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Regular breakfast consumption is associated with higher blood vitamin status in adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study

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Abstract

Objective: The present study aimed to examine the association between different breakfast consumption patterns and vitamin intakes and blood vitamin concentrations in European adolescents.

Design: Breakfast consumption was assessed by a questionnaire. Vitamin intake was calculated from two 24 h recalls. Blood vitamin and total homocysteine (tHcy) concentrations were analysed from fasting blood samples.

Setting: The European Commission-funded HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study.

Subjects: Participants were 1058 (52·8 % females) European adolescents (aged 12·5–17·5 years) from ten cities.

Results: Lower vitamin D and vitamin C concentrations were observed in male and female breakfast skippers than in consumers ($P<0·05$). Female breakfast consumers presented higher holo-transcobalamin and lower tHcy ($P<0·05$), while males had higher cobalamin concentrations, compared with skippers ($P<0·05$). Higher vitamin D and total folate intakes were observed in adolescents who consumed breakfast compared with skippers ($P<0·05$). Likewise, female consumers had higher intakes of vitamin B_6 and vitamin E than occasional consumers ($P<0·05$).

Conclusions: Regular breakfast consumption is associated with higher blood vitamin D and cobalamin concentrations in males and with higher vitamin D and holo-transcobalamin and lower tHcy concentrations in females. Moreover, breakfast consumption is associated with high intakes of vitamin D and total folate in both sexes, and with high intakes of vitamin B_6 and vitamin E in females.

Keywords
Breakfast
Adolescents
Vitamin D

Although breakfast is widely promoted as essential for the nutritional well-being of young people, breakfast skipping is relatively common among adolescents in developed countries[1,2]. The breakfast meal and the frequency with which it is consumed, together with genetic and environmental factors, may influence appetite, dietary intake and food choices. These factors may have important implications for body weight regulation and to prevent diseases[3].

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There is some evidence that breakfast skipping behaviour is related to higher BMI in adolescents[1,4–6]. In addition, regular breakfast consumption has been associated with a healthier cardiovascular profile in European adolescents[5].

Breakfast has been documented to make an essential contribution to nutrient intakes[1,7,8], due to its contribution of approximately 20 % to total daily energy intake and content of specific nutrients such as vitamins[9]. Adolescents who consume breakfast are more likely to meet recommended intakes of Ca, Fe, Mg, Zn, Cu, folate, vitamins A, D, B_6, B_1, C and E, and total energy than those who skip breakfast[1]. Likewise, breakfast consumption may reduce the risk of chronic diseases due to its potential impact on overall diet quality[10–13]. Consequently, breakfast is considered an important key component of a healthy diet contributing to adequate growth and adolescent development.

On the other hand, previous reports from the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study have stated that deficient blood vitamin concentrations, at least at the subclinical level, are prevalent in European adolescents: plasma folate (PF; 15 %), vitamin D (25-hydroxyvitamin D (25(OH)D); 27 %), vitamin B_6 (pyridoxal phosphate (PLP); 5 %), β-carotene (25 %) and vitamin E (5 %)[14–16]. Likewise, some vitamin deficiencies could be associated with fatness and other diseases[17]. In addition, in Europe, there is a lack of comparable data on vitamin intakes and blood concentrations[18]. Therefore, a balanced breakfast could be a good strategy to avoid these blood vitamin deficiencies. However, there are no studies analysing the relationship of blood vitamin levels with breakfast patterns. Thus, the aim of the present study was to examine the association between vitamin intakes, blood vitamin concentrations and different patterns of breakfast consumption in European adolescents participating in the HELENA Study.

Participants and methods

Study design, recruitment and participants

The HELENA Study was a multicentre cross-sectional study aiming at obtaining reliable and comparable data from a random sample of 3000 European adolescents, aged between 12·5 and 17·5 years, from ten different cities, on a broad range of nutrition and health-related parameters[19,20]. Selection of cities was based on two criteria: regional distribution and presence of an active research group assuring sufficient expertise and resources to successfully perform epidemiological studies. Within the study, Stockholm (Sweden), Athens and Heraklion (Greece), Rome (Italy), Zaragoza (Spain), Pécs (Hungary), Ghent (Belgium), Lille (France), Dortmund (Germany) and Vienna (Austria) were included. On a regional basis, diversity of the sample with respect to cultural and socioeconomic aspects was achieved by selecting a random proportional distribution of all schools taking into account the site of the school (district/zone of the city) and the type of school (public or private). One partner centrally performed the school and class random selection procedure for all study centres, including the subset of classes for blood sampling. In case a selected school refused to participate, a school with comparable characteristics from a list of substitutes was chosen. The number of adolescents to be studied was estimated at 3000 using variance of BMI. BMI has the greatest dispersion in the study population with regard to the hypotheses under consideration. The sample size was calculated with a confidence level of 95 % and with ±0·3 error in the parameter BMI. Error of 0·3 was chosen as a worst-case scenario for precision level as described by the HELENA Study[19,20]. One-third of the classes were randomly selected for blood collection, resulting in a total of 1089 blood samples for the subsequent clinical biochemistry assays. After validating data on breakfast assessment and blood samples, 1058 (52·8 % females) adolescents were included in the present study.

Exclusion criteria were limited to adolescents who were not able to speak the local language, those who were participating in another clinical trial at the same time, those aged <12·5 or >17·5 years and adolescents having suffered from infection one week before the visit. Exclusions from the study were done *a posteriori*, without the knowledge of the affected individuals. All procedures involving human participants were approved by the ethics committee of each city involved and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed written consent was obtained from adolescents and parents or guardians. A complete description of ethical issues and good clinical practice within the HELENA Study is given elsewhere[21].

Breakfast consumption patterns assessment

The 'Food Choices and Preferences' questionnaire was developed based on forty-four focus groups which explored attitudes and issues of concern among adolescents regarding food choices, preferences, healthy eating and lifestyle[22]. This questionnaire has been used in previous research[5]. Adolescents reported their breakfast habits by responding to the following statement: 'I often skip breakfast'. There were seven possible answers ranging as 'strongly disagree' (=1), 'moderately disagree' (=2), 'slightly disagree' (=3), 'neither agree nor disagree' (=4), 'slightly agree' (=5), 'moderately agree' (=6) and 'strongly agree' (=7). Adolescents were categorized into three groups: (i) consumers (answered '1' or '2'); (ii) occasional consumers (answered '3', '4' or '5'); and (iii) skippers (answered '6' or '7').

Supplement use

Information on vitamin supplement use was obtained via the clinical recall of the adolescents (case report form).

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Adolescents were asked about taking any micronutrient supplement and were classified into two groups: (i) supplement users; and (ii) non-supplement users.

Socio-economic status

The complete description of the self-reported socio-economic questionnaire is provided elsewhere[23]. The Family Affluence Scale (FAS) is based on the concept of material conditions in the family as the basis for the selection of items. Currie *et al*. chose a set of items which reflected family expenditure and consumption that were relevant to family circumstances[24]. FAS was used in the present study as an index of socio-economic status (SES)[25] and included four questions answered by the adolescent: (i) 'Do you have your own bedroom?' ('no' (=0); 'yes, one' (=1)); (ii) 'How many cars are there in your family?' ('no' (=0); 'yes, one' (=1); 'yes, two' (=2); 'yes, more than two' (=3)); (iii) 'How many PCs [personal computers] are there in your home?' ('none' (=0); 'one' (=1); 'two' (=2); 'more than two' (=3)); and (iv) 'Do you have Internet access at home?' ('no' (=0); 'yes, one' (=1)). We defined low, medium and high SES based on the final score obtained from the four questions. We gave a numerical value to each possible answer in the four questions and then we summed the final score from all the questions, which ranged from 0 to 8. Finally, we grouped these scores into three levels: low (from 0 to 2), medium (from 3 to 5) and high (from 6 to 8).

Family structure

We obtained information about family structure through the aforementioned questionnaire. Family structure was defined as 'traditional family' when the adolescent was living at home with two parents (parents and/or step-parents) or 'single/shared-care' when the adolescent was living in a single-parent family or had 'shared care' between parents. Those living in other family structures (e.g. in a foster home or with grandparents) were categorized into the 'single/shared-care' family structure[5].

Dietary intake assessment

Dietary consumption was assessed using the self-administered, computerized 24 h recall (24HR), HELENA Dietary Assessment Tool (DIAT), which is based on the Young Adolescents' Nutrition Assessment software validated in European adolescents[26] (r_s = 0·86–0·91) for all nutrient and energy intakes. The adolescents completed the 24HR twice (within 2 weeks) during school time; both times, trained staff including a dietitian were present. The HELENA-DIAT used special techniques to support and enhance respondents' memory, which allowed a more detailed description and quantification of the foods consumed. The European Food Consumption Survey Method project indicated the repeated 24HR as the most suitable method to obtain population means and distributions[27]. To calculate energy and nutrient intakes, data from HELENA-DIAT were linked to the German Food Code and Nutrient Database (BLS (Bundeslebensmittelschlüssel), version II.3.1, 2005)[28]. The use of the German Food Code and Nutrient Database has been evaluated and was considered equally valid as using the local food composition databases from each individual country[29]. The Multiple Source Method was used to estimate the habitual dietary intake of nutrients and foods[30]. This statistical modelling technique takes into account within-person variability. Likewise, this technique calculates habitual intakes taking into account age, sex and study centre. Participants from Heraklion and Pécs were excluded from these analyses as no nutrient intake information was calculated for these two cities due to logistical problems (insufficient local staff available). Therefore, eight out of the ten study centres were included in the 24HR analyses, resulting in a sample size decrease. Moreover, under-reporters were excluded from all analyses. The BMR was calculated from age-specific FAO/WHO/United Nations University equations[31]. Under-reporting was considered when the ratio of energy intake to estimated BMR was <0·96, as proposed by Black[32].

Medical examination

The day prior to the study day, participants were asked to abstain from eating and drinking after 20.00 hours. On the study day, a medical doctor visited the school classes and interviewed all participants for medical history and acute diseases. A blood sampling questionnaire was used to assess fasting status, acute infections, allergies, smoking, vitamin and mineral supplements, and medication. Maturity was assessed by means of Tanner stage[33].

Specimen collection and biochemical analyses

A specific handling, transport and traceability system for biological samples was developed for the HELENA Study as described by González-Gross *et al.*[34]. Blood samples were obtained at the same time as dietary intake assessment. Fasting blood samples were collected by venepuncture at school between 08.00 and 10.00 hours in the morning. PF, cobalamin (CBL) and red-blood-cell (RBC) folate were measured by competitive immunoassay (Immulite 2000; DPC Biermann GmbH, Bad Nauheim, Germany; CV for PF = 5·4 %, RBC folate = 10·7 %, CBL = 5·0 %)[35]. PLP was measured by HPLC (Varian Deutschland GmbH, Darmstadt, Germany; CV = 1 %) with the modified method of Kimura *et al.*[36]. Serum holotranscobalamin (a marker of vitamin B_{12} status; holo-TC) was measured by microparticle enzyme immunoassay (Active B12; Axis-Shield Ltd, Dundee, UK; CV = 5·1 %) with the use of an AxSYM analyser (Abbott Diagnostics, Abbott Park, IL, USA)[37].

Plasma 25(OH)D was analysed using an ELISA kit (OCTEIA 25(OH)D; IDS Immunodiagnostic Systems

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Deutschland GmbH, Frankfurt am Main, Germany) and measured with a Sunrisee photometer (TECAN, Männedorf, Germany). The CV for the method was less than 1 %[14]. Plasma vitamin C, retinol, α-tocopherol and β-carotene were analysed using reversed-phase HPLC (Sykam GmbH, Gilching, Germany) with UV detection (UV-Vis 205; Merck, Darmstadt, Germany). The CV of the method was 2·9 % for retinol, α-tocopherol and β-carotene, and 1·7 % for vitamin C[16].

Body composition measurements

The anthropometric methods used within the HELENA Cross-Sectional Study were described by Nagy *et al.*[38]. Briefly, body weight was measured in kilograms using a standard beam balance scale (type SECA 861, UK; precision 100 g, range 0–150 kg). Height was measured in centimetres using a precision stadiometer (type SECA 225, UK; precision 0·2 cm, range 70–200 cm). BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2). Adolescents were classified according to the international BMI cut-off values as non-overweight or overweight/obese[39].

Statistical analyses

Associations between sex and BMI status (non-overweight and overweight/obese), SES (low, medium and high), family structure (traditional family and single/shared-care) and breakfast consumption categories (consumer, occasional consumer and skipper) were assessed by the χ^2 test. Mean vitamin intakes and serum vitamin levels were compared across breakfast consumption categories using one-way ANCOVA with breakfast consumption category as the fixed factor, vitamin intakes or blood vitamin concentrations as the dependent variables and age, BMI, supplement use, centre, sex, SES and family structure as covariates[22]. The analyses were stratified by sex. On the other hand, the associations between breakfast pattern categories and different statuses of vitamin concentrations (sufficiency, insufficiency or deficiency) were analysed by the χ^2 test. Likewise, adolescents were grouped in relationship with their vitamin blood status according to accepted reference values and as previously published by Moreno *et al.*[40]. All analyses were performed using the statistical software package IBM SPSS Statistics for Windows Version 22.0 and the level of significance was set at 5 %.

Results

Table 1 presents anthropometric and body composition data, blood vitamin status and vitamin intake data; and also displays BMI status, SES, family structure and distribution of breakfast consumption patterns in the study sample. There were significant differences between sexes in serum PLP, CBL, vitamin C, β-carotene, α-tocopherol, retinol and tHcy ($P < 0·05$). In addition, there were differences between breakfast consumers compared with occasional consumers and breakfast skippers according to sex ($P < 0·001$).

Table 2 presents vitamin intake data according to different breakfast patterns, by sex, after controlling for centre, age, BMI, supplement use, SES and family structure. Higher vitamin D (cholecalciferol) and total folate intakes were observed in adolescent breakfast consumers compared with breakfast skippers ($P < 0·05$). Likewise, female breakfast consumers had higher intakes of vitamin B_6 (pyridoxine) and vitamin E (tocopherol equivalents) than skippers ($P < 0·05$).

Table 3 presents differences in blood vitamin concentrations across breakfast consumption categories, stratified by sex, after controlling for centre, age, BMI, supplement use, SES and family structure. Male adolescents who regularly consumed breakfast presented higher blood concentrations of 25(OH)D, CBL and vitamin C than breakfast skippers. Higher BMI was also observed in male breakfast skippers compared with occasional consumers and consumers ($P < 0·05$). Female breakfast consumers had higher blood concentrations of 25(OH)D, holo-TC and vitamin C compared with skippers ($P < 0·05$). Moreover, BMI and tHcy were lower in female breakfast consumers in contrast to skippers ($P < 0·05$).

Table 4 presents the frequency and percentage of different blood vitamin concentration statuses by breakfast consumption pattern in the study sample. Adolescent breakfast skippers presented higher 25(OH)D, CBL and PF deficiency prevalence compared with habitual breakfast consumers.

Discussion

To the best of our knowledge, the current study is the first to analyse the associations among vitamin intakes, blood vitamin concentrations and different patterns of breakfast consumption in European adolescents, taking into account several potential influential factors such as centre, age, sex, BMI, supplement use, family structure and SES. The main results showed that regular breakfast consumption is associated with a healthier profile for only some blood vitamins in European adolescents and for tHcy only in females.

In agreement with our data, other articles have shown that only half of European adolescents are breakfast consumers, while 25 % of males and 33 % of females are breakfast skippers[40,41], similar to other areas in the world[42]. Age influences breakfast consumption, with the older adolescents being more likely to skip breakfast (4–8 years: 2 %; 9–13 years: 9 %; 14–18 years: 18 %)[2,43]. Another interesting fact is that although habitual breakfast consumers have higher energy intake, those skipping breakfast are more likely to be obese[1]. In agreement, our data showed that male and female breakfast skippers had higher BMI compared with consumers.

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Table 1 Mean anthropometric indices, mean blood vitamin concentrations, BMI status, socio-economic status, sociodemographic status and breakfast consumption patterns of the study sample by sex; adolescents (*n* 1058) aged 12·5–17·5 years from ten European cities, HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study

	Males (*n* 499)		Females (*n* 559)		
	Mean	SD	Mean	SD	*P*
Age (years)	14·9	1·3	14·9	1·2	0·519
Height (cm)	169·6	9·8	161·7	7·1	**<0·001**
Body mass (kg)	61·6	13·7	55·9	10·3	**<0·001**
BMI (kg/m^2)	21·5	4·0	21·4	3·4	0·492
25-Hydroxyvitamin D (nmol/l)	56·6	21·2	58·7	21·8	0·154
Pyridoxal phosphate (nmol/l)	66·3	46·5	60·6	56·0	**<0·001**
Cobalamin (pmol/l)	327·8	122·8	370·8	155·7	**<0·001**
Holo-transcobalamin (pmol/l)	62·7	30·9	62·7	30·8	0·967
Plasma folate (nmol/l)	18·4	10·1	19·0	10·1	0·304
Whole-blood folate (nmol/l)	349·2	141·7	315·8	128·4	**<0·001**
Red-blood-cell folate (nmol/l)	791·8	335·3	758·7	299·5	0·299
Vitamin C (mg/l)	10·0	3·2	10·6	3·2	**0·028**
β-Carotene (ng/ml)	226·9	152·7	251·8	150·4	**<0·001**
α-Tocopherol (μg/ml)	9·5	2·0	10·2	2·1	**<0·001**
Retinol (ng/ml)	355·8	99·4	352·4	108·6	**0·039**
Total homocysteine (μmol/l)	7·7	3·8	6·8	2·5	**<0·001**
	n	%	*n*	%	
BMI status					
Non-overweight	427	85·5	482	86·3	
Overweight/obese	72	14·5	77	13·7	
Socio-economic status					0·760
Low	60	11·9	96	16·5	
Medium	291	57·3	316	55·0	
High	156	30·8	164	28·4	0·076
Family structure					
Traditional family	403	80·7	459	82·0	
Single/shared-care	96	19·3	100	18·0	
Breakfast pattern					0·572
Consumer	310	62·2	293	52·4	
Occasional consumer	77	15·5	102	18·2	
Skipper	112	22·2	164	29·4	**<0·001**

Significant *P* values are shown in bold font.

Breakfast consumption has been consistently associated with a more favourable nutrient intake profile and improved diet quality (i.e. favourable nutrient and energy intakes) in children and adolescents[43], as we have observed in the present study. Thus, skipping breakfast has been proposed to indirectly influence BMI by over-compensating intake later in the day, consuming more snacks and fewer meals[44]. Further, including low-fat dairy foods, wholegrain breads and cereals, and citrus fruits, other fruits and juices for breakfast could possibly have a positive influence on body mass[45].

Moreover, in order to assess an individual's nutritional status, vitamin, food and nutrient intake data should be complemented with biochemical data[46], owing to the fact that micronutrient intake data are not always associated with blood concentrations[47]. Our results showed an association of regular breakfast consumption with greater vitamin D (cholecalciferol), vitamin B_9 (total folate) and vitamin B_6 (pyridoxine) intakes and blood vitamin C, vitamin D (25(OH)D), vitamin B_{12} (CBL, holo-TC), folate (PF, RBC folate) and tHcy concentrations, after controlling for centre, age, BMI, supplement use, SES and family structure. However, both vitamin D and folate intakes were considerably below average requirements for adolescents (e.g. as estimated for the USA and Canada in the Dietary Reference Intakes). Specifically, the average requirements for vitamin D and folate for adolescents are ~10 μg/d and 320 μg/d, respectively, whereas mean intakes were only 2–3 μg/d (vitamin D) and <250 μg/d (folate), even among breakfast consumers[48,49]. Thus, the intakes of breakfast consumers cannot be considered to be optimal. Nevertheless, this association between breakfast eating, higher vitamin intakes and higher concentrations of some vitamins and tHcy in females may be due to some specific foods consumed at breakfast, rather than eating breakfast *per se*[6]. In addition, intakes of other foods in the remaining meals consumed during the rest of the day could explain the association.

Breakfast is an ideal opportunity for adolescents to begin the day by eating bread, other cereals, dairy products and fruits, which have been associated with better dietary intakes of the aforementioned micronutrients[45]. Likewise, some of these breakfast products, especially packaged foods, are fortified with vitamins and minerals that also can contribute to achieving recommended

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Table 2 Mean energy and vitamin intakes of the study sample by breakfast consumption pattern and sex; adolescents (*n* 1058) aged 12·5–17·5 years from ten European cities, HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study

	Males (*n* 499)			Females (*n* 559)		
	Mean	SD	*P*	Mean	SD	*P*
Energy (kJ/d)						
Consumer	11 035·3	4258·1	0·307	8011·5	2888·6	0·087
Occasional consumer	11 248·3	5137·1		8060·5	2515·4	
Skipper	10 101·0	5610·4		7194·8	3002·0	
Energy (kcal/d)						
Consumer	2637·5	1017·7	0·307	1914·8	690·4	0·087
Occasional consumer	2688·4	1227·8		1926·5	601·2	
Skipper	2414·2	1340·0		1719·6	717·5	
Vitamin D (cholecalciferol; μg/d)						
Consumer	2·9	4·8	**0·012**	2·2	4·2	**0·043**
Occasional consumer	2·9	4·6		1·6*	1·3	
Skipper	2·1*,†	3·3		1·7*	2·9	
Vitamin B_6 (pyridoxine; μg/d)						
Consumer	1926·0	838·3	0·325	1543·2	805·6	**0·001**
Occasional consumer	1909·7	756·0		1468·4	634·2	
Skipper	1713·5	1123·5		1217·7*	625·4	
Vitamin B_{12} (cobalamin; μg/d)						
Consumer	6·8	5·1	0·309	5·0	7·0	0·234
Occasional consumer	6·4	3·9		7·2	15·6	
Skipper	5·7	4·8		4·9	10·4	
Vitamin B_9 (total folate; μg/d)						
Consumer	224·7	95·9	**0·041**	188·9	76·0	**0·046**
Occasional consumer	236·8	104·4		186·2	88·3	
Skipper	192·4*	105·5		162·2	92·7*	
Vitamin C (mg/d)						
Consumer	103·9	78·9	0·106	104·1	86·7	0·438
Occasional consumer	106·3	72·0		94·9	73·1	
Skipper	80·0	71·2		86·1	87·4	
Vitamin A (β-carotene; μg/d)						
Consumer	2257·4	2366·9	0·775	2516·1	2568·0	0·117
Occasional consumer	2580·7	2605·9		2117·2	2051·3	
Skipper	2320·5	2859·9		1855·5	2790·7	
Vitamin E (tocopherol equivalents; μg/d)						
Consumer	10 849	6285	0·348	8796	6559	**0·045**
Occasional consumer	11 303	6328		9113	4092	
Skipper	9604	6701		7366†	4324	
Vitamin E (α-tocopherol; μg/d)						
Consumer	8545	5355	0·418	7472	6297	0·065
Occasional consumer	9536	5421		7399	3657	
Skipper	7921	6952		6161	3721	
Vitamin A (retinol equivalents; μg/d)						
Consumer	1200·4	1570·1	0·373	1097·7	2370·4	0·203
Occasional consumer	1159·0	813·3		2087·9	8255·0	
Skipper	1009·0	876·5		1147·9	3712·4	
Vitamin A (retinol; μg/d)						
Consumer	748·2	1477·3	0·303	619·1	2335·2	0·180
Occasional consumer	664·5	554·7		1673·4	8213·4	
Skipper	527·3	520·5		780·0	3671·3	

All analyses were adjusted for centre, age, BMI, supplement use, family structure and socio-economic status (covariables).
P values from one-way ANCOVA indicate statistical significance among different breakfast patterns; significant *P* values are shown in bold font.
Significant differences among breakfast consumption groups ($P < 0·05$) by Bonferroni *post hoc* test: **v.* consumers; †*v.* occasional consumers.

intakes of these micronutrients. Based on our results, vitamin D concentrations are highly and positively affected by breakfast in both sexes. Furthermore, vitamin D has a major role in bone mass development. Adolescence is a critical life stage for bone mineral accrual: peak bone mass is acquired at approximately 18 years of age[50] and 50 % of adult total bone mass is achieved during this period of life[51]. Thus, 25(OH)D deficiency at these early ages could be considered a risk factor for bone health (i.e. osteoporosis), but also other diseases including diabetes, cancer, CVD and metabolic syndrome[14,52]. Moreover, 25(OH)D is identified as one of the vitamins most highly at risk, with almost 80 % of adolescents below optimal levels[14–16,40]. Dairy products at breakfast could be an important target for improving vitamin D concentrations, especially if these dairy products are fortified[51]. Likewise, the other very important vitamin D source is sunlight exposure. In our study we took into account the latitude of the adolescent's residence, with study centre being added as a confounding factor. However, other confounders

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Table 3 Mean age, BMI and blood vitamin concentrations of the study sample by breakfast consumption pattern and sex; adolescents (*n* 1058) aged 12·5–17·5 years from ten European cities, HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study

	Males (*n* 499)			Females (*n* 559)		
	Mean	SD	*P*	Mean	SD	*P*
Age (years)						
Consumer	14·8	1·2	**0·008**	15·0	1·1	**0·046**
Occasional consumer	14·9	1·2		14·7*	1·2	
Skipper	15·3*	1·3		14·9	1·4	
BMI (kg/m^2)						
Consumer	21·0	3·7	**<0·001**	21·1	3·5	**0·012**
Occasional consumer	21·4	3·6		21·1	2·9	
Skipper	22·9†	4·7		22·0*	3·4	
25-Hydroxyvitamin D (nmol/l)						
Consumer	59·2	21·1	**0·003**	61·7	22·0	**0·006**
Occasional consumer	54·2	23·4		58·5	23·3	
Skipper	50·5*	18·9		53·5*	19·7	
Pyridoxal phosphate (nmol/l)						
Consumer	66·0	48·1	0·603	62·8	67·3	0·610
Occasional consumer	71·1	47·6		59·6	45·2	
Skipper	63·9	41·4		57·3	35·5	
Cobalamin (pmol/l)						
Consumer	341·1	123·8	**0·001**	371·9	149·2	0·638
Occasional consumer	313·4	115·6		373·1	168·0	
Skipper	293·6*	113·7		358·5	156·0	
Holo-transcobalamin (pmol/l)						
Consumer	64·8	32·4	0·087	66·2	34·0	**0·009**
Occasional consumer	56·7	18·2		60·2	25·4	
Skipper	59·7	35·4		57·2*	25·7	
Plasma folate (nmol/l)						
Consumer	18·5	9·7	0·488	19·7	10·9	0·074
Occasional consumer	18·8	9·4		18·6	9·8	
Skipper	17·2	11·4		17·4	9·4	
Whole-blood folate (nmol/l)						
Consumer	349·3	135·2	0·530	327·3	133·8	0·183
Occasional consumer	341·5	151·2		303·8	117·4	
Skipper	365·4	201·3		309·7	128·3	
Red-blood-cell folate (nmol/l)						
Consumer	795·3	325·2	0·996	788·0	303·4	0·139
Occasional consumer	794·5	383·8		732·4	289·2	
Skipper	798·7	425·8		739·8	299·7	
Vitamin C (mg/l)						
Consumer	10·3	3·2	**0·048**	10·9	3·4	**0·024**
Occasional consumer	10·2	3·2		10·4	3·0	
Skipper	9·4*	3·2		10·0*	3·3	
β-Carotene (ng/ml)						
Consumer	236·2	167·9	0·191	267·6	160·5	0·113
Occasional consumer	229·4	144·2		245·9	160·6	
Skipper	202·6	124·9		235·8	136·3	
α-Tocopherol (μg/ml)						
Consumer	9·5	1·9	0·648	10·4	2·3	0·153
Occasional consumer	9·5	2·0		10·0	1·8	
Skipper	9·3	2·0		10·1	1·9	
Retinol (ng/ml)						
Consumer	364·1	103·8	0·069	354·2	114·2	0·843
Occasional consumer	339·7	95·4		346·4	100·2	
Skipper	376·6	109·0		354·0	112·0	
Total homocysteine (μmol/l)						
Consumer	7·6	3·6	0·156	6·5	2·2	**<0·001**
Occasional consumer	8·0	4·2		6·7	2·0	
Skipper	8·6	5·7		7·5*,†	3·1	

All analyses were adjusted for centre, age, BMI, supplement use, family structure and socio-economic status (covariables).
P values from one-way ANCOA indicate statistical significance among different breakfast patterns; significant *P* values are shown in bold font.
Significant differences among breakfast consumption groups ($P < 0·05$) by Bonferroni *post* hoc test: **v.* consumers; †*v.* occasional consumers.

regarding vitamin D such as physical activity were not accounted for.

Furthermore, our results illustrate that adolescents consuming breakfast habitually had higher concentration levels for some B-vitamin markers. CBL was higher only in male breakfast consumers and holo-TC was higher only in female breakfast consumers compared with breakfast skippers. On the other hand, tHcy was lower in female

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Table 4 Frequency and percentage of different blood vitamin concentration statuses by breakfast consumption pattern in the study sample; adolescents (*n* 1058) aged 12·5–17·5 years from ten European cities, HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study

	Total (*n* 1058)		Consumer (*n* 603)		Occasional consumer (*n* 179)		Skipper (*n* 276)		
	Frequency	%	Frequency	%	Frequency	%	Frequency	%	*P*
25-Hydroxyvitamin D									
Deficiency (<49·99 nmol/l)	399	37·8	204	33·8	78	43·8	117	42·7	**0·012**
Insufficiency (50–75 nmol/l)	446	42·1	266	44·1	77	42·9	103	37·2	
Sufficiency (>75 nmol/l)	213	20·1	133	22·1	24	13·4	56	20·1	
Pyridoxal phosphate									
Deficiency (<20 nmol/l)	55	5·2	31	5·2	8	4·3	16	5·0	0·872
Insufficiency (20–30 nmol/l)	124	11·7	66	11·0	23	13·1	35	12·4	
Sufficiency (>30 nmol/l)	879	83·1	506	83·8	148	82·6	225	82·6	
Cobalamin									
Deficiency (<149 pmol/l)	23	2·2	5	0·9	6	3·1	12	4·4	**0·002**
Sufficiency (≥149 pmol/l)	1035	97·8	598	99·1	173	96·9	264	95·6	
Holo-transcobalamin									
Deficiency (<30 pmol/l)	47	4·4	21	3·5	12	6·6	14	4·8	0·155
Sufficiency (≥30 pmol/l)	1011	95·6	582	96·5	167	93·4	262	95·2	
Plasma folate									
Deficiency (<10·2 nmol/l)	160	15·1	69	11·4	29	16·2	62	22·4	**<0·001**
Insufficiency (13·6–10·2 nmol/l)	214	20·2	131	21·7	27	15·3	56	20·2	
Sufficiency (>13·6 nmol/l)	684	64·7	400	66·8	123	68·5	161	57·4	
Red-blood-cell folate									
Deficiency (<566 nmol/l)	26	2·5	13	2·1	5	3·0	8	3·1	0·846
Insufficiency (566–906 nmol/l)	729	68·9	411	68·1	123	68·8	195	70·9	
Sufficiency (>906 nmol/l)	303	28·6	179	29·8	51	28·2	73	26·0	
Vitamin C									
Deficiency (<1 mg/l)	3	0·2	0	0·0	1	0·5	2	0·5	0·306
Insufficiency (1–14·99 mg/l)	69	6·5	39	6·4	14	7·6	16	6·1	
Sufficiency (<15 mg/l)	986	93·2	564	93·6	164	91·9	258	93·4	
β-Carotene									
Deficiency (<0·3 mmol/l)	345	32·6	182	30·2	61	34·2	102	36·9	0·124
Sufficiency (>0·3 mmol/l)	713	67·4	421	69·8	118	65·8	174	63·1	
α-Tocopherol									
Deficiency (<12 mmol/l)	4	0·3	4	0·6	0	0·0	0	0·0	0·220
Sufficiency (12–46 mmol/l)	1054	99·7	599	99·4	179	100·0	276	100·0	

Reference values used to analyse different blood vitamin concentration statuses in adolescents aged 12·5–17·49 years were from the HELENA Study[40].
Significant *P* values are shown in bold font.

breakfast consumers than in skippers. There is increasing evidence of sub-deficient PF, PLP and CBL status in several population groups, including children and adolescents. Concretely, in a previous study, subclinical deficiency of PF and PLP in about 20% of European adolescents was reported[15]. Results from the present study also showed higher frequency of CBL and PF deficiency in breakfast skippers than in habitual breakfast consumers. Vitamin B_6, vitamin B_{12} and folic acid contribute to healthy growth and development, thus deficiency during adolescence is related to irreversible neurological damage and several diseases, such as CVD and cancer[18]. In addition, low blood CBL concentrations have been associated with suppressed osteoblast activity[53] and low concentrations of PF, PLP and CBL have also been associated with stimulated osteoclast activity[53]. Moreover, these vitamins are determinants of tHcy, which may also have an independent effect on different diseases[54–57]. Likewise, several studies have shown an association between increased total tHcy concentration and the risk of osteoporotic fracture[54]. Better tHcy profile and higher blood B-vitamin concentrations have been reported in young people aged 4–18 years[58] and adults who consume breakfast cereals[59].

Moreover, retinol, α-tocopherol, vitamin C and β-carotene are considered antioxidant nutrients and are widely found in plant or plant-derived foods[60]. It is well recognized that dietary antioxidants play an active role in affording protection against chronic diseases like cardiovascular and cerebrovascular disease, cancer and diabetes in adulthood[16,61]. Retinol and vitamin C deficiency are not common; however, β-carotene and α-tocopherol show significant deficiency prevalence in adolescents[40]. In our study, blood vitamin C concentration was associated with breakfast habits. Breakfast consumers presented higher vitamin C concentrations compared with the breakfast skippers. However, this made no difference to the prevalence of deficiency or sufficiency in both groups. It should be noted that the association between vitamin C intake and plasma concentration in the general population shows that in order to achieve protective plasma concentrations, it is necessary to increase the intake of vitamin C up to the RDA levels[62]. Daily breakfast consumption

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containing fruit seems to be an ideal strategy to achieve this goal. Further to the above, β-carotene concentrations below the normal range have been identified in European adolescents (25 %)[40].

The large sample size and the standardized methodology are notable strengths of the present study. On the other hand, the use of the statement 'I often skip breakfast' for the assessment and categorization of breakfast consumption habits of adolescents could be a possible limitation of our study. The term 'breakfast consumers' in the literature includes a variety of definitions, such as consuming breakfast every day, every weekday, on the dietary survey day, or usual or habitual consumption[1,63], which makes comparisons difficult. In addition, there is no consensus regarding the definition of breakfast consumption. A study conducted by Dialektakou and Vranas found that the percentage of breakfast skippers varied greatly according to how breakfast was categorized[63]. An important limitation is that we are comparing total vitamin concentrations from only one meal per day (i.e. breakfast; requirements for the vitamins could be completed with other meals during the day). In addition, we are analysing consuming breakfast or not, instead of the quality of the breakfast. Furthermore, because of the cross-sectional nature of the study design, no conclusion can be drawn about the directionality and causality of the associations observed between breakfast consumption and serum vitamin concentrations.

Conclusions

Regular breakfast consumption is associated with higher intakes of some vitamins, higher blood concentrations of some vitamins and lower blood concentration of tHcy in European adolescents. Particularly, skipping breakfast is associated with lower blood vitamin D (25(OH)D) and CBL concentrations in males and with lower vitamin D (25(OH)D) and holo-TC, and higher tHcy, concentrations in females; and with low intakes of vitamin D (cholecalciferol) and vitamin B_9 (total folate) in both sexes, and with low intakes of vitamin B_6 (pyridoxine) and vitamin E (tocopherol equivalents) in females. Consuming breakfast regularly could be an indicator for a healthier dietary pattern overall.

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References

1. Rampersaud GC, Pereira MA, Girard BL *et al.* (2005) Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *J Am Diet Assoc* **105**, 743–760.
2. Alexy U, Wicher M & Kersting M (2010) Breakfast trends in children and adolescents: frequency and quality. *Public Health Nutr* **13**, 1795–1802.
3. Giovannini M, Agostoni C & Shamir R (2010) Symposium overview: Do we all eat breakfast and is it important? *Crit Rev Food Sci Nutr* **50**, 97–99.
4. Diethelm K, Huybrechts I, Moreno L *et al.* (2014) Nutrient intake of European adolescents: results of the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutr* **17**, 486–497.
5. Hallström L, Labayen I, Ruiz JR *et al.* (2013) Breakfast consumption and CVD risk factors in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutr* **16**, 1296–1305.
6. Cho S, Dietrich M, Brown CJ *et al.* (2003) The effect of breakfast type on total daily energy intake and body mass index: results from the Third National Health and Nutrition Examination Survey (NHANES III). *J Am Coll Nutr* **22**, 296–302.
7. Rampersaud GC (2009) Benefits of breakfast for children and adolescents: update and recommendations for practitioners. *Am J Lifestyle Med* **3**, 86–103.
8. Miller GD, Forgac T, Cline T *et al.* (1998) Breakfast benefits children in the US and abroad. *J Am Coll Nutr* **17**, 4–6.
9. Wyon DP, Abrahamsson L, Järtelius M *et al.* (1997) An experimental study of the effects of energy intake at breakfast on the test performance of 10-year-old children in school. *Int J Food Sci Nutr* **48**, 5–12.
10. Matthys C, De Henauw S, Bellemans M *et al.* (2007) Breakfast habits affect overall nutrient profiles in adolescents. *Public Health Nutr* **10**, 413–421.
11. Serra-Majem L, Ribas L, Perez-Rodrigo C *et al.* (2002) Determinants of nutrient intake among children and adolescents: results from the enKid Study. *Ann Nutr Metab* **46**, Suppl. 1, 31–38.
12. van den Boom A, Serra-Majem L, Ribas L *et al.* (2006) The contribution of ready-to-eat cereals to daily nutrient intake and breakfast quality in a Mediterranean setting. *J Am Coll Nutr* **25**, 135–143.
13. Viteri FE & Gonzalez H (2002) Adverse outcomes of poor micronutrient status in childhood and adolescence. *Nutr Rev* **60**, 5 Pt 2, S77–S83.

14. González-Gross M, Valtueña J, Breidenassel C *et al.* (2012) Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr* **107**, 755–764.
15. González-Gross M, Benser J, Breidenassel C *et al.* (2012) Gender and age influence blood folate, vitamin B_{12}, vitamin B_6, and homocysteine levels in European adolescents: the Helena Study. *Nutr Res* **32**, 817–826.
16. Breidenassel C, Valtuena J, Gonzalez-Gross M *et al.* (2011) Antioxidant vitamin status (A, E, C, and β-carotene) in European adolescents – the HELENA Study. *Int J Vitam Nutr Res* **81**, 245–255.
17. Kremer R, Campbell PP, Reinhardt T *et al.* (2009) Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *J Clin Endocrinol Metab* **94**, 67–73.
18. Al-Tahan J, González-Gross M & Pietrzik K (2006) B-vitamin status and intake in European adolescents. A review of the literature. *Nutr Hosp* **21**, 452–465.
19. De Henauw S, Gottrand F, De Bourdeaudhuij I *et al.* (2007) Nutritional status and lifestyles of adolescents from a public health perspective. The HELENA Project – Healthy Lifestyle in Europe by Nutrition in Adolescence. *J Public Health* **15**, 187–197.
20. Moreno L, De Henauw S, Gonzalez-Gross M *et al.* (2008) Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. *Int J Obes (Lond)* **32**, Suppl. 5, S4–S11.
21. Beghin L, Castera M, Manios Y *et al.* (2008) Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. *Int J Obes (Lond)* **32**, Suppl. 5, S12–S18.
22. Hallstrom L, Vereecken CA, Ruiz JR *et al.* (2011) Breakfast habits and factors influencing food choices at breakfast in relation to socio-demographic and family factors among European adolescents. The HELENA Study. *Appetite* **56**, 649–657.
23. 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.
24. Currie CE, Elton RA, Todd J *et al.* (1997) Indicators of socioeconomic status for adolescents: the WHO Health Behaviour in School-aged Children Survey. *Health Educ Res* **12**, 385–397.
25. Currie C, Molcho M, Boyce W *et al.* (2008) Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. *Soc Sci Med* **66**, 1429–1436.
26. Vereecken CA, Covents M, Sichert-Hellert W *et al.* (2008) Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. *Int J Obes (Lond)* **32**, Suppl. 5, S26–S34.
27. Brussaard JH, Lowik MR, Steingrimsdottir L *et al.* (2002) A European food consumption survey method – conclusions and recommendations. *Eur J Clin Nutr* **56**, Suppl. 2, S89–S94.
28. Dehne LI, Klemm C, Henseler G *et al.* (1999) The German food code and nutrient data base (BLS II.2). *Eur J Epidemiol* **15**, 355–358.
29. Julian-Almarcegui C, Bel-Serrat S, Kersting M *et al.* (2016) Comparison of different approaches to calculate nutrient intakes based upon 24-h recall data derived from a multicenter study in European adolescents. *Eur J Nutr* **55**, 537–545.
30. Harttig U, Haubrock J, Knüppel S *et al.* (2011) The MSM program: web-based statistics package for estimating usual dietary intake using the Multiple Source Method. *Eur J Clin Nutr* **65**, Suppl. 1, S87–S91.
31. World Health Organization (1985) *Energy and Protein Requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. WHO Technical Report Series* no. 724. Geneva: WHO.
32. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**, 1119–1130.
33. Tanner JM & Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* **51**, 170–179.
34. Gonzalez-Gross M, Breidenassel C, Gómez-Martínez 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.
35. Ueland PM, Refsum H, Stabler SP *et al.* (1993) Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem* **39**, 1764–1779.
36. Kimura M, Kanehira K & Yokoi K (1996) Highly sensitive and simple liquid chromatographic determination in plasma of B_6 vitamers, especially pyridoxal 5′-phosphate. *J Chromatogr A* **722**, 295–301.
37. Brady J, Wilson L, McGregor L *et al.* (2008) Active B12: a rapid, automated assay for holotranscobalamin on the Abbott AxSYM analyzer. *Clin Chem* **54**, 567–573.
38. 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.
39. Cole T, Bellizzi M, Flegal K *et al.* (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* **320**, 1240–1243.
40. Moreno LA, Gottrand F, Huybrechts I *et al.* (2014) Nutrition and lifestyle in European adolescents: The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Adv Nutr* **5**, issue 5, 615S–623S.
41. Hallstrom L, Vereecken CA, Labayen I *et al.* (2012) Breakfast habits among European adolescents and their association with sociodemographic factors: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. *Public Health Nutr* **15**, 1879–1889.
42. Nicklas TA, O'Neil C & Myers L (2004) The importance of breakfast consumption to nutrition of children, adolescents, and young adults. *Nutr Today* **39**, 30–39.
43. Barr SI, DiFrancesco L & Fulgoni VL (2014) Breakfast consumption is positively associated with nutrient adequacy in Canadian children and adolescents. *Br J Nutr* **112**, 1373–1383.
44. Affenito SG (2007) Breakfast: a missed opportunity. *J Am Diet Assoc* **107**, 565–569.
45. Utter J, Scragg R, Mhurchu CN *et al.* (2007) At-home breakfast consumption among New Zealand children: associations with body mass index and related nutrition behaviors. *J Am Diet Assoc* **107**, 570–576.
46. Moreno L, Gonzalez-Gross M, Kersting M *et al.* (2008) Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutr* **11**, 288–299.
47. Brady H, Lamb MM, Sokol RJ *et al.* (2007) Plasma micronutrients are associated with dietary intake and environmental tobacco smoke exposure in a paediatric population. *Public Health Nutr* **10**, 712–718.
48. Food and Nutrition Board, Institute of Medicine (2010) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academies Press.
49. Food and Nutrition Board, Institute of Medicine (2006) *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC: National Academies Press.

50. Vicente-Rodríguez G, Ezquerra J, Mesana MI *et al.* (2008) Independent and combined effect of nutrition and exercise on bone mass development. *J Bone Miner Metab* **26**, 416–424.
51. Peters B, Verly E Jr, Marchioni D *et al.* (2012) The influence of breakfast and dairy products on dietary calcium and vitamin D intake in postpubertal adolescents and young adults. *J Hum Nutr Diet* **25**, 69–74.
52. Parker J, Hashmi O, Dutton D *et al.* (2010) Levels of vitamin D and cardiometabolic disorders: systematic review and meta-analysis. *Maturitas* **65**, 225–236.
53. Carmel R, Lau KW, Baylink DJ *et al.* (1988) Cobalamin and osteoblast-specific proteins. *N Engl J Med* **319**, 70–75.
54. Gjesdal CG, Vollset SE, Ueland PM *et al.* (2007) Plasma homocysteine, folate, and vitamin B_{12} and the risk of hip fracture: the Hordaland Homocysteine Study. *J Bone Miner Res* **22**, 747–756.
55. Haan MN, Miller JW, Aiello AE *et al.* (2007) Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. *Am J Clin Nutr* **85**, 511–517.
56. Saposnik G, Ray JG, Sheridan P *et al.*; Heart Outcomes Prevention Evaluation 2 Investigators (2009) Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. *Stroke* **40**, 1365–1372.
57. Murakami H, Iemitsu M, Sanada K *et al.* (2011) Associations among objectively measured physical activity, fasting plasma homocysteine concentration, and MTHFR C677T genotype. *Eur J Appl Physiol* **111**, 2997–3005.
58. Gibson S (2003) Micronutrient intakes, micronutrient status and lipid profiles among young people consuming different amounts of breakfast cereals: further analysis of data from the National Diet and Nutrition Survey of Young People aged 4 to 18 years. *Public Health Nutr* **6**, 815–820.
59. Tucker KL, Olson B, Bakun P *et al.* (2004) Breakfast cereal fortified with folic acid, vitamin B-6, and vitamin B-12 increases vitamin concentrations and reduces homocysteine concentrations: a randomized trial. *Am J Clin Nutr* **79**, 805–811.
60. Dybkowska E, Waszkiewicz-Robak B & Piekot E (2014) Evaluation of vitamins A, C and E content in diets of adolescents living in Warsaw, Poland. *Rocz Panstw Zakl Hig* **65**, 21–25.
61. Nothlings U, Schulze MB, Weikert C *et al.* (2008) Intake of vegetables, legumes, and fruit, and risk for all-cause, cardiovascular, and cancer mortality in a European diabetic population. *J Nutr* **138**, 775–781.
62. Krajcovicova-Kudlackova M, Babinska K, Valachovicova M *et al.* (2007) Vitamin C protective plasma value. *Bratisl Lek Listy* **108**, 265–268.
63. Dialektakou KD & Vranas PB (2008) Breakfast skipping and body mass index among adolescents in Greece: whether an association exists depends on how breakfast skipping is defined. *J Am Diet Assoc* **108**, 1517–1525.