Nutrition, Metabolism & Cardiovascular Diseases (2014) **24**, 883–890

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Dietary patterns and fatty acids levels of three European populations. Results from the IMMIDIET study☆

G. Pounis [a], M. de Lorgeril [b], P. Salen [b], F. Laporte [c], V. Krogh [d], A. Siani [e], J. Arnout [f], F.P. Cappuccio [g], M. van Dongen [h], M.B. Donati [a], G. de Gaetano [a], L. Iacoviello [a,*], On behalf of the European Collaborative Group of the IMMIDIET Project

[a] *Laboratory of Molecular and Nutritional Epidemiology, Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Via Dell'Elettronica, 86077 Pozzilli, IS, Italy*
[b] *TIMC-IMAG, Coeur et Nutrition, PRETA, CNRS 5525, Université Joseph Fourier-CNRS, Faculté de Médecine, La Tronche, France*
[c] *Département de Biochimie, Pharmacologie et Toxicologie, Hôpital Universitaire, Grenoble, France*
[d] *Istituto Nazionale dei Tumori, Milan, Italy*
[e] *Institute of Food Sciences, CNR, Avellino, Italy*
[f] *Katholieke Universiteit Leuven, Belgium*
[g] *Warwick Medical School, Coventry, United Kingdom*
[h] *Department of Epidemiology, Maastricht University, Maastricht, The Netherlands*

Received 18 October 2013; received in revised form 4 January 2014; accepted 7 January 2014
Available online 29 January 2014

KEYWORDS
n–3 Fatty acids;
n–6 Fatty acids;
Dietary patterns;
Plasma fatty acids;
Plasma *n*–3 fatty acids;
Plasma *n*–6 fatty acids;
Cardiovascular disease;
Neurological disease

Abstract *Background and aims:* Differences in blood fatty acids (FAs) profile among populations with different lifestyle have partly been attributed to differences in food intake. A holistic approach in dietary guidance through dietary patterns is essential. This study aimed at evaluating the main plasma and red blood cell (RBC) FAs in three European populations and assessing the role of dietary patterns in explaining variation in their levels.
Methods: In the framework of the IMMIDIET Project, 1604 subjects (802 male–female pairs) aged 26–65 years were enrolled in Italy, Belgium and UK. Plasma and RBC FAs were measured. One year recall food frequency questionnaires were used to evaluate dietary habits of each individual.
Results: Italian cohort showed lower plasma and RBC $n-3$ levels than participants of the other two populations ($P < 0.001$). Both plasma and RBC arachidonic acid were higher in Italian cohort as compared to Belgian and English. Reduced rank regression analysis indicated two dietary patterns explaining 35% and 17% of the total variation of the sum of plasma and RBC $n-3$, respectively. In a holistic dietary analysis, neither fish nor mollusks intake seemed to contribute to $n-3$ variation as compared to vegetable oils and polyphenol-rich foods.
Conclusion: The Italian cohort presented significant lower plasma and RBC $n-3$ FA levels compared to Belgians and English. A holistic approach in dietary analysis seemed to explain a relatively high proportion of plasma and RBC $n-3$ FAs variability. Dietary pattern analysis may contribute to the study of the association of human diet with FAs levels.

Abbreviations: RRR, reduced rank regression; FA, fatty acid; CVD, cardiovascular disease; RBC, red blood cell; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; NAF, nutrition analysis of FFQ; FCT, food composition tables; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; LDL, low-density lipoprotein.

☆ IMMIDIET Project Investigators are listed in the Supplementary material.

* Corresponding author. Tel.: +39 0865929664; fax: +39 0865927575.
E-mail addresses: licia.iacoviello@moli-sani.org, licia.iacoviello@neuromed.it (L. Iacoviello).

0939-4753/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.numecd.2014.01.012

Introduction

The majority of studies evaluating possible associations of dietary habits with health outcomes focus on the effects of single nutrients or foods on health or illness. However, the complexity of the human diet, especially the interactions between foods or nutrients, makes it more difficult to evaluate their effects on diseases or on biological predictors or risk factors. Recently, new statistical methods such as reduced rank regression (RRR) analysis have been proposed to study the combined effect of foods on health outcomes. As an "a posteriori" method, it includes the construction of dietary patterns derived from the data at hand; thus determining combinations of food intake that explain as much response variation as possible in specific populations [1].

Growing evidence suggests that blood fatty acids (FAs) are major biomarkers-predictors and even causal factors for many diseases, in particular cardiovascular disease (CVD), cancer and nervous system diseases [2–5]. In that context, omega-3 FAs ($n-3$) and their interactions with omega-6 FAs ($n-6$) are obviously critical [2,3].

Consumption of $n-3$, EPA ($20{:}5n-3$) and DHA ($22{:}6n-3$), may improve cardiovascular health by altering FA metabolism, inducing haemodynamic changes, decreasing arrhythmias, modulating platelet function, improving endothelial function and inhibiting inflammatory pathways [2,3]. $n-3$ Deficiency or insufficiency and low blood/tissue $n-3$ levels were associated with increased disease risks [2,3,6].

Most population differences in prevalence and severity of CVD such as between Northern European vs. Mediterranean populations could in part be explained by population differences in lifestyle and dietary habits [7]. Types of fats consumed are among the possible factors explaining the relations between dietary habits and CVD risk [8].

Despite the relation of FAs levels with the consumption of some food groups, there is limited knowledge on the possible association of whole dietary patterns with blood/tissue FA profiles. Moreover, there is apparently no comparative data on the levels of main FAs in blood/tissue across different populations such as Northern vs. Southern Europeans neither on dietary patterns that may explain differences in FA profiles.

This study aimed at evaluating the main plasma and red blood cell (RBC) FAs levels in three European populations at different dietary habits and risk of CVD. We also evaluated the role of dietary patterns in relation to the observed variation in $n-3$ blood/tissue (plasma/RBC) levels.

Subjects and methods

Participants

The IMMIDIET Project and the recruitment of subjects were previously described [7]. Between October 2001 and October 2003, 271 male–female pairs in the Abruzzo region in Italy, 268 pairs in the Flemish territory of Belgium and 263 in South-West London in England, aged 26–65 years were enrolled in this cross-sectional study. Eligible pairs were randomly selected in each country, by local general practitioners. The recruitment strategies were carefully defined and standardised across the recruiting centres (see Supplementary file). Exclusion criteria for all groups were as follows: history of cardiovascular disease, diabetes mellitus, familial hypercholesterolaemia, malignancies, chronic diseases like heart, liver or renal failure, hypo/hyperthyroidism and epilepsy. The participation rates were 85% in Italy, 70% in Belgium and 90% in UK. The ethical committees of all participating institutions approved the study. All study participants agreed by written informed consent.

Dietary and lifestyle assessment

Interviews were taken using a standardised questionnaire previously adopted in the Olivetti Prospective Heart Study [9].

The validated Italian and English European Prospective Investigation into Cancer and Nutrition (EPIC) food frequency questionnaires (FFQ) [10] were used to evaluate dietary intake of 164 food items over the past year. Since a valid EPIC-FFQ version was not available for the Belgian subpopulation a new 322-item FFQ was developed to assess the food intake of the Belgian–Belgian couples [11]. A computer programme, nutrition analysis of FFQ (NAF) [12] converted questionnaire dietary data into frequencies of consumption and average daily quantities of foods, energy and nutrients consumed. NAF was linked to the McCance food composition tables (FCT) [13], the Italian FCT for Epidemiological studies [14], and the Dutch NEVO [15] and Flemish-Belgian Nubel FCTs [16]. For the purpose of this study, the164 food items of EPIC-FFQ formed the 20 basic food groups that were included in the dietary analysis.

Clinical assessment and biochemical measurement of FAs

Trained research personnel in the different recruitment centres performed blood pressure (BP) and anthropometric measurements. Systolic and diastolic BP was measured with an automated device (OMRON-HEM-705CP; OMRON Corporation, Amsterdam, Netherlands) [17]. Body weight and height were measured in subjects without shoes and wearing light clothing with the use of a standard beam balance scale and attached ruler. BMI (kg/m^2) was calculated.

Blood samples were obtained between 7.00 and 9.00a.m. from patients who had been fasting overnight and had refrained from smoking for at least 6 hours. Measurements of serum lipids and blood glucose levels were performed at the Institute of Food Sciences, CNR, Avellino, Italy, by an automated analyser (Cobas-Mira-Plus, Roche, Milano Italy). LDL-cholesterol was estimated by the Friedewald formula [18].

FA measurements were performed at the Université Joseph Fourier-CNRS, Faculté de Médecine, La Tronche,

France. Blood samples (collected into EDTA), were separated into plasma and packed cells by centrifugation and kept frozen at −70 °C until FAs could be extracted. The FA composition in red blood cells was shown to remain stable for a long time at this temperature [19].

Plasma and RBC FAs were measured by gas chromatography [8]. Lipids were extracted by a monophasic method that uses a solution of hexane to isopropanol (3:2; vol:vol) after adding heptadecanoic acid as an internal standard. Extracted lipids were saponified and methylated with 14% boron trifluoride in alcohol. After extraction, the methylated FAs were quantified by gas chromatography with flame ionization detection on a capillary column. Hydrogen was the carrier gas. FA peaks were identified and quantified by comparison with known standards, and FA composition is reported as weight percentage of total FAs. RBC EPA and DHA reported as weight percentage of total FAs gave the "Omega 3 Index" [4].

Statistical analysis

The normality of continuous variables was tested graphically and presented as mean (standard deviation). Plasma or RBC FA levels and dietary data were skewed so they are presented as median (1st—3rd quartiles). Comparisons of plasma or RBC FA levels between countries were performed using the one-way ANOVA *F*-test on log-transformed levels. After Bonferroni correction for multiple comparisons alpha = 0.003 (17-tests) was established as significance cut-off point for both plasma and RBC FA differences between countries. Comparisons of clinical and anthropometric measurements between countries were performed using one-way ANOVA *F*-test; while comparisons of dietary data were done using Kruskal—Wallis test.

RRR was used in order to derive dietary patterns that better describe high blood $n-3$ levels. RRR works with two different sets of variables, called predictors and responses; it extracts linear functions of predictors (named factors) that explain as much response variation as possible [1]. In this study RRR-analysis was performed two times. In each of both analyses the predictors were 21 dietary components and the responses were the log-transformed total plasma $n-3$ FAs (ALA, EPA, DPA, DHA) values for the 1st model, and the log-transformed total RBC $n-3$ FAs (ALA, EPA, DPA, DHA) values for the 2nd model. RRR-analysis produced each time one factor model-dietary pattern. The correlations between each extracted factor and dietary components are called factor loadings; as a rule of thumb, we characterized the factor using the dietary components with an absolute factor loading ≥ 0.20. Each subject received, for each pattern, a factor score (RRR-score), calculated by summing the observed intakes of the 21 dietary components, each weighted by its factor loading [1]. A higher value of this score represents increased adherence to the factor-dietary pattern that describes better the high total plasma or RBC $n-3$ level.

Spearman's rho was used to quantify the association between each dietary component indicated by the two extracted dietary patterns with total plasma or RBC $n-3$ levels. All tested hypotheses were two-sided. A *P*-value<0.05 was considered to reflect statistical significance. STATA version 9, software was used for all calculations except from RRR where SAS software version 9.1 was used. PASW statistics software version 18.0 was used to produce histograms and normal curves.

Results

Table 1 presents the clinical and anthropometric characteristics of study participants according to country of residence. Italian cohort seemed to be at lower age compared to Belgian and British. In addition, BMI, SBP, DBP and triglycerides presented higher in Italy compared to Belgium or UK. However, Italian participants seemed to have lower serum levels of LDL-cholesterol and fasting glucose.

Plasma FAs levels

Table 2 shows the distribution of individual plasma FAs according to country. A significant difference between the three countries was observed for the majority of them. Italian cohort showed higher plasma levels of myristic and palmitic FAs but lower levels of arachidic acid as compared to Belgian (*P* for all pair differences <0.001). No significant difference was observed between Italian and English cohorts for any saturated FAs (*P* for all >0.003).

Plasma $n-3$ ALA, EPA, and DPA levels were lower in Italian cohort compared to either Belgian or English

Table 1 Distribution of individual clinical and anthropometric characteristics according to country of residence.[a]

	Country of residence			*P*[b]
	Italy ($n=542$)	Belgium ($n=536$)	UK ($n=526$)	
Age (years)	44.4 (7.1)[1,2]	46.2 (8.5)[1]	48.0 (7.5)[2]	<0.001
BMI (kg/m^2)	27.2 (4.4)[1,2]	26.1 (4.3)[1,3]	26.4 (4.3)[2,3]	<0.001
SBP (mmHg)	126 (18)[1]	123 (16)[2]	120 (18)[1,2]	<0.001
DBP (mmHg)	80 (11)[1]	78 (10)	77 (10)[1]	0.002
Total cholesterol (mg/dl)	215 (37)[1]	227 (39)[1,2]	215 (39)[2]	<0.001
LDL-cholesterol (mg/dl)	138 (33)[1]	149 (36)[1,2]	141 (35)[2]	<0.001
HDL-cholesterol (mg/dl)	50 (13)[1]	56 (15)[1,2]	53 (14)[2]	<0.001
Triglycerides (mg/dl)	132 (94)[1,2]	110 (68)[1]	109 (66)[2]	<0.001
Fasting glucose (mg/dl)	76.1 (19.6)[1,2]	78.7 (11.4)[1,3]	88.9 (13.0)[2,3]	<0.001

Superscript numbers (1, 2, 3) indicated pairs of countries presenting significant differences on individual clinical and anthropometric characteristics, according to Bonferroni comparisons. Bonferroni correction for multiple comparisons (9 tests and significance level $a=0.05$) yield a corrected $\acute{a}=0.006$ which indicated significant differences at the aforementioned univariate analysis.

[a] Continuous data are presented as mean (standard deviation).

[b] *P*-values derived through one-way ANOVA *F*-tests for country differences on normally distributed continuous characteristics.

Table 2 Distribution of individual plasma FAs according to country of residence.

	Country of residence			P^b
	Italy ($n = 542$)	Belgium ($n = 536$)	UK ($n = 526$)	
FAs (% of total FAs)[a]				
Saturated				
14:0 (myristic)	1.20 (0.83–1.61)[1]	1.06 (0.76–1.39)[1,2]	1.17 (0.87–1.55)[2]	<0.001
16:0 (palmitic)	22.0 (20.7–23.7)[1]	21.6 (20.2–23.1)[1]	21.7 (20.5–23.2)	<0.001
18:0 (stearic)	5.67 (5.13–6.22)	5.85 (5.25–6.48)	5.72 (5.26–6.24)	0.004
20:0 (arachidic)	0.04 (0.03–0.05)[1]	0.05 (0.04–0.07)[1,2]	0.04 (0.03–0.05)[2]	<0.001
Unsaturated				
18:3$n-3$ (α-linolenic)	0.44 (0.36–0.56)[1,2]	0.83 (0.66–1.01)[1,3]	0.89 (0.73–1.06)[2,3]	<0.001
20:5$n-3$ (Eicosapentaenoic)	0.60 (0.46–0.82)[1,2]	0.92 (0.69–1.25)[1,3]	1.27 (0.99–1.65)[2,3]	<0.001
22:5$n-3$ (Docosapentaenoic)	0.41 (0.34–0.51)[1,2]	0.54 (0.45–0.63)[1,3]	0.63 (0.55–0.73)[2,3]	<0.001
22:6$n-3$ (Docosahexaenoic)	1.88 (1.51–2.26)[1,2]	2.06 (1.72–2.53)[1,3]	2.25 (1.79–2.94)[2,3]	0.003
18:2$n-6$ (Linoleic)	29.8 (26.4–33.0)[1,2]	33.5 (30.0–36.7)[1,3]	31.3 (27.7–34.0)[2,3]	<0.001
18:3$n-6$ (γ-linolenic)	0.59 (0.48–0.77)[1,2]	0.50 (0.39–0.64)[1]	0.54 (0.42–0.67)[2]	<0.001
20:2$n-6$ (Eicosadienoic,)	0.18 (0.15–0.23)[1]	0.23 (0.20–0.26)[1,2]	0.20 (0.17–0.23)[2]	<0.001
20:3$n-6$ (Dihomo-γ-linolenic)	1.67 (1.45–1.92)[1,2]	1.46 (1.22–1.70)[1]	1.49 (1.31–1.73)[2]	<0.001
20:4$n-6$ (Arachidonic)	8.23 (6.95–9.26)[1,2]	7.45 (6.44–8.46)[1,3]	6.82 (6.00–7.73)[2,3]	<0.001
22:4$n-6$ (ardenic)	0.21 (0.18–0.25)[1]	0.21 (0.17–0.25)[2]	0.18 (0.15–0.23)[1,2]	<0.001
16:1$n-7$ (palmitoleic)	2.13 (1.69–2.92)[1]	2.13 (1.66–2.88)[2]	2.70 (2.16–3.37)[1,2]	<0.001
18:1$n-7$(cis-vaccenic)	1.63 (1.43–1.90)[1]	1.56 (1.38–1.75)[1,2]	1.64 (1.44–1.91)[2]	<0.001
18:1$n-9$ (oleic)	21.8 (19.7–24.0)[1,2]	18.4 (16.8–20.4)[1,3]	19.9 (18.1–21.7)[2,3]	<0.001
20:3$n-9$ (mead)	0.14 (0.11–0.19)[1,2]	0.10 (0.08–0.13)[1,3]	0.12 (0.09–0.16)[2,3]	<0.001

Superscript numbers (1, 2, 3) indicated pairs of countries with significantly different levels on each FA according to Bonferroni comparisons. Bonferroni correction for multiple comparisons (17 tests and significance level a = 0.05) yield a corrected $\acute{a}$=0.003 which indicated significant differences at the aforementioned univariate analysis.

[a] Plasma FAs were presented as median (1st–3rd quartiles) because their distributions were skewed.

[b] *P*-values derived through one-way ANOVA *F*-tests for country differences on log-transformed plasma FAs levels.

participants (*P* for all pair differences $< 0.001 < \acute{a} = 0.003$, Bonferroni correction for multiple comparisons) (Table 2). Plasma DHA was lower in Italian cohort compared to Belgian or English; while levels in UK were the highest (*P* for all pair differences $< 0.001 < \acute{a} = 0.003$). The trend of Italian participants to have lower plasma $n-3$ than the participants of the other two populations is also illustrated in Supplementary Fig. S1.

The distribution of plasma $n-6$, $n-7$ and $n-9$ presented significant differences among the three countries with no specific trends (Table 2). $n-6$ LA was lower and AA and oleic acid were higher in Italy compared to Belgium or UK (*P* for all pair differences $< 0.001 < \acute{a} = 0.003$).

RBC FAs levels

The distribution of RBC FA levels according to country is illustrated in Table 3. Most of saturated and unsaturated RBC FAs followed the same trend observed for plasma FAs. RBC myristic acid levels were lower in Italian cohort compared to Belgian and English. On the contrary, palmitic acid levels were in Italy higher than in Belgium and lower than in UK (P for all pair differences $< 0.001 < \acute{a} = 0.003$, Bonferroni correction for multiple comparisons). No significant differences were observed for stearic and arachidic acids (*P* for all $>0.003 = \acute{a}$).

RBC $n-3$ ALA, EPA, and DPA were lower in Italian cohort compared to Belgian and English (*P* for all pair differences $< 0.001 < \acute{a} = 0.003$, Bonferroni correction for multiple comparisons) (Table 3), while DHA levels differed only between English and Belgian cohorts (*P* for all pair differences$<0.001<\acute{a} = 0.003$) and no significant difference was observed with Italian participants (P for pair differences$>\acute{a} = 0.003$). This was also evident for the omega-3 index, which was significantly lower in Italian cohort compared to Belgian or English (P for all pair differences $< 0.001 < \acute{a} = 0.003$). The trend of Italian cohort to have lower $n-3$ RBC compared with the two other populations is also illustrated in Supplementary Fig. S2.

The distribution of $n-6$, $n-7$ and $n-9$ FA in RBC presented also significant differences among the three populations, although without specific trends (Table 3). N-6 LA (the substrate for the endogenous synthesis of the $n-6$ AA) levels were lower in Italy compared to Belgium and UK whereas AA levels were higher in Italy (P for all pair differences$<0.001<\acute{a} = 0.002$).

Dietary patterns in relation to n – 3 FAs levels

RRR-analysis was used in order to derive dietary patterns that could fully describe the high plasma and RBC $n-3$ levels (Table 4).

Model 1 identified a factor-dietary pattern explaining 35% of the total variation of total plasma $n-3$. This pattern was described by high consumption of cabbages and root vegetables, dairies, vegetable oils, chocolate, coffee and tea and mayonnaise; and low intake of cheese, pasta and rice, red meat and processed products and olive oil.

The model 2 revealed a factor-dietary pattern explaining 17% of the total variation of total RBC $n-3$. This dietary

Table 3 Distribution of individual RBC FAs according to country of residence.

	Country of residence			P^b
	Italy ($n = 542$)	Belgium ($n = 536$)	UK ($n = 526$)	
FAs (% of total FAs)[a]				
Saturated				
14:0 (myristic)	0.27 (0.22–0.35)[1,2]	0.29 (0.24–0.36)[1,3]	0.32 (0.26–0.39)[2,3]	<0.001
16:0 (palmitic)	21.4 (20.6–22.1)[1,2]	21.0 (20.4–21.9)[1,3]	22.1 (21.2–23.2)[2,3]	<0.001
18:0 (stearic)	15.8 (15.0–16.6)	16.0 (15.1–16.8)	16.1 (15.2–16.9)	0.006
20:0 (arachidic)	0.08 (0.07–0.09)	0.09 (0.08–0.10)	0.09 (0.07–0.10)	0.48
Unsaturated				
18:3$n-3$ (α-linolenic)	0.09 (0.07–0.11)[1,2]	0.19 (0.16–0.23)[1]	0.19 (0.16–0.23)[2]	<0.001
20:5$n-3$ (Eicosapentaenoic)	0.63 (0.52–0.78)[1,2]	1.00 (0.82–1.27)[1,3]	1.21 (0.97–1.57)[2,3]	<0.001
22:5$n-3$ (Docosapentaenoic)	2.54 (2.92–2.24)[1,2]	3.09 (2.83–3.40)[1,3]	3.30 (2.92–3.65)[2,3]	<0.001
22:6$n-3$ (Docosahexaenoic)	6.16 (5.35–6.91)	6.40 (5.49–7.22)[1]	6.15 (5.03–7.27)[1]	<0.001
18:2$n-6$ (Linoleic)	10.8 (10.1–11.9)[1,2]	12.4 (11.6–13.4)[1,3]	11.8 (10.9–12.7)[2,3]	<0.001
18:3$n-6$ (γ-linolenic)	0.06 (0.05–0.07)	0.06 (0.04–0.08)	0.06 (0.05–0.08)	0.03
20:2$n-6$ (Eicosadienoic)	0.22 (0.20–0.25)[1]	0.26 (0.23–0.30)[1,2]	0.22 (0.20–0.26)[2]	<0.001
20:3$n-6$ (Dihomo-γ-linolenic)	1.91 (1.67–2.19)[1,2]	1.77 (1.57–2.03)[1]	1.80 (1.56–2.06)[2]	<0.001
20:4$n-6$ (Arachidonic)	19.3 (18.2–20.4)[1,2]	18.3 (17.3–19.3)[1,3]	16.6 (15.3–17.8)[2,3]	<0.001
22:4$n-6$ (ardenic)	3.66 (3.32–4.05)[1,2]	3.30 (2.90–3.73)[1,3]	2.76 (2.37–3.20)[2,3]	<0.001
16:1$n-7$ (palmitoleic)	0.30 (0.23–0.41)[1,2]	0.35 (0.27–0.46)[1,3]	0.40 (0.31–0.53)[2,3]	<0.001
18:1$n-7$(cis-vaccenic)	1.65 (1.41–1.88)[1,2]	1.24 (1.13–1.49)[1,3]	1.48 (1.22–1.81)[2,3]	<0.001
18:1$n-9$ (oleic)	14.4 (13.5–15.2)[1]	13.4 (12.7–14.1)[1,2]	14.5 (13.8–15.2)[2]	<0.001
20:3$n-9$ (mead)	0.08 (0.06–0.10)[1,2]	0.06 (0.05–0.07)[1,3]	0.07 (0.05–0.09)[2,3]	<0.001
Omega-3 index (EPA + DHA)	6.82 (5.93–7.71)[1,2]	7.44 (6.33–8.48)[1]	7.42 (6.06–8.72)[2]	<0.001

Superscript numbers (1, 2, 3) indicated pairs of countries with significantly different levels on each FA according to Bonferroni comparisons. Bonferroni correction for multiple comparisons (17 tests and significance level a = 0.05) yield a corrected $\acute{a} = 0.003$ which indicated significant differences at the aforementioned univariate analysis.

[a] RBC FAs were presented as median (1st–3rd quartiles) because their distributions were skewed.

[b] *P*-values derived through one-way ANOVA *F*-tests for country differences on log-transformed RBC FAs levels.

Table 4 Results from RRR-analyses that evaluated the dietary patterns associated with blood $n-3$ FAs levels of IMMIDIET population.

$N = 1604$	Factor loadings	
	Model 1[a]	Model 2[b]
Food product groups (g/day)		
Starches (bread, potatoes, legumes)		
Cabbages and root vegetables	0.48	0.42
Leafy vegetables and tomatoes		
Fruits and fruit or vegetable juices		
Diaries	0.29	
Cheese	−0.22	
Pasta, rice	−0.31	−0.28
Red meat and products	−0.27	−0.24
White meat and products		
Fishes		0.49
Mollusks		0.25
Vegetable oils	0.21	0.20
Olive oil	−0.29	−0.26
Nuts and seeds		
Fats and pizza		
Sweets and sugars		
Snacks		
Chocolate, coffee and tea	0.40	0.27
Mayonnaise	0.20	0.22
Alcoholic drinks		
Alcohol contained in all food groups		

[a] Model 1 derived through RRR-analysis with main outcome the log-transformed total plasma $n-3$ FAs. Factor loadings with absolute values lower than 0.20 were not presented for simplicity.

[b] Model 2 derived through RRR-analysis with main outcome the log-transformed total RBC $n-3$ FAs. Factor loadings with absolute values lower than 0.20 were not presented for simplicity.

pattern was described by high intake of cabbages and root vegetables, fishes, mollusks, vegetable oils, chocolate, coffee and tea and mayonnaise and low intake of pasta and rice, red meat and processed products and olive oil.

Population differences in dietary patterns related to $n-3$ FAs

Italian cohort had lower RRR-scores in both models compared with Belgian and English (Table 5) ($P < 0.001$). The analysis of food group consumption confirmed the low adherence to the two dietary patterns identified by RRR-analysis. In particular, the intake of several food groups such as cabbages and root vegetables, diaries, vegetable oils, chocolate, coffee, tea and mayonnaise that should be consumed mostly according to these patterns were significantly lower in Italy compared to Belgium or UK ($P < 0.001$).

Discussion

The main finding of this study comparing three European study populations is that there are striking differences in plasma and RBC FA profiles and, in particular, that Italian participants have lower plasma and RBC $n-3$ FA levels compared to Belgians and English. Apparently for the first time, dietary pattern analysis identified two dietary patterns associated with high plasma and RBC levels of $n-3$ FA, respectively. These patterns, explained the 35% and the

Table 5 Dietary habits of IMMIDIET population according to country of residence.[a]

$N = 1604$	Country of residence			P[b]
	Italy ($n = 542$)	Belgium ($n = 536$)	UK ($n = 526$)	
RRR-score from patterns analysis 1[c]	−1.23 (−1.70, −0.74)	0.09 (−0.43, 0.56)	1.07 (0.50–1.65)	<0.001
RRR-score from patterns analysis 2[c]	−0.79 (−1.28, −0.16)	0.13 (−0.42, 0.72)	0.36 (−0.12, 0.97)	<0.001
Food groups (g/day)				
Starches (bread, potatoes, legumes)	169 (112–251)	326 (251–412)	198 (139–265)	<0.001
Cabbages and root vegetables	40 (26.3–69.0)	95 (69–132)	165 (107–224)	<0.001
Leafy vegetables and tomatoes	125 (85–167)	83 (116–154)	103 (68–151)	<0.001
Fruits and fruit or vegetable juices	365 (248–530)	226 (126–398)	243 (142–381)	<0.001
Diaries	125 (14–224)	98 (26–208)	285 (351–507)	<0.001
Cheese	43 (28–68)	49 (24–80)	17 (5–27)	<0.001
Pasta and rice	71 (44–101)	44 (29–64)	30 (18–56)	<0.001
Red meat and products	106 (72–154)	148 (105–209)	45 (29–80)	<0.001
White meat and products	45 (29–62)	52 (35–69)	50 (25–59)	<0.001
Fishes	26 (16–38)	22 (14–35)	11 (5–22)	<0.001
Mollusks	7.6 (3.6–16.2)	10.5 (0–18.2)	6.7 (3.8–12.0)	0.10
Vegetable oils	0.8 (0.4–0.8)	19 (9–36)	15 (4–28)	<0.001
Olive oil	25 (19–33)	3.6 (2.3–5.8)	0 (0–22)	<0.001
Nuts and seeds	0.2 (0.2–0.7)	1.8 (0–7.0)	1.1 (0.7–3.4)	<0.001
Fats and pizza	47 (33–67)	49 (19–74)	23 (11–36)	<0.001
Sweets and sugars	96 (49–177)	230 (120–486)	101 (47–198)	<0.001
Snacks	14 (5–30)	14 (7–25)	3 (1–10)	<0.001
Chocolate, coffee and tea	130 (90–195)	671 (391–1062)	964 (681–1358)	<0.001
Mayonnaise	0.1 (0–0.5)	7.0 (3.5–10.5)	2.6 (1.0–6.9)	<0.001
Alcoholic drinks	84 (7–258)	134 (35–346)	94 (19–240)	<0.001
Alcohol contained in all food groups	6.0 (0.3–23.8)	8.6 (2.6–23.5)	12.7 (10.2–14.9)	<0.001
Total energy intake (kcal/d)	2324 (1940–2852)	2644 (2210–3291)	2114 (1756–2603)	<0.001
Total protein (%kcal/d)	15.9 (14.6–17.3)	17.0 (15.6–18.5)	14.5 (13.1–16.0)	<0.001
Total carbohydrates (%kcal/d)	49.3 (44.0–54.3)	42.4 (39.0–46.7)	47.8 (43.9–52.1)	<0.001
Total fibres (g/d)	16 (13.2–19.8)	22.8 (18.7–27.6)	14.1 (11.0–17.5)	<0.001
Total fat (%kcal/d)	33.6 (30.5–37.6)	36.6 (33.1–40.1)	36.9 (33.4–40.5)	<0.001
Total saturated lipids (%kcal/d)	11.8 (10.2–13.2)	14.4 (12.9–16.3)	11.9 (10.0–14.0)	<0.001
Total monounsaturated lipids (%kcal/d)	16.2 (14.2–18.2)	12.2 (11.0–13.4)	13.1 (10.8–15.3)	<0.001
Total polyunsaturated lipids (%kcal/d)	3.7 (3.3–4.1)	6.6 (5.7–8.0)	7.5 (5.3–7.5)	<0.001

[a] Results of dietary variables were presented as median (1st–3rd quartiles) because their distributions were skewed.
[b] *P*-values derived through Kruskal–Wallis test for country differences.
[c] Each of the RRR-scores 1 and 2 was derived through RRR-analysis with main outcome the log-transformed total plasma and RBC $n-3$ FAs, correspondingly.

17% of the variation of plasma and RBC $n-3$ FA, correspondingly. Italian cohort showed lower adherence compared to Belgian and English to both patterns, as confirmed by food group consumption analysis.

Plasma and RBC $n-3$ FA levels in Italians appeared to be low in absolute values too and close to those observed in patients with CVD [8]. These levels were similar to those observed in Italian patients enrolled in the GISSI-HF trial [20] and lower than those measured in the English population of the EPIC-Norfolk study [21]. The *omega-3 index* of these apparently healthy Italians would classify them as "at intermediate risk" for CVD [4].

The diet of Italians included in our study was by one side characterized by relatively high intakes of olive oil, fruits and leafy vegetables, tomatoes, pasta, rice and fish as compared with English and Belgians; on the other side, they were lower consumers of cabbages and root vegetables, diaries, vegetable oils, chocolate, coffee and tea and mayonnaise. In both $n-3$-inducing dietary patterns identified in this study, the consumption of these foods was associated with higher $n-3$ levels despite their low content in $n-3$ (except nuts), suggesting important interactions among substances present in these foods that may regulate the metabolism and the levels of $n-3$.

Some of the relations between foods and $n-3$ FAs observed in this study could be in part explained by differences in oil consumption. The three populations actually consumed different vegetable oils. Italians almost exclusively used olive oil, which is poor in both $n-3$ and $n-6$ but is rich in oleic acid. In contrast, in UK and Belgium, participants consumed greater quantities of vegetable oils, margarines and mayonnaises that are all relatively high in $n-6$ and $n-3$. As ALA and LA are the precursors of the other long-chain $n-3$ and $n-6$ through endogenous elongation/desaturation [2], this implies that all the other $n-3$ and $n-6$ FA would be different across the three populations.

Other observed associations between food intake and FAs blood levels are to be mentioned. Fish and mollusks are reportedly the main sources of EPA, DPA and DHA [23]. Italian participants consume more fish, twice the amount eaten by English, yet they show levels of plasma EPA, DPA and DHA about 50% lower. Thus the most reasonable explanation for the low EPA and DPA levels in Italian study

population is that the synthesis of both these FAs from ALA is partly inhibited [24,25]. This is in line with their high AA levels. There is indeed competition between $n-3$ and $n-6$ for the respective synthesis of EPA and AA, and low ALA is considered a factor for the preferred synthesis of AA from LA instead of EPA from ALA [24,25].

When the whole dietary habits were taken into account through RRR-analysis, fish intake was a significant food component in the model explaining variations in RBC $n-3$ but not in the model explaining variations in plasma $n-3$. RBC FAs levels reflect a more stable condition [8] and the concentration may represent a long-term nutritional situation and the results of complex metabolic interactions. On the other hand, the variation in $n-3$ content of different species of fish and "farmed" or "wild" ones [13,14,26] could not be considered in the present analysis. Only the total intake of fishes was taken in account. By this way the inside food group variation in EPA and DHA content could not be expressed.

In contrast, other food groups belonging to the two dietary patterns, such as cabbages, root vegetables, chocolate, coffee and tea, were significantly associated with variations in both plasma and RBC $n-3$, although these foods do not contain significant amounts of $n-3$. However, they contain high amounts of antioxidant vitamins and phytochemicals, which were shown to increase plasma and RBC $n-3$ in both human and animal studies [27–29].

Some of the above mentioned food group consumption such as cabbages and vegetables is associated with vegetable oils intake, at high content of $n-3$ FA, as an internationally typical cooking and serving habit. This may overestimate their high contribution in dietary pattern extraction.

Further studies are needed to examine whether the findings reported here for Italians also apply to some other Mediterranean populations.

Despite the importance of the present work limitations still exist. The cross-sectional design of this study does not enable determination of causality. This study was not aimed at investigating biological mechanisms and further studies are necessary to investigate at which level(s) the foods identified in the two dietary patterns interfere with the metabolism of $n-3$ and $n-6$ FA. Furthermore, the FFQ used in this study does not allow precise evaluation of each specific $n-3$ and $n-6$ FA and the kind of fish eaten, as fatty vs. non-fatty fish. Possible errors by misreporting in the evaluation of the dietary habits of each individual should be acknowledged. Finally, the adherence of dietary patterns extracted by the present setting has not been evaluated in association with FAs levels in other study samples. This is limiting the generalization of our conclusions and makes important the confirmation of present results by other similar studies.

Concluding, the Italian cohort presented significant lower plasma and RBC $n-3$ FA levels compared to Belgians and English. A holistic approach in dietary analysis seemed to explain a relatively high proportion of plasma and RBC $n-3$ FAs variability. Dietary pattern analysis may contribute to the study of the association of human diet with FAs levels.

Acknowledgements

The study was supported by European Union grant no QLK1-2000-00100.

The European Collaborative Group of the *IMMIDIET* Project is listed in the Supplementary material.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2014.01.012.

References

[1] Hoffman K, Schulze MB, Schienkiewitz A, Nöthlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. Am J Epidemiol 2004;159(10):935–44.
[2] De Caterina R. *N*-3 fatty acids in cardiovascular disease. N Engl J Med 2011;364(25):2439–50.
[3] de Lorgeril M, Salen P, Defaye P, Rabaeus M. Recent findings on the health effects of omega-3 fatty acids and statins, and their interactions: do statins inhibit omega-3? BMC Med 2013;11:5.
[4] Harris WS, Von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease? Prev Med 2004;39(1):212–20.
[5] Samieri C, Maillard P, Crivello F, Proust-Lima C, Peuchant E, Helmer C, et al. Plasma long-chain omega-3 fatty acids and atrophy of the medial temporal lobe. Neurology 2012;79(7):642–50.
[6] Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 2002;346(15):1113–8.
[7] Iacoviello L, Arnout J, Buntinx F, Cappuccio FP, Dagnelie PC, de Lorgeril M, et al. Dietary habit profile in European communities with different risk of myocardial infarction: the impact of migration as a model of gene-environment interaction. The IMMIDIET Study. Nutr Metab Cardiovasc Dis 2001;11(4 Suppl):122–6.
[8] de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 1999;99(6):779–85.
[9] Cappuccio FP, Strazzullo P, Farinaro E, Trevisan M. Uric acid metabolism and tubular sodium handling: results from a population-based study. JAMA 1993;270(3):354–9.
[10] Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002;5(6B):1125–45.
[11] vanDongen MC, Lentjes MA, Wijckmans NE, Dirckx C, Lemaître D, Achten W, et al. Validation of a food-frequency questionnaire for Flemish and Italian-native subjects in Belgium: The IMMIDIET study. Nutrition 2011;27(3):302–9.
[12] Pala V, Sieri S, Palli D, Salvini S, Berrino F, Bellegotti M, et al. Diet in the Italian EPIC cohorts: presentation of data and methodological issues. Tumori 2003;89(6):594–607.
[13] McCance RA, Widdowson EM. The composition of foods. 5th ed. Cambridge, United Kingdom: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food; 1991.
[14] Salvini S, Parpinel M, Gnagnarella P, Maisonnneuve P, Turrini A. Banca Dati di Composizionede gli Alimenti per Studi Epidemiologici in Italia. Milan, Italy: Istituto Europeo di Oncologia; 1998.
[15] Stichting NEVO. NEVO: Dutchfood database 2001 [NEVO-tabel: NederlandsVoedingsstoffenbestand 2001]. Den Haag: Voedingscentrum; 2001.

[16] van Havere R, Muls E, Seeuws C. Belgian food composition table [BelgischeVoedingsmiddelentabel]. 3rd ed. Brussel: NUBEL v.z.w. (NutriëntenBelgië); 1999.
[17] O'Brien E, Waeber B, Parati G, Staessen J, Myers MG. Blood pressure measuring devices: recommendations of the European Society of Hypertension. BMJ 2001;322(7285):531–6.
[18] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18(6):499–502.
[19] Hodson L, Skeaff CM, Wallace AJ, Arribas GL. Stability of plasma and erythrocyte fatty acid composition during cold storage. Clin Chem 2002;321(1-2):63–7.
[20] Masson S, Marchioli R, Mozaffarian D, Bernasconi R, Milani V, Dragani L, et al. Plasma $n-3$ polyunsaturated fatty acids in chronic heart failure in the GISSI-heart failure trial: relation with fish intake, circulating biomarkers, and mortality. Am Heart J 2013; 165(2):208–15.
[21] Khaw KT, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. PLoS Med 2012;9(7):e1001255.
[23] Chowdhury R, Stevens S, Gorman D, Pan A, Warnakula S, Chowdhury S, et al. Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis. BMJ 2012;345: e6698.
[24] Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 2002;88(4):411–20.
[25] Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. Br J Nutr 2002;88(4):355–63.
[26] Hossain MA. Fish as source of $n-3$ polyunsaturated fatty acids (PUFAs), which one is better-farmed or wild? Adv J Food Sci Technol 2011;3(6):455–66.
[27] de Lorgeril M, Salen P, Martin JL, Boucher F, de Leiris J. Interactions of wine drinking with omega-3 fatty acids in coronary heart disease patients: a fish-like effect of moderate wine drinking. Am Heart J 2008;155(1):175–81.
[28] di Giuseppe R, de Lorgeril M, Salen P, Laporte F, Di Castelnuovo A, Krogh V, et al. Alcohol consumption and n-3 polyunsaturated fatty acids in healthy men and women from 3 European populations. Am J Clin Nutr 2009;89(1):354–62.
[29] Toufektsian MC, Salen P, Laporte F, Tonelli C, de Lorgeril M. Dietary flavonoids increase plasma very long-chain ($n-3$) fatty acids in rats. J Nutr 2011;141(1):37–41.