Use of a common food frequency questionnaire (FFQ) to assess dietary patterns and their relation to allergy and asthma in Europe: Pilot study of the GA2LEN FFQ

Vanessa Garcia Larsen, Marta Luczynska, Marta Luczynzka, Marek L Kowalski, Helena Voutilainen, Matti Alhstrom, Tari Haahtela, Elina Toskala-Hannikainen, Angelina Bockelbrink, Hae-Hyuk Lee, et al.

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[1]Vanessa Garcia-Larsen PhD, [2]Marta Luczynska BSc, [2]Marek L Kowalski PhD, [3]Helena Voutilainen MSc, [3]Matti Ahlström BSc(c), [3]Tari Haahtela PhD, [3]Elina Toskala MD, [4]Angelina Bockelbrink PhD, [5]Hae-Hyuk Lee PhD, [6]Emilia Vassilopoulou PhD, [6]Nikolaos G Papadopoulos MD, [7]Renata Ramalho MSc, [7]André Moreira MD PhD, [7]Luís Delgado MD PhD, [7]María G Castel-Branco MD, [8]Philip C Calder PhD, [8]Caroline E Childs PhD, [1]Ioannis Bakolis MSc, [1]Richard Hooper PhD, [1]Peter G Burney MD

1 National Heart & Lung Institute, Imperial College London, United Kingdom; 2 Department of Immunology, Rheumatology and Allergy, Medical University of Łódź , Poland; 3 Skin and Allergy Hospital, Helsinki University Central Hospital, Finland; 4 Institute for Social Medicine, Epidemiology, and Health Economics, Charité University Medical Center, Berlin, Germany; 5 Department of Allergy, Charité University Medical Centre, Berlin, Germany ; 6 Allergy Department, University of Athens, Greece; 7 Faculdade de Medicina da Universidade do Porto, and Hospital São João, Porto, Portugal; 8 School of Medicine, University of Southampton, Southampton General Hospital, United Kingdom

For the working group of GA^{2}LEN-WP 1.2 'Epidemiological and clinical studies'

Corresponding author:

Dr Vanessa Garcia-Larsen

Respiratory Epidemiology & Public Health Group, NHLI, Imperial College London,

Emmanuel Kaye Building

Manresa Road, London SW3 6LR, United Kingdom

Phone: 0044 020 7352 8121   Fax: 0044 0207352 8133 E-mail:

v.garcialarsen@imperial.ac.uk

Running title: Validation of the GA^{2}LEN Food Frequency Questionnaire

Abstract

Background

Comparable international data on food and nutrient intake is often hindered by the lack of a common instrument to assess food intake.

Objective

Within the Global Allergy and Asthma European Network of Excellence (GA^2LEN) we developed and piloted a food frequency questionnaire (FFQ) to assess its validity in Europe.

Design

Five countries participating in GA^2LEN took part in the pilot. 200 adults aged 31 to 75 years were invited to complete a FFQ in 2 occasions and to give a blood sample. The intra-class correlation coefficient (ICC) was used to assess repeatability of the FFQ. Plasma phospholipid fatty acids were analysed by gas chromatography. Pearson correlation was used to analyse the correlation between estimated dietary fatty acid intake and plasma phospholipid fatty acid levels.

Results

177 participants (89%) had complete data on FFQ_1 and plasma phospholipid fatty acids. 152 participants (76%) completed both FFQs. ICCs between macronutrients ranged from 0.70 (saturated fatty acids) to 0.78 (proteins) and between 0.70 (retinol) to 0.81 (vitamin D) for micronutrients. Dietary n-3 fatty acids showed a good correlation with total plasma phospholipid n-3 fatty acids and with docosahexaenoic acid in the whole sample (0.40) and in individual countries. Poor correlations were observed for other fatty acids.

Conclusion

The GA^2LEN FFQ is an appropriate tool to estimate dietary intake for a range of nutrients across Europe regardless of cultural and linguistic differences. The FFQ seems to be useful to estimate the intake of n-3 fatty acids but not other fatty acids.

Key words: FFQ, validation, Ga^{2}len, asthma, fatty acids, diet

Introduction

Diets are complex mixtures of foods providing a wide variety of nutrients. The most common methods for assessing food and nutrient intakes are through recall of all foods eaten over a fixed period, most commonly 24 hours, or by estimates of the frequency with which specified foods are eaten over a longer period, usually days to a year (Food Frequency Questionnaires (FFQ)) (Subar, 2004). From a list of foods eaten and information on the nutrient composition of foods listed in food composition tables, individual intake of nutrients can be estimated. Nutrients can also be measured in biological samples such as blood and urine and such data may be more precisely related to nutrient intakes than information gained from dietary recall or the use of FFQs. However blood concentrations of nutrients may not represent intake as they are also influenced by digestion, absorption, metabolism and excretion (Jenab et al, 2009).

Traditionally, diets have been assessed as individual foods and nutrients, but more recently there has been a trend to look at dietary patterns instead (Michels et al, 2005). It has been argued that this may better represent the complexity of different diets and allows for the possibility that interactions among different foods may be of importance.

Internationally comparable data on the types of dietary patterns and their association with nutrition-related chronic diseases are hindered by the heterogeneity of the dietary surveys. Attempts have been made in few studies (Tunstall-Pedoe et al 2000; Kuulasmaa et al 2000; Slimani et al 2002) to make international comparisons between dietary habits in relation to chronic diseases.

The Epidemiological Prospective Study in Cancer (EPIC) has reported dietary patterns in adults and elderly individuals in 10 European countries (Touvier et al 2009; Masala et al 2007; Bamia et al 2007) using locally designed FFQs. Comparisons of dietary patterns were usually studied with a single 24 hour recall questionnaire, standardised for all participant countries. To date no data are available on dietary patterns across Europe using a common FFQ.

The Global Allergy and Asthma Network of Excellence (GA^{2}LEN) survey includes a nutritional component designed to study the relation between diet and allergy and asthma in adults across Europe. The GA^{2}LEN FFQ pilot study developed and tested a method for determination of dietary intake patterns in Europe, targeting allergic disease and asthma. The aim of the pilot was to evaluate whether the FFQ would yield measurements with a sufficient level of accuracy between very different countries; and to assess the validity and reproducibility of the FFQ.

Methods

Population and study design

7 centres participating as partners or collaborators in the GA^2LEN Network were invited to take part in the study, of which 5 centres took part: Poland (Lodz), Germany (Berlin), Portugal (Porto), Greece (Athens) and Finland (Helsinki).

Each centre invited 40 males and females aged 21 to 75 years to complete the FFQ and to provide a fasting blood sample. Exclusion criteria were minimal in order to allow for a sample as representative as possible of the general population: having a severe gastrointestinal disease or mental illness, having changed diet in the past 12 months or being pregnant.

Individuals were selected from hospital or university centres through posted invitations, and from direct recruitment from patient clinics. Selected participants answered the FFQ on two separate occasions within a time frame of 3 months and provided a blood sample to assess blood levels of fatty acids after answering the first FFQ.

Food frequency questionnaire (FFQ)

The GA^2LEN FFQ was designed to include a wide range of foods and nutrients that could be representative of the average intake in national populations. Foods were also included to allow the investigation of potential associations between intake of specific food items or nutrients and allergic diseases across Europe.

In order to create a representative dietary questionnaire for most countries in Europe, information was collected from national dietary surveys that were used as part of the

EPIC study (Slimani et al 2002) and the FFQ used in the European Community Respiratory Health Survey II (ECRHS II). After combining items that had a very similar nutritional composition, a final list of 200 foods was included.

Classification into food groups

Food groups were classified using the European Food Group (EFG) classification system (Brussaard et al 2002). This is an international classification based on The European Food Consumption Survey Method (EFCOSUM) designed to "define a method for the monitoring of food consumption in nationally representative samples of all age-sex categories in Europe in a comparable way".

The EFG classification seems sufficiently extensive and clear to allow international comparisons as well as between national nutritional surveys (Ireland et al 2002) but it has not been implemented in standard international nutritional studies until now. The GA^{2}LEN FFQ was therefore designed with 32 food sections. To improve the accuracy in the collection on data on fat, animal and vegetable spreads were asked separately. Following the example in the Dutch EPIC FFQ, we included various percentage of fat to ascertain the different types of spreads. Local brand names were also included wherever possible in order to facilitate their identification by the participants. Similarly, a broad range of oils was included in the FFQ, and an additional section specifically asked for types of oils or fats used for cooking.

Translation of the FFQ

The standard operational procedure for translation of documents as advised by the World Health Organisation was used.

Nutritional composition and portion sizes

The GA²LEN FFQ was comprised of 7 options to report frequency of food consumption, namely 2+ per day; once per day; 5-6 per week; 2-4 per week; once per week; 1-3 times per week; and rarely or never. Dietary intake reported in the FFQ was registered in standard portion sizes following the Food Standard Agency Food Portion Sizes Guidelines (Food Standard Agency 2006). The GA²LEN FFQ includes these recommended portion sizes across all the food sections. Nutritional composition was calculated using the 6th Edition of the McCance and Widdowson's Composition of Foods (McCance & Widdowson 2006).

Biomarkers of fatty acid (FA) intake: Determination of fatty acid composition of plasma phosphatidylcholine

Samples were analysed at the University of Southampton. Blood (2 ml) was taken into lithium heparin, and centrifuged to separate cells from plasma (3000 rpm for 10 min in a standard bench top centrifuge). Plasma was stored at minus 70°C until shipping on dry ice. The fatty acid composition of plasma phosphatidylcholine (PC), the major circulating phospholipid, was determined using gas chromatography. In brief, total lipid was extracted from plasma using chloroform/methanol (2:1 vol/vol). PC was isolated from the total lipid extract using aminopropylsilica solid phase extraction columns; PC was eluted with chloroform/methanol (3:2 vol/vol). Fatty acid methyl esters were prepared from the isolated PC by incubation with methanol containing 2% sulphuric acid for 2 hr at 50°C. Fatty acid methyl esters were extracted using hexane and then separated on a Hewlett Packard 6890 gas chromatograph. Running conditions were as described elsewhere (Thies et al 2001). The fatty acid methyl esters were identified by comparison with standards run previously and data are expressed as % of total fatty acids present in the PC fraction.

Statistical analyses

Agreement between nutrient intakes in FFQ_1 and FFQ_2 was assessed using the intra-class correlation coefficient (ICC). Nutrient intakes were log transformed to make their distributions less skewed (a small constant was added to each intake so that zero values could be transformed). Agreement was also calculated using the log transformed nutrient density (nutrient intake in milligrams divided by total energy intake (TEI) in kcal) as a way of adjusting for energy intake. Dietary intake of total saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), n-3 PUFAs, n-6 PUFAs and trans fatty acids (TFAs) was validated against the corresponding levels in plasma PC using the Pearson correlation coefficient. Strength and direction of association between intake of specific foods rich in fatty acids with individual and total fatty acids in plasma PC was measured as the Pearson correlation coefficient. Statistical analyses were performed using Stata 10.1

Ethics

Each participant gave written informed consent and ethical approval was obtained by each participant centre.

Results

A total of 177 participants took part in the study. Mean age was 36.7 years (range 21 to 75) (Table 1). The distribution of dietary intakes in the participating countries is described in Table 2, which includes data for those that completed the FFQ on both occasions (FFQ_{t1} and FFQ_{t2}) (n=162). In Greece, Portugal, Poland and Germany, the TEI was higher for FFQ_{t1} than FFQ_{t2}, and macro-nutrient intakes were typically higher for FFQ_{t1} than FFQ_{t2}, while in Finland the reverse was true. Intake of micro-nutrients was also slightly higher in FFQ_{t1} than FFQ_{t2} (data not shown).

Repeatability of the FFQ at the nutrient level

The ICCs for TEI as well as macro- and micro-nutrient intakes for FFQ_{t1} compared to FFQ_{t2} are shown in Table 3. ICCs for macronutrients in all participants (i.e. all countries) ranged from 0.65 (n-3 fatty acids) to 0.78 (proteins and TFAs). ICCs for micronutrients ranged from 0.70 (Retinol) to 0.80 (vitamin D). Analyses within individual countries showed ICCs in a similar range. Agreements were generally lower after energy adjustment (using nutrient densities).

A Bland Altman plot (on a log scale) of n-3 fatty acid intake is shown in Figure 1. The 95% limits of agreement for the ratio of intakes in FFQ_{t2} and FFQ_{t2} were 0.41 to 2.33

Validation against plasma PC fatty acids

Total dietary intake (FFQ_{t2}) of SFAs, MUFAs, PUFAs, n-6 PUFAs and n-3 PUFAs was compared against plasma PC levels of the corresponding fatty acids. Table 4 shows the correlations between fatty acid estimates from FFQ_{t2} and plasma PC levels of those fatty acids for all participants (all countries). Total dietary PUFAs had a correlation of 0.11

with total plasma PC PUFAs across all participants (all countries) and ranged between -0.22 and 0.16 within individual countries.

Dietary n-6 PUFAs showed negative correlations with plasma PC n-6 PUFAs in all countries. Dietary n-3 PUFAs showed a correlation of 0.40 with the corresponding plasma PC level in the whole sample and ranged between 0.16 and 0.45 across countries. Good correlations were also observed between dietary n-3 PUFA intake and several individual n-3 PUFAs in plasma (data not shown). Table 5 shows the correlation between estimated dietary intakes of n-6 and n-3 PUFAs and plasma PC levels of individual fatty acids of potential interest for asthma and allergy. Dietary n-3 PUFAs and plasma PC EPA showed a correlation of 0.31 in the total sample, ranging from 0.21 to 0.43 across countries. Dietary n-3 PUFAs showed a correlation of 0.54 with plasma PC DHA in the total sample which ranged between 0.19 to 0.54 across countries. The correlations for dietary SFAs and MUFAs and the corresponding plasma PC fatty acids showed generally poor correlations in all participant countries.

Discussion

The objective of this pilot study was to assess the repeatability and validity of a FFQ designed to collect typical dietary intake information using a common instrument in different countries across Europe. The study aimed specifically at validating the intake of n-3 fatty acids, as these have a potential role in asthma and allergic diseases (Kremmyda et al 2010). We found a good level of repeatability in the FFQ, although we found evidence that intakes from FFQ_{t1} were systematically and significantly higher than intakes from FFQ_{t2} in most centres and in the total sample. There was generally poorer agreement after adjusting for TEI. Nevertheless the 95% limits of agreement were still fairly wide: n-3 fatty acid intake estimated from FFQ_{t1} could still be half or double the intake estimated from FFQ_{t2}. Intake of major food groups such as meats, cereals, vegetables, fruits, and fish showed a high repeatability (data not shown).

We included a number of food items that were only relevant to one country but not to others. This was intended to ascertain local consumption as well as general intake of foods. The average TEI reported from the FFQ indicates that the questionnaire is able to capture general nutritional intake within the usual range of energy consumed by adults.

The assignment of standard portion sizes was preferred in order to facilitate the interpretation and completion of the FFQ. In large epidemiological studies that involve many different geographical and cultural areas a questionnaire should be kept simple (Noethlings et al 2003). Studies looking at the effect of including portion size questions have shown small changes in estimated nutrient intakes (Block 1990). Tjonneland and colleagues reported that correlation coefficients for food groups and nutrients changed only slightly, indicating that little extra information could be obtained by additional questions about portion size (Tjonneland 1992). Cade et al observed that correlation coefficients of nutrient intakes tended to be higher in subjects who reported their own portion sizes compared to standard portion size specified in the questionnaire (Cade et al 2004). Bearing in mind the objective of our FFQ which is to ascertain food intake and variations in dietary habits across countries, we opted to include standard food portion sizes.

We used a nutrient density approach to adjust for energy intake, rather than looking at residuals from a regression adjusting for energy intake. Both approaches are in common use, and a high degree of correlation between intakes adjusted by these two methods (Willet & Stampfer 1986). Calculating nutrient density meant that we were using the same adjustment for each FFQ assessment, and thus comparing the same quantity each time.

A major element of interest in the design of the FFQ was to include a representative variety of foods rich in n-3 and n-6 fatty acids because of their potential roles in allergic disease risk (Sala-Vila et al 2008). Measurement of plasma levels of fatty acids is considered a good biomarker of the intake of some fatty acids (Ashley et al 1996; Hodson et al 2008). Although all diet-related biomarkers carry a measurement error and may be poor indicators of intake due to variations in digestion, absorption and metabolic changes after consumption, it is suggested that PUFA status in biological samples including plasma is a useful marker of their regular dietary intake (Arab et al 2003; Hodson et al 2008).

The GA^{2}LEN FFQ includes food groups with separate questions for the varieties of foods with a high content of n-3 and n-6 fatty acids. These include fish, shellfish, eggs and egg based dishes, margarine, meats and cereals (Meyer et al 2003). Our study found a correlation between dietary intake of n-3 PUFAs and plasma PC levels of n-3 PUFAs of 0.40 for all participants and a range between 0.16 and 0.45 across countries. Similar ICCs were observed between dietary n-3 PUFAs and plasma PC DHA in all the participants. Our results are in agreement with those reported by other studies validating dietary n-3 PUFA estimated intakes from FFQs with corresponding plasma levels (Ma et al 1995; McNaughton et al 2007; Sullivan et al 2006; Woods et al 2002). In the GA^{2}LEN FFQ fatty fish showed the best correlation with plasma DHA and EPA in analyses per country and with the total sample (data not shown). Correlations between lean fish and plasma fatty acids were less strong, while shellfish and other seafood showed poor correlations.

In our study, correlations between dietary intake of SFAs and MUFAs and plasma levels of these fatty acids were poor. This is likely to be explained by the capacity for endogenous synthesis of SFAs and MUFAs. It is also plausible that differences might be due in part to the fact that plasma reflects more recently intake of fatty acids than other tissues such as red blood cells and adipose tissue (Hodson et al 2008).

The overall aim of the GA^{2}LEN FFQ is the assessment of the relation between dietary habits, food intake and nutrient intakes with asthma and allergy in adults across Europe, using a single common dietary instrument. The pilot study indicates that the FFQ can be answered without major difficulties by individuals from very different countries and cultures. We chose two Mediterranean countries (Greece and Portugal), a central European country (Germany), an Eastern European country (Poland) and a Nordic country (Finland) in an attempt to represent the diversity of the people that will participate in the survey where the FFQ will be used.

There is little evidence so far accumulated in international comparisons of dietary patterns in relation to chronic disease, and none using a common dietary questionnaire or a common food classification system that facilitates comparisons. With few exceptions (Bamia et al 2003), most of the data published on comparisons of dietary patterns across the participant countries in EPIC have been analysed using a standardised 24 hour recall questionnaire (Slimani et al 2002), which gave a generally accurate measurement of intake in the short term (Kaaks 1996).

The correlations between foods rich in fatty acids or total dietary n-3 and n-6 PUFAs with plasma PC levels of AA, EPA, DHA and total plasma PC levels of n-3 and n-6 PUFAs found in this study indicate that the FFQ captures moderately well dietary intake of these fatty acids. We are aware of the limitation that using a country specific Table in this validation study with several European countries might not always reflect nutrient composition relevant to each of these countries. An advantage of using the British Table is that it is one of the most comprehensive Food Composition Tables and is being used as a reference method by countries where national Tables are less complete. We are currently working with the EuroFir Network to use the updated nutritional composition databases from all the available tables in Europe to analyse the nutritional information obtained from the GA^{2}LEN follow-up.

In summary, the GA^{2}LEN FFQ is an appropriate tool to estimate dietary intake of a varied range of foods across Europe regardless of cultural and idiomatic differences. The

FFQ is a reasonably good indicator of the intake of n-3 fatty acids, but seems not to be useful for estimating intakes of other fatty acids.

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Conflict of interest

All authors declare no conflict of interest.

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Table 1 General characteristics of the participants

Centre (n)	Age (years) Mean (SD)	Males (%)
All (177)	36.7 (13.2)	35.0
Greece (39)	35.7 (7.0)	28.2
Portugal (40)	38.0 (14.3)	25.0
Poland (42)	42.0 (14.5)	47.6
Germany (31)	34.9 (10.6)	26.1
Finland (25)	29.8 (12.3)	44.0

Table 2 Daily intake of macronutrients and selected micronutrients by country in participants who responded to the FFQ in both occasions (Median (IQR))

Nutrient	All countries (n=162)		Greece (n=24)		Portugal (n=40)	
	FFQ_{t1}	FFQ_{t2}	FFQ_{t1}	FFQ_{t2}	FFQ_{t1}	FFQ_{t2}
TEI (kcal)	2463.2 (1921.7 – 3169.1)	2457.5 (1680.9 – 3154.4)	2092.0 (1629.3 – 2420.0)	1772.8 (1375.2 – 2160.5)	3225.2 (2292.5-3683.1)	3177.3 (2289.0-3674.2)
Protein (g)	103.0 (77.5 – 138.5)	98.9 (67.2 – 137.7)	83.5 (64.8 -100.3)	70.6 (55.5 – 90.0)	143.8 (114.9-165.7)	144.9 (112.6-171.1)
Total fat (g)	117.7 (87.4 – 154.5)	115.7 (78.6 – 152.4)	95.7 (66.9 – 110.5)	83.3 (58.0 – 97.4)	137.0 (95.0-181.0)	142.8 (108.7-166.7)
Total n-6 PUFAs (g)	9.7 (6.9 – 13.4)	9.5 (6.4 – 13.3)	7.9 (6.5 – 11.9)	7.8 (5.7 – 9.5)	10.2 (7.7-15.4)	12.3 (8.9-15.0)
Total n-3 PUFAs (g)	1.3 (1.0 – 1.8)	1.3 (0.9 – 1.9)	1.0 (0.9 – 1.5)	1.0 (0.8 – 1.4)	1.8 (1.3-2.4)	1.9 (1.5-2.4)
SFAs (g)	43.3 (31.5 – 54.9)	40.4 (28.0 – 52.3)	33.1 (23.2 – 41.9)	29.6 (19.8 – 35.2)	47.3 (32.5-60.8)	47.0 (36.4-54.9)
MUFAs (g)	37.3 (28.3 – 48.9)	35.9 (25.2 – 49.0)	30.8 (21.6 – 35.8)	27.2 (19.3 – 27.2)	43.2 (32.3-59.2)	45.6 (34.9-55.0)
*PUFAs	10.9 (7.9 – 14.5)	10.7 (7.2 – 14.7)	8.9 (7.3 – 13.2)	8.8 (6.5 – 10.8)	11.9 (9.2 – 17.6)	14.0 (10.4 – 15.0)
Cholesterol (mg)	333.3 (248.7 – 447.7)	309.3 (228.1 – 416.2)	257.0 (167.2 -358.5)	230.7 (180.3 – 305.5)	395.9 (297.9-487.3)	384.8 (270.9-509.1)
Carbohydrates (g)	250.4 (209.7 – 324.8)	245.6 (192.1 – 327.8)	212.7 (188.4 – 243.0)	194.9 (126.9 – 225.4)	331.2 (276.3-380.5)	314.7 (249.4-385.2)

Table 2 *(continued)* Intake of macro and micro nutrients by country (Median (IQR))

Nutrient	Poland (n=42)		Germany (n=31)		Finland (n=25)	
	FFQ_{t1}	FFQ_{t2}	FFQ_{t1}	FFQ_{t2}	FFQ_{t1}	FFQ_{t2}
TEI (kcal)	2487.9 (1893.6-2973.4)	2319 (1671.0-2899.6)	2169.0 (1827.9-2834.4)	1958.9 (973.7-2963.9)	2464.5 (2021-3034.7)	2915.5 (2215.9-3348.4)
Protein (g)	103.6 (70.8-133.1)	92.3 (62.3-121.1)	82.8 (68.1-105.3)	66.7 (47.2-109.5)	91.8 (73.0-131.4)	124.4 (90.4-141.8)
Total fat (g)	124.4 (85.6-156.7)	115.4 (74.4-152.4)	101.2 (83.0-149.6)	102.0 (54.0-148.3)	124.2 (88.1-145.8)	140.2 (101.5-166.6)
Total n-6 PUFAs (g)	9.5 (5.7-12.4)	9.1 (5.9-12.5)	9.8 (7.0-13.9)	7.9 (6.4-12.1)	10.9 (7.2-12.9)	11.2 (9.2-16.4)
Total n-3 PUFAs (g)	1.3 (0.9-1.7)	1.2 (0.9-1.5)	1.1 (0.8-1.6)	0.8 (0.5-1.3)	1.5 (1.1-1.6)	1.6 (1.2-1.9)
SFAs (g)	46.3 (35.4-56.1)	41.2 (26.8-51.7)	36.8 (30.8-50.6)	32.8 (27.6-46.4)	44.7 (30.4-52.2)	46.8 (33.5-57.5)
MUFAs (g)	38.4 (28.2-47.3)	35.5 (21.4-46.4)	33.5 (27.2-49.4)	28.4 (22.7-39.4)	38.7 (30.7-48.3)	45.2 (32.2-55.3)
*PUFAs	10.6 (6.3 – 13.4)	10.2 (6.5 – 13.6)	10.9 (7.9 – 15.0)	8.9 (3.4 – 13.2)	11.9 (8.1 – 14.5)	12.5 (10.3 – 17.9)
Cholesterol (mg)	395.1 (308.2-498.7)	351.7 (238.3-468.5)	278.8 (213.8-426.0)	215.7 (156.8-347.0)	266.8 (245.6-397.1)	301.1 (249.1-394.5)
Carbohydrates (g)	266.1 (202.7-310.4)	229.6 (176.4-285.6)	236.3 (185.8-268.3)	218.3 (70.8-294.8)	239.7 (220.7- 297.9)	259.9 (231.9-327.8)

* total n-6 and n-3 fatty acids

Table 3 Intra class correlation coefficients (ICCs) for log-transformed nutrient intake (1) and log-transformed nutrient density (2) in FFQ_1 and FFQ_2

Nutrient	All countries (n=152)		Greece (n=25)		Portugal (n=40)		Poland (n=42)		Germany (n=31)		Finland (n=25)	
	1	2	1	2	1	2	1	2	1	2	1	2
TEI	0.76	---	0.76	---	0.79	---	0.87	---	0.89	---	0.86	---
Protein	0.78	0.65	0.78	0.47	0.74	0.51	0.84	0.43	0.86	0.72	0.84	0.75
Total fat	0.73	0.56	0.73	0.61	0.74	0.29	0.86	0.70	0.81	0.38	0.82	0.46
Total n-6 PUFAs	0.65	0.53	0.65	0.72	0.67	0.39	0.69	0.48	0.56	0.15	0.84	0.74
Total n-3 PUFAs	0.75	0.60	0.75	0.63	0.71	0.44	0.69	0.45	0.73	0.46	0.84	0.81
SFAs	0.70	0.70	0.70	0.40	0.70	0.33	0.86	0.83	0.80	0.70	0.81	0.80
MUFAs	0.72	0.52	0.72	0.67	0.71	0.38	0.84	0.59	0.78	0.47	0.80	0.40
PUFAs	0.73	0.61	0.73	0.61	0.73	0.52	0.79	0.54	0.74	0.45	0.85	0.68
Cholesterol	0.79	0.72	0.79	0.54	0.77	0.53	0.84	0.59	0.83	0.73	0.90	0.90
Carbohydrates	0.74	0.64	0.74	0.67	0.78	0.34	0.85	0.66	0.89	0.74	0.81	0.70
Total sugars	0.71	0.67	0.71	0.57	0.73	0.66	0.81	0.82	0.77	0.57	0.84	0.72
Sodium	0.75	0.65	0.75	0.41	0.74	0.45	0.89	0.73	0.88	0.69	0.81	0.68
Potassium	0.77	0.80	0.77	0.74	0.73	0.65	0.80	0.78	0.90	0.72	0.87	0.75
Calcium	0.75	0.78	0.75	0.50	0.74	0.71	0.83	0.73	0.87	0.88	0.89	0.88

Table 3 *(continued)* Intra class correlation coefficients (ICCs) for log-transformed nutrient intake (1) and log-transformed nutrient density (2) in FFQ_1 and FFQ_2

Nutrient	All countries (n=162)		Greece (n=24)		Portugal (n=40)		Poland (n=42)		Germany (n=31)		Finland (n=25)	
	1	2	1	2	1	2	1	2	1	2	1	2
Magnesium	0.78	0.83	0.78	0.80	0.79	0.68	0.83	0.78	0.92	0.71	0.85	0.68
Phosphorus	0.78	0.78	0.78	0.79	0.77	0.76	0.83	0.55	0.89	0.79	0.86	0.78
Iron	0.78	0.67	0.78	0.56	0.80	0.24	0.87	0.75	0.91	0.45	0.89	0.81
Copper	0.76	0.58	0.76	0.47	0.80	0.33	0.76	0.57	0.92	0.62	0.86	0.61
Zinc	0.71	0.67	0.71	0.47	0.57	0.60	0.87	0.65	0.75	0.77	0.64	0.72
Selenium	0.80	0.54	0.80	0.09	0.78	0.45	0.81	0.60	0.88	0.62	0.84	0.56
Vitamin D	0.81	0.73	0.81	0.51	0.80	0.75	0.62	0.30	0.87	0.81	0.86	0.78
Vitamin E	0.73	0.65	0.73	0.64	0.79	0.59	0.74	0.62	0.78	0.60	0.84	0.68
Riboflavin	0.78	0.76	0.78	0.74	0.76	0.71	0.80	0.60	0.85	0.73	0.88	0.82
Thiamine	0.75	0.64	0.75	0.45	0.65	0.46	0.86	0.68	0.92	0.67	0.79	0.71
Vitamin C	0.66	0.68	0.66	0.49	0.63	0.48	0.72	0.63	0.81	0.76	0.86	0.80
Retinol	0.70	0.68	0.70	0.46	0.81	0.72	0.55	0.44	0.86	0.74	0.94	0.86

Legend to Figure 1

Bland Altman plot of n-3 PUFA intake (mg/day) (on a *log* scale) in FFQ_{t2} compared with FFQ_{t1} (n=162)

Table 4 Correlations between fatty acid intakes reported from FFQ_{t1} and plasma PC levels of fatty acids in participant countries

Participant countries (n)	**Correlations (r) of fatty acids in diet and plasma PC***				
	Total SFAs	**Total MUFAs**	**Total PUFAs**	**Total n-6 PUFAs**	**Total n-3 PUFAs**
All countries (177)	0.10	-0.16 [0.03]	0.11	-0.20 [0.008]	0.40 [0.0008]
Greece (39)	0.05	-0.02	-0.03	-0.14	0.25
Portugal (40)	0.14	0.06	-0.22	-0.17	0.16
Poland (42)	0.13	-0.17	0.11	-0.13	0.45 [0.003]
Germany (31)	-0.12	-0.05	0.16	-0.17	0.45 [0.01]
Finland (25)	0.25	-0.18	0.05	-0.41	0.55 [0.006]

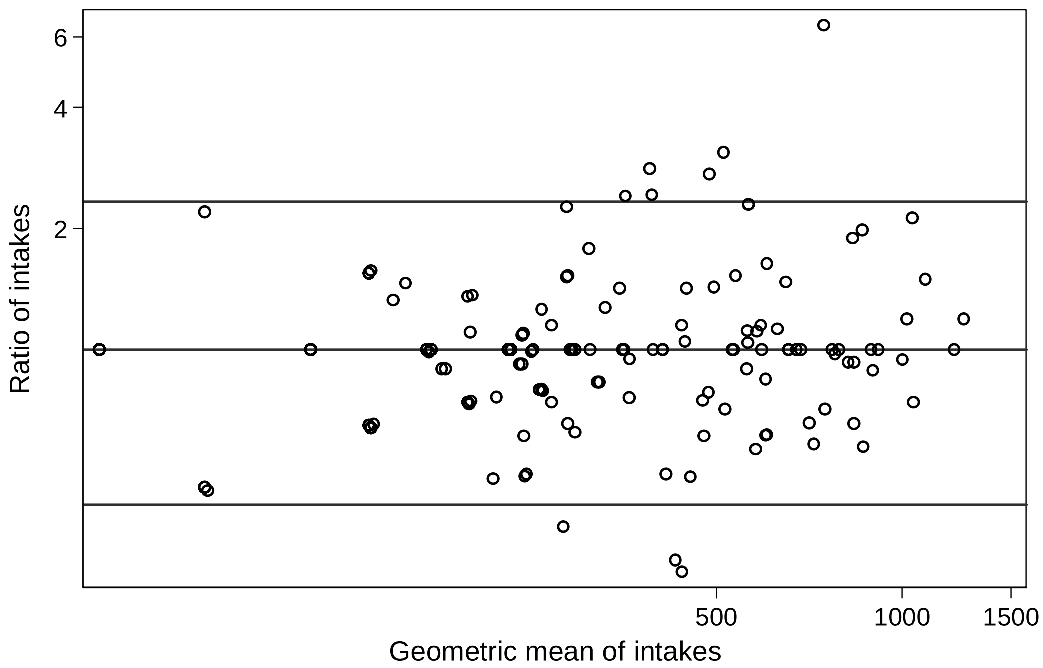
* *log*-transformed scale –when correlations statistically significant p-values shown in brackets

Table 5 Correlations between fatty acid intakes reported from FFQ_{t1} and plasma PC levels of fatty acids in participant countries

Participant countries (n)	**Correlations between n-6 and n-3 from diet and selected individual plasma fatty acids ***		
	n-6	**n-3**	
	20:4n-6 (AA)	**20:5n-3 (EPA)**	**22:6n-3 (DHA)**
All countries (177)	-0.01	0.31 [<0.0001]	0.43 [<0.0001]
Greece (39)	0.02	0.21	0.19
Portugal (40)	-0.02	0.23	0.20
Poland (41)	0.002	0.40 [0.009]	0.36 [0.02]
Germany (29)	0.24	0.43 [0.02]	0.34
Finland (29)	-0.28	0.43 [0.04]	0.54 [0.007]

* *log*-transformed scale –when correlations statistically significant p-values shown in brackets

Figure 1


6
4
2
Ratio of intakes
500
1000
1500
Geometric mean of intakes