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ORIGINAL ARTICLE

Biomarker-based evaluation of two 24-h recalls for comparing usual fish, fruit and vegetable intakes across European centers in the EFCOVAL Study

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Background/Objectives: A standardized methodology is important to enable consistent monitoring of dietary intake across European countries. For this reason, we evaluated the comparability of the assessment of usual food intake collected with two non-consecutive computerized 24-h dietary recalls (24-HDRs) and a food propensity questionnaire (FPQ) among five European centers.

Subjects/Methods: Two 24-HDRs using EPIC-Soft (the software developed to conduct 24-HDRs in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study) were performed to determine fish, fruit and vegetable (FV) consumed by 600 adults in Belgium (BE), the Czech Republic (CZ), France (FR), the Netherlands (NL) and Norway (NO) in a validation study. An FPQ was used to identify non-consumers. Information from the 24-HDRs and FPQ were used to estimate individual usual food intake by the Multiple Source Method (MSM). Blood samples were drawn to determine fatty acids in phospholipids and serum carotenoids as biomarkers of fish, and FV intake, respectively.

Results: The pooled correlation between usual fish intake and eicosapentaenoic acid plus docosahexaenoic acid in phospholipids was 0.19 in men and 0.31 in women (P for heterogeneity >0.50) and center-specific correlations ranged between 0.08 (CZ) and 0.28 (BE and NO) in men, and between 0.19 (BE) and 0.55 (FR) in women. For usual FV intake, the pooled correlation with serum carotenoids was 0.31 in men and 0.40 in women (P for heterogeneity >0.10); the center-specific correlations varied between 0.07 (NO) and 0.52 (FR) in men, and between 0.25 (NL) and 0.45 (NO) in women.

Conclusion: Two standardized 24-HDRs using EPIC-Soft and an FPQ appeared to be appropriate to rank individuals according to their fish and FV intake in a comparable way among five European centers.

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Introduction

Dietary data from national food consumption surveys are useful to develop and evaluate policies on nutrition and food safety. In Europe, national food consumption data are important to assess the variability of food patterns among different countries. However, European countries performing national surveys use different methodologies such as 24-h

dietary recalls (24-HDRs) and food diaries to collect dietary data (EFSA, 2009). In addition, differences exist in a number of aspects such as the food classification system used across countries. For instance, olives can be considered as a fruit in one food classification, and as a vegetable in another (Ireland *et al*., 2002).

European countries are expected to provide similar dietary indicators if harmonized food consumption data are collected in future national surveys (Brussaard *et al*., 2002a). For this reason, the European Food Consumption Survey Method (EFCOSUM) Consortium recommended the collection of food consumption data using two non-consecutive standardized 24-HDRs as the most appropriate method in future pan-European surveys (Brussaard *et al*., 2002b). Furthermore, the consortium recommended the use of EPIC-Soft (the software developed to conduct 24-HDRs in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study) for standardization, and defined a set of dietary components, including, besides specific nutrients, vegetables, fruits, bread, fish and shellfish (Biro *et al*., 2002; Slimani and Valsta, 2002; Steingrimsdottir *et al*., 2002; Brussaard *et al*., 2002b) to serve as nutritional indicators.

Because the use of a standardized and valid methodology is crucial to enable consistent monitoring of diet across European countries, the European Food Consumption Validation (EFCOVAL) Consortium aimed to further develop and validate the methodology proposed for pan-European dietary monitoring. To that end, our previous work showed that two non-consecutive days of dietary intake collected with 24-HDRs (EPIC-Soft) were considered sufficiently valid for comparing usual protein and potassium intake among five European centers (Crispim *et al*., 2011). In the present study, we intended to further evaluate the dietary intake collected with respect to the comparability of food group assessment across different European populations. A food propensity questionnaire (FPQ) was included in the assessment to offer covariate information in complementing the 24-HDRs during the estimation of usual intake of food groups (Subar *et al*., 2006).

Assessment of intake of fruits and vegetables (FVs) and fish and shellfish can be evaluated using, serum carotenoids (Yeum *et al*., 1996; Polsinelli *et al*., 1998; Macdonald *et al*., 2009) and *n*-3 fatty acids, respectively, in, for example, phospholipids (Leaf *et al*., 1995; Katan *et al*., 1997; Zock *et al*., 1997) as concentration biomarkers. Concentration biomarkers are related to dietary intake, but not as directly as recovery biomarkers because their concentrations are the result of complex metabolic processes (Freedman *et al*., 2010). Therefore, their use in validation studies is restricted to their associations, commonly as correlations, with self-reported dietary intakes. The strength of these correlations is often lower (<0.6) than that of recovery biomarkers (Jenab *et al*., 2009).

This paper aims to evaluate and compare the assessment of ranking of individuals according to their usual fish and FV consumption estimated with two non-consecutive standardized 24-HDRs and an FPQ between five selected centers in Europe, using fatty acids in phospholipids and serum carotenoids as biomarkers of intake, respectively.

Subjects and methods

Study population

The study population consisted of 297 men and 303 women, in the age group between 45 and 65 years, from five selected centers from Belgium, the Czech Republic, France (Southern part), the Netherlands and Norway. These centers were chosen to represent the large diversity of food patterns across Europe. Participants were recruited by convenience sampling, and were healthy individuals representing all educational levels. Eligible participants were able to read and speak the national language, not following prescribed dietary therapy, not pregnant or lactating and not enrolled in another study at the same period. In addition, we did not allow subjects in the study who were donating blood or plasma during or <4 weeks before the study, institutionalized persons or more than one member of the same household. More details about the study populations, including recruitment and sampling procedures are described elsewhere (Crispim *et al*., 2011).

Study design

The period of data collection was from April to July 2007 in the Netherlands and from October or November 2007 to April 2008 in the other four centers. Ethics committees in each center approved the research protocol, and participants signed an informed consent. At the beginning of the study, each participant filled out a screening and a general questionnaire, with questions about lifestyle and food habits, including type and frequency of supplements used during the previous 3 months. Participants were then weighed and had their height measured in the study centers following standardized procedures. They also underwent a non-fasting venipuncture. Thereafter, we collected two non-consecutive 24-HDRs with approximately 1 month gap in between. The time interval between blood sampling and the first 24-HDR was on average less than a week for all centers, except in the Czech Republic where the average was 2 weeks.

Dietary data

We collected the two 24-HDRs using EPIC-Soft, version 9.16 (Slimani *et al*., 1999, 2000). In brief, EPIC-Soft is a computer-assisted dietary tool that follows a standardized procedure to minimize measurement errors when describing, quantifying, probing and calculating food intakes across countries (Slimani *et al*., 1999). The two 24-HDRs were collected using two modes of administration: one through phone and one

by conducting face-to-face interviews. A randomization schedule was created to consider a random order of the two modes of administration, as well as the inclusion of all days of the week equally among the subjects. This randomization allowed the same person to have the same day of the week recalled for both interviews by chance.

Interviewers in each center were nutritionists or dietitians who were trained by qualified local trainers in interviewing skills and working with EPIC-Soft. Centers were allowed to organize their data collection in the same way as they would in a future performance of their national monitoring survey. For example, dietary recalls in Belgium, the Czech Republic and the Netherlands were not conducted on Sundays and, therefore, intakes from Saturday were recalled 2 days later, that is, on Mondays. Furthermore, interviewees were permitted to check food packages and household measures in their home for detailed information during the phone interview, whereas this was not possible during the face-to-face interview at the study center. All centers used an existing version of EPIC-Soft, which had already been used in a national survey or within the EPIC study, except the Czech Republic for which a new version was developed. Methods of estimation of portion size included household measures, weight/volume, standard units and portions, as well as photographs in a picture book. Furthermore, dietary supplement-use information of the recalled day was collected at the end of the 24-HDR interview. If a supplement was taken, subjects reported on the physical state (for example, capsule), the number of units per consumption occasion and the frequency. If known, the brand name was also reported. In addition, an FPQ including one question per food group was used to identify frequency of usual consumption of fish, FVs over the past year.

Food groups were defined as suggested by European Food Consumption Survey Method (Ireland *et al*., 2002). To this end, the foods, as reported by the recalls, were regrouped by including or excluding specific subgroups of the EPIC-Soft food classification (Slimani *et al*., 1999). Fruit intake was defined not to include nuts, seeds, olives and fruit juices other than freshly squeezed juices. Vegetable intake was defined to include herbs but not pulses and potatoes. Fish intake was defined to include shellfish. Fish was classified in lean fish (<4 g of fat/100 g of edible part such as cod, tuna, tilapia and carp) or fatty fish ($\geqslant 4$ g of fat/100 g of edible part such as salmon, herring and mackerel) using country-specific food composition tables.

Venipuncture and biomarkers

We provided participants with guidelines to consume a low-fat breakfast before blood sampling. We requested subjects to rest before a trained lab technician drew blood (2×9 ml) from the antecubital vein. The blood was then allowed to clot for 30 min at room temperature (20–22 °C) and centrifuged for 15 min at 1200 *g*. Serum samples from each subject were aliquoted into cryotubes for storage at −80 °C, until shipment on dry ice to the central laboratory at Wageningen University, where analyses were carried out.

After thawing and mixing the samples, fatty acids in the phospholipid fraction were measured by extracting and separating the lipid classes. Briefly, the phospholipid fraction was separated from the other lipid classes on an aminopropyl column according to the procedure described by Kaluzny *et al*. (1985). Fatty acid methyl ester profile was prepared according to the procedure described by Metcalfe *et al*. (1966). Serum carotenoids were analyzed as described by Khan *et al*. (2007). This method does not adequately separate lutein and zeaxanthin; consequently, these two carotenoids are presented together. Total cholesterol was measured spectrophotometrically on a Synchron LX20 clinical analyzer (Beckman Coulter, Mijdrecht, The Netherlands).

The fatty-acid composition of phospholipids was used as concentration biomarker of fish intake, namely the percentage of eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) in relation to the total area of measured fatty acids (36 fatty acids). The sum of serum carotenoids, including α-carotene, β-cryptoxanthin, β-carotene, lutein and zeaxanthin, was used as marker of FV intake. To further explore the correlations across centers, both α-carotene and β-carotene were used as biomarkers of FVs. Similarly, β-cryptoxanthin was used as a biomarker of fruit intake (Polsinelli *et al*., 1998; Jansen *et al*., 2004) and lutein plus zeaxanthin of vegetable intake (Jansen *et al*., 2004; Al-Delaimy *et al*., 2005b). The intraassay precision, expressed as coefficient of variation, of EPA and DHA was $<4\%$ and of individual carotenoids between 5 and 8%.

Supplement use

Supplement users were identified as those who reported taking any supplements containing EPA, DHA or carotenoids on one of the recalled days or during the past 3 months according to the general questionnaire. To identify the presence of fatty acids and carotenoids in the reported supplements, we (1) searched websites of companies, (2) visited drugstores and (3) searched other sources such as national databases.

Statistical analyses

The statistical analyses were carried out separately for men and women. For evaluating the ranking of individuals, we calculated Pearson's correlation coefficients between the average intake of foods groups, based on the 2 days and their respective biomarkers per center. In addition, adjusted Pearson's correlation coefficients were estimated between usual intake of food groups and respective biomarker using partial correlations. For adjusted correlations, we used usual intake corrected for within-person variability together with the information from the FPQ (Adjusted_1), as estimated by the Multiple Source Method (MSM; German Institute of

Human Nutrition, 2009). We further corrected for the following covariables that were expected to be associated with the intake or excretion on the basis of pre-existing knowledge: age, body mass index, educational level, alcoholic beverage intake and smoking status (Adjusted$_2$). Fruit and vegetable intake analyses were also corrected for total serum cholesterol (Brady *et al*., 1996). For the calculation of the correlations, intake of foods and concentrations of biomarkers were log-transformed to improve normality of the observed distributions. Considering the fact that improvements in the normality of the distribution may have not been achieved, Spearman's correlations were also computed. However, only when conclusions based on Pearson's and Spearman's correlations differed, we presented the latter. Confidence intervals of the correlations were obtained using Fisher's *Z*-transformation (Kleinbaum *et al*., 2007).

The MSM is a statistical method to estimate usual dietary intake of nutrients and foods, including episodically consumed foods, for populations as well as individuals. In contrast to many other statistical methodologies, MSM first estimates individual usual intakes rather than constructing directly the population distributions of usual intake. The method can make use of covariate information such as consumption frequency information from an FPQ to improve the modeling of consumption probability and intake amount (German Institute of Human Nutrition, 2009).

Pooled correlations of the five centers were calculated by first converting correlations into a standard normal metric (Fisher's *r*-to-*Z* transformation). Next, the pooled average was calculated, in which each transformed correlation coefficient was weighted by its inverse variance, followed by the back transformation (Kleinbaum *et al*., 2007). Cochrane *Q*-test was used for testing heterogeneity of the pooled correlation (Field, 2005).

The estimated intake did not include the amounts of EPA, DHA or carotenoids, originating from supplement use. To help interpreting the main results, biomarker levels were presented separately for the total sample, users and non-users of supplements. Given the small number of subjects in each group, men and women were grouped together to optimize this part of the analysis.

Analyses were performed using SAS statistical package, version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

With regard to the characteristics of our study population, the mean body mass index of the French men and women was somewhat lower than those of the other four centers (Table 1). A larger prevalence of smokers was observed in Czech men (33%) and Norwegian women (23%) than in subjects of the other centers. Moreover, subjects with a low educational level were less represented than subjects with a moderate or high educational level, especially in Norwegian men. Belgian men reported the highest intake of alcoholic beverages (average of 30.2 g/day) and Czech women the lowest (average of 6.3 g/day). Furthermore, the total serum cholesterol concentration of the subjects did not vary substantially across the five centers in both genders.

In all centers, each day of the week was represented by between 12 and 17% of the 24-HDRs, except in France, where Saturday was less representative (8%) and Thursday more (19%), and in the Czech Republic, where almost 19% of the interviews were about the intake of a Sunday. For all centers, the interval between the first and the second 24-HDRs was at least 3 weeks.

Table 1 Characteristics of subjects from the five European centers in the EFCOVAL Study

	Men					*Women*				
	BE	*CZ*	*FR*	*NL*	*NO*	*BE*	*CZ*	*FR*	*NL*	*NO*
n	63	58	54	60	62	60	60	59	62	62
Age (years)	54 ± 0.7	55 ± 0.9	56 ± 0.7	57 ± 0.6	55 ± 0.8	55 ± 0.7	55 ± 0.8	55 ± 0.8	55 ± 0.7	54 ± 0.8
BMI (kg/m^2)	27.2 ± 0.5	27.9 ± 0.5	25.5 ± 0.4	26.5 ± 0.5	26.4 ± 0.3	25.2 ± 0.5	25.0 ± 0.5	23.2 ± 0.4	25.5 ± 0.6	24.8 ± 0.5
Smoking (% of total)										
Current	15.9	32.8	14.8	11.7	19.4	13.3	10.0	8.5	3.2	22.6
Former	47.6	17.2	25.9	61.6	40.3	28.3	21.7	23.7	37.1	38.7
Never	36.5	50.0	59.3	26.7	40.3	58.4	68.3	67.8	59.7	38.7
Education (% of total)										
Low	15.9	20.7	25.9	20.0	3.2	16.7	16.6	35.6	24.2	16.1
Intermediate	23.8	24.1	24.1	20.0	30.7	25.0	46.7	27.1	40.3	19.4
High	60.3	55.2	50.0	60.0	66.1	58.3	36.7	37.3	35.5	64.5
Alcoholic beverage intake (g/day)	30.2 ± 4.2	17.8 ± 3.4	15.1 ± 2.5	27.1 ± 3.4	16.5 ± 2.8	17.3 ± 2.7	6.3 ± 1.3	6.9 ± 1.3	12.3 ± 2.0	10.7 ± 2.1
Total serum cholesterol (mmol/l)	5.5 ± 0.1	5.5 ± 0.1	5.8 ± 0.1	5.5 ± 0.1	5.8 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.9 ± 0.1

Abbreviations: BE, Belgium; BMI, body mass index; CZ, the Czech Republic; EFCOVAL, European Food Consumption Validation; FR, France; NL, the Netherlands; NO, Norway.
Results are shown as mean ± s.e., unless otherwise stated.

Table 2 Intakes of fish, fruits and vegetables estimated from the 2 × 24-HDRs and related biomarkers (mean ± s.e.) of 600 participants in the EFCOVAL Study

	Men					*Women*				
	BE	*CZ*	*FR*	*NL*	*NO*	*BE*	*CZ*	*FR*	*NL*	*NO*
Fish intake (g/day)	52 ± 7.6	20 ± 5.4	47 ± 8.0	25 ± 4.8	82 ± 10.5	32 ± 4.7	24 ± 5.0	43 ± 6.6	22 ± 4.6	65 ± 8.8
% Fish consumers	63	26	59	42	77	65	40	64	40	82
% EPA + DHA of total fatty acids in phospholipids	5.3 ± 0.2	4.4 ± 0.1	5.2 ± 0.2	4.5 ± 0.2	7.3 ± 0.2	5.3 ± 0.2	4.1 ± 0.1	5.8 ± 0.2	4.8 ± 0.2	7.0 ± 0.3
Ratio fatty/lean fish intake[a]	1.0	0.2	0.3	1.1	0.9	1.2	0.8	0.8	4.3	0.9
Fruit intake (g/day)	163 ± 18.4	207 ± 23.2	228 ± 27.5	198 ± 21.4	199 ± 23.7	206 ± 18.2	226 ± 20.1	265 ± 21.7	257 ± 19.0	194 ± 18.7
Vegetable intake (g/day)	220 ± 13.7	162 ± 15.9	222 ± 19.4	194 ± 12.1	168 ± 13.5	215 ± 14.7	157 ± 12.8	254 ± 18.5	174 ± 10.0	166 ± 12.6
% Cooked fruits and vegetables	50	27	35	38	31	43	24	34	29	25
Subgroups[b] *(g/day)*										
Citrus fruits	39 ± 7.7	61 ± 10.8	55 ± 11.6	22 ± 6.4	43 ± 8.9	49 ± 10.6	67 ± 10.5	82 ± 11.8	41 ± 9.6	49 ± 8.1
Non-citrus fruits	107 ± 15.7	132 ± 16	139 ± 17.9	159 ± 17.2	154 ± 20.1	150 ± 14.3	157 ± 17.2	162 ± 16.5	180 ± 14.6	131 ± 16
Leafy vegetables	34 ± 5.2	7 ± 3.8	39 ± 7.1	35 ± 5.6	10 ± 2.4	41 ± 8.0	6 ± 2.3	59 ± 8.1	29 ± 4.4	17 ± 3.9
Fruiting vegetables	63 ± 9.3	54 ± 10	77 ± 10.3	69 ± 7.9	56 ± 7.7	60 ± 7.3	51 ± 6.7	77 ± 10.1	63 ± 5.9	61 ± 8.2
Root vegetables	26 ± 4.4	29 ± 4.4	38 ± 6.6	10 ± 2.7	30 ± 6.4	29 ± 4.7	29 ± 5.0	45 ± 6.9	21 ± 5.3	28 ± 5.2
Cabbages	25 ± 6.6	44 ± 8.5	18 ± 5.4	27 ± 7.0	35 ± 6.4	29 ± 6.8	42 ± 7.1	16.4 ± 4.1	27 ± 5.5	25 ± 5.7
Onion and garlic	33 ± 5.3	14 ± 2.1	20 ± 3.4	24 ± 4.0	9 ± 1.6	38 ± 5.7	12 ± 1.7	16.3 ± 2.4	13 ± 2.5	9 ± 1.7
Sum of serum carotenoids[c] (mcg/100 ml)	77 ± 4.8	60 ± 3.1	121 ± 8.5	87 ± 5.4	77 ± 3.2	102 ± 6.1	81 ± 5.4	151 ± 8.6	108 ± 5.9	100 ± 5.8
Lutein + zeaxanthin (mcg/100 ml)	28.5 ± 1.3	26.3 ± 1.4	42.2 ± 2.7	31.5 ± 1.7	29.5 ± 2.2	37.0 ± 2.2	26.7 ± 1.7	49.0 ± 2.6	36.2 ± 2.1	33.4 ± 2.0
β-cryptoxanthin (mcg/100 ml)	15.3 ± 1.1	12.8 ± 1.1	22.4 ± 2.1	16.5 ± 1.3	13.3 ± 0.8	21.3 ± 1.9	14.4 ± 1.3	32.6 ± 3.7	24.5 ± 1.9	14.7 ± 0.8
α-carotene (mcg/100 ml)	7.2 ± 0.7	5.0 ± 0.5	12.9 ± 1.2	6.4 ± 1.6	7.3 ± 0.6	9.4 ± 1.0	8.8 ± 1.2	15.0 ± 1.2	7.1 ± 0.6	11.0 ± 1.0
β-carotene (mcg/100 ml)	26.0 ± 2.7	15.9 ± 1.4	42.9 ± 3.7	32.7 ± 2.9	26.7 ± 1.4	34.1 ± 2.9	30.8 ± 3.2	54.5 ± 3.7	39.8 ± 2.9	40.6 ± 3.1

Abbreviations: BE, Belgium; CZ, the Czech Republic; DHA, docosahexaenoic acid; EFCOVAL, European Food Consumption Validation; EPA, eicosapentaenoic acid; FR, France; NL, the Netherlands; NO, Norway.
[a]Excluding fish products.
[b]Fruit and vegetable subgroups that are main contributors to the main food group intake. Other fruits not presented, included mixed fruits (for example, dry fruits). Other vegetables not presented, included mushrooms, mixed, grain and stalk vegetables.
[c]α-Carotene + β-cryptoxanthin + β-carotene + lutein + zeaxanthin.

Mean fish intake was highest in the Norwegian center; it was 3–4 times higher than the mean intakes in the Czech Republic or the Netherlands (Table 2). Similarly, the highest mean percentage of EPA plus DHA in phospholipids was seen in Norway and the lowest in the Czech Republic and the Netherlands. A low proportion of fatty to lean fish intake (excluding fish products) was observed in the Czech and French men (ratio $\leqslant 0.3$) as compared with the other three centers, in which the ratio ranged between 0.9 and 1.1. A high proportion of fatty to lean fish (ratio 4.3) was observed in Dutch women. Shellfish and roe products contributed between 13 and 26% of total fish intake in Belgium and France, whereas the Czech Republic did not report any consumption of it (data not shown). The percentage of fish consumers identified by 24-HDRs was lower (Table 2) than that identified by FPQ, which showed nearly 95% of consumers in all centers (data not shown).

The lowest crude correlation between fish intake and the biomarker (Table 3) was observed in the Czech Republic in both genders ($r = -0.04$ in men and 0.24 in women). When we analyzed usual fish intake by adjusting intakes for within-person variability and including FPQ data (see Table 3, Adjusted$_1$), evident improvement of correlations was seen across the centers, with the exception of the correlation coefficients in Belgian and Czech women that decreased, respectively, from 0.34 to 0.19 and from 0.24 to 0.21. Further adjustment of the correlations for possible confounders did not explain the differences across centers (see Table 3, Adjusted$_2$). Nevertheless, although the adjusted correlation for fish intake was still considerably lower in Czech men ($r = 0.08$) than in the other centers, no statistically significant heterogeneity of correlations was found between the centers ($P > 0.20$ in both gender).

The largest average intake of both FVs was seen in France for both men and women (Table 2). Although the lowest fruit intake was reported in Belgium for men and in Norway for women, the lowest vegetable intake was observed in the Czech Republic and Norway for both genders. In addition, there were large differences in the types of FV consumed across centers for both men and women. Cooked FVs were less consumed by Czech subjects (~25%) and more by French (~45%). The average amounts of citrus fruits consumed were larger in the Czech Republic and France than in the other three centers. Leafy vegetables were clearly less consumed in the Czech Republic and Norway than in Belgium, France and the Netherlands. The percentage of FV consumers identified by the 24-HDRs was the same as that identified by the FPQ, and was nearly 100%. In relation to the biomarker, the highest mean concentration of carotenoids was observed in France and the lowest in the Czech Republic.

Table 3 Pearson's correlation coefficients (*r*) with confidence intervals (CIs) between intakes of fish, fruit and vegetable from 24-HDRs and related biomarkers of participants in the EFCOVAL Study

Center[a]	*Men*						*Women*					
	Crude		*Adjusted$_1$*[b]		*Adjusted$_2$*[c]		*Crude*		*Adjusted$_1$*		*Adjusted$_2$*	
	r	*CI*	r	*CI*	r	*CI*	r	*CI*	r	*CI*	r	*CI*
Fish vs EPA + DHA												
BE	0.11	(−0.14, 0.35)	0.32	(0.07, 0.52)	0.28	(0.03, 0.50)	0.34	(0.09, 0.54)	0.19	(−0.07, 0.42)	0.19	(−0.09, 0.43)
CZ	−0.04	(−0.29, 0.23)	0.05	(−0.21, 0.30)	0.08	(−0.19, 0.34)	0.24	(−0.02, 0.46)	0.21	(−0.04, 0.44)	0.28	(0.02, 0.51)
FR	0.22	(−0.05, 0.46)	0.34	(0.08, 0.56)	0.27	(−0.02, 0.51)	0.37	(0.12, 0.57)	0.54	(0.32, 0.69)	0.55	(0.33, 0.71)
NL	0.13	(−0.13, 0.37)	0.23	(−0.03, 0.46)	0.21	(−0.06, 0.46)	0.30	(0.06, 0.51)	0.34	(0.10, 0.54)	0.35	(0.09, 0.56)
NO	0.22	(−0.04, 0.44)	0.27	(0.02, 0.49)	0.28	(0.01, 0.50)	0.31	(0.07, 0.52)	0.48	(0.25, 0.65)	0.46	(0.22, 0.64)
Pooled[d]	0.13	(0.01, 0.25)	0.24	(0.13, 0.36)	0.19	(0.07, 0.30)	0.31	(0.20, 0.43)	0.36	(0.24, 0.47)	0.31	(0.20, 0.41)
Fruit and vegetables vs sum of carotenoids[e]												
BE	0.38	(0.15, 0.57)	0.38	(0.14, 0.57)	0.36	(0.11, 0.57)	0.49	(0.27, 0.66)	0.52	(0.30, 0.68)	0.36	(0.10, 0.57)
CZ	0.54	(0.32, 0.70)	0.47	(0.24, 0.65)	0.52	(0.28, 0.69)	0.33	(0.08, 0.54)	0.39	(0.14, 0.58)	0.35	(0.09, 0.56)
FR	0.43	(0.18, 0.62)	0.50	(0.26, 0.67)	0.43	(0.16, 0.64)	0.37	(0.13, 0.57)	0.42	(0.18, 0.61)	0.44	(0.19, 0.64)
NL	0.32	(0.06, 0.53)	0.20	(−0.06, 0.44)	0.16	(−0.12, 0.41)	0.42	(0.18, 0.60)	0.35	(0.11, 0.55)	0.25	(−0.02, 0.48)
NO	0.05	(−0.20, 0.30)	0.06	(−0.20, 0.31)	0.07	(−0.20, 0.33)	0.44	(0.21, 0.62)	0.46	(0.23, 0.64)	0.45	(0.21, 0.64)
Pooled	0.35	(0.23, 0.46)	0.33	(0.21, 0.45)	0.31	(0.20, 0.41)	0.41	(0.30, 0.53)	0.43	(0.31, 0.54)	0.40	(0.29, 0.49)
Vegetable intake vs sum of carotenoids												
BE	0.21	(−0.04, 0.44)	0.24	(−0.02, 0.45)	0.20	(−0.06, 0.44)	0.34	(0.09, 0.54)	0.36	(0.11, 0.56)	0.39	(0.14, 0.59)
CZ	0.47	(0.23, 0.65)	0.55	(0.34, 0.71)	0.63	(0.42, 0.77)	0.19	(−0.07, 0.42)	0.23	(−0.03, 0.46)	0.21	(−0.06, 0.45)
FR	0.37	(0.11, 0.58)	0.31	(0.04, 0.53)	0.33	(0.04, 0.56)	0.44	(0.21, 0.63)	0.47	(0.23, 0.64)	0.58	(0.36, 0.73)
NL	0.09	(−0.17, 0.33)	0.01	(−0.24, 0.27)	−0.01	(−0.28, 0.26)	0.46	(0.23, 0.63)	0.34	(0.09, 0.54)	0.16	(−0.10, 0.41)
NO	0.16	(−0.10, 0.39)	0.12	(−0.14, 0.36)	0.06	(−0.21, 0.32)	0.44	(0.22, 0.62)	0.53	(0.31, 0.68)	0.50	(0.26, 0.67)
Pooled	0.26	(0.14, 0.38)	0.26	(0.14, 0.37)	0.24	(0.12, 0.34)	0.38	(0.26, 0.49)	0.39	(0.27, 0.50)	0.38	(0.28, 0.47)
Fruit intake vs sum of carotenoids												
BE	0.27	(0.02, 0.48)	0.21	(−0.04, 0.43)	0.22	(−0.04, 0.46)	0.22	(−0.03, 0.45)	0.40	(0.16, 0.59)	0.18	(−0.10, 0.42)
CZ	0.14	(−0.12, 0.39)	0.28	(0.02, 0.50)	0.30	(0.02, 0.53)	0.31	(0.06, 0.52)	0.32	(0.07, 0.53)	0.27	(0.00, 0.50)
FR	0.27	(0.01, 0.50)	0.49	(0.25, 0.67)	0.54	(0.29, 0.72)	0.21	(−0.05, 0.44)	0.29	(0.03, 0.51)	0.26	(−0.01, 0.50)
NL	0.29	(0.03, 0.50)	0.23	(−0.03, 0.46)	0.16	(−0.12, 0.41)	0.24	(−0.01,0.46)	0.32	(0.07, 0.52)	0.23	(−0.03, 0.47)
NO	−0.16	(−0.39, 0.10)	−0.07	(−0.32, 0.19)	−0.07	(−0.33, 0.20)	0.12	(−0.13, 0.36)	0.25	(−0.01, 0.47)	0.21	(−0.06, 0.45)
Pooled	0.16	(0.05, 0.28)	0.23	(0.11, 0.34)	0.17	(0.06, 0.28)	0.22	(0.11, 0.34)	0.32	(0.20, 0.43)	0.25	(0.14, 0.36)
Fruit and vegetables vs α−carotene												
BE	0.41	(0.18, 0.60)	0.45	(0.23, 0.63)	0.39	(0.14, 0.59)	0.44	(0.21, 0.62)	0.47	(0.24, 0.64)	0.34	(0.08, 0.56)
CZ	0.46	(0.23, 0.64)	0.43	(0.19, 0.61)	0.48	(0.23, 0.66)	0.14	(−0.12, 0.38)	0.16	(−0.10, 0.39)	0.14	(−0.14, 0.39)
FR	0.55	(0.33, 0.71)	0.59	(0.38, 0.74)	0.50	(0.24, 0.69)	0.38	(0.13, 0.58)	0.42	(0.17, 0.61)	0.41	(0.16, 0.62)
NL	0.23	(−0.03, 0.46)	0.16	(−0.11, 0.40)	0.11	(−0.17, 0.37)	0.52	(0.30, 0.68)	0.44	(0.22, 0.62)	0.38	(0.12, 0.58)
NO	0.11	(−0.15, 0.35)	0.12	(−0.14, 0.36)	0.09	(−0.19, 0.34)	0.43	(0.20, 0.61)	0.47	(0.25, 0.65)	0.43	(0.19, 0.62)
Pooled	0.36	(0.27, 0.44)	0.36	(0.24, 0.48)	0.31	(0.20, 0.41)	0.37	(0.27, 0.50)	0.40	(0.28, 0.51)	0.34	(0.23, 0.44)
Fruit and vegetables vs β-carotene												
BE	0.44	(0.22, 0.62)	0.43	(0.21, 0.61)	0.38	(0.13, 0.58)	0.41	(0.17, 0.60)	0.44	(0.21, 0.62)	0.30	(0.03, 0.52)
CZ	0.46	(0.22, 0.64)	0.41	(0.17, 0.60)	0.42	(0.16, 0.62)	0.22	(−0.04, 0.45)	0.26	(0.01, 0.48)	0.24	(−0.03, 0.48)
FR	0.45	(0.20, 0.64)	0.52	(0.29, 0.69)	0.39	(0.11, 0.61)	0.33	(0.08, 0.54)	0.38	(0.13, 0.58)	0.39	(0.12, 0.59)
NL	0.17	(−0.09, 0.41)	0.03	(−0.23, 0.29)	−0.05	(−0.32, 0.23)	0.21	(−0.04, 0.44)	0.14	(−0.11, 0.38)	−0.03	(−0.29, 0.23)
NO	0.18	(−0.07, 0.41)	0.13	(−0.13, 0.37)	0.07	(−0.20, 0.33)	0.44	(0.21, 0.62)	0.46	(0.24, 0.64)	0.51	(0.27, 0.68)
Pooled	0.33	(0.21, 0.44)	0.31	(0.20, 0.43)	0.26	(0.15, 0.37)	0.34	(0.23, 0.46)	0.34	(0.23, 0.46)	0.30	(0.19, 0.40)
Vegetable intake vs lutein + zeaxanthin												
BE	−0.11	(−0.35, 0.14)	−0.07	(−0.31, 0.18)	−0.06	(−0.32, 0.20)	0.39	(0.15, 0.59)	0.45	(0.21, 0.63)	0.48	(0.24, 0.66)
CZ	0.37	(0.12,0.57)	0.48	(0.25, 0.66)	0.52	(0.28, 0.69)	0.15	(−0.11, 0.39)	0.15	(−0.11, 0.39)	0.17	(−0.10, 0.42)
FR	0.23	(−0.04, 0.47)	0.24	(−0.03, 0.48)	0.22	(−0.07, 0.48)	0.26	(0.01, 0.48)	0.29	(0.03, 0.51)	0.43	(0.17, 0.63)
NL	0.15	(−0.11, 0.39)	0.07	(−0.19, 0.32)	0.04	(−0.23, 0.31)	0.45	(0.22, 0.63)	0.36	(0.12, 0.56)	0.21	(−0.06, 0.45)
NO	0.08	(−0.18, 0.32)	0.06	(−0.19, 0.31)	0.01	(−0.25, 0.28)	0.32	(0.07, 0.52)	0.41	(0.17, 0.60)	0.33	(0.07, 0.55)
Pooled	0.14	(0.02, 0.26)	0.16	(0.04, 0.28)	0.17	(0.06, 0.29)	0.32	(0.20, 0.43)	0.34	(0.22, 0.45)	0.29	(0.18, 0.39)
Fruit intake vs β-cryptoxanthin												
BE	0.38	(0.14, 0.57)	0.28	(0.03, 0.49)	0.35	(0.09, 0.55)	0.20	(−0.06, 0.43)	0.41	(0.17, 0.60)	0.19	(−0.08, 0.43)
CZ	0.17	(−0.09, 0.41)	0.31	(0.05, 0.52)	0.35	(0.09, 0.57)	0.57	(0.36, 0.72)	0.57	(0.36, 0.72)	0.59	(0.38, 0.74)
FR	0.16	(−0.12, 0.41)	0.41	(0.15, 0.61)	0.48	(0.22, 0.67)	0.28	(0.03, 0.50)	0.29	(0.03, 0.51)	0.22	(−0.06, 0.46)

Table 3 *Continued*

Center[a]	*Men*						*Women*					
	Crude		*Adjusted$_1$*[b]		*Adjusted$_2$*[c]		*Crude*		*Adjusted$_1$*		*Adjusted$_2$*	
	r	*CI*	r	*CI*	r	*CI*	r	*CI*	r	*CI*	r	*CI*
NL	0.26	(0.01, 0.48)	0.18	(−0.09, 0.42)	0.17	(−0.10, 0.43)	0.27	(0.02, 0.49)	0.40	(0.17, 0.59)	0.32	(0.06, 0.54)
NO	0.18	(−0.08, 0.41)	0.03	(−0.23, 0.28)	0.08	(−0.19, 0.34)	0.05	(−0.21, 0.29)	0.15	(−0.11, 0.39)	0.09	(−0.18, 0.35)
Pooled	0.23	(0.12, 0.35)	0.24	(0.12, 0.36)	0.21	(0.09, 0.32)	0.28	(0.17, 0.40)	0.37	(0.26, 0.49)	0.35	(0.26, 0.46)

Abbreviation: EFCOVAL, European Food Consumption Validation.
[a]BE, Belgium; CZ, the Czech Republic; FR, France; NL, the Netherlands; NO, Norway.
[b]Adjusted for within-person variability by Multiple Source Method, taking into account the food propensity questionnaire.
[c]Adjusted$_1$ + adjusted for age, body mass index, educational level, alcoholic beverage, smoking status using partial Pearson correlations. Cholesterol level was also included in the fruit and vegetable analysis.
[d]*P*-value for heterogeneity was >0.10 for all analyses in the table.
[e]α-Carotene + β-cryptoxanthin + β-carotene + lutein + zeaxanthin.

Table 4 Biomarker levels (mean ± s.e.) of the total sample, users and non-users of specific supplements in the EFCOVAL Study

	BE		*CZ*		*FR*		*NL*		*NO*	
	% EPA + DHA[a]	n	*% EPA + DHA*	n	*% EPA + DHA*	n	*% EPA + DHA*	n	*% EPA + DHA*	n
All subjects	5.3 ± 0.1	123	4.2 ± 0.1	118	5.5 ± 0.2	111	4.7 ± 0.1	120	7.2 ± 0.2	121
Supplement users[b]	6.5 ± 0.5	6	4.7 ± 0.3	17	5.8 ± 0.8	10	5.6 ± 0.5	13	7.9 ± 0.2	76
Non-supplement users	5.2 ± 0.1	117	4.1 ± 0.1	101	5.4 ± 0.1	101	4.6 ± 0.1	107	5.9 ± 0.3	45
	Serum carotenoids[c]	n	*Serum carotenoids*	n	*Serum carotenoids*	n	*Serum carotenoids*	n	*Serum carotenoids*	n
All subjects	89.1 ± 4.0	123	70.6 ± 3.3	118	136.8 ± 6.3	111	98.2 ± 4.1	120	88.4 ± 3.5	121
Supplement users[d]	79.1 ± 19.2	2	94.4 ± 11.4	11	140.2 ± 62	5	127.5 ± 17.4	11	197	1
Non-supplement users	89.3 ± 4.1	121	68.1 ± 3.3	107	136.6 ± 6.1	106	95.2 ± 4.1	109	87.5 ± 3.4	120

Abbreviations: BE, Belgium; CZ, the Czech Republic; EFCOVAL, European Food Consumption Validation; FR, France; NL, the Netherlands; NO, Norway.
[a]Of total fatty acids in phospholipids.
[b]Fish oil supplements.
[c]α-Carotene + β-cryptoxanthin + β-carotene + lutein + zeaxanthin.
[d]Supplements containing carotenoids.

Crude correlation coefficients of FV intake with the sum of carotenoids were between 0.05 in the Norwegian men and 0.54 in the Czech men (Table 3). A smaller range of correlations was observed in women (0.33 in the Czech Republic to 0.49 in Belgium). Pearson's correlations, based on usual intakes, as estimated by the MSM method, barely differed from the crude ones (Adjusted$_1$). Adjusted correlations including possible confounders varied in different directions and did not explain the differences across the centers (Adjusted$_2$). The adjusted correlation of FV intake in the Norwegian men was 0.07, whereas all other centers presented correlations ranging from 0.16 to 0.52. However, we did not identify deviating correlations when assessing the heterogeneity of the pooled correlations ($P>0.10$ for all comparisons). Overall, the correlations of the combined intake of FVs with the sum of carotenoids were higher than that of FVs separately, especially for fruit. Correlations between FV intake with α-carotene were higher than that with the sum of carotenoids in some sub-populations but lower in others such as in the Dutch men, who happened to have the lowest consumption of carrots (results not shown). When using β-carotene as a biomarker of FV intake, correlations were often lower than the sum of carotenoids, particularly in the Netherlands. The correlations between vegetable intake and luteine plus zeaxanthine, and the correlations between fruit intake and β-cryptoxanthin did not explain the low correlation observed in Norway. Nevertheless, when using Spearman's correlations, there was an improvement of those correlations in Norway. Furthermore, after including all types of juices in the FV group, the correlations modestly increased between FV intake and the sum of carotenoids in some centers, but not for all (data not shown). The major changes were seen in Norway for men, where for instance the correlation between fruit intake and β-cryptoxanthin increased from 0.03 to 0.32.

The percentage of fish oil supplement users was high in Norway (63%) as compared with the other four centers (<14%; Table 4). In line with this, the percentage of EPA plus DHA in phospholipids of subjects, who reported not taking any fish oil supplement, was substantially lower than that of the supplement users and the total group in Norway. Supplements containing carotenoids were less often consumed

than those with fish oil, with the highest number of users in the Czech Republic and the Netherlands (11 subjects each) and the lowest in Norway (1 subject). As a result, mean serum carotenoid concentrations of non-supplement users were similar to those of the total group.

Discussion

We compared the assessment of usual fish and FV consumption of adults estimated with two non-consecutive standardized 24-HDRs in combination with a FPQ among five centers in Europe. Overall, we observed weak-to-moderate associations between fish and FV intake and respective biomarkers. In men, correlations for fish intake in the Czech Republic and for FV intake in Norway were distinctly lower than those in the other centers. In women, the correlations across centers were rather comparable.

One of the major strengths of this study is the replicate collection of 24-HDRs, allowing the application of statistical adjustment to obtain an estimate of the individual usual intake. Welch *et al*. (2006) have shown that one 24-HDR was less consistent in providing an association between fish intake and serum fatty acids than food frequency questionnaires or a 7-day diary. This can be explained by the fact that 24-HDRs are not able to reflect usual intakes of infrequently consumed foods. Indeed, we have observed that correlations substantially improved when considering usual intakes including FPQ data for fish. Another important strength of this study is the unique setting of data collection that has provided standardized dietary intake and biomarker information for different countries.

One of the limitations of our study is that given the limited number of participants, large confidence intervals were observed. The sample size may also have limited the interpretation of the Cochrane *Q*-test. We found no statistically significant heterogeneity between correlations, but this could be caused by the relatively small sample size. Nevertheless, this is not very likely because the observed *P*-values for that test were rather high, especially in the assessment of fish intake ($P>0.50$). In addition, data collection was performed in a different season in the Netherlands than in the other four centers. Considering the fact that carotenoid contents in FVs may differ between seasons (Maiani *et al*., 2009), this could have led to a different performance of the method in the Dutch population. Nevertheless, we expect that both intake and biomarker assessment have been affected, thus minimizing the possible influence of seasonality on the correlations. Another potential limitation may be that the five center populations are not representative of their respective country populations, because they can be expected to consist of health-conscious subjects. This hampers the extrapolation of our results to the general population. Furthermore, the individual usual intakes of foods estimated with MSM can be questioned. A study by Souverein *et al*. (2011) showed that when applying methods such as MSM to groups of small sample size, the estimates of usual intake distributions are highly uncertain. However, the accuracy of individual usual intakes estimated by MSM remains unclear, as it has not been investigated.

Apart from sampling variation, the differences in correlations between centers can be attributed to differences in the range of food intake, different compositions of the foods consumed and the presence of other determinants or modifiers of the concentration biomarkers. The range of food intakes differed across centers, especially for fish intake. The low correlation for fish in Czech men may be explained by their very low amounts of fish consumption (on average 20 g/day) by only few subjects (26%). In the Czech population, the fish consumption is traditionally very low and our finding agrees with the low intake of 13 g/day according to a household budget survey (Dofkova *et al*., 2001). In terms of differences in types of foods consumed, studies have shown that the correlation between fatty fish with serum and plasma fatty acids was stronger than that for total fish (Hjartaker *et al*., 1997; Welch *et al*., 2006). We were unable to present these correlations for our data given the high number of non-consumers for fish subgroups and the lack of specific FPQ data. Even so, we observed a very low ratio between fatty and lean fish intake in Czech men and in France. However, in France, a considerable amount of shellfish and roe products was reported, which also contributes to *n*-3 fatty acids. The low consumption of shellfish and roe products together with the low ratio between fatty and lean fish intake might explain why the Czech Republic presented a very low correlation between fish intake and the biomarker, and France did not. Furthermore, substantial differences were observed in the types of FVs consumed across centers. Because the contents and bioavailability of carotenoids in foods can differ depending on harvest conditions, degree of maturity, storage and physical state (Maiani *et al*., 2009), populations with different FV intakes, being more represented by one specific carotenoid than another, may have different carotenoid profiles as well. As a consequence, the sum of carotenoids may not sensibly represent the carotenoid content of FVs of a specific population. We did observe different correlations across centers when using specific carotenoids as biomarkers of specific FV intake, but these did not substantially explain any observed differences in the comparison of FV intake vs sum of carotenoids across centers. Furthermore, other dietary sources may contribute to *n*-3 fatty acids or carotenoids in the blood. For example, some oils are rich sources of α-linolenic acid, which may, to a low extent, be converted to EPA and DHA (Connor, 2001), and colored foods, such as cheeses, can contain carotenoids (Gordon and Bauernfeind, 1982; Jones *et al*., 2005). Non-fresh FV juices, which were not included in the FV group, and fortified foods may also contribute to concentrations of carotenoids. These sources may partly explain the low associations between intake and biomarker, as observed in Norway.

In addition, the interpretation of our results demands understanding of aspects influencing not only the assessment of intake, but also that of the biomarker. Concentration biomarkers do not reflect absolute intake and their quantitative relationship with diet may vary between populations, depending on the presence and relative impact of determinants, such as genetic variation and lifestyle factors (Kaaks *et al*., 1997; Jenab *et al*., 2009). For instance, smoking and alcohol consumption have been inversely associated with levels of *n*-3 fatty acids (Simon *et al*., 1996; Galan *et al*., 2005; Pawlosky *et al*., 2007) and serum carotenoids (Rock *et al*., 1999; Northrop-Clewes and Thurnham, 2007). Nevertheless, when adjusting our correlations for a number of potential confounders such as alcohol intake, smoking status and total serum cholesterol concentrations, the outcomes were quite similar.

In addition, the association between food intake and biomarkers may have been influenced by the use of supplements (Hjartaker *et al*., 1997; Welch *et al*., 2006). Although the Norwegian sample was not the center with the most deviating association between fish intake and its biomarker, the percentage EPA plus DHA in phospholipids in supplement users was markedly higher than that in non-supplement users and the total population in Norway. Nevertheless, it is questionable whether participants were able to recall the exact type and brand name of their supplements (Skeie *et al*., 2009). Therefore, a degree of uncertainty remains in the evaluation of supplement use in relation to food intake assessment and in the explanations of differences in correlations across centers.

The correlations in this paper are consistent with the results of other studies that have found weak to moderate correlations between fish intake and *n*-3 fatty acids in the blood (Welch *et al*., 2006; Saadatian-Elahi *et al*., 2009) and between FV and serum carotenoids (Resnicow *et al*., 2000; Al-Delaimy *et al*., 2005a) when using 24-HDRs (*r* for fish intake between 0.11 and 0.22 and for FV intake between 0.30 and 0.42).

Despite the limitations, we conclude that two standardized 24-HDRs using EPIC-Soft in combination with an FPQ appeared to be appropriate to rank subjects according to their usual fish and FV intake within the five European centers in a comparable manner.

Conflict of interest

JR received consulting fees from the Czech Technology Platform for food and healthy lifestyle. The remaining authors declare no conflict of interest.

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