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Plasma phospholipid fatty acid profiles and their association with food intakes: results from a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition^{1–3}

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ABSTRACT

Background: Plasma phospholipid fatty acids have been correlated with food intakes in populations with homogeneous dietary patterns. However, few data are available on populations with heterogeneous dietary patterns.

Objective: The objective was to investigate whether plasma phospholipid fatty acids are suitable biomarkers of dietary intakes across populations involved in a large European multicenter study.

Design: A cross-sectional study design nested to the European Prospective Investigation into Cancer and Nutrition (EPIC) was conducted to determine plasma fatty acid profiles in >3000 subjects from 16 centers, who had also completed 24-h dietary recalls and dietary questionnaires. Plasma fatty acids were assessed by capillary gas chromatography. Ecological and individual correlations were calculated between fatty acids and select food groups.

Results: The most important determinant of plasma fatty acids was region, which suggests that the variations across regions are largely due to different food intakes. Strong ecological correlations were observed between fish intake and long-chain n–3 polyunsaturated fatty acids ($r = 0.78$, $P < 0.01$), olive oil and oleic acid ($r = 0.73$, $P < 0.01$), and margarine and elaidic acid ($r = 0.76$, $P < 0.01$). Individual correlations varied across the regions, particularly between olive oil and oleic acid and between alcohol and the saturation index, as an indicator of stearoyl CoA desaturase activity.

Conclusions: These findings indicate that specific plasma phospholipid fatty acids are suitable biomarkers of some food intakes in the EPIC Study. Moreover, these findings suggest complex interactions between alcohol intake and fatty acid metabolism, which warrants further attention in epidemiologic studies relating dietary fatty acids to alcohol-related cancers and other chronic diseases.

INTRODUCTION

Assessment of dietary fat intake by commonly used dietary methods is in part limited by recall biases and inherent measurement errors (1). The latter are mostly due to factors such as the widespread distribution of different fats throughout the diet and the intrinsic difficulty that subjects have recognizing and

reporting the consumption of hidden fats, which leads to under-reporting, particularly by overweight and obese individuals (2, 3).

In light of these limitations, much research has been undertaken to identify biomarkers of habitual fat intake as alternative or complementary independent measures of dietary exposures to better understand the potential role of fats in the etiology of chronic diseases. However, no biomarker can accurately reflect the absolute habitual fat intake, largely because of endogenous fatty acid synthesis and complex fatty acid metabolism. Of the blood lipid fractions, triglycerides are influenced by the type and amount of fat consumed during recent meals; therefore, they are not the most appropriate markers of usual dietary fat intake. The fatty acid profile of serum, plasma, or erythrocyte membrane phospholipids reflects medium-term (weeks to months) intakes of

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some fatty acids and may be used as biomarkers of habitual intake of dietary fatty acids in large-scale epidemiologic studies (4, 5).

Although the plasma phospholipid composition of some fatty acids has been correlated with intake of various food groups in homogeneous populations (6–11), it is unclear whether such correlations may exist across different populations with heterogeneous dietary and lifestyle patterns. Thus, the main aim of this study was to analyze the relation between plasma phospholipid fatty acid concentrations and dietary intakes of major food groups within a cross-sectional substudy of the European Prospective Investigation into Cancer and Nutrition (EPIC)—a large cohort with >520,000 subjects from 23 different centers in 10 European countries (12, 13). Secondary objectives were to evaluate the potential of 2 different dietary assessment methods to predict plasma fatty acid concentrations at the population level and to examine the influence of different endogenous [age, sex, and body mass index (BMI; in kg/m²)] and exogenous (region and laboratory variables) factors on plasma fatty acid concentrations.

SUBJECTS AND METHODS

Subjects

The rationale and design of the EPIC Study was previously detailed (12). Briefly, the EPIC cohort consists of 500,000 subjects distributed among 23 centers in 10 European countries (Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom). Between 1992 and 1998, country-specific food-frequency questionnaires (FFQs; previous year's intake) and standardized lifestyle and personal history questionnaires were completed, and anthropometric data and blood samples were collected. In addition, detailed and standardized 24-h dietary recalls (24-HDRs) were collected from a stratified and representative subset (37,000 subjects) of the EPIC Study by using computerized EPIC-SOFT software (14, 15).

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In each of the 23 recruitment centers, blood samples of ≥ 30 mL were drawn from most participants, stored at 5–10°C, protected from light, and transported to local laboratories for processing and preparing aliquots (16). The only exceptions were the EPIC-Oxford center (United Kingdom), where blood samples were collected from a network of general practitioners and transported to a central laboratory and centers in Sweden and Denmark and prepared as aliquots within 1 h of being collected. In all countries, except Denmark and Sweden, blood was separated into 0.5-mL fractions (serum, plasma, red blood cells, and buffy coat for DNA extraction). Each fraction was placed into straws, which were heat-sealed and stored under liquid nitrogen. In Denmark, 1.0-mL blood fraction aliquots were stored locally in Nunc tubes at -150°C under nitrogen vapor. In Sweden, samples were stored in freezers at -80°C .

For the purposes of the present analysis, 16 geographic areas (regions) were designated by grouping some of the 23 EPIC centers together: France (Paris and surrounding areas; in this study, most French participants came from northern France), Florence (central Italy), Varese/Turin (northern Italy), Ragusa/Naples (southern Italy), northern Spain (San Sebastian, Pamplona, and Oviedo), Granada (southern Spain), Murcia (southeastern Spain), Cambridge, the health conscious group in Oxford, the Netherlands (Utrecht and Bilthoven), Greece (Athens and other regions), Heidelberg (southwest Germany), Potsdam (former East Germany), Malmö (southern Sweden), Umeå (northern Sweden), and Denmark (Aarhus and Copenhagen).

Except for France, where only women were recruited, 100 men and 100 women were chosen from each of the 16 regions, which resulted in a total of 3100 subjects. In total, 3089 subjects were selected for participation in the study from those who completed the FFQs and 24-HDRs. Laboratory results for fatty acids were available for 3009 subjects, either because no biological material was available (4 aliquots missing) or because of the failure to extract fatty acids (76 subjects). Six additional subjects were dropped because they were not part of the EPIC central database, which left a total of 3003 subjects for the present study.

For Oxford, 65 of the 100 men selected were vegans (eating no animal products) and 35 were ovo-lacto-vegetarians (eating no meat or meat products); 88 of the 99 women selected were vegans and 11 were ovo-lacto-vegetarians. Subject selection was stratified by 4 age groups (45–49, 50–54, 55–59, and 60–64 y) with an equal number of men and women in each group. As far as possible, equal numbers of subjects were selected for each season in which the blood samples were collected.

Laboratory analysis

Straws containing citrated plasma were extracted from the EPIC biological bank, and samples of the same sex and age category were ordered randomly within analysis batches. Each batch included one subject from each participating center and one sample from a standard pool for the quality control (injected twice at the middle and end of each series). Samples from men and women were analyzed in separate batches by using a gas chromatography method that was detailed elsewhere (17). Briefly, on the day of analysis, straws of citrated plasma were unfrozen at room temperature, and total lipids were extracted from a 200- μL aliquot with 6 mL chloroform-methanol (2:1, vol:vol). Phospholipids were purified by adsorption chromatography on silica tubes and then

transmethylated at room temperature to fatty acid methyl esters (FAMES) with the use of 25 μ L methyl-prep II. FAMES were separated on a gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with an on-column injector and a capillary column (length: 30 m; diameter: 0.32 mm; Supelco, Bellefonte, PA). Helium was used as a carrier gas at a flow rate of 1 mL/min, with nitrogen as a make-up gas for the flame ionization detector. Identification of individual FAMES was based on the comparison of their relative retention time with authentic standard methyl ester fatty acids (Sigma, St Louis, MO). The relative amount of each fatty acid was expressed as the percentage of total area. Chromatograms were integrated automatically and then checked by a laboratory technician.

The analysis method allowed for the analysis of 22 individual fatty acids with a chain length between 14 and 22 carbons belonging to different fatty acid classes: saturated fatty acids (SFAs: 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0), monounsaturated fatty acids (MUFAs: 16:1n-7, *cis* 18:1n-9, *trans* 18:1n-9, *cis* 18:1n-7, and 20:1n-9), n-3 polyunsaturated fatty acids (PUFAs: 18:3, 20:5, 22:5, and 22:6), and n-6 polyunsaturated fatty acids (PUFAs: 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, and 22:5). Analytic quality control was carried out by the daily use of a standard quality-control plasma sample ($n = 137$). The CVs for the major fatty acids were as follows: 3.35% for 16:0, 1.91% for 18:0, 6.43% for *cis* 18:1n-9, 5.38% for *trans* 18:1n-9, 0.93% for 18:2n-6, 3.96% for 20:4n-6, 12.75% for 18:3n-3, 3.86% for 20:5n-3, and 4.55% for 22:6n-3. The within-day CVs, which were calculated by analyzing the fatty acid composition of 10 plasma samples during the same day, ranged from 0.29% for large peaks to 9.34% for the smallest peaks. All laboratory analyses were performed at the International Agency for Research on Cancer–World Health Organization.

Dietary data

Information on food consumption was assessed by FFQ for all subjects at study enrollment (18). Extensive quantitative or semi-quantitative dietary questionnaires developed and validated locally were used in most centers, whereas a mixed method combining a semiquantitative FFQ and 14-d record was adopted in Malmö (19). The 24-HDR was obtained from a representative sample of each center by means of a standardized computerized interview program (EPIC-SOFT) (20). Individual portion sizes were estimated by using a common picture book throughout all centers (21) and other tools, such as depictions of household measurements, bread shapes, standard units, and portions. For this analysis, the same specific food groups to be correlated with biological measurements were considered for both the FFQ and the 24-HDR: meat (red and processed), fish (fatty, lean and total, including fish and shellfish), dairy products (milk, yogurt, and cheese), nuts and seeds, olive oil, margarine, and other vegetable oils.

Statistical analysis

All statistical analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc, Cary, NC).

Plasma fatty acids

Means (\pm SD) plasma fatty acid concentrations by region and sex are presented on nontransformed data for descriptive pur-

poses. Multivariate regression analysis was used to determine the influence of different factors (region, sex, BMI, age at blood collection, alcohol intake, season, smoking status, column, life time of column, and storage time since blood sampling) on plasma fatty acid concentrations; a backward elimination procedure was used to estimate the sum of squares for each independent variable, given other dependent variables in the model. The partial r^2 value was calculated as the ratio of each variable's sum of squares, over the residual sum of squares of the model excluding that independent variable. This indicates the proportion of the variation explained by the model that can be attributed to each independent variable. The R^2 value for the model was calculated by dividing the uncorrected total sum of squares by the corrected total sum of squares of the model.

Dietary data

The same food classification was used to classify foods reported through the FFQ and 24-HDR, after their quantities were expressed as finally consumed (ie, cooked and/or without wastes, where relevant) (20). For dietary questionnaires, the conversion of food quantities into nutrient intakes was obtained by means of country-specific nutrient databases. For the 24-HDR, used as reference calibration measurements in EPIC, an ad hoc nutrient database was compiled and standardized between the EPIC participating countries (22).

Ecological correlations

The crude means of select plasma phospholipid fatty acids and the crude mean intake of select food groups, as recorded by 24-HDR and FFQ, were calculated for each region and Spearman's rank-correlation coefficients were computed. The 24-HDR means were weighted by the season and day of the week (weekday compared with weekend) during which the recall took place to correct for day-to-day and seasonal variability of a single 24-HDR (23, 24). For the present analysis, 8 relevant food groups (dairy products, fish, meat, processed meat, nuts and seeds, olive oil, margarine, and other vegetable oils) were chosen on the basis of their high contribution to dietary fat, along with alcohol for its effect on fat metabolism. FFQ values for olive oil were unavailable for the EPIC-Umeå center, because its consumption was marginal: average intakes of 0.4 g in women and 1.2 g in men (25).

Individual correlations

Although a single 24-HDR is not appropriate for an analysis of correlations at the individual level, because of the large day-to-day variability in dietary consumption, individual Pearson correlation coefficients were calculated between plasma phospholipid fatty acid concentrations and the select food groups and for both dietary measurements (ie, 24-HDRs and FFQs) to confirm or not confirm observations made at the ecological level. All measurements were adjusted for sex, age, center, BMI, storage time, and total energy and weighted for season and day of the 24-HDR.

RESULTS

Plasma phospholipid fatty acid concentrations

Crude mean (\pm SD) plasma phospholipid fatty acid concentrations for men and women are provided in detail for all regions in **Table 1**. Five fatty acids accounted for more than two-thirds

TABLE 1
Crude plasma phospholipid fatty acids by region¹

Fatty acid	Common name	Region															
		Athens (n = 91 M, 100 W)	Granada (n = 93 M, 100 W)	Murcia (n = 96 M, 100 W)	N Spain (n = 94 M, 100 W)	Ragusa/Naples (n = 90 M, 99 W)	Florence (n = 94 M, 99 W)	Varese/Turin (n = 96 M, 100 W)	France (n = 96 W)	Heidelberg (n = 95 M, 96 W)	Potsdam (n = 96 M, 99 W)	Netherlands (n = 95 M, 100 W)	Cambridge (n = 95 M, 100 W)	Oxford (n = 95 M, 100 W)	Denmark (n = 96 M, 100 W)	Malmö (n = 100 M, 95 W)	Umeå (n = 94 M, 99 W)
		% of total area															
Men																	
SFA		40.0 ± 1.1	40.2 ± 1.3	40.2 ± 1.2	40.2 ± 1.3	40.7 ± 1.2	40.4 ± 1.1	41.0 ± 0.9	—	41.2 ± 1.2	41.4 ± 0.9	41.2 ± 1.3	40.7 ± 1.1	39.3 ± 1.9	41.3 ± 1.0	40.3 ± 1.3	40.9 ± 1.1
14:0	Myristic	0.21 ± 0.08	0.20 ± 0.09	0.19 ± 0.07	0.20 ± 0.10	0.25 ± 0.09	0.24 ± 0.08	0.26 ± 0.10	—	0.30 ± 0.11	0.27 ± 0.10	0.29 ± 0.11	0.29 ± 0.12	0.22 ± 0.19	0.26 ± 0.09	0.27 ± 0.09	0.28 ± 0.10
15:0	Pentadecanoic	0.14 ± 0.04	0.11 ± 0.03	0.10 ± 0.03	0.12 ± 0.04	0.15 ± 0.05	0.15 ± 0.04	0.14 ± 0.04	—	0.16 ± 0.05	0.15 ± 0.05	0.15 ± 0.05	0.16 ± 0.05	0.10 ± 0.05	0.15 ± 0.04	0.14 ± 0.04	0.16 ± 0.04
16:0	Palmitic	25.2 ± 1.4	24.7 ± 1.6	25.0 ± 1.4	25.2 ± 1.4	26.1 ± 1.7	25.9 ± 1.3	26.5 ± 1.3	—	26.4 ± 1.6	26.5 ± 1.6	26.1 ± 2.0	25.8 ± 1.4	24.2 ± 2.2	26.9 ± 1.6	25.5 ± 1.6	26.6 ± 1.5
17:0	Heptadecanoic	0.34 ± 0.07	0.32 ± 0.06	0.31 ± 0.07	0.32 ± 0.08	0.33 ± 0.08	0.31 ± 0.06	0.29 ± 0.06	—	0.29 ± 0.07	0.28 ± 0.06	0.29 ± 0.07	0.32 ± 0.07	0.25 ± 0.07	0.30 ± 0.07	0.32 ± 0.06	0.30 ± 0.05
18:0	Stearic	14.0 ± 1.0	14.7 ± 1.0	14.5 ± 1.0	14.3 ± 1.2	13.9 ± 1.1	13.8 ± 1.0	13.7 ± 0.9	—	14.0 ± 1.0	14.2 ± 1.3	14.4 ± 1.4	14.1 ± 1.0	14.5 ± 1.0	13.7 ± 1.1	14.0 ± 0.9	13.6 ± 0.9
20:0	Arachidic	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	—	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.05 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
MUFA		14.4 ± 2.2	14.5 ± 2.3	13.9 ± 2.3	13.4 ± 2.6	14.5 ± 2.1	15.3 ± 1.7	14.3 ± 2.1	—	13.0 ± 1.7	13.3 ± 1.9	11.8 ± 1.8	13.2 ± 1.6	12.7 ± 1.8	13.2 ± 1.7	13.6 ± 1.4	14.6 ± 1.4
16:1n-7	Palmitoleic	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	—	0.7 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	0.6 ± 0.2	0.4 ± 0.2	0.7 ± 0.3	0.6 ± 0.2	0.7 ± 0.2
cis 18:1n-7	cis-Vaccenic	1.6 ± 0.3	1.6 ± 0.3	1.7 ± 0.3	1.5 ± 0.3	1.8 ± 0.3	1.7 ± 0.3	1.7 ± 0.3	—	1.6 ± 0.3	1.7 ± 0.3	1.6 ± 0.9	1.6 ± 0.2	1.7 ± 0.4	1.7 ± 0.3	1.7 ± 0.3	1.9 ± 0.3
cis 18:1n-9	Oleic	12.1 ± 2.1	12.2 ± 2.1	11.5 ± 2.2	11.2 ± 2.3	12.0 ± 2.0	12.7 ± 1.5	11.7 ± 1.8	—	10.4 ± 1.4	10.6 ± 1.6	9.3 ± 1.8	10.5 ± 1.4	10.3 ± 1.6	10.6 ± 1.7	10.9 ± 1.3	11.5 ± 1.2
trans 18:1n-9	Elaeidic	0.14 ± 0.07	0.08 ± 0.04	0.08 ± 0.06	0.09 ± 0.06	0.09 ± 0.05	0.09 ± 0.04	0.09 ± 0.04	—	0.13 ± 0.06	0.10 ± 0.04	0.16 ± 0.09	0.21 ± 0.10	0.23 ± 0.16	0.12 ± 0.04	0.26 ± 0.13	0.18 ± 0.09
20:1n-9	Gadoleic	0.20 ± 0.08	0.17 ± 0.06	0.16 ± 0.05	0.18 ± 0.07	0.19 ± 0.08	0.19 ± 0.07	0.20 ± 0.07	—	0.24 ± 0.09	0.22 ± 0.10	0.21 ± 0.08	0.31 ± 0.11	0.23 ± 0.09	0.24 ± 0.08	0.19 ± 0.06	0.26 ± 0.09
Slm-7		63.2 ± 17.6	62.5 ± 17.9	64.1 ± 19.6	62.5 ± 18.6	48.4 ± 16.3	52.5 ± 16.6	46.8 ± 13.8	—	39.6 ± 12.2	42.5 ± 16.5	46.1 ± 15.1	44.9 ± 13.3	80.9 ± 46.1	42.5 ± 12.3	44.5 ± 12.6	41.3 ± 9.5
Slm-9		1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	1.2 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	—	1.4 ± 0.2	1.4 ± 0.3	1.7 ± 0.8	1.4 ± 0.2	1.4 ± 0.3	1.3 ± 0.3	1.3 ± 0.2	1.2 ± 0.2
n-6 PUFA		38.4 ± 2.4	38.0 ± 2.6	37.5 ± 2.7	38.3 ± 3.5	38.1 ± 2.8	38.1 ± 2.3	38.4 ± 2.6	—	39.3 ± 2.5	38.0 ± 2.7	40.4 ± 2.8	37.7 ± 3.4	43.7 ± 3.5	35.8 ± 2.9	36.6 ± 2.6	35.7 ± 2.5
18:2n-6	Linoleic	23.3 ± 2.9	21.8 ± 2.9	22.0 ± 3.1	22.6 ± 3.2	20.8 ± 2.8	21.1 ± 2.7	21.3 ± 3.1	—	22.8 ± 3.4	22.5 ± 2.9	24.7 ± 2.9	23.3 ± 3.5	27.7 ± 4.1	21.4 ± 3.0	22.2 ± 2.7	21.8 ± 2.6
18:3n-6	γ-Linolenic	0.09 ± 0.05	0.10 ± 0.09	0.09 ± 0.06	0.09 ± 0.06	0.11 ± 0.06	0.11 ± 0.07	0.10 ± 0.06	—	0.11 ± 0.06	0.09 ± 0.04	0.10 ± 0.05	0.09 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	0.06 ± 0.07	0.07 ± 0.04
20:2n-6		0.32 ± 0.06	0.35 ± 0.05	0.35 ± 0.06	0.34 ± 0.07	0.35 ± 0.05	0.32 ± 0.05	0.35 ± 0.06	—	0.35 ± 0.06	0.36 ± 0.06	0.35 ± 0.07	0.35 ± 0.06	0.46 ± 0.13	0.31 ± 0.04	0.34 ± 0.06	0.35 ± 0.06
20:3n-6	Dihomo-γ-linolenic	3.6 ± 0.8	3.6 ± 0.7	3.3 ± 0.8	3.3 ± 0.8	4.1 ± 0.8	3.9 ± 0.7	3.9 ± 0.8	—	3.4 ± 0.7	3.3 ± 0.7	3.4 ± 0.8	3.4 ± 0.8	3.3 ± 0.9	2.9 ± 0.6	3.0 ± 0.6	3.3 ± 0.6
20:4n-6	Arachidonic	10.5 ± 2.0	11.5 ± 1.9	11.3 ± 1.9	11.5 ± 2.0	12.0 ± 2.1	11.9 ± 1.8	12.1 ± 1.9	—	11.9 ± 2.1	11.2 ± 1.8	11.2 ± 2.0	10.1 ± 1.7	11.4 ± 2.4	10.6 ± 1.7	10.5 ± 1.6	9.7 ± 1.4
22:4n-6		0.32 ± 0.10	0.32 ± 0.08	0.29 ± 0.07	0.31 ± 0.10	0.41 ± 0.10	0.40 ± 0.12	0.41 ± 0.10	—	0.40 ± 0.09	0.34 ± 0.08	0.38 ± 0.10	0.31 ± 0.10	0.53 ± 0.19	0.29 ± 0.07	0.30 ± 0.08	0.29 ± 0.07
22:5n-6		0.23 ± 0.08	0.24 ± 0.07	0.21 ± 0.06	0.22 ± 0.08	0.34 ± 0.09	0.34 ± 0.09	0.34 ± 0.11	—	0.28 ± 0.08	0.24 ± 0.08	0.25 ± 0.08	0.19 ± 0.07	0.27 ± 0.08	0.16 ± 0.05	0.16 ± 0.06	0.19 ± 0.05
n-3 PUFA		7.3 ± 1.8	7.4 ± 1.6	8.4 ± 1.9	8.1 ± 2.4	6.5 ± 1.6	6.2 ± 1.4	6.3 ± 1.8	—	6.4 ± 1.3	7.3 ± 1.6	6.6 ± 1.8	8.4 ± 2.7	4.1 ± 1.4	9.6 ± 2.7	9.6 ± 2.4	8.8 ± 2.1
18:3n-3	α-Linolenic	0.13 ± 0.06	0.11 ± 0.05	0.12 ± 0.05	0.11 ± 0.05	0.16 ± 0.06	0.13 ± 0.05	0.14 ± 0.06	—	0.20 ± 0.08	0.23 ± 0.10	0.20 ± 0.10	0.23 ± 0.11	0.24 ± 0.10	0.23 ± 0.07	0.28 ± 0.10	0.29 ± 0.09
20:5n-3	EPA	1.1 ± 0.7	1.0 ± 0.6	1.4 ± 0.7	1.4 ± 1.2	0.9 ± 0.6	0.9 ± 0.4	0.9 ± 0.5	—	1.1 ± 0.5	1.3 ± 0.6	1.1 ± 0.6	1.7 ± 1.1	0.6 ± 0.4	2.2 ± 1.2	1.9 ± 1.0	1.8 ± 1.0
22:5n-3		0.84 ± 0.18	0.76 ± 0.14	0.85 ± 0.16	0.78 ± 0.17	0.93 ± 0.19	0.88 ± 0.15	0.92 ± 0.18	—	1.16 ± 0.20	1.15 ± 0.19	1.13 ± 0.21	1.29 ± 0.26	1.18 ± 0.40	1.28 ± 0.22	1.23 ± 0.20	1.29 ± 0.20
22:6n-3	DHA	5.2 ± 1.2	5.6 ± 1.1	6.1 ± 1.2	5.8 ± 1.3	4.5 ± 1.2	4.4 ± 1.1	4.4 ± 1.3	—	4.0 ± 0.9	4.6 ± 1.1	4.2 ± 1.3	5.2 ± 1.7	2.1 ± 0.9	5.9 ± 1.6	6.2 ± 1.6	5.4 ± 1.2
Women																	
SFA		39.2 ± 1.2	38.8 ± 1.2	39.2 ± 1.2	39.0 ± 1.3	39.5 ± 1.3	39.5 ± 1.4	39.8 ± 1.0	40.0 ± 1.2	40.6 ± 1.2	40.5 ± 1.1	40.1 ± 1.2	39.7 ± 1.5	37.8 ± 1.6	40.2 ± 1.2	40.9 ± 1.1	40.0 ± 1.2
14:0	Myristic	0.20 ± 0.08	0.17 ± 0.05	0.17 ± 0.06	0.18 ± 0.07	0.21 ± 0.09	0.24 ± 0.07	0.24 ± 0.08	0.26 ± 0.09	0.31 ± 0.10	0.28 ± 0.10	0.28 ± 0.10	0.25 ± 0.09	0.15 ± 0.06	0.25 ± 0.08	0.29 ± 0.11	0.28 ± 0.08
15:0	Pentadecanoic	0.14 ± 0.04	0.11 ± 0.03	0.11 ± 0.03	0.13 ± 0.04	0.15 ± 0.04	0.15 ± 0.04	0.15 ± 0.04	0.18 ± 0.04	0.17 ± 0.05	0.17 ± 0.05	0.16 ± 0.04	0.15 ± 0.04	0.08 ± 0.03	0.15 ± 0.03	0.17 ± 0.05	0.16 ± 0.03
16:0	Palmitic	23.8 ± 1.7	22.8 ± 1.5	22.8 ± 1.5	23.1 ± 1.8	24.0 ± 1.7	24.5 ± 1.7	24.6 ± 1.5	24.8 ± 1.9	25.6 ± 1.4	25.3 ± 2.2	24.6 ± 1.9	24.9 ± 2.1	22.0 ± 2.1	25.1 ± 1.9	26.3 ± 1.8	25.3 ± 1.6
17:0	Heptadecanoic	0.38 ± 0.08	0.36 ± 0.05	0.36 ± 0.07	0.39 ± 0.07	0.38 ± 0.07	0.35 ± 0.07	0.36 ± 0.06	0.38 ± 0.06	0.33 ± 0.06	0.34 ± 0.06	0.33 ± 0.05	0.34 ± 0.07	0.27 ± 0.06	0.33 ± 0.06	0.32 ± 0.06	0.33 ± 0.05
18:0	Stearic	14.6 ± 1.2	15.4 ± 1.1	15.7 ± 1.1	15.1 ± 1.2	14.7 ± 1.2	14.3 ± 1.1	14.4 ± 1.1	14.3 ± 1.1	14.2 ± 1.1	14.4 ± 1.7	14.7 ± 1.4	14.1 ± 1.4	15.2 ± 1.2	14.3 ± 1.3	13.9 ± 1.4	13.9 ± 1.1
20:0	Arachidic	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.06 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
MUFA		13.7 ± 2.6	13.7 ± 2.2	12.4 ± 2.4	12.5 ± 2.5	13.8 ± 2.0	15.2 ± 2.2	14.0 ± 1.7	12.0 ± 1.7	12.7 ± 1.7	12.7 ± 1.7	12.2 ± 1.8	13.3 ± 1.7	12.5 ± 1.7	13.0 ± 1.6	13.9 ± 1.5	14.5 ± 1.5
16:1n-7	Palmitoleic	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
cis 18:1n-7	cis-Vaccenic	1.5 ± 0.3	1.5 ± 0.2	1.5 ± 0.3	1.4 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	1.7 ± 0.4	1.7 ± 0.3	1.8 ± 0.3	1.8 ± 0.3
cis 18:1n-9	Oleic	11.4 ± 2.4	11.5 ± 2.0	10.3 ± 2.3	10.4 ± 2.3	11.2 ± 1.8	12.6 ± 2.2	11.5 ± 1.5	9.4 ± 1.5	10.1 ± 1.4	10.1 ± 1.4	9.6 ± 1.4	10.4 ± 1.4	10.0 ± 1.5	10.3 ± 1.3	11.0 ± 1.3	11.5 ± 1.4
trans 18:1n-9	Elaeidic	0.22 ± 0.08	0.13 ± 0.06	0.12 ± 0.07	0.18 ± 0.09	0.15 ± 0.06	0.15 ± 0.06	0.15 ± 0.06	0.20 ± 0.08	0.19 ± 0.07	0.19 ± 0.07	0.24 ± 0.09	0.31 ± 0.11	0.32 ± 0.20	0.19 ± 0.06	0.18 ± 0.08	0.26 ± 0.08
20:1n-9	Gadoleic	0.16 ± 0.05	0.14 ± 0.04	0.14 ± 0.04	0.14 ± 0.05	0.15 ± 0.04	0.15 ± 0.05	0.16 ± 0.05	0.19 ± 0.07	0.21 ± 0.08	0.21 ± 0.08	0.21 ± 0.08	0.25 ± 0.08	0.19 ± 0.06	0.17 ± 0.05	0.25 ± 0.08	0.22 ± 0.06
Slm-7		53.7 ± 12.9	56.5 ± 12.9	56.6 ± 14.3	55.9 ± 16.3	44.8 ± 11.7	41.4 ± 10.7	41.2 ± 11.0	40.5 ± 9.5	35.2 ± 9.							

TABLE 1 (Continued)

Fatty acid	Common name	Region															
		Athens (n = 91 M, 100 W)	Granada (n = 93 M, 100 W)	Murcia (n = 96 M, 100 W)	N Spain (n = 94 M, 100 W)	Ragusa/Naples (n = 90 M, 99 W)	Florence (n = 94 M, 99 W)	Varese/Turin (n = 96 M, 100 W)	France (n = 96 W)	Heidelberg (n = 95 M, 96 W)	Potsdam (n = 96 M, 99 W)	Netherlands (n = 95 M, 100 W)	Cambridge (n = 95 M, 100 W)	Oxford (n = 95 M, 100 W)	Denmark (n = 96 M, 100 W)	Malmö (n = 100 M, 95 W)	Umeå (n = 94 M, 99 W)
20:3n-6	Dihomo- γ -linolenic	3.8 \pm 0.8	3.9 \pm 0.9	3.5 \pm 0.8	3.3 \pm 0.8	4.1 \pm 0.8	4.1 \pm 0.9	4.1 \pm 0.8	3.3 \pm 0.8	3.6 \pm 0.9	3.4 \pm 0.6	3.6 \pm 0.7	3.4 \pm 0.8	3.2 \pm 0.9	3.0 \pm 0.7	3.1 \pm 0.8	3.3 \pm 0.8
20:4n-6	Arachidonic	11.5 \pm 1.9	12.2 \pm 1.9	12.3 \pm 1.9	12.4 \pm 2.3	13.2 \pm 2.0	12.6 \pm 1.9	12.6 \pm 1.8	12.5 \pm 1.9	12.2 \pm 2.0	11.8 \pm 1.7	11.8 \pm 2.1	11.1 \pm 2.2	13.2 \pm 2.38	10.9 \pm 1.8	9.9 \pm 1.6	10.2 \pm 1.5
22:4n-6	n-3 PUFA	0.34 \pm 0.08	0.35 \pm 0.10	0.32 \pm 0.08	0.35 \pm 0.09	0.44 \pm 0.11	0.44 \pm 0.10	0.43 \pm 0.09	0.37 \pm 0.08	0.41 \pm 0.08	0.36 \pm 0.08	0.40 \pm 0.10	0.35 \pm 0.10	0.55 \pm 0.14	0.29 \pm 0.07	0.27 \pm 0.08	0.30 \pm 0.06
22:5n-6		0.24 \pm 0.08	0.24 \pm 0.09	0.20 \pm 0.06	0.24 \pm 0.08	0.34 \pm 0.13	0.38 \pm 0.12	0.35 \pm 0.11	0.25 \pm 0.07	0.30 \pm 0.11	0.25 \pm 0.07	0.26 \pm 0.08	0.21 \pm 0.10	0.28 \pm 0.11	0.14 \pm 0.04	0.18 \pm 0.06	0.18 \pm 0.06
18:3n-3		7.7 \pm 2.0	8.4 \pm 2.0	9.2 \pm 2.4	8.3 \pm 1.9	7.5 \pm 2.1	6.7 \pm 1.7	6.7 \pm 1.5	9.0 \pm 2.2	7.0 \pm 1.8	7.7 \pm 2.2	7.0 \pm 1.7	8.6 \pm 2.4	4.7 \pm 2.2	10.4 \pm 2.5	9.0 \pm 2.3	9.6 \pm 2.2
20:5n-3		α -Linolenic	0.13 \pm 0.05	0.11 \pm 0.04	0.13 \pm 0.05	0.11 \pm 0.04	0.13 \pm 0.05	0.16 \pm 0.07	0.15 \pm 0.06	0.16 \pm 0.06	0.20 \pm 0.08	0.22 \pm 0.07	0.21 \pm 0.07	0.23 \pm 0.09	0.23 \pm 0.10	0.25 \pm 0.09	0.29 \pm 0.09
20:5n-3	EPA	1.0 \pm 0.9	1.1 \pm 0.7	1.6 \pm 1.2	1.2 \pm 0.7	1.0 \pm 0.6	1.0 \pm 0.7	0.9 \pm 0.4	1.4 \pm 0.8	1.1 \pm 0.8	1.4 \pm 1.0	1.1 \pm 0.6	1.6 \pm 0.8	0.7 \pm 0.9	2.3 \pm 1.2	1.9 \pm 1.0	1.9 \pm 0.8
22:5n-3	DHA	0.86 \pm 0.19	0.78 \pm 0.16	0.85 \pm 0.16	0.82 \pm 0.16	0.97 \pm 0.20	0.93 \pm 0.18	0.95 \pm 0.18	1.14 \pm 0.24	1.09 \pm 0.20	1.09 \pm 0.23	1.18 \pm 0.24	1.21 \pm 0.27	1.15 \pm 0.29	1.26 \pm 0.20	1.16 \pm 0.19	1.30 \pm 0.20
22:6n-3		5.7 \pm 1.4	6.4 \pm 1.4	6.6 \pm 1.3	6.3 \pm 1.3	5.4 \pm 1.6	4.6 \pm 1.1	4.7 \pm 1.2	6.3 \pm 1.6	4.6 \pm 1.2	5.0 \pm 1.4	4.6 \pm 1.3	5.6 \pm 1.6	2.7 \pm 1.3	6.6 \pm 1.5	5.7 \pm 1.4	6.1 \pm 1.5

[†] All values are means ± SDs. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SIn-7, standardized index for the ratio of 16:0 to 16:1n-7; SIn-9, standardized index for the ratio of 18:0 to 18:1n-9.

of total plasma phospholipid fatty acids: palmitic acid (16:0), linoleic acid (18:2n-6), stearic acid (18:0), arachidonic acid (20:4n-6), and oleic acid (*cis* 18:1n-9). Minor fatty acids were also measured, such as n-3 long-chain PUFAs: eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and *trans* elaidic acid (18:1n-9).

We also determined the saturation index (SI) as the ratio of 18:0 to *cis* 18:1n-9 and the ratio of 16:0 to 16:1n-7. The SI is an indicator of activity of the rate-limiting enzyme stearoyl CoA desaturase (Δ^9 desaturation), which transforms the SFAs 16:0 and 18:0 into the MUFAs 16:1n-7 (SIn-7) and *cis* 18:1n-9 (SIn-9), respectively. Overall, we observed weak differences between men and women: SFA and MUFA concentrations were higher, whereas n-6 and n-3 PUFA concentrations were lower in men than in women (Table 1 and Table 2).

Multiple regression analysis of predictors of main plasma fatty acids for men and women combined in all regions together showed that sex, BMI, season of blood drawing, age, smoking, alcohol intake, and storage time after blood sampling had small effects, even significant, compared with diet (Table 2). The more pronounced effect of alcohol intake concerned SFAs (16:0, 17:0, and 18:0), MUFAs (16:1n-7 and *cis* 18:1n-9), and the ratios of 16:0 to 16:1n-7 and of 18:0 to *cis* 18:1n-9. Geographic region appeared to be the most important factor of variability for fatty acids and had a strong effect on *trans* 18:1n-9, *cis* 18:1n-9, 18:3n-3, 20:5n-3, and 22:6n-3, but less of an effect on 18:0, *cis* 18:1n-7, and 18:3n-6 (Figure 1). The values represent the means of fatty acids, by center, expressed as a percentage of the total area for the fatty acids in each family, adjusted for age at blood donation, BMI, storage time, season of blood collection, smoking status, alcohol intake and sex after stratification by sex. SFAs did not vary across regions (Figure 1A). In contrast, MUFAs varied widely across regions with a clear geographic gradient, particularly for *trans* 18:1n-9 (Figure 1B). In general, southern Europe had the highest percentages of *cis* 18:1n-9 compared with northern Europe, except in Umeå where the percentage was almost the same as in Northern Spain. In contrast, the northern and central centers, particularly the 2 British centers, had the highest percentages of *trans* 18:1n-9 compared with the southern centers. Similarly to *trans* 18:1n-9, percentages of the essential fatty acid 18:3n-3 were globally higher in northern than in southern Europe, with the highest concentration in Sweden (Figure 1C). In contrast, the essential fatty 18:2n-6 did not vary substantially across regions (Figure 1C). The percentages of the n-3 long-chain PUFAs 20:5n-3 and 22:6n-3 were highest in Denmark and Sweden, lowest in the health conscious group in Oxford, and remained relatively constant across the other regions (Figure 1D). Concentrations of the n-6 long-chain PUFA 20:4n-6 did not vary widely across regions (Figure 1E).

Mean intakes of select food groups

Mean intakes of select food groups containing fatty acids, estimated from the 24-HDRs and FFQs, are presented in Table 3. Southern countries (Greece, Italy, and Spain) were characterized by a higher consumption of olive oil and a lower consumption of margarine compared with the other EPIC countries (France, Germany, Denmark, the Netherlands, the United Kingdom, and Sweden). Spain reported the highest fish and shellfish consumption, \approx 3–5 times higher than the lowest intake reported in

TABLE 2
Multiple regression analysis of predictors of main plasma phospholipid fatty acids in the cross-sectional European Prospective Investigation into Cancer and Nutrition (EPIC) Study¹

Fatty acid (% of total fatty acids)	Region		BMI		Sex		Season		Age		Alcohol intake		Smoking		Storage time	
	Partial r^2	P^2	Partial r^2	P^2	Partial r^2	P^2	Partial r^2	P^2	Partial r^2	P^2	Partial r^2	P^2	Partial r^2	P^2	Partial r^2	P^2
SFA	16.5	<0.0001	4.8	<0.0001	4.8	<0.0001	0.1	0.26	0.0	0.42	3.0	<0.0001	0.8	<0.0001	0.9	<0.0001
14:0	16.4	<0.0001	0.8	<0.0001	0.1	0.09	0.3	0.04	1.7	<0.0001	0.5	<0.01	0.1	0.44	0.1	0.20
15:0	26.8	<0.0001	1.0	<0.0001	0.3	0.01	0.1	0.62	0.0	1.00	4.9	<0.0001	1.0	<0.0001	0.0	0.87
16:0	18.9	<0.0001	0.2	0.01	6.3	<0.0001	0.0	0.89	0.3	<0.01	6.9	<0.0001	0.7	<0.01	0.8	<0.0001
17:0	16.8	<0.0001	4.5	<0.0001	1.1	<0.0001	0.1	0.53	0.0	0.28	12.5	<0.0001	1.6	<0.0001	0.3	<0.01
18:0	8.8	<0.0001	3.1	<0.0001	1.2	<0.0001	0.1	0.65	1.4	<0.0001	4.7	<0.0001	0.1	0.61	0.1	0.06
20:0	13.4	<0.0001	0.8	<0.0001	0.1	0.15	0.0	0.76	3.7	<0.0001	0.4	<0.01	0.4	0.01	0.1	0.08
MUFA	16.3	<0.0001	0.9	<0.0001	0.1	0.20	0.1	0.37	0.7	<0.0001	2.0	<0.0001	0.8	<0.0001	0.2	0.03
16:1n-7	31.7	<0.0001	2.2	<0.0001	3.4	<0.0001	0.4	0.01	1.2	<0.0001	6.9	<0.0001	0.8	<0.0001	0.1	0.22
cis 18:1n-7	10.5	<0.0001	1.6	<0.0001	1.6	<0.0001	0.3	0.02	0.0	0.57	0.0	0.59	0.2	0.19	0.1	0.15
cis 18:1n-9	17.8	<0.0001	0.9	<0.0001	0.1	0.10	0.1	0.30	0.7	<0.0001	1.6	<0.0001	0.9	<0.0001	0.2	0.02
trans	26.4	<0.0001	0.8	<0.0001	7.8	<0.0001	0.5	<0.01	0.4	<0.01	2.7	<0.0001	0.3	0.05	2.7	<0.0001
18:1n-9																
20:1n-9	14.7	<0.0001	0.2	0.02	3.0	<0.0001	0.4	0.01	1.5	<0.0001	0.1	0.13	0.1	0.38	0.0	0.60
Sn-7	29.0	<0.0001	2.3	<0.0001	6.7	<0.0001	0.4	<0.01	1.8	<0.0001	5.2	<0.0001	0.6	<0.01	0.0	0.86
Sn-9	15.9	<0.0001	2.2	<0.0001	0.5	<0.01	0.1	0.41	0.1	0.30	3.5	<0.0001	0.7	<0.0001	0.2	<0.01
n-6 PUFA	30.4	<0.0001	0.0	0.71	0.2	0.01	0.3	0.03	1.6	<0.0001	1.2	<0.0001	0.8	<0.0001	0.2	0.02
18:2n-6	17.6	<0.0001	1.1	<0.0001	0.6	<0.0001	0.2	0.14	1.0	<0.0001	3.5	<0.0001	0.1	0.47	0.3	0.01
18:3n-6	4.1	<0.0001	2.6	<0.0001	0.1	0.04	0.3	0.06	1.5	<0.0001	0.9	<0.0001	0.2	0.09	0.3	<0.01
20:2n-6	14.8	<0.0001	0.0	0.37	0.7	<0.0001	0.0	0.80	0.0	0.76	0.7	<0.0001	0.0	0.79	0.2	0.01
20:3n-6	15.8	<0.0001	7.6	<0.0001	0.3	<0.01	0.2	0.11	0.3	<0.01	0.5	<0.01	0.1	0.34	0.0	0.57
20:4n-6	13.7	<0.0001	0.2	0.02	2.4	<0.0001	0.1	0.31	0.2	0.02	0.1	0.04	1.0	<0.0001	0.0	0.45
22:4n-6	28.3	<0.0001	0.1	0.20	1.0	<0.0001	0.0	0.79	0.1	0.04	0.1	0.18	0.2	0.10	0.0	0.97
22:5n-6	36.5	<0.0001	0.1	0.19	0.2	0.02	0.2	0.17	1.3	<0.0001	0.0	0.69	0.1	0.37	0.2	0.01
n-3 PUFA	38.1	<0.0001	0.0	0.34	1.3	<0.0001	0.3	0.02	0.6	<0.0001	0.2	0.02	0.3	0.02	0.1	0.09
18:3n-3	37.5	<0.0001	0.3	<0.01	0.2	0.03	0.0	0.93	0.0	0.32	0.0	0.37	0.0	0.75	0.1	0.07
20:5n-3	31.2	<0.0001	0.0	0.30	0.2	0.01	0.5	<0.01	0.7	<0.0001	1.7	<0.0001	0.1	0.60	0.0	0.92
22:5n-3	34.6	<0.0001	0.7	<0.0001	0.1	0.21	0.4	<0.01	0.4	<0.01	0.0	0.30	0.5	<0.01	0.0	0.89
22:6n-3	43.4	<0.0001	0.0	0.72	2.1	<0.0001	0.2	0.15	0.5	<0.01	0.0	0.99	0.4	<0.01	0.2	0.01

¹ SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Sn-7, standardized index for the ratio of 16:0 to 16:1n-7; Sn-9, standardized index for the ratio of 18:0 to 18:1n-9.

² P value from F test on type III sum of squares estimate.

the Netherlands and in Germany. Red and processed meat intake was the highest in the northern countries (Denmark, Sweden, Germany, the Netherlands, and France) and in northern Spain and the lowest in Greece. Dietary intake of dairy products was the highest in the Netherlands and the lowest in Italy. The highest consumption of alcohol was reported in Denmark, Germany, northern Spain, and Italy (Varese, Turin, and Florence) and the lowest in Athens and Umeå.

Correlations between plasma phospholipid fatty acid concentrations and select food groups at the ecological level

Correlations between mean plasma phospholipid fatty acid concentrations and mean dietary food groups are presented in

detail, by center, in **Table 4**. Some strong correlations were found between individual fatty acids and intake of specific food groups, such as fish intake and 22:6n-3 concentration (**Figure 2A**), olive oil intake and *cis* 18:1n-9 concentration (**Figure 2B**), and margarine intake and *trans* 18:1n-9 concentration (**Figure 2C**).

Some potential fatty acid patterns that appear to be associated with specific dietary groups are highlighted in **Figure 3**. For example, a high consumption of fatty fish is characterized by a high concentration of 22:6n-3, 20:5n-3, and 17:0 and by a low concentration of n-6 PUFAs in plasma phospholipids (**Figure 3A**). The same profile was associated with lean fish (**Figure 3B**). A high consumption of olive oil was characterized by a high

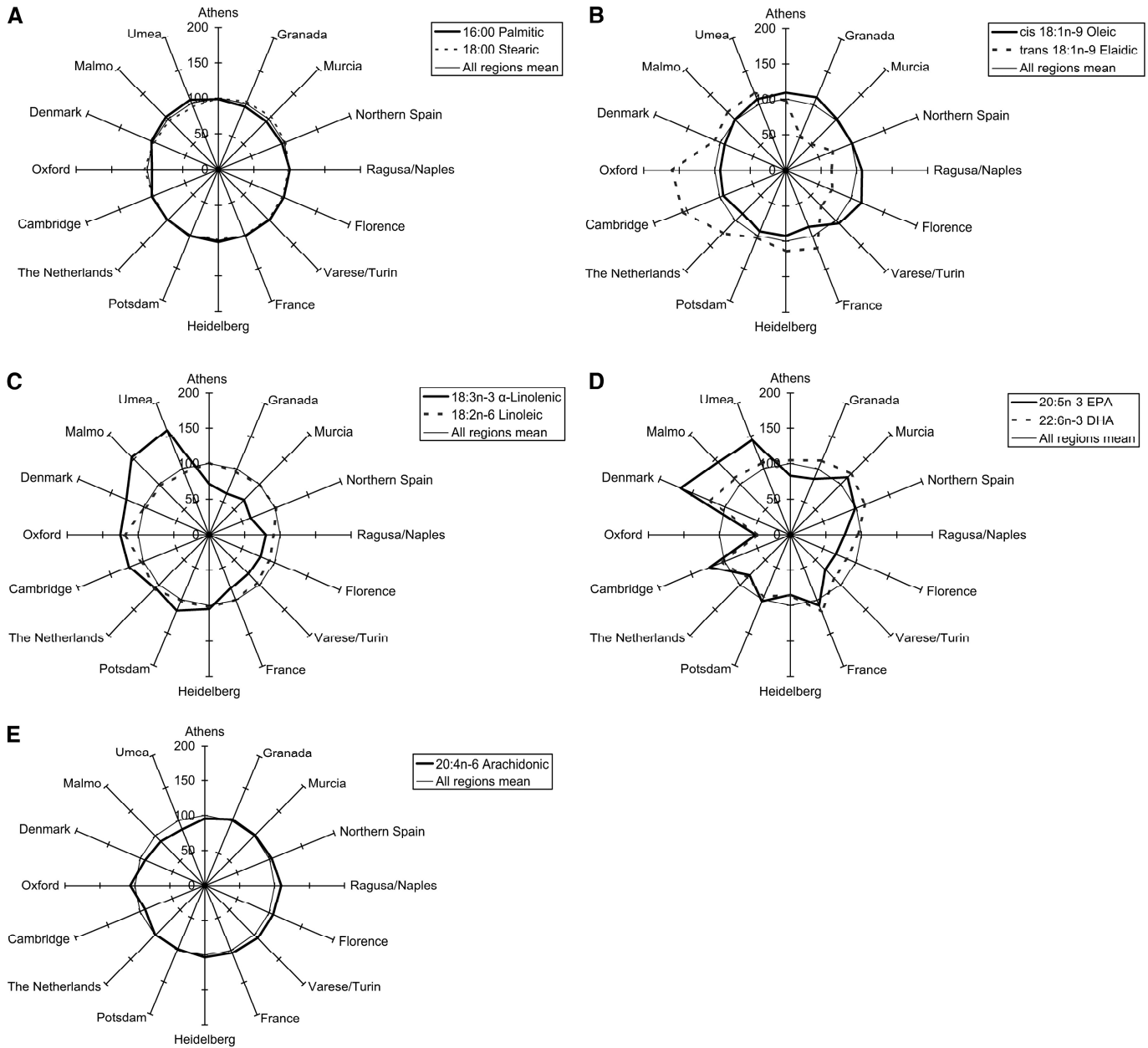


FIGURE 1. Mean deviation (%) in the center-adjusted (by age at blood donation, storage time, BMI, season of blood collection, smoking status, alcohol intake, and sex) major fatty acids in each family from the mean of all regions. The reference circle of the radius (100%) indicated in each panel (A–E) corresponds to the global mean of all centers, and the spikes indicate the deviation of a given center mean from the mean of all centers. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

TABLE 3
Intake of main food groups supplying fatty acids, estimated from 24-h dietary recalls (24-HDRs) and food-frequency questionnaires¹

Food group and method	Region																All regions Combined (n = 3003)
	Athens (n = 191)	Granada (n = 193)	Murcia (n = 196)	N Spain (n = 194)	Ragusa Naples (n = 189)	Florence (n = 193)	Varese Turin (n = 196)	France (n = 96)	Heidelberg (n = 191)	Potsdam (n = 195)	Netherlands (n = 195)	Cambridge (n = 195)	Oxford (n = 195)	Denmark (n = 196)	Malmö (n = 195)	Umeå (n = 193)	
	g/d																
Fish and shellfish																	
Questionnaire	24.5 ± 1.1	63.3 ± 3.1	56.8 ± 2.7	68.6 ± 3.2	34.3 ± 2.2	32.5 ± 1.7	23.7 ± 1.3	40.2 ± 2.3	17.8 ± 1.1	25.7 ± 2.1	12.4 ± 1.2	40.6 ± 1.8	0.4 ± 0.3	47.4 ± 2.0	42.7 ± 2.7	18.6 ± 1.0	34.4 ± 0.6
24-HDR	35.2 ± 5.5	67.2 ± 6.1	59.5 ± 6.7	100.7 ± 9.5	33.3 ± 5.9	28.1 ± 4.4	19.9 ± 3.5	45.7 ± 7.3	13.3 ± 3.5	10.0 ± 2.2	15.6 ± 3.5	38.0 ± 4.8	0.0 ± 0.0	46.0 ± 5.1	25.9 ± 3.9	28.1 ± 4.1	35.6 ± 1.4
Fatty fish																	
Questionnaire	4.8 ± 0.3	19.2 ± 1.3	15.0 ± 1.3	23.5 ± 1.7	11.3 ± 0.8	12.1 ± 0.8	13.3 ± 0.8	12.2 ± 1.0	3.2 ± 0.5	6.7 ± 0.8	3.5 ± 0.5	13.9 ± 1.0	0.3 ± 0.3	21.5 ± 1.2	15.7 ± 1.7	7.5 ± 0.7	11.5 ± 0.3
24-HDR	9.9 ± 3.4	23.4 ± 3.6	20.2 ± 2.8	32.5 ± 5.3	9.7 ± 2.7	11.6 ± 2.8	4.4 ± 1.3	8.6 ± 2.8	8.4 ± 3.0	9.0 ± 2.1	5.6 ± 2.0	19.0 ± 3.7	0.0 ± 0.0	19.0 ± 3.2	10.9 ± 2.3	13.9 ± 2.7	13.2 ± 0.8
Lean fish																	
Questionnaire	16.4 ± 0.8	19.1 ± 1.7	11.5 ± 1.4	37.3 ± 2.3	7.5 ± 0.7	13.3 ± 1.0	8.9 ± 0.7	16.6 ± 1.4	2.1 ± 0.1	1.2 ± 0.1	7.4 ± 0.7	23.9 ± 1.1	0.0 ± 0.0	18.8 ± 1.0	18.0 ± 1.6	9.2 ± 0.7	13.1 ± 0.3
24-HDR	18.3 ± 4.2	34.6 ± 5.1	32.5 ± 5.6	60.6 ± 8.3	14.0 ± 3.8	8.3 ± 2.7	8.8 ± 2.6	29.5 ± 6.4	2.2 ± 1.3	0.9 ± 0.6	8.4 ± 2.7	16.3 ± 3.5	0.0 ± 0.0	14.7 ± 3.2	9.8 ± 2.9	8.8 ± 2.8	16.5 ± 1.0
Olive oil																	
Questionnaire	51.2 ± 1.8	21.8 ± 0.9	20.1 ± 0.9	20.3 ± 1.2	29.0 ± 1.2	29.1 ± 1.0	23.8 ± 1.0	1.5 ± 0.3	2.2 ± 0.2	1.2 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	1.1 ± 0.2	0.4 ± 0.2	—	13.8 ± 0.4
24-HDR	35.7 ± 2.1	26.6 ± 1.8	28.3 ± 1.7	20.2 ± 1.7	17.2 ± 1.6	21.5 ± 1.4	15.9 ± 1.1	2.0 ± 0.5	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.8 ± 0.2	3.9 ± 0.6	0.8 ± 0.3	0.8 ± 0.3	0.4 ± 0.2	11.2 ± 0.4
Vegetable oils ²																	
Questionnaire	5.5 ± 0.8	2.0 ± 0.4	5.6 ± 0.7	9.9 ± 1.0	6.9 ± 0.6	4.1 ± 0.3	6.0 ± 0.7	4.6 ± 0.4	4.9 ± 0.2	2.8 ± 0.1	2.9 ± 0.3	4.4 ± 0.3	2.4 ± 0.2	2.0 ± 0.2	1.7 ± 0.2	—	4.4 ± 0.1
24-HDR	1.1 ± 0.4	2.7 ± 0.7	5.4 ± 0.9	8.0 ± 1.1	2.4 ± 0.8	1.8 ± 0.7	3.2 ± 0.6	3.2 ± 0.7	1.7 ± 0.4	1.8 ± 0.4	1.0 ± 0.3	2.8 ± 0.5	4.9 ± 0.7	0.6 ± 0.2	1.2 ± 0.4	1.1 ± 0.3	2.7 ± 0.2
Margarine																	
Questionnaire	2.3 ± 0.2	3.5 ± 0.8	1.0 ± 0.3	1.6 ± 0.4	0.7 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	3.6 ± 0.6	4.5 ± 0.5	15.3 ± 1.0	17.5 ± 0.9	16.6 ± 1.1	15.0 ± 1.2	15.4 ± 1.1	36.6 ± 2.0	27.7 ± 1.3	10.4 ± 0.3
24-HDR	2.2 ± 0.5	3.1 ± 0.9	1.0 ± 0.3	1.6 ± 0.4	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	5.2 ± 1.1	4.6 ± 0.8	27.1 ± 2.5	24.7 ± 1.9	16.2 ± 2.0	21.3 ± 1.9	18.8 ± 1.8	33.6 ± 2.6	32.6 ± 2.4	12.2 ± 0.4
Dairy products																	
Questionnaire	237.2 ± 10.3	333.4 ± 16.7	270.9 ± 12.6	335.8 ± 13.6	176.6 ± 9.8	244.8 ± 15.1	222.8 ± 11.6	304.4 ± 17.6	240.8 ± 14.8	256.5 ± 15.1	453.2 ± 21.7	451.6 ± 13.8	78.8 ± 12.7	346.6 ± 20.7	404.6 ± 19.0	386.7 ± 17.3	296.6 ± 4.3
24-HDR	228.3 ± 14.6	376.9 ± 15.7	284.7 ± 15.2	394.2 ± 18.2	158.9 ± 10.3	208.6 ± 12.6	206.4 ± 13.1	264.1 ± 20.4	225.3 ± 18.6	188.7 ± 15.1	421.6 ± 23.0	409.8 ± 17.5	86.2 ± 14.6	259.3 ± 20.8	319.8 ± 18.2	388.8 ± 18.7	279.9 ± 4.6
Red meat																	
Questionnaire	46.2 ± 2.5	40.1 ± 2.3	43.3 ± 2.2	56.2 ± 2.7	36.5 ± 1.6	59.9 ± 2.6	50.2 ± 2.4	52.3 ± 3.7	32.5 ± 2.0	29.6 ± 1.4	59.8 ± 2.5	40.6 ± 2.2	0.6 ± 0.1	81.0 ± 3.0	48.1 ± 2.4	25.3 ± 1.4	43.6 ± 0.7
24-HDR	36.3 ± 5.6	37.8 ± 4.8	36.1 ± 6.6	61.5 ± 7.1	43.4 ± 5.9	49.1 ± 5.3	53.4 ± 5.4	56.5 ± 7.4	41.9 ± 5.4	47.2 ± 5.1	50.7 ± 4.7	39.3 ± 5.6	0.0 ± 0.0	53.7 ± 4.7	47.6 ± 4.7	29.4 ± 3.5	42.9 ± 1.4
Processed meat																	
Questionnaire	2.3 ± 0.3	38.7 ± 3.9	38.5 ± 3.3	33.6 ± 2.1	23.3 ± 1.5	23.2 ± 1.4	28.9 ± 1.9	39.0 ± 2.9	50.1 ± 3.0	64.5 ± 3.0	31.5 ± 2.2	25.4 ± 1.4	0.3 ± 0.1	28.0 ± 1.6	49.3 ± 3.0	31.8 ± 1.8	31.6 ± 0.6
24-HDR	9.2 ± 2.4	36.7 ± 3.9	33.6 ± 3.5	39.8 ± 3.8	21.2 ± 3.1	25.6 ± 2.8	30.5 ± 3.2	28.9 ± 4.3	65.0 ± 6.4	61.7 ± 5.6	52.0 ± 4.9	27.8 ± 3.5	0.4 ± 0.3	36.8 ± 3.7	53.4 ± 4.6	56.2 ± 5.4	37.1 ± 1.1
Red and processed meats																	
Questionnaire	48.5 ± 2.6	78.8 ± 5.0	81.8 ± 4.4	89.7 ± 3.6	59.9 ± 2.6	83.0 ± 3.2	79.0 ± 3.8	91.3 ± 4.8	82.6 ± 4.2	94.1 ± 3.7	91.3 ± 4.0	66.0 ± 3.0	0.9 ± 0.1	108.9 ± 4.1	97.4 ± 4.1	57.1 ± 2.7	75.2 ± 1.0
24-HDR	45.5 ± 6.1	74.5 ± 6.6	69.8 ± 7.7	101.3 ± 7.7	64.6 ± 6.7	74.7 ± 5.6	83.9 ± 6.1	85.5 ± 7.6	106.9 ± 8.1	108.9 ± 7.6	102.7 ± 6.3	67.2 ± 6.2	0.4 ± 0.3	90.6 ± 5.9	100.9 ± 6.2	85.6 ± 5.9	80.0 ± 1.7
Nuts and seeds																	
Questionnaire	5.8 ± 0.5	7.7 ± 1.3	9.0 ± 1.0	2.3 ± 0.5	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.1	6.3 ± 0.9	3.6 ± 0.5	3.1 ± 0.5	9.8 ± 0.9	4.3 ± 0.5	19.0 ± 1.8	1.9 ± 0.3	1.7 ± 0.3	0.1 ± 0.0	4.8 ± 0.2
24-HDR	2.5 ± 0.8	1.5 ± 0.5	4.8 ± 1.0	1.9 ± 0.7	0.9 ± 0.3	0.5 ± 0.3	0.3 ± 0.2	2.3 ± 0.9	1.1 ± 0.4	0.3 ± 0.2	1.5 ± 0.6	0.8 ± 0.5	6.6 ± 1.3	0.4 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	1.5 ± 0.1
Alcohol																	
Questionnaire	8.3 ± 0.9	9.1 ± 1.4	17.8 ± 1.6	18.6 ± 2.1	8.8 ± 0.9	16.6 ± 1.5	17.2 ± 1.4	11.9 ± 1.2	17.5 ± 1.3	16.4 ± 1.8	12.6 ± 1.4	11.1 ± 1.4	7.5 ± 0.9	20.0 ± 1.5	12.4 ± 1.0	3.9 ± 0.3	—
24-HDR	9.6 ± 1.5	11.7 ± 1.6	17.6 ± 1.9	18.6 ± 2.6	10.1 ± 1.1	15.2 ± 1.6	19.8 ± 1.8	11.0 ± 1.5	22.0 ± 2.2	16.6 ± 1.7	14.9 ± 2.1	16.6 ± 2.4	8.4 ± 1.8	23.3 ± 2.1	12.5 ± 1.6	7.6 ± 1.3	—

¹ All values are means ± SEs.

² Does not include olive oil.

TABLE 4

Ecological Spearman correlation coefficients between mean 24-h dietary recalls (24-HDRs) and food-frequency questionnaires (FFQs) for select food group intakes and mean plasma fatty acid concentrations by region ($n = 16$)¹

Fatty acid	Food group															
	Fatty fish				Lean fish				Total fish				Olive oil			
	FFQ	24-HDR	FFQ	24-HDR	FFQ	24-HDR	FFQ	24-HDR	FFQ	24-HDR	FFQ	24-HDR	FFQ	24-HDR	FFQ	24-HDR
SFA	-0.16	-0.18	-0.28	-0.41	-0.33	-0.43	-0.32	-0.84 ³	-0.25	-0.70 ³	0.50	0.34	0.08	0.11	0.36	0.52
15:0 + 17:0	0.03	-0.04	0.26	0.32	0.16	0.24	0.36	0.01	0.55	0.19	-0.16	-0.26	-0.08	0.16	0.34	-0.03
14:0	-0.30	-0.23	-0.22	-0.41	-0.43	-0.44	-0.37	-0.86 ³	-0.28	-0.71 ³	0.60	0.57	0.44	0.29	0.04	0.75 ³
15:0	-0.18	-0.16	-0.12	-0.23	-0.24	-0.26	-0.27	-0.77 ³	-0.12	-0.47	0.49	0.46	0.33	0.15	0.04	0.71 ³
16:0	-0.09	-0.08	-0.14	-0.34	-0.28	-0.32	-0.25	-0.79 ³	-0.24	-0.68 ³	0.46	0.45	0.30	0.07	0.12	0.71 ³
17:0	0.54	0.40	0.63 ³	0.85 ³	0.73 ³	0.80 ³	0.64 ³	0.69 ³	0.64 ³	0.55	-0.52	-0.61	-0.08	0.13	0.41	-0.22
18:0	-0.10	-0.05	-0.19	0.16	0.11	0.09	0.08	0.42	0.24	0.52	-0.35	-0.27	-0.29	-0.09	-0.27	-0.35
20:0	0.10	0.08	0.24	0.31	0.24	0.30	0.13	0.76 ³	0.20	0.58	-0.36	-0.40	-0.25	-0.05	-0.06	-0.86 ³
MUFA	0.20	0.17	0.20	0.05	0.09	0.14	0.61	0.33	0.10	-0.11	-0.33	-0.36	-0.15	-0.07	-0.07	-0.31
16:1n-7	-0.19	-0.07	-0.16	-0.38	-0.34	-0.37	-0.35	-0.85 ³	-0.25	-0.69 ³	0.54	0.50	0.40	0.19	-0.01	0.25
cis 18:1n-7	-0.08	-0.16	-0.25	-0.44	-0.33	-0.40	-0.15	-0.23	-0.39	-0.31	0.19	0.21	-0.15	-0.28	-0.37	-0.27
cis 18:1n-9	0.30	0.27	0.31	0.24	0.27	0.36	0.73 ³	0.61	0.14	0.04	-0.52	-0.53	-0.24	-0.09	0.02	-0.28
trans 18:1n-9	-0.39	-0.23	-0.08	-0.31	-0.48	-0.34	-0.68 ³	-0.49	-0.40	-0.29	0.76 ³	0.72 ³	0.31	0.17	-0.19	-0.14
20:1n-9	-0.42	-0.29	-0.24	-0.50	-0.56	-0.52	-0.73 ³	-0.80 ³	-0.42	-0.49	0.77 ³	0.73 ³	0.37	0.21	-0.27	0.12
Sn-7	0.19	0.08	0.16	0.36	0.34	0.37	0.35	0.86 ³	0.25	0.70 ³	-0.56	-0.51	-0.40	-0.19	0.04	-0.23
Sn-9	-0.08	-0.08	-0.15	0.06	0.04	-0.04	-0.60	-0.21	-0.05	0.25	0.26	0.29	0.19	0.14	0.05	0.11
n-6 PUFA	-0.51	-0.61	-0.46	-0.22	-0.34	-0.35	0.02	0.09	0.38	0.39	-0.29	-0.20	-0.46	-0.29	-0.09	0.05
18:2n-6	-0.37	-0.18	-0.11	-0.10	-0.28	-0.14	-0.54	-0.16	-0.13	0.09	0.43	0.44	0.17	0.18	-0.20	-0.17
18:3n-6	-0.25	-0.37	-0.32	-0.26	-0.08	-0.24	0.51	0.19	0.47	0.10	-0.66 ³	-0.67 ³	-0.38	-0.29	0.12	0.17
20:2n-6	-0.46	-0.41	-0.57	-0.41	-0.36	-0.49	-0.51	-0.31	-0.07	0.19	0.17	0.24	-0.09	-0.06	-0.61	-0.46
20:3n-6	-0.28	-0.30	-0.21	-0.10	-0.11	-0.12	0.69 ³	0.39	0.43	0.11	-0.65 ³	-0.69 ³	-0.43	-0.23	-0.14	-0.19
20:4n-6	-0.21	-0.49	-0.36	-0.16	-0.02	-0.21	0.43	0.35	0.50	0.56	-0.75 ³	-0.68 ³	-0.74 ³	-0.61	0.02	0.14
22:4n-6	-0.60	-0.76 ³	-0.62 ³	-0.48	-0.43	-0.55	0.23	0.10	0.26	0.32	-0.52	-0.43	-0.71 ³	-0.63 ³	-0.19	-0.02
22:5n-6	-0.53	-0.70 ³	-0.57	-0.46	-0.35	-0.48	0.40	0.14	0.32	0.25	-0.62 ³	-0.54	-0.67 ³	-0.59	-0.06	0.10
n-3 PUFA	0.66 ³	0.72 ³	0.59	0.57	0.51	0.57	-0.23	-0.16	-0.18	-0.22	0.50	0.41	0.66 ³	0.55	0.21	0.10
18:3n-3	-0.25	-0.18	-0.23	-0.55	-0.51	-0.52	-0.78 ³	-0.77 ³	-0.64 ³	-0.58	0.78 ³	0.78 ³	0.32	0.04	-0.19	-0.06
20:5n-3	0.52	0.65 ³	0.48	0.35	0.37	0.39	-0.43	-0.41	-0.29	-0.37	0.63	0.54	0.72 ³	0.54	0.15	0.13
22:5n-3	-0.20	-0.16	-0.14	-0.39	-0.44	-0.44	-0.79 ³	-0.73 ³	-0.52	-0.53	0.79 ³	0.73 ³	0.40	0.18	-0.09	-0.02
22:6n-3	0.81 ³	0.80 ³	0.71 ³	0.83 ³	0.78 ³	0.84 ³	0.15	0.30	0.09	0.15	0.05	-0.02	0.40	0.41	0.38	0.17

¹ SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Sn-7, standardized index for the ratio of 16:0 to 18:0; Sn-9, standardized index for the ratio of 18:0 to 18:1n-9c.

² Does not include olive oil.

³ $P \leq 0.01$.

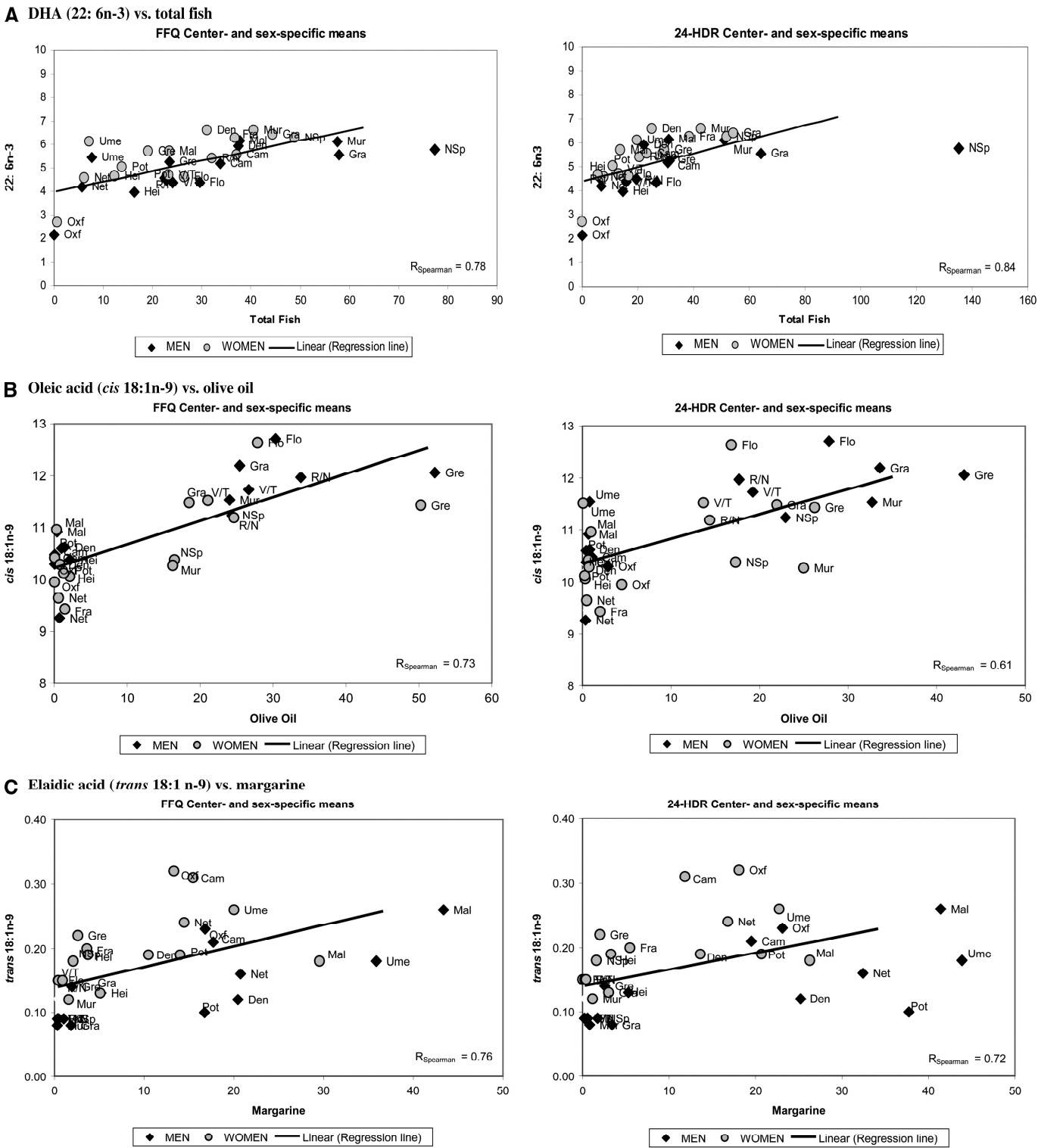


FIGURE 2. A–C: Ecological correlations between mean individual plasma fatty acid concentrations and mean intakes of specific food groups estimated from food-frequency questionnaires (FFQs) and 24-h dietary recalls (24-HDRs). Cam, Cambridge (United Kingdom); Den, Denmark; Flo, Florence (Italy); Fra, France; Gra, Granada (Spain); Gre, Greece (Athens); Hei, Heidelberg (Germany); Oxf, Oxford (United Kingdom); Mal, Malmö (Sweden); Mur, Murcia (Spain); Net, Netherlands; NSp, northern Spain; Pot, Potsdam (Germany); R/N, Ragusa/Naples (Italy); Ume, Umeå (Sweden); and V/T, Varese, (Milan)/Turin (Italy).

concentration of *cis* 18:1n-9 and 17:0 and a low concentration of *trans* 18:1n-9 and 18:3n-3 in plasma phospholipids (Figure 3C). Other vegetable oils were associated with a high concentration of 17:0 and 20:4n-6 and with a low concentration of 18:3n-3 (Figure 3D). A high consumption of margarine was associated with a high

concentration of *trans* 18:1n-9 and 18:3n-3 and with a low concentration of 20:4n-6 and 17:0 (Figure 3E). Dairy products were associated with a high concentration of 20:5n-3 and with a low concentration of 20:4n-6 (Figure 3F). No strong association was observed between meat intake and fatty acid concentrations

(Figure 3G). Processed meat intake was associated with a high concentration of the SFAs 16:0 and 17:0 (Figure 3H). Intake of nuts and seeds was associated with a high concentration of 18:0 and 18:2n-6 and with a low concentration of *trans* 18:1n-9 (Figure 3I).

We also determined correlations between plasma phospholipid fatty acid concentrations and their respective estimated dietary intakes available for all countries in the EPIC nutrient databases. Mean plasma phospholipid SFA concentrations, by center, were significantly correlated (although weakly) with estimated mean dietary intakes of SFAs (FFQ: $r = 0.09$, $P < 0.0001$; 24-HDR: $r = 0.12$, $P < 0.0001$), MUFA concentrations with estimated dietary intakes of MUFAs (FFQ: $r = 0.25$, $P < 0.0001$; 24-HDR: $r = 0.18$, $P < 0.0001$), and PUFA concentrations with estimated dietary intakes of PUFAs (FFQ: $r = 0.23$, $P < 0.0001$; 24-HDR: $r = 0.18$, $P < 0.0001$).

Correlations between plasma phospholipid fatty acid concentrations and select food groups at the individual level

Although it was not the initial purpose of this article, because of the use of a single 24-HDR for individual estimates, correlations between food groups and plasma phospholipid concentrations performed at the individual level to confirm observations made at the aggregated level. The strongest correlations that we found at the ecological level remained at the individual level, but at much lower values: fatty fish intake and 22:6n-3 (FFQ: $r = 0.29$, $P < 0.01$; 24-HDR: $r = 0.11$, $P < 0.01$), olive oil and *cis* 18:1n-9 (FFQ: $r = 0.26$, $P < 0.01$; 24-HDR: $r = 0.17$, $P < 0.01$), and margarine and *trans* 18:1n-9 (FFQ: $r = 0.08$, $P < 0.05$; 24-HDR: $r = 0.02$, $P < 0.05$).

Some associations that were not found at the ecological level appeared at the individual level. Particularly, dairy product intake was associated with the sum of 15:0 + 17:0 (FFQ: $r = 0.27$, $P < 0.01$; 24-HDR: $r = 0.21$, $P < 0.01$) and with *trans* 18:1n-9 (FFQ: $r = 0.17$, $P < 0.01$; 24-HDR: $r = 0.13$, $P < 0.01$). Alcohol consumption was associated with 16:0 (FFQ: $r = 0.30$, $P < 0.01$; 24-HDR: $r = 0.23$, $P < 0.01$), *cis* 18:1n-9 (FFQ: $r = 0.10$, $P < 0.01$; 24-HDR: $r = 0.09$, $P < 0.01$), and 20:5n-3 (FFQ: $r = 0.17$, $P < 0.01$; 24-HDR: $r = 0.14$, $P < 0.01$), but was inversely associated with 18:0 (FFQ: $r = -0.24$, $P < 0.01$; 24-HDR: $r = -0.18$, $P < 0.01$) and with the ratio of 16:0 to 16:1n-7 (FFQ: $r = -0.19$, $P < 0.01$; 24-HDR: $r = -0.18$, $P < 0.01$) and of 18:0 to *cis* 18:1n-9 (FFQ: $r = -0.17$, $P < 0.01$; 24-HDR: $r = -0.14$, $P < 0.01$).

Interestingly, some correlations between diet and fatty acids varied by center. Olive oil intake was associated with *cis* 18:1n-9 concentration only in centers where olive oil consumption was high (Athens, Italy, and Spain), whereas no significant correlation remained in centers where olive oil consumption was low (Heidelberg, France, Potsdam, Denmark, the Netherlands, Malmö, Oxford, and Cambridge) (Figure 4). In centers where olive oil consumption was low, plasma phospholipid *cis* 18:1n-9 was not significantly correlated with other food groups, except for a positive correlation with meat intake in Cambridge. However, alcohol intake was associated with *cis* 18:1n-9 concentration only in centers where olive oil was low (Figure 4). Similarly, alcohol intake was inversely associated with the ratio

of 18:0 to *cis* 18:1n-9 in countries where olive oil intake was low, whereas no association was found in countries where olive oil intake was high (data not shown).

DISCUSSION

This study was the first large cross-sectional study providing data on plasma phospholipid fatty acid concentrations and their ecological and individual correlations with intake of select foods across European EPIC populations. Except for SFAs, "region" was the determinant factor of variability in plasma fatty acid concentrations, which suggests that the observed variations were likely largely due to different food habits and lifestyle. Strong ecological correlations indicated that plasma fatty acid profiles could be used as suitable biomarkers of dietary patterns. Finally, specific correlations between alcohol intake and some fatty acids suggest interactions with fatty acid metabolism.

Essential fatty acids that cannot be endogenously synthesized, such as 18:2n-6 and 18:3n-3, are expected to show direct relations between plasma concentrations and dietary intakes. Ecological analyses showed that 18:2n-6 correlated with intake of nuts and seeds, whereas 18:3n-3 correlated with margarine intake. The plasma concentration of 18:3n-3 was significantly higher in northern than in southern Europe, probably because of the addition of rapeseed oil to margarine in Swedish. The inverse ecological correlation between plasma 18:3n-3 and consumption of fish and olive oil might be related to particular dietary habits and regimens (ie, use of margarine compared with use of olive oil). The inverse correlation between 18:3n-3 and the estimated intake of vegetable oils might be explained by the fact that vegetable oils are a source of 18:2n-6, which competes with 18:3n-3 for absorption (26).

EPA and DHA are found mainly in fatty fish and fish oil, and their endogenous synthesis from α -linolenic acid is limited in vivo (27). The strongest association was found between fatty fish and 22:6n-3, whereas weaker associations were found with 20:5n-3, as previously reported in the EPIC-United Kingdom cohort (11). Furthermore, we found an association between alcohol intake and 20:5n-3, but not with 22:6n-3, at the individual level. An association with both fatty acids would have been expected from the positive correlation that we found between fish and alcohol intake (data not shown). The absence of an association with 22:6n-3 may suggest that alcohol enhances 22:6n-3 peroxidation or the retroconversion of 22:6n-3 to 20:5n-3 (28).

In a comparison of UK vegetarians and vegans with the other EPIC populations, the most striking difference observed was for 18:2n-6, which was higher in the plasma of the vegans, whereas n-3 PUFAs (20:5n-3 and 22:6n-3) and SFAs were lower. Other authors have reported similar findings (29, 30). The ratio of n-6 to n-3 PUFAs was 2-3 times that of vegans in the rest of the EPIC populations (13.71 and 11.65 for male and female vegans, respectively, compared with 3.89-6.42 for the other EPIC centers). The low concentrations of 20:5n-3 and 22:6n-3 in vegetarians and vegans may be further explained by the reduced conversion of 18:3n-3 to long-chain n-3 PUFAs because of the high ratio of n-6 to n-3 fatty acids. These findings agree with those reported earlier (29, 31). However, unlike Phinney et al (32), we did not observe a reduced concentration of 20:4n-6 in plasma associated with a vegetarian diet. In contrast with

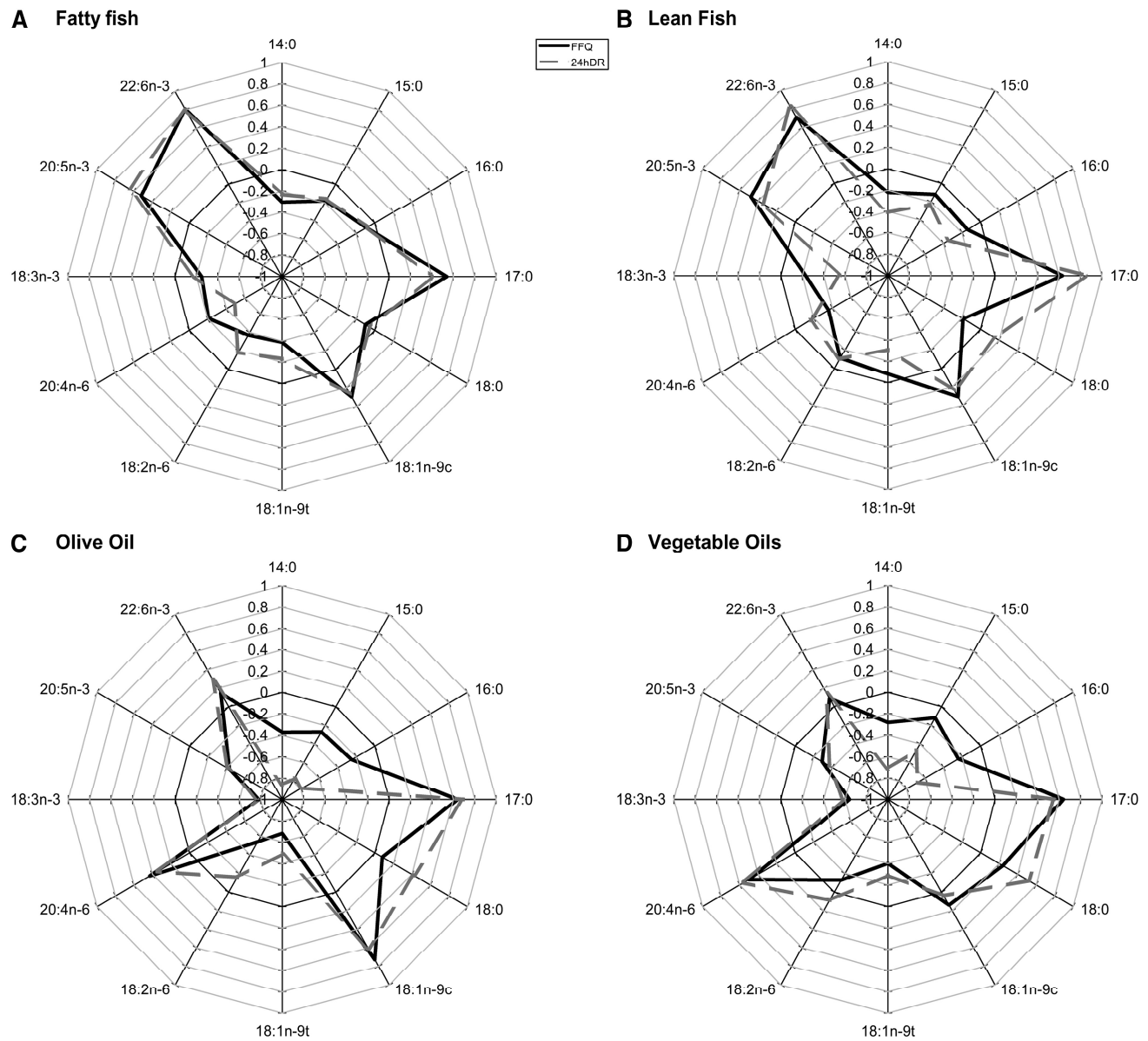


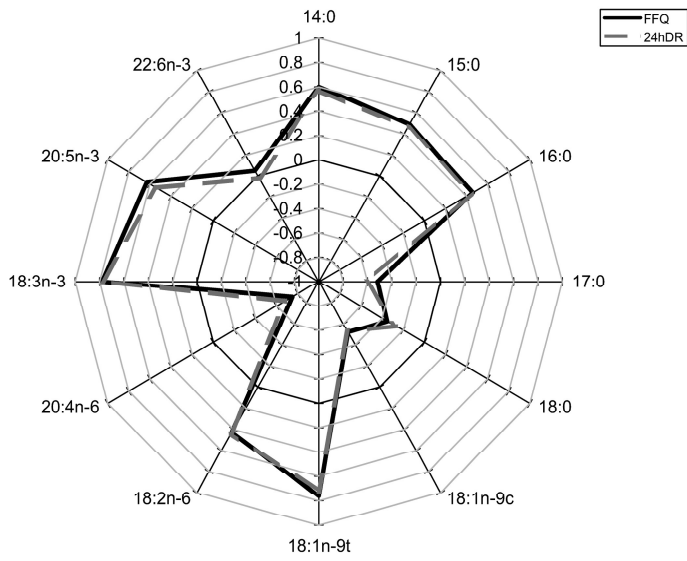
FIGURE 3. A–I: Ecological Spearman correlations between mean plasma phospholipid fatty acid concentrations and intakes of specific food groups. FFQ, food-frequency questionnaire; 24 hDR, 24-h dietary recall; *t*, *trans*; *c*, *cis*. (Continued on next page.)

essential fatty acids, we found weaker associations between diet, SFAs, and MUFAs, probably because they are endogenously synthesized (33).

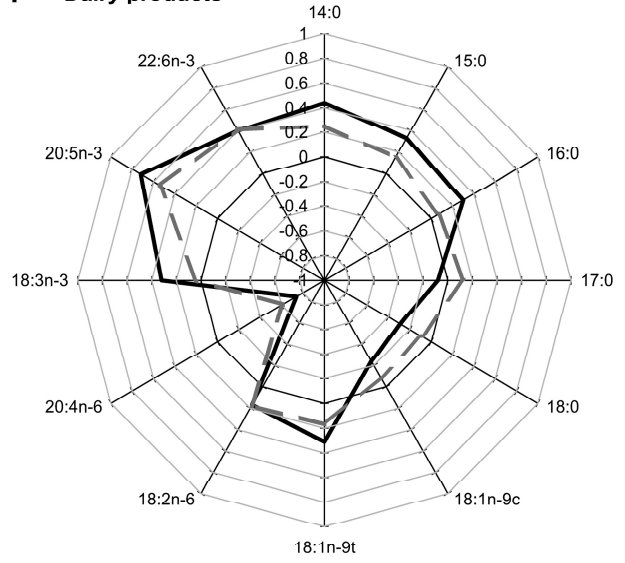
Of the saturates, fatty acids containing an odd number of carbon atoms (eg, 15:0 and 17:0) cannot be synthesized *de novo* and can only be derived from ruminant products (34). Many studies have reported a positive association between the 15:0 + 17:0 concentration in serum or plasma phospholipids and dairy products intake (9, 10, 35). In our study, the concentration of 15:0 + 17:0 was not associated with dairy products intake at the ecological level but an association was found at the individual level, which may be the consequence of a relatively low variability in the mean dietary intake of dairy products across centers. Our analyses at the individual level indicated that plasma phospholipid 15:0 + 17:0 may be considered a biomarker of dairy product intake.

Ecological analyses showed a strong correlation between the plasma concentration of *cis* 18:1n–9 and the estimated intake of olive oil. The highest concentration of *cis* 18:1n–9 was found in countries where the intake of olive oil, considered the major source of this fatty acid, was the highest (Greece, Italy, and Spain). The lowest concentration of *cis* 18:1n–9 along with a low intake of olive oil were found in France, probably because the majority of French participants originated from the northern part of the country, where olive oil consumption is low (25). However, we noted that the concentration of *cis* 18:1n–9 was high in Umeå, where the consumption of olive oil was low. Individual analyses showed that olive oil intake was strongly associated with plasma *cis* 18:1n–9 in centers where olive oil was high, >20 g/d (southern centers), whereas no significant association remained in centers where olive oil is low, <2 g/d (northern centers). In these centers, *cis* 18:1n–9 was not significantly associated with other

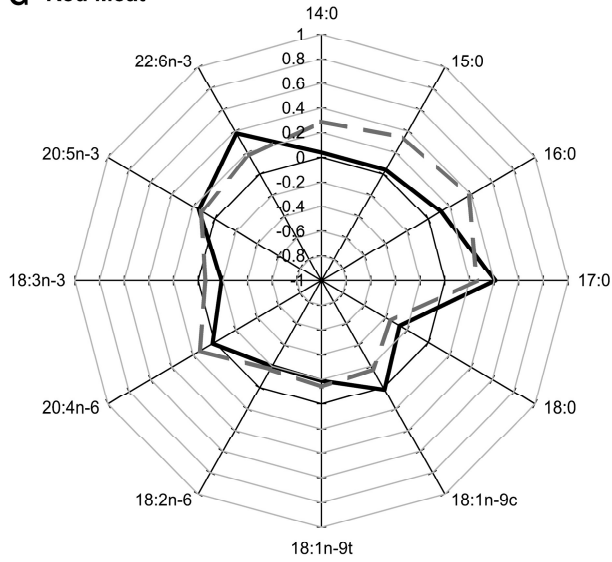
E Margarine



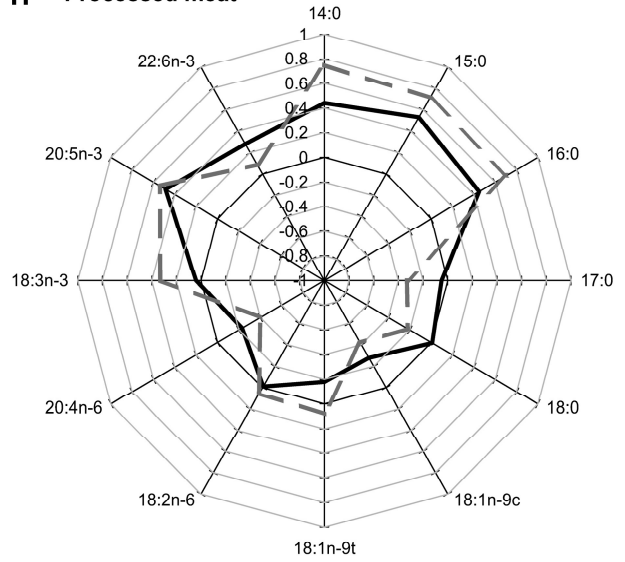
F Dairy products



G Red Meat



H Processed meat



I Nuts & Seeds

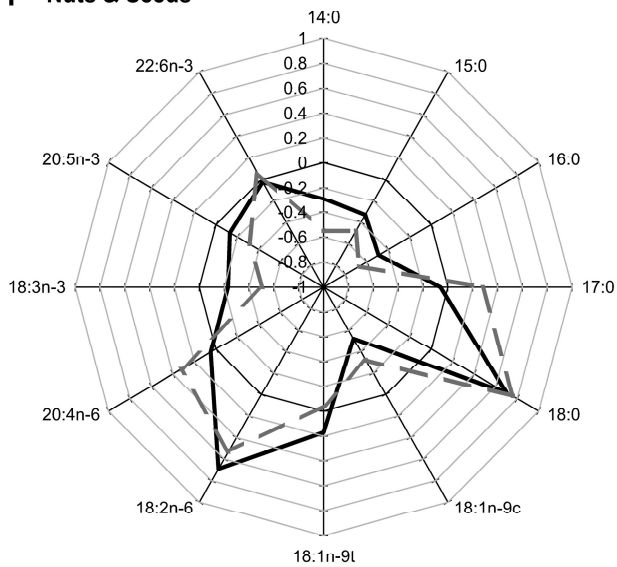


FIGURE 3. (Continued).

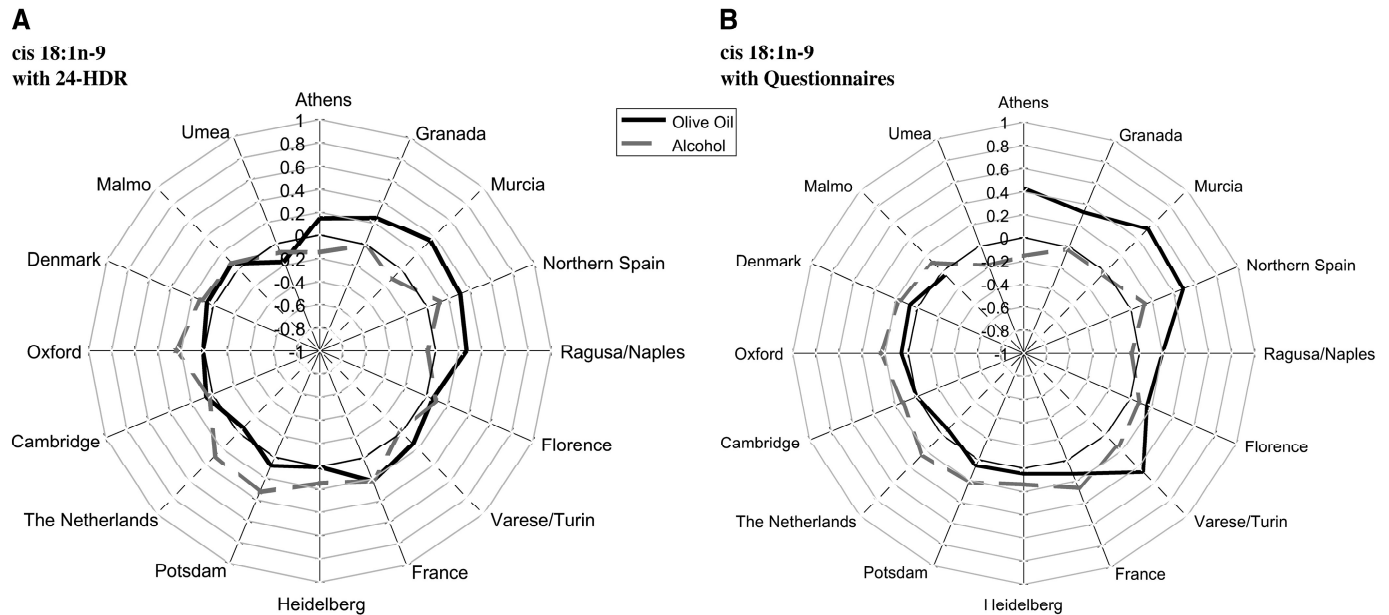


FIGURE 4. A, B: Individual Pearson correlations between plasma phospholipid *cis* 18:1n-9 concentrations and intakes of olive oil and alcohol estimated from food-frequency questionnaires (FFQs) and 24-h dietary recalls (24-HDRs) at all centers.

food groups, which suggested that dietary contributors of *cis* 18:1n-9 may not be a determinant compared with endogenous hepatic synthesis from 18:0. In contrast, a high dietary intake of olive oil in southern Europe may be considered a major determinant of plasma *cis* 18:1n-9.

Individual analyses also showed that alcohol intake was positively associated with 16:0. The direct association between plasma phospholipid 16:0 and alcohol intake was also reported for erythrocyte membrane 16:0 in the Italian EPIC Study (10). As an underlying mechanism, alcohol may increase the activity of acetyl-CoA carboxylase and fatty acid synthase—the key enzymes in 16:0 synthesis (10). Alcohol was also inversely associated with the ratio of 16:0 to 16:1n-7 and with the ratio of 18:0 to *cis* 18:1n-9, as an indicator of activity of the stearoyl CoA desaturase, but these associations varied across centers. Inverse associations with alcohol were only found in centers where olive oil intake was low, whereas no significant inverse association remained in southern countries, where olive oil intake was high (data not shown). The inverse association with alcohol may reflect complex interactions with MUFA synthesis via Δ^9 desaturation when the dietary supply of *cis* 18:1n-9 is low. As a potential mechanism, alcohol may enhance the conversion of SFAs to MUFAs via stearoyl CoA desaturase. When the dietary supply of *cis* 18:1n-9 is high, stearoyl CoA desaturase activity may be down-regulated via a feedback regulation. This finding warrants further interest because both alcohol consumption (36) and a low SI in serum (17, 37) or in erythrocyte membranes (38) have been associated with an increased risk of breast cancer.

We found a positive correlation between plasma *trans* 18:1n-9 concentration and margarine intake. *trans* Fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. Because humans do not synthesize *trans* fatty acids, the occurrence of these fatty acid isomers in blood depends on their availability in the diet. *trans* Fatty acids occur naturally in fat from ruminant-animal meat (mainly vaccenic

acid, *trans* 18:1n-7), milk and dairy fat and unnaturally in industrially hardened vegetable oils (mainly elaidic acid, *trans* 18:1n-9) (39, 40). Dietary exposure to partially hydrogenated vegetable oils occurs through consumption of margarine and of industrially processed foods (41). A higher plasma concentration of *trans* 18:1n-9 was found in northern than in southern Europe, probably because of the higher consumption of margarine in northern Europe, which is the primary fat ingredient in a myriad of industrial products. Indeed, results of the EPIC dietary measurement showed that consumption of margarine ranges between 15 and 36.6 g/d in northern countries and between 0.4 and 4.5 g/d in southern countries of Europe (25). Thus, our data showed that the plasma phospholipid *trans* 18:1n-9 concentration can be considered a valid biomarker of margarine intake. The same data will be used in the near future to investigate the contribution of processed foods to the *trans* 18:1n-9 concentration.

Some fatty acids, particularly fatty acids not synthesized endogenously, are good biomarkers of diet estimated by FFQs and 24-HDRs. With few exceptions, correlations at the ecological level between plasma phospholipid fatty acids and diet are generally higher when estimated from 24-HDRs than when estimated from FFQs. This can be explained by the fact that, in contrast with FFQs, 24-HDRs are highly standardized across countries and provide a much higher level of detail and precision in estimating food intakes, particularly (added) fats. This finding is in line with findings reported in previous similar studies that used other independent biomarkers, such as urinary nitrogen and several plasma carotenoids (42, 43).

In summary, this study provides unique data on plasma phospholipid fatty acid concentrations and their correlation with food intakes in a large population with heterogeneous dietary habits. The dietary heterogeneity observed in European regions involved in this cross-sectional study will be a good basis in the future for investigating the relation between dietary fat intake and the incidence of cancer and cardiovascular diseases.

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