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Long-chain *n*-3 fatty acids and classical cardiovascular disease risk factors among the Catalan population

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ABSTRACT

The protective cardiovascular effect of long-chain *n*-3 fatty acids has been firmly established in populations with high fish consumption, like those from Mediterranean countries. The current fish consumption in a representative sample from Catalonia, a Mediterranean region, and its relationship with plasma concentrations of eicosapentaenoic (EPA) and docosahexaenoic (DHA) and some classical cardiovascular disease risk factors was evaluated. Mean fish and seafood intake was 78.5 ± 51.4 g/day. Mean plasma concentrations of EPA and DHA were respectively 0.48% and 1.99% of total fatty acids. Consumption of marine foods among the Catalan population, the main source of *n*-3 fatty acids, appears to beneficially affect some cardiovascular disease risk factors. Our results show that both EPA and DHA are negatively associated with triacylglycerol (TG) concentrations and the ratio of total cholesterol (TC) to HDL-cholesterol. Furthermore, EPA but not DHA has a beneficial effect on plasma HDL-cholesterol among the Catalan population. There were no significant associations between long-chain *n*-3 fatty acids and LDL-cholesterol, TC, glucose, insulin or blood pressure. Oily fish intake, which is richest in EPA and DHA, is currently at an order of only 1 serving per week in the Catalan population and its increase should therefore be promoted.

1. Introduction

The protective cardiovascular effect of fish consumption has been firmly established by numerous scientific studies (Harris, Miller, Tighe, Davidson, & Schaefer, 2008; Lee, O'Keefe, Lavie, Marchioli, & Harris, 2008; Psota, Gebauer, & Kris-Etherton, 2006; Wang et al., 2006). High intakes of the long-chain *n*-3 fatty acids eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) have been found to be associated with reduced coronary artery disease risk through several potential mechanisms. They include antithrombotic (Din et al., 2008; Robinson & Stone, 2006) and anti-arrhythmic effects (Chrysohoou et al., 2007; Leaf, 2007; London et al., 2007; Reiffel & McDonald, 2006), reduced inflammatory responses (Calder, 2006), decreased heart rate variability (Mozaffarian, Stein, Prineas, & Siscovick, 2008; Mozaffarian et al., 2005), reduced blood pressure (Geleijnse, Giltay, Grobbee, Donders, & Kok, 2002; Ueshima, Stamler, & Elliott, 2007), decreased triglyceride (TG) concentrations (Harris, 1997), and increased insulin sensitivity.

International associations recommend a fish intake of at least twice a week for healthy adults. For patients with documented coronary heart disease (CHD), 1 g of EPA and DHA per day is desirable (Gebauer, Psota, Harris, & Kris-Etherton, 2006; Lichtenstein et al., 2006). However, if cardiac effects of fish consumption are primarily related to effects of *n*-3 LC-PUFA, then associations may vary, depending not only on the quantity but also on the type of fish consumed (Mozaffarian et al., 2003), as *n*-3 PUFA content can vary by an order of magnitude when comparing fatty fish with lean fish.

A recent review (Calder, 2004), in which 25 major studies investigating associations between fish or long-chain *n*-3 fatty acids and cardiovascular disease (CVD) were revised, concluded that long-chain *n*-3 fatty acid consumption should be promoted for all individuals, especially those at risk of developing CVD. It suggests that there is a wide gap between current intakes of long-chain *n*-3 PUFA and many of the recommendations given from international organisations.

Nowadays a shift away from traditional lifestyles and diets in several populations, especially among young people, is being associated with an increased prevalence of risk factors for CVD, such as

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high blood pressure, elevated blood lipids, diabetes, and obesity (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006; Moreno, Sarría, & Popkin, 2002).

This fact must be also taken into account in populations with healthy dietary patterns, such as those followed in the Mediterranean area. There is therefore a need of verifying and promoting their main characteristic food components, such as fish and seafood.

The aim of this study was to examine the current marine-derived *n*-3 fatty acid status of a representative sample of the adult population in Catalonia, a Mediterranean region, and to evaluate the relationship of the current Catalan fish intake with plasma concentrations of EPA and DHA and some classical cardiovascular disease risk factors.

2. Materials and methods

2.1. Study design

A cross-sectional nutritional survey among the Catalan adult population ($n = 1600$; 18–80 years) was carried out in 2002–2003 (Juncà et al., 2003). Its primary objective was to collect relevant information on the dietary habits of the Catalan population and assess their food consumption patterns. Dietary habits were assessed by means of a quantitative food-frequency questionnaire (FFQ). The sampling technique included stratification according to geographical area and municipality size, age and gender of inhabitants. The participation rate (65%) in the present study can be regarded as representative of the adult population of Catalonia. A total of 550 participants agreed to have blood drawn and underwent physiological and anthropometric measurements in a clinical session after informed consent. Only people who did not under-report their energy intake (EI/BMR $\geqslant$ 1.14, according to Goldberg et al. (1991)) were considered for analysis. From the resulting 533 Catalans, 17 did not fast for >12 h before blood sampling and were therefore excluded. The final sample consisted of 516 subjects aged 18–77 years, 203 men and 313 women. The study protocol was approved following Declaration of Helsinki 1975 standards.

2.2. Laboratory measurements

Blood samples were collected after the subjects had fasted for 12 h. Plasma was stored at −80 °C before being analysed. Standardised enzymatic methods were used for the analysis of serum lipids (Juncà et al., 2003). The fatty acid (FA) profile in total plasma and in plasma phospholipids was determined by fast gas chromatography (fast-GC) with two different methods (Bondia-Pons, Castellote, & López-Sabater, 2004a; Bondia-Pons, Morera-Pons, Castellote, & López-Sabater, 2004b) in the first 300 samples. In the rest of the samples only total plasma fatty acid profile was determined. This was due to the fact that the Pearson's correlation coefficients (r) between EPA and DHA determined by both methods were high enough to ensure reliable results with total plasma FA determination. The calculated r was 0.91 ($p < 0.001$) for EPA and 0.83 for DHA ($p < 0.001$). Results were expressed as relative percentages of total FA.

2.3. Lifestyle assessment and anthropometry

Smoking status was assessed by questionnaire during a face-to-face interview. Height, weight, and waist and hip circumference were measured during the clinical session. Waist circumference was measured by positioning the measuring tape horizontally at the level of noticeable waist narrowing and recording the circumference to the nearest centimetre. The mean (±SD) body mass index (BMI; in kg/m^2) of the subjects was 26.4 ± 4.8 and their mean waist circumference was 86.8 ± 13.6 cm. In this study, the accumulation of adipose tissue in the abdominal area as measured by waist circumference was used to measure abdominal obesity. A waist circumference (WC) $\geqslant$ 102 cm for men and $\geqslant$ 88 cm for women was defined as abdominal obesity.

2.4. Dietary assessment

Data on food intake were obtained with the use of a quantitative FFQ, which was previously validated (Martin-Moreno et al., 1993) and applied to other Spanish regions (Serra-Majem, Armas-Navarro, Ribas-Barba, 1999; Tur, Romaguera, & Pons, 2004). The FFQ, which asked the subject to recall average intake over the past year, consisted of 92 food items. The FFQ was arranged by food type and meal pattern. Frequency categories where based on the number of times that items were consumed per day, week or month. Consumption less than once a month was considered no consumption. Daily consumption in grams was determined by dividing the reported amount of the intake by the frequency in days. The relevant period of consumption of seasonal items was also taken into account. Edible fractions of foods were recorded in the database.

Marine food was categorised into six groups. Examples of the species were included for each group in the diet questionnaire, as well as the amount of the raw edible serving size. Categories were established as: freshwater fish (trout; 150–180 g/portion); saltwater lean fish (hake, sole, angler fish, grouper, sea bream; 150 g/portion); saltwater oily fish (mackerel, sardine, tuna, anchovy, bonito; 120 g/portion); cephalopods (octopus, cuttlefish, squid; 120–150 g/portion); mollusc seafood (mussel, clam, razor shell; 60–70 g/portion) and crustacean seafood (prawn, king prawn; 70 g/portion). Food values were converted into nutrient values by validated software developed by the Centre of Nutrition and Dietetics CESNID, based on Spanish tables of food composition (Cervera, 2006).

2.5. Statistical analysis

The statistical distribution of plasma fatty acid concentrations was checked and was found to be skewed. Therefore, geometric means were used to describe FA concentrations. Pearson correlations were used to compare EPA and DHA fatty acid values in total plasma and in plasma phospholipids. Analysis of variance (ANOVA) on the logarithm of plasma FA and the Duncan test (for variables with $\geqslant$3 categories) were used to determine effect comparisons among groups. Mean daily intakes of marine foods and mean values of CVD risk factors were calculated according to age and sex. The chi-squared test was used to compare the prevalence of CVD risk factors according to sex and age.

The associations between the plasma concentrations of EPA and DHA and values for CVD risk factors were assessed by use of multiple linear regression analysis. Variables with a skewed distribution were logarithmically transformed. The regression analyses were conducted for subjects who were not taking prescribed drugs for hypercholesterolaemia, high blood pressure, or diabetes. The CVD risk factor values were considered as dependent variables and the relative concentrations of EPA and DHA in plasma as predictor variables. Adjustments were made for potential confounding effects of age, sex, waist circumference, body mass index, SFA intake, MUFA intake, and smoking.

We also calculated conditional odds ratios, to examine association between the prevalence of low HDL concentrations and quintiles of EPA and DHA in plasma. Logistic regression was performed to control for the same confounding variables as described above and excluded the same subjects. All results were processed with

the SPSS 12.0 statistical package (SPSS; Chicago, IL). Statistical significance was set at $p = 0.05$.

3. Results

The study population was composed of 203 men (mean ± SD age: 49.3 ± 14.7) and 313 women (mean ± SD age: 45.5 ± 15.5) aged 18–77. EPA and DHA accounted for 78% of total plasma *n*-3 fatty acids (Table 1). The geometric concentration of total *n*-6 FA was 41.3% by weight and arachidonic acid (AA) accounted for 16.2% of *n*-6 FA. 97% of the sample had a ratio of plasma PUFA/SFA > 1.0 and the *n*-6 to *n*-3 ratio of total plasma FA was 12.5/1. Mean energy intake per kg body weight was 123.5 ± 42.6 kJ/kg (29.5 ± 10.2 kJ/kg). The mean contribution of the total fat intake to energy was 38.0 ± 5.8%. According to the gender of the sample, no significant differences were observed in PUFA intake (7.7 ± 2.5 (men) *vs.* 7.4 ± 2.3 (women); values in% of total energy; $p = 0.336$). However, the SFA and MUFA intake was higher for women than men (12.6 ± 2.7% *vs.* 12.0 ± 2.7%, $p = 0.02$/18.5 ± 3.5% *vs.* 17.4 ± 4.1%, $p = 0.001$).

Table 2 summarises the relation between total fish intake, relative concentrations of *n*-3 fatty acids and characteristics of the Catalan population. Concentrations of EPA, DHA, EPA + DHA and the ratios of EPA to AA and of *n*-3 to *n*-6 fatty acids did not varied significantly according to sex. However, long-chain *n*-3 fatty acid concentrations varied significantly according to age, with those aged >40 having higher values than subjects aged 18–40. Subjects with elevated waist circumference showed higher concentrations of EPA and higher EPA to AA ratio than subjects with normal WC. Non-smokers had higher concentrations of EPA, DHA, EPA + DHA and a higher ratio of EPA to AA and of *n*-3 to *n*-6 than smokers and occasionally smokers. A similar behaviour was found between groups of subjects using or not using medication for hypertension. Higher values of the ratios EPA:AA and *n*-3:*n*-6 were observed in subjects medicated for hypertension compared to those not using medication, but no significant differences were found between those who were diabetic or not. Regarding fish intake, there were only significant differences between groups using or not using medication for hypertension.

Daily intakes of the different marine foods, by sex and age, are shown in Table 3. Mean marine food consumption (±SD) was 78.5 ± 51.4 g/day. Quantitatively the most popular variety of fish consumed by the sample was saltwater lean fish. Saltwater lean fish and oily fish constitute 70% of the total marine source consumption in the Catalan sample. Saltwater lean fish daily intake was significantly higher in women than in men, while we did not find significant differences in fish consumption according to age.

Table 4 shows the EPA and DHA content in plasma according to each category of marine food consumption. Plasma EPA content significantly increased according to the frequency of saltwater lean fish, saltwater oily fish, mollusc, cephalopods and crustaceans consumption, but not for other kind of marine source. In the case of DHA, significant differences in plasma DHA were found according to the frequency of consumption of saltwater lean fish, oily fish, cephalopods and molluscs. When comparing inside a given category, consumption greater than twice a week is necessary to obtain an EPA concentration significantly higher in the case of saltwater lean fish, saltwater oily fish and cephalopods, with the exception of molluscs, where a consumption of twice a week is sufficient. Regarding DHA, consumption greater than twice a week (freshwater fish and saltwater lean fish) or at least twice a week (saltwater oily fish, cephalopods, and molluscs) is necessary to obtain concentrations significantly higher than those who consume less fish.

To perform regression analyses, those subjects who reported using medication for problems associated with CVD (hypercholesterolaemia, high blood pressure, or diabetes) were excluded (103 of the 516). From the resultant 413 subjects, the prevalence of high-risk concentrations of total cholesterol (TC) did not vary according to sex but significantly increased with age, although only in the case of women (Table 5). This behaviour according to age is shared by all CVD risk factors. Thus, CVD risk factors do not vary significantly with age in men. On the contrary, excepting for HDL-cholesterol (HDL-C), higher values were more prevalent in women as age increased. The prevalences of high-risk concentrations of HDL-C, TG, and the ratio of TC:HDL-C, were the only ones that varied significantly according to sex.

Table 6 shows the regression coefficients (β) from the multiple linear regression analysis. EPA was positively associated with HDL-C concentrations. We found a negative association between EPA and the ratio TC:HDL-C and TG. Even though DHA also showed a negative association with the ratio TC:HDL-C and with TG, it was not associated with HDL-C concentrations. When accounting for both *n*-3 FAs, EPA + DHA was also negatively associated with the ratio TC:HDL-C and TG and positively associated with HDL-C. Neither long-chain *n*-3 FA was associated with TC, LDL-C, diastolic blood pressure (DBP), systolic blood pressure (SBP), glucose or insulin.

Separate regression analyses were conducted by sex and age on those CVD risk factors where a residual modifying effect due to these factors was found. Thus, additional regression analysis was performed by sex and age for HDL-C, TC:HDL-C and TG, the only CVD risk factors that presented a modification effect with sex and age. However, no additional information was obtained.

Additional analyses were performed to examine the association between the prevalence of low HDL-C ($\leqslant$0.9 mM) concentrations and quintiles of EPA and DHA by using conditional odd ratios and comparing subjects in quintiles 2–5 with those in quintile 1. In what refers to EPA quintiles, the higher prevalence of low HDL-C concentrations was found in quintile 1 (27.5%), and it decreased significantly according to the several quintiles ($p = 0.002$), with the lowest prevalence found in quintile 5 (6.0%) (Table 7). On the contrary, the prevalence of low HDL-C did not vary significantly according to DHA quintiles ($p = 0.999$).

When modelling the probability of HDL-C concentrations <0.9 mM, the odds ratio decreased significantly according to quintiles of EPA, excepting for quintile 4, which presents the highest value from quintiles 2–5 but is the only one which is not significant (Table 7). The odds ratio for the group with the highest EPA

Table 1
Relative concentrations of plasma fatty acids in the sample population (% by wt of total fatty acids).[a]

Fatty acids	Geometric mean	Minimum	Maximum
SFA	28.55	19.12	38.75
C16:0	20.38	11.79	27.89
C18:0	6.55	3.39	11.47
MUFA	26.05	15.65	43.27
C18:1	23.73	14.38	40.84
PUFA	44.61	24.33	58.74
Total *n*-6[b]	41.30	21.40	55.70
C18:2 *n*-6	32.28	14.83	48.60
AA	6.70	1.24	12.76
Total *n*-3[c]	3.15	1.27	7.11
EPA	0.48	0.07	2.55
DHA	1.99	0.33	3.72
n-3/*n*-6	0.08	0.03	0.19
EPA/AA	0.07	0.01	0.63

[a] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid (C20:4 *n*-6); EPA, eicosapentaenoic acid (C20:5*n*-3); DHA, docosahexaenoic acid (C22:6*n*-3).
[b] C18:2 + C18:3 + C20:2 + C20:3 + C20:4.
[c] C18:3 + C20:5 + C22:5 + C22:6.

Table 2
Relative concentrations of *n*-3 fatty acids in plasma according to characteristics of the sample population.

Potential confounding variables	Fish intake g/day	EPA % by wt of total FA	DHA	EPA + DHA	EPA:AA	*n*-3:*n*-6
Sex						
Men (*n* = 203)	75.15 ± 51.55	0.47 ± 0.38	1.93 ± 0.59	2.45 ± 0.87	0.07 ± 0.07	0.08 ± 0.03
Women (*n* = 313)	80.72 ± 51.30	0.48 ± 0.37	2.03 ± 0.54	2.56 ± 0.79	0.07 ± 0.06	0.08 ± 0.02
p	0.230	0.811	0.065	0.141	0.392	0.260
Age						
18–40 years (*n* = 192)	81.91 ± 0.56.24	0.39 ± 0.31	1.91 ± 0.49	2.34 ± 0.69	0.06 ± 0.06	0.07 ± 0.02
>40 years (*n* = 324)	76.52 ± 48.31	0.54 ± 0.39	2.03 ± 0.59	2.62 ± 0.87	0.08 ± 0.07	0.08 ± 0.03
p	0.251	0.0001	0.024	0.0001	0.0001	0.0001
Waist circumference						
Elevated (*n* = 158)	78.18 ± 56.83	0.52 ± 0.33	2.01 ± 0.50	2.58 ± 0.72	0.08 ± 0.06	0.08 ± 0.03
Normal (*n* = 358)	80.63 ± 51.75	0.45 ± 0.34	1.98 ± 0.55	2.48 ± 0.79	0.07 ± 0.06	0.07 ± 0.02
p	0.558	0.038	0.658	0.252	0.033	0.017
Smoking status						
Smoker (*n* = 142)	74.53 ± 48.36	0.42 ± 0.36	1.84 ± 0.55	2.32 ± 0.80	0.06 ± 0.05	0.07 ± 0.03
Occasionally smoker (*n* = 22)	79.60 ± 53.86	0.42 ± 0.23	2.02 ± 0.57	2.47 ± 0.70	0.06 ± 0.04	0.07 ± 0.03
Non-smoker (*n* = 352)	79.76 ± 51.87	0.50 ± 0.38	2.05 ± 0.55	2.60 ± 0.82	0.08 ± 0.07	0.08 ± 0.02
p	0.584	0.010	0.001	0.001	0.006	0.018
Medication for hypertension						
Yes (*n* = 56)	89.09 ± 50.60	0.61 ± 0.31	2.20 ± 0.52	2.85 ± 0.71	0.10 ± 0.07	0.09 ± 0.03
No (*n* = 460)	77.28 ± 51.15	0.47 ± 0.38	1.97 ± 0.70	2.49 ± 0.83	0.07 ± 0.06	0.08 ± 0.02
p	0.045	0.003	0.016	0.004	0.0001	0.0001
Medication for CVD problems						
Yes (*n* = 30)	76.81 ± 56.32	0.58 ± 0.40	2.15 ± 0.65	2.78 ± 0.90	0.10 ± 0.08	0.09 ± 0.03
No (*n* = 486)	78.48 ± 51.00	0.47 ± 0.37	1.99 ± 0.55	2.51 ± 0.82	0.07 ± 0.06	0.08 ± 0.02
p	0.881	0.130	0.220	0.130	0.020	0.008
Diabetic						
Yes (*n* = 35)	75.29 ± 53.37	0.51 ± 0.34	1.91 ± 0.75	2.46 ± 0.99	0.09 ± 0.14	0.03 ± 0.03
No (*n* = 481)	78.52 ± 51.13	0.48 ± 0.37	2.00 ± 0.55	2.52 ± 0.82	0.07 ± 0.06	0.08 ± 0.03
p	0.793	0.524	0.432	0.670	0.101	0.243

Geometric mean ± SD. *p* By one-way ANOVA.

Table 3
Daily intakes of marine foods in the sample population by sex and age, according to the food-frequency questionnaire.

Marine food category	Men (*n* = 203)	Women (*n* = 313)	18–39 years (*n* = 192)	40 years (*n* = 324)	All (*n* = 516)
1	4.48 ± 10.88	4.45 ± 12.80	5.41 ± 13.10	3.91 ± 11.40	4.46 ± 12.07
2	30.19 ± 30.73	38.27 ± 29.50[a]	35.84 ± 32.04	34.64 ± 29.13	35.09 ± 30.22
3	20.25 ± 20.05	19.53 ± 21.31	20.36 ± 22.56	19.49 ± 19.73	19.81 ± 20.81
4	11.20 ± 10.71	10.06 ± 9.53	11.62 ± 10.36	9.85 ± 9.77	10.51 ± 10.02
5	4.24 ± 4.36	3.78 ± 3.65	3.86 ± 3.64	4.02 ± 4.13	3.96 ± 3.95
6	4.79 ± 7.31	4.38 ± 5.14	4.82 ± 6.63	4.38 ± 5.73	4.54 ± 6.08

Mean value (g/day) ± SD.
Marine food category: (1) freshwater fish, (2) saltwater lean fish, (3) saltwater oily fish, (4) cephalopods, (5) molluscs, (6) crustaceans.
[a] Significantly different from men (two-factor ANOVA): $p \leqslant 0.05$.

quintile was 0.12 (95% CI: 0.03, 0.45) (Fig. 1). The threshold value of EPA (in the highest quintile) that afforded this protective effect was 0.77. Conversely, the odds ratio did not vary significantly according to quintiles of DHA.

4. Discussion

Fish is one of the characteristic food groups of healthy Mediterranean dietary patterns, which has traditionally been consumed in Catalonia (Cucó, Fernández-Ballart, Martí-Hennenberg, & Arija, 2002; Serra-Majem, Ngo de la Cruz, Ribas, & Tur, 2003), possibly being one of the factors contributing to the mortality rates for CVD being relatively low in Spain, compared with those in other developed countries (Moreno et al., 2002).

The mean daily fish and shellfish intake in Catalonia (78.5 ± 51.4 g/day) was in the same range of data reported for the Spanish population six years ago. However, the EPA + DHA median concentration was lower due the higher intake of saltwater lean fish intake (45%) than oily fish (25%) in the Catalan population.

Women declared significant higher daily intakes of lean fish than men, but it seemed that this intake was not high enough to cause significant differences in plasma EPA, DHA or total *n*-3 fatty acid contents. The high intake of saltwater lean fish in women could be compensated by the intakes of other marine sources in men, or it could mean that saltwater lean fish intake does not contribute to the increase of plasma levels of EPA and DHA if the frequency of consumption is not higher than two servings a week. This suggests that in addition to the quantity of fish consumed, the type of fish and its *n*-3 FA content must be taken into account.

According to the results, smoking status showed significantly lower levels of EPA and DHA fatty acids in smokers than in non-smokers. Having in mind that smokers fish intakes did not significantly differ from those of non-smokers, the lower *n*-3 FA levels could be explained by the high free radical levels involved in body oxidation processes in smokers.

Levels of EPA were found to be elevated in subjects medicated for hypertension compared to healthy subjects, in concordance with the clinical advice of increasing their *n*-3 fatty acid status as

Table 4
EPA and DHA plasma content (%) according to the frequency of marine food intake.

Marine food category		>2 Per week	2 Per week	1 Per week	2–3 Per month	1 Per month	Never	*p*
1	EPA	0.41 ± 0.24	0.48 ± 0.28	0.53 ± 0.39	0.51 ± 0.45	0.49 ± 0.36	0.47 ± 0.38	0.928
	DHA	2.81 ± 0.26[a]	2.13 ± 0.26[b]	1.99 ± 0.55[b]	2.09 ± 0.61[b]	2.14 ± 0.54[b]	1.95 ± 0.55[b]	0.047
	n	3	8	15	29	65	293	
2	EPA	0.68 ± 0.48[a]	0.47 ± 0.31[b]	0.46 ± 0.33[b]	0.44 ± 0.37[b]	0.37 ± 0.20[b]	0.46 ± 0.63[b]	0.0001
	DHA	2.29 ± 0.61[a]	2.01 ± 0.47[b]	1.99 ± 0.52[b]	1.85 ± 0.57[b]	1.90 ± 0.61[b]	1.86 ± 0.58[b]	0.002
	n	57	114	130	57	31	24	
3	EPA	0.75 ± 0.36[a]	0.61 ± 0.60[b]	0.47 ± 0.34[c]	0.43 ± 0.31[c]	0.38 ± 0.23[c]	0.40 ± 0.20[c]	0.0001
	DHA	2.33 ± 0.52[a]	2.24 ± 0.47[a]	2.02 ± 0.55[b]	2.05 ± 0.51[b]	1.76 ± 0.44[c]	1.65 ± 0.55[c]	0.0001
	n	37	57	140	79	50	50	
4	EPA	0.93 ± 0.27[a]	0.55 ± 0.25[b]	0.51 ± 0.46[b]	0.48 ± 0.36[b]	0.44 ± 0.37[b]	0.42 ± 0.30[b]	0.019
	DHA	2.67 ± 0.52[a]	2.14 ± 0.44[a,b]	2.06 ± 0.60[b]	2.02 ± 0.49[b]	1.94 ± 0.55[b]	1.84 ± 0.53[b]	0.020
	n	5	21	113	119	95	60	
5	EPA	–	0.80 ± 0.36[a]	0.51 ± 0.38[b]	0.50 ± 0.31[b]	0.45 ± 0.44[b]	0.43 ± 0.34[b]	0.001
	DHA	–	2.44 ± 0.57[a]	2.05 ± 0.54[b]	2.06 ± 0.54[b]	2.00 ± 0.54[b]	1.83 ± 0.53[b]	0.005
	n	0	15	95	95	118	90	
6	EPA	0.71 ± 0.40	0.59 ± 0.17	0.55 ± 0.40	0.51 ± 0.33	0.45 ± 0.43	0.42 ± 0.31	0.011
	DHA	2.53 ± 0.51[a]	2.14 ± 0.59[a,b]	2.07 ± 0.53[a,b]	2.01 ± 0.50[a,b]	1.99 ± 0.60[a,b]	1.89 ± 0.50[a,b]	0.146
	n	6	3	79	99	149	77	

Geometric mean (%) ± SD.
Values in the same row with different superscript letters are significantly different $p < 0.05$.
Marine food category: (1) freshwater fish, (2) saltwater lean fish, (3) saltwater oily fish, (4) cephalopods, (5) molluscs, (6) crustaceans.

Table 5
Prevalences of cardiovascular disease risk factors in the sample population, by sex and age.[a]

CVD risk factors	Men (%)				Women (%)				All subjects (*n* = 413)	*p*[c]
	18–40 years (*n* = 55)	>40 years (*n* = 102)	All (*n* = 157)	*p*[b]	18–40 years (*n* = 118)	>40 years (*n* = 138)	All (*n* = 256)	*p*[b]		
TC ⩾ 6.2 mM	12.7	11.9	12.2	0.240	5.4	18.8	12.8	0.02	12.6	0.854
LDL-C ⩾ 4.1 mM	16.7	14.6	15.3	0.728	4.2	19.4	12.4	0.001	13.5	0.404
HDL-C ⩽ 0.9 mM	14.3	20.4	18.2	0.341	5.9	5.8	5.8	0.965	10.6	0.001
TC:HDL-C ⩾ 6	12.7	10.9	11.5	0.732	0.0	3.6	2.0	0.042	5.7	0.001
TG ⩾ 2.3 mM	8.9	8.7	8.8	0.968	0.0	3.6	1.9	0.037	4.6	0.001
SBP:DBP ⩾ 140/90	51.9	53.5	52.9	0.842	31.6	53.0	43.0	0.001	46.8	0.053
Glucose ⩾ 6.1 mM	10.7	20.8	17.2	0.109	3.4	17.4	10.9	0.001	13.3	0.069
Insulin ⩾ 90 pM	32.1	29.1	30.2	0.692	26.9	27.3	27.1	0.936	28.3	0.501

[a] TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[b] Chi-squared test by age.
[c] Chi-squared test by sex.

Table 6
Standardised regression coefficients from multiple linear regression analysis.

CVD risk factors	β (Log EPA)	*p*	β (Log DHA)	*p*	β (Log EPA + DHA)	*p*
TC	0.045	0.400	−0.025	0.646	0.006	0.905
LDL-C	0.049	0.351	0.006	0.915	0.027	0.602
HDL-C	0.170	0.001	0.100	0.054	0.134	0.010
Log TC:HDL-C	−0.118	0.018	−0.117	0.019	−0.122	0.015
Log TG	−0.165	0.001	−0.192	0.0001	−0.198	0.0001
Log SBP	−0.056	0.211	−0.075	0.092	−0.070	0.116
Log DBP	0.015	0.765	−0.054	0.261	−0.036	0.462
Log glucose	0.022	0.665	−0.058	0.253	−0.036	0.474
Log insulin	−0.096	0.056	−0.044	0.372	−0.065	0.195

Each model included the CVD risk factor as the dependent variables, the relative concentrations of plasma FA as the predictor variable; and age, sex, waist circumference, body mass index, SFA intake, MUFA intake and smoking status as fixed and covariate variables.

a risk population, which was reflected in their fish consumption being higher than in the rest of participants.

It is interesting to point out that only the consumption of saltwater lean fish, saltwater oily fish, cephalopods and molluscs was responsible for changes in the plasma levels of long-chain *n*-3 fatty acids, but in a different pattern. While EPA and DHA plasma contents with saltwater fish, cephalopods and molluscs were significantly higher for consumptions of 2 or more servings per week, they remained constant within the range of lower servings. However, the EPA and DHA plasma contents with saltwater oily fish showed a gradual increase along greater frequencies of consumption. Crustaceans and freshwater fish consumption showed a similar behaviour to the former group regarding plasma contents of DHA, although it was not reflected in plasma concentrations of EPA, probably due to their low intake among the population under study. In any case, consumptions of 2 or more servings a week are necessary to obtain EPA and DHA plasma contents different from those obtained at lower consumptions. Thus, these results are in agreement with international recommendations of consuming two fish meals per week, preferably fatty fish (Gebauer et al., 2006).

Table 7
Prevalence and odds ratio of low HDL-C concentration (⩽0.9 mM) by quintiles of EPA and DHA.

Quintiles	Q1	Q2	Q3	Q4	Q5
EPA					
Prevalence[a] (%)	27.5	10.2	7.6	10.6	6.0
Odds	1	0.25	0.17	0.27	0.12
95% CIs	–	(0.08, 0.66)	(0.05, 0.53)	(0.07, 0.97)	(0.03, 0.45)
p	0.009	0.007	0.002	0.055	0.002
DHA					
Prevalence[b] (%)	11	10.2	11.2	10.5	11.3
Odds	1	0.94	1.05	0.94	1.08
95% CIs	–	(0.31, 2.79)	(0.35, 3.13)	(0.29, 3.09)	(0.36, 3.53)
p	0.998	0.906	0.937	0.924	0.884

[a] Prevalence for all 10.8% and *p* among groups by one-way ANOVA = 0.002.
[b] Prevalence for all 10.8% and *p* among groups by one-way ANOVA = 0.999.

Classical CVD risk factors showed differences according to sex and age in the Catalan sample. Younger women were the participants that presented the healthiest lipoprotein and TG profiles and the lowest blood pressure (BP) values. Neither EPA nor DHA were associated with TC or LDL-C. Although some studies showed that *n*-3 PUFA tend to lower plasma TC and LDL-C, there are others showing no evidence for any association between fish consumption and TC or LDL-C levels (Harris, 1997; Sidhu, 2003). On the other hand, in a critical review about fish oils and plasma lipid and lipoprotein metabolism in humans, it was concluded that TC and LDL-C levels are usually not affected, unless compared to a diet high in saturated fat, in which case they will decrease, while LDL-C levels are often increased by 5–10% with fish oil supplementation (Harris, 1997).

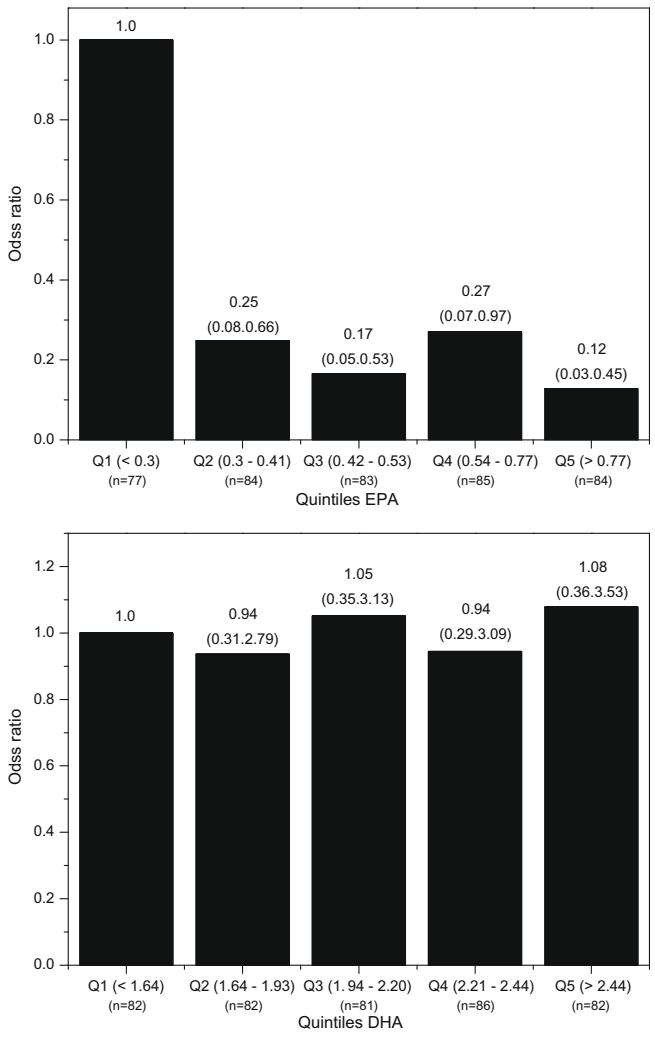

Fig. 1. Odd ratios (95% CIs) of a prevalent high-risk concentration of plasma HDL-C by quintiles of EPA (upper) and DHA (lower), expressed as % of total FA.

Long-chain *n*-3 FA may also slightly increase plasma HDL-C levels as observed in a cross-sectional study with healthy European men (Dallongeville et al., 2003), in which fish consumption was associated with decreased heart rate and in which HDL-C was significantly higher among fish consumers than among non-consumers. In the critical review of Harris (1997) about fish oil it was concluded that HDL-C concentrations also increased, but only by 1–3%, while in the study of Laidlaw and Holub (2003), a 4 g dose of fish oil resulted in a 7–10% increase in HDL-C in healthy women. Fish oils were also found to increase HDL-C in men with abdominal obesity (Chan, Watts, Nguyen, & Barrett, 2006). However, the HDL-C raising effect of fish oils has not been shown in all studies. For example, in subjects with familial combined hyperlipidaemia, a 1.88 g of EPA + 1.48 g of DHA dose did not increase significantly the HDL-C (Calabresi et al., 2004). In other studies with *n*-3 LC-PUFA-enriched foods mixed results have also been shown (Junker et al., 2001; Lovegrove, Brooks, Murphy, Gould, & Williams, 1997). In the Catalan sample, EPA was positively associated with HDL-C, but no significant association was found for DHA. However, when considering both *n*-3 FA (EPA + DHA), the beneficial effect on HDL-C profile was found again.

According to our results on HDL-C, it could be of interest to determine the effects of EPA and DHA when used separately. There are a few studies comparing the effects of EPA and DHA. In some studies EPA and DHA were reported to be equally effective in reducing serum TG, but only DHA raised HDL-C (Grimsgaard, Bonaa, Hansen, & Nordoy, 1997; Mori & Woodman, 2006). Moreover, the LDL-increasing effect appears to be due to DHA, but not EPA (Von Shacky, 2006). On the contrary, other studies showed that both EPA and DHA decreased TG, but there were no significant effects on TC, LDL-C or HDL-C (Mori et al., 2000; Woodman et al., 2002). Moreover, in a study examining the cross-sectional relationship between the frequency of habitual fish consumption and serum lipoproteins, it was concluded that EPA correlated positively with HDL-C, while DHA showed a negative association (Bonaa, Bjerve, & Nordoy, 1992). In a similar way, in the Catalan sample EPA was unique in increasing significantly the HDL-C, although DHA was not associated negatively with HDL-C. Taken together, there appears to be some diversity as to the effects of EPA and DHA on TC, LDL-C and HDL-C. This may be due to the differences in the populations studied. However, since results obtained with EPA + DHA/*vs.* results obtained with either EPA or DHA diverge, there may be even other sources of heterogeneity that should be clarified.

Owing to differences found in the association between HDL-C and EPA or DHA we decided to investigate their relation more thoroughly using conditional odds ratio of prevalent high-risk concentration of plasma HDL-C by quintiles of EPA. Our results showed a protective effect of EPA on plasma HDL-C. In our study, a threshold value of 0.77% of the total plasma FA, corresponding to an average fish intake of 126 g/day, afforded this protective effect. In contrast, the odds ratios did not present any significant difference among the DHA quintiles, resulting in no protective effect of DHA on plasma HDL-C, confirming the results found in the regression analysis. Thus, our results show that EPA but not DHA has a beneficial effect on plasma HDL-C among the Catalan population.

Both EPA and DHA were negatively associated with the ratio TC:HDL-C, considered a strong marker of cardiovascular risk, and to TG concentration. The reduction in fasting and postprandial serum TG is one of the most consistent effects of *n*-3 FA. Harris (1997) reported that ≈4 g/day of *n*-3 FA from fish oil decreased serum triglyceride concentrations by 25–30% and that this reduction is dose-dependent. However, reductions have been reported at much lower doses (≈1 g/day) in either hypertriglyceridaemic subjects (Junker et al., 2001) or normotriglyceridaemic subjects (Roche & Gibney, 1996; Visioli et al., 2000). It has been estimated that a minimum dose of 1 g/day of *n*-3 LC-PUFA is the minimum necessary to significantly lower TG (Weber & Raederstorff, 2000). In the Catalan population, the fish intake was 78 g/day, equivalent to ≈900 mg EPA + DHA. Therefore, it seems likely that this range of fish intake is sufficient to observe the lowering TG effects of EPA + DHA. Furthermore, according to previous results (Grimsgaard et al., 1997; Mori & Woodman, 2006; Mori et al., 2000; Woodman et al., 2002), both EPA and DHA significantly decreased TG levels.

n-3 FA seem to have a small, dose-dependent hypotensive effect, which depends on the degree of hypertension. Hence, in a recent meta-analysis of 36 trials, Geleijnse et al. (2002) showed that fish oil intake (median dose 3.7 g/day EPA + DHA) reduced SBP by 2.1 mm Hg and DBP by 1.6 mm Hg. These effects tended to be greater in populations that were older (>45 years) and in hypertensive populations ($\geqslant$140/90 mmHg). In a recent cross-sectional epidemiological study, *n*-3 FA intake was inversely related to blood pressure, including in non-hypertensive persons, with small estimated effect size (Ueshima et al., 2007). However, most studies that targeted healthy individuals with no clinical manifestation of hypertension did not detect a hypotensive effect of *n*-3 FA on BP (Covington, 2004). In our study, only a small portion of the sample suffered from diagnosed hypertension. In view of the high doses required to lower blood pressure it is possible that the *n*-3 LC-PUFA intake of hypertensive patients was not high enough to show a significant relationship with the blood pressure.

The effect of *n*-3 FA on glycaemia, insulinaemia, and types 1 and 2 diabetes is not clear. We found no association between *n*-3 FA and plasma glucose and insulin. Most studies concerning the effect of *n*-3 FA on plasma glucose and insulin were conducted among patients with type 1 or type 2 diabetes. A prospective study provided evidence for an inverse association between fish and LC-PUFA *n*-3 intake, and risk of CHD and total mortality among diabetic women. It finally concluded that regular fish consumption should be considered as part of a healthy diet for diabetic management (Hu, Cho, Rexrode, Albert, & Manson, 2003). Other authors have also found high plasma *n*-3 FA positively associated with plasma glucose (Dewailly et al., 2001), but the weak associations found in this study do not allow for conclusions to be formed towards any trend referring to this issue. A recent meta-analysis of 23 randomised controlled trials (mean dose 3.5 g/day of *n*-3 FA) concluded that neither EPA + DHA nor EPA nor DHA had a meaningful effect on fasting glucose or fasting insulin (Hartweg et al., 2008). If considering such a great dose of *n*-3 FA, there was no significant change in glucose and insulin, it was expected that the fish intake of the Catalan population was not high enough to show a protective effect of *n*-3 FA on plasma glucose and insulin.

It would be therefore recommended to look for public health strategies to promote and increase the fish intake among the Catalan population in the context of the "traditional" Mediterranean diet and healthy lifestyle habits. Special attention should be given to oily fish, which, according to the present study, is currently consumed only once a week.

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