Socioeconomic differences in micronutrient intake and status in Europe Romana Novaković

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Romana Novaković

PROPOSITIONS

1. Low socioeconomic status goes together with poor intake of calcium, vitamin C and folate. (this thesis)

2. Differences in micronutrient intake between countries are larger than between socioeconomic strata. (this thesis)

3. To improve health of populations, physical activity prescribed by general practitioners is likely to be more cost-effective than promotion via mass-media.

4. Antibiotic resistance is a catastrophic threat that deserves more scientific attention than global warming.

5. If performance enhancing drugs get legal, athletes will have no choice, but society should take responsibility for their health hazard.

6. Multiculturalism is not only about finding out how long others cook their vegetables, but it also helps you to discover your identity.

Propositions belonging to the thesis entitled 'Socioeconomic differences in micronutrient intake and status in Europe'

Romana Novaković,
Wageningen, July 2nd, 2013

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Romana Novaković

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Socioeconomic differences in micronutrient intake and status in Europe

Romana Novaković

Thesis

Submitted in fulfilment of the requirements for the degree of doctor

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in the presence of the

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I dedicate this thesis to my '3 M' (Mirjana Gurinović, Marija Glibetić, Marina Nikolić) and Adrienne Cavelaars for their outstanding support and care.

Romana

Contents

Chapter 1

Introduction

Introduction[1]

In Europe, data collected by nutritional surveys are used to identify population groups who are either at risk of deficiency or malnourished. These surveys provide information on the level and distribution of the populations' micronutrient intake and status and look for factors that possibly influence these, such as age, sex, socioeconomic status, etc.

Potentially vulnerable groups that should be monitored for the risk of low micronutrient intake have been identified by the European Commission: children and adolescents, pregnant and lactating women, elderly, and socioeconomically disadvantaged groups (1). Because dietary intake and nutritional status surveys are demanding in terms of cost and time, it is important to prioritize which micronutrients should be evaluated for the risk of low intake and status among populations. A common purpose in nutritional surveillance is to evaluate collected data in relation to dietary reference values (DRVs) and to cutoff levels for the status markers. However, wide disparities in dietary reference values across Europe make evaluation and cross-country comparison of population nutritional health very challenging (2).

The EURopean micronutrient RECommendations Aligned (EURRECA) Network of Excellence (NoE) explored the process of setting micronutrient recommendations aiming to harmonize DRVs (3). Thereby the attention was given to the assessment of the relationship between micronutrient intake and status which is essential information for underpinning DRVs.

One of the initial steps was to produce a list of public health priority micronutrients for which reference values should be urgently re-evaluated. From this process, 5 micronutrients were prioritized for intake and status (iron, folate, vitamin B12, zinc and iodine) and 5 for intake (calcium, vitamin C, vitamin D, selenium and copper). The rationale for this choice was based on: 1) the need for alignment of their DRVs which considerably vary across countries, 2) an indication for inadequate intake or status and an association with health outcome, and 3) the amount of new scientific evidence that should be accounted for in the estimation of micronutrient requirements (4). Evaluation of intake and status of resulting micronutrients of concern was recognized as a prerequisite to estimate the populations' nutritional health and to identify groups at risk of nutritional inadequacies.

Figure 1 provides the schematic framework that relates micronutrient intake and status data for the evaluation of populations' nutritional health, for deriving dietary reference values and for the integration of evidence into policy and practice (3).

The aforementioned points - prioritizing micronutrients and assessment of population nutritional status- are bases for *Stage 1 (Defining the problem)* and *Stage 2 (Monitoring and evaluating),* respectively. The relationship between micronutrient intake and status is one of the building blocks for *Stage 3 (Deriving dietary reference values).* Finally, policy implementation and evaluation

acknowledge the role of DRVs in the wider public health nutrition context: research and applications in nutritional policy and practice (*Stage 4*).

[1]*This thesis focuses on indications for low, rather than excess micronutrient intake.*

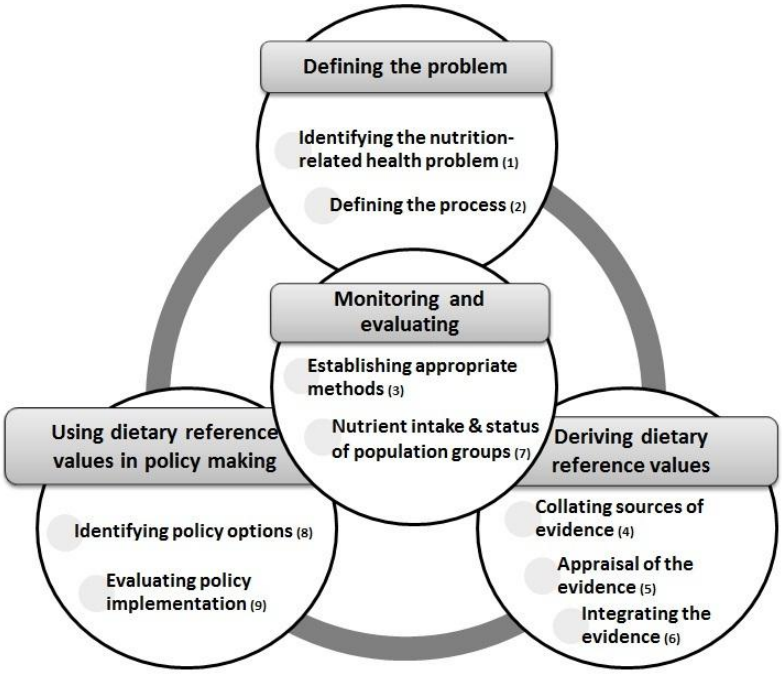

Figure 1 The nine activities of the EURRECA framework clustered into four stages: 1. Defining the problem, 2. Monitoring and evaluating, 3. Deriving dietary reference values and 4. Using dietary reference values in policy making (3).

Monitoring and evaluation of populations' micronutrient intake and status

The 2008-2013 WHO response to address European nutrition challenges in improving nutrition and preventing diet-related diseases advocates establishment of national and regional surveillance systems on nutritional status and dietary intake of micronutrients. It underlines the need for monitoring the micronutrient adequacy for different age and socioeconomic groups (5).

Data on micronutrient intake in Europe from national surveys have been collated and evaluated in the European Nutrition Health Report II (6). The results show that the intakes of vitamin D and folate were generally low in nearly all age groups. Also, intakes of calcium, magnesium, iron in women, and, in some age groups of iodine were below the reference values. It has been recognized that intake and status of public health priority micronutrients for life stage groups other than adults and elderly have been poorly addressed so far (7). The above mentioned source is currently the most comprehensive overview of nutritional intake in Europe. Yet, it didn't address micronutrient intake and status in

Central and Eastern Europe (CEE), as nutritional data from CEE for all age ranges have been insufficiently studied (8,9), or have not been published in the open access literature. Therefore, grey literature written in national languages might be a valuable source of information that could be used to report on the nutritional status of CEE populations. More specifically, data from national surveys, country reports, theses and dissertations might add to the existing evidence, provided that scientific quality and translation of the data are not barriers.

Nevertheless, a comprehensive Europe-wide overview of intake and status of public health priority micronutrients in all age ranges including socioeconomically disadvantaged groups is still lacking.

Socioeconomic status and micronutrient intake and status

Many studies show that there are large differences between individuals in quality and quantity of foods consumed (10-12). Generally, unhealthy diets that are energy rich and nutrient poor are more consumed among groups of low socioeconomic status (SES) (10, 13-15). At the same time, these groups are more prone to diet-related diseases than affluent groups. SES, conventionally measured as education, occupation or income, has been studied for its association with dietary quality or health outcome (16). Systematic reviews of the data from several European countries suggest that socioeconomically disadvantaged groups have dietary profiles that do not comply with dietary guidelines, for example they consume diets that are low in fruit and vegetables, fish and high-fibre products and therefore have an increased risk of poor health (13, 14). Indications for low micronutrient intakes have been found in general European populations (5, 6); therefore, we hypothesized that low SES groups with unfavorable dietary patterns are at the highest risk of inadequacy. Moreover, the WHO reports that deficiencies in several micronutrients such as calcium, zinc, vitamin B12 and vitamin D are not only found in developing countries, but are likely to be common among disadvantaged groups in industrialized nations (17). Despite the growing interest in the relationship between diet, SES and health, the link between SES and intake of micronutrients in Europe has not been studied enough. So far, research is orientated toward intake of one (or few) micronutrient(s) within a defined life stage group, or has been carried out in one country only (7-9, 18). The heterogeneity in SES measures challenges the evaluation of their association with nutritional data. Studies use education, occupation and income interchangeably even though each of them might add in a different way to the association with diet, particularly in socioeconomically and culturally diverse settings. Often, studies apply the one which is easily obtainable or most commonly used in national datasets, e.g. occupation has traditionally been used to describe ‘inequalities’ of health in Great Britain (16). This diversity in classification of SES makes the evidence on SES-diet relationships very challenging to compare between studies. Unfortunately, no individual participant-based multi-country study on the association between SES and intake of public health prioritized micronutrients in Europe is currently available. Furthermore, the data that explicitly address the association between

micronutrient intake and SES have not yet been aggregated into a systematic review that would summarize all available evidence and enlighten this issue, or report on knowledge gaps and opportunities for further research. An overview of the level and distribution of public health prioritized micronutrients' intake by SES indicators across all age ranges in Europe is urgently needed as it is a prerequisite for clarifying this association and for delivering data for nutrition policies.

Relationship between folate intake and status

For the evaluation of micronutrient intake distribution of populations, dietary reference values (DRVs) are essential. This evaluation is hampered by different DRVs by country, which reflects uncertainties in the inference from the scientific evidence-base. Folate is one of the top five public health priority micronutrients for which dietary reference values need to be critically evaluated (2, 4). Folate plays a central role in the synthesis and methylation of nucleotides that intervene in cell multiplication and tissue growth (17). Low intakes of folate are associated with a higher risk of giving birth to infants with neural tube defects and possibly other birth defects, and with an increased risk of cardiovascular diseases, cancer and impaired cognitive function in adults (17). The WHO report shows that the evidence from the available countries' national surveys indicates that folate deficiency may be a public health problem across a variety of population groups and countries that are at different stages of their development. In addition, the WHO highly recommends that the assessment of folate intake and status in representative national surveys should be done accounting for the determinants of folate intake, such as socioeconomic status (19). However, the effort of assessing the likely prevalence of deficiency in a population is hampered by the diversity in dietary reference values across countries.

Changes in folate intake are reflected by biomarkers of folate status (20). Serum folate is a good indicator of recent dietary folate intake, and the most widely used method of assessing folate status. Erythrocyte folate is, however, the better indicator of long-term status and of tissue folate stores. Elevated plasma homocysteine concentration is a strong predictor of inadequate folate status although other vitamin deficiencies (e.g. vitamins B2, B6 and B12) also increase homocysteine values (4, 10).

To strengthen the basis for the derivation of folate requirements and recommended values, the relationship between folate intake, status and health outcomes provides relevant information (I-S-H relationship). The analytical framework for deriving folate requirements and reference values requires information on folate intake and biomarkers of folate status reported in randomized controlled trials (RCTs) and observational studies. These data are needed to estimate the dose-response relationship between intake and status that may be used for deriving folate reference values which outline the levels of folate needed to maintain or eventually achieve optimal health (H). Such information is of use for international bodies that are charged with the responsibility of setting DRVs.

Thesis objective

Preamble

In this section, the objectives of this thesis are positioned along a simplified model of the causal chain between the determinants of micronutrient intake and status (education, occupation and income) and eventually health outcomes. This includes the micronutrient intake-status relationship needed for supporting DRVs, but not their relationship to specific health outcomes (Figure 2).

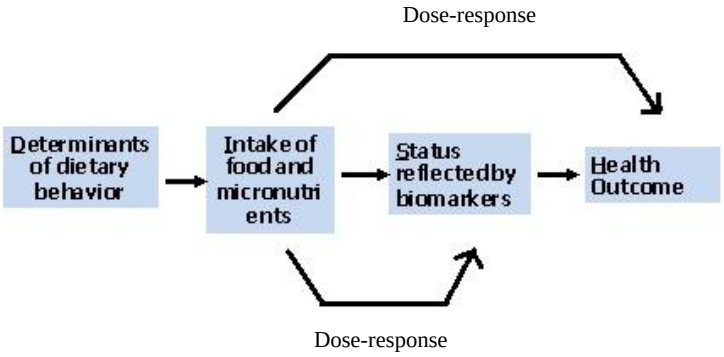

Figure 2 The DISH model for food, nutrition and health research describes the relationship between determinants of dietary behaviour (D), intake of foods and nutrients (I), biomarkers of status and function (S) and health outcome of interest (H) (21). The EURRECA Network of Excellence emphasized the right side of the framework (I-S-H) whereas the present thesis addresses the left part (D-I-S) by studying socio economic factors as determinant of intake.

The primary objective of this thesis was to study available evidence on the association between SES and micronutrient intake and status in Europe, and to identify knowledge gaps. It also addresses the relation between the intake and status markers of folate as one of the micronutrients of concern which is relevant to deriving dietary reference values for the evaluation of the intake of populations. Thus:

- Chapter 2 focuses on the box “Intake” in Figure 2. It describes micronutrient intake and status data available in Europe by giving a summary of the evidence from a systematic literature review of open access and grey literature of CEE populations in comparison to more affluent European countries.

- Chapter 3 and 4 address the relation between "Determinants" and "Intake", by studying indicators of SES as a potential determinant of micronutrient intake and status. Chapter 3 addresses inequalities in micronutrient intake and status associated with different levels of SES (education, occupation and income) among Europeans of all age ranges, as based on a systematic literature review. Chapter 4 focuses on education as a proxy for SES in adults and elderly and evaluates its relationship with micronutrient intake using the individual-participant data from 10 European countries participating in the EPIC cohort.

- Chapter 5 studies "Intake" as related to biomarkers of folate "Status'' in adults and elderly, using systematically collected literature from observational studies followed by meta- analysis.

The general discussion addresses the results from the former studies and their potential implications for policy and future research.

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Chapter 2

Micronutrient intake and status in Central and Eastern Europe compared with other European countries, results from the EURRECA network

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Abstract

Objective: Compare micronutrient intake and status in Central and Eastern European (CEE) to other European countries, and to reference values.

Design: Review of the micronutrient intake/status data from open access and grey literature sources from CEE.

Setting: Micronutrients studied were folate, iodine, iron, vitamin B12 and zinc (for intake and status) and calcium, copper, selenium, vitamin C, vitamin D (for intake). Intake data were based on validated dietary assessment methods; mean intakes were compared to average nutrient requirement set by the Nordic countries or Institute of Medicine, USA. Nutritional status was assessed using the status biomarkers and cut-off levels recommended primarily by the World Health Organization.

Subjects: For all population groups in CEE, the mean intake and mean/median status levels were compared between countries and regions: CEE, Scandinavia, Western Europe and Mediterranean countries.

Results: Mean micronutrient intake of adults in the CEE region were in the same range as those from other European regions, with exception of calcium (lower in CEE). CEE children and adolescents had poorer iodine status, and intakes of calcium, folate and vitamin D were below the reference values.

Conclusion: CEE countries are lacking comparable studies on micronutrient intake/status across all age ranges, especially in children. Available evidence showed no differences in micronutrient intake/status in CEE populations in comparison to other European regions except for calcium intake in adults, and iodine and iron status in children. The identified knowledge gaps urge further research on micronutrient intake/status of CEE populations to make a basis for evidence-based nutrition policy.

Introduction

Epidemiological research has shown socio-economic differences in health at all ages throughout Europe[1]. These inequalities in health have been reported to vary between countries and between socio-economic indicators [2, 3]. A possible explanation for these inequalities is a less optimal nutritional status in disadvantaged groups. For example, those with lower income suffer from higher rates of obesity, CVD, and from certain cancers that are linked to nutrition and diet [3, 4]. This may be due to the fact that groups with limited economic mean consume unhealthy foods that are cheap, energy-dense and nutrient-poor. Socio-economic position, as measured by income for instance, has been found to be one of the most important predictors of diet quality [4].

Since the number of (economically) disadvantaged populations is more prevalent in Central and Eastern European countries [5-9] it is highly relevant to evaluate whether differences in nutritional status may contribute to higher morbidity and mortality in CEE as compared to other European countries.

Assessment of dietary intake and nutritional status for European populations has been an emerging focus for the last two decades. However, current nutritional data are lacking information for Central and Eastern Europe populations [10, 11]. This hinders research on nutritional health, which is needed to underpin nutrition policies for CEE populations with sizable disadvantaged groups.

To fill the abovementioned knowledge gap, an additional approach is needed to deal with the standard searches conducted in known (open access) literature data bases, i.e. the identification of commonly overlooked grey literature sources from CEE countries. So far, available nutritional data in CEE have often only been used for local health policies and have remained largely unexploited because they are either not published in an accessible manner, or not available in English [1, 12, 13].

In this study, we compare micronutrient intake and status in Central and Eastern Europe to that in other European countries using both open access sources and grey literature sources. A better use of the latter data from CEE and its further exploitation will enable the evaluation of the current nutritional situation of CEE populations to enhance regional policy making.

Methods

EURRECA

This study was carried out within the context of EURRECA (EURopean micronutrient RECommendations Aligned) Network of Excellence (http://www.eurreca.org). This network aims to advance methodology for setting micronutrient requirements and recommendations, and to identify vulnerable groups regarding micronutrient intake and status [14, 15]. To answer our research question, we used the standardized dietary intake/status methodology recommended by EURRECA (more details are below). We focused on the EURRECA top ten micronutrients, prioritized on the basis of the amount of new scientific evidence, their relevance to public health and the variations in current micronutrient recommendations (vitamin B_{12}, folate, iron, zinc and iodine (intake and status) and vitamin C, vitamin D, calcium, selenium and copper (intake only))[14].

Search methodology

Data on CEE countries were collected from PubMed and grey literature for intake and status, and from the WHO Vitamin and Mineral Nutrition Information System (VMNIS) database (http://www.who.int/vmnis/database/en/) for status. For other European countries, we used available comprehensive reviews, primarily ENHR II for intake [16] and PubMed and WHO VMNIS for status only. Review of the European Food Safety Authority (EFSA) Concise and the Comprehensive European Databases did not result in additional data.

We searched for studies on intake and/or status in PubMed published from January 1990 till April 2010 using common medical subject headings (MeSH) and free text search terms: Micronutrients (listed) OR Biomarkers of status (listed) AND General intake/status terms with terms for adequacy, i.e. intake/status (requirement* or recommend* or adequacy or inadequacy or adequate or inadequate or cut point or threshold- in title and abstract) AND Country (CEE and non- CEE countries, listed).

The WHO Global Micronutrient Databases on Iodine deficiency and Anaemia were used to collate information on iodine and iron status.

To collect grey literature data on intake and or status from CEE countries, we cooperated with the United Nations University/Standing Committee on Nutrition Network for Capacity development in Nutrition in Central and Eastern Europe (NCDN CEE) www.srbnutrition.info/?page=Network [17]. This Network gathers nutritional researchers and public health specialists from all CEE countries, whose representatives were introduced with the purpose of this study and with the search criteria for collection of grey literature at their NCDN annual meeting in November 2008. They were asked to identify potentially relevant data from their countries, translate the data to English if necessary and send them to us for further screening.

Title and abstracts from every source were screened, and for potentially relevant papers and reports, full texts were retrieved. Subsequently, we assessed which studies were eligible for our study using the following inclusion criteria:

- The population studied should be representative for the country, apparently healthy, and data gathered should have had a sample size of at least 100 per gender group [18].
- Include information on mean usual micronutrient intake, and applied one of the following dietary intake methods (EURRECA best practice dietary intake methods): a validated Food Frequency Questionnaire or validated diet history, a food diary/register with at least seven days, three or more 24 Recall of registers, or < 3 recalls with an adjustment for intra-individual variability [19]. Initially, these criteria were applied to both CEE and non- CEE data; however, keeping this condition for CEE data would have resulted in very few studies for assessment. For this reason, CEE studies on intake that used one or more 24h recalls (with no adjustments for intra-individual variability) or other dietary assessment methods were included as well. If more than one country representative study that fulfils the inclusion criteria was available, the choice of the study to be graphically presented was made by giving the preference to one that was the most recently published.
- Regarding nutritional status, data gathered should include information on mean or median status using best practise biomarkers defined in the context of EURRECA: hemoglobin, serum/plasma ferritin or serum/plasma transferrin receptor for iron; erythrocyte(red cell) folate, serum/plasma folate or serum/plasma homocysteine for folate; serum/plasma total B_{12}, serum/plasma methylmalonic acid or serum/plasma holotranscobalamin for vitamin B_{12}; serum/plasma zinc for zinc, and urinary iodine (24h or spot), serum or dried whole blood spot thyroglobulin or serum thyroid-stimulating hormone for iodine [20-27].

Data analyses

We compared mean intakes and median (or mean) status levels between countries, between regions, and with external reference values to evaluate differences between countries and indications for inadequacies. When several studies were available for one country, studies were ranked for their eligibility considering: representativeness of the sample for the country, dietary assessment method used, year of the study (most recent were given a preference).

To study regional variations in micronutrient intake and/or status, Europe was divided in 4 geographical regions: Central and Eastern Europe (Albania, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Latvia, Lithuania, Hungary, Macedonia, Montenegro, Poland, Romania, Serbia, Slovakia and Slovenia), Mediterranean countries (Greece, Italy, Portugal and Spain), Western Europe (Austria, Belgium, France, Germany, Ireland, The Netherlands, Switzerland and United Kingdom) and Scandinavian countries (Denmark, Finland, Iceland, Norway and Sweden).

Regional comparisons were made when data for a specific micronutrient and population group were available for at least 3 CEE countries. This comparison included the calculation of weighed mean regional intakes and median status levels, graphic plotting of country means (medians) and subsequently the description of: i) differences in intake and/or status across European regions and ii) observed mean intake and/or status in contrast to nutritional reference values. Variables considered in the description were differences in dietary assessment method (checked also by differences in mean daily energy consumption) and study population.

In cases where data were available for less than three CEE countries, data from CEE countries were compared with the data from other European countries published in the ENHR II [16], or from single studies, selecting those with the most comparable data (regarding age group, status biomarker, dietary assessment method, year of the study).

To evaluate whether there is an indication for inadequacy in micronutrient intake, the mean usual intake was compared to the ANR (Average Nutrient Requirement: amount of nutrient which is enough for 50% of the apparently healthy population) derived for the Nordic countries (NNR) [28, 29], as these are the most recent references values set for a series of European countries [1]. If ANRs for micronutrients were not set by NNR (calcium and vitamin D in adults, all micronutrients in children), ANRs published for USA/Canada by the Institute of Medicine of the National Academies, Food and Nutrition Board, were used [22]. Relevant ANRs are bellow each figure (1-6). To assess adequacy of levels of status markers, we used guidance on cut-off values from EURRECA experts [30- 33]. These cut-off values were based on key references mostly published by the World Health Organization in cooperation with other institutions [20-27]. The specific values used are added to figures, tables or text where applicable.

[1]Although more sophisticated methods are available for assessment of inadequacy such as the EAR cut point method (27), we only compared mean levels with a reference value, as the more advanced methods use SDs. SDs vary considerable by assessment method and request a higher comparability of measurement between countries.

Results

Literature search and data availability

Search on micronutrient intake/status for all age ranges in CEE in PubMed database resulted in 1949 titles and abstracts out of which 121 left after screening for potential relevance. The key reason for exclusion was that studies did not report mean intake or status. For one hundred thirteen, full manuscripts were obtained and checked for compliance with the inclusion criteria; 8 studies were inaccessible. Finally, thirty-eight studies were kept for further analysis, of which the grey literature added nine studies in total, and contributed primarily data on micronutrient intake in adults. WHO VMNIS database provided 34 studies on iodine and iron status. A detailed overview of the study characteristics is given in Table 1.

Comparison of micronutrient intake

Micronutrient intake data were most abundant for calcium, folate, iron, vitamin B12, vitamin C and vitamin D in adults. In figures 1-6 the mean micronutrient intakes (with 95% confidence intervals) for males and females are plotted geographically (by country in 4 regions), and information is given on: the dietary assessment method, the age range of the study population, the number of subjects, and mean energy intake for males and females.

Observed mean calcium intakes (Figure 1) among females in CEE countries (estimated mean: 869 mg/d) were in general lower than in other European countries. The pooled means were 978, 1006 and 881 mg/d for Scandinavian, Western and Mediterranean countries, respectively. The observed mean intake among females in Estonia, Hungary and Lithuania were below the average nutrient requirement (ANR, source Nordic Nutrition Recommendations) which indicates that there is a risk of inadequacy. Similar results were observed for CEE males (pooled estimate 862 mg/d); the pooled mean calcium intakes for Scandinavian, Western and Mediterranean countries were as follows: 1130, 1129 and 885 mg/d. In CEE, four out of eight countries had a mean intake below the ANR among males, and in three countries among females. Within CEE, relatively high levels of calcium intake were observed for Croatia and Serbia. This can likely be explained by the different age range of the study population in the Croatian study (university students, 18-30 years), and the different dietary assessment method used in the Serbian study (intake data were collected per household and calculated per person using the consumption units). This explanation is supported by the fact that mean energy intakes of these studies are also relatively high. This remark holds for the levels of intake of other micronutrients from these two studies.

Figure 2 shows the observed mean intakes for folate. In CEE the mean pooled intake (307mg) was slightly higher than in the other regions (Scandinavia: 235 mg, Western Europe: 278 mg, Mediterranean 275 mg) which was due to the relatively high value for Croatia. In CEE, Hungarian and Estonian females had mean intakes were below the average nutrient requirement, indicating a risk of

inadequacy. Among CEE males, the estimated mean intake (302 mg) was in range to those in other regions- Scandinavia: 268 mg, Western Europe: 322 mg, Mediterranean: 287 mg. Hungarian males had the folate intake below the ANR.
These results correspond with the previous publication by de Bree A *et al* [34] which indicated that mean dietary folate intake in Europe is in line with recommendations.

The observed mean iron intake is shown in Figure 3. The pooled mean intake among females from CEE (13 mg) was similar to those from the other European regions (range 11-13mg). For all CEE countries the mean intake of females was above the ANR with exception of Hungary. For males, the mean estimated intake in CEE (16 mg) was slightly higher than in other regions: range from 13-15 mg; all countries reported the observed mean intakes higher than the average daily requirements.

The mean intakes of vitamin B_{12} are shown in Figure 4. The pooled levels for both females and males in CEE, 4µg and 7µg respectively, were similar to the pooled means for Scandinavia and Western Europe, whereas the highest means were observed in the Mediterranean countries (7 µg in females, 8µg in males). For both genders in all countries, mean intake values were above the ANR.

The mean observed vitamin C intake (Figure 5) in CEE females (the pooled estimate 134 mg) was similar to Western countries (136 mg), and it was higher than in Scandinavia and in the Mediterranean: 107 and 119 mg, respectively. The same held for CEE males: a pooled estimate for CEE was 118mg, for other regions it ranged from 93-142 mg. Both genders in CEE countries had the mean intakes above the ANR.

The pooled mean vitamin D intake for females and males of CEE countries was 3 and 4 µg, respectively (Figure 6). These values were comparable to those of the other regions; pooled estimates for females and males were as follows: Scandinavia 7.8 µg; Western Europe: 2.4 µg and Mediterranean: 3. 3 µg. The mean intakes in CEE and in other regions were below the ANR with exception of Norway.

Data for the other micronutrients: copper, iodine, selenium and zinc were limited and available for one or two CEE countries, and for some population groups only (see Table 2).

The mean copper intake in CEE countries was comparable to other European countries 1.2 - 1.9 mg [16], except for Serbia, which reported the highest intake. The mean intakes were above the reference values set for copper. Iodine intake data was only available for Serbia, and the mean intake was in range with other regions (108-253 µg for males; 101- 194 µg for females) [16] being above the ANR. Selenium intake data for CEE adults was available for Croatia, and the mean intake values were above the range observed in the other regions: (36-73 µg for males; 31-55µg for females) [16]. The mean intakes for both genders were well above the ANR. Data on mean zinc intake were available for 3

CEE countries, and the values were similar to those for non-CEE countries (7.8-13.6 mg for males; 7.8-10.3 mg for females) [16] -the average intakes were considerably above the ANR.

Data on micronutrients intake in CEE children were available from Croatia, Hungary and Serbia. The mean micronutrient intakes in these countries (Table 3) were in range of intakes in children in other European countries [16]: calcium (600-1381 mg), copper (0.8-1.9 mg), folate (138-428 μg), iodine (94-209 μg), iron (7.7-17.9 mg), selenium (28-110 μg), vitamin C (78- 197 mg), vitamin D (1.2- 4.8 μg) and zinc (6.5- 14.6 mg), except for intake of vitamin B_{12} in Hungary, which was lower in comparison to other European regions (2.9-11.8 μg). In these 3 CEE countries, mean daily intakes below the ANR were observed for calcium (except in boys in Serbia), folate, vitamin D and for selenium in Croatia.

Comparison of micronutrient status

For micronutrient status most CEE data were available for iodine and iron status in children. Figures 7 and 8 show the median urinary iodine concentrations and the mean haemoglobin concentrations and SD, respectively, for children in Europe. The data on iodine and iron status were mainly retrieved from the WHO VMNIS database. Exceptions are the data for The Republic of Srpska and Serbia. CEE countries had lower median urinary iodine levels than other European regions: mild iodine deficiency (urinary iodine <100μg/L) in CEE was found in children and adolescents from Estonia, Hungary, Latvia and Lithuania, whereas children in Albania suffered from moderate iodine deficiency (urinary iodine <50μg/L). Data from other European countries showed an adequate iodine status (median urinary level >100 μg/L) for all countries, except for Italy and Belgium.

The mean haemoglobin levels in CEE children and adolescents were lower than in other European countries, for which the data were scarce. Mean levels were in general above the cut off values; only infants in Lithuania were at risk of iron deficiency anaemia (haemoglobin level< 110g/L), whereas children in Romania had borderline concentration.

CEE status data on iodine and iron in adults, and on folate, vitamin B_{12} and zinc status for all population groups were too limited to allow between region comparisons. An overview of the available status data for CEE populations is presented in Table 4.

Median urinary iodine concentrations in CEE adults ranged from 51- 158 μg/day, which was in similar to other European regions (source WHO VMNIS database for non-CEE countries not included in the table). In CEE, mild iodine deficiency (UI<100μg/L) was observed in Romanian females. In other regions, both males and females from Italy, France and Germany had mean urinary iodine level below the cut off.

Prevalence of iodine deficiency in Europe was recently outlined by Zimmerman et al [35]. For CEE countries (data on school age children- if not available, preschool children, adolescents or adults) iodine deficiency was reported in Albania, Estonia, Hungary, Latvia and Lithuania.

For iron status, CEE data were only available for Serbia [36] and for females from Macedonia [12]. Reported mean haemoglobin concentrations were in range with those of other European countries included in the WHO Global data base (e.g Spain, France, Denmark and Finland), and above the cut off value 120/130 g/L.

Overall, the results on iron status (the haemoglobin levels above the cut-offs) confirm the results on iron intake for all countries in this study (except Hungary).

Data on folate status were available for adults in Croatia, Czech and Hungary. Mean serum folate levels in CEE were comparable to those from other European countries (range mean: 16 to 18 nmol/L) [40, 41, 43], with an exception for Norwegian adults (7 nmol/L) [42]. The mean serum folate levels in CEE populations and in other regions were above the cut-off values for both genders, except for Roma mothers in Czech Republic. The findings on folate status are in accordance to observed results on folate intake from this study for all European regions (except Hungary and Estonian females).

For vitamin B_{12} status, only one study from CEE was identified. In the Czech Republic reported mean levels of serum vitamin B_{12} were comparable to other European countries, such as Norway[42], and above the reference value (150 pmol/L). These findings are consistent with the vitamin B12 intake results from this study (Figure 4).

Data on zinc status were available for adults and children in three CEE countries. CEE adults from Czech Republic and Hungary had mean zinc status concentrations (13-17 µmol/L) comparable to Western European countries (range mean: 13-14 µmol/L) [45-47], and higher than the reference value. Mean status levels for Polish and Czech children were in range from 12 to 15 µmol/L (above the cut offs), and similar to findings from other European countries such as the UK (15 µmol/L) [44].

Discussion

This is the first comprehensive overview of CEE nutritional data using both open access and grey literature sources with a twofold objective: evaluation of intake and status for targeted micronutrients in CEE in comparison to those of other European countries, and to the reference values.

From the limited data available, the results of this review show no differences in micronutrient intake and status in CEE populations in comparison to other European regions except for intake of calcium in adults, and iodine and iron status in children (intake and status levels lower in CEE than in non-CEE countries). Comparisons with the intake/status reference values suggest highest risk of inadequacy in intake of vitamin D in all age ranges, and calcium, folate and iodine in children.

We collated data using EURRECA's best practice guidance on dietary assessment methods and status biomarkers [30]. In general, CEE studies on micronutrient intake and status for all age ranges were scarce - even after being less strict in inclusion criteria- only a few intake studies could be added to the current comprehensive overview. The largest knowledge gap regarding intake refers to children, whereas data on status were scarce for all population groups, with exception of iron and iodine in children. The available studies on micronutrient intake and status studies in CEE countries are diverse with regard to design: they differ in dietary assessment, food composition databases, sampling procedures, range of age groups, and this may confound regional and between country comparisons. Cooperation with the nutritional network from CEE [17] resulted in obtaining nine studies from grey literature. Even though it was not sufficient to fill an evident knowledge gap in nutritional data from CEE, it added to the existing open sources. However, despite a paucity of data and the variations in methodologies that can influence the true differences in intake [82], some regional variations were observed: it seems that nutritional health in CEE in comparison to other European countries is less favorable but only for certain micronutrients.

Since CEE is less affluent in comparison to other European regions, it would be interesting to examine what are the variations in micronutrient intake and status within CEE countries across different socioeconomic layers. That would indicate specific subgroups that are most at risk of poor nutritional health.

In addition, for future research, we recommend reviewing grey literature, and its accessibility and reliability need further attention. However, to bring a comprehensive conclusion on the nutritional situation in CEE countries much work is required: for developing tailored, sound evidence-based nutritional policy, the knowledge gaps and establishing nutritional surveys of comparable quality, covering the diversity of population groups need to be addressed. The inclusion of CEE countries in pan European nutritional surveys is highly essential to achieve this objective.

Figure 1. Mean calcium intake in milligrams per day (standard deviation) and daily energy intake in kilojoules per day (standard deviation) by country, for males (M) and females (F). Dietary intake method: EFR- estimated food record; 24h R- twenty four hour recall; DH- diet history; FFQ- food frequency questionnaire; HFCS- household food consumption survey. NA: not available.

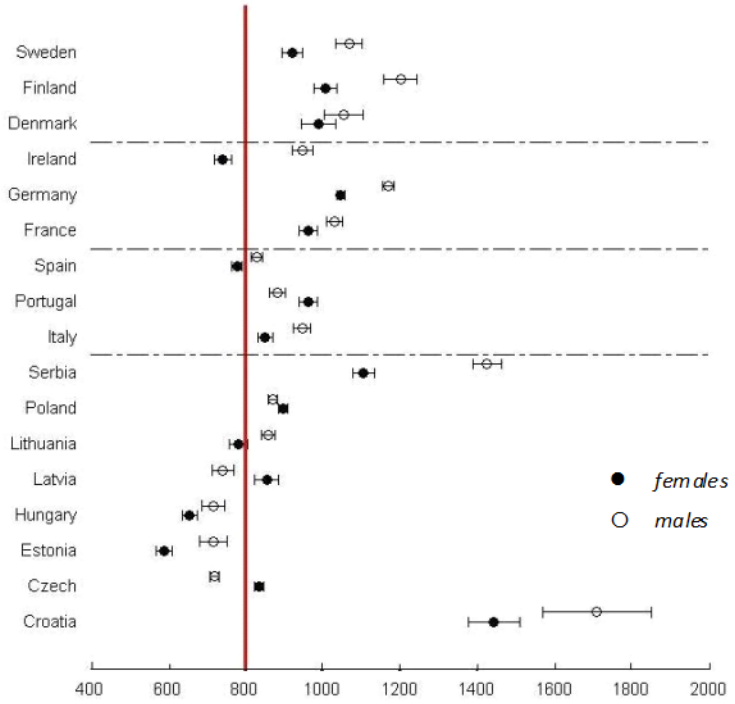


Figure 1. Mean Calcium intake (mg/day)

Country	Dietary intake method	Number of subjects; sex	Age range (years)	Mean (mg/day)	SD	Energy (kJ/day)	SD
Sweden[16]	EFR	500 M	19-64	1069	395	9933	
		500 F		922	300	7841	
Finland[16]	24h R	730 M	19-64	1202	592	9265	2960
		846 F		1007	450	6804	2028
Denmark[16]	EFR	300 M	19-64	1055	448	10638	2910
		300 F		990	389	8232	2209
Ireland [16]	EFR	650 M	19-64	949	354	11033	3108
		650 F		742	299	7623	2007
Germany[16]	DH	5000 M	19-64	1171	558	11041	4112
		5000 F		1047	389	8131	2511
France[16]	EFR	954 M	52-68	1032	325	NA	
		800 F		964	339		
Spain[16]	2x24h R	750 M	19-59	830	200	8925	
		750 F		778	170	7047	
Portugal[16]	FFQ	1200 M	19-64	883	354	9937	2305
		1200 F		963	359	8731	2108
Italy[16]	EFR	700 M	19-64	947	309	10336	1906
		700 F		851	264	8433	1604
Serbia[36]	HFCS	1173 M	30-60	1426	643	11415 (M&F)	
		1227 F		1105	501		
Poland[20]	FFQ	4815 M	45-69	872	425	9529	2910
		5044 F		899	438	8727	2608
Lithuania[66]	24h R	2606 M	19-64	858	468	10945	4317
		1132 F		782	420	8202	3494
Latvia[66]	24h R	1065 M	19-64	742	456	10848	5023
		1235 F		855	547	7522	3355
Hungary[69]	24h R	473 M	18-60+	717	319	11734	
		706 F		656	276	9227	
Estonia[60]	24h R	900 M	19-64	716	544	9567	4804
		115 F		589	362	6888	3217
Czech R.[21]	FFQ	3690 M	51-65	721	362	8727	3007
		4223 F		835	411	4867	3007
Croatia[31]	FFQ	183 M	18-30	1711	965	15955	7534
		480 F		1444	740	11982	5720

*The Average Nutrient Requirement (ANR): 800mg/day for females and males

**Graph shows mean intake with 95% confidence intervals

Figure 2. Mean folate intake in micrograms per day (standard deviation) and daily energy intake in kilojoules per day (standard deviation) by country, for males (M) and females (F). Dietary intake method: EFR- estimated food record; 24h R- twenty four hour recall; DH- diet history; FFQ- food frequency questionnaire; HFCS- household food consumption survey. NA: not available.

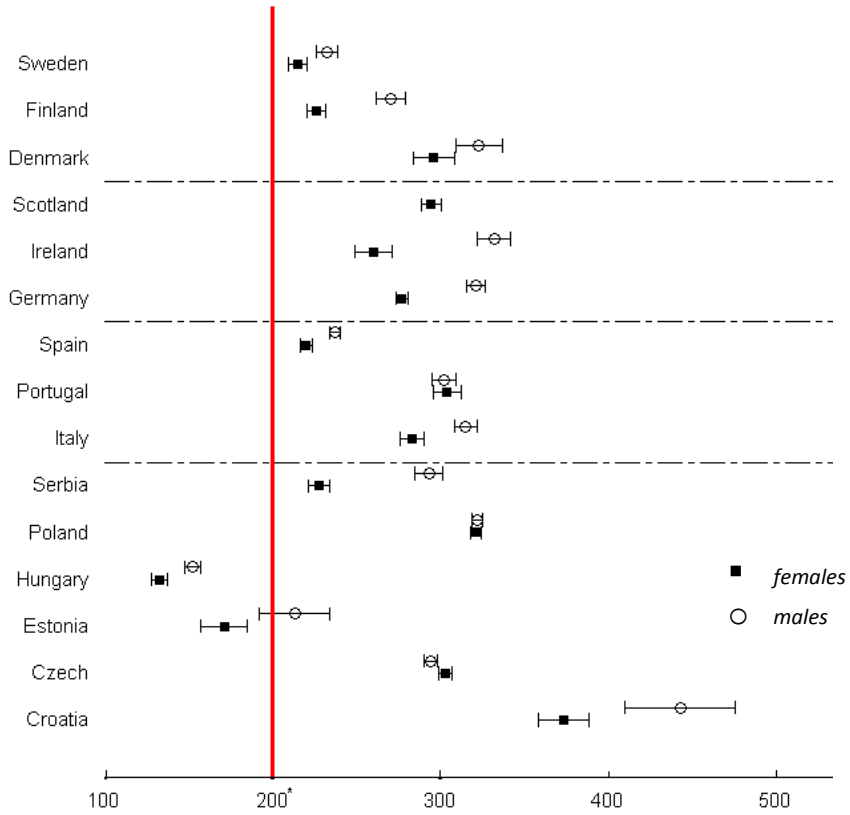


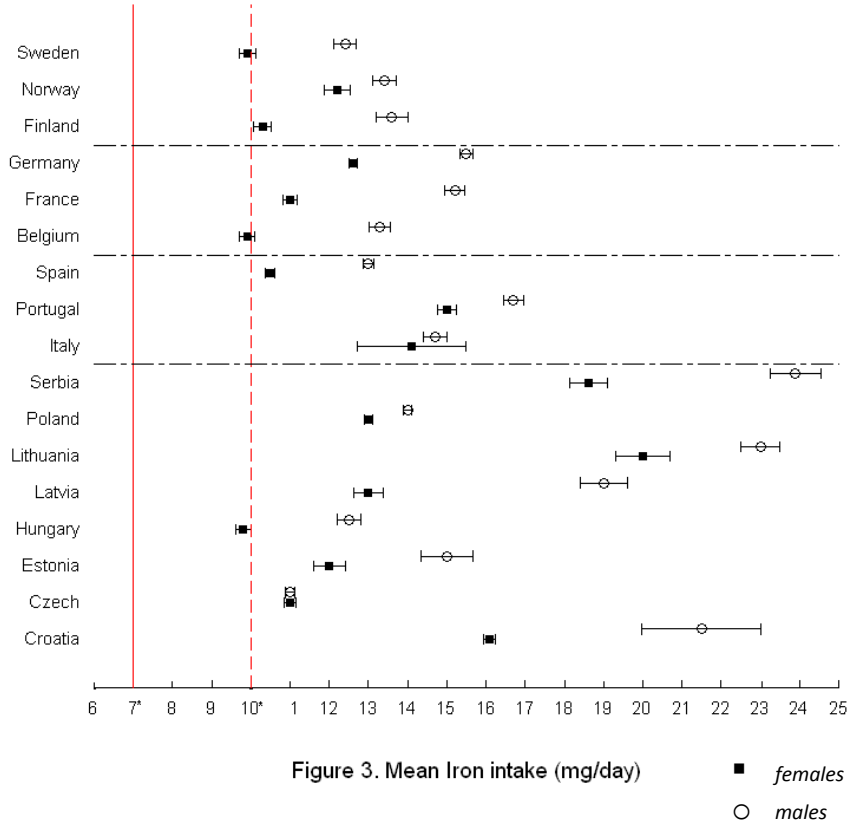
Figure 2. Mean Folate intake (µg/day)

*The Average Nutrient Requirement (ANR): 200µg/day for females and males

**Graph shows mean intake with 95% confidence intervals

Country	Dietary intake method	Number of subjects; sex	Age range (years)	Mean (µg/day)	SD	Energy (kJ/day)	SD
Sweden[16]	EFR	500 M	19-64	232	73	9933	
		500 F		215	65	7841	
Finland[16]	24h R	730 M	19-64	270	120	9265	2960
		846 F		226	88	6804	2028
Denmark[16]	EFR	300 M	19-64	323	120	10638	2910
		300 F		296	111	8232	2209
Scotland[16]	FFQ	898 F	45-54	294	89	7925	
Ireland[16]	EFR	650 M	19-64	332	128	11033	3108
		650 F		260	144	7623	2007
Germany[16]	DH	5000 M	19-64	321	202	11041	4112
		5000 F		277	124	8131	2511
Spain[16]	2x24h R	750 M	19-59	237	45	8925	
		750 F		220	48	7047	
Portugal[16]	FFQ	1200 M	19-64	302	130	9937	2305
		1200 F		304	144	8731	2108
Italy[16]	EFR	700 M	19-64	315	91	10336	1906
		700 F		283	100	8433	1604
Serbia[36]	HFCS	1173 M	30-60	293	148	11415 (M&F)	
		1227 F		228	116		
Poland[56]	FFQ	4815 M	45-69	322	118	9529	2910
		5044 F		321	124	8727	2608
Hungary[69]	24h R	473 M	18-60+	152	53	11734	
Hungary[68]	3x24h R	352 F	18-59	132	47	9227	
Estonia[52]	24h R	126 M	55-65	213	121	9567	4804
		190 F		171	99	6888	3217
Czech R.[57]	FFQ	3690 M	51-65	294	115	8727	3007
		4223 F		303	135	4867	3007
Croatia[37]	FFQ	183 M	18-30	443	226	15955	7534
		480 F		373	168	11982	5720

Figure 3. Mean iron intake in milligrams per day (standard deviation) and daily energy intake in kilojoules per day (standard deviation) by country, for males (M) and females (F). Dietary intake method: EFR- estimated food record; 24h R- twenty four hour recall; DH- diet history; FFQ- food frequency questionnaire; HFCS- household food consumption survey. NA: not available.



*The Average Nutrient Requirement (ANR): 7mg/day for males, 10mg/day for females

**Graph shows mean intake with 95% confidence intervals

Country	Dietary intake method	Number of subjects; sex	Age range (years)	Mean (mg/day)	SD	Energy (kJ/day)	SD
Sweden[16]	EFR	500 M	19-64	12	3	9933	
		500 F		10	2	7841	
Norway[16]	FFQ	1100 M	16-79	13	5	11142 (3914)	
		1100 F		12	6	1936 (645)	
Finland[16]	24h R	730 M	19-64	14	6	9265	2960
		846 F		10	3	6804	2028
Germany[16]	DH	5000 M	19-64	16	6	11041	4112
		5000 F		13	4	8131	2511
France[16]	EFR	954 M	52-68	15	4	NA	
		800 F		11	3		
Belgium[16]	24h R	500 M	19-64	13	3	NA	
		500 F		10	2		
Spain[16]	2x24h R	750 M	19-59	13	2	8925	
		750 F		11	2	7047	
Portugal[16]	FFQ	1200 M	19-64	17	5	9937	2305
		1200 F		15	4	8731	2108
Italy[16]	EFR	700 M	19-64	15	4	10336	1906
		700 F		14	19	8433	1604
Serbia[36]	HFCS	1173 M	30-60	24	11	11415 (M&F)	
		1227 F		19	9		
Poland[56]	FFQ	4815 M	45-69	14	4	9529	2910
		5044 F		13	4	8727	2608
Lithuania[66]	24h R	2606 M	19-64	23	13	10945	4317
		1132 F		20	12	8202	3494
Latvia[66]	24h R	1065 M	19-64	19	10	10848	5023
		1235 F		13	7	7522	3355
Hungary[69]	24h R	473 M	18-60+	13	3	11734	
		706 F		10	3	9227	
Estonia[66]	24h R	900 M	19-64	15	10	9567	4804
		1115 F		12	7	6888	3217
Czech R.[57]	FFQ	3690 M	51-65	11	4	8727	3007
		4223 F		11	5	4867	3007
Croatia[37]	FFQ	183 M	18-30	22	10	15955	7534
		480 F		16	2	11982	5720

Figure 4. Mean vitamin B12 intake in micrograms per day (standard deviation) and daily energy intake in kilojoules per day (standard deviation) by country, for males (M) and females (F). Dietary intake method: EFR- estimated food record; 24h R- twenty four hour recall; WFR- weighed food record; DH- diet history; FFQ- food frequency questionnaire; HFCS- household food consumption survey.
NA: not available.

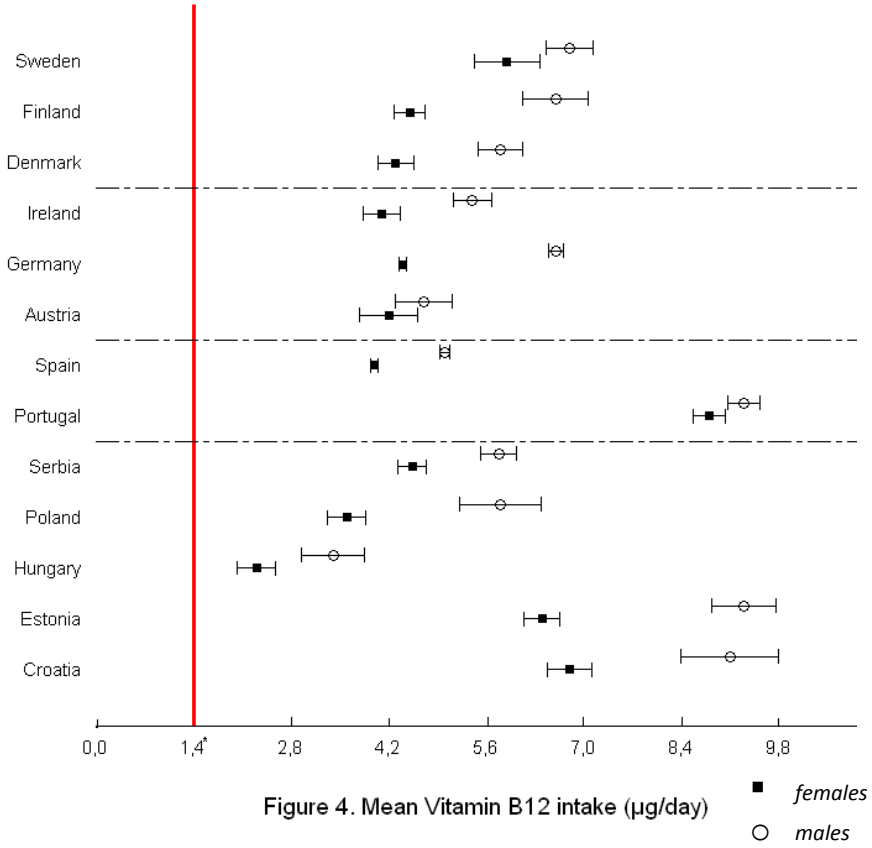


Figure 4. Mean Vitamin B12 intake (µg/day)

*The Average Nutrient Requirement (ANR): 1.4µg/day for females and males

**Graph shows mean intake with 95% confidence intervals

Country	Dietary intake method	Number of subjects; sex	Age range (years)	Mean (mg/day)	SD	Energy (kJ/day)	SD
Sweden[16]	EFR	500 M	19-64	7	4	9933	
		500 F		6	5	7841	
Finland[16]	24h R	730 M	19-64	7	6	9265	2960
		846 F		5	3	6804	2028
Denmark[16]	WFR	400 M	19-64	6	3	10638	2910
		400 F		4	3	8232	2209
Ireland[16]	EFR	700 M	19-64	5	4	11033	3108
		700 F		4	4	7623	2007
Germany[16]	DH	5000 M	19-64	7	4	11041	4112
		5000 F		4	2	8131	2511
Austria[16]	EFR	125 M	>64	5	2	8026	
		125 F		4	2	6921	
Spain[16]	2x24h R	750 M	19-59	5	1	8925	
		750 F		4	1	7047	
Portugal[16]	FFQ	1200 M	19-64	9	4	9937	2305
		1200 F		9	4	8731	2108
Serbia[36]	HFC	1173 M	30-60	6	4	11415 (M&F)	
		1227 F		5	4		
Poland[75]	24h R	3132 M	20-74	6	17	10386	
		3529 F		4	8	7060	
Hungary[52]	24h R	93 M	>65	3	2	10432	2205
		159 F		2	2	8828	1705
Estonia[52]	24h R	126 M	55-65	9	3	9567	4804
		190 F		6	2	6888	3217
Croatia[37]	FFQ	183 M	18-30	9	5	15955	7534
		480 F		7	4	11982	5720

Figure 5. Mean vitamin C intake in milligrams per day (standard deviation) and daily energy intake in kilojoules per day (standard deviation) by country, for males (M) and females (F). Dietary intake method: EFR- estimated food record; 24h R- twenty four hour recall; WFR- weighed food record; DH- diet history; FFQ- food frequency questionnaire; HFCS- household food consumption survey. NA: not available.

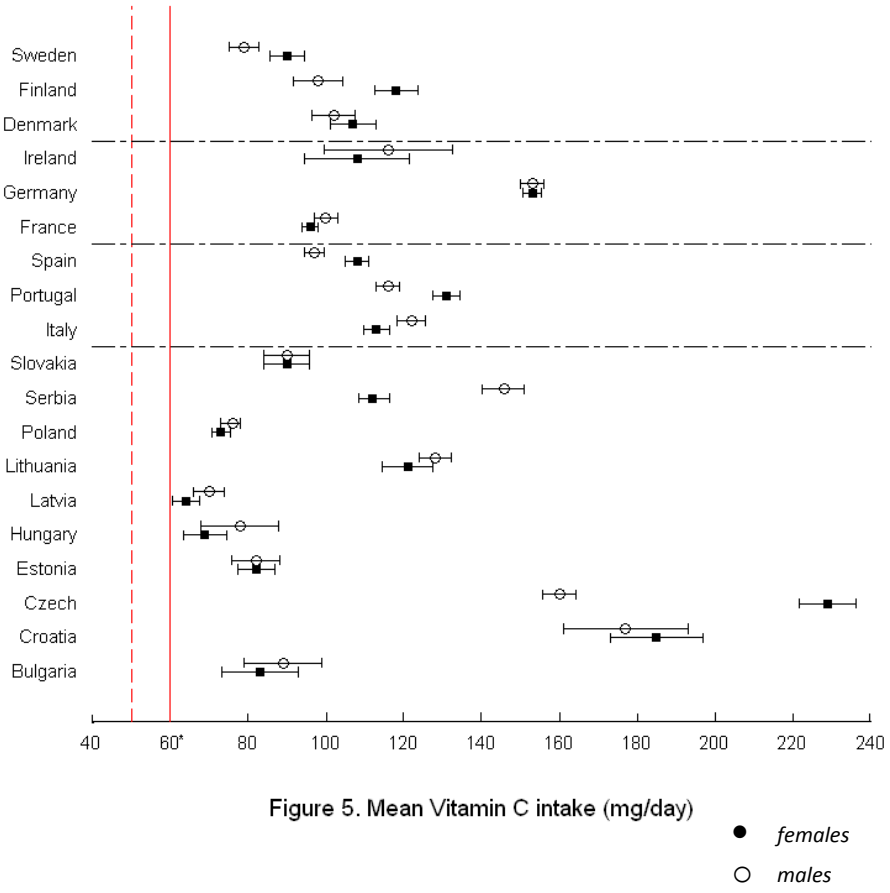


Figure 5. Mean Vitamin C intake (mg/day)

*The Average Nutrient Requirement (ANR): 50mg/day for females, 60mg/day for males

**Graph shows mean intake with 95% confidence intervals

Country	Dietary intake method	Number of subjects; sex	Age range (years)	Mean (mg/day)	SD	Energy (kJ/day)	SD
Sweden[16]	EFR	500 M	19-64	79	45	9933	
		500 F		90	50	7841	
Finland[16]	24h R	730 M	19-64	98	88	9265	2960
		846 F		118	82	6804	2028
Denmark[16]	WFR	400 M	19-64	102	56	10638	2910
		400 F		107	61	8232	2209
Ireland[16]	EFR	700 M	19-64	116	223	11033	3108
		700 F		108	183	7623	2007
Germany[16]	DH	5000 M	19-64	153	106	11041	4112
		5000 F		153	84	8131	2511
France[16]	EFR	954 M	52-68	100	49	NA	
		1369 F		96	43		
Spain[16]	2x24h R	750 M	19-59	830	200	8925	
		750 F		778	170	7047	
Portugal[16]	FFQ	1200 M	19-64	883	354	9937	2305
		1200 F		963	359	8731	2108
Italy[16]	EFR	700 M	19-64	947	309	10336	1906
		700 F		851	264	8433	1604
Slovakia[80]	FFQ	68 M	19-68	90		NA	
		109 F		90			
Serbia[36]	HFC	1173 M	30-60	146	93	11415 (M&F)	
		1227 F		112	71		
Poland[75]	24h R	3132 M	20-74	76	74	10386	
		3529 F		73	75	7060	
Lithuania[66]	24h R	2606 M	19-64	128	108	10945	4317
		1132 F		121	112	8202	3494
Latvia[66]	24h R	1065 M	19-64	70	65	10848	5023
		1235 F		64	63	7522	3355
Hungary[52]	24h R	93 M	>65	78	49	10432	2205
		159 F		69	36	8828	1705
Estonia[66]	24h R	900 M	19-64	82	96	9567	4804
		1115 F		82	81	6888	3217
Czech R.[57]	FFQ	3690 M	51-65	160	135	8727	3007
		4223 F		229	242	4867	3007
Croatia[37]	FFQ	183 M	18-30	177	111	15955	7534
		480 F		185	132	11982	5720
Bulgaria[52]	24h R	186 M	60-75	89	70	10231	4116
		194 F		83	70	8127	2608

Figure 6. Mean vitamin D intake in micrograms per day (standard deviation) and daily energy intake in kilojoules per day (standard deviation) by country, for males (M) and females (F). Dietary intake method: EFR- estimated food record; 24h R- twenty four hour recall; WFR- weighed food record; DH- diet history; FFQ- food frequency questionnaire; HFCS- household food consumption survey. NA: not available.

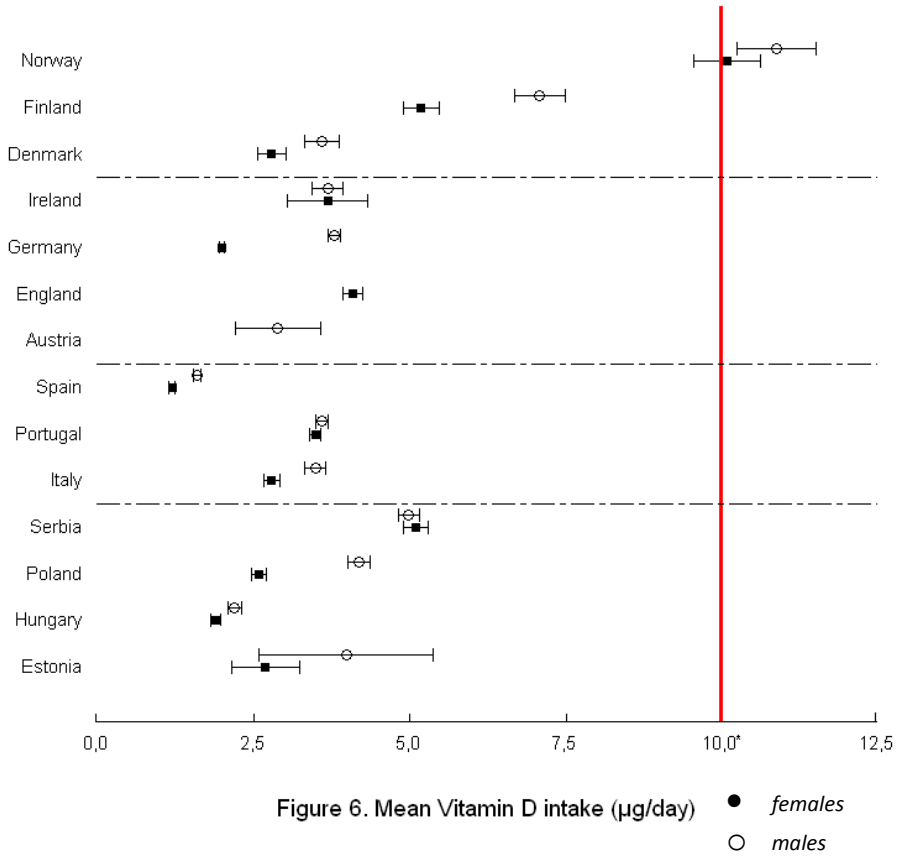


Figure 6. Mean Vitamin D intake (µg/day)

*The Average Nutrient Requirement (ANR): 10µg/day for females and males

**Graph shows mean intake with 95% confidence intervals

Country	Dietary intake method	Number of subjects; sex	Age range (years)	Mean (µg/day)	SD	Energy (kJ/day)	SD
Norway[16]	FFQ	1100 M	16-79	11	11	11142	3914
		1100 F		10	9	1936	645
Finland[16]	24h	730 M	19-64	7	6	9265	2960
		846 F		5	4	6804	2028
Denmark[16]	WFR	400 M	19-64	4	3	10638	2910
		400 F		3	2	8232	2209
Ireland[16]	EFR	700 M	19-64	4	3	11033	3108
		700 F		4	9	7623	2007
Germany[16]	DH	5000 M	19-64	4	4	11041	4112
		5000 F		2	1	8131	2511
Austria[16]	EFR	170 M	>64	3	5	NA	
England[16]	FFQ	898 F	45-54	4	2	7925	2205
Spain[16]	2x24hR	750 M	19-59	2	1	8925	
		750 F		1	1	7047	
Portugal[16]	FFQ	1200 M	19-64	4	2	9937	2305
		1200 F		4	2	8731	2108
Italy[16]	EFR	700 M	19-64	4	2	10336	1906
		700 F		3	2	8433	1604
Serbia[36]	HFC	1173 M	30-60	5	3	11415 (M&F)	
		1227 F		5	3		
Poland[75]	24h R	3132 M	20-74	4	5	10386	
		3529 F		3	4	7060	
Hungary[69]	24h R	473 M	18-60+	2	1	11734	
		706 F		2	1	9227	
Estonia[52]	24h R	126 M	55-65	4	8	9567	4804
		190 F		3	4	6888	3217

Figure 7. Median urinary iodine concentration in micrograms per liter per day by country, in children and adolescents

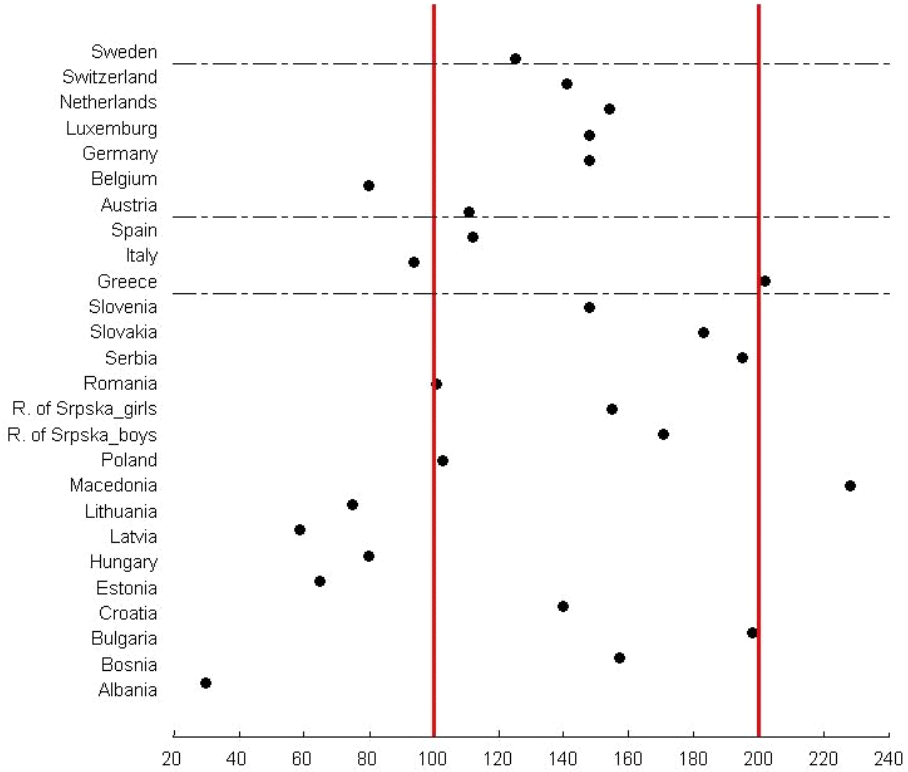


Figure 7. Median urinary Iodine in children and adolescents (µg/L)

*The optimal range for median urinary iodine concentration: 100-199 µg/L

** Source of data: WHO Vitamin and Mineral Nutrition Information System, except for studies from Republic of Srpska[39] and Serbia[80]

Figure 8. Mean haemoglobin (Hb) concentration (with 95% confidence intervals) in gram per liter per day by country, in children and adolescents

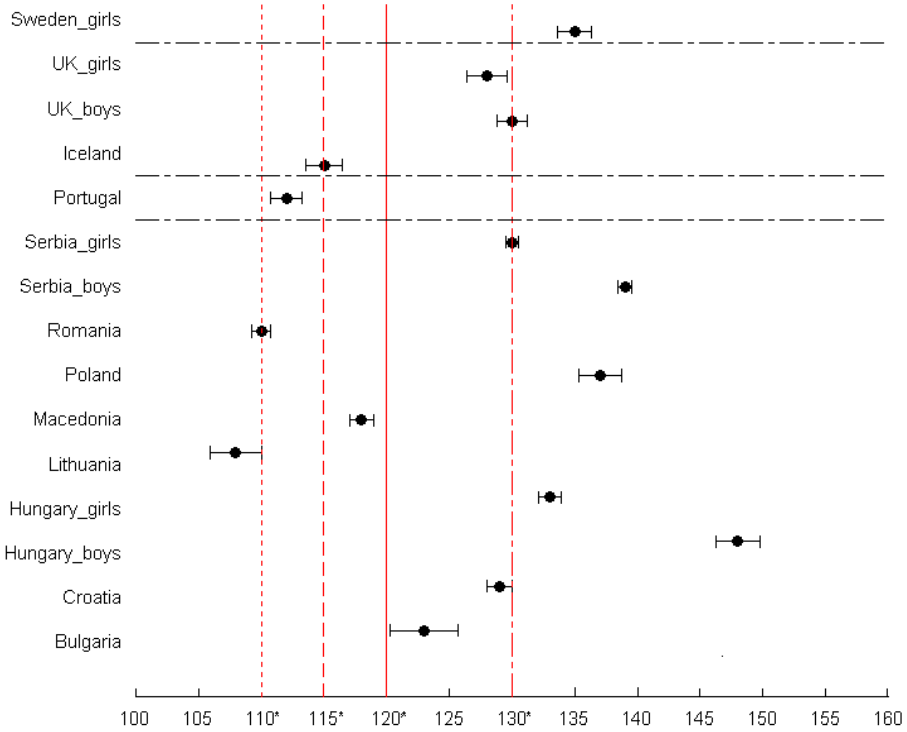


Figure 8. Mean Hb concentrations in children (g/L)

* The age range (years) of the subjects: Bulgaria (2-4y), Croatia (7-8y), Hungary (15-19y), Lithuania (0.5-2y), Macedonia (0.5-5y), Poland (10-13y), Romania (1y), Serbia (15y), Portugal (1y), Iceland (1y), UK (7-10y), Sweden (15-16y)

**The haemoglobin concentrations below which anemia is present: children 0.5-5 years 110 g/L, 5-11 years 115 g/L, 12-14 years 120 g/L; females above 15 years 120 g/L; males above 15 years 130 g/L

*** Source of data: WHO Vitamin and Mineral Nutrition Information System, except for study from Serbia (36)

Table 1 Dietary survey methods used for the present study

Country	Study name	Year of the study	Population group	Number of subjects (M=males, F=females)	Food intake method OR Status measure	Micronutrients included in the study
Albania	Iodine Treatment in Children with Subclinical Hypothyroidism Due to Chronic Iodine Deficiency Decreases Thyrotropin and C-Peptide Concentrations and Improves the Lipid Profile[48]	2009	10+/-2y Children	133	Urinary iodine	Iodine
	Iodine supplementation improves cognition in iodine-deficient school children in Albania: a randomized, controlled, double-blind study[49]	2006	10-12y Children	303	Urinary iodine	Iodine
Bosnia	Thyroid volume and urinary iodine excretion in school children in North-Eastern Bosnia[50]	2008	7-14y Children	513	Urinary iodine	Iodine
Bulgaria	Evaluation of Endemic Goiter Prevalence in Bulgarian School children Results from National Strategies for Prevention and Control of Iodine-Deficiency Disorders[51]	2007	8-15y Boys and Girls	274M; 209F	Urinary iodine	Iodine
	*Aging nutrition[52]	2006	>55y	186M; 194F	24h Recall	Vitamin C
	*National study of urinary Iodine excretion-biomarker of Iodine nutrition[53]	2003	7-11y Children; Pregnant women	809; 355F	Urinary iodine	Iodine
Croatia	Diet quality in Croatian university students: Energy, macronutrient intakes according to gender[37]	2007	18-30y Males and Females	183M; 480F	Food Frequency Questionnaire (FFQ) quantified	Calcium, Copper, Folate, Iron, Selenium, Vitamin B_{12}, Vitamin C, Zinc
	Dietary Habits and Folate Status in Women of Childbearing Age in Croatia[54]	2006	20-30y Females	100F	2x24h Recall; Serum Folate	Folate
	Calcium Intake, Food Sources and Seasonal Variations in Eastern Croatia[55]	2005	18-55y Males and Females	46M; 115F	FFQ	Calcium
	Comparison of dietary habits in the urban and rural Croatian schoolchildren[38]	2004	8-16y Children	315 (urban) 163 (rural)	FFQ quantified	Calcium, Folate, Iron, Selenium, Vitamins B_{12}, C and D, Zinc
	Ultrasound bone measurement in children and adolescents: Correlation with nutrition, puberty, anthropometry, and physical activity[56]	2003	7-10y Boys and girls; 15-17y Boys and girls	120M, 122F; 112M, 147F	Semi quantitative FFQ	Calcium

Table 1 Dietary survey methods used for the present study (continued)

Country	Study name	Year of the study	Population group	Number of subjects (M=males, F=females)	Food intake method OR Status measure	Micronutrients included in the study
Czech Republic	Dietary habits in three Central and Eastern European countries: the HAPIEE study[57]	2009	45-69y Males and Females	3690M; 4223F	FFQ validated	Calcium, Folate, Iron , Vitamin C
	Health behaviors, nutritional status, and anthropometric parameters of Roma and non-Roma mothers and their infants in the Czech Republic[58]	2009	Lactating women	151F	Serum folate	Folate
	Iodine in early pregnancy- is there enough?[59]	2008	17-41y Pregnant women	168F	Urinary iodine	Iodine
	Actual levels of soy phytoestrogens in children correlate with thyroid laboratory parameters[60]	2006	8-15y Boys and girls	129M; 139F	Urinary iodine	Iodine
	Mild hyperhomocysteinaemia is associated with increased aortic stiffness in general population[61]	2006	25-65y Males and Females	126M; 125F	Homocysteine; serum folate; serum vitamin B12	Folate; Vitamin B_{12}
	Genetic Determinants of Folate Status in Central Bohemia[62]	2005	18-65y Males and Females	250M; 261F	Serum folate; serum homocystein; erythrocitefolate; serum vitamin B12	Folate; Vitamin B_{12}
	INAA of Serum Zinc of Inhabitants in Five Regions of the Czech Republic[63]	1999	10y Boys and Girls; 36-49y Males; 36-49y, 50-65y Females	90M, 104F; 118M; 118F, 106F	Serum zinc	Zinc
Estonia	The reference limits and cut-off value for serum soluble transferrin receptors for diagnosing iron deficiency in infants[64]	2008	9-12month	146	Serum transferrin	Iron
	Prevalence and causes of iron deficiency anemias in infants aged9 to 12 months in Estonia[65]	2007	9-12month	171	Haemoglobin; transferrin; ferritin	Iron
	*Aging nutrition[52]	2006	>55y	126M; 190F	24h Recall	Folate, Vit. B_{12}, C ,D
	*Nutrition and lifestyle in the Baltic republics - summary report[66]	1999	19-64y Males and Females	900M; 1115F	24h Recall	Calcium, Iron, Folate

Table 1 Dietary survey methods used for the present study (continued)

Country	Study name	Year of the study	Population group	Number of subjects (M=males, F=females)	Food intake method OR Status measure	Micronutrients included in the study
Hungary	Dietary habits of schoolchildren: representative survey in metropolitan elementary schools: Part 2[67]	2007	11-14y Boys and girls	124M; 111F	3days Diet record	Calcium, Copper, Folate, Iron, Vitamin B_{12}, Vitamin C, Vitamin D, Zinc
	*Report on Periconceptional Folic Acid Supplementation for Hungary[68]	2007	18-59y Females	352F	3x24h Recall	Folate
	*Aging nutrition[52]	2006	>55y	93M; 159F	24h Recall	Vitamin B_{12}, Vitamin C
	Dietary survey in Hungary, 2003-2004[69]	2005	18- 60+y, Males and Females	473M; 706F	24h Recall	Calcium, Copper, Folate, Iron, Vitamin B_{12}, Vitamin C, Vitamin D, Zinc
	Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in community dwelling postmenopausal Hungarian women[70]	2003	41-91y Females	319F	FFQ validated	Calcium
	*Nutrition survey of the Hungarian population in a randomized trial between 1992-1994[71]	1996	18-34y, 35-59y Males 18-34y, 35-54y, >55y Females	338M, 730M; 343F, 938F, 105F	24h Recall/ Haemoglobin; serum folate; serum zinc	Calcium, Copper, Folate, Iron, Vitamin C, Zinc
Latvia	*Nutrition and lifestyle in the Baltic republics - summary report[66]	1999	19-64y Males and Females	1065M; 1235F	24h Recall	Calcium, Iron, Vitamin C
Lithuania	Prognostic value of reticulocyte haemoglobin content to diagnose iron deficiency in 6–24-month-old children[72]	2008	6-24 month	180	Haemoglobin	Iron
	*Nutrition and lifestyle in the Baltic republics - summary report[66]	1999	20-65y Males and Females	2606M; 1132F	24h Recall	Calcium, Iron, Vitamin C

Table 1 Dietary survey methods used for the present study (continued)

Country	Study name	Year of the study	Population group	Number of subjects (M=males, F=females)	Food intake method OR Status measure	Micronutrients included in the study
Poland	Iodine excretion with urine and thyrotrophic hormone concentration in normal and complicated pregnancies in the industrial region of iodine deficiency[73]	2006	28.2+/-6.4y Pregnant women	104F	Urinary Iodine	Iodine
	*Food consumption of low income groups in Poland and Belgium[74]	2007	19-59y Males and Females	240M&F	24h Recall and FFQ	Calcium, Copper, Iron, vitB_{12}, Vitamin C, Zinc
	*Wartość energetyczna i odżywcza diety dorosłych mieszkańców Polski. Wyniki programu WOBASZ (unpublished results, obtained by personal correspondence with dr ElzbietaSygnowska, Nov10th, 2010)[75]	2010	20-74y Males and Females	3132M; 3529F	24h Recall/ Homocystein	Calcium, Copper, Folate, Iron, Vitamin B_{12}, Vitamin C, Vitamin D, Zinc
	Dietary habits in three Central and Eastern European countries: the HAPIEE study[56]	2009	45-69y Males and Females	4815M; 5044F	FFQ validated	Calcium, Folate, Iron, Vitamin C
	Polish Food Consumption and Anthropometric Survey 2000: Comparison between household budget survey and 24-hour recall data in a nationally representative sample of Polish households [76]	2005	All age groups (0-96y)	3716M&F	24h Recall	Calcium, Copper, Iron, Vitamin C, Zinc
	Effectiveness of the iodine prophylaxis model adopted in Poland[77]	2008	6-12y Boys and girls	1450M;1563F	Urinary Iodine	Iodine
	Increased prevalence of hyperthyroidism as an early and transient side-effect of implementing iodine prophylaxis[78]	2007	>16 y Males and Females	491M; 933F	Urinary Iodine	Iodine
	Comparative analysis of zinc status, food products' frequency intake and food habits of 11-year-old healthy children[79]	2002	11y Children	157	Serum Zinc	Zinc

Table 1 Dietary survey methods used for the present study (continued)

Country	Study name	Year of the study	Population group	Number of subjects (M=males, F=females)	Food intake method OR Status measure	Micronutrients included in the study
Serbia	*National survey of the biological impact of universal salt iodinization 2007. Institute of Public Health of Serbia[80]	2007	6-14y Children	1745	Urinary Iodine	Iodine
	*Yugoslav study of atherosclerosis precursors in schoolchildren in Serbia from 1998-2003[36]	2003	15y Boys and Girls;	1984M; 1859F;	Haemoglobin	Iron
			10-15; 30-59; 60-75 Males and Females	1225M, 1173M,147M; 1228F, 1227F, 246F	Household food consumption survey (7 days record)	Calcium, Copper, Folate, Iodine, Iron, Vitamin B_{12}, Vitamin C, Vitamin D, Zinc
Slovakia	Vitamin C protective plasma value[81]	2007	19-68y Males and females	78M; 109F	FFQ	Vitamin C
The Republic of Srpska	The Republic of Srpska Iodine Deficiency Survey 2006[39]	2008	7-10y Boys and Girls	599M; 592F	Urinary Iodine	Iodine

* Grey literature

** For all studies: supplements are not included in the assessment

Table 2 Energy and micronutrient intake (copper, iodine, selenium and zinc) by CEE country in males (M) and females (F)- means and standard deviations (SD)

Country	Dietary Intake method	Number of subjects; sex	Age range (years)	Mean	SD	Energy (SD) (kJ/day)
Copper (mg/day)						
Croatia[37]	FFQ	183 M	18-30	2	1	15955 (7534)
		480 F		2	1	11982 (5720)
Hungary[69]	24h R	473 M	18-60+	1	1	11734
		706 F		1	0.5	9227
Poland[75]	24h R	3132 M	20-74	1	0.5	10386
		3529 F		1	0.4	7060
Serbia[36]	HFCS	1173 M	30-60	5	7	11415 (M&F)
		1227 F		4	5	11415 (M&F)
Iodine (µg/day)						
Serbia[36]	HFCS	1173 M	30-60	184	139	11415 (M&F)
		1227 F		144	112	11415 (M&F)
Selenium (µg/day)						
Croatia[37]	FFQ	183 M	18-30	215	106	15955 (7534)
		480 F		141	65	11982 (5720)
Zinc (mg/day)						
Croatia[37]	FFQ	183 M	18-30	18	9	15955 (7534)
		480 F		13	6	11982 (5720)
Hungary[69]	24h R	473 M	18-60+	10	3	11734
		706 F		8	2	9227
Serbia[36]	HFCS	1173 M	30-60	15	9	11415 (M&F)
		1227 F		12	8	11415 (M&F)

* Abbreviations: FFQ (Food Frequency Questionnaire); 24h R (24 hour recall); HFCS (Household food consumption survey)

** Reference values used: ANR (Nordic Nutritional Recommendations (NNR) and Institute of Medicine of the National Academies, Food and Nutrition Board, USA (IOM)) for adults per day: copper 0.7 mg; iodine 100µg; selenium 30µg for females, 35µg for males; zinc 5 mg for females, 6mg for males.

Table 3 Energy and micronutrient intake by CEE country in boys and girls: means and standard deviations (SD), dietary intake method, age range (years) and number of subjects (n).

	Ca (mg)	SD	Co (mg)	SD	Folate (µg)	SD	Iodine (µg)	SD	Fe (mg)	SD	Se (µg)	SD	Vit. B12 (µg)	SD	Vit. C (mg)	SD	Vit. D (µg)	SD	Zn (mg)	SD	Energy (kJ)	SD
Croatia[38], FFQ; (8-16y)																						
Boys and Girls (n=315)	927	306			162	63			19	7	22	8	5	2	135	68	2	1	12	4	9017	2604
Hungary[67], FFQ; (11-14y)																						
Boys (n=124)	798	288	1	0.3	151	58			11	3			3	2	99	79	2	1	9	2	10453	1902
Girls (n=111)	696	238	1	0.3	140	65			10	3			2	1	94	70	2	1	7	2	9219	1503
Serbia[36], HFCS; (10-15y)																						
Boys (n=1225)	1123	499	4	7	230	117	147	112	19	9			5	4	114	72	4	3	12	8	11533	
Girls (n=1228)	958	428	3	4	196	97	124	94	16	7			4	3	97	60	3	2	10	7	9769	

* Abbreviations: y (years); n (number); FFQ (Food Frequency Questionnaire); HFCS (Household food consumption survey)

** Reference values used: Average nutrient requirement (IOM) for children per day: calcium (Ca) 1100 mg; copper (Co) 0.5 mg; folate 250 µg; iodine (I) 73µg; iron (Fe)6 mg; selenium (Se)35 µg; vitamin B12 1.5 µg; vitamin C 39 mg; vitamin D 10 µg; zinc (Zn)7 mg.

Table 4 Micronutrient status by country: folate, vitamin B12 and zinc in adults and children, iodine and iron in adults- medians or means and standard deviations (SD)

Country	Number of subjects; sex	Age range (years)	Means of status marker level (or median where indicated)	SD
Serum Folate (Mean(SD)/Median) (nmol/L)				
Croatia[54]	100 F	20-30	23	9
Czech R. (Lactation)[58]	227 F	20-35	20 (median) Polish; 7(median) Roma	
Czech R.[61]	126 M	25-65	14	0.4
	125 F		14	0.4
Czech R.[62]	250 M	18-65	14 (median)	
	261 F	18-64	14 (median)	
Hungary[71]	1173 M	>18	20	9
	1386 F		21	10
Urinary Iodine (Median) (μg/L)				
Poland[78]	491 M	>16	121	
	933 F		106	
Romania (WHO database)	1387 F	15-46	51	
Czech R. (WHO database)	254 M&F	18-66	114	
Bulgaria (pregnancy)[53]	355 F	26+/-5	165	
Czech R. (pregnancy)[59]	168 F	17-41	367	
Serbia (pregnancy)[80]	347 F	20-35	158	
Haemoglobin (Mean) (SD) (g/L)				
Serbia[36]	544 M	20-21	148	10
	725 F		128	11
Macedonia (WHO database)	1018 F	15-46	134	14
Serum Vitamin B12 (Mean(SD)/Median) (pmol/L)				
Czech R.[61]	126 M	25-65	239	7
	125 F		239	7
Czech[62]	250 M	18-65	278 (median)	
	261 F		278 (median)	
Serum Zinc (Mean(SD) (μmol/L)				
Czech R.[63]	118 M	36-49	13	3
	118 F		13	3
Hungary[71]	1173 M	>18	17	3
	1386 F		17	3
Czech R.[63]	90 boys	10	15	3
	194 girls		14	3
Poland[79]	157 boys and girls	11	12	2

Cut off values as proposed in references (20-27) and (30-33): serum folate 10 nmol/L for adults; urinary iodine 100 μg/L for adults, 150 μg/L for pregnant women; haemoglobin 130 g/L for males, 120 g/L for females, 110 g/L for pregnant women; serum vitamin B12 150 pmol/L for adults; serum zinc 10 μmol/L for children and females, 10.7 μmol/L for males.

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Chapter 3

Socioeconomic determinants of micronutrient intake and status in Europe: a systematic review

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Abstract

Objective: Provide the evidence base for targeted nutrition policies to reduce the risk of micronutrient diet-related diseases among disadvantaged populations in Europe, by focusing on: folate, vitamin B12, iron, zinc, iodine for intake and status; vitamin C, vitamin D, calcium, selenium, copper for intake.

Design: Medline and Embase databases were searched to collect original studies that: i) were published from 1990 to 2011, ii) involved >100 subjects, iii) dietary intake was assessed on individual level and/or iv) included best practice biomarkers reflecting micronutrient status. We estimated relative differences in mean micronutrient intake and/or status between the lowest and highest socio-economic group to: i) evaluate variation in intake and status between SES groups, and ii) report on data availability.

Setting: Europe.

Subjects: Children, adults and elderly.

Results: Data from 18 publications originating primarily from Western Europe showed that there is a positive association between SES indicators and micronutrient intake and/or status. The largest differences were observed for intake of vitamin C in 11 out of 12 studies (5- 47%) and for vitamin D in total of 4 studies (4-31%).

Conclusions: Observed positive association between micronutrient intake and SES should complement existing evidence on socio-economic inequalities in diet-related diseases among disadvantaged populations in Europe. These findings could provide clues for further research and have implications for public health policy aimed at improving the intake of micronutrients and diet related diseases.

Introduction:

Many studies have shown that socioeconomic status (SES), conventionally measured as education, occupation or income, contributes to inequalities in health[1,2]. Those in lower socioeconomic groups have a higher incidence of premature death and suffer more from heart diseases and some forms of cancer, than those in more socioeconomic advantageous groups[2]. One important risk factor for these diseases may be the quality of the diet[1].

Adequate consumption of micronutrients is one of the key elements of diet quality. Within the EURRECA Network of Excellence (www.eurreca.org) there was shown that the prevalence of micronutrient inadequacies in Europe ranged from 11-30% for copper, folate, selenium, iodine, vitamin B12 and vitamin C in adults[3]. A recent study has shown that diet cost mediates the relation between socioeconomic position (education and income) and diet quality[4]. Since energy-dense and nutrient-poor diets are cheap and more consumed by those with limited economic means and lower educational level[1, 2], it is likely that SES could be associated with variation in micronutrient intake and status. Currently, a knowledge gap exists as to an overview of public health prioritized micronutrients intake and status by SES groups. Available data that stem from methodologically comparable nutritional surveillances have studied intake and/or status of a few micronutrients within limited life stages, whereas literature reviews have predominantly focussed on the relationship between SES and intake of macronutrients or specific food groups and food patterns (e.g. fat, fruits and vegetables) [1, 5-12]. Furthermore, the studies were performed primarily in adults, while children and elderly might be more vulnerable to insufficiencies. The aim of this study is to provide the evidence base for targeted nutrition policies to reduce the risk of micronutrient diet-related diseases among disadvantaged populations in Europe, by focusing on intake and status of folate, vitamin B12, iron, zinc and iodine, and intake of vitamin C, vitamin D, calcium, selenium and copper.We carried out a systematic review on socioeconomic differences in micronutrient intake and status in adults, elderly and children in Europe using education, occupation and income as SES indicators. In addition, we reported on availability of the data that addressed our research question.

Methods

Literature search and data extraction

Prioritization of the micronutrients under study was evidence based[13] and accounted for: (A) the amount of new scientific evidence, particularly from randomized controlled trials; (B) the public health relevance of micronutrients; (C) variations in current micronutrient recommendations for folate, vitamin B_{12}, iron, zinc, and iodine for intake and status, and for vitamin C, vitamin D, calcium,

selenium and copper for intake. Socio- economic indicators of interest were educational level, occupational status and income as these are the most conventionally used[14].

A systematic literature search (SLR) was carried out in Medline and Embase databases to collect studies published from January 1990 up to November 2011 that were performed in apparently healthy populations of all life stages in Europe. Both databases were searched using database-specific indexing terms for dietary intake and/or nutritional status of at least one of the micronutrients under study, SES (education, occupation or income) and Europe: [micronutrients (listed) OR biomarkers of status (listed)] AND general intake/status terms (diet/ or eating/nutritional support/ or dietary supplements/ or food, fortified/ or deficiency diseases/ nutritional requirements/ or nutritional status/ or nutritive value/ or nutrition assessment/ or diet records) AND socioeconomic status terms (socioeconomic factors/ or poverty/ or social class/ or educational status/ or employment/ or unemployment/ or income/ or occupations/ or social conditions) - in title and abstract AND country (European countries listed). The search strategy can be obtained from the authors upon request.

Studies were considered eligible if they reported on micronutrient intake and/or status across different levels of education, occupation or income. For the final analysis, we used the data where dietary intake was assessed at the individual level and reported as mean intake. Nutritional status was reflected by the level of status markers defined as best practice biomarkers within the context of EURRECA[15, 16].

The search results were screened on the basis of title and abstract by two independent reviewers. For articles that seemed to comply with the study criteria, full texts were obtained and checked for relevance by two independent reviewers. When necessary, the discrepancies in abstract and study selection were discussed, and further processing on inclusion or exclusion was made based on consensus.

Study characteristics (country, life stage and number of subjects, sampling procedure, descriptors of SES) and levels of micronutrient intake/status (mean, standard deviations and/or standard errors) and energy consumption were extracted into a database.

Data analysis

We calculated the relative difference (%) in intake and status between the lowest and highest SES groups as a percentage of the value of the highest SES group, for males and females separately unless the paper only reported data for both genders together.

In addition, if studies reported a standard deviation (SD) or standard error (SE), 95% confidence intervals (CI) of the mean intakes/status for SES groups were calculated. T-test was performed to evaluate if the difference in intake/status between the lowest and highest SES groups was statistically significant (the level of statistical significance was set at $p < .05$).

Means and 95% confidence intervals (when SD/SE were available) of intake and status of the lowest and highest SES were plotted per micronutrient for males and females separately. Where the results of t-test showed statistically significant difference in intake/status between the lowest and highest SES groups, study was marked with asterisk (*) (figure 1- 10).

If more than one SES indicator was available in one study, we showed the level of intake/status for one indicator only, i.e. education was given the preference over occupation, and the latter was prioritized over income.

Results

The systematic search in Medline and Embase resulted in 8460 abstracts in total. After screening on the basis of title and abstract, 230 potentially relevant references were identified: 132 on intake and 98 on status. Retrieval of the full papers and their examination disqualified 212 articles mainly because they did not include an internal comparison of intake and/or status between different SES groups, e.g. manual workers vs. non-manual in the same population group. The remaining eighteen studies included ten studies on intake, three studies on status and one on intake and status in adults, plus four studies on intake in children. Not a single study on status in children was found.

Table 1 gives an overview of data availability per country and study characteristics.

Most data available were for adults, studying the relationship between vitamin C or calcium intake and education, occupation or income. No single study was found on intake of copper in adults. Data on biochemical indices of status were very limited and available for adults only: for iron and zinc only two studies were found per micronutrient, and for iodine status one study was found. The studies included for analysis were performed either in Western Europe or Scandinavia with the exception of one study from Eastern Europe[17]. Figures 1-10 show the mean/median intake and status level (with 95% confidence intervals of the mean where available) in lowest and highest SES groups in adults/elderly and children. Following each figure, the relative differences between SES groups for intake/status per micronutrient are presented.

Calcium (figure 1)

N^{o} of identified studies: 10 studies on intake

As from six studies on calcium intake in adults, the mean calcium intake in low SES groups was lower than in high SES groups except for males from Spain[20] and males[25] and females [24, 25] from the Netherlands (see figure 1). The relative difference ranged from 2% in Swiss females[18] to 14% in UK elderly[27]. Results on children were more heterogeneous. Higher calcium intake in low SES as compared to high SES groups was found in Belgian boys[28] and in Turkey[17]: relative differences were

1% and 19-54%, respectively. Belgian girls and the children from Spain[29] and the UK[33] had higher intake in the high SES groups: the relative difference ranged from 3-29%. Statistically significant differences in calcium intake between SES groups were observed in Irish females[23] and Belgian girls[28] ($p < .01$).

Vitamin C (figure 2)

N° of identified studies: 12 studies on intake

Eight studies on vitamin C intake in adults reported lower mean intake in low SES groups in comparison to high SES, except in studies in Dutch females[24, 25] and Spanish males[20] where relative differences ranged from 3-8%. In other studies where higher intake was found in high SES in comparison to low SES, the relative difference was the lowest in Dutch males[25] (5%), whereas in the other six studies[20, 21, 25, 26, 2, 27] it ranged from 10-48%, the largest difference found in UK[27] for intake by income.

Four studies on vitamin C intake in children showed lower mean intake in low SES groups in comparison to high SES, except in Belgian boys[28] where the relative difference between SES groups was 12%. In other studies where lower mean intake in low SES groups in comparison to high SES was reported, relative differences ranged from 6% in Turkish boys[18] up to 33% for Spanish children[29].

Statistically significant differences between SES groups were found in studies from Finland[21], Ireland[23] and Scotland[26] for adults and in the study from Spain[29] for children ($p < .01$).

Iron (figure 3)

N° of identified studies: 9 studies on intake, 2 studies on status

Four[20, 23, 24, 27] out of five studies on iron intake in adults reported lower intake in low SES than in high SES groups (relative differences ranged 1-12%), whereas one study from Spain[21] reported no difference between SES groups. In children, reported intake was consistently lower in low SES than in high SES (relative differences ranged 2-14%).

Data on iron status was available only for adults and showed higher values in low SES than in high SES: relative differences were 2 and 7% for Spain[21] and Norway[32], respectively.

Significant differences in intake/status between SES groups were found in Dutch[24] and Irish[23] females and Belgian girls[28] ($p < .01$).

Folate, vitamin B_{12}, vitamin D, selenium, iodine, zinc, copper (figures 4-9)

N^{o} of identified studies: folate, vitamin B_{12} and vitamin D= 4 studies on intake per each micronutrient; selenium= 2 studies on intake; iodine= 3 studies on intake, 1 study on status; zinc= 4 studies on intake, 2 studies on status; copper= 1 study on intake

The two available studies on folate intake in adults[23, 27] and two for children[29, 33] reported lower intake in the low as compared to the high SES group (relative differences ranged 7-22%). The Irish study in adults and Spanish study in children, both studying folate intake by educational level reported significant differences between SES groups ($p < .01$).

For vitamin B_{12} four studies were found (see Figure 5). The two studies from UK for elderly[27] and children[33] showed higher intake in the high than in low SES group and with the relative difference in intake of 6-21%. In contrast, the study in Irish adults[23] and the study in Spanish children[29] found higher mean intakes in low SES groups than in high SES: relative differences were 8-29% and 2%, respectively. Difference in intake by education between SES groups in Irish study was statistically significantly different ($p < .01$).

Results for vitamin D that come from four studies showed consistently lower intakes for the low SES group (see Figure 6). In adults, relative differences were more apparent in Switzerland[18] (13-31%) than in Ireland[23] (4-7%). In children, relative differences in intake between high and low SES groups were 4% in UK[33] and 30% in Spain[29], with an observed statistically significant difference in the latter study ($p < .05$).

Data on selenium, which were reported in one study among Irish adults[23] and one study in UK children[33], showed a slightly lower mean intake in the low SES group (relative differences 2-5%).

The only study on iodine intake in adults showed no difference in intake in males and a slightly lower intake (relative difference 4%) in the low SES group in females in France[22]. For children, two studies were found and they reported lower intake in the low SES groups than in high SES: relative differences were 4 and 11% for UK[33] and Spain[29], respectively. Data on iodine status were identified only for Spanish adults[30]: the median urinary iodine level in the low SES group was lower than in the high SES group; the relative difference between the two SES groups was 37%.

Three[19, 29, 33] out of four studies on intake of zinc for adults, elderly and children showed somewhat lower intake in low than in high SES groups (relative difference ~3%). The Irish study[23] showed inconsistent results on variation in intake between SES groups, depending on gender and SES indicator. Zinc status data was available only for adults: two studies[19, 31] that were found showed 2-5% higher serum zinc level in the low SES group in comparison to high SES.

The only study on intake of copper was in children in the UK[33]; the intake was higher in low SES group than in high SES (relative difference was 6%).

Of the 18 studies, 10 studies used education as an indicator of SES, 3 applied income and 2 occupational criteria; 3 studies used more than one indicator.

Reviewing the results of the different indicators showed that education (especially in females) and income were associated with the largest variations in intake.

Discussion

This is the first systematic review addressing differences in levels of micronutrient intake and status between low and high socio-economic groups in European children, adults and elderly using education, occupation and income as indicators of socioeconomic status. The results support our hypothesis that social variations are associated with differences in intake and status of certain micronutrients.

This review included all age groups and differences in intake/status were studied separately for each gender where applicable. The subjects were recruited either by stratified random sampling procedure or were representative of the national population, and as such can be considered as exemplary for their countries (Table 1). As one of the outcomes of the systematic review process is observed diversity in study characteristics, there are several aspects to consider in the interpretation of the results. First, the studies differed in the exact categorization and number of groups used for the three SES indicators: for education studies defined 2- 4 groups, for occupation 2-3, and for income 2-4 groups were specified. The size of the absolute and relative differences may be affected by the exact definition of the low and high SES group. Being aware of the inherent differences among studies and after their careful investigating, we have made the decision to integrate the results by taking into account both those differences and the same methodological backbone across studies, that is, a variation between SES groups. The comparisons are made between only two groups, by choosing extreme groups or broad groups[14]. Thus, comparing broad SES groups rather than the outer groups in society may result in an underestimation of existing differences. Another aspect to consider is the impact of the dietary intake methods on observed differences in intake. The dietary intake methods used in the identified studies were heterogeneous: food frequency questionnaire, 24 hour recall, food record; also, the intake from supplements was included only in three studies [27, 30, 33] , all of which could contribute to variation in reported mean nutrient intakes. These diverse methods may vary in the extent to which they reflect true usual intake of micronutrients as some of these methods, e.g. non validated food frequency questionnaires, diet recorded for less than 3 days are generally considered inappropriate to estimate population's usual dietary intake. This is of importance when estimating the prevalence of inadequacy (comparison of absolute mean intake/status levels with reference values).On the other hand, this is less significant for the aim of the present study, i.e. for observing the variation between SES groups per

study as we focused on differences within studies. However, as mentioned in certain publications on socio-economic differences in food consumption, we acknowledge the potential of misreporting of micronutrient intake for subjects with higher educational levels as they may be more conscious of nutrition and health issues and possibly exaggerate the consumption of healthy foods, e.g. fruit and vegetables, thus giving rise to elevated mean vitamin and mineral intake levels[7, 34]. The issue of reporting errors does not affect the results on micronutrient status; therefore, the information on iron, zinc and iodine status strengthens the evidence from the observed differences between intake in low and high SES.

Results of systematic literature search showed that the studies on micronutrient intake and/or status associated with SES indicators for children from all European countries and for children, adults and elderly from Central and Eastern Europe (CEE) are very limited. For example, our review included the data for Western European populations with the exception of one study on children from Turkey[17]. Inclusion of studies from other parts of Europe such as CEE is needed to draw a pan-European conclusion on variations in micronutrient intake/status between groups of different education, occupation and income levels. It is disputable whether similar results would be found for CEE because of the widely divergent socioeconomic position within these countries: larger social inequalities, diversity in food costs and availability etc. A recent publication that compared micronutrient intake/status in CEE with other European regions showed no striking differences, with an exception for calcium[35]. More research is however needed to fill this gap of available evidence. An explanation for the limited studies we identified throughout Europe is that we focused on electronic databases Medline and Embase only. It is of particular importance for CEE, as it is shown that reliable nutritional data from these countries can be found as grey literature: country reports for governmental or academic institutions, thesis and dissertations[35]. The inclusion was, however, not feasible due to the large number of languages to cover. Nevertheless, it is apparent that even though the relationship between socio-economic position and diet has been notably studied in the last few decades, current evidence from key databases is still lacking data on SES related micronutrient intake and/or status for many European countries and particularly for children and elderly.

Existing reviews in Europe have not addressed the association between multiple socio-economic indicators and intake and/or status of more than one micronutrient. The largest study[36] to date which described intake of five water-soluble vitamins across several European countries reported the association between education and intake: it was positive for the intake of riboflavin, vitamin C, vitamin B6 (in women) and no clear conclusion for vitamin B_{12}. These results are in accordance with the findings for vitamins B_{12} and C from the present study.

Still, there is a body of evidence on disparities in food habits in association with SES indicators. A review on consumption of fruit and vegetables in 15 European countries reported lower consumption

in low SES in comparison to high SES groups[9]; the same was observed in 9 European countries for the consumption of cheese, but not for milk[10]. These findings support the results on vitamin C and calcium from our study. To estimate if similar results could be found for other vitamins and minerals under study, we examined the studies evaluating the variation in intake of nutrient-rich healthy foods in groups with different socioeconomic backgrounds. Likewise, whole grains, lean meats, fish, low-fat dairy products, and fresh vegetables and fruit were found to be preferably consumed by high SES groups[1]. The most recent study on the relationship between SES and frequency of consumption of seven predefined healthy foods (consumption of fruit, vegetables, wholegrain bread, vegetable-fat spread, vegetable cooking fat, low-fat milk and low-fat cheese) in CEE and Western Europe reported healthier food choices among those with higher education, occupational position and fewer economic difficulties, as well as heterogeneity in the association between SES and healthy food habits across countries[6]. This study suggests that the differences are the most apparent in SES groups of different educational background, especially in females. On the other hand, Table 1 shows that for both intake and status for all age groups, it is education that is the most studied indicator: 10 studies on education, 2 studies on education and occupation, 1 study on education and income, 2 studies on occupation and 3 on income. Where studies reported on intake by two indicators[18, 21, 23], the relative differences between SES groups observed for education were confirmed for occupation[18, 23] and income[21], but the variation for the latter two indicators was lower than for intake by education.

More pronounced effect of education on variation in intake/status in comparison to occupation and income may be due to the fact that the level of education is considered to remain rather stable through the life course[37]. Furthermore, the education attained in early life is not only a determinant of occupation and income, but also affects the ability to understand and implement dietary guidance messages and risk-reducing dietary behaviors[38]. Despite that the studies in this review used different procedures in assessing SES, most methods have the same underlying principle: difference in micronutrient intake/status between SES groups. To the extent that similar principles are addressed indeed, the heterogeneity of results between different studies might be a reflection of these methodological shortcomings, but as long as no clear gold standard is available, the pooling of results in the way we have done is the best possible option. On the other hand, the presence of dissimilarities in SES categorization among studies can have advantages by increasing the generalization of the conclusion[39] that there is observed positive association between, in most cases, all SES indicators under study and micronutrient intake and/or status.

To support policy makers to develop targeted nutritional policies to reduce the risk of diet-related diseases among disadvantaged populations in Europe, a comprehensive overview of nutritional intake and status across different socioeconomic strata is needed. However, this review showed that contrary to the large body of evidence on socio-economic inequalities in health, very little data on socio-economic differences in micronutrient intake and status is available in scientific literature. This applies

to the whole of Europe and for all age groups, but especially to CEE countries and to children, adolescents and elderly. In conclusion, when intake and/or status of priority micronutrients (folate, vitamin B_{12}, iron, zinc, and iodine for intake and status; vitamin C, vitamin D, calcium, selenium and copper for intake) between low and high SES groups are compared, lower values for all micronutrients (except vitamin B_{12}) are found in the low SES category. The largest relative differences between SES groups are observed for intake of calcium, vitamin C, folate and vitamin D (2-29, 5-47, 7-22 and 4-31% respectively), although the data for the latter two were fewer than for the former two nutrients. The results presented in this review stem from the analysis of the original studies as from 1990 up to November 2011. Current evidence suggests that there are inadequate micronutrient intakes in Europe[3]. Since the relationship between SES and health is dynamic and evolves throughout the life course[40], it should be regarded that the changes in observed SES inequalities in micronutrient intake and status should be monitored over time. This can be realized via analyses of high quality nutritional data with EU coverage that apply comparable measures of SES (e.g. from HELENA and EPIC) or via the inclusion of comparable socio-economic determinants and measures in currently running and forecasted nutritional surveillance programs.

Table 1 Characteristics of the study populations and methodology, and the description of the socioeconomic determinants

Study reference:	Age range (years):	Study population characteristics (sampling frame):	Number of participants and gender-males (M) and females (F):	Micronutrients:	Dietary intake/ Nutritional status assessment method:	Description of the determinant:
Intake in adults						
CH (18), 2001	35-74y	A community based random sample of adults in Geneva canton	2929M; 2767F	Calcium, Vitamin D	Food Frequency Questionnaire (FFQ)	Education: 3 groups (≤8y of education; 9-12y and ≥13y); Occupation (3 groups as from British Registrar Classification: high, medium, low)
ES (19), 2009	25-60y	Randomly selected adults in Andalusia, Spain	1747M; 1674F	Iron, Zinc	2x 24h Recall	Education: 3 groups (no schooling or primary; secondary; university)
ES (20), 2001	65-95y	Institutionalized in urban nursing home and randomly selected free living subjects	130M; 218F	Calcium, Iron, Vitamin C	FFQ	Education: 2 levels (primary or less; partial secondary to completed university)
FI (21), 1996	25-64y	Subjects were a subsample of 3rd FINMONICA risk factor survey (1992)	870M; 991F	Vitamin C	3 day food record (FR)	Education: 3 groups (0-9y of education; 10-12y and >13y); Income: household income per consumption unit divided into quartiles
FR (22), 2009	35-60y	SUVIMAX study (1994-96): representative sample of the national population in terms of geographical density and SES	2117M; 1885F	Iodine	6x 24h Recall	Education: 3 groups (<13y of education, 13-14y, ≥14y)

Table 1 Characteristics of the study populations and methodology, and the description of the socioeconomic determinants (continued)

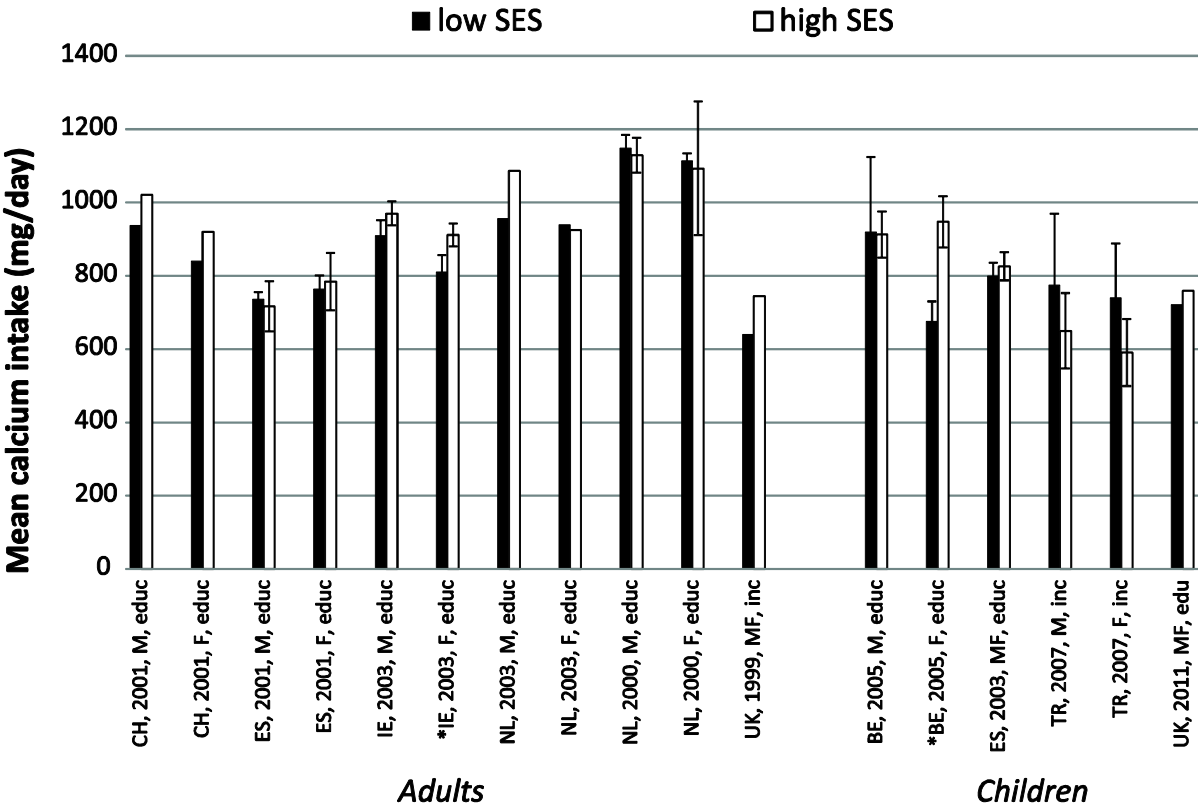
Study reference:	Age range (years):	Study population characteristics (sampling frame):	Number of participants and gender-males (M) and females (F):	Micronutrients:	Dietary intake/ Nutritional status assessment method:	Description of the determinant:
Intake in adults						
IE (23), 2003	≥18y	Stratified probability sampling design on the Electoral Register drawn across the Republic's 26 counties	5979MF	Calcium, Iron, Vitamin C, Vitamin D, Vitamin B_{12}, Zinc, Folate, Selenium	FFQ	Education: 3 groups (non/primary; second level; third level) Occupation: 3 groups (semi-skilled and unskilled labor; non-manual and skilled manual; professional, managerial and technical)
NL (24), 2003	≥19y	Dutch National Food Consumption Survey 1997/98, obtained from a panel by a stratified probability sample of the non-institutionalized population	2020M; 2345F	Calcium, Iron, Vitamin C (only females)	2 days FR	Education: 3 groups (SES was based on education and occupation and categorized into (very) low, middle and high)
NL (25), 2000	≥55y	The Rotterdam study (subject living in a district of Rotterdam, The Netherlands)	2213M; 3193F	Calcium, Vitamin C	FFQ	Education: 4 groups (primary; lower/Intermediate general and lower vocational; higher gen. and interm. vocat.; higher vocat. and university)
SCT (26), 1991	40-59y	Data collected from the Scottish Heart Health Study from 22 districts in Scotland, recruited through 260 general practitioners	>10000MF	Vitamin C	FFQ	Occupation: divided into 2 groups- manual and non-manual (by husband's occupation for women)

Table 1 Characteristics of the study populations and methodology, and the description of the socioeconomic determinants (continued)

Study reference:	Age range (years):	Study population characteristics (sampling frame):	Number of participants and gender-males (M) and females (F):	Micronutrients:	Dietary intake/ Nutritional status assessment method:	Description of the determinant:
Intake in adults						
UK (27), 1999	65-95y	Subjects were free-living people drawn from 80 randomly-selected postcode sectors, geographically representative of mainland Britain	1000MF	Calcium, Iron, Vitamin C, Vitamin B_{12}, Folate	4 days Dietary Record (DR)	Income: 2 groups (low and high)
UK (2), 1997	All life stages	National food survey, 1980-1995(3 stage stratified random sample)	7000 households	Vitamin C	7 day DR conducted by the household member mostly responsible for domestic arrangements	Income: 2 groups (low and high)
Intake in children						
BE (28), 2005	13-18y	Random sample of adolescents was drawn on the basis of a multistage cluster sampling technique from the private and public schools in Ghent region, Belgium	129M; 212F	Calcium, Vitamin C, Iron	Estimated FR 7 days	Education of subjects: 2 groups (general vs. vocational school)
ES (29), 2003	2-5y	Infants from two randomly selected day-care centers in Madrid	110MF	Calcium, Vitamin C, Iron, Vitamin D, Vitamin B_{12}, Folate, Iodine, Zinc	FR 7 days	Education of mothers: 3 groups (no studies or low graduate level; high school graduate or trade training; not full/full university degree studies)

Table 1 Characteristics of the study populations and methodology, and the description of the socioeconomic determinants (continued)

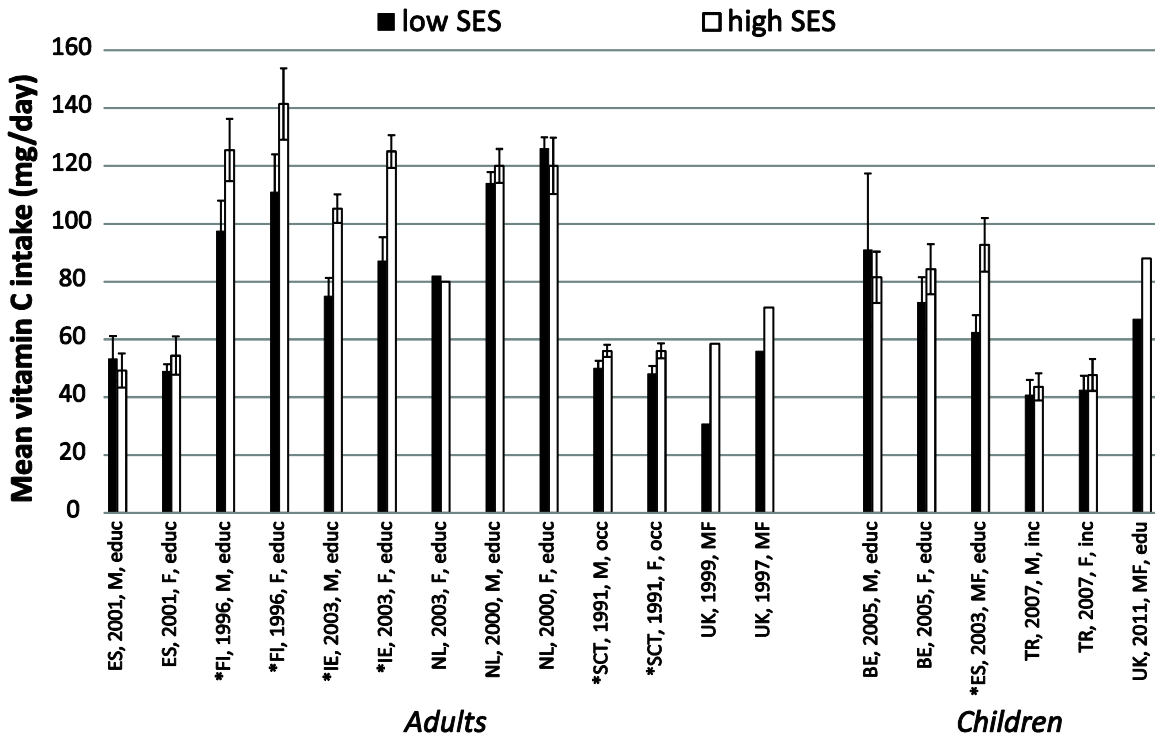
Study reference:	Age range (years):	Study population characteristics (sampling frame):	Number of participants and gender-males (M) and females (F):	Micronutrients:	Dietary intake/ Nutritional status assessment method:	Description of the determinant:
Intake in children						
TR (17), 2007	12-13y	Schoolchildren attending 3 primary schools, located in 2 districts of Istanbul with higher and lower socio-economic level (multi stage sampling method)	256M; 248F	Calcium, Vitamin C, Iron	3x24h Recall	Income: 2 groups(annual household income of students from lower and higher socioeconomic level school districts)
UK (33), 2011	10 y	Children participating in the Avon Longitudinal Study of Parents and Children, an ongoing cohort study; sample similar to National Census data.	3735M; 3828F	Calcium, Vitamin C, Iron, Vitamin D, Vitamin B_{12}, Folate, Iodine, Zinc, Copper, Selenium	3-day diet diaries (child completed with parental help)	Education of mothers: 3 groups (no schooling or primary; secondary; university)
Status in adults						
ES (19), 2009	25-60y	Randomly selected subjects in Andalusia, Spain	170M; 184F	Iron, Zinc	Haemoglobin level; Plasma zinc	Education: 3 groups (no schooling or primary; secondary; university)
ES (30) ,2007	18-104y	Randomly selected adults in Galicia, Spain	1326M; 1551F	Iodine	Urinary iodine	Education: 5 groups (illiterate; uncompleted primary; primary school; high school; university)
FR (31), 2010	35-60y	SUVIMAX study (1994-96): representative sample of the national population in terms of geographical density and SES	3127M; 4425F	Zinc	Plasma zinc	Education: 3 groups (primary; high school; university or equivalent)
NO (32), 2006	30-69y	Randomly selected non-smoking adults in Northern Norway, an iron mining municipality	690M	Iron	Serum ferritin	Occupation: miners and non-miners



Relative differences in intake of calcium for males and females
(mean intake in low SES- mean intake in high SES)/mean intake in high SES

	Study (country, year) and SES indicator:								
Adults									
	CH[18] (2001)		**ES[20] (2001)**	**IE[23] (2003)**		**NL[24] (2003)**	**NL[25] (2000)**	**UK[27] (1999)**	
	Educ	Occup	Educ	Educ	Occup	Educ	Educ	Income	
M	-8.3%	-6.0%	2.6%	-6.2%	-6.5%	-12.0%	1.7%		
F	-8.7%	-2.3%	-2.6%	-11.0%	-2.8%	1.5%	1.9%		
MF								-14.0%	
Children									
	BE[28] (2005)	**ES[29] (2003)**	**TR[17] (2007)**	**UK[33] (2011)**					
	Educ	Educ	Income	Educ					
M	0.7%		19.3%						
F	-28.7%		54.0%						
MF		-3.2%		-5.0%					

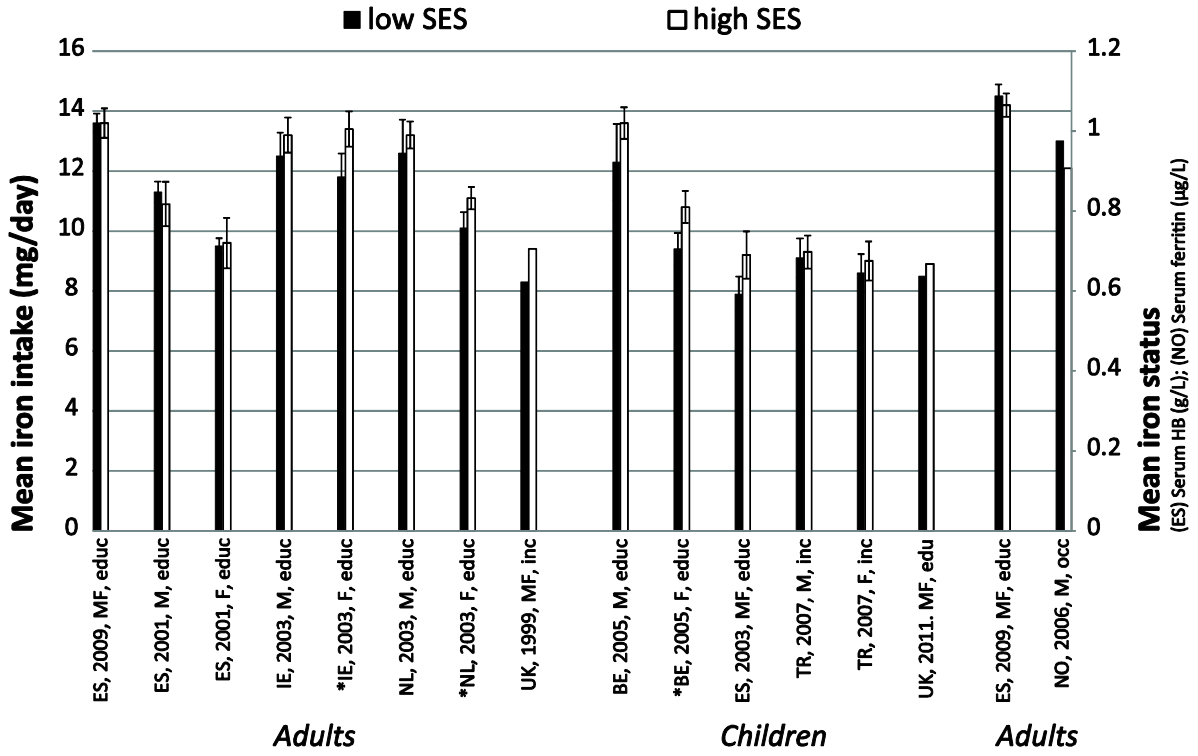
Figure 1 Mean calcium intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: BE, Belgium; CH, Switzerland; ES, Spain; IE, Ireland; NL, Netherlands; TR, Turkey; UK, United Kingdom



Relative differences in intake of vitamin C for males and females
(mean intake in low SES- mean intake in high SES)/mean intake in high SES

	Study (country, year) and SES indicator:									
Adults										
	ES[20] (2001)	**FI[21] (1996)**		**IE[23] (2003)**		**NL[24] (2003)**	**NL[25] (2000)**	**SCT[26] (1991)**	**UK[27] (1999)**	**UK[2] (1997)**
	Educ	Educ	Income	Educ	Occup	Educ	Educ	Occup	Income	Income
M	8.3%	-27.7%	-16.5%	-28.7%	-18.8%		-5.0%	-10.7%		
F	-10.1%	-20.3%	-13.0%	-30.3%	-16.4%	2.5%	5.0%	-14.3%		
MF									-47.4%	-23.3%
Children										
	BE[28] (2005)	**ES[29] (2003)**	**TR[17] (2007)**	**UK[33] (2011)**						
	Educ	Educ	Income	Educ						
M	11.7%		-6.4%							
F	-13.8%		-11.0%							
MF		-32.6%		-23.9%						

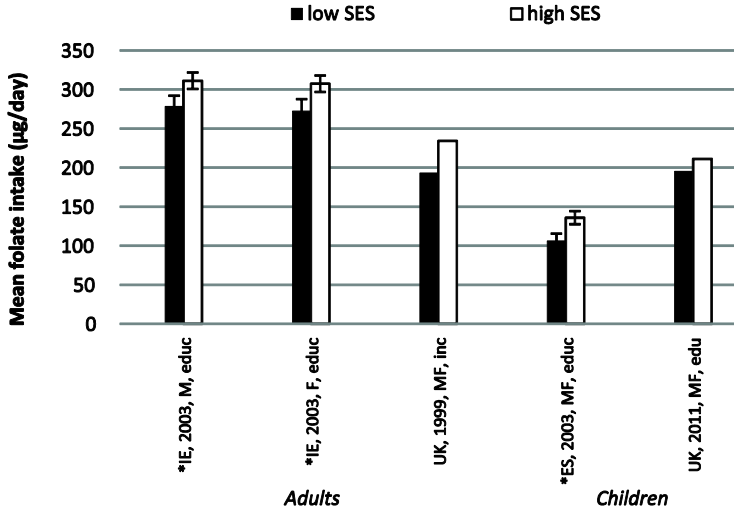
Figure 2 Mean vitamin C intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: BE, Belgium; ES, Spain; FI, Finland; IE, Ireland; NL, Netherlands; SCT, Scotland; TR, Turkey; UK, United Kingdom



Relative differences in intake and status of iron for males and females
(mean intake/status in low SES- mean intake/status in high SES)/mean intake/status in high SES

	Study (country, year) and SES indicator:						
Adults Intake							
	ES[19] (2009)	**ES[20] (2001)**	**IE[23] (2003)**		**NL[24] (2003)**	**UK[27] (1999)**	**ES[31] (2007)**
	Educ	Educ	Educ	Occup	Educ	Income	Occup
M		3.7%	-5.3%	-7.5%	-4.5%		
F		-1.0%	-11.9%	1.5%	-9.0%		
MF	0.0%					-11.7%	
Adults Status							
	ES[19] (2009)						**NO[32] 2006**
	Educ						Occup
M							7.40%
MF	2.10%						
Children Intake							
	BE[28] (2005)	**ES[29] (2003)**	**TR[17] (2007)**	**UK[33] (2011)**			
	Educ	Educ	Income	Educ			
M	-9.5%		-2.2%				
F	-12.5%		-4.4%				
MF		-14.1%		-4.5%			

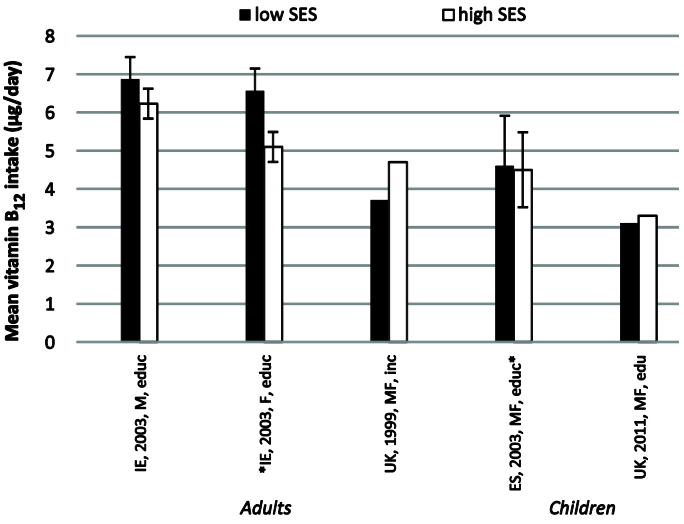
Figure 3 Mean iron intake and status (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake/status between the lowest and highest SES group. Countries with studies available: BE, Belgium; ES, Spain; IE, Ireland; NL, Netherlands; NO, Norway; TR, Turkey; UK, United Kingdom



Relative differences in intake of folate for males and females (mean intake in low SES- mean intake in high SES)/mean intake in high SES

Study (country, year) and SES indicator:			
Adults			
	IE[23] (2003)		**UK[27] (1999)**
	Educ	Occup	Income
M	-10.5%	-7.3%	
F	-11.4%	0.6%	
MF			-17.5%
Children			
	ES[29] (2003)	**UK[33] (2011)**	
	Educ	Educ	
MF	-21.9%	-7.6%	

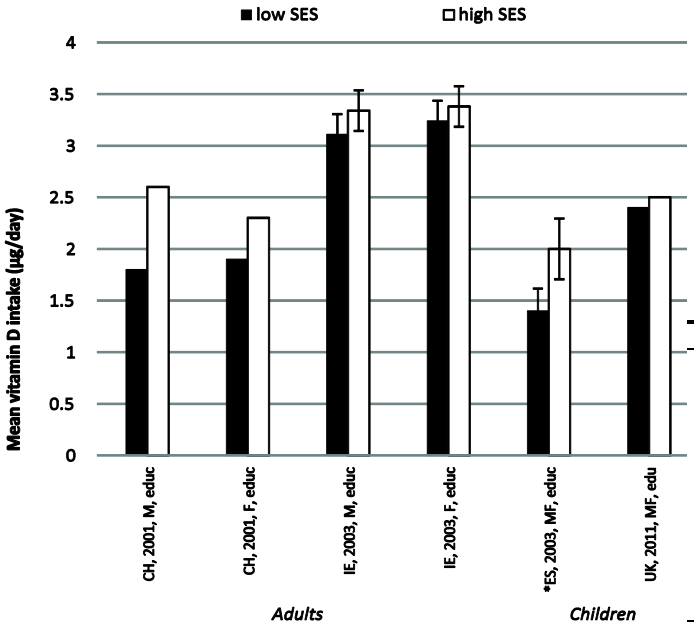
Figure 4 Mean folate intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: ES, Spain; IE, Ireland; UK, United Kingdom



Relative differences in intake of vitamin B12 for males and females (mean intake in low SES- mean intake in high SES)/mean intake in high SES

Study (country, year) and SES indicator:			
Adults			
	IE[23] (2003)		**UK[27] (1999)**
	Educ	Occup	Income
M	10.1%	7.5%	
F	28.6%	20.7%	
MF			-21.3%
Children			
	ES[29] (2003)	**UK[33] (2011)**	
	Educ	Educ	
MF	2.2%	-6.0%	

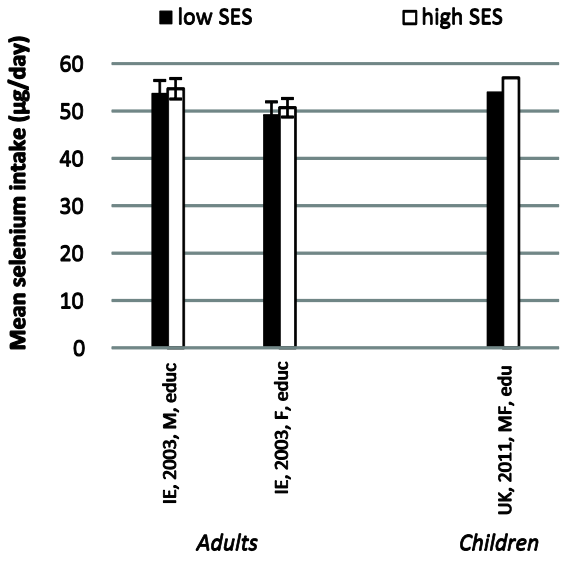
Figure 5 Mean vitamin B12 intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/ elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: ES, Spain; IE, Ireland; UK, United Kingdom



Relative differences in intake of vitamin D for males and females (mean intake in low SES- mean intake in high SES)/mean intake in high SES

Study (country, year) and SES indicator:				
Adults				
	CH[18] (2001)		**IE[23] (2003)**	
	Educ	Occup	Educ	Occup
M	-30.8%	-13.0%	-6.9%	-7.0%
F	-17.4%	-17.4%	-4.1%	5.7%
Children				
	ES[29] (2003)	**UK[33] (2011)**		
	Educ	Educ		
MF	-30.0%	-4.0%		

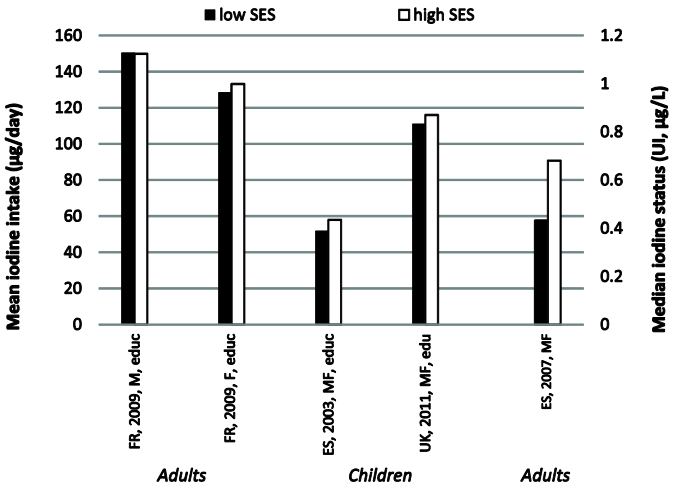
Figure 6 Mean vitamin D intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/ elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: CH, Switzerland; ES, Spain; IE, Ireland; UK, United Kingdom



Relative differences in intake of selenium for males and females (mean intake in low SES- mean intake in high SES)/mean intake in high SES

Study (country, year) and SES indicator:		
Adults		
	IE[23] (2003)	
	Educ	Occup
M	-1.8%	-3.4%
F	-3.0%	2.9%
Children		
	UK[33] (2011)	
	Educ	
MF	-5.3%	

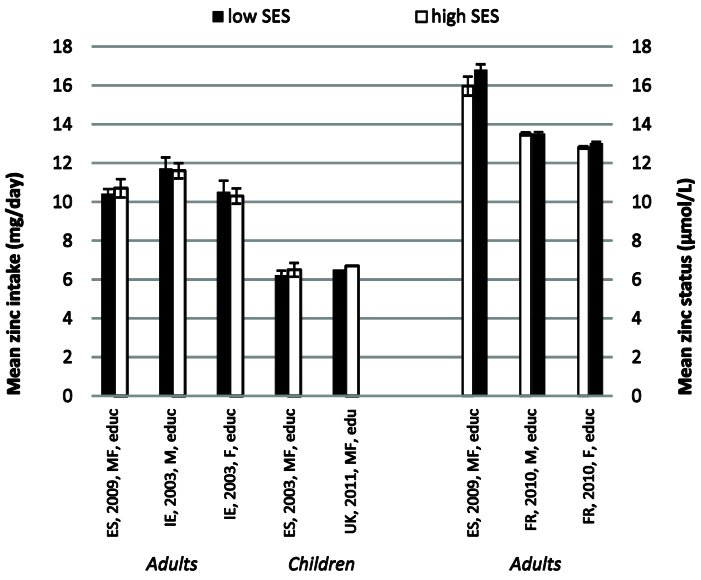
Figure 7 Mean selenium intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: IE, Ireland; UK, United Kingdom



Relative differences in intake and status of iodine for males and females (mean intake/status in low SES- mean intake/status in high SES)/mean intake/status in high SES

Study (country, year) and SES indicator:		
Adults Intake		
	FR[22] (2009)	
	Educ	
M	0.3%	
F	-3.6%	
Adults status		
	ES[30] (2007)	
	Educ	
MF	-36.6%	
Children Intake		
	ES[29] (2003)	**UK[33] (2011)**
	Educ	Educ
MF	-10.8%	-4.3%

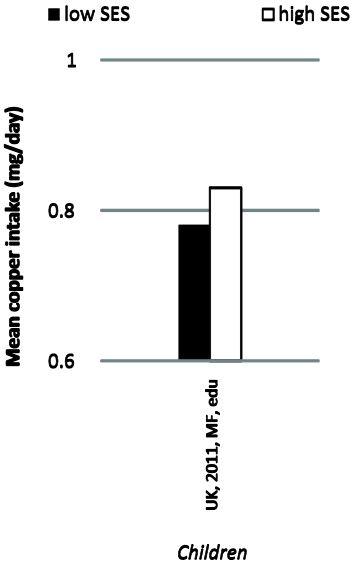
Figure 8 Mean iodine intake and status (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: ES, Spain; FR, France; UK, United Kingdom



Relative differences in intake and status of zinc for males and females
(mean intake/status in low SES- mean intake/status in high SES)/mean intake/status in high SES

Study (country, year) and SES indicator:						
Adults Intake						
	ES[19] (2009)		**IE[23] (2003)**			
	Educ		Educ		Occup	
M				0.9%		-3.3%
F				1.9%		10.2%
MF		-2.8%				
Adults status						
	ES[19] (2009)		**FR[31] (2010)**			
	Educ		Educ			
M				0%		
F				1.5%		
MF		5.2%				
Children Intake						
	ES[29] (2003)	**UK[33] (2011)**				
	Educ		Educ			
MF		-4.6%	-3.0%			

Figure 9 Mean zinc intake and status (with 95% confidence interval, where available, represented by vertical bars) of European adults/elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: ES, Spain; FR, France; IE, Ireland; UK, United Kingdom



Relative differences in intake of copper for males and females
(mean intake in low SES- mean intake in high SES)/mean intake in high SES

	Study (country, year) and SES indicator:
Children Intake	**UK[33] (2011)**
	Educ
MF	-6.0%

Figure 10 Mean copper intake (with 95% confidence interval, where available, represented by vertical bars) of European adults/ elderly and children (M, males; F, females; MF, males and females) in groups of lowest and highest socio-economic status (SES) by different SES indicators (educ, education; occup, occupation; inc, income). * denotes a statistically significant difference (P< 0.05) in mean intake between the lowest and highest SES group. Countries with studies available: UK, United Kingdom

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Chapter 4

Socio-economic differences in micronutrient intake: variation by education, results from the European Prospective Investigation into Cancer and Nutrition cohort

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The final list of EPIC co-authors to be included is under consideration by the EPIC Steering Committee.The final list of EPIC co-authors to be included is under consideration by the EPIC Steering Committee.)

Abstract

Background: Limited data and the diversity of research methodologies hamper the understanding of the association between socio-economic status (SES) and micronutrient intake in adults and elderly in Europe. Using education as a proxy for SES, this study evaluates intake of calcium, iron, folate, vitamins B12, C and D in 10 Western European countries participating in the EPIC cohort.

Methods: Data originate from 35,596 men and women aged 35-74 years. Based on single 24-h dietary recall data, generalized linear models were used to obtain micronutrient intakes adjusted for age, total energy intake, height and weight, and weighted by season and day of recall. Results were presented by country, gender and education level.

Results: The mean intake of calcium, folate and vitamin C was lower in the lowest compared to the highest education group: relative differences ranged up to 12, 13 and 23%, respectively. The intake of iron varied marginally (differences between SES groups ranged up to ± 9%), whereas differences in intake of vitamins B12 and D between the lowest and the highest education category varied widely: they ranged from -19% to 45% for the former, and from -25% to 26% for the latter micronutrient. These patterns were similar for men and women.

Conclusion: SES, defined by educational level, is an important determinant of intake of calcium, folate and vitamin C in European adults and elderly. This evidence should be considered when developing public health nutrition programs aiming to reduce unfavourable dietary habits among low SES groups.

Introduction:

The prevalence of some diet-related chronic diseases varies greatly across different socioeconomic groups (1-4). In addition, energy-low and nutrient-dense foods are likely to be consumed less in low socioeconomic groups (1-8). According to the World Health Organization (WHO), even in developed countries, these groups are reported to have low intake of calcium, vitamin B12, and vitamin D (9). Socio-economic status (SES) is a multidimensional concept that covers several social, financial and material circumstances that are interlinked and are found to be associated with food choice (10). Commonly used SES indicators, such as "education", "occupation" and "income", appear to have similar impact on nutrition and diet (1), although they are not directly interchangeable as each of them can have its own explanatory pathway to influence health (10-12). Education can be seen as a useful indicator of SES from early adult life on, since formal education is usually completed in young adulthood, and because it enables people to obtain certain occupations and related income (3, 13). In addition, it has been shown that education may be the most important social predictor for a healthy diet, as it is associated with nutritional knowledge and health-related attitude (10, 14). A comprehensive overview of differences in micronutrient intake between socioeconomic groups in Europe is currently lacking. Available published studies differed with respect to methodology and study designs which hamper comparison between countries and interpretation of the results. Data collected in a harmonized way with comparable procedures in assessing dietary data and SES have been collected in the European Prospective Investigation into Cancer and Nutrition (EPIC) (15). The aim of this study is to assess the relation between education level and the intake of public health prioritized micronutrients (16) (i.e. calcium, iron, folate, vitamin B12, vitamin C and vitamin D) in adults and elderly across ten participating countries, using data with uniform definitions and methodology from the EPIC cohort.

Methods:

Study population

The EPIC study is an ongoing multi-centre prospective cohort study in 10 European countries (Greece, Spain, Italy, France, Germany, Netherlands, UK, Denmark, Sweden and Norway). From 1992 to 2000, more than 500,000 individuals (in majority 35 to 74 years of age) were recruited from the general population residing in given geographical areas. Recruitment procedures have been described in detail by Riboli et al (15). The cohort of France is based on female members of a health insurance scheme for school employees. Parts of the Italian and Spanish cohorts included members of local blood donors associations. The cohorts from Utrecht (the Netherlands) and Florence (Italy) recruited participants of breast cancer screening programs. The Oxford cohort consisted of vegetarians, vegans and other health-conscious individuals. In France, Norway, Utrecht (the Netherlands) and Naples (Italy) only

women were recruited. Baseline information on medical history and socio-demographic and lifestyle factors including educational level were assessed using self-reported questionnaires or face-to-face interviews. Baseline dietary intake was measured by country-specific assessment instruments. Data analyzed in the current study stem from the EPIC calibration subsample (n = 36,994 subjects, nearly 8% of the entire EPIC prospective cohort),using a standardized computerized 24hour dietary recall (24-HDR) programme, which was designed to correct for measurement error in the baseline dietary intake measurement (17). Subjects under 35 or over 74 years of age were excluded because of low participation in these age categories, so the analytical sample in this study involved12,937 men and 22,659 women.

Dietary and nutrient intake data

The 24-h dietary recall (24-HDR) interviews were standardized across countries using the computerized interview software program EPIC-Soft® (International Agency for Research on Cancer, Lyon, WHO, France). More details on the concept of standardization and the structure of EPIC-Soft® are described in detail elsewhere (18, 19). All subjects were administered the 24-HDR in a face-to-face interview, except in Norway where a telephone interview was conducted. Nutrient intakes, without dietary supplements, were estimated using standardized food composition tables developed to improve cross-country comparability. Compilation of these food composition tables resulted in the EPIC Nutrient Database (ENDB), a standardized reference instrument for calibrating the EPIC measurements at the nutrient level (20).

Educational level

Educational level based on the highest vocational training reached was categorized into: (1) primary education comprising ‘no education’ and ‘primary school completed ’, (2) secondary education compiling the subjects with ‘technical/professional’ and ‘secondary’ school, and (3) higher education including those with a vocational training higher than secondary school, i.e. college, university or higher. The subjects with missing information or not specified educational level were excluded from the analyses. Comparisons of socio-economic gradients based on categorical variables may be biased if the proportionate allocation of subjects across the strata differs (21). Therefore, in this study the three-level educational variable was treated as relative measure, called “Relative Index of Inequality” (RII), resulting from a computation already applied in EPIC (22, 23). The distribution of the population across the educational categories was stratified by country, gender and two age-groups (age at 24-HDR interview $< / \geq 60$ years). The cut- point of 60 years was chosen because the proportion of educational levels was unequally distributed in subjects below and above 60 (table 1) and it was previously applied in the literature (24). The midpoint of each proportion was used to calculate the score of RII. For example, for Italian males aged < 60 years with the primary educational level that constituted 30% of the total Italian males within the same age group, a RII score of 15 (30/2) was assigned. If the secondary educational level for Italian males aged < 60 years involved 40%, the RII

score would be 50 (30+(40/2)). The calculation of the RII for the higher education group was analogous: 30+40+(30/2)=85. Based on these RII scores, all participants were assigned to one of three educational level categories referring to primary (1), secondary (2) and higher education (3) based on the tertiles of the RII scores in each country (22). This procedure was applied to the overall study population. In Norway, in women <60y, the RII categorization resulted in two educational groups (primary and higher).

Data analysis

Baseline characteristics of the study population were reported per educational level as mean ± SD for continuous variables or as percentage for categorical variables. Differences in the intake of calcium, iron, folate, vitamin B12, vitamin C and vitamin D in relation to educational level were examined and reported by country,as well as for all countries together (pooled analysis).Statistical analysis for the cohort from UK was done for all subjects and additionally by dividing them into 'general population group' and 'health- conscious' group, the latter involving a heterogeneous sub-population of vegans, vegetarians, fish eaters and meat eaters (17). All analyses were carried out stratified by gender.

Generalized linear models were used to compute least square means in nutrient intake ± standard errors (SE) and 95% confidence intervals (CI), adjusted for age at recruitment (continuous), total energy intake, weight and height (self-reported by participants during the 24-HDR interview), and weighted by season and day of the 24-HDR to control for different sampling procedures of the 24-HDR interviews across seasons (spring, summer, autumn and winter) and days of the week. The optimal generalized linear model to assess micronutrient intake was applied in several steps. At first, the full generalized linear model used micronutrient intake as the dependent variable, whereas country, educational level, sex and the corresponding two-way interaction terms (education*sex, education*country, sex*country) and the three-way interaction term (education *country*sex) were explanatory variables. After removing the non-significant three-way interaction, and the non-significant education*sex interaction in the subsequently refitted model, it appeared that the optimal model for all six micronutrients was the one with three significant main effects (country, education, sex) and two interaction terms (education*country and sex*country). Least squares means (LS-means) were computed for each education*country effect and presented by sex. Statistical tests for trend across education levels were carried out by assigning equally spaced scores to the categories and treating the variables as continuous in the regression analysis (p trend); the level of statistical significance was set at $p < .05$ for 2-sided testing. All analyses were performed with SAS statistical software (version 9.1, SAS Institute Inc, Cary, NC, USA).

Finally, the relative differences (%) in micronutrient intake between primary and higher education level were calculated using the latter as reference category: (%) = ([micronutrient intake in primary

group– micronutrient intake in higher group]/ micronutrient intake in higher group)* 100; the lowest and the highest values of the relative differences for each micronutrient are indicated below.

Results:

The final EPIC calibration study population comprised 35,596 participants with a higher percentage of subjects > 60 years in men (38.5%) than in women (30.7%). In total, the proportional distribution of the educational level for primary, secondary and higher education was as follows: 33.3, 34.9 and 31.8% for men and 33.7, 35.2 and 31.1% for women, respectively (table 1). The mean BMI was 26.8 ± 0.6 for men and 25.3 ± 0.7 kg/m^2 for women, and decreased with increasing educational level. Mean energy intake was 2,500± 17kcal/day for men and 1,857±35kcal/day for women. The proportion of current smokers was higher in the primary education group than in the other two groups in both genders. The proportion of physically inactive people was more prevalent among higher educated subjects than in the other two education groups in both genders.

Micronutrient intakes:

The adjusted mean micronutrient intakes (with 95% CI) ordered by country from south to north are presented in Figures 1-6, by gender and by educational level. In addition, the mean micronutrient intakes (SE) are presented in the Appendix.

Calcium (figure 1):

Mean calcium intake (SE) in male EPIC participants was 1000 (8.4), 1043 (13.3) and 1050 (8.5) mg/day for primary, secondary and higher education, respectively (p-trend <.0001). In all countries, with the exception of the UK, the calcium intake in males of the highest education group was higher than the intake in the lowest education group. Only in Greece a statistically significant, positive trend over the three education categories was seen (p-trend= .04). The relative difference in mean calcium intake between the lowest and highest education category varied between countries from -10.8% (Greece) to 5.3% (UK); (p for interaction < .0001).

Compared to men, mean calcium intake (SE) in women was lower: 873 (5.0), 895 (4.4) and 912 (4.9) mg/day, for primary, secondary and higher education, respectively (p-trend <.0001). In all countries, with the exception of France and the UK, higher intake was found in the highest education group in comparison to the lowest education group. Similarly to men, a statistically significant positive trend over the three education categories was only found in Greece (p-trend= .004). The relative difference in mean calcium intake between lowest and highest education group ranged from -12.0% (the Netherlands) to 0.5% (France); (p for interaction <.0001).

Folate (figure 2):

Mean folate intake in the three education groups in men was 297 (2.3), 295 (3.7) and 307 (2.8) µg/day for primary, secondary and higher education group, respectively (p-trend= .004). Lower mean folate

intake in the lowest in comparison to the highest education group was seen in men from all countries, but Greece. A statistically significant positive trend over the three education categories was observed in Italy and Sweden. The relative difference in mean folate intake between the lowest and highest education category varied between countries from -7.0% (Denmark) to 6.3% (Greece); (p for interaction < .0001).

In women, mean folate intake was 245 (1.5), 249 (0.8) and 254 (1.5) µg/day for the primary, secondary and higher education group, respectively (p-trend <.0001). As for men, the mean folate intake was lower in the lowest than in the highest education group among women from all countries, but Greece. A borderline significant positive trend was found in the UK (p-trend= .06). Across countries, the relative difference in mean folate intake between lowest and highest education group ranged from -12.6% (Sweden) to 13.3% (Greece); (p for interaction < .0001).

Iron (figure 3):

In men, no differences in mean iron intake (SE) by educational level were found: 15.4 (0.09), 15.2 (0.15) and 15.5 (0.10) mg/day for primary, secondary and higher education, respectively (p-trend= .88). Lower intakes in the lowest education groups than in the highest education groups were found in all countries except in Italy, Spain and Greece. A borderline significant positive trend was found in Germany (p-trend= .06). The relative difference in mean iron intake between lowest and highest education group ranged from -8.6% (UK) to 6.7% (Spain); (p for interaction <.0001).

In women, no differences in mean iron intake (SE) between primary, secondary and higher education groups were found: 11.4 (0.1), 11.4 (0.2) and 11.4 (0.1) mg/day (p-trend= .92). Five out of ten countries reported lower mean iron intake in the lowest than in the highest education groups: the UK, the Netherlands, Germany, Sweden and Denmark. No statistically significant trend over the three education categories was observed in any of the countries. The relative differences ranged from -4.7% (Germany) to 9.1% (Greece); (p for interaction <.0001).

Vitamin B12 (figure 4):

In men, mean vitamin B12 intake was 7.6 (0.2), 7.3 (0.3) and 7.0 (0.2) µg/day for primary, secondary and higher group, respectively (p-trend= .03). In Greece, Sweden and Denmark, men from the lowest educated groups had lower mean vitamin B12 intake than men from the highest education groups, in the other five countries an opposite trend was observed. However, no statistically significant trend over the three education categories was found in any of the countries. Yet, the relative differences in mean vitamin B12 intake between the lowest and highest education category substantially varied between countries: -17.1% (Greece) to 27.1% (UK); (p for interaction <.0001).

Mean vitamin B12 intakes in women were 5.4 (0.1), 5.6 (0.1) and 5.2 (0.1) µg/day for primary, secondary and higher education, respectively (p-trend= .08). In Greece, Germany and Denmark

women from the lowest education groups had lower vitamin B12 intake than women from the highest education groups, while in the other seven countries a higher intake was seen in the lower educated. No statistically significant trend over the three education categories was observed in any of the countries. Relative difference in mean vitamin B12 intake between the lowest and highest education category ranged from -18.9% in Greece to 44.7% in Italy; (p for interaction <.0001).

Vitamin C (figure 5):

Mean intake of vitamin C in men was 108 (1.6), 112 (2.6) and 126 (1.7) mg/day for primary, secondary and higher education, respectively (p-trend <.0001). In all countries, men from the lowest education groups had lower mean vitamin C intake than men from the highest education groups. A statistically significant positive trend over the three education categories was observed in Germany. The relative differences in mean vitamin C intake between the lowest and the highest education group ranged from -6.8% in Germany to -20.8% in Denmark;(p for interaction <.0001).

In women, the mean intake for primary, secondary and higher education was: 108 (1.1), 113 (0.6) and 120 (0.1) mg/day (p-trend <.0001). As for men, in all countries, women from the lowest education groups had lower mean vitamin C intake than women from the highest education groups. No statistically significant trend over the three education categories was observed in any of the countries. The lowest relative difference in mean vitamin C intake between the lowest and the highest education group was found in France (-0.4%) and the highest in Sweden (-22.6%); (p for interaction <.0001).

Vitamin D (figure 6):

In men, no differences in mean intake of vitamin D between educational groups were found: 5.2 (0.1), 5.2 (0.2) and 5.0 (0.1) µg/day for primary, secondary and higher education (p-trend= .33). Only in Greece and Denmark men from the lowest education groups had lower mean vitamin D intake than men from the highest education groups. Higher intakes in the lowest than in the highest education groups were seen in the other six countries with a statistically significant negative trend over the three education categories in the UK. The relative difference in mean vitamin D intake between the lowest and the highest education group varied considerably across countries, from -24.9% (Greece) to 26.2% (UK); (p for interaction <.0001).

Mean intake of vitamin D in women was 3.7 (0.07), 3.4 (0.04) and 3.5 (0.07) µg/day for primary, secondary and higher education, respectively (p-trend= .10). Only in Germany and Norway women from the lowest education groups had lower mean vitamin D intake than women from the highest education groups, while intakes were higher in the lowest than in the highest education groups in the other eight countries. No statistically significant trend over the three education categories was observed in any country. The relative difference in mean vitamin D intake between the lowest and the highest education group ranged from -5.4% in Germany to 15.8% in Spain; (p for interaction <.0001).

Discussion:

The results of this study show that individuals of lowest SES (defined by educational attainment) have a lower intake of calcium, folate and vitamin C than their highest SES counter parts. In all countries, intake of calcium (except in France and the UK distinctive 'health- conscious' group), folate (except in Greece), and vitamin C was lower in the lowest than in the highest education groups, with relative differences ranging up to 12, 13 and 23%, respectively. The intake of iron marginally differed between the lowest and the highest education groups (differences between SES groups ranged up to ± 9%), while the intake of vitamins D and B12 was higher in the lowest than in the highest education groups in more than half of the countries. When data for all countries were combined, we found a statistically significant positive association between educational level and intake of calcium, folate and vitamin C in both genders, whereas a statistically significant inverse association was found only for the intake of vitamin B12 in males. Generally, the observed association between educational level and intake of micronutrients was the same for men and women, but differed between countries.

The UK 'health conscious' group, a distinctive EPIC population group, reported the highest intake of vitamin C in men (~155mg/day) and the lowest intake of vitamin B12 in both genders (~3µg/day), which was expected as this group involved mainly vegans and vegetarians.

The comparison of the results that stem from publications with heterogeneous study methodologies is hampered by different classification of SES and dietary assessment instruments. In the present study we used a standardized measure of educational categorization which is crucial for making cross-country comparisons. This standardized measure, the RII, takes into account the size and relative position of each educational group, which is appropriate for comparing populations with different educational distributions (23). Also, uniformity in food composition databases reduced the possibility of distorted observed mean intakes that can occur if food composition databases with different structures and content are used (20). An additional strength of the present study is the large sample size with high cultural diversity. However, diet was assessed by only one 24-HDR measurement per study subject, which limits the accuracy for estimating intakes of individuals (no information on intra-individual variability), but does not affect estimation of population mean intakes which was the main objective of our cross-sectional study. The choice of micronutrients under study was based on the prioritization of micronutrients of public health importance as recently reported from the EURRECA project (15) and on data availability within the EPIC-project.

With respect to limitations of this study, several issues should be considered. First, the EPIC calibration subsample fairly represents the overall EPIC cohort (15), but the question might arise if the inferences from this study might be generalized to other populations. The study sample stems from developed Western European countries, so the observed associations could be different in less affluent societies with a wider socio-economic gradient where educational level might have different impact on

the dietary pattern. It should be added that no contribution of dietary supplements to total micronutrient intakes has been taken into account due to unavailability of quantitative data. Current data indicates consumption of supplements to vary between educational groups and regions, e.g. a higher level of education was a strong predictor of supplement use (25, 26). As for the EPIC calibration study populations, a clear north-south gradient was reported with higher supplement use in northern countries and in women than in men (25). This could be of interest as exclusion of the contribution of dietary supplements to nutrient intake estimates leads to an underestimation of micronutrient intake in supplement users and therefore reduces overall estimates of mean intakes. Elimination of supplements leaves the interpretation of the education-micronutrient intake association to the level of traditional food patterns which are shown to vary across the EPIC participating countries. (27) Results from this study point to substantial between-country variation in micronutrient intake, too. Yet, overall, the intake of calcium, vitamin C and folate clearly followed an educational gradient.

Nutritional data from European country surveys were recently collated and analyzed. The results suggest inadequate intakes of vitamins C and D, folic acid, calcium, selenium and iodine in adults and elderly (28). Given the SES-related differences in intake of calcium, vitamin C and folate in the present study, it could be that lowest educated groups are less likely to meet micronutrient recommendations than their more educated counterparts. Available publications on the association between micronutrient intake and education among European populations stem from Western European countries (29). Those findings are, overall, consistent with the results from the present study: if variation was observed, lower micronutrient intakes were found among those with lower education than in higher educated groups for all micronutrients under study except for vitamin B12. For example, available data on intake of calcium showed lower mean intakes in lower than in higher educated groups in Switzerland and Ireland, and in Dutch males (30, 31, 32), while negligible variation was observed in Spain (33) and in Dutch females (34). The intake of iron was less in lower versus higher education groups in Ireland and the Netherlands (31, 32). A dietary survey from Belgium reported a positive association between intake of iron and educational level when adolescents from general and vocational schools were compared. These findings were confirmed within the same survey when the intake was related to the educational level achieved by the adolescents' parents (35). Data from Spain showed almost no difference in intake of calcium (33) and iron (36) between educational levels. Data on intake of vitamin C associated with education was most available, and the results were consistent: lower intake in the lowest than in the highest education groups in Finland, Ireland, Spanish females and Dutch males. Lower intakes in lower than in higher educated groups were reported for the intake of folate and vitamin D in Switzerland and for folate in Ireland.

The studies mentioned above have diverse study characteristic such as categorization of education levels, dietary intake assessment methods, etc, which might induce biased reporting of differences in

micronutrient intake if two broad groups from one study are compared with two extreme groups from another (12). Also, the variability in intake is shown to be higher in the studies using a FFQ when compared to food records or diaries (28). Still, these studies had the common approach for presenting the results: variation in micronutrient intake between low and high SES groups. It seems that the heterogeneity among study designs did not alter the association between SES and micronutrient intake, and thus might be considered as supporting fact rather than shortcoming in generalization of the conclusion. Recent data from the USA showed that education was positively and significantly associated with a nutrient density measure, although this association was attenuated after inclusion of the food cost variable in the model (37).

Results from the present study indicate that educational attainment is an important socioeconomic indicator of differences in micronutrient intake, particularly for calcium, folate and vitamin C, with lower intakes in the lowest than in the highest education groups, and for vitamins B12 and D for which most of the countries reported higher intakes in the lowest than in the highest education groups. In line with the WHO 2008-2013 Action Plan (40), this suggests that socioeconomic differences in micronutrient intakes might partially explain the known health inequality between SES groups because of their role in preventing chronic diseases (38, 39). People consume foods, not micronutrients. Smith and Bruner (14) suggested that socially patterned differences in food sources determine the differences in the level of micronutrient intake. Indeed, food choice is known to be influenced by a wide range of socioeconomic factors, education being one of them (3, 10, 41). Building on the results from this study, it seems that educational level and intake of calcium on the one hand and folate and vitamin C on the other hand reflect the association between education and the consumption of dairy products and of fruits/vegetables, respectively. Evidence on SES differences in consumption of fruit and vegetables (important sources of vitamin C and folate) showed that a higher SES was associated with a greater consumption of both fruit and vegetables in the adult population across European countries (4, 42). Dairy products supply 50–80% of dietary calcium in most industrialized countries (39); a review on consumption of cheese and milk in nine European countries showed that consumption of cheese is likely to be higher among subjects belonging to higher socioeconomic levels (43). Results on educational differences in the consumption of meat and meat products, main sources of iron and vitamins B12 and D, were inconsistent (44).

Given that the disparity in micronutrient intake by education was found in socioeconomically affluent Western European countries, it would be interesting to examine whether similar results would be found in Central and Eastern Europe as these countries are expected to have a larger socioeconomic gap possibly associated with considerable variation in micronutrient intake. The variation in micronutrient intake and their determinants should be monitored over time to estimate inadequacy in intake and evaluate if SES inequalities are widening (12). It is also important to assess the efficacy of nutritional campaigns and policy. Although there may be some generalized socioeconomic gradients

for micronutrient intake across Europe, this does not imply that a particular strategy is effective under all conditions (45). Given the herein observed between-country variation, we recommend policy makers to account for the country specific setting when using this evidence in planning intervention strategies.

In conclusion, for European adults and elderly, lowest educated groups have lower intake of calcium, folate and vitamin C than their highest educated counterparts. Since diet is an important and modifiable life-style determinant of human health and our findings are consistent with existing literature, they should be accounted for when planning targeted nutrition intervention programs for socioeconomically disadvantaged groups.

Table 1 Baseline characteristics of the participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (n = 35596)[1]

Education:		Primary	Secondary	Higher
No of subjects	men	4313	4517	4107
	women	7626	7983	7050
Mean age, years (SD)	men	56.8 (7.8)	57.2 (7.6)	56.3 (8.3)
	women	55.3 (7.6)	55.8 (7.6)	55.0 (8.6)
>60 y, % (n)	men	36.1 (1557)	42.2 (1904)	36.9 (1514)
	women	28.8 (2193)	29.0 (2312)	34.5 (2459)
Mean weight, kg, (SD)	men	82.2 (12.1)	81.6 (12.0)	81.4 (11.3)
	women	68.6 (12.2)	66.3 (11.5)	66.0 (11.1)
Mean height, cm, (SD)	men	172.9 (7.4)	174.1 (7.2)	175.9 (7.2)
	women	161.9 (6.5)	161.8 (6.7)	163.3 (6.4)
Mean BMI, kg/m^2, (SD)	men	27.4 (3.8)	26.9 (3.7)	26.2 (3.3)
	women	26.0 (4.7)	25.2 (4.4)	24.6 (4.1)
Mean energy intake,	men	2505.0 (901.8)	2510.7 (842.3)	2482.0 (800.3)
kcal/day (SD)	women	1823.1 (637.9)	1852.8 (639.5)	1890.3 (624.6)
Carbohydrates, g/day (SD)	men	253.0 (103)	259.7 (99.4)	254.0 (93.8)
	women	200.5 (76.7)	202.7 (76.4)	207.2 (76.3)
Protein, g/day (SD)	men	100.6 (43.5)	98.6 (38.4)	95.8 (37.7)
	women	74.7 (28.5)	74.5 (27.9)	75.6 (27.6)
Fat, g/day (SD)	men	103.0 (49.2)	101.5 (47.0)	101.6 (45.5)
	women	74.2 (35.5)	74.7 (36.2)	76.1 (35.4)
Current Smokers, %, (n)	men	30.0 (1293)	27.6 (1245)	24.5 (1004)
	women	21.4 (1634)	15.7 (1250)	17.0 (1201)
Physically inactive, %, (n)	men	9.7 (372)	19.7 (775)	28.3 (1082)
	women	9.1 (558)	15.3 (1114)	17.1 (998)

[1]Abbreviations: SD: standard deviation; n: numbers, %: percentages

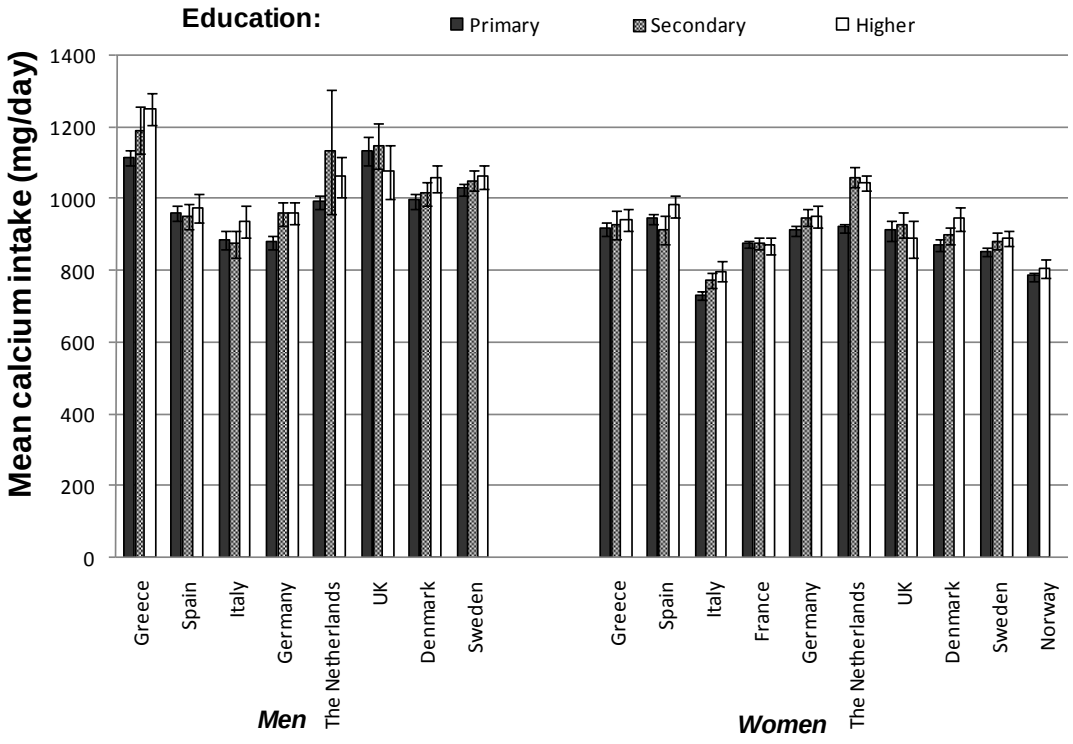

Figure 1 Mean calcium intake (in mg/day, with 95% confidence interval)† in men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study ordered by country from south to north, according to educational level.

†Adjusted for age, total energy intake, weight and height and weighted by season and day of recall.

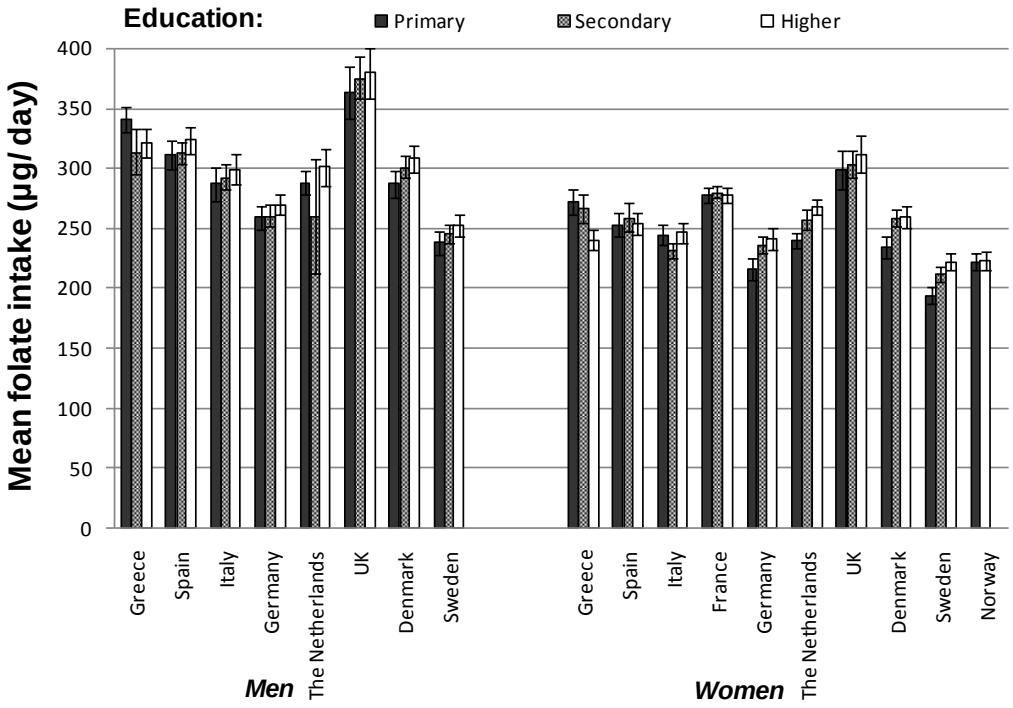

Figure 2 Mean folate intake (in µg /day, with 95% confidence interval)† in men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study ordered by country from south to north, according to educational level.

†Adjusted for age, total energy intake, weight and height and weighted by season and day of recall.

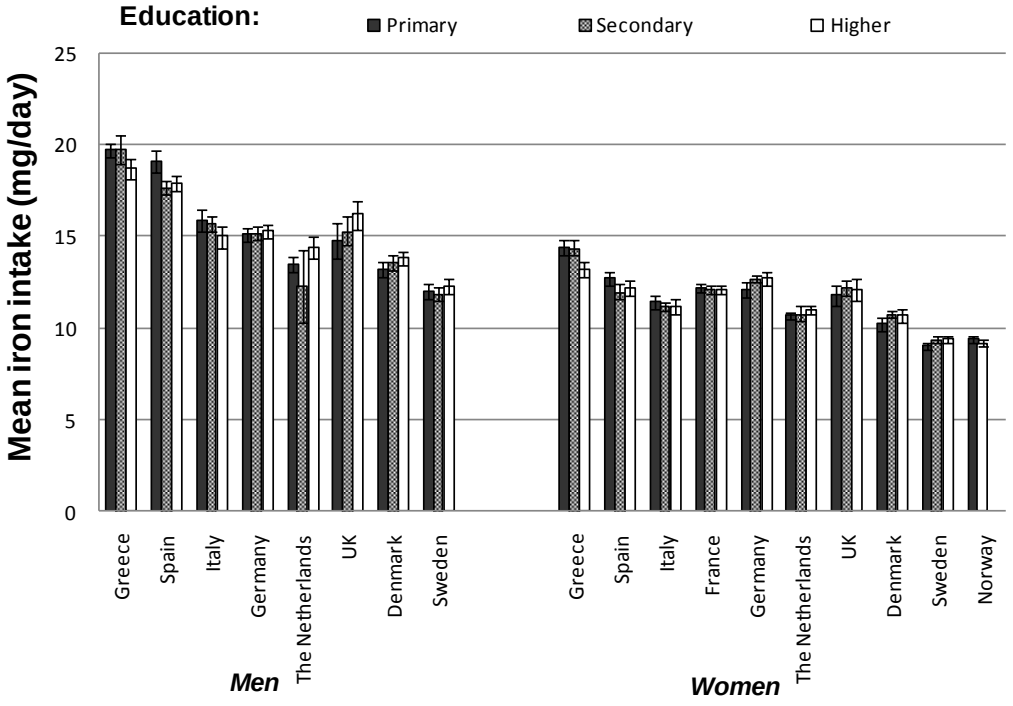

Figure 3 Mean iron intake (in mg/day, with 95% confidence interval)† in men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study ordered by country from south to north, according to educational level.

†Adjusted for age, total energy intake, weight and height and weighted by season and day of recall.

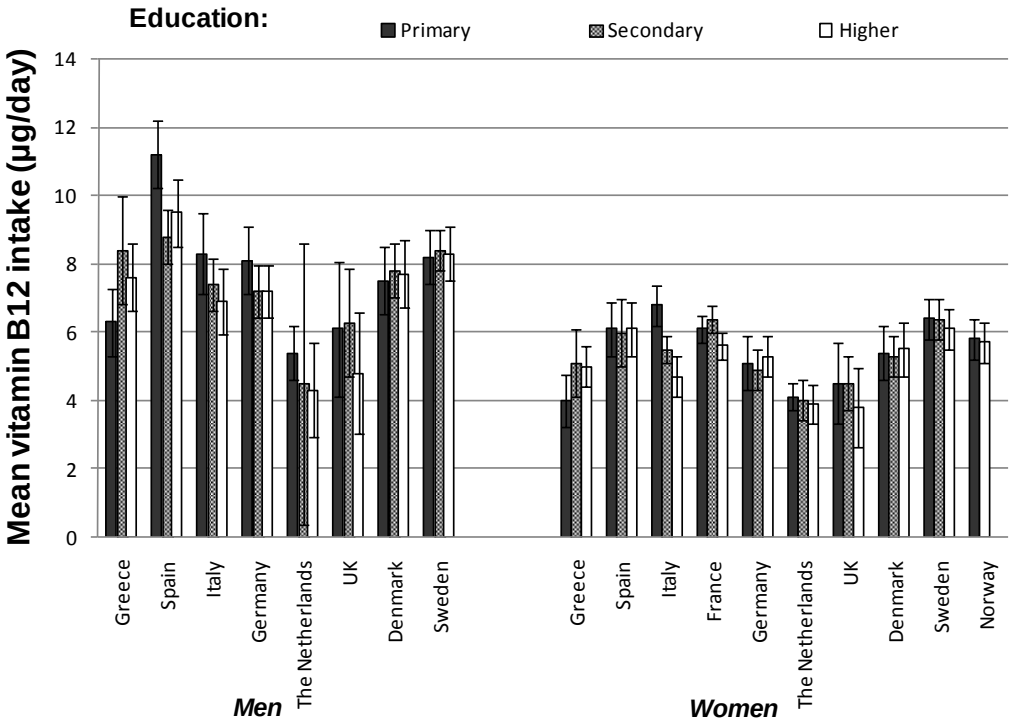

Figure 4 Mean vitamin B12 intake (in µg/day, with 95% confidence interval)† in men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study ordered by country from south to north, according to educational level.

†Adjusted for age, total energy intake, weight and height and weighted by season and day of recall.

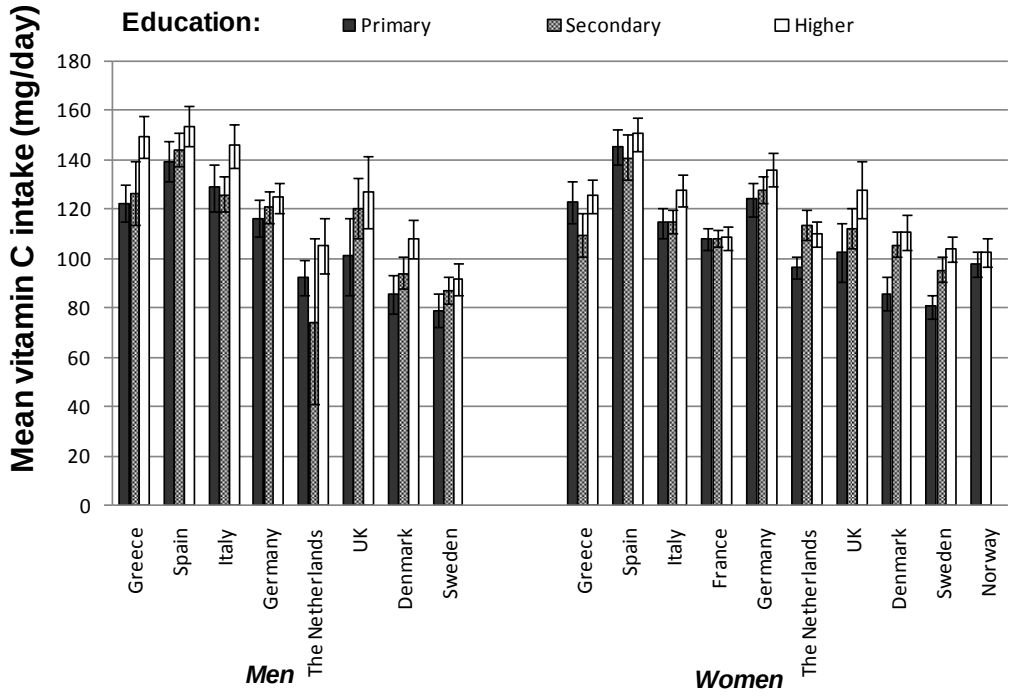

Figure 5 Mean vitamin C intake (in mg/day, with 95% confidence interval)† in men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study ordered by country from south to north, according to educational level.

†Adjusted for age, total energy intake, weight and height and weighted by season and day of recall.

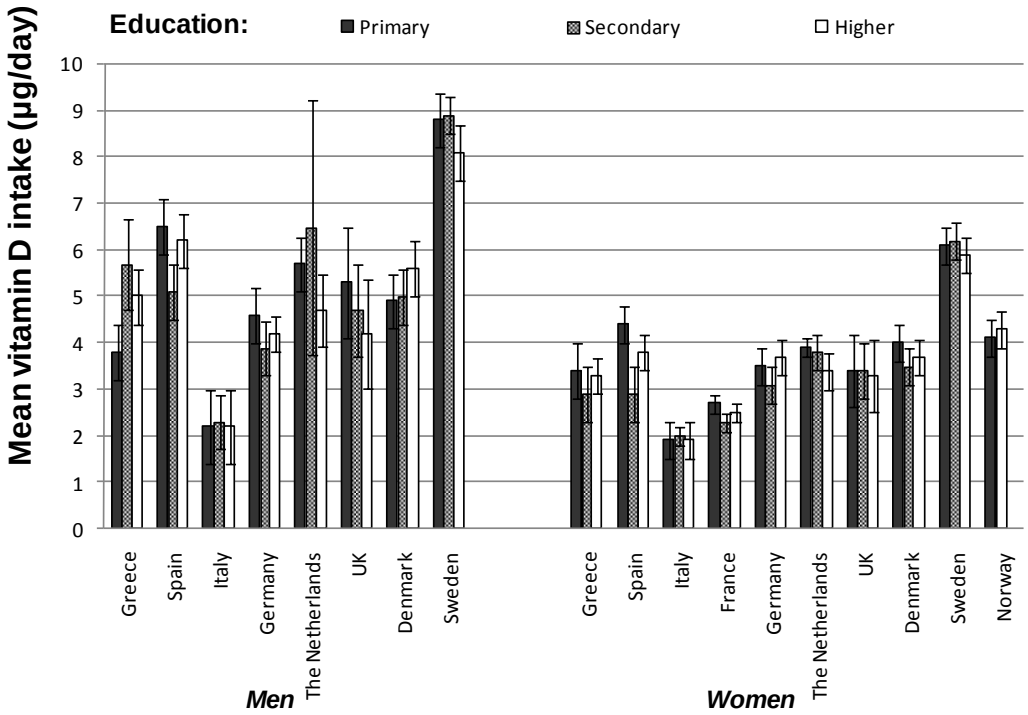

Figure 6 Mean vitamin D intake (in µg/day, with 95% confidence interval)† in men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study ordered by country from south to north, according to educational level.

†Adjusted for age, total energy intake, weight and height and weighted by season and day of recall.

Appendix Mean intake (SE) of calcium and folate by country, gender and educational level (primary, secondary and higher), adjusted for age at recruitment (continuous), total energy intake, weight and height, and weighted by season and day of the 24-HDR. The level of statistical significance for test for trend was set at $p < .05$ for 2-sided testing. Note: a,b,c Values containing the same superscript are not significantly different within the country ($p > .05$).

Calcium (mg/day)

Men

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	1117 (19.4)[a]	962 (21.7)[a]	885 (21.5)[ab]		880 (19.3)[a]	993 (18.3)[a]	1134 (40.4)[a]	996 (20.5)[a]	1028 (17.7)[a]		*1000 (8.4)[a]*
secondary	1192 (33.8)[b]	952 (17.6)[a]	877 (18.7)[b]		961 (17.3)[b]	1134 (88)[ab]	1150 (31.8)[a]	1017 (16.6)[ab]	1052 (14.1)[a]		*1043 (13.3)[b]*
higher	1252 (22.2)[b]	976 (21.2)[a]	937 (22.9)[a]		961 (15.8)[b]	1063 (28.6)[b]	1077 (38.1)[a]	1057 (20.0)[b]	1064 (16.7)[a]		*1050 (8.5)[b]*
p-trend	*0.04*	*0.62*	*0.42*		*0.33*	*0.67*	*0.46*	*0.12*	*0.13*		*<.0001*

Women

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	***pooled estimate***
primary	919 (19.0)[a]	946 (15.9)[ab]	732 (13.7)[a]	875 (10.4)[a]	912 (15.5)[a]	921 (10.4)[a]	913 (27.5)[a]	873 (15.7)[a]	853 (11.2)[a]	784 (11.8)[a]	*873 (5.0)[a]*
secondary	930 (20.4)[a]	915 (20.5)[a]	776 (11)[b]	876 (8.1)[a]	948 (11.9)[a]	1063 (14.3)[b]	929 (18.7)[a]	900 (11.9)[a]	884 (11.3)[b]		*895 (4.4)[b]*
higher	941 (15.2)[a]	981 (15.6)[b]	798 (14.4)[b]	870 (10.9)[a]	953 (15)[a]	1046 (11.5)[b]	889 (26.1)[a]	945 (16.4)[b]	890 (11.1)[b]	807 (13.3)[a]	*912 (4.9)[c]*
p-trend	*0.004*	*0.64*	*0.12*	*0.53*	*0.27*	*0.40*	*0.60*	*0.09*	*0.23*		*<.0001*

Folate (µg/day)

Men

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	341 (5.4)[a]	311 (6)[a]	287 (7)[a]		259 (5.3)[a]	288 (5.1)[a]	363 (11.2)[a]	287 (5.7)[a]	237 (4.9)[a]		*297 (2.3)[a]*
secondary	314 (9.4)[b]	313 (4.9)[a]	293 (5.2)[a]		260 (4.8)[a]	260 (24.4)[a]	375 (8.8)[a]	301 (4.6)[ab]	245 (3.9)[ab]		*295 (3.7)[a]*
higher	321 (6.1)[b]	323 (5.9)[a]	299 (6.3)[a]		269 (4.4)[a]	301 (7.9)[a]	379 (7.6)[a]	308 (5.6)[b]	252 (4.6)[b]		*307 (2.8)[b]*
p-trend	*0.49*	*0.24*	*0.004*		*0.24*	*0.80*	*0.18*	*0.12*	*0.04*		*0.004*

Women

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	272 (5.7)[a]	253 (4.8)[a]	244 (4.1)[a]	278 (3.1)[a]	216 (4.6)[a]	239 (3.1)[a]	298 (8.2)[a]	234 (4.7)[a]	194 (3.3)[a]	222 (3.5)[a]	*245 (1.5)[a]*
secondary	266 (6)[a]	259 (6.1)[a]	231 (3.3)[b]	280 (2.4)[a]	236 (3.5)[b]	258 (4.3)[b]	304 (5.6)[a]	259 (3.6)[b]	212 (3.4)[b]		*249 (0.8)[a]*
higher	240 (4.5)[b]	254 (4.7)[a]	246 (4.3)[a]	279 (3.3)[a]	241 (4.5)[b]	268 (3.4)[b]	312 (7.8)[a]	259 (4.9)[b]	222 (3.3)[c]	223 (4.0)[a]	*254 (1.5)[b]*
p-trend	*0.23*	*0.92*	*0.93*	*0.95*	*0.21*	*0.11*	*0.06*	*0.33*	*0.10*		*<.0001*

Appendix (continued). Mean intake (SE) of iron and vitamin B12 by country, gender and educational level (primary, secondary and higher), adjusted for age at recruitment (continuous), total energy intake, weight and height, and weighted by season and day of the 24-HDR. The level of statistical significance for test for trend was set at $p < .05$ for 2-sided testing. Note: a,b,c Values containing the same superscript are not significantly different within the country ($p > .05$).

Iron (mg/day)

Men

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	19.7 (0.2)[a]	19.1 (0.3)[a]	15.9 (0.3)[a]		15.1 (0.2)[a]	13.5 (0.2)[a]	14.8 (0.5)[a]	13.2 (0.2)[a]	12.0 (0.2)[a]		*15.4 (0.1)[a]*
secondary	19.8 (0.4)[a]	17.7 (0.2)[b]	15.7 (0.2)[ab]		15.2 (0.2)[a]	12.3 (1)[ab]	15.3 (0.4)[ab]	13.6 (0.2)[a]	11.9 (0.2)[a]		*15.2 (0.2)[a]*
higher	18.7 (0.3)[b]	17.9 (0.2)[b]	15.0 (0.3)[b]		15.3 (0.2)[a]	14.4 (0.3)[b]	16.2 (0.4)[b]	13.8 (0.2)[a]	12.3 (0.2)[a]		*15.5 (0.1)[a]*
p-trend	*0.41*	*0.40*	*0.17*		*0.06*	*0.72*	*0.10*	*0.14*	*0.45*		*0.88*

Women

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	***pooled estimate***
primary	14.4 (0.2)[a]	12.7 (0.2)[a]	11.4 (0.2)[a]	12.2 (0.1)[a]	12.1 (0.2)[a]	10.7 (0.1)[a]	11.8 (0.3)[a]	10.2 (0.2)[a]	9.02 (0.1)[a]	9.41 (0.1)[a]	*11.4 (0.1)[a]*
secondary	14.4 (0.2)[a]	12 (0.2)[b]	11.2 (0.1)[a]	12.1 (0.1)[a]	12.7 (0.1)[b]	10.8 (0.2)[a]	12.2 (0.2)[a]	10.8 (0.1)[b]	9.37 (0.1)[ab]		*11.4 (0.2)[a]*
higher	13.2 (0.2)[b]	12.2 (0.2)[b]	11.2 (0.2)[a]	12.1 (0.1)[a]	12.7 (0.2)[b]	11 (0.1)[a]	12.1 (0.3)[a]	10.7 (0.2)[b]	9.37 (0.1)[b]	9.25 (0.1)[b]	*11.4 (0.1)[a]*
p-trend	*0.36*	*0.49*	*0.34*	*0.46*	*0.31*	*0.13*	*0.44*	*0.35*	*0.33*		*0.92*

Vitamin B12 (µg/day)

Men

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	6.29 (0.5)[a]	11.19 (0.5)[a]	8.34 (0.6)[a]		8.07 (0.5)[a]	5.39 (0.4)[a]	6.10 (1)[a]	7.55 (0.5)[a]	8.18 (0.4)[a]		*7.64 (0.2)[a]*
secondary	8.42 (0.8)[b]	8.79 (0.4)[b]	7.44 (0.4)[a]		7.21 (0.4)[a]	4.54 (2.1)[a]	6.33 (0.8)[a]	7.76 (0.4)[a]	8.44 (0.3)[a]		*7.37 (0.3)[ab]*
higher	7.60 (0.5)[ab]	9.51 (0.5)[b]	6.92 (0.5)[a]		7.17 (0.4)[a]	4.33 (0.7)[a]	4.82 (0.9)[a]	7.69 (0.5)[a]	8.30 (0.4)[a]		*7.04 (0.2)[b]*
p-trend	*0.58*	*0.52*	*0.10*		*0.31*	*0.21*	*0.42*	*0.54*	*0.70*		*0.03*

Women

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	4.04 (0.4)[a]	6.13 (0.4)[a]	6.78 (0.3)[a]	6.14 (0.2)[ab]	5.11 (0.4)[a]	4.15 (0.2)[a]	4.51 (0.6)[a]	5.42 (0.4)[a]	6.44 (0.3)[a]	5.78 (0.3)[a]	*5.45 (0.1)[a]*
secondary	5.20 (0.5)[a]	5.99 (0.5)[a]	5.51 (0.2)[a]	6.44 (0.2)[b]	4.89 (0.3)[a]	4.00 (0.3)[a]	4.52 (0.4)[a]	5.32 (0.3)[a]	6.40 (0.3)[a]		*5.61 (0.1)[b]*
higher	4.98 (0.3)[a]	6.14 (0.4)[a]	4.66 (0.3)[b]	5.58 (0.2)[a]	5.32 (0.3)[a]	3.91 (0.3)[a]	3.75 (0.6)[a]	5.53 (0.4)[a]	6.09 (0.3)[a]	5.71 (0.3)[a]	*5.17 (0.1)[c]*
p-trend	*0.41*	*0.95*	*0.08*	*0.55*	*0.67*	*0.09*	*0.35*	*0.68*	*0.26*		*0.08*

Appendix (continued). Mean intake (SE) of vitamins C and D by country, gender and educational level (primary, secondary and higher), adjusted for age at recruitment (continuous), total energy intake, weight and height, and weighted by season and day of the 24-HDR. The level of statistical significance for test for trend was set at $p < .05$ for 2-sided testing. Note: a,b,c Values containing the same superscript are not significantly different within the country ($p > .05$).

Vitamin C (mg/day)

Men

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	122 (3.8)[a]	139 (4.2)[a]	129 (4.9)[a]		116 (3.8)[a]	92.0 (3.6)[a]	101 (7.9)[a]	85.3 (4)[a]	78.7 (3.5)[a]		*108 (1.6)[a]*
secondary	127 (6.6)[a]	144 (3.4)[ab]	126 (3.6)[a]		121 (3.4)[a]	74.4 (17.2)[ab]	121 (6.2)[b]	94.1 (3.2)[a]	86.9 (2.8)[ab]		*112 (2.6)[a]*
higher	149 (4.3)[b]	154 (4.1)[b]	146 (4.5)[b]		125 (3.1)[a]	105 (5.6)[b]	127 (7.4)[b]	108 (3.9)[b]	91.6 (3.3)[b]		*126 (1.7)[b]*
p-trend	*0.24*	*0.12*	*0.41*		*0.04*	*0.72*	*0.18*	*0.08*	*0.10*		*<.0001*

Women

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	123 (4.3)[a]	145 (3.6)[a]	115 (3.1)[a]	108 (2.3)[a]	124 (3.5)[a]	96.4 (2.3)[a]	102 (6.2)[a]	85.5 (3.5)[a]	80.3 (2.5)[a]	97.5 (2.7)[a]	*108 (1.1)[a]*
secondary	109 (4.5)[b]	141 (4.6)[a]	115 (2.5)[a]	108 (1.8)[a]	128 (2.7)[ab]	114 (3.2)[b]	112 (4.2)[a]	106 (2.7)[b]	95.3 (2.6)[b]		*113 (0.6)[b]*
higher	125 (3.4)[a]	150 (3.5)[a]	127 (3.3)[b]	108 (2.5)[a]	136 (3.4)[b]	111 (2.6)[b]	128 (5.9)[b]	110 (3.7)[b]	104 (2.5)[c]	103 (3)[a]	*120 (0.1)[c]*
p-trend	*0.90*	*0.63*	*0.31*	*0.24*	*0.14*	*0.46*	*0.08*	*0.22*	*0.10*		*<.0001*

Vitamin D (µg/day)

Men

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	3.78 (0.3)[a]	6.49 (0.3)[a]	2.18 (0.4)[a]		4.56 (0.3)[a]	5.67 (0.3)[a]	5.30 (0.6)[a]	4.94 (0.3)[a]	8.83 (0.3)[ab]		*5.22 (0.1)[a]*
secondary	5.67 (0.5)[b]	5.06 (0.3)[b]	2.26 (0.3)[a]		3.94 (0.3)[a]	6.48 (1.4)[a]	4.68 (0.5)[a]	5.00 (0.3)[a]	8.87 (0.2)[b]		*5.24 (0.2)[a]*
higher	5.03 (0.3)[b]	6.24 (0.3)[a]	2.18 (0.4)[a]		4.21 (0.2)[a]	4.70 (0.4)[a]	4.15 (0.6)[a]	5.58 (0.3)[a]	8.16 (0.3)[a]		*5.03 (0.1)[a]*
p-trend	*0.55*	*0.90*	*0.98*		*0.61*	*0.64*	*0.04*	*0.28*	*0.36*		*0.33*

Women

	Greece	**Spain**	**Italy**	**France**	**Germany**	**The Netherlands**	**UK**	**Denmark**	**Sweden**	**Norway**	*pooled estimate*
primary	3.40 (0.3)[a]	4.37 (0.2)[a]	1.90 (0.2)[a]	2.69 (0.1)[a]	3.52 (0.2)[ab]	3.88 (0.1)[a]	3.42 (0.3)[a]	3.97 (0.2)[a]	6.06 (0.2)[a]	4.13 (0.2)[a]	*3.73 (0.1)[a]*
secondary	2.92 (0.3)[a]	2.89 (0.3)[b]	2.00 (0.1)[a]	2.34 (0.1)[a]	3.11 (0.2)[b]	3.76 (0.2)[ab]	3.44 (0.3)[a]	3.51 (0.2)[a]	6.17 (0.2)[a]		*3.44 (0.1)[a]*
higher	3.31 (0.2)[a]	3.81 (0.2)[a]	1.92 (0.2)[a]	2.47 (0.1)[a]	3.74 (0.2)[a]	3.37 (0.2)[b]	3.29 (0.2)[a]	3.65 (0.2)[a]	5.86 (0.2)[a]	4.31 (0.2)[a]	*3.57 (0.1)[a]*
p-trend	*0.89*	*0.76*	*0.86*	*0.57*	*0.78*	*0.18*	*0.41*	*0.53*	*0.56*		*0.10*

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Chapter 5

Systematic review of observational studies and dose–response meta-analysis for folate intake and folate biomarkers in adults and elderly

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Abstract

Background and objectives: Dietary reference values for folate intake vary widely across Europe. Data from observational studies on the association between folate intake and biomarkers of folate status provide estimates of a dose-response relationship which may help to substantiate folate intake reference values.

Methods: A systematic review was conducted of prospective cohort, nested case–control and cross-sectional studies in healthy adults and elderly which provided data on folate intake and folate status biomarkers (serum/plasma folate, red blood cell folate and plasma homocysteine). The regression coefficient of biomarkers of status on intake (β) was extracted from each study, and the overall and stratified pooled β and SE (β) were obtained by random effects meta-analysis on a double log scale.

*Results:*Based on19 estimates from 14 observational studies a pooled estimate of β =0.33 (95%CI: 0.22–0.44, p< 0.0001) resulted for the relationship between folate intake and serum/plasma folate; that is, doubling folate intake increased folate concentration in serum/plasma by 26%; nine estimates for red blood cell folate yielded a pooled estimateβ = 0.27 (95%CI: 0.15–0.38, p< 0.0001; +21% for each doubling of intake); seven estimates for plasma homocysteine yielded β = –0.26 (95%CI: –0.39 to –0.13, p< 0.0001; –16% for each doubling of intake).For serum/plasma folate, the associations were stronger when the intake was assessed as folate from the diet than as folate from the diet and supplements (β = 0.49vs 0.16,p< 0.0001, respectively) and when FFQs were used rather than 24hour recalls combined with food records (β =0.36 vs. 0.19, p = 0.31, respectively).

Conclusions: The quantified relationships between between folate intake and folate status markers are similarly positive for plasma and erythrocytes and inverse for homocysteine. The large heterogeneity between studies is partially attributable to dietary assessment methodology. Results may aid in setting folate intake recommendations for adults and elderly.

Key words: folate intake, biomarkers of folate; dose-response; dietary recommendations

Introduction

Adequate folate intake is necessary to achieve and maintain optimal health in all life stages. The most common problems associated with this deficiency among adults and elderly are anaemia, cancer, neural tube defects, CVD, depression and dementia (1, 2). Dietary reference values (DRVs) provide guidance on optimal dietary folate intake that should prevent deficiency and development of health problems.

The EURopean micronutrient RECommendations Aligned network (EURRECA; http://www. eurreca.org) has been developing approaches to derive and update micronutrient reference values, including folate (3).This process requires reliable data on the association between folate intake, status and health outcomes, which would allow estimating the intakes to achieve preset concentrations of relevant biomarkers. The EURRECA network has proposed three biomarkers of folate status: plasma or serum folate, red blood cell (RBC) folate and plasma homocysteine (tHcy) (4). Using data from randomized controlled trials (RCTs), the relation between folate intake and plasma biomarkers has recently been summarized in a dose-response meta- analysis in adults and elderly (5). This systematic review included data from observational studies in which folate intake represents intakes from its natural dietary matrix (6), and in which populations are not confined to those strictly selected by the RCT's in- and exclusion criteria (6, 7).This paper fills the knowledge gap by systematic reviewing all available observational studies which investigated the relationship between folate intake and biomarkers of folate status in adults and elderly, followed by meta-analyses in order to model biomarkers of folate status as a function of folate intake.

Methods

Search strategy

Within the EURRECA framework, systematic reviews were performed to explore the associations between intakes, status, and selected health outcomes for the public heath priority micronutrients (iron, zinc, folate, vitamin B12 and iodine) (8, 9). This process followed a harmonized search strategy, aimed to collect the data published up to and including June 2012 using MEDLINE and Embase search terms for ['study designs in humans'] AND [folate] AND [intake OR status]. Both indexing and text terms were used and languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian). The summary of the general search strategy developed for a search in EMBASE (Ovid) is shown in Table 1. Search strategies for Ovid MEDLINE and Cochrane library were adapted based on this strategy. The reference lists of retrieved articles and of published reviews were also checked for relevant studies. Where necessary, authors were contacted to provide missing data or clarify methods or results.

Table 1 Summary of the EURRECA general search strategy (http://www. eurreca.org) including the search terms specific for folate, developed for a search in EMBASE (Ovid).

#1 Study design in Humans	1) Cohorts 2) Systematic reviews 3) Cross-sectional
#2 Intake or Status	1) supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or infant nutrition/ or artificial milk/ or breast milk/ or bottle feeding/ or breast feeding/ or lactation/ OR 2) exp nutritional status/ or nutritional deficiency/ or expfolate deficiency/ or expfolate blood level/ OR 3) (intake* or diet* or supplement* or deplet* or status or concentration* or expos* or fortif* or plasma or serum or "red blood cell*" or red blood cell or RBC or RCF or plasma homocysteine or hcy).mp.
#3 Micronutrient	Folate or folic acid or vitamin B9
# 1 AND #2 AND #3	

Study selection

For the current paper, studies that were considered potentially relevant were prospective cohort studies, nested case–control studies and cross-sectional studies in apparently healthy adults and elderly. For a study to be included, it had to report on both folate intake and status, following the EURRECA guidance on best practice micronutrient intake and status methods (10). Studies were included if folate intake was measured with a validated food frequency questionnaire (FFQ), a dietary history method, a 24-h recall method (24hR) or a food record/diary (FR) for at least 3 days. Folate status was reported by the plasma or serum folate, red blood cell folate, or it was measured as total plasma homocysteine.

With respect to design, studies were excluded if they were retrospective cohort studies, non-nested case-control studies, were performed in non-healthy other than adults and elderly populations, or were commentaries, reviews, or duplicate publications from the same study.

Of 1832 identified articles in the wider search on folate intake, status and priority health outcomes in all populations, 1710 were excluded following screening of the title and abstract. The remaining 122 were screened by the two independent reviewers (RN and MN) and all discrepancies on study inclusion were discussed. This process resulted in 46 potentially relevant papers, out of which 17 remained for the final analysis, in addition to 1 study that was identified by hand searching. Papers with incomplete data not obtainable from the authors were excluded. The flow diagram of the articles screened, assessed, and excluded at various stages for this paper is shown in figure 1.

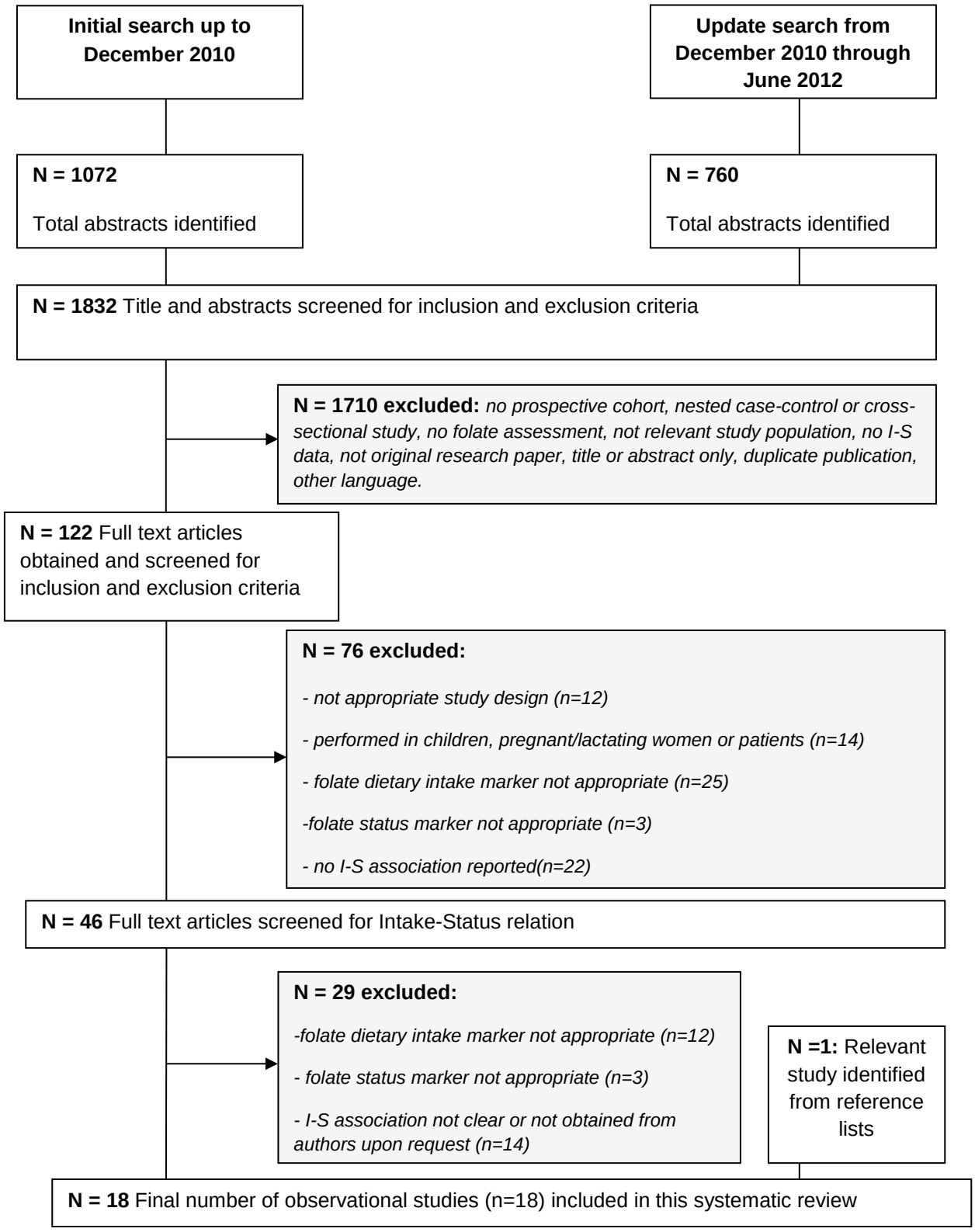

Figure 1 Flow diagram of the study selection process

Abbreviations: I, intake; S, status, referring to a biomarker of intake

Data extraction

For each of the identified manuscripts, data were extracted into a standardized database. This was done by one (RN) and checked by another reviewer (MN).

Extracted data included population characteristics, mean and SD of folate intake and dietary assessment method, levels of folate related biomarkers and analytical method, the association and type of association between folate intake and folate related biomarkers (Spearman rank correlation coefficient, Pearson correlation coefficient, linear regression coefficient), and information on any transformations applied to obtain the reported associations. Serum/plasma folate concentrations were converted to μmol/L when applicable. Table 2 presents the characteristics of the included studies.

Data synthesis

Each study provided at least one independent estimate; for some studies two or three estimates were obtained because of stratification by gender (11, 12) or by (13). Where data on folate intake and status were available for males and females separately but there was only one estimate of the association (14-17), the pooled estimates of mean folate intake and status were calculated and used in the analysis.

Pre-Specified Potential Factors Modifying the Association

We investigated the intake-status relationship for each of the three biomarkers separately. In addition, we investigated whether mean age (continuous), dietary intake method (categories: 1. food frequency questionnaire, and 2. 24hour recall and food record),and folate matrix (categories: 1. folate from the diet, 2. folate from the diet and supplements) were variables that modified the association.

Statistical analysis

The intake-status relationship was assumed to be linear on the $\log_e$-$\log_e$ scale (natural logarithm of dependent and independent variable). It was assumed that this relationship would be linear on this scale with a coefficient between -1 and +1, e.g. positive values would imply on the arithmetic scale a monotonic concave curve that steeply increases at low intakes and less so at higher levels, which is a common shape in biology (5, 6, 18).

Assuming this model, summary statistics from each study were transformed into estimates of dose-response relationship (6): a regression coefficient (beta) and the standard error (SE (beta)) of the linear regression coefficient of micronutrient status on micronutrient intake (5). The individual estimates of the dose–response regression coefficients were combined into an overall pooled beta and SE (beta) by means of random effects meta-analysis, which incorporates the between study variation using the method of DerSimonian and Laird (19) to obtain the weights required for the summary estimate. Residual

heterogeneity between studies was evaluated using the I^2 statistic. Pre-specified factors that could modify the association were explored using meta-regression. The statistical transformations to obtain β and SE (β) were performed using GenStat version 13-SP2 (VSN International Ltd., http://www.vsni.co.uk/) and the meta-analysis was performed using STATA version 10.0 (College Station, TX), with statistical significance defined as $P<0.05$.

Results

Serum/Plasma Folate

We identified 19 estimates from 14 observational studies of folate intake and serum/plasma folate status that were eligible for our meta-analyses. One paper reported results separately for 3 different genotypes and 3 papers reported results for males and females separately. In total 7,308 subjects were included with mean age of 48.9 years and sample sizes that ranged from 53 to 1275 subjects (Table 2). The mean folate intake ranged from 152 to 468 μg/day. All associations were obtained from cross sectional analyses, although some were derived from nested case-control studies and from a prospective cohort study.

Combining these estimates in one meta-analysis yielded an overall pooled beta-coefficient of 0.33 (95%CI: 0.22-0.44; I^2=94%) (Figure 2). This implies that for every doubling in folate intake, the difference in serum/plasma folate concentration increases by 2^{β} or 26% ($2^{0.33}$= 1.26) Thus an average person with a folate intake of 100 μg/day has a serum/plasma folate status concentration that is 26% higher than a person who has a folate intake of 50 μg/day, or an average person with a folate intake of 200 μg/day has a serum/plasma folate status concentration that is 26% higher than a person who has a folate intake of 100 μg/day.

As the estimates showed substantial heterogeneity between the studies in the overall meta-analysis, the meta-analysis was subsequently stratified for folate matrix, dietary intake method and mean population age.

Stratification according to the matrix of provided folate (categories: 1. folate from the diet, 2. folate from the diet and supplements, whether or not converted to folate equivalents) yielded significantly different estimates of 0.49 (95% CI: 0.34-0.64) for dietary folate and 0.16 (95% CI: 0.07-0.26) for dietary folate plus supplements (either expressed as dietary folate equivalents or as folate μg/day). Although this difference was statistically significant ($p< 0.0001$), the heterogeneity within the strata remained high (I^2= 86 and 49%, respectively).

Stratification for the dietary intake method yielded different estimates for FFQs (0.36, 95% CI: 0.22-0.49) and 24hR and FR combined (0.19, 95% CI: -0.02-0.38), although this may be attributed to chance as the difference was not statistically significant (p=0.31). The between study heterogeneity remained high: within 24hR and FR it was I^2= 57%, and it remained high in the FFQ subgroup I^2=94%.

Each of these potential sources of heterogeneity was evaluated continuously and simultaneously in a meta-regression model. The results show that the matrix of provided folate was statistically significant determinants of the overall beta (p< 0.0001), unlike the dietary intake method (p=0.31). The age of the study participants was not statistically significant determinant of the overall association.

Red Blood Cell Folate

We identified 8 observational studies of folate intake and red blood cell folate status that were eligible for our meta-analyses and four of the studies on serum/plasma folate status provide red blood cell folate data, too. As one paper reported results for males and females separately, a total of 9 estimates was available for red blood cell folate, including a total of 1,670 subjects and sample sizes ranging from 53 to 439 subjects (Table 2). The mean folate intake ranged from 188 to 524 µg/day. All studies were cross sectional with the exception of one study which was originally prospective cohort and for that study data were analysed cross-sectionally at baseline. Combining the 9 estimates in one meta-analysis yielded an overall pooled beta-coefficient of 0.27 (95%CI: 0.15-0.38; I^2=90%) (Figure 3).

This means that for every doubling in folate intake, the difference in red blood cell folate concentration is 2^{β} or 21% ($2^{0.27}$= 1.21). Stratifying the analysis for the matrix of provided folate (categories: 1. folate from the diet, 2. folate from the diet and supplements) yielded different estimates (0.30, 95%CI: 0.05-0.55 and 0.23, 95%CI: -0.04- 0.51, respectively) although they did not differ significantly and did not reduce heterogeneity within the strata (I^2= 80% and 95%, respectively). Stratifying the analysis for the dietary intake method (categories: 1. FFQ and 2. 24hR and FR combined) yielded estimates of 0.16, 95%CI: 0.10-0.22 for FFQ and 0.35, 95%CI: 0.09-0.60 for 24hR and FR combined, and reduced the between study heterogeneity in the FFQ subgroup, but not in the latter subgroup (I^2= 0% and 87%, respectively). Each of these potential sources of heterogeneity was evaluated continuously and simultaneously in a meta-regression model. The results show that neither the dietary intake method (p=0.13) nor the matrix of provided folate (p=0.60) were statistically significant determinants of the overall beta. The age of the study participants was not statistically significant determinant of the overall association.

Combined analysis serum/plasma and red blood cell folate.

It was postulated that factors that explain heterogeneity in plasma/serum and red blood cell folate would be similar. Since both biomarkers yielded similar overall estimates, we combined the 19 estimates on folate serum/plasma and 9 estimates on red blood cell folate simultaneously in meta-regression model, with the biomarker medium (serum/plasma or red blood cell folate) as an additional covariable. The biomarker medium provided no statistically significant explanation (p=0.49). The combined estimates in one meta-analysis yielded an overall pooled beta-coefficient of 0.31 (95%CI: 0.23-0.39). In this analysis the estimates for dietary matrix were 0.42 (CI%: 0.31-0.54) and 0.19 (CI%: 0.12-0.26) for diet versus diet plus supplements, respectively, and 0.31 (CI%: 0.21-0.42) and 0.29 (CI%: 0.15-0.43) for FFQ versus 24hR and FR combined, respectively.

Plasma homocysteine

We identified 5 observational studies of folate intake and plasma homocysteine status that were eligible for our meta-analyses; these studies gave information on serum/plasma folate status, too. One paper reported results separately for 3 different genotypes. In total, we had 7 estimates for plasma homocysteine from 5 observational studies including a total 2,821 subjects with sample sizes ranging from 99 to 938 subjects (Table 2). The mean folate intake ranged from 206 to 330 µg/day. All studies were cross sectional with the exception of two studies which were nested case-control studies and for those studies data were analysed cross-sectionally at baseline. Combining the 5 observational studies in one meta-analysis yielded an overall pooled beta of -0.26 (95%CI: -0.39 to-0.13; I^2=92.4%) (Figure 4). Thus, a doubling of folate intake goes together with a difference in plasma homocysteine concentration of 2^{β} ($2^{-0.26}$= 0.84), which is -16%. Stratifying the analysis for the matrix of provided folate (categories: 1. folate from the diet, 2. folate from the diet and supplements) yielded different estimates (-0.32, 95%CI: -0.66-0.01 and -0.13, 95%CI: -0.48- 0.22, respectively).They did not differ significantly and the heterogeneity was reduced only in the latter subgroup (I^2= 0%), whereas in the former it remained high (I^2= 93.3%).

We did not perform stratified meta-analyses or meta-regression for the dietary intake method and total homocysteine because of the small number of studies.

The association between total folate intake (folic acid from supplements and/or fortified foods plus dietary folate) and its biomarkers in plasma/serum, red blood cells, and plasma homocysteine levels in adults and elderly, stratified by folate matrix and dietary intake method is summarized in Table 3.

Table 2 General characteristics of the included observational studies reporting the association between folate intake and the biomarkers of folate status: red blood cell folate, plasma or serum folate and plasma homocysteine

First author, year	N	Sex	Mean age (years)	Folate intake (µg/day)	Folate intake (source)[1]	Folate intake (assessment)	Folate status biomarker (analytical method)
Plasma or serum folate							
Arnaud et al., 2001 (20)	106	M	39	152.0	Diet	Food record (7 days)	Protein-binding assay
Ashfield-Watt et al., 2003 (21)	133	M&F	41	242.0	Diet	FFQ	Protein-binding assay
Chew et al., 2010 (17)	100	M&F	28	296.0	Diet	1x24HDR	Microbiological assay
Colic Baric et al., 2009 (22)	99	F	53	252.6	Diet& Supplements[1]	FFQ	Ion-Capture-Assay
de Bree et al., CC genotype, 2003 (13)	938	M&F	41	205.0	Diet	FFQ	Lact. caseimicrob. aasay
de Bree et al., CT genotype, 2003 (13)	907	M&F	41	203.0	Diet	FFQ	Lact. caseimicrob. aasay
de Bree et al., TT genotype, 2003 (13)	206	M&F	41	201.0	Diet	FFQ	Lact. caseimicrob. aasay
Drogan et al., 2004 (15)	363	M&F	52	226.9	Diet & Supplements[1]	FFQ	Ion-Capture-Assay
Glyn et al., 1996 (23)	249	M	60	320.0	Diet& Supplements	FFQ	Radioassay
Johansson et al., males, 2010 (24)	96	M	45	196.0	Diet& Supplements	10x24HDR	Radioassay
Johansson et al., females, 2010 (24)	99	F	45	181.0	Diet& Supplements	10x24HDR	Radioassay
Melse-Bunstra et al., males, 2002 (12)	1275	M	41	232.0	Diet	FFQ	Microbiological assay
Melse-Bunstra et al., females, 2002 (12)	1160	F	41	186.0	Diet	FFQ	Microbiological assay
Sedjo et al., 2002 (14)	179	F	27	418.6	Diet& Supplements	FFQ	Radioassay
Shuaibi et al., 2008 (25)	70	F	21	447.9	Diet& Supplements[1]	Food record (3 days)	Radioassay
Van Guelpen et al., males, 2009 (11)	704	M	51	249.0	Diet& Supplements	FFQ	Radioassay
Van Guelpen et al., females, 2009 (11)	293	F	60	249.0	Diet& Supplements	FFQ	Radioassay
Verkleij-Hagoort et al., 2007 (26)	53	F	32	177.0	Diet	FFQ	EDTA Hemat. Anal.
Weinstein et al., 2008 (16)	278	M	58	330.7	Diet	FFQ	Radioassay

Table 2 (continued) General characteristics of the included observational studies reporting the association between folate intake and the biomarkers of folate status: red blood cell folate, plasma or serum

folate and plasma homocysteine

First author, year	N	Sex	Mean age (years)	Folate intake (µg/day)	Folate intake (source)[1]	Folate intake (assessment)	Folate status biomarker (analytical method)
Red blood cell folate							
Chew et al., 2010 (17)	100	M&F	28	296.0	Diet	1x24HDR	Microbiological assay
Colic Baric et al., 2009 (22)	99	F	52	252.6	Diet& Supplements[1]	FFQ	Ion-Capture-Assay
Drogan et al., 2004 (15)	363	M&F	52	226.9	Diet & Supplements[1]	FFQ	Ion-Capture-Assay
Fayet et al., 2010 (27)	53	F	22	383.0	Diet	3x24HDR	Chemiluminescent assays
Hoey et al., 2007 (28)	439	M&F	62	250.3	Diet& Supplements	Food diary (4 days)	Microbiological assay
Knutsen et al., 2001, nonhispanic blacks (29)	97	M&F	47	306.6	Diet	8x 24HDR	Radioimunoassay
Knutsen et al., 2001, nonhispanic whites (29)	96	M&F	53	372.7	Diet	8x 24HDR	Radioimunoassay
Owens et al., 2007 (30)	370	M&F	44	524.0	Diet& Supplements[1]	FFQ	Chemiluminescent assays
Verkleij-Hagoort et al., 2007 (26)	53	F	32	177.0	Diet	FFQ	EDTA Hemat. Anal.
Plasma homocysteine							
Chew et al., 2010 (17)	100	M&F	28	296.0	Diet	1x24HDR	HPLC
Colic Baric et al., 2009 (22)	99	F	53	252.6	Diet& Supplements[1]	FFQ	Fluor. Polar. Imm.
de Bree et al., CC genotype, 2003 (13)	938	M&F	41	205.0	Diet	FFQ	Protein-bound assay
de Bree et al., CT genotype, 2003 (13)	907	M&F	41	203.0	Diet	FFQ	Protein-bound assay
de Bree et al., TT genotype, 2003 (13)	206	M&F	41	201.0	Diet	FFQ	Protein-bound assay
Van Guelpen et al., 2009 (11)	293	F	60	249.0	Diet& Supplements	FFQ	Immunoassay
Weinstein et al., 2008 (16)	278	M	58	330.7	Diet	FFQ	HPLC

[1]Studies reported total folate intake as folate from the diet and supplements converted into dietary folate equivalents DFE/day, other studies reported folate µg/day

Abbreviations: N, number; SD, standard deviation; M, males; F, females; 24HDR, 24 hour diet recall; FFQ, food frequency questionnaire; Lact. caseimicrob. aasay, Lactobacillus casei microbiological assay; EDTA Hemat. Anal., Ethylene-diamine-tetra-acetate (EDTA) hematological analyses; Fluor. Polar. Imm. Fluorescence Polarization Immunoassay HPLC, High Pressure Liquid Chromatography.

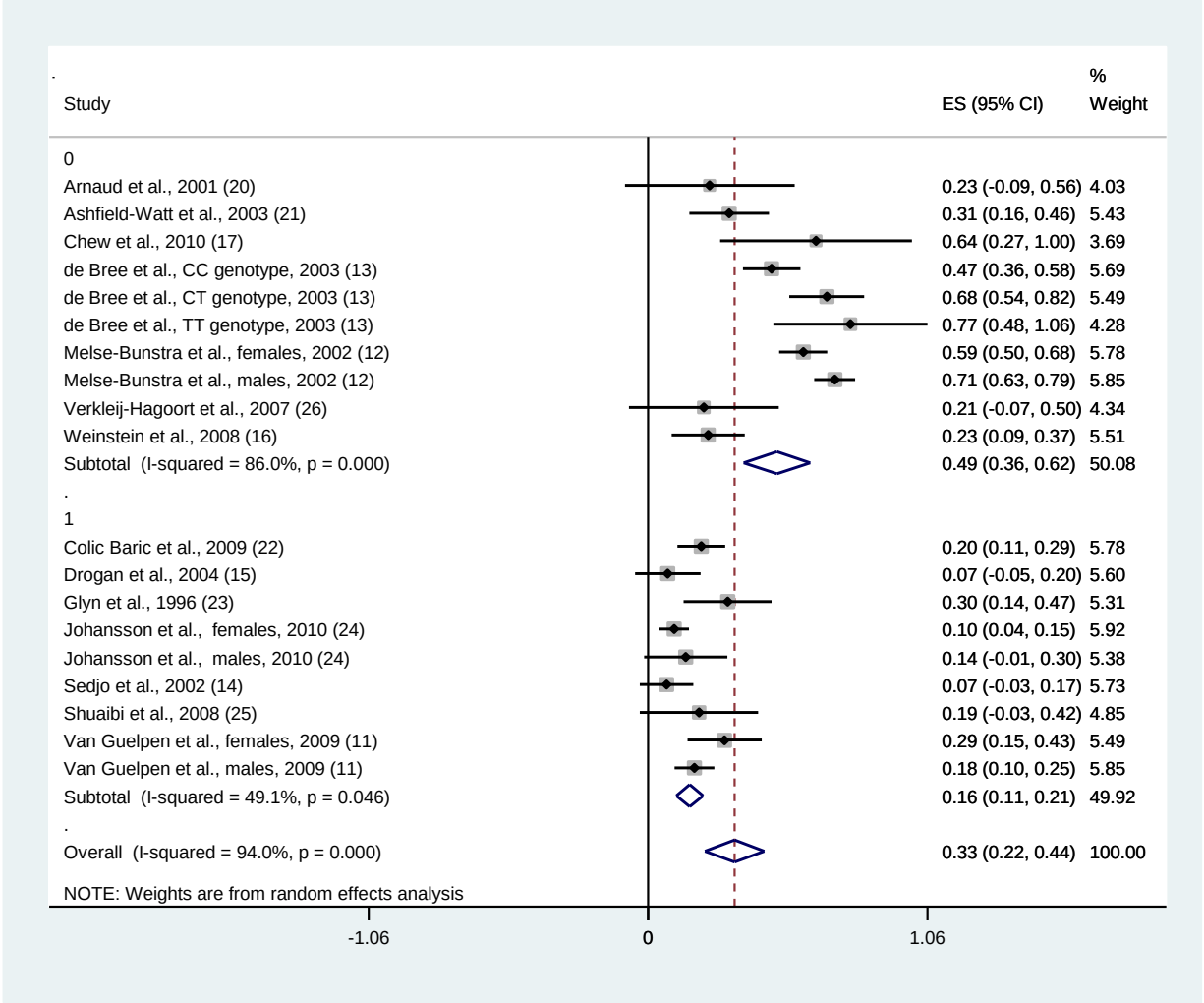

Figure 2 Random effects meta-analyses of observational studies stratified for the matrix of provided folate (dietary folate vs. dietary folate including folic acid) evaluating the effect of dietary folate on serum/plasma folate in adults and elderly. Beta's represent the regression coefficients for the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed serum/plasma folate status (lines represent the confidence intervals of each study).

0= Subgroup with dietary folate. (In all studies folate intake was estimated as natural food folate expressed in µg/day.)
Heterogeneity: Tau-squared = 0.0317; Chi-squared = 64.13, d.f. = 9 (p< 0.0001); I-squared = 86%
Test for overall effect: Z = 7.57 (p <0.0001)

1= Subgroup with dietary folate including folic acid. (In all studies folate intake was estimated as natural food folate plus folic acid from supplements and fortified foods. In studies (15), (22) and (25) it was expressed in DFE, whereas in other studies it was expressed in folate µg/day.) Heterogeneity: Tau-squared = 0.0027; Chi-squared = 15.73, d.f. = 8 (p= 0.05); I-squared = 49%
Test for overall effect: Z = 6.01 (p <0.0001)

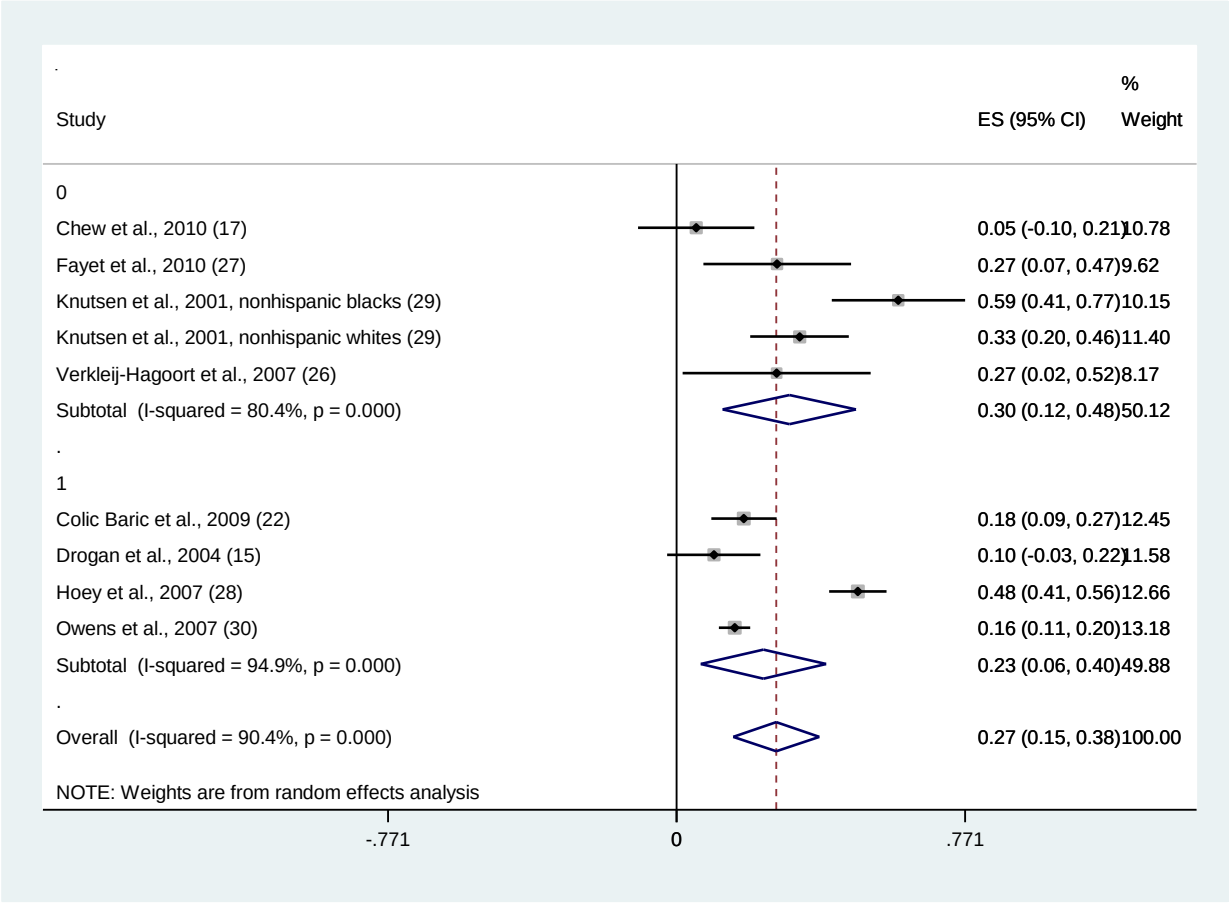

Figure 3 Random effects meta-analyses of observational studies stratified for the matrix of provided folate (dietary folate vs. dietary folate including folic acid) evaluating the effect of total dietary folate on red blood cell folate (RBC) in adults and elderly. Beta's represent the regression coefficients for the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed RBC folate status (lines represent the confidence intervals of each study).

0= Subgroup with dietary folate. (In all studies folate intake was estimated as natural food folate expressed in µg/day.)
Heterogeneity: Tau-squared = 0.0325; Chi-squared = 20.41, d.f. = 4 (p< 0.0001); I-squared = 80%
Test for overall effect: Z = 3.32 (p=0.001)

1= Subgroup with dietary folate including folic acid. (In all studies folate intake was estimated as natural food folate plus folic acid from supplements and fortified foods. In studies (15), (22) and (30) it was expressed in DFE, whereas in (28) it was expressed in folate µg/day.)
Heterogeneity: Tau-squared = 0.0274; Chi-squared = 59.20, d.f. = 3 (p< 0.0001); I-squared = 95%
Test for overall effect: Z = 2.71 (p=0.007)

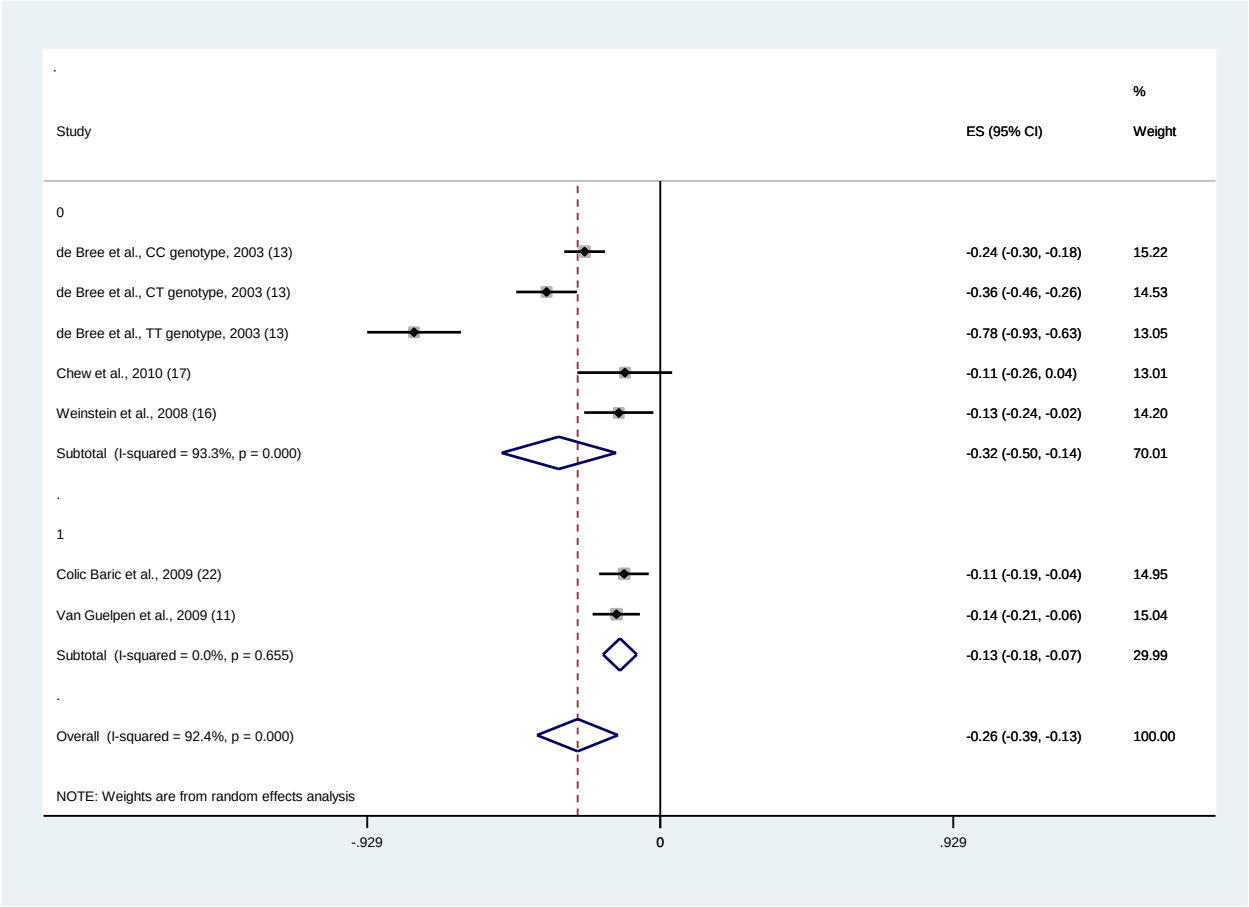

Figure 4 Random effects meta-analyses of observational studies stratified for the matrix of provided folate (dietary folate vs. dietary folate including folic acid) evaluating the effect of total dietary folate on plasma homocysteine folate in adults and elderly. Beta's represent the regression coefficients for the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed plasma homocysteine folate status (lines represent the confidence intervals of each study).

0= Subgroup with dietary folate. (In all studies folate intake was estimated as natural food folate expressed in µg/day.)
Heterogeneity: Tau-squared = 0.0394; Chi-squared = 59.39, d.f. = 4 (p< 0.0001); I-squared = 93%
Test for overall effect: Z = 3.46 (p=0.001)

1= Subgroup with dietary folate including folic acid. (In all studies folate intake was estimated as natural food folate plus folic acid from supplements and fortified foods. In study (22) it was expressed in DFE, whereas in study (11) it was expressed in folate µg/day.)
Heterogeneity: Tau-squared = 0.0000; Chi-squared = 0.20, d.f. = 1 (p = 0.66); I-squared = 0%
Test for overall effect: Z =4.63 (p < 0.0001)

Table 3 Associations between total folate supply (i.e. natural food folate plus folic acid from supplements and/or fortified foods) and folate in plasma/serum and in red blood cells, as well as on plasma homocysteine levels in adults and elderly, stratified by folate matrix and dietary intake method.

	Folate in serum/ plasma			Folate in red blood cells			Plasma homocysteine		
Stratum for analysis	No. of estimates (*n* participants)	Regression coefficient[1] (95% CI)	Heterogeneity I^2 (%)	No. of estimates (*n* participants)	Regression coefficient[1] (95% CI)	Heterogeneity I^2 (%)	No. of estimates (*n* participants)	Regression coefficient[1] (95% CI)	Heterogeneity I^2 (%)
All studies	19 (7308)	0.33 (0.22, 0.44) p<0.0001	94	9 (1670)	0.27 (0.15, 0.38) p=0.002	90	7 (2821)	-0.26 (-0.39 to -0.13) p=0.03	92
Folate matrix									
Diet (folate µg/day)	10 (5156)	0.49 (0.34, 0.64) p<0.0001	86	5 (399)	0.30 (0.05, 0.55) p=0.03	80	5 (2429)	-0.32 (-0.66, 0.01) p=0.06	93
Diet & supplements (folate µg/day)	9 (2152)	0.16 (0.07, 0.26) p=0.007	49	4 (1271)	0.23 (-0.04, 0.51) p=0.08	95	2 (392)	-0.13 (-0.48, 0.22) p=0.13	0
Dietary intake method									
Food Frequency Questionnaire	14 (7035)	0.36 (0.22, 0.49) p<0.0001	94	4 (885)	0.16 (0.10, 0.22) p=0.003	0	6 (2721)	-0.29 (-0.55, -0.03) p=0.04	93
24-hour Recall and Food Record	5 (471)	0.19 (-0.02, 0.38) p=0.06	57	5 (785)	0.35 (0.09, 0.60) p=0.02	87	1 (100)	*-0.11 (-0.26, 0.04)* p = 0.05	n.a.

[1] Regression coefficients for the linear association between $\log_e$ transformed folate intake and $\log_e$ transformed folate status markers

Abbreviations: N.A= not available

Discussion

This systematic review gives a summary of available data on the association between folate intake and its biomarkers for adults and elderly. This evidence can be used as complementary data in underpinning folate DRVs which vary greatly across countries. For example, the Average Nutrient Requirement (ANR) for folate for adults and elderly in both genders ranges from 200 μg/day in the Nordic nutrition recommendations to 320 μg/day as proposed by the Institute of Medicine, USA and the World Health Organization (31, 32, 33). The main effect found by pooling data from the observational studies showed that total folate intake (i.e. folic acid plus dietary folate) is significantly associated with folate status markers: serum/plasma folate, red blood cell folate and total plasma homocysteine concentrations in adults and elderly. The estimate of the dose- response relationship of folate intake and folate serum or plasma concentration of 0.33 means that for every doubling in folate intake, the increase in folate serum or plasma concentration amounts to 26%. Meta-analysis of observational studies on folate in red blood cells provided an overall beta of 0.27, meaning that doubling the folate intake increases the concentration of the folate in red blood cells by 21%. Meta-analysis of observational studies on total plasma homocysteine yielded an overall beta of -0.26, meaning that doubling the folate intake decreases the total plasma homocysteine concentration in 16%.

The studies included in this meta-analysis were different in terms of study objectives, populations, analytical methods that were used to measure levels of biomarkers and dietary assessment including the quantification of folate in either μg of folate/day or as DFE/day). Therefore, it is no surprise that, when combining these studies in a meta-analysis, a large heterogeneity between the studies is observed (I^2 ranged from 90-94%).The matrix of provided folate was a statistically significant determinant of the association between folate intake and serum/plasma folate, so we examined it more in detail. Dietary folate intake can be expressed as DFE, which takes differences in bioavailability in account, as synthetic folate is 1.7 times more bioavailable than naturally occurring folate (22, 34).Thus, to obtain the DFE, the folate content of foods fortified with folate and the folate content of dietary supplements should be multiplied by 1.7. For studies that assessed folate intake from the diet including the use of supplements, the conversion into DFE was done in 4 out of 12 studies. Also, some studies used 100-200μg/day of folic acid as a conservative estimate based on available supplement and added that amount with no conversion to DFE to total dietary folate (11, 23, 24). The lack of standardization in reporting total folate intake as DFE might have induced bias in the estimation of the associations. However, as reported in the studies included in this review (11, 16, 20, 21, 23, 25), the proportion of regular and occasional supplement users was between 5-26%, with average supplement dose up to 200 μg/day, so we assume that inconsistency in reporting folate intake in terms of DFE may have influenced our estimates. Further to this, we found statistically significant stronger association for the estimate based on studies that included diet without supplement use in comparison to those in

which folate supply was from diet and supplements. This is in line with results from a similar systematic review of RCTs (rather than observational studies) among women in childbearing age and in pregnancy and lactation (18). The reason might be that despite its lower bioavailability, other factors make naturally occurring folate as effective as or even more effective than folic acid in preserving folate status (18). Also, it should be mentioned that since the key source of folate is fruit and vegetables, for the estimation of usual intake of folate in a population, the seasonal variation in periods when studies were conducted might affect the observed intake values. This observation can be of importance for serum/plasma folate as it reflects short term folate intake, whereas it has less influence on folate in red blood cells as it mirrors long term folate intake. This is consistent with our estimates as the association between folate intake and serum/plasma based on long term folate intake (reference period in FFQ >1 year) gives estimates similar to red blood cell folate.

The diversity in dietary assessment is another aspect that should be taken into consideration when examining the heterogeneity between studies, as it is known that dietary intake measurement errors differ considerably by dietary assessment method (35). For example, in general, FFQs are designed to rank individuals rather than to assess their absolute intake levels. Also, a small number of replicates of 24hR in the studies with no adjustment for intra-individual variability can give different estimates in comparison to that with multiple replicates. The FFQ was the most commonly used dietary instrument in this review; it was used to reflect folate intake for 3 months or 1 year. Although stratified meta-analysis for the dietary intake method gave different estimates between the FFQ subgroup and 24hR and FR combined, dietary intake method did not statistically significant predict the overall beta for any of the biomarkers. To some extent, the reason for high heterogeneity in meta analysis of serum/plasma folate for the studies with dietary assessment FFQ=1 year might be genetic variation in the 677C→T mutation in the gene which is known to significantly influence concentrations of folate status markers at folate intake levels <250 μg/day (13). Three estimates out of eight provided the information on gene mutation; however, the low number of studies and lack of gene mutation information from the other studies did not allow us to divide them into subgroups and perform further analysis.

Overall, when the pooled estimates from stratified subgroup analysis for folate matrix and for dietary intake method are looked at in detail, it seems that folate matrix, i.e. natural food folate is the most influencing factor on overall beta for each biomarker.

The relationship between folate intake and status in adults and elderly has also been analyzed in RCTs (5). The association in RCTs was stronger than in the observational studies in this paper, i.e. for every doubling in folate intake, the difference increase in folate serum or plasma concentration was 48%. A similar publication by Berti et al based on RCTs in women of reproductive age showed the relationship for folate in red blood cells and for total plasma homocysteine similar to those reported in the present study: 0.33 (CI%: 0.23-0.44) and -0.12 (CI%: -0.15 to -0.08), respectively, whereas the association for serum/plasma folate was stronger 0.65 (CI%: 0.39-0.93) than in our data (18).

The differences in assessment of folate intake and other (non-estimable) factors that induce between study heterogeneity are of relevance to the interpretation of the results from this study and for their application in applied nutritional research. Still, this study contributes to existing quantified dose-response relationships between folate intake, status and relevant health outcomes in adults and elderly. It enables assessment of intake-status relationship across a broad range of intakes in a large number of subjects in very diverse natural settings which should be considered when using this evidence in underpinning DRVs. Specifically, it can be used in further modeling together with the estimates from randomized controlled trials (6). The principle of that methodology has been developed with the EURRECA NoE, aiming to harmonize DRVs which vary greatly across countries (10).

The observed mean folate intake in this review (251 μg/day) is in line with intakes from individual observational studies that assessed folate intake in adults and elderly in Europe. For example, the mean folate intake for adults and elderly in the European Nutrition and Health Report (25 countries) was in range from130-370 μg/day in women and from 150-440 μg/day in men (36), whereas the results from the EPIC cohort (10 countries) reported 200 to 300 μg/day in women and 250 to 350 μg/day in men (37). The levels of serum/plasma folate reported in recent publication on micronutrient intake and status is Europe were 7-18 nmol/L, which are lower than those observed in our review (5-31 nmol/L) (38). The reason for the discrepancy in status levels between the two studies might be that the former included different study populations, e.g. studies were limited and performed in European populations only, whereas our review compiled data from Europe and worldwide.

The present study summarizes available I-S evidence from observational studies. It confirms the need for methodologically comparable data on food composition and folate intake, taking into account intakes of both natural food folate and folic acid obtained through supplements and fortified foods, and corresponding biomarker status (2). It also shows that dietary folate intake and the levels of serum/plasma folate as shown in this study are similar to those reported in individual studies, and therefore reasonably represent folate intake and status in adults and elderly. The quantified estimates of folate intake-status relationship stem from the evidence base with high heterogeneity, partially explained by diverse dietary assessment methods, and that should be taken into account when using present findings for future research.

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Chapter 6

General discussion

Main findings

The observed micronutrient intake and status in Central and Eastern Europe (CEE) are in the same ranges as those in other European regions with the exception of calcium intake in adults and iodine status in children, which were lower in CEE countries. When micronutrient intake and status in European populations (as from the individual participant data) were examined for their association with education, the results showed a positive association with the intake of calcium, vitamin C and folate, e.g. the lowest educated groups had 12-23% lower intake than the highest education groups. The association with intake of zinc and vitamins D and B12 was not consistent, whereas the intake of iron marginally differed between the lowest and the highest education groups. When the associations between education and micronutrient intake and status were examined at the country level, the differences in micronutrient intake between the countries were larger than between education groups within these countries. Data from individual studies showed that the largest differences by educational level were observed for intake of calcium, vitamin C, folate and vitamin D (up to 47% lower for low socioeconomic status (SES) level). Finally, the intake of folate, which is identified as one of the micronutrients of concern, was clearly associated with folate status markers in adults and elderly: doubling folate intake from e.g. 50 to 100 µg/day, increases the folate level in serum/plasma and in red blood cells by 26% and 21%, respectively, whereas total plasma homocysteine was –16% lower for doubling the intake.

Table 1 summarizes the results by micronutrient and identifies the remaining knowledge gaps.

Evaluation of micronutrient intake and status relies on assessment of the distribution of usual micronutrient intake and status. The intake data are then compared to DRVs to estimate the prevalence of inadequacy and to identify groups at risk of deficiency. Ideally, assessment of nutritional status on the country level is based on individual participant data collected from a country representative sample of all age ranges, using the best practice methodology for assessment of dietary intake and biomarkers of status. The Scientific Panel of the European Food Safety Authority recommends that nutritional surveys should cover two non-consecutive days and use the dietary record method for infants and children and the 24-hour recall method for adults (1). Unfortunately, country representative nutritional surveys are expensive and not common practice. Some countries have surveys carried out at the regional or local level only (2). Since methodologically comparable pan-European nutrition surveillance has not been established yet, current research has to rely on existing but not harmonized data. The variability in study designs of existing surveys prohibits between study and cross country comparisons. However, lack of uniformity in study designs doesn’t mean there’s no benefit in comparing. First, a systematic review provides an overview of data availability and thereby it indicates the knowledge gaps and research needs. An analysis of such data establishes whether the findings of the included studies are consistent and can be generalized to different populations, whether there may

be methodological differences or other population characteristics that explain the heterogeneity, or whether the heterogeneity remains unexplained by the postulated factors.

In this general discussion, we will discuss methodological issues relevant to Chapters 2-5, and come to directions for future research and possible public health implications.

Table 1 Main findings and knowledge gaps on micronutrient intake and status in populations in the European region.

Micronutrient[1]	Chapter 2	Chapter 3	Chapter 4	Chapter 5
	a) Do CEE countries differ in micronutrient intake and status in comparison to other European regions? b) What are remaining knowledge gaps?	a) Are micronutrient intake and status lower in low than in high SES groups? b) What are remaining knowledge gaps?	Is micronutrient intake lower in low than in high educated groups?	a) What is the relationship between folate intake and status? b) What are remaining knowledge gaps?
Calcium	a) Yes- lower in adults in CEE than in other European regions b) Intake data for CEE children	a) Yes b) Intake data for CEE countries	Yes	--
Vitamin C	a) No b) Intake data for CEE children	a) Yes b) Intake data for CEE countries	Yes	--
Folate*	a) No b) Status data for CEE populations of all age ranges	a) Yes b) Intake and status data for all age ranges Europe-wide	Yes	a) doubling of folate intake increases serum/plasma folate by 26% and red blood cell folate by 21%, and it decreases total plasma homocysteine by 16%; b) dietary data that includes detailed information on folic acid obtained through supplements and fortified foods
Vitamin D	a) No b) Intake data for CEE children	a) Yes b) Intake data for all age ranges Europe-wide	Inconsistent findings	--
Vitamin B12*	a) No b) Intake and status data for CEE populations of all age ranges	a) Inconsistent findings b) Status data for all age ranges Europe-wide	Inconsistent findings	--
Iron*	a) No differences in females. Males in CEE had slightly higher intake than those in other European regions b) Status data for CEE adults	a) Yes for intake, No for status b) Status data for all age ranges Europe-wide	No difference	--
Iodine*	a) Yes- CEE children had lower iodine status b) Status data for CEE adults	a) Yes for intake, No for status b) Status data for all age ranges in CEE	--	--
Zinc*	a) No b) Status data for CEE populations of all age ranges	a) Yes for intake, No for status b) Intake and status data for all age ranges Europe-wide	--	--
Selenium	a) No b) Intake and status data for CEE populations of all age ranges	a) Yes b) Intake and status data for all age ranges Europe-wide	--	--
Copper	a) No c) Intake and status data for CEE populations of all age ranges	a) Yes b) Intake and status data for all age ranges Europe-wide	--	--

[1] Results in the Table 1 refer to data on micronutrient intake; dashes (--) indicate that these micronutrients were not addressed. For micronutrients indicated with a * the research in chapters 2 and 3 did not only address intake data, but also data on micronutrient status (biomarkers); Abbreviations: CEE, Central and Eastern Europe; SES, socioeconomic status

Micronutrient intake and status of populations

Comparison of micronutrient intake and status in CEE countries with that in other European regions was done in Chapter 2. Data from CEE were collected from open access and grey literature whereas the studies from Western Europe, Scandinavia and Mediterranean countries were nutritional country reports gathered in the European Nutrition and Health Report 2009 (ENHR II) (2). As the objective of the ENHR II was to collect existing data, collated studies showed methodological differences in nutritional assessment and age ranges. The individual studies from CEE differed in terms of studies' designs, too. Therefore, study methodologies of data both from CEE and non-CEE countries were diverse with respect to dietary intake methods, food composition databases, recruitment method and age distribution of the subjects. The heterogeneity in study designs limits comparability across studies, and causes variation in reported mean intakes. For example, application of the 24h recall method conducted over a short period of time or with multiple passes through the preceding day may produce different estimates (3). Also, FFQs might underestimate or overestimate nutrient intake, depending on the number of food items included in the food list and how portion sizes were quantified. As from our review, the duplicate 24 hour recall (the preferred dietary assessment method proposed by EFSA) was not consistently used: studies applied one or more replicates of 24h recalls, validated and non-validated FFQs and food diaries. Challenges with comparing studies with different methodologies compiled in ENHR II are already addressed in a review by Roman Vinas et al (4). Basically, countries that were using the food diary as dietary intake instrument had mean intake values for vitamin C and iron lower than countries using an FFQ.

Mensink et al. (3) recently evaluated the prevalence of low micronutrient intakes in Europeans of all age ranges based on country representative individual participant data. Currently, that is the most comprehensive evidence of micronutrient intake based on a compilation of single studies from 8 European countries, with only one CEE country (Poland). Except for vitamin D, the results showed low risk of low micronutrient intakes in all age and sex groups. The surveys included in that review differed with respect to study methodology, but access to raw data enabled estimation of mean intakes for uniform age groups. The levels of micronutrient intake reported in Chapter 2 were within the ranges of those reported by Mensink et al, were based on estimates form the ENHR II from 25 country studies, peer reviewed published data (38 studies) and included grey literature (9 studies) and 34 studies from the WHO VMNIS database (6). Based on the results from Chapter 2, it is likely that the prevalence of low micronutrient intakes is low among CEE countries as well, with the exception of calcium. When the limited data on micronutrient intake and status in children in CEE countries were compared to the review of 23 studies on dietary intake in European children and adolescents by Lambert et al (5), no differences were observed between CEE and other European countries.

As from this thesis, the results on micronutrient status point at a risk of iodine deficiency in children in CEE countries and in adults in Romania. We compiled data on iodine status from the grey literature and the WHO database, therefore it is no surprise that our results are similar to those in the publication by Zimmerman et al (7) which were based on the WHO sources only. Because the WHO reports that iodine deficiency is a continuing public health problem that affects health outcome across all age ranges, monitoring iodine status in CEE is indeed essential to prevent iodine deficiency related disorders. It should be noted that the WHO VMNIS database (6) is the most comprehensive source of iodine and iron status data for European children and adolescents, but not for other life stages.

To our knowledge, this was the first time that national studies and nutrition reports written in the original languages from Central and Eastern Europe were collected, translated if necessary, checked for compliance with study inclusion criteria, and if relevant further analysed. Even though future reviews should consider grey literature when assessing nutritional population health, they will tend to be hampered by methodological differences, which make it unlikely that this approach can fill the substantial knowledge gaps on micronutrient intake and status in CEE populations, especially in children. For example, country representative surveys for all life stages for Albania, Bosnia, Croatia, and the Republic of Srpska are still lacking, and no single study on the regional or local level that reports micronutrient intake was found for Romania. Although no differences in micronutrient intake and status between CEE and other European regions were found (except lower intake of calcium in CEE adults and iodine status in CEE children), it should be noted that this thesis addressed mean intakes and that the differences in methodology did not allow the evaluation of the distribution of micronutrient intake and status and estimation of prevalence of inadequacy.

In conclusion, data on micronutrient intake show no difference in intake of the 10 public health priority micronutrients between CEE populations and other European regions. However, given the apparent width of socioeconomic inequalities in CEE countries (8), it might be that the micronutrient intake and status might be lower than anticipated. Because there is an evident lack of data on children in CEE countries, it would be worthwhile that future surveys fill this knowledge gap.

Micronutrient intake and status in socioeconomically disadvantaged groups

Chapters 3 and 4 describe the extent to which micronutrient intake and status differ across socioeconomic layers. The data that were analysed in Chapter 3 were heterogeneous with respect to study methods, i.e. dietary intake methods, description of SES categories, selection of participants. The influence of varying dietary assessment on between- study and cross-country comparison has already been addressed in the former section of this chapter. However, it is worthwhile adding that SES may play a role in self-reported dietary intake, since it was found that the likelihood of biased

reporting is higher in persons of low SES (9, 10). On the other hand, selective over-reporting toward social desirability was found among higher social classes (11). Therefore, it might be that the observed variation in micronutrient intakes, for example, for micronutrients that are supplied from fruit and vegetables is under- or overestimated, but we have no data that can help us to evaluate the quantitative impact of this type of reporting bias.

For the assessment of SES, we followed guidance from available literature which suggests that education, occupation and income are the most relevant indicators. It has been suggested that these parameters cover different aspects of the socio-economic structure and thus may each contribute individually to the relationship between SES and diet (12). However, some evidence points out that occupation, income and education as social class markers behave uniformly with regard to eating patterns (13). Our decision to report the results on micronutrient intake/status by each of these indicators allows evaluation to what extent the findings for one indicator are confirmed by the other(s). In the systematic review in Chapter 3, it was observed that similar associations were found when SES differences in micronutrient intake were related to education and occupation (especially among women), and that data on income was least available. In addition, systematic reviewing enables to examine data availability and where future research should be targeted.

Because SES indicators were not used in a standardized way in individual studies, it might be that comparing broad SES groups rather than the most extreme groups resulted in an underestimation of existing differences. This methodological limitation was bypassed using the individual participant data with uniform measurement of SES from the EPIC cohort. Another advantage of this dataset was the possibility to use the relative index of inequality which accounts for unequal SES group sizes in different countries. In addition, because countries applied the same dietary assessment method, cross-country comparisons in mean micronutrient intakes were not affected by different dietary assessment methods. The analysis of 6 public health priority micronutrients available in the EPIC cohort (Chapter 4) confirmed results obtained from the systematic review (Chapter 3), with exception of iron: in the EPIC cohort there were no differences between education groups, but a lower iron intake was found in low versus high SES groups in the systematic review. This discrepancy might be caused by the methodological diversity among individual studies collated in the review in comparison to results from homogenous measurements of both dietary assessment and SES in the EPIC dataset.

To our knowledge, no other studies on micronutrient intake and SES in Europe other than those compiled in the review in Chapter 3 were available for comparison with our results. The recent results from non-European countries, such as a study from the USA by Aggarwal et al. showed that persons who consumed cheaper, lower quality diets were more likely to be from lower SES groups with lowest intakes of dietary fibre, vitamins A, C, D, E, and B12, beta carotene, folate, iron, calcium, potassium, and magnesium (13). However, the SES differences in intake of iron and folate were small, possibly

because of the relatively low cost of grain products fortified with iron and folate in the USA. The findings for SES differences in intake of calcium, vitamin C and folate in the US are in line with those reported in the present thesis.

Finally, it should be added that the range of differences in micronutrient intake between SES groups is lower than that between countries. It was in particular evident for those micronutrients which are mainly supplied by fruit and vegetables and dairy products, and for vitamin D which is mostly supplied by fish and meat. Obviously, the culturally inherited variation in regional dietary patterns, already shown in the analysis of the EPIC cohort and in the review by Roos et al. (14-16), has a strong impact on food choice and, therefore, on the level of micronutrient intake in all SES groups. For example, in Mediterranean countries, fruit and vegetables are a major source of folate, whereas in Northern Europe it is cereal products (15). Overall, these findings can help to understand what aspects of diversity in European food patterns could be of interest for achieving adequate dietary patterns in public health nutrition strategies.

The association between folate intake and biomarkers of folate status in adults and elderly

Folate is a public health priority micronutrient which was studied more in detail in this thesis. The reason for that is twofold. At first, because folate dietary reference values vary greatly, their re-evaluation is urgently needed. The Average Nutrient Requirement (ANR) from the Nordic nutrition recommendations is 200 μg/day, whereas the Institute of Medicine, USA and the World Health Organization propose 320 μg/day for adults and elderly in both genders (17-19). Secondly, the reviewed cross-sectional data at the ecological level and the individual participant data showed broad ranges of mean folate intake in European adults and elderly: e.g. from 130-370 μg/day in women and from 150-440 μg/day in men (2, 20, 21). Moreover a positive association was observed between SES and folate intake which implies that those at the bottom of the SES gradient are at higher risk of low folate intake than those of high SES (Chapters 3 and 4). Diversity in folate reference values hampers evaluation of adequacy in folate intake and brings uncertainty to public health policy makers. In addition, the fact that natural food folate and folic acid from supplements and fortified foods do not have equal bioavailability is not considered in some countries' recommendations. For example, in the USA, folate intake is expressed as μg of 'Dietary Folate Equivalents' (DFEs= the amount of natural food folate plus 1.7 times the amount of added folic acid in food), whereas in the research reported from most European countries this conversion factor is not applied and folate intakes and recommendations are expressed simply as total folate in μg/d, thus disregarding the known differences in bioavailability between the natural food forms and folic acid (22). Further to this, studies that report on mean folate intake use the conversion of folic acid to DFE inconsistently. These issues make comparison of folate intake and folate dietary values even more challenging.

By definition, the ANR is the amount of folate that would suffice to meet the needs of 50% of apparently healthy individuals (18). Current estimates of the ANR for folate are based on the concentration of folate in blood or tissues which is associated with no deficiency symptoms, and the role of folate in the prevention of neural tube defects has played in a major role in advice to women who anticipate a pregnancy. Folate in red blood cells and serum/plasma folate are the biomarkers of folate status which were most often used as primary health indicators for establishing an ANR, although cut-off values for adequacy varied across countries' recommendations (22). In the EURRECA Network of Excellence, it was proposed to derive the ANR as the intake at the intersection of the intake-status relationship with the clinically relevant cutpoint for serum/plasma folate or folate in red blood cells (23). For that purpose, data on the association between folate intake and status are needed. These can be obtained from dose response data from either RCTs or from observational studies, the first hampered by unnaturally high intake levels, the latter by measurement errors in exposure assessment.

The study in Chapter 5 fills part of the knowledge gap on the association between folate intake and biomarkers of folate status. The estimates reported in Chapter 5 stem from observational studies which are diverse in their study designs and methodologies. Although large heterogeneity remains, part of the between-study heterogeneity could be explained. Data on folate intake and status from less heterogeneous (methodologically comparable) surveys with low measurement error would give more valid and precise estimates of this relationship. Nevertheless, the methodology applied in this thesis uses best quality available data and gives valuable quantitative estimates that can be incorporated into on-going strategies aimed at harmonizing dietary recommendations. Specifically, it complements a series of systematic reviews that included only those studies which used the most robust measures to assess folate intake and status, briefly summarized in Table 2 (all originating from the EURRECA Network of Excellence). Apart from the intake-status relationship, folate intake and status were also reviewed as related to a number of health outcomes relevant to specific stages of the lifecycle. The RCT-based association among women in childbearing age (24) and those from adults and elderly (25) are very similar, although they used only 3 same studies in their analysis. The weaker associations between intake and status among pregnant and lactating women (24) as compared to those in women of reproductive age and adults and elderly (25) in RCTs might be due to the fact that the regression approach assumes a dynamic steady state of intake and status, which is disturbed owing to the anabolic needs of pregnancy and the loss via lactation. For plasma folate, the stronger association in RCTs in comparison to observational studies in adults and elderly might be attributable to both measurement errors in the intake data as well as to bioavailability of high doses of folic acid. In RCTs it appears unaltered in the circulation and gives rise to serum/plasma folate levels (Chapter 5).

Table 2 Overview of intake-status associations for folate intake and biomarkers of folate, in different population groups, based on similar dose-response meta-analyses of RCTs and observational studies

Reference	Study design	Number of studies (number of subjects)	Population group	Estimate of dose-response for serum/plasma folate (95% CI)	Estimate of dose-response for folate in red blood cells (95% CI)	Estimate of dose-response for plasma homocysteine (95% CI)
Berti et al. (20)	RCTs	4-7 (343-486)	Women in childbearing age	0.65 (0.39, 0.92)	0.33 (0.23, 0.44)	-0.12 (-0.15, -0.08)
		1-3 (30-204)	Pregnant women	0.52 (0.30, 0.75)	0.26 (0.15, 0.37)	-0.42 (-0.58, -0.25)
		2 (85)	Lactating women	0.36 (0.08, 0.79)	0.19 (0.09, 0.28)	0.03 (-0.04, 0.11)
Duffy et al. (21)	RCTs	19 (2341)	Adults and elderly	0.64 (0.51, 0.76)	0.43 (0.23, 0.62)	-0.09 (-0.12, -0.07)
Chapter 5 (Novakovic et al (26))	Observational studies	18 (1670-7308)	Adults and elderly	0.33 (0.22, 0.44)	0.27 (0.15, 0.38)	-0.26 (-0.39, -0.13)

Further analysis and modelling of these estimates from RCTs and observational studies for the three different biomarkers and incorporating observed levels of folate intake and status from dietary surveys will eventually contribute to evidence-based folate reference values. The EURRECA Network of Excellence has contributed with systematic literature searches and harmonized meta-analyses to advance this evidence-base. Nevertheless, harmonizing the dietary reference values, as for folate, remains a complex process at the cutting-edge of nutritional epidemiology, folate metabolism and societal concerns on population health outcomes.

Directions for future research

This thesis gives an overview of micronutrient intake and status in Europe, with a focus on the role of SES indicators. The results illustrate that cross country comparisons are hampered by: i) variation in the methodologies of available studies and ii) lack of intake and status data for many population groups and countries, especially in the CEE region. This underlines the need for pan-European Nutrition Surveillance with uniform methodology to fill the current knowledge gaps. Also, efforts are required to facilitate cross country comparisons of nutritional status data in Europeans of all age ranges, or in the identified risk groups. Since we found diversity in countries' study methodologies, we underline this necessity and suggest future surveys to monitor total food intake including detailed information on the consumption of fortified foods and supplements. Observed variation in micronutrient intake and status between SES groups requires continuous monitoring to detect trends and SES inequalities in micronutrient intake and status.

As we found low folate intake in European populations and because folate related health outcomes appear as pressing issue in the public health domain, we gave special attention to examining the folate intake and status relationship; the methodology of systematic searches should be further developed towards modeling to obtain estimates of nutrient intake reference values, and be applied to other micronutrients and trace elements, preferably by pooling the original data from the underlying studies. It is indeed essential to derive the optimal level of folate intake that leads to the most favorable health outcomes. In conclusion, we recommend to further develop the statistical modeling to derive micronutrient intake requirements and to further exploit the European diversity in food patterns as a major determinant of micronutrient intake in different countries and SES groups.

Public health implications

Because we observed a broad range of micronutrient intakes in European populations, we focused on low socioeconomic groups assuming that they might have low micronutrient intake, which, in turn, could be linked with poor health outcomes. The finding that low SES is associated with low intake of calcium, vitamin C and folate is important to identify priorities and design strategies to improve health in low SES groups within countries. Also, the extent to which low SES groups meet dietary recommendations should be further examined. Delivering these micronutrients of concern to target populations through fortification procedures or via supplement use are of relative importance, and the priority should be given to increasing dietary diversity because it is generally regarded as the most desirable and sustainable option to improve dietary habits (27). Specifically, promotion of intake of fruit and vegetables and dairy products among low SES groups should be considered in on-going and future public health strategies, accounting for the observed variation in traditional country specific dietary patterns across Europe. For example, translation of findings on micronutrient intake into foods can be done by formulating Food Based Dietary Guidelines that incorporate relevant parts of e.g. the Mediterranean region into the Western European food pattern, and vice versa. Food-based approaches usually take longer to implement than supplementation programs, but once established they are likely to be more sustainable (27).

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Et cetera

Summary

The aim of this thesis was to evaluate micronutrient intake and status of socioeconomic disadvantaged populations, such as from Central and Eastern European (CEE) as compared to other European populations, and low socioeconomic status (SES) groups as compared to high SES groups within European countries. We addressed the micronutrients that have been prioritized because of their relevance for nutritional health by the EC-funded EURRECA Network of Excellence. Moreover, we assessed the association between folate intake and status which can be used in the process of setting folate DRVs.

Micronutrient intake and status of CEE countries versus other European countries

CEE countries have recently experienced rising income inequalities over a period of economic transition. There is some evidence that these reforms have been accompanied by health inequalities. Inadequacy in micronutrient intake and status may contribute to these inequalities. Because in more affluent Western European countries wide ranges in micronutrient intake and status are observed, we studied if low micronutrient intake and status levels are prevailing in CEE. The findings from this thesis signal no differences in micronutrient intake and status between CEE populations in comparison to those of other European regions with the exception of calcium intake in adults and iodine status in children that were lower in CEE than in other European regions. Since data from Western Europe indicate that inadequacies do exist among SES strata, more insight in the nutritional situation of lower SES populations in CEE and an understanding of both its determinants and consequences is needed. It is important to mention that evidence from grey literature added to that from open access sources. Fundamental to further studying of nutritional health in CEE, is suitable data. We underline the necessity for conducting nutritional surveillances on micronutrient intake and status in CEE as we have identified significant knowledge gaps for many life-stage groups.

Differences in micronutrient intake between SES groups

Considering that not enough studies have addressed the relationship between SES and micronutrient intake and status in their analyses of nutritional health, we performed a systematic review on this topic and we used data from the large European EPIC cohort to address that issue.

To be able to conclude on socioeconomic, i.e. educational, occupational and income, inequalities associated with intake and status of prioritized micronutrients for all life stages in Europe, substantial knowledge gaps should be filled. Currently, data are mostly available for the intake of calcium, vitamin C and iron as collected from adults in Western European countries. When either of the above mentioned SES indicators was applied to estimate relative differences in micronutrient intake and status between the lowest and the highest SES category within one study, the results often, but not

consistently, indicated a lower intake and/or status in low versus high SES groups. For example, in eight out of ten studies a lower intake for calcium intake was found with relative differences ranging from -2 to -14%. Similar patterns were found for vitamin C and iron: in eleven out of twelve studies relative differences ranged from -5 to -48% for vitamin C, whereas in nine of ten studies on iron relative differences went up to -14%. Studies on intake and/or status of folate, vitamin B12, zinc, iodine, and intake of vitamin D, selenium and copper were limited. Still, when differences were observed, it appeared that lower intake in low than in high SES groups was found except for vitamin B12 and zinc for which the findings were inconsistent.

Furthermore, using education as a proxy for SES, we assessed differences in micronutrient intake between educational levels using the individual-participant data on European adults and elderly from the EPIC cohort. Based on data from 10 Western European countries it appeared that intake of calcium (except in France and a distinctive 'health-conscious' group in the UK), folate (except in Greece), and vitamin C was lower in the lowest than in the highest education groups: relative differences ranged up to 12, 13 and 23%, respectively. The intake of iron differed marginally, whereas the variation in intake of vitamins D and B12 was inconsistent. The observed association between educational level and intake of micronutrients was the same for men and women. Furthermore, differences in micronutrient intake were found to be larger between countries than between SES groups.

With respect to SES differences in micronutrient intake and status, there are significant gaps in the open source literature for many life-stage groups in Europe, but particularly in CEE countries. There is a clear need for cross-country and within country comparative research and for the monitoring of trends in dietary intake across different SES groups and European countries.

Relationship between folate intake and status to add complementary evidence for deriving folate dietary reference values (DRVs)

DRVs are under continuous review and periodic revision as the cumulative evidence base and body of knowledge evolve. Folate is considered a public health priority micronutrient for which re-evaluation of DRVs is needed. For this micronutrient, a systematic review of observational studies on the relationship between intake and status was done followed by meta-analysis. The intake of folate was significantly associated with markers of folate status. The results of our meta-analysis showed that an average person with a folate intake of 100 µg/day has a serum/plasma folate status concentration that is 26% higher and a red blood cell folate status that is 21% higher than a person who has a folate intake of 50 µg/day; plasma homocysteine was found to be 16% lower. The difference between natural food folate and that from supplements and fortified foods (folic acid) significantly influenced the estimated relationship between folate intake and serum/plasma status. Associations were stronger when assessed as folate from the diet than as folate from diet and supplements. Dietary assessment method did not significantly influence the association, although pooled estimates were somewhat

higher when FFQs were used as compared to 24-hour recalls combined with food records. To focus on the impact of poor intakes on related health outcomes, data modelling can be conducted to produce estimates for Average Nutrient Requirements. For this analysis datasets and statistical models developed within the EURRECA NoE are available and can be used.

Overall, further research would benefit from methodologically comparable data on food intake in all age ranges, especially on so far understudied CEE populations. Both intakes obtained through diet and from supplements and fortified foods should be assessed. Monitoring of trends across SES strata should be done with standardized SES measurements that would also facilitate cross-country comparative research. The findings on the level and distribution of micronutrient intake and status could be used for development of food based dietary guidelines. To make them effective in meeting populations' micronutrient needs, they should be created accounting for the country specific dietary patterns giving consideration to the socioeconomic context.

Samenvatting (Summary in Dutch)

Het doel van dit proefschrift was om de micronutriëntinname en -status van sociaal-economisch achtergestelde bevolkingsgroepen, zoals die uit Midden en Oost-Europa in vergelijking tot andere Europese populaties, en die van lage sociaal-economische status (SES) groepen in vergelijking tot hoge SES-groepen binnen Europese landen te evalueren. Hierbij hebben we ons gericht op de micronutriënten die vanwege hun relevantie voor een goede voedingskundige gezondheid prioriteit hebben gekregen van het door de EU gefinancierde Network of Excellence EURRECA. Tevens onderzochten we het verband tussen de inname van folaat en de folaatstatus welke kan worden gebruikt voor het opstellen van voedingsnormen voor folaat.

Inname en status van micronutriënten in Midden- en Oost-Europese landen in vergelijking tot andere Europese landen

Midden- en Oost-Europese landen hebben recent, in een periode waarbij sprake was van een economische transitie, te maken gekregen met een stijgende inkomensongelijkheid. Er is bewijs dat dergelijke hervormingen gepaard zijn gegaan met een toename in gezondheidsverschillen. Een ontoereikendheid inname van micronutriënten en lage nutriënt status kan bijdragen aan deze ongelijkheden. Eerder onderzoek heeft een grote spreiding in de inname en status van micronutriënten aangetoond in welvarende West-Europese landen. Wij bestudeerden of lagere innames en statuswaarden vaker voorkwamen in Midden- en Oost Europese landen. De bevindingen beschreven in dit proefschrift tonen geen verschillen in de inname en status van micronutriënten tussen populations uit Midden- en Oost-Europse landen en populaties uit andere Europese regio's. Een uitzondering betrof de calciuminname van volwassenen en de jodiumstatus van kinderen die lager waren in Midden- en Oost Europe dan in andere Europese regio's. Omdat gegevens van West-Europa aantonen dat er tekorten aan micronutriënt bestaan in sommige SES strata binnen hun bevolking is het belangrijk dat er meer inzicht komt in de voedingssituatie van de lagere SES groepen in Midden- en Oost Europese landen en is zowel begrip van de determinanten en gevolgen van deze verschillen nodig. Het is hierbij belangrijk om gegevens die beschikbaar zijn uit open access bronnen aan te vullen met bewijs uit de grijze literatuur. Fundamenteel voor verder onderzoek naar de voedingskundige gezondheid in Midden-en Oost-Europese landen is de beschikbaarheid van goede data. Het is van belang dat in Midden-en Oost-Europese landen een goede monitoring van de voedingstoestand wordt opgezet en uitgevoerd omdat ons onderzoek laat zien dat er grote hiaten zijn in gegevens voor diverse leeftijdsgroepen.

Verschillen in de inname van micronutriënten tussen SES groepen

Omdat weinig onderzoeken naar de voedingskundige gezondheid van de bevolking rapporteren over het verband tussen de inname en status van micronutrienten en de SES van de groepen, hebben wij een systematische review uitgevoerd om deze verbanden in kaart te brengen en te evalueren. Tevens hebben we, gebruik makend van de gegevens van de Europese EPIC cohorten, zelf deze verbanden in een groot aantal landen bestudeerd.

Om te kunnen concluderen of sociaal-economische ongelijkheiden, gemeten als opleidings, beroeps en inkomens ongelijkheden, in verband staan met de inname en de status van de geprioriteerde micronutriënten zullen eerst voor de verschillende leeftijdsgroepen in Europa, de aanzienlijke lacunes in kennis moet worden gevuld. Momenteel zijn voornamelijk gegevens beschikbaar over de inname van calcium, vitamine C en ijzer voor volwassenen in West-Europese landen. Wanneer gebruikmakende van een van de bovengenoemde SES indicatoren relative verschillen tussen SES groepen werden geschat, werd vaak, maar niet consistent, een relatief lagere inname en / of status gevonden in de lage SES groepen in vergelijking tot hoge SES groepen. Bijvoorbeeld, in acht van de tien studies werd een lagere inname voor calcium gevonden met relatieve verschillen variërend van -2 tot -14%. Vergelijkbare patronen werden gevonden voor vitamine C en ijzer: in elf van de twaalf studies varieerden de relatieve verschillen van -5 tot -48% voor vitamine C, terwijl in negen van de tien studies de relatieve verschillen voor ijzer opliepen tot -14%. Het aantal studies naar de inname en / of de status van folaat, vitamine B12, zink, jodium en naar de inname van vitamine D, selenium en koper was beperkt. Toch, als er verschillen werden gevonden, was er sprake van een geringere inname in de lagere SES groepen met uitzondering voor vitamine B12 en zink waarvoor de bevindingen inconsistent waren.

Met opleiding als indicator voor SES hebben we verder onderzocht of er verschillen waren in de inname van micronutriënten tussen SES strata gebruikmakende van individuelele gegevens van Europese volwassenen en ouderen van de bestaande EPIC-cohorten. Gebaseerd op de EPIC gegevens van 10 West-Europese landen bleek dat de inname van calcium (behalve in Frankrijk en een kenmerkende 'gezondheids-bewuste' groep in het Verenigd Koninkrijk), foliumzuur (behalve in Griekenland), en vitamine C in de laagste opleidingsniveau lager was dan in het hoogste opleidingsniveau: de relatieve verschillen varieerde tot 12%, 13% en 23%, respectievelijk. De inname van ijzer verschilde marginaal, terwijl de variatie in de inname van vitamine D en B12 inconsistent was. De waargenomen verbanden tussen opleidingsniveau en de inname van micronutriënten was hetzelfde voor mannen en vrouwen. Bovendien bleken de verschillen in de inname van micronutriënten tussen landen groter te zijn dan tussen SES groepen.

Wat betreft de kennis van SES verschillen in de inname en status van micronutriënten zijn er aanzienlijke lacunes in de open access literatuur. Dit geldt voor diverse leeftijd- en leeffasegroepen in

Europa als geheel, maar vooral voor de Midden- en Oost Europese landen. Er is een duidelijke behoefte aan vergelijkend onderzoek tussen en binnen landen en aan het volgen van trends in voedingsinname in de verschillende leeftijds- en SES-groepen in de Europese landen.

Relatie tussen inname van folaat (foliumzuur) en de status als aanvullend bewijs voor het afleiden van voedingsnormen voor folaat

Voedingsnormen worden voortdurend beoordeeld en periodiek herzien als gevolg van de steeds toenemende hoeveelheid wetenschappelijk bewijs. Folaat wordt beschouwd als een voor de volksgezondheid zeer belangrijk micronutriënt waarvoor de herbeoordeling van de voedingsnormen een hoge prioriteit heeft. Voor dit micronutriënt is een systematische review, inclusief meta-analyse van observationele studies naar de relatie tussen de inname en de status uitgevoerd. De inname van folaat was significant gerelateerd met indicatoren van folaatstatus. De resultaten van onze meta-analyse toonden aan dat een gemiddeld persoon met een inname van van 100 μg/dag een serum / plasma concentratie heeft die 26% hoger is en een rode bloedcel folatestatus heeft die 21% hoger is dan een persoon die een folaat inname heeft van 50 μg/dag. Het verschil in plasma homocysteine bleek 16% lager te zijn. De bron van folaat, natuurlijke folaat uit de voeding, uit supplementen (foliumzuur) of uit met foliumzuur verrijkte voedingsmiddelen beïnvloedde de geschatte relatie tussen de inname en de serum / plasma-status significant. De verbanden waren sterker wanneer folate gemeten werd als de hoeveelheid inname via de voeding dan wanneer de inname werd geschat als de inname via de voeding en die van supplementen. De methoden van voedselconsumptieonderzoek had geen significante invloed op de gevonden relaties, hoewel de gepoolde schattingen iets hoger waren wanneer FFQs werden gebruikt in plaats van 24-uur recalls in combinatie met voedingsdagboekjes. Om de impact van lage innames van folate op de gezondheid verder te evalueren kan gebruik gemaakt worden van datamodellering. Met behulp van de datasets en statistische modellen die ontwikkeld en beschikbaar zijn binnen het Network of Excellence EURRECA kan op basis van de waargenomen associaties de gemiddelde dagelijkse behoefte aan folaat worden geschat.

Tot slot, verder onderzoek zou kunnen profiteren van methodologisch vergelijkbare gegevens over de voedselinname in alle leeftijdscategorieën in Europe, met name voor de tot nu toe weinig bestudeerde bevolking van Midden- en Oost Europa. Zowel de inname via de voeding als die uit supplementen en verrijkte voedingsmiddelen moeten worden beoordeeld. Voor de monitoring van trends over SES strata moet worden gebruik gemaakt van gestandaardiseerde indicatoren voor SES zodat ook vergelijkingen tussen landen makkelijker kunnen worden gemaakt. Bevindingen over de hoogte en verdeling van de micronutriënt inname en status kan worden gebruikt voor de ontwikkeling van op voedsel gerichte voedingsrichtlijnen. Om deze richtlijnen effectief te laten bijdragen aan het toegemoet komen van de micronutriëntenbehoeften van bevolkingen, moet bij het opstellen rekening gehouden worden met de voor het land specifieke voedingspatronen en dient er de nodige aandacht voor de sociaal-economische context te zijn.

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About the author

Romana Novaković was born on April 11th, 1976 in Benkovac, Croatia. After completing gymnasium in 1994, she started her studies at the Faculty of pharmacy, University of Belgrade, in Belgrade, Serbia. She earned her bachelor degree in 2002 and worked as registered pharmacist until August 2005 when she enrolled in the Master program in Applied Public Health Nutrition and Physical Activity at Karolinska Institute, in Stockholm, Sweden. In June 2006 she was given an award for achieving the highest grades in the Master program. In 2008 she started to work in nutritional research within the EU funded EURRECA project (EURopean Micronutrient RECommendations Aligned) at the Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Serbia. She was appointed as a PhD candidate at the division of Human Nutrition of Wageningen University in January 2010, and her PhD project was part of EURRECA. As from 2008 Romana is involved in capacity development activities under the frame of the United Nation University Standing Committee on Nutrition, Regional Network for Capacity Development in Nutrition in Central and Eastern Europe. She had a student visit to the WHO/International Agency for Research on Cancer in Lyon, France (from January to May 2012) for the research collaboration.

Romana joined the educational program of the graduate school VLAG, the European Nutrition Leadership Program, and aattended several international meetings and conferences.

Currently, she is employed as a researcher at the Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Serbia.

List of publications

Original research papers

Novaković R, Cavelaars AEJM, Geelen A, Marina Nikolić, Iglesia Altaba I, Roman Viñas B, Ngo J, Golsorkhi M, Warthon Medina M, Brzozowska A, Szczecinska A, de Cock D, Vansant G, Renkema M, Serra Majem L, Aznar Moreno L, Glibetić M, Gurinović M, van't Veer P, de Groot L. Socioeconomic determinants of micronutrient intake and status in Europe: a systematic review. Accepted for publishing in Public Health Nutr (2013)

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Overview of completed training activities

Discipline specific courses and activities

2nd Integrating meeting EURRECA (oral presentation), 2008 (Sveti Stefan, Republic of Montenegro)

VLAG course: Evidence-based nutrition: From Requirements to Recommendations and Policies, 2008 (Warsaw, Poland)

EURRECA workshop: ” Workshop for alignment of activities and training in systematic review techniques”, 2008 (Norwich, United Kingdom)

EURRECA training: ’’ Nutrition software tool for implementing micronutrient recommendations’’, 2009 (Belgrade, Serbia)

3rd Integrating meeting EURRECA (oral and 3 poster presentations), 2009 (Barcelona, Spain)

The 5th Meeting/Workshop of the UNU/SCN Network for Capacity Development in Nutrition in Central and Eastern Europe (NCDNCEE), (oral presentation), Belgrade, Serbia, 2009

EFCOVAL closing conference (poster presentation), Utrecht, The Netherlands, 2009

VLAG course: ’’Master class Exposure assessment in nutrition research’’, 2010 (Wageningen, The Netherlands)

VLAG course: ’’International Master Class Public health interventions in real-life settings’’, 2010 (Wageningen, The Netherlands)

2nd World Congress of Public Health Nutrition (oral and poster presentation), 2010 (Porto, Portugal)

4th Integrating meeting EURRECA (oral presentation), 2010 (Copenhagen, Denmark)

3rd Annual European Public Health Conference (oral presentation), 2010 (Amsterdam, The Netherlands)

VLAG course: ’’Masterclass multilevel analysis’’, 2011(Wageningen, The Netherlands)

The 6th Meeting/Workshop of the UNU/SCN Network for Capacity Development in Nutrition in Central and Eastern Europe (NCDNCEE) (oral presentation), 2011 (Belgrade, Serbia)

11th European Conference on Nutrition of the Federation of the European Nutrition Societies (FENS) (poster presentation), 2011 (Madrid, Spain)

6th Central European Food Congress, 2012 (Novi Sad, Serbia) (oral and 2 poster presentations)

Master class Longitudinal data analysis, Wageningen University, February 2013

General courses and activities

EURRECA course: ''Training on communication skills for young scientists'', 2009 (Rome, Italy)

EURRECA training: '' Using of Grey literature in research'', by prof. Primoz Juznic, University of Ljubljana, Faculty of Arts, Department of Library and Information Science and Book Studies, 2009 (Ljubljana, Slovenia)

VLAG PhD week October 2010

VLAG course: ''Scientific writing'', 2011(Wageningen, The Netherlands)

Erasmus Summer Programme, NIHES, 2012 (Rotterdam, The Netherlands)

Optional courses and activities

FOCUS Balkans Project training in Food consumer sciences: ''Food with health claims'', 2009 (Becici, Montenegro)

4th Annual Review of EURRECA project; DG Research (oral presentation), 2011 (Brussels, Belgium)

Systematic literature review in CHANCE project: ''Identification of nutritional criticalities'', University of Leeds 2011(Leeds, The UK)

Preparation research proposals and research presentations, 2008-2013

Staff seminars Institute for Medical Research (Belgrade, Serbia), Division of human nutrition Wageningen UR (Wageningen, The Netherlands)

COLOPHON

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156