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Relating fatty acid composition in human fingertip blood to age, gender, nationality and *n*-3 supplementation in the Scandinavian population

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Abstract
This study investigated data obtained from whole blood fatty acid (FA) composition of 3476 Norwegian and Swedish individuals, which provided background information including age, gender, nationality and self-motivated *n*-3 supplement consumption. The aim of this paper was to statistically relate this background information on the subjects to their whole blood FA profile, focusing mainly on the *n*-3 polyunsaturated FA (PUFA). Results showed that age had significant effects on the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in blood lipids for the Norwegian individuals, while *n*-3 PUFA supplementation had a positive effect on EPA and DHA content in whole blood for the investigated population. Gender differences were also found for individual FA. A correlation also exists with previous studies on the FA profiling of blood lipids, further validating the test procedure.

Keywords: *diet, fatty acid composition, home test kit, whole blood*

Introduction

In the human body, fatty acids (FA) are incorporated in blood lipids, depot fats and structural lipids in membranes (Risé et al. 2008). Hence the distribution of FA can be measured in various blood fractions and tissues (Baylin and Campos 2006). Dietary intake, lifestyle, intestinal absorption and metabolism are various factors that affect the FA profile of circulating lipids in humans (Risé et al. 2007; Panagiotakos et al. 2009).

In the Scandinavian countries, citizens have access to a home test kit for voluntary testing of blood for lipid profiling. The test involves absorption of whole blood from the prick of a finger on a special paper. In addition, a requisition form seeking information regarding gender, age, nationality and *n*-3 polyunsaturated FA (PUFA) supplementation is provided. The blood samples are analyzed by gas chromatography of FA methyl esters (FAME) by an accredited laboratory in Norway, after arrival by postal delivery. In this study, a dataset was obtained with results from whole blood FA analysis and background information of 3476 individuals in Norway and Sweden.

The aim of this study was to statistically relate the background information on the subjects to their whole blood FA profile, focusing especially on the *n*-3 PUFA.

Materials and methods

Subjects

The blood test was employed by individuals who were interested in a screening of their blood FA composition through the use of a home test kit (developed by University of Milano (Italy), St. Olavs Hospital in Trondheim (Norway) and is distributed by Itogha AS, Norway, as Oil4Life™ Test). Individuals acquired the test kit through therapists, health clinics, sport stores, a web shop and other sales channels in the two Nordic

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countries, Norway and Sweden. The results presented in this study are from tests taken during the period January 2009–July 2011.

Test kit

The content of the home test kit was as follows: user manual (Grako AS, Norway), requisition form (Grako AS, Norway) for providing necessary information (gender, age, nationality, use of *n*-3 supplements and date), the individual test ID to access results online (Grako AS, Norway), an absorption paper for the blood spot samples (Grako AS), a disposable automatic lancing device (Med-Kjemi AS, Norway), aluminium bag (Whatman, Denmark) for preservation and storage of the test paper and an enclosed envelope (Grako AS, Norway) addressed to St. Olav's Hospital, Trondheim University Hospital, Norway.

Self-administered blood sampling

Drops of blood were obtained from a fingertip by use of the automatic lancing device. Subsequently the blood drops were absorbed on a special piece of paper and thereafter added into a sealed aluminium bag for preservation and storage. A filled out requisition form was sent together with the aluminium bag containing the blood spot sample in an enclosed envelope addressed to St. Olavs Hospital, Trondheim University Hospital, Norway. The anonymous tests were gathered and processed by the accredited laboratory personnel at St.Olavs Hospital, after arrival. The postal delivery may vary from 1 day to 1 week depending on the place of departure. The process from the blood sampling to dataset is illustrated in Figure 1.

GC FA analysis

The blood spot samples were stored at 4°C after arrival at the hospital laboratory, and were then prepared and analyzed within a week. The transesterification and extraction procedure was based on the method published by Marangoni et al. (2004) with slight modifications. Briefly, the pieces of absorbent paper containing the blood samples were transferred to screw-capped glass vials and treated with 1 ml of 0.5 M HCl in MeOH. Samples were then stored at 70°C in a dry bath for 1 h to achieve transesterification of FA to FAME. After cooling, 1 ml of H_2O and 1 ml of saturated KCl were added, before FA were extracted using 2 ml of hexane. The solvent was then evaporated by N_2 and the samples were re-dissolved in 50 µl of hexane. Resultant FAME were analyzed on a gas chromatography mass spectrometry system (HP Agilent 6890 GC unit; HP Agilent 5973 MS detector; Agilent Technologies, Santa Clara, CA, USA). The GC unit was equipped with a 15-m capillary column (Supelco Omegawax 100; Sigma-Aldrich, St. Louis, MO, USA). Samples were injected with a split ratio of 100:1, and the column was heated from 150 to 260°C with a rate of 40°C/min followed by 15 min of isothermal conditions at 260°C. Pure reference compounds were used as standards and all chemical and gases were of analytical grade. Heptadecanoic acid and tricosanoic acid were used as internal standards. Eleven FA were reported semi-quantitatively and expressed as percentage of the total measured FA content in whole blood. The measured FA were; palmitic acid C16:0, stearic acid C18:0, oleic acid C18:1*n*-9, linoleic acid (LA) C18:2*n*-6, γ-linolenic acid C18:3*n*-6, α-linolenic acid C18:3*n*-3, dihomo-γ-linolenic acid C20:3*n*-6, arachidonic acid (AA) C20:4*n*-6, eicosapentaenoic acid (EPA) C20:5*n*-3, docosapentaenoic acid C22:5*n*-3 and docosahexaenoic acid (DHA) C22:6*n*-3. The absorbent paper was tested with regard to the stability of FA in the blood spot samples by storage and analysis of blood spot samples from type 0 during a time interval of 3 months with an internal standard as reference. Only minor changes in stability of the eleven FA were shown at the end of the storage period, indicating stability of more than 2 weeks as demonstrated by Marangoni et al. (2004).

Statistical analysis

The statistical analysis was performed in R ver. 2.14.1, a free software environment maintained by the R Development Core Team (http://www.r-project.org/). Analysis of variance, and simple and multiple regressions were performed directly on un-processed

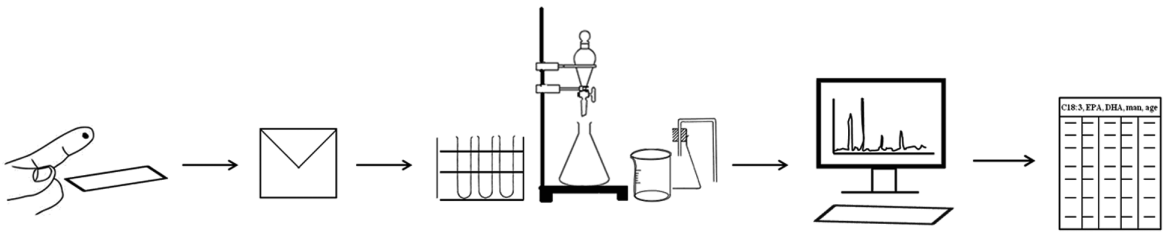

Figure 1. Schematic overview of the sample giving and analysis process of whole blood obtained through a home test kit. (1) Blood spot sample from a fingertip absorbed on paper, (2) sample and a requision form sent by post to laboratory, (3) gas chromatographic analysis of blood samples and (4) statistical analysis of dataset.

data to find differences between groups, e.g. nationalities, and linear trends, e.g. age effects. Age groups equally spaced with 5 year intervals from 10 to 80 years of age were formed to give impressions of age dependent differences, e.g. between males and females, and to indicate distributions of the subjects. Because of the limited number of subjects below 10 years and above 80 years of age, these were all taken out from the analysis. The responses were transformed using the natural logarithm to obtain normally distributed residuals and improve the explained variance. The FA EPA and DHA were selected as markers for *n*-3 PUFA content in whole blood.

Results

Characteristics of individuals

The investigated population included 3244 Norwegians (1885 women and 1359 men) and 232 Swedes (128 women and 104 men). The mean age of the Norwegian individuals was 46 years (for women) and 41 years (for men). Of the Norwegian subjects, 56% declared a supplementation of *n*-3 PUFA. The mean age of the Swedish individuals was 51 years (for women) and 46 years (for men) with 45% of the Swedes declaring PUFA supplementation.

Blood FA composition, age and n-*3 PUFA supplementation*

Figure 2 illustrates incidences of *n*-3 PUFA supplementation within and between age clusters of the investigated Norwegian population. The most prevalent users of the home test kit and also of *n*-3 PUFA supplements in Norway were in the age range of 45–54 years. The higher use of *n*-3 PUFA supplementation seen in this group was not observed in the younger age groups (20–34 years).

A multiple linear regression relating *n*-3 long-chain PUFAs EPA (20:5*n*-3) and DHA (22:6*n*-3) in whole blood of Norwegian individuals, to age, *n*-3 supplementation and their interactions were constructed. The results are shown in the effect plot in Figure 3. As expected (Visioli et al. 2003; Sands 2005; Barceló-Coblijn et al. 2008), our findings show that proportions of EPA and DHA in blood were significantly higher among Norwegian individuals using *n*-3 FA supplements, compared to individuals not consuming *n*-3 supplements (Figure 3). The average whole blood EPA + DHA of the Norwegian individuals not consuming *n*-3 PUFA supplements was 4.9%. Sands et al. (2005) reported the same average value for individuals not consuming *n*-3 FA, when FA in red blood cells were analyzed. The multivariate analysis reveals that age has a significant effect on EPA + DHA content in whole blood of the investigated Norwegian population, also illustrated in Figure 3. Individuals in the age groups < 10 and > 70 years were not included in the multivariate analysis due to the small number of samples. A regular increase in EPA + DHA with rising age is seen for individuals who do not consume *n*-3 PUFA supplements (Figure 3). The interaction between age and usage of *n*-3 PUFA supplements also gave a significant effect on EPA + DHA content in whole blood.

The data obtained from the Swedish population regarding EPA + DHA values showed higher variations compared with the dataset of the Norwegian individuals (Figure 4), also expected in a smaller control group. The effect of age was also significant for the Swedish individuals, as found for the Norwegians. *n*-3 PUFA supplementation had a significant effect on EPA + DHA in blood lipids with a *p*-value of 0.03. No interaction effects were found.

Concerning the proportions of FA in the Norwegian individuals, palmitic acid (16:0), oleic acid (18:1*n*-9) and LA (18:2*n*-6) account for the highest percentage of total FA in both groups. Palmitic and oleic acids show higher proportions in the group not consuming *n*-3 FA on a daily basis. By contrast LA shows the highest proportions in those consuming *n*-3 FA supplements. AA (20:4*n*-6) was the predominant

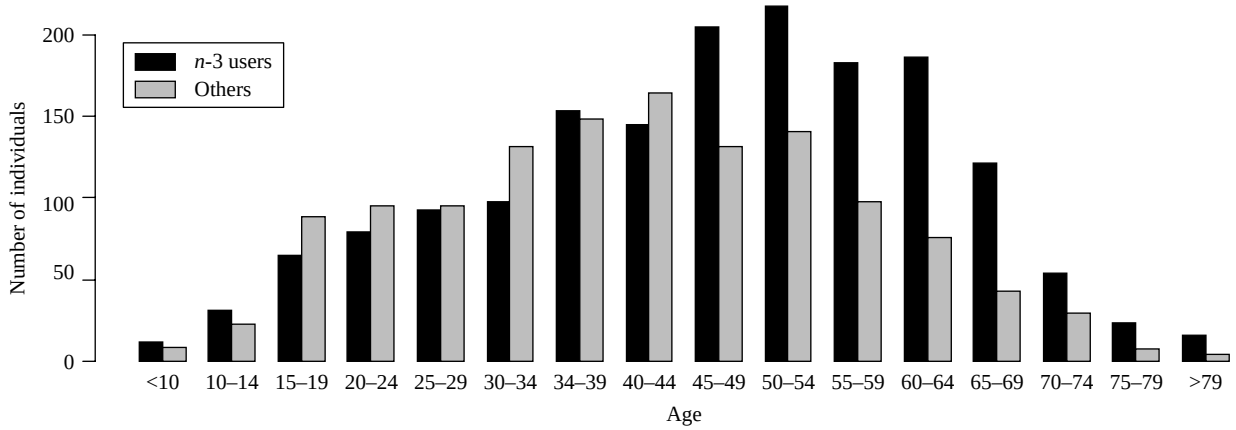

Figure 2. Overview of numbers of Norwegian individuals consuming (black columns) or not consuming (gray columns) *n*-3 FA supplements, according to age.

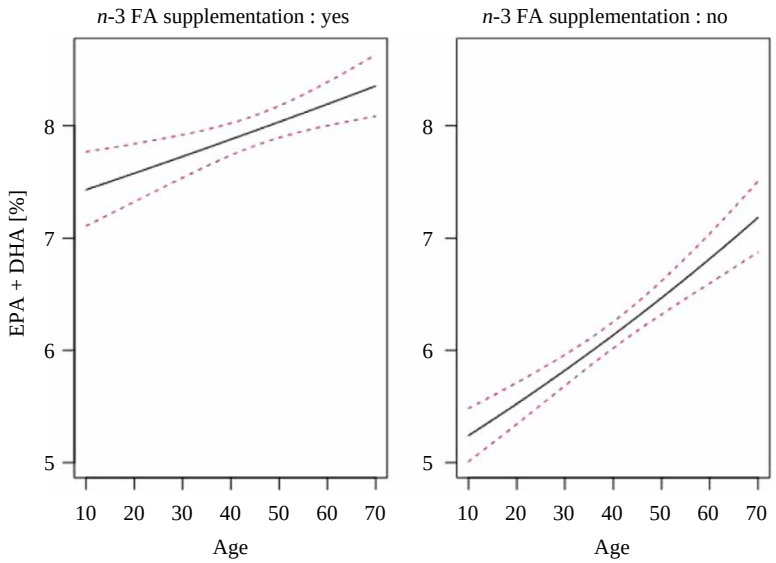

Figure 3. Effect plot presenting EPA and DHA content in the blood of Norwegian individuals at different ages consuming and not consuming *n*-3 FA supplements. (Effects of age, *n*-3 FA supplementation and the interaction effect, gave *p*-values < 0.01.)

LC-PUFA in both groups, but individuals not consuming *n*-3 FA supplementations had a higher proportion (9.30%) in whole blood lipids. As a result, the total *n*-6 PUFAs were significantly higher in the group not consuming *n*-3 FA supplements.

Proportions of FA found in the Swedish individuals were the same as for the Norwegian individuals, with palmitic, oleic and LA accounting for the highest FA percentage composition (Table I). Proportions of oleic acid were generally significantly higher among the Swedish compared to the Norwegian individuals (Tables I and II). Higher proportions of the *n*-6 PUFA AA, as well as higher total *n*-6 PUFAs were found in Swedish individuals not consuming *n*-3. EPA and DPA and, hence, total *n*-3 PUFAs were significantly higher in Swedish individuals consuming *n*-3 supplements.

Blood FA composition and gender

Table II gives the FA composition of blood samples from individuals in the Norwegian and Swedish population, subdivided into men and women. Fifty-seven percent of the Norwegian and 48% of the Swedish women declared a daily intake of *n*-3 FA supplements, while 51% of the Norwegian and 40% of the Swedish men declared the same.

The main difference in FA profile between men and women among the Norwegian individuals in this study concerns the significantly higher monounsaturated oleic acid in men. Higher levels of the *n*-6 FA, LA

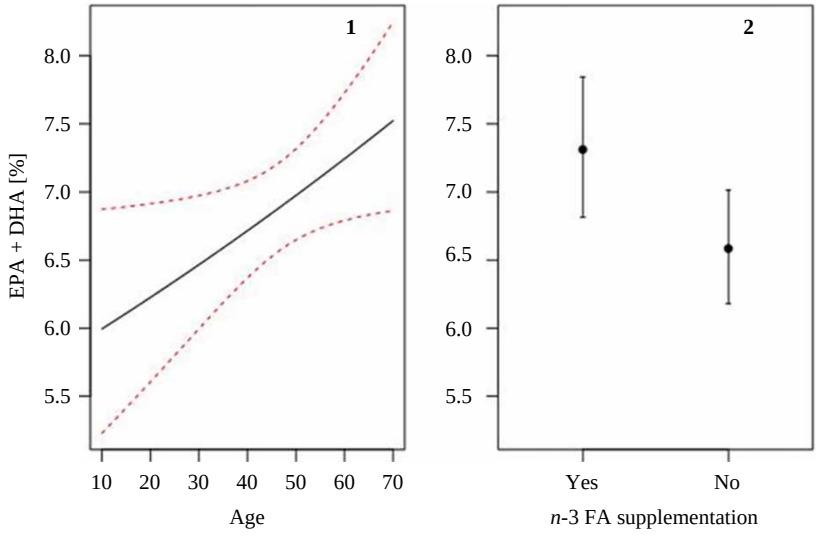

Figure 4. Effect plots presenting the Swedish individuals. 1, Effect of age on EPA and DHA levels in whole blood; 2, effect of *n*-3 FA supplementation on EPA and DHA levels in whole blood. (Effects of age and *n*-3 FA supplementation gave *p*-values < 0.05.)

Table I. Whole blood FA composition (%) of 3244 Norwegian and 232 Swedish individuals, subdivided by daily *n*-3 FA supplementation.

	Norwegian		Swedish	
Fatty acids	Using *n*-3 supplements	Not using *n*-3 supplements	Using *n*-3 supplements	Not using *n*-3 supplements
SFA				
16:0[a]	22.0 ± 3.0	22.7 ± 3.1	22.0 ± 3.0	22.2 ± 2.5
18:0	12.6 ± 1.8	12.6 ± 2.0	12.9 ± 2.0	12.4 ± 1.6
MUFA				
18:1*n*-9[a]	23.8 ± 4.0	24.5 ± 4.4	25.9 ± 3.7	26.2 ± 4.0
PUFA				
18:2*n*-6 (LA)[a]	22.8 ± 3.4	22.2 ± 3.4	21.1 ± 3.2	21.2 ± 3.2
18:3*n*-6 (GLA)	0.5 ± 0.4	0.5 ± 0.3	0.4 ± 0.4	0.5 ± 0.3
18:3*n*-3 (ALA)	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.4	0.5 ± 0.5
20:3*n*-6 (DHGLA)[ab]	1.1 ± 0.4	1.3 ± 0.4	1.1 ± 0.3	1.3 ± 0.4
20:4*n*-6 (AA)[ab]	8.6 ± 2.0	9.3 ± 2.2	8.3 ± 2.1	9.1 ± 2.3
20:5*n*-3 (EPA)[ab]	2.2 ± 1.3	1.3 ± 1.0	2.1 ± 1.1	1.5 ± 0.7
22:5*n*-3 (DPA)[ab]	1.5 ± 0.4	1.3 ± 0.4	1.5 ± 0.4	1.3 ± 0.4
22:6*n*-3 (DHA)[a]	4.3 ± 1.5	3.6 ± 1.3	3.7 ± 1.5	3.6 ± 1.3
Total *n*-6 [ab]	33.1 ± 2.0	33.4 ± 2.1	31.0 ± 2.0	32.1 ± 2.0
Total *n*-3 [ab]	8.4 ± 1.0	6.7 ± 0.9	7.8 ± 1.0	7.0 ± 1.0

Note: Results are expressed as mean (%) ± SD. Significant ($p < 0.05$) differences in individual FA between daily *n*-3 supplementation and non-daily supplementation are indicated by superscript letters (a) for Norwegian individuals and (b) for Swedish individuals. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

and AA were observed in women. On the other hand, women also had significantly higher proportions of the LC-PUFAs, EPA and DHA in whole blood lipids compared to men. It is also of interest that the levels of DPA are higher in men than in women. GLA, ALA, and DHGLA are substantially the same in the two genders. Total *n*-6 and *n*-3 PUFAs were both significantly higher in women. Profiles of individual FA in the Swedish men and women mainly show the same trend as for the Norwegian men and women. Except for the significant difference in LA and total *n*-6 proportions between Swedish men and women, no significant differences could be detected.

Discussion

This study describes the blood FA composition of Norwegian and Swedish individuals who have used a simplified analytical approach for voluntary testing of blood lipids. The use of a home test kit for obtaining a blood spot sample is an easy-to-use option which is applicable to all population groups (Marangoni et al. 2004). Mean values of the FA composition of the Norwegian individuals are not to be viewed as cross sections of the Norwegian population but the connection between blood lipids and the background information of the population should be representative regarding relative changes, considering the high number of samples. The cost of the test which is to be paid for by the purchaser and the individual's personal interest in acquiring such a test are limiting factors that do not make the home test accessible to everyone.

Important background information for interpretation of the data, such as the description of the sample givers' dietary habits is not reported. In this respect, suppositions of the background for the FA results are difficult to assume. Several studies have indicated positive correlations between dietary intake and FA composition in diverse blood fractions when dietary habits have been investigated (Garland et al. 1998; Hibbeln et al. 2003; Kuriki et al. 2003).

Correlations between age and the content of *n*-3 PUFA in blood lipids were observed for Norwegian individuals in this study. Similar observations were seen in plasma concentrations of Japanese women, and were also related to dietary intakes (Kuriki et al. 2002).

Table II. FA composition of whole blood lipids in Norwegian and Swedish individuals; men and women.

	Norwegian		Swedish	
Fatty acids	Men	Women	Men	Women
SFA				
16:0[a]	22.6 ± 3.0	22.0 ± 3.0	22.4 ± 2.3	21.9 ± 3.1
18:0[a]	12.8 ± 2.0	12.7 ± 1.8	12.8 ± 1.7	12.5 ± 1.8
MUFA				
18:1*n*-9[a]	24.9 ± 4.5	23.7 ± 3.9	26.8 ± 3.7	25.7 ± 3.7
PUFA				
18:2*n*-6 (LA)[ab]	21.7 ± 3.4	23.2 ± 3.3	20.6 ± 2.9	21.7 ± 3.3
18:3*n*-6 (GLA)	0.6 ± 0.4	0.5 ± 0.4	0.5 ± 0.3	0.5 ± 0.4
18:3*n*-3 (ALA)	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.5	0.5 ± 0.5
20:3*n*-6 (DHGLA)	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4
20:4*n*-6 (AA) [a]	8.8 ± 2.2	9.1 ± 2.1	8.6 ± 2.1	8.9 ± 2.3
20:5*n*-3 (EPA)[a]	1.7 ± 1.3	1.9 ± 1.3	1.7 ± 0.9	1.9 ± 1.1
22:5*n*-3 (DPA)[a]	1.5 ± 0.5	1.4 ± 0.4	1.5 ± 0.4	1.4 ± 0.4
22:6*n*-3 (DHA)[a]	4.0 ± 1.6	4.1 ± 1.4	3.5 ± 1.3	3.8 ± 1.5
Total *n*-6[ab]	32.2 ± 2.0	34.0 ± 2.0	30.9 ± 1.8	32.3 ± 2.0
Total *n*-3[a]	7.5 ± 1.1	7.7 ± 1.0	7.1 ± 0.9	7.6 ± 1.0

Noe: Norwegian; men: $n = 1359$, women: $n = 1885$. Swedish; men: $n = 104$, women: $n = 128$. Results expressed as mean (%) ± SD. Significant ($p < 0.05$) differences in individual FA between men and women are indicated by superscript letters (a) for Norwegian individuals and (b) for Swedish individuals. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

According to Hjartåker and Lund (1998) dietary habits usually vary between different age groups with Norwegian older women tending to have a healthier diet than Norwegian younger women. Regarding intake of the long chain PUFAs EPA and DHA from fish, Johansson et al. (1998) reported that daily intake was higher among the middle aged and older subjects in Norway compared with the younger age groups. This observation might explain some of the observed increase in EPA + DHA levels in blood with increasing age for individuals not consuming *n*-3 PUFA supplements.

Gender differences in the FA profiles of circulating lipids found in this study were also demonstrated when blood was collected from fingertip by Marangoni et al. (2007). Suggested attributes for the differences were metabolic and dietary diversities between genders.

Conclusions

The results from this study confirm the findings reported in previous studies regarding FA composition in different blood fractions and the effects of age, gender and *n*-3 supplementation, validating the test procedure used in this study as the unexplained variation would not be distinguishable from the effects we confirmed. As shown in this study, supplementation of *n*-3 FA has a direct effect on blood lipids, but factors such as diet and metabolism have a higher effect. Since only relative concentrations of lipids are reported, no assumptions of health effects of the increased EPA and DHA content in whole blood by *n*-3 supplementation are taken in this paper. For future analysis regarding comparisons of whole blood FA composition, more background information about the sample givers would be necessary, including food-frequency questionnaires and lifestyle parameters.

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