs. medium and high position groups), maternal education (high vs. medium-low and medium-high position groups), and paternal education (medium-low vs. high position group), all at p <0.05 level. In females, differences were observed between high and middle

and low position groups of FAS, parental migration background (low position group vs middle and high), household composition, maternal education (high position group vs all other groups), paternal education (high position group vs medium-low and medium-high position groups, and low position group vs medium-high position group), and maternal occupation (high position group vs medium-low and low position groups) ($p < 0.05$) (table 2 supplemental material).

Moreover, serum cobalamin concentrations in females were significantly different between high and medium-high and low position groups of paternal education ($p < 0.05$). Significant differences in holoTC were observed in females, by paternal education groups (low *vs.* high position) (table 2 supplemental material).

3.3. Associations between SEF and folate intakes and biomarkers

Table 2 present observed associations between folate intakes and biomarkers and SEF through unstandardized beta coefficients. What these unstandardized betas explain, is the change in units of measurements of the intakes and biomarkers when compared any of the categories of the SEF with the category denoting higher socioeconomic level. In males, lower folate intakes were associated with single/shared-care, and lower maternal and paternal education levels ($p < 0.05$). In females, lower folate intakes were observed for lower maternal and paternal education levels, and lower paternal occupation ($p < 0.05$). Concerning plasma folate, lower concentrations were associated with single/shared-care and paternal occupation in males ($p < 0.05$). No associations were found for females, or for both males and females with RBC-folate concentrations.

3.4. Associations between SEF and vitamin B_{12} intakes and biomarkers

In table 3,vitamin B_{12} lower intakes, both in males and females, were associated with lower FAS, and in females, also with migrant background, lower levels of maternal education, and lower maternal occupation ($p < 0.05$) In males, this association were also observed for parental education, and in females for single/shared-care ($p<0.05$).

Table 3, also presents associations between lower serum cobalamin and holoTC, and lower maternal education only in females ($p < 0.05$). In addition, in females, lower serum cobalamin concentrations were significantly associated with lower maternal occupation, and lower holoTC concentrations with lower paternal occupation ($p < 0.05$).

4. Discussion

The results showed that SEF are associated with folate and vitamin $B_{12,}$ intakes and biomarkers in both males and females. Maternal education and paternal occupation seem to be the most related SEF with folate and vitamin B_{12} intakes and biomarkers among all the studied. In general, the results of this study indicated that SEF are more associated with intakes than with biomarkers, and are more relevant for females than for males. Following the model that was proposed in the introduction, the main possible explanation is that SEF is an important determinant of the dietary habits and consequently, of the vitamins intake and nutritional biomarkers. However, these biomarkers could be also influenced by genetics, physiologic status, interactions between other nutrients [37-39]. These differences in associations obtained between intakes and biomarkers, evidences the importance of measuring the biomarkers in these kinds of studies to better understand how SEF really affect

to health. Moreover, this claims the need of further research about what are the main food contributors to these vitamin biomarkers,

Our findings support the hypothesis that the socioeconomic gradient affecting a number of health outcomes applies to folate and vitamin B_{12} intakes and blood-related biomarkers in this population of European adolescents. To our knowledge, this is the first study to examine the associations between this complete set of SEF and folate and vitamin B_{12} intakes and their related blood biomarkers concentrations in a large sample of adolescents in eight European countries.

Folate and the metabolically related B vitamins, such us vitamin B_{12} are an important priority throughout childhood and adolescence. An optimal status of folate and vitamin B_{12} must be a priority because of its role in the prevention of neural tube defects and in cardiovascular diseases (CVD) [5], and with cognitive functions, megaloblastic anemia and growth, respectively [40]. Folate is recognized by a recent review [6] as a possible risky micronutrient on healthy European adolescents in terms of intake and status adequacy. Nevertheless, vitamin B_{12} deficiency is uncommon in young populations unless they are vegan, live in a developing region, or have a congenital malabsorption syndrome [41], but the prevalence could be higher than formerly recognized [42]. The manifestations of both deficiencies are indistinguishable as hematological complications are concerned, so it is important to have a reliable methods to discriminate between the two vitamin deficiencies, and to monitor intervention programs designed to avoid their deficiencies, normally based on the use of fortification or supplements [43].

Comparability with other studies is limited due to the lack of available studies during adolescence. At European level, a review of B-vitamin intakes and status in European adolescents pointed out that nutritional status was closely related with SEF [6]. However, the

findings of a recently conducted systematic review [44], developed within the EURRECA (EURopean RECommendations Aligned) frame, failed to identify high quality studies showing a consistent association between folate or vitamin B_{12} intakes or biomarkers concentrations and SEF in Western European countries.

Associations between folate intakes, were observed with maternal and paternal education for both males and females and for males, also with household composition (for males) and paternal occupation (for females). For biomarkers, concentrations, associations were only found with household composition, and paternal occupation (plasma folate), and only in males. All the shown associations confirmed that adolescents in lower socioeconomic positions have lower intakes and biomarkers concentrations. In addition, the issue that most of the sample have unhealthy values of folate intakes (<400µg/d) [45] and healthy values of folate biomarkers (>13.6 nmol/L of plasma folate, and >06 nmol/L of RBC-folate) [31, 35], could be due to the high dependency of the retention of folate, both on the food in question and the method of cooking [46]. Also, it could be explained by the supplements use, which was no controlled by in this study [47].

For vitamin B_{12} intakes, associations were observed with FAS, migrant background, maternal education, and maternal occupation, both in males and females. In males, associations with paternal education were also found, and in females, with household composition. In case of biomarkers concentrations, associations were only found for females. They were shown that maternal education is associated with both serum vitamin B_{12} and holoTC, and with maternal occupation in case of serum B_{12}, and with paternal occupation in case of holoTC.

In spite of the scarce literature available, previous findings on micronutrient intakes and SEF in young populations have showed also this socioeconomic gradient. Studies during adolescence suggested decreased vitamin B_{12} intakes with decreasing wealth in regions of the

developed and developing world [4, 8, 48]. The results reported by the UK National Diet and Nutrition Survey (NDNS) of young people (4-18 y) showed that participants in households of the lower socioeconomic position had lower intakes of most vitamins and minerals studied, including folate and vitamin B_{12} [8]. Similarly, the results of a Norwegian study suggested healthier food habits with higher levels of parental education [48], similarly to those of a US and a Spanish studies [49, 50] where higher parental education level and higher income positions respectively were consistently associated with adequate levels of nutrient intakes, including folate and vitamin B_{12}. Finally, a study of Brazilian adolescents indicated that participants in the lower income and parental educational positions were at highest risk of having inadequate intakes for vitamin B_{12} [51].

In young population groups, very few studies have addressed the relationship between folate and vitamin B_{12} intakes and biomarkers concentrations and SEF [52]. In our sample, higher concentrations of folate and vitamin B_{12} biomarkers were associated with higher parental socioeconomic status, contrary to the results of a recent Greek study [53] which showed no association with parental education. However, in that study, homocysteine was the biomarker used to relate with parental education, and it is possible that homocysteine is not the most appropriate biomarker in reflecting vitamin B_{12} status because it is influenced by complex interactions between B-group vitamins [54].

As socioeconomic variables may be all related in minor or major degree, the effect of interactions between SEF was also studied based on ANCOVA´s models (results not shown). However, most of the times, the effect on the current dependent variables, point at the same direction and their isolated effect is more or less the same than when we check the effect for all the possible interactions between different socioeconomic variables. For this reason, the present results were finally presented.

An important strength of this study is the relatively large sample of adolescents representing various European regions and the application of tested and standardised procedures [20]. Similarly, the study offered the opportunity to examine relations using a large pool of accurate SEF [22, 24, 28, 55, 56], measured with reliable and validated questionnaires [22]. The clustered sample was representative for the participating cities but not for the countries [20]. Moreover, the use of self-reported data in questionnaires should be considered also as a limitation of this study [57].

The method of two dietary 24-hour recalls, is the most appropriated for population mean analysis of participants aged 10 years and over in different European countries [29], while it allows statistical adjustments for within-person variation. However, the influence of the method´s limitations reflecting accurate assessment should be considered [58]. In absence of the frequency of consumption used as a covariate, the assessment of two 24-hours recalls within two weeks might misrepresent the food consumption over the four seasons [59]. Bias in the calculation of dietary estimates might have been introduced by the use of the German food composition table due to absence of a European one. However, preliminary analyses of estimates obtained using both the BLS and national food composition tables indicated small, and for most nutrients negligible, differences using the two approaches (unpublished results). Adolescents identified as under-reporters were also included in the analysis as groups at risk of inadequate intakes are slightly biased by true under reporters [60]. Although correlations between biomarkers and usual food intakes obtained from the recalls were low in this sample [31], the use of biochemical markers strengthened the investigation, and provided a deeper understanding of observed associations by avoiding the typical measurement error in dietary assessment [61]. However, dietary study limitation is that supplement use was not used as a covariable, which is a covariable between SEF and vitamin intakes [47, 62], which could have

explained some of the differences obtained in the results between intakes and biomarkers. Furthermore, blood biomarkers were all analysed together in the same centre, strengthening the reliability of the results.

Our study makes an important contribution in providing evidence on folate and vitamin B_{12} intakes and status of European adolescents. Intakes of folate and vitamin B_{12} were consistently more associated with SEF than their biomarkers, mainly because biomarkers are determined for other several factors, such as genetics, physiologic status, and interactions with other nutrients. Considering SEF, maternal education and paternal occupation, were those most times associated with folate and vitamin B_{12}, through the results. The homogenising effect regarding habits among adolescents, as a defining feature (such as school, peers, youth culture), can mediate in these associations and further investigations need to assess it.

As part of a strategy to prevent adult diseases, we should aim at initiatives to improve adolescents' dietary habits, and consequently, assuring adequate levels of vitamins. As they are associated with various serious health problems such us megaloblastic anemia, cardiovascular disease, or cognitive impairment, special considerations should be given, in this sense, to adolescents in lower socioeconomic positions, and the future researches regardless the social patterning of adolescents' eating habits should be a priority.

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Table 1. Socioeconomic factors in the dietary and biomarker samples of European adolescents

Characteristic	Dietary intake sample		Biomarkers sample	
	Males N (%)	Females N (%)	Males N (%)	Females N (%)
Gender	1029 (46.0)	1224 (54.0)	457 (47.0)	520 (53.0)
Age (mean, SE, CI 95%)	14.8, 0.4, 14.7-14.9	14.7, 0.0, 14.7-14.8	14.8, 0.1, 14.7-14.9	14.7, 0.1, 14.6-14.8
BMI (mean, SE, CI 95%)	21.3, 0.1, 21.1-21.5	21.2, 0.1, 21.1-21.4	21.5, 0.2, 20.7-21.7	21.2, 0.1, 20.9-21.6
Energy in kcal (mean, SE, CI 95%)	2531.03, 26.8, 2478.5-2583.6	1941.06, 17.4, 1907.0-1975.2	2600.29, 51.8, 2498.4-2702.2	1950.90, 31.9, 1888.2-2013.6
FAS				
Low FAS	83 (8.1)	140 (11.4)	49 (10.7)	78 (15.0)
Medium FAS	589 (57.2)	664 (54.2)	267 (58.4)	291 (56.0)
High FAS	351 (34.1)	141 (33.8)	141 (30.9)	150 (28.8)
Migrant background				
Both parents born abroad	65 (6.3)	72 (5.9)	18 (3.9)	22 (4.2)
One parent born abroad	50 (4.9)	65 (5.3)	21 (4.6)	19 (3.7)
Both parents born in survey´s country	887 (86.2)	1050 (85.8)	413 (90.4)	468 (90.0)
Household composition				
Single/shared-care	297 (28.9)	355 (29.0)	116 (25.4)	13 (26.3)
Traditional family	732 (71.1)	869 (71.0)	341 (74.6)	383 (73.7)
Maternal education				
Low	62 (6.0)	80 (6.5)	32 (7.0)	52 (10.0)
Medium-low	269 (26.1)	300 (24.5)	113 (24.7)	124 (23.8)
Medium-high	286 (27.8)	368 (30.1)	142 (31.1)	174 (33.5)
High	356 (34.6)	416 (34.0)	150 (32.8)	155 (29.8)
Paternal education				
Low	40 (3.9)	84 (6.9)	34 (7.4)	42 (8.1)
Medium-low	284 (27.6)	330 (27.0)	123 (26.9)	146 (28.1)
Medium-high	258 (25.1)	297 (24.3)	130 (28.4)	156 (30.0)
High	365 (35.5)	403 (32.9)	145 (31.7)	156 (30.0)
Maternal occupation				
Low	208 (20.2)	246 (20.1)	95 (20.8)	110 (21.2)
Medium-low	196 (19.0)	204 (16.7)	88 (19.3)	77 (14.8)
Medium-high	390 (37.9)	494 (40.4)	186 (40.7)	196 (37.7)
High	142 (13.8)	180 (14.7)	62 (13.6)	99 (19.0)
Paternal occupation				
Low	63 (6.1)	62 (5.1)	31 (6.8)	31 (6.09
Medium-low	312 (30.3)	323 (26.4)	143 (31.3)	135 (26.0)
Medium-high	304 (29.5)	344 (28.1)	143 (31.3)	128 (24.6)
High	220 (21.4)	322 (26.3)	110 (24.1)	180 (34.6)
Centre (cities)				
Athens	123 (12.0)	140 (11.4)	38 (8.1)	56 (10.8)
Dortmund	218((21.2)	165 (13.5)	62 (13.6)	40 (7.7)
Gent	139 (13.5)	165 (13.5)	56 (12.3)	50 (9.6)
Lille	82 (8.0)	124 (10.1)	33 (7.2)	49 (9.4)
Heraklion	-	-	39 (8.5)	42 (8.1)
Rome	95 (9.2)	152 (12.4)	46 (10.1)	48 (9.2)
Stockholm	107 (10.4)	184 (15.0)	44 (9.6)	47 (9.0)
Vienna	157 (15.3)	177 (14.5)	42 (9.2)	59 (11.3)
Zaragoza	108 (10.5)	117 (9.6)	47 (10.3)	58 (11.2)
Pecs	-	-	49 (10.7)	71 (13.7)

Abbreviations: SD, standard deviation; BMI, Body Mass Index; FAS, Family Affluence Scale

Table 2. Linear regression analysis assessing the associations between folate intakes, plasma folate and RBC-folate and socioeconomic factors after adjusting by age, body mass index, and total energy intake (kcal).

INDICATORS	FOLATE INTAKES *(µg/d)*				PLASMA FOLATE *(nmol/L)*				RBC FOLAT	
	Males		Females		Males		Females		Males	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
FAS										
Low FAS	0.0	-0.0, 0.1	0.0	-0.0, 0.1	0.1	-0.2, 0.4	0.1	-0.0, 0.3	-0.0	-0.3, 0.2
Medium FAS	-0.0	-0.0, 0.0	0.0	-0.0, 0.0	0.0	-0.1, 0.2	-0.0	-0.1, 0.1	0.0	-0.1, 0.1
Migrant background										
Both parents born abroad	0.0	-0.1, 0.1	0.0	-0.0, 0.1	0.0	-0.3, 0.4	0.2	-0.1, 0.4	-0.1	-0.4, 0.2
One parent born abroad	-0.0	-0.1, 0.0	0.0	-0.1, 0.1	-0.2	-0.5, 0.1	-0.0	-0.3, 0.2	-0.0	-0.3, 0.2
Household composition										
Single/shared-care	-0.0**a**	-0.1, -0.0	-0.0	-0.0, 0.0	-0.2**a**	-0.3, -0.0	-0.0	-0.1, 0.1	-0.0	-0.2, 0.1
Maternal education										
Low	-0.0**a**	-0.1, -0.0	-0.1**a**	-0.1, -0.0	0.0	-0.1, 0.1	-0.1	-0.2, 0.0	0.0	-0.1, 0.1
Paternal education										
Low	-0.0**a**	-0.1, -0.0	-0.1**a**	-0.1, -0.0	-0.1	-0.2, 0.0	-0.1	-0.2, 0.0	-0.1	-0.2, 0.0
Maternal occupation										
Low	0.0	-0.0, 0.0	-0.0	-0.0, 0.0	-0.1	-0.2, 0.1	-0.1	-0.2, 0.0	-0.0	-0.2, 0.1
Paternal occupation										
Low	-0.0	-0.1, 0.0	-0.0**a**	-0.1, -0.0	-0.2**a**	-0.3, -0.0	-0.1	-0.2, 0.1	-0.1	-0.2, 0.0

Abbreviations: B, unstandardized B coefficients; CI, confidence interval; FAS, Family Affluence Scale.
High position categories for each indicator were used as a reference.
Significant differences ($p<0.05$) between groups are indicated by letters (always in comparison with the high position group).

Table 3. Linear regression analysis assessing the associations between cobalamin intakes, serum cobalamin and Holotranscobalamin and socioeconomic factors after adjusting by age, body mass index, and total energy intake (kcal).

INDICATORS	VITAMIN B_{12} INTAKES *(µg/d)*				SERUM VITAMIN B_{12} *(nmol/L)*				HOLOTRANSCOBALAMIN *(nmol/L)*			
	Males		Females		Males		Females		Males		Females	
	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI
FAS												
Low FAS	-0.2 **a**	-0.3, -0.1	-0.1 **a**	-0.2, -0.1	0.1	-0.2, 0.3	-0.1	-0.2, 0.2	-0.1	-0.3, 0.2	0.0	-0.1, 0.2
Medium FAS	-0.0	-0.1, 0.0	-0.0 **a**	-0.1, -0.0	-0.0	-0.1, 0.1	-0.0	-0.1, 0.1	-0.0	-0.1, 0.1	-0.0	-0.1, 0.1
Migrant background												
Both parents born abroad	-0.1 **a**	-0.2, -0.0	-0.2 **a**	-0.3, -0.1	-0.1	-0.3, 0.2	0.0	-0.2, 0.2	-0.1	-0.4, 0.2	-0.2	-0.4, 0.1
One parent born abroad	-0.1 **a**	-0.2, -0.0	-0.0	-0.1, 0.0	0.0	-0.2, 0.3	-0.1	-0.3, 0.1	-0.1	-0.4, 0.1	-0.1	-0.3, 0.2
Household composition												
Single/shared-care	0.0	-0.0, 0.1	-0.1 **a**	-0.1, -0.0	0.1	-0.0, 0.2	-0.0	-0.1, 0.1	0.1	-0.1, 0.2	-0.0	-0.1, 0.1
Maternal education												
Low	-0.1 **a**	-0.1, -0.0	-0.1 **a**	-0.1, -0.0	-0.1	-0.2, 0.0	-0.1 **a**	-0.2, 0.0	-0.1	-0.2, 0.1	-0.1 **a**	-0.2, -0.0
Paternal education												

	Low	-0.1 **a**	-0.1, -0.0	-0.0	-0.1, 0.0	-0.1	-0.2, 0.0	-0.1	-0.2, 0.0	-0.1	-0.2, 0.1	-0.1	-0.2, 0.0
Maternal occupation													
	Low	-0.1 **a**	-0.1, -0.0	-0.1 **a**	-0.1, -0.0	-0.1	-0.1, 0.0	-0.1 **a**	-0.2, -0.0	-0.0	-0.1, 0.1	-0.1	-0.2, 0.0
Paternal occupation													
	Low	-0.0	-0.1, 0.0	-0.0	-0.1, 0.0	-0.0	-0.2, 0.1	-0.1	-0.2, 0.0	-0.0	-0.1, 0.1	-0.1 **a**	-0.2, -0.0

Abbreviations: B, unstandardized B coefficients; CI, confidence interval; FAS, Family Affluence Scale.
High position categories for each indicator were used as a reference.
Significant differences ($p<0.05$) between groups are indicated by letters (always in comparison with the high position group).

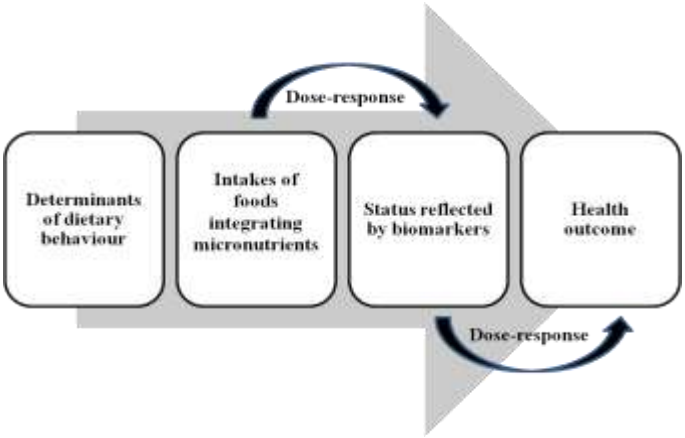

Figure 1. The DISH model for food, nutrition and health research describes the relationship between determinants of dietary behaviour (D), intake of foods and nutrients (I), biomarkers of status and function (S) and health outcome of interest (H). Adapted from Romana Novakovic´s thesis [18].

Supplementary table 1. Estimates of folate intakes and plasma and RBC-folate concentrations by socioeconomic factors, adjusted for age, body mass index, and total energy intake (kcal) in European adolescents.

INDICATORS	***FOLATE INTAKES* (µg/d)**				***PLASMA FOLATE* (nmol/L)**			
	Males		**Females**		**Males**		**Females**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
FAS								
Low FAS	220.0	5.9	177.6	3.9	17.9	3.1	21.1	1.56
Medium FAS	209.8	2.2	177.3	1.8	18.7	0.8	17.5	0.74
High FAS	211.6	2.9	176.7	2.3	17.4	1.0	18.4	0.90
P for trend								
Migrant background								
Both parents born abroad	216.2	6.7	180.9	5.4	18.1	3.4	21.9	2.5
One parent born abroad	203.8	7.6	181.5	5.7	14.8	2.8	17.6	2.6
Both parents born in survey´s country	212.2	1.8	176.1	1.4	18.3	0.6	18.1	0.6
P for trend								
Household composition								
Single/shared-care	204.8a	3.1	177.6	2.4	15.8a	1.2	18.5	1.1
Traditional family	213.8a	2.0	177.0	1.6	19.0a	0.7	18.2	0.6
P for trend	**0.02**				**0.01**			
Maternal education								
Low	218.6	6.8	177.3	5.1	14.3	2.5	15.6	1.9
Medium-low	206.6	3.3	170.5	2.7	19.3	1.3	18.3	1.2
Medium-high	212.0	3.2	177.9	2.4	17.5	1.1	19.2	1.0
High	215.6	2.9	181.8	2.3	18.7	1.0	18.4	1.0
P for trend								
Paternal education								
Low	221.5	8.6	173.9	5.1	15.9	3.0	15.0	2.0
Medium-low	205.8a	3.2	171.4ab	2.5	17.7	1.2	18.0	1.1
Medium-high	211.0	3.4	181.9a	2.7	17.9	1.2	18.9	1.0
High	216.9a	2.9	181.4b	2.3	19.5	1.0	18.9	1.0
P for trend	**0.01**		**0.00**					
Maternal occupation								
Low	217.7	3.8	178.0	2.9	18.1	1.4	17.5	1.2
Medium-low	209.4	4.5	173.2	3.4	15.6	1.9	17.8	1.2
Medium-high	210.6	2.7	176.5	2.0	18.8	0.9	18.1	0.9
High	213.7	3.9	177.3	3.2	18.2	1.3	20.0	1.4
P for trend								

Paternal occupation								
Low	213.1	6.9	170.1	5.8	16.0	2.4	19.3	2.1
Medium-low	207.2	3.7	174.5	2.6	16.9	1.4	17.4	1.0
Medium-high	209.8	3.1	180.1	2.5	19.0	1.0	17.5	1.0
High	216.9	3.1	180.8	2.6	18.9	1.1	19.6	1.0
P for trend			**0.05**					

Abbreviations: RBC-folate, red blood cell folate; SE, Standard Error; FAS, Family Affluence Scale

Low position categories denote lower well-being and subsequently lower position of inadequacy of both intakes and biomarkers levels.

Significant differences ($p<0.05$) between groups are indicated by the same superscripts letters.

P for trend based on F test are stated in bold only when significant (p-values$\leq$0.05).

Supplementary table 2. Estimates of vitamin B_{12} intakes and serum cobalamin and Holotranscobalamin by socioeconomic indicators, adjusted for age, body mass index, and total energy intake (kcal) in European adolescents.

INDICATORS ***VITAMIN B12 INTAKES* (µg/d)** ***SERUM VITAMIN B12* (pmol/L)**

	Males		**Females**		**Males**		**Females**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
FAS								
Low FAS	5.4ab	0.2	4.2ab	0.1	342.7	40.2	378.5	25.5
Medium FAS	6.0a	0.1	4.5a	0.1	331.0	10.1	380.9	12.2
High FAS	6.1b	0.1	4.8b	0.1	338.9	13.0	395.0	14.9
P for trend	**0.00**		**0.00**					
Migrant background								
Both parents born abroad	5.6	0.3	3.9ab	0.2	320.3	44.7	403.7	40.4
One parent born abroad	5.4	0.3	4.6a	0.2	339.6	37.1	347.0	42.1
Both parents born in survey´s country	6.1	0.1	4.6b	0.1	335.6	8.2	386.4	9.4
P for trend	**0.02**		**0.00**					
Household composition								
Single/shared-care	6.0	0.1	4.4a	0.1	351.5	15.5	374.3	17.3
Traditional family	6.0	0.1	4.6a	0.1	328.4	90.4	389.6	10.3
P for trend			**0.02**					
Maternal education								
Low	6.3	0.3	4.6a	0.2	308.0	30.9	362.0	30.4
Medium-low	5.7a	0.1	4.3b	0.1	308.7	16.1	357.7	18.7
Medium-high	5.8b	0.1	4.3c	0.1	328.1	13.6	378.0	15.8
High	6.3ab	0.1	5.0abc	0.1	355.0	12.6	415.3	15.9
P for trend	**0.00**		**0.00**					
Paternal education								
Low	6.2	0.3	4.8a	0.2	344.8	38.8	318.0a	31.9
Medium-low	5.8a	0.1	4.4b	0.1	308.7	15.8	385.3	17.0
Medium-high	5.9	0.1	4.3ac	0.1	337.7	15.0	356.0b	16.7
High	6.3a	0.1	4.9bc	0.1	347.3	13.1	427.3ab	15.6
P for trend	**0.00**		**0.00**				**0.00**	
Maternal occupation								
Low	5.8	0.1	4.5a	0.1	330.2	17.4	363.0	19.2
Medium-low	5.8	0.2	4.4b	0.1	312.0	24.8	350.8	20.4
Medium-high	6.1	0.1	4.6	0.0	334.3	11.9	400.0	14.3

High	6.2	0.1	4.6ab	0.1	354.0	17.1	418.3	22.2
P for trend	0.04		0.00					
Paternal occupation								
Low	5.8	0.3	4.3	0.2	358.7	31.7	386.4	35.0
Medium-low	5.9	0.1	4.5	0.1	319.7	18.3	362.7	16.2
Medium-high	6.0	0.1	4.6	0.1	327.9	13.0	418.6	16.7
High	6.1	0.1	4.8	0.1	350.0	13.7	380.1	17.1
P for trend								

Abbreviations: SE, Standard Error; FAS, Family Affluence Scale.

Low position categories denote lower well-being and subsequently lower position of inadequacy of both intakes and biomarkers levels.

Significant differences ($p<0.05$) between groups are indicated by the same superscripts letters.

P for trend based on F test are stated in bold only when significant (p-values$\leq$0.05).