Original Article

The atherosclerotic risk profile is affected differently by fish flesh with a similar EPA and DHA content but different n-6/n-3 ratio

Francesco Sofi MD, PhD[1-3], Gianluca Giorgi MSc, PhD[4], Francesca Cesari MSc, PhD[1], Anna Maria Gori MSc[1,3], Lucia Mannini MD[1], Giuliana Parisi MSc, PhD[4], Alessandro Casini MD[2], Rosanna Abbate MD[1], Gian Franco Gensini MD[1], Bianca Maria Poli MSc[4]

[1]*Department of Clinical and Experimental Medicine, Thrombosis Centre*
[2]*Agency of Nutrition, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy*
[3] *Don Carlo Gnocchi Foundation Italy, Florence, Italy*
[4]*Department of Agricultural Biotechnology, Section of Animal Sciences, University of Florence, Italy*

The aim of this study was to evaluate the effect of consuming gilthead sea bream fillets, with different n-6/ n-3 ratios, on atherosclerotic biomarkers. Twenty healthy subjects were included in a randomised single-blinded cross-over trial. Participants were randomized into 2 groups, both of which received approximately 630 g per week of gilthead sea bream fed with either 100% fishmeal (FM) or partial replacement with plant proteins (PP) over two consecutive 10 week periods, respectively. Group A consumed firstly the FM fillets followed by the PP fillets, whereas the reverse order was adopted for group B. Group A reported a significant decrease of 29.3% (Δ = –26 mg/dL) in total cholesterol after the first phase of the intervention, before returning to baseline levels after the dietary intervention with fish fed with PP. Similarly, in group A, both LDL-cholesterol and triglycerides decreased significantly by 21.6% (Δ = –19 mg/dL) and 11.7% (Δ = –10.7 mg/dL), respectively, before increasing again after the intervention. Improvements in the inflammatory cytokines, interleukin-6 and -8 were also noted. Moreover, whole blood viscosity appeared significantly improved in group A, as seen by a significant increase of 7.59% (Δ = +4.59 mPA) for erythrocyte filtration rate. In conclusion, similar EPA+DHA content with different n-6/n-3 ratio fish flesh intake was shown to have varied affects on lipid, inflammatory and haemorheological parameters in a group of healthy subjects.

Key Words: fish intake, cardiovascular disease, inflammation, diet, omega-3

INTRODUCTION

During the past decades numerous epidemiological and observational studies have been published on the cardiovascular and metabolic benefits of fish consumption.[1,2] More than 30 years ago, when Bang and Dyerberg reported that, despite a diet low in fruit and vegetables and complex carbohydrates, the Greenland Inuit had lower risk of myocardial infarction than that shown for age-matched residents in Denmark, considerable attention has been directed towards the benefits of fish consumption and n-3 PUFA on several aspects of human health. These aspects have included lipid profiles, blood pressure, heart rate, and a healthy modulation of the coagulation pathway.[3-5]

As shown by both epidemiological and interventional studies, the fatty acids present influence the *in vivo* production of inflammatory components, blood rheology and membrane functionality, thereby demonstrating a preventive effect on various diseases.[6] However, the importance of the n-6/n-3 ratio should be empahsised.[7] The dietary ingestion of n-3 PUFA helps to replace, at least partially, n-6 fatty acids, especially arachidonic acid, in the cell membranes, and most importantly in the membranes of platelets, erythrocytes and neutrophils.[7] Over the past decades, global fish stocks have declined rapidly, whereas pressure on fish stocks have increased enormously along with the number of recommendations for an increased consumption of fish and fish oil.[8,9] Nowadays, because of limited wild fish stocks, fish farming is becoming the major contributor of global fish supplies.[8,9] Hence, with the production of farmed fish eclipsing that of wild fish, the global need for fish oil and fishmeal for aquaculture has increased the demand for more in depth insight on the potential for alternative protein sources in aquafeeds[9,10]. The interest in the substitution of fishmeal by more sustainable and renewable protein sources was initiated in

Corresponding Author: Dr Francesco Sofi, Department of Medical and Surgical Critical Care, University of Florence. Largo Brambilla 3, 50134 Florence, Italy.
Tel: +39-055-7949420; Fax: +39-055-7949418
Email: francescosofi@gmail.com
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the 1970s, but there is limited knowledge on the nutritional value and physiological consequences of plant protein replacement of fishmeal in the diet of fish species.[11]

It is also important to investigate the nutritional and healthy characteristics of farmed fish products, because the diet of the fish and the health and quality of the final product are closely linked- in particular as far as lipid and fatty acid composition are concerned. Researchers and industries need to use the knowledge of these diet-product quality relationships in order to provide effective amounts of the health promoting n-3 PUFA to consumers.[11] Some examples of dietary intervention studies that attempted to modify the quality of feed for fish in aquaculture have been undertaken. In 2005, an intervention study carried out in Norway evaluated the effect of consuming salmon containing different amounts of n-3 PUFA on biomarkers of inflammation and atherosclerosis.[12] The diets of this intervention study were composed of salmon that had been fed with different lipid regimens (fish oil, fish oil/rapeseed oil, rapeseed oil) to modulate the n-3 PUFA content in the fish. Patients with stable coronary heart disease consumed a weekly quota of 700 g weekly of salmon with varying content of n-3 PUFA over a period of 6 weeks. Significant reductions in serum triglycerides, vascular cell adhesion molecules and interleukin-6 were observed in patients consuming the fillets from fish oil fed salmon in comparison to the other two groups.

The aim of the present research was to study the effects of a short-term intake of fillets from gilthead sea bream that were different in terms of n-6/n-3 ratio, due to the presence of fish or plant protein sources in the feed, on biomarkers related to the atherosclerotic process.

MATERIAL AND METHODS

Study population

Twenty clinically healthy subjects (8 women; 12 men; with a median age of 52.5 years) volunteered to participate in the study. Individuals taking any prescribed medication, fish oil supplements, or following special diets were excluded from the study. Participants were subjects who reported that they were fond of fish and willing to undertake eating fish over the required period. In order to identify symptom-free subjects, and to exclude those who were suspected of having any form of vascular disease, a detailed interview addressing personal and familial history was performed. Current smoking status was determined at the time of the physical examination, and BMI was calculated as weight (kg)/height $(m)^2$. All participants submitted signed informed consent. The study was approved of by the local Ethics Committee, and conformed to the Declaration of Helsinki.

Fish rearing and final product characteristics

Two groups of sea bream were reared in duplicate (2×2) in square floating cages (7×7×5 m) for 460 days in Cala Saccaia, Olbia, Italy. The two groups of fish were fed two isoenergetic and isoproteic diets, either 100% fish meal (FM group), or 50% fish meal replaced by a mixture of plant protein sources (soybean meal, corn gluten meal, pea protein isolate) (PP group). Diet composition and fatty acid profile are reported in Table 1. After 460 days of rearing, fish from both groups reached 601±96.7 g body weight. A subsample of one hundred and twenty sea bream for each dietary group were respectively sampled and analysed for body/fillet quality traits, while the other 400 from each dietary group were collected for the inter-

Table 1. Main nutritional parameters of the two fish diets and relative fish fillets

	DietFM†	DietPP‡	FilletsFM†	FilletsPP‡	rsd§
Moisture, (g/100g)	5.0	5.2	67.9	68.0	1.83
Protein, (g/100g)	49.9	48.5	18.3	18.5	1.30
Ash, (g/100g)	10.7	7.45	1.33	1.34	0.16
Total lipids, (g/100g)	18.6	20.1	12.0	11.9	2.5
Gross Energy (kJ/100g)	2,285	2,365	781	779	10.1
Fatty acids, (%)					
C14:0	5.1	3.4	5.1**	4.0**	0.2
C16:0	15	13.3	16.8**	15.6**	0.6
C16:1 n-7	5.8	3.8	6.3**	4.4**	2.4
C18:0	3.2	3.4	3.4	3.4	0.2
C18:1 n-9	19.6	21.6	21.6**	22.8**	1.0
C18:2 n-6	13.1	27.3	11.3**	20.2**	0.9
C18:3 n-3	2.1	3.0	1.2**	2.1**	0.1
C20:1 n-9	2.1	1.3	1.2**	1.0**	0.2
C20:5 n-3	11.8	7.7	8.6**	6.3**	0.5
C22:5 n-3	2.0	1.3	3.6**	2.9**	0.2
C22:6 n-3	11.2	8.5	14.2**	12.6**	0.6
SFA¶	24.3	20.8	25.8**	23.4**	0.9
MUFA††	29.8	28.1	29.9*	28.8*	1.9
n-6 PUFA	14.2	28.1	12.3**	21.1**	0.9
n-3 PUFA	29.5	21.9	29.5**	25.1**	0.9
n-6 PUFA/n-3 PUFA	0.44	1.27	0.42**	0.84**	0.2

The fatty acids C12:0, C13:0, C14:1, C15:0, C16:2n-4, C17:0, C17:2, C16:3 n-4, C16:4n-1, C18:2n-4, C18:3n-4, C18:3n-6, C18:4 n-3, C18:4 n-1, C20:0, C20:1n-7, C20:2n-6, C20:3n-6, C20:3 n-3, C20:4n-6, C20:4 n-3,,C21:5 n-3, C22:0, C22:1n-9, C22:1 n-11, C22:4n-6, C22:5n-6, resulted lower than 1%, and were considered only in the composite fractions.

* $p<0.05$ and ** $p<0.01$ represent significant differences among the fillets.

†Fish Meal, ‡Plant Protein: the parameters were calculated on dry matter for the diets and on wet basis for the fillets. §Residual standard deviation of fillets. ¶ Saturated Fatty Acids; ††MonoUnsaturated Fatty Acids.

vention trial.[13]

Total skinned fillets taken from the subsamples for each dietary group were analysed for proximate analysis,[14] total lipids [15] and quantitative fatty acid composition (Table 1). A Varian gas chromatograph 430-GC equipped with a flame-ionization detector and capillary column (Omegawax, 30 m 0.32 mm i.d., 0.25 μm film; split ratio, 100:1; Supelco 24152) was used for analyzing Fatty Acid Methil Esters (FAME).[16] Parameters of the Gas Chromatographic GC system were set as follows: injector and detector temperatures, 220 and 300°C, respectively; the column temperature was set to 160 °C for 1 min and gradually heated to 220°C at a rate of 2°C/min, and then maintained isothermal for 9 min (40 min of total run). Helium was used as a carrier and auxiliary gas. The fatty acid concentrations were calculated by comparison of their retention times with those of the reference standards (Supelco, Bellefonte, PA, USA). An internal standard (C23:0) was used to obtain absolute quantification.

The 800 fish were filleted in a commercial processing plant and each fillet was packed under vacuum and identified by fish number and weight, and stored at –80 °C until use for the intervention trial.

Experimental design

A single-blinded, randomised, cross-over, clinical trial was performed. During the first 15 days no intervention was undertaken and subjects were only monitored for diet (*run-in phase*). During the next period of 10 weeks, the participants entered the first intervention phase, according to the group they had been assigned to (*1st intervention phase*). Following the first intervention phase, the subjects were crossed over in order to obtain the second intervention phase (*2nd intervention phase*) during the second 10 week period. The subjects were randomly allocated in two groups, with each group consuming a weekly quota of about 630 g gilthead sea bream fed with either fishmeal (FM) or partial replacement (50%) with plant proteins (PP). In group A, subjects were randomized to consume firstly fish fed with FM diet followed by those fed with PP diet (Group A), whereas group B subjects firstly consumed fish fed with PP diet and then those fed with FM diet (Group B). At baseline, a physical examination, a dietary questionnaire, and blood sampling were obtained from each participant. The sea bream fillets that had been packed under vacuum (and identified for fish number and weight to be consumed each week) were provided to each participant at no cost after the end of the physical examination. All participants were instructed to eat 630 g of fish each week, and were advised by an expert dietician to consume the fish four times per week and to maintain a regular eating pattern during the intervention period. Guidelines were given to cook sea bream fillets in a few minutes in a simple way (microwave or oven), using only salt and olive oil.

Blood measurements

Blood samples were collected from the antecubital vein into evacuated plastic tubes (Vacutainer), after an overnight fasting. Samples, obtained by centrifuging at 2000 g for 10 minutes at 4 °C, were stored in aliquots at –80 °C until analysis.

Lipid variables and liver enzymes were assessed by conventional methods. Interleukin-6, interleukin-8, interleukin-10, and tumour necrosis factor-α (TNF-α) levels were determined by using the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to manufacturer's instructions. With regard to hemorheological variables, whole blood viscosity (WBV) and plasma viscosity (PLV) were measured using the Rotational Viscosimeter LS 30 (Contraves, Zurich, Switzerland), whereas erythrocyte filtration was measured by a microcomputer-assisted filtrometer, model MF4 (Myrenne GmbH, Roetgen, Germany). Erythrocyte deformability was estimated by a curve indicating erythrocyte filtration throughout the 10-minute recording period in order to determine rheological properties of erythrocytes, by passing them through polycarbonate filters with 5-μm micropores (Nucleopore, Pleasanton, CA, USA). The initial flow rate from the microcomputer-generated curves was used for assessing the erythrocyte deformability index. WBV was analysed at shear rates of 0.512 sec^{-1} and 94.5 sec^{-1}.

Statistical analysis

Statistical analysis was performed by using the PASW Statistics (SPSS Inc, Chicago, IL, USA) software for Macintosh (Version 18.0). Results are expressed as mean±SD. Data on the effect of diet on chemical analysis of fish flesh were submitted to one-way ANOVA. A general linear model for repeated measurements was performed to compare the effect of the two different treatments with sea bream. A model with adjustment for age, gender, and smoking habit, was performed. Data for the general linear model are reported as geometric mean and standard error. A p-value <0.05 were considered to indicate statistical significance.

RESULTS

Fillet characteristics and flesh intake

Diet did not significantly influence both percentage of fillets with skin[13] and flesh proximate analysis and energy. However, differences emerged between fillets for fatty acid composition. As shown in Table 1, the flesh of FM fillets was characterized by a higher (p<0.01) content of saturated, monounsaturated, n-3 PUFA fatty acids, and above all, lower values (p<0.01) of n-6 PUFA and n-6/n-3 PUFA ratio than the PP fillets. These differences are primarily due to the higher content of EPA, DHA, C22:5 n-3 in the FM group, and to the higher level of linoleic acid in the PP fillets. On the basis of the quantitative fatty acid composition (data not reported for brevity), the FM fillets contained a greater amount of the sum of EPA+DHA (2.07 vs. 1.82 g/100 g) in comparison to the PP fillets. Therefore, the intake of 3.5 g of EPA+DHA per week, as recommended by ISSFAL [17] and the American Hearth Association[18] could be satisfied by a weekly consumption of either 169 g of the FM fillets or 192 g of the PP fillets. The same flesh quantity would also provide a different amount of C18:2 n-6, 1.74 g and 3.74, respectively.

The fatty acid content of the subsample fillets from the different dietary groups consumed by each subject was estimated, taking into account the fillet weight. In Table 2,

the weekly average intake of flesh, lipids, individual fatty acids, principle fatty acid groups and the n-6/n-3 PUFA ratio of main nutritive significance for each dietary group were reported. These values were related both to the fillet fatty acid composition and to the flesh intake that was higher in the PP group (on average, FM 534 vs PP 667 g weekly). The higher PP flesh intake was related to an average higher fillet weight in absolute value reported for this last group (FM 146 vs PP 172 g). From the higher PP flesh intake, the result was a higher weekly intakes of total lipids (on average FM 64 vs PP 79.1 g) and energy, which was not reported in table 2 for brevity (on average FM 1088 vs PP 1289 kcal/100g). Moreover, the higher flesh intake resulted in an increase of total saturated fatty acids (on average FM 13.8 vs PP 15.6 g), in the linoleic acid content (C18:2 n-6) (on average FM 6 vs PP 13.79 g), and in the total PUFA n-6 consumed by the PP group. There was a similar EPA+DHA weekly intake in both groups (on average FM 12.1 vs PP 12.5 g), in the presence of a higher n-6/n-3 ratio (on average FM 0.48 vs PP 0.91).

Characteristics of the study population

Twenty subjects (12 men; 8 women) were enrolled in the study with a median age of 52.5 years (range: 23-67) and a mean±SD BMI of 26.8±3.6. No differences according to gender were observed. Two participants were current smokers. At the end of the intervention programme, BMI did not change significantly with respect to baseline. Moreover, no significant differences were observed between nutrient intake before and after each intervention.

No significant differences between the two groups of subjects at baseline were observed (Table 3).

Group A

In order to analyse the changes in the parameters investigated after the dietary intervention, a general linear model for repeated measurements, adjusted for age, gender, and smoking habit was performed. In Table 4, adjusted values for all the variables investigated before and after the dietary interventions in the group A were reported. Subjects, consuming firstly fish fed with FM were reported to have significant amelioration with regard to lipid variables such as total cholesterol, LDL cholesterol and triglycerides. Indeed, total cholesterol significantly decreased by 29.3% (Δ = –26 mg/dL) in the first phase of intervention, returning to similar baseline values after the dietary intervention with fish fed with PP. Similarly, after the first phase of the dietary intervention, LDL-cholesterol decreased significantly by 21.6% (Δ = –19 mg/dL), whereas triglycerides decreased by 11.7% (Δ = –10.7 mg/dL). Thereafter, the levels of both LDL-cholesterol and triglycerides increased, returning to baseline.

With regards the liver enzymes, the dietary intervention with fish fed with FM reduced AST and ALT values, to a lesser extent (Δ = –1.5 UI/L; Δ = –4.9 UI/L, for AST and ALT, respectively), not followed by a significant increased in the second phase of intervention, with the exclusion of AST values. Similarly, with regard to inflammatory cytokines, interleukin-6 and interleukin-8, showed a significant reduction in the first phase of intervention.

Finally, whole blood viscosity appeared significantly improved after the FM phase of intervention, with erythrocyte filtration rate showing a significant increase by 7.59% (Δ = +4.59 mPA).

Group B

Data regarding variables investigated in this group are reported in Table 5. Concerning the results of the subjects who started to consume fish fed with PP, no significant differences emerged either after the first phase or after the second phase of intervention. The only exceptions were total cholesterol and interleukin-6, that both showed a significant reduction after the cross-over intervention with FM.

DISCUSSION

In the present study, for the first time, the effect of consuming differently fed gilthead sea bream on several atherosclerotic risk parameters in healthy subjects was evaluated. We observed that the intake of fillets with a similar EPA+DHA content but a different n-6/n-3 ratio determines different changes in the biochemical profile in terms of the primary prevention of cardiovascular diseases.

We carried out a single-blinded cross-over intervention study with two groups of gilthead sea breams fed with different diets: one group of gilthead sea bream was fed with a diet containing 100% fish meal as source of protein, whereas the other group was fed with a diet where fish meal was partially replaced with a mixture of plant sources (50% fish meal; 50% plant proteins). The subjects consumed both groups of fish, crossing over from one group to another. At the end of the intervention period the two groups of subjects were shown to have consumed a similar quantity of EPA+DHA, but with a significant difference in the n-6/n-3 PUFA ratio between the two intervention phases. The amount of EPA+DHA consumed was greater than the quantity of 1 g/day suggested by Levis *et al* [19] for humans with disease accidents, as well as the 3.5 g/week suggested by the American Heart Association [18] and by ISSFAL [17] for people without cardiovascular disease. On the contrary the n-6 PUFA quantity and the n-6/n-3 PUFA ratio were, respectively, on average more than double for the PP fillet consumers in comparison to the FM fillet consumers. There was a higher weekly consumption of 24% lipids and 13% saturated fatty acids by PP fillet consumers in comparison to the FM fillet consumers. A relationship between high intake of lipid and SFAs with risks of cardiovascular disease development is generally accepted. These relation may have contributed to the lack of decrease of the atherosclerotic biomarker levels following the PP diet consumption

As a result, while group A subjects, consuming the FM fillets first, showed significant beneficial biochemical changes, with improvement in lipid, inflammatory and haemorheological parameters, group B subjects, who consumed the PP flesh first (with a higher ratio of n-6/n-3), reported an unchanged biochemical profile with regard to all blood parameters investigated. Moreover, the modest beneficial effect on the blood parameters, derived from FM fillets consumed during the second intervention,

Table 2. Weekly intake per participant of fillets and flesh components

Intake[†]	Flesh (g)	Lipids (g)	C20:5n-3 EPA[‡] (g)	C22:6n-3 DHA[§] (g)	C18:2n-6 (g)	EPA+DHA (g)	SFA[¶] (g)	MUFA[††] (g)	n-3PUFA (g)	n-6 PUFA (g)
Group A										
FM	528±17.9*	63.4±2.2*	4.55±0.15*	7.45±0.25*	5.94±0.20**	12±0.44	13.6±0.46*	15.8±0.54*	15.6±0.53*	6.50±0.22**
PP	678±43.5*	80.4±5.2*	4.23±0.27*	8.52±0.55*	13.6±0.87**	12.7±0.87	15.9±1.01*	19.6±1.26*	17±1.10*	14.3±0.91**
Group B										
PP	663±43.9*	78.5±3.5*	4.13±0.18*	8.32±0.37*	13.3±0.59**	12.4±0.55	15.5±0.68*	19.1±0.62*	16.6±0.73*	13.9±0.62**
FM	536±18.4*	64.3±2.9*	4.61±0.19*	7.56±0.34*	6.09±0.27**	12.2±0.54	13.8±0.62*	16±0.71*	15.8±0.70*	6.59±0.29**
Means										
PP	533±22.0*	64.0±2.6*	4.59±0.19*	7.52±0.31*	6.00±0.24**	12.1±0.50	13.8±0.56*	15.9±0.66*	15.7±0.65*	6.57±0.27**
FM	667±33.7*	79.1±3.9*	4.16±0.21*	8.38±0.42*	13.8±0.57**	12.5±0.63	15.6±0.78*	19.3±0.97*	16.7±0.84*	14.1±0.71**

* $p<0.05$ and ** $p<0.01$ among the fillets in the same group.
[†]All values are mean±SDd, [‡]Eicosapentaenoic acid; [§]Docosahexaenoic acid, [¶] Saturated fatty acids; [††]Monounsaturated fatty acids.

Table 3. Baseline characteristics of the two groups of subjects

	Group A[†] (n=10)	Group B[†] (n=10)	*p*-value	Normal value
Age, median (range)	55 (32-67)	56.5 (23-62)	0.2	
Gender, M/F	6 / 4	6 / 4	-	
Smoking habit, n (%)	1 (1)	1 (1)	-	
BMI, kg/m^2) [§]	24.8±4.9	25.2±5.3	0.3	
Total cholesterol (mg/dL) [v]	233±28.6	215.8±49	0.4	<200
LDL-cholesterol (mg/dL) [§]	152±31.9	139±38.9	0.5	<130
Triglycerides (mg/dL) [§]	117±47.6	93.8±45.2	0.2	<150

[†]Group A consumed first the fish meal fillets and then the plant protein ones, group B consumed first the plant protein fillets and then the fish meal ones.
[§]Data are reported as mean ± SD.

Table 4. Variables investigated in the group A (1st: FM – 2nd: PP)

Variables†	Baseline (T0)	End of 1st intervention (T1)	*p* (T1-T0)	End of 2nd intervention (T2)	*p* (T2-T1)
Lipid variables					
Total cholesterol, mg/dL	232 (215-249)	206 (193-219)	0.002	232 (226-238)	0.06
LDL[1]-cholesterol, mg/dL	149 (131-170)	131 (114-151)	0.005	153 (143-164)	0.04
HDL[2]-cholesterol, mg/dL	56.1 (46.9-67.1)	51.6 (42.9-61.9)	0.09	52.3 (45-60.7)	0.6
Triglycerides, mg/dL	107 (73.8-156)	96.5 (70.4-132)	0.02	105 (86.7-128)	0.3
Liver enzymes					
AST[3], UI/L	22.2 (18.9-26.1)	20.7 (17.8-24.1)	0.04	23.8 (19.5-28.9)	0.04
ALT[4], UI/L	25.2 (16.6-37.8)	20.3 (14.1-29.1)	0.002	22.9 (16.2-32.4)	0.1
Inflammatory variables					
Interleukin-6, pg/mL	8.2 (3.1-12.5)	6.1 (4.4-10.5)	0.02	7.5 (5.2-9.8)	0.4
Interleukin-8, pg/mL	18.1 (12.1-23.1)	12.9 (10.4-15.5)	0.003	13.5 (11.2-17.2)	0.3
Interleukin-10, pg/mL	19.7 (13.3-25.1)	17.5 (13.2-22.3)	0.3	17.4 (12.7-21.2)	0.6
TNF[5]-alpha, pg/mL	34.6 (28.5-42.4)	32.5 (27.6-41.3)	0.8	31.4 (28.7-35.4)	0.7
Haemorheological variables					
WBV[6] 0.512 sec^{-1}, mPA.s	25 (21.9-28.6)	23 (22.1-24)	0.6	24.2 (19.6-27.8)	0.2
WBV[6] 94.500 sec^{-1}, mPA.s	4.5 (4.1-4.9)	4.6 (4.4-4.8)	0.5	4.7 (4.3-5.1)	0.6
PLV[7], mPA.s	4.1 (3.9-4.3)	3.9 (3.7-4.0)	0.05	4.0 (3.9-4.2)	0.7
EDI[8]	7.01 (4.03-12.2)	11.6 (9.87-13.7)	0.008	8.3 (7.36-9.39)	0.01

†Values are expressed as geometric mean and confidence interval; general linear model adjusted for age, gender and smoking habit.
[1]LDL = low-density lipoprotein; [2]HDL = high-density lipoprotein. [3]AST = aspartate amino transferase; [4]ALT = alanine amino transferase; [5]TNF = tumor necrosis factor; [6]WBV = whole blood viscosity; [7]PLV = plasma viscosity; [8]EDI = erythrocyte deformability index

Table 5. Variables investigated in the group B (1st: PP – 2nd: FM)

Variables†	Baseline (T0)	End of 1st intervention (T1)	*p* (T1-T0)	End of 2nd intervention (T2)	*p* (T2-T1)
Lipid variables					
Total cholesterol, mg/dL	211 (171-259)	216 (176-266)	0.3	204 (165-254)	0.009
LDL[1]-cholesterol, mg/dL	134 (102-176)	139 (108-179)	0.2	131 (98.6-173)	0.1
HDL[2]-cholesterol, mg/dL	56.9 (48.3-67.1)	55.9 (49.6-62.9)	0.5	54.6 (48.6-61.3)	0.6
Triglycerides, mg/dL	105 (81.3-139)	103 (73.1-129)	0.8	103 (75.9-122)	0.9
Liver enzymes					
AST[3], UI/L	25.1 (21.2-29.7)	23.6 (19.7-28.2)	0.3	24.8 (20.7-29.8)	0.4
ALT[4], UI/L	26.4 (18-38.6)	23.8 (19.7-28.7)	0.3	24.5 (18.7-32.1)	0.6
Inflammatory variables					
Interleukin-6, pg/mL	7.4 (4.8-10.4)	7.1 (5.1-12.7)	0.6	5.5 (3.2-9.8)	0.03
Interleukin-8, pg/mL	13.1 (9.5-15.6)	12.9 (10.4-15.5)	0.3	12.5 (11.2-16.2)	0.07
Interleukin-10, pg/mL	18.4 (14.2-22.5)	17.8 (12.5-20.3)	0.3	17.1 (13.2-20.5)	0.8
TNF[5]-alpha, pg/mL	32.1 (27.8-38.1)	32.5 (26.5-39.3)	0.7	31.4 (27.4-33.2)	0.9
Haemorheological variables					
WBV[6] 0.512 sec^{-1}, mPA.s	23.2 (20.2-26.7)	23.9 (21.3-26.8)	0.2	22 (19.6-22.8)	0.4
WBV[6] 94.500 sec^{-1}, mPA.s	4.4 (4.2-4.7)	4.5 (4.2-4.8)	0.4	4.3 (4.0-4.7)	0.4
PLV[7], mPA.s	3.9 (3.8-4.1)	4.1 (3.9-4.3)	0.06	3.9 (3.7-4.1)	0.08
EDI[8]	7.41 (4.20-13.1)	7.29 (4.70-11.3)	0.9	9.6 (8.56-10.8)	0.2

[1]LDL = low-density lipoprotein; [2]HDL = high-density lipoprotein. [3]AST = aspartate amino transferase; [4]ALT = alanine amino transferase; [5]TNF = tumor necrosis factor; [6]WBV = whole blood viscosity; [7]PLV = plasma viscosity; [8]EDI = erythrocyte deformability index

was limited to the decrease of total cholesterol and interleukin 6. This may be explained by a residual effect of the high level of PUFA n-6 flesh consumed during the 1st intervention study. On the other hand, a change in the composition of erythrocyte fatty acids requires several weeks of dietary change.[20] This lag in the awaited positive effect could suggest a sort of persistence of n-6 fatty acids, especially arachidonic acid, in the cell membranes [7] with a delay in the n-6 PUFA washing out that inhibited the potential beneficial effect of the abundant EPA+DHA n-3 PUFA supply from the FM fillet intake. To be remembered in that regard is that even if AA (20:4 n-6) and EPA (20:5 n-3) are parent compounds for the production of eicosanoids, those compounds derived from AA are antagonistic to those from EPA. These results show that modification of fish feeding is able to determine the level of beneficial biochemical changes in subjects who consume the final product.

Over the last decades, thousands of epidemiological, observational, and experimental studies and randomized controlled trials have documented the positive cardiovascular effects of fish consumption, as well as that of long-chain n-3 PUFA.[1-3] These beneficial effects seem to result primarily from DHA and EPA enrichment of membrane phospholipids, which leads to improved arterial and endothelial function, reduced platelet aggregation, blood rheology and suppressed production of pro-inflammatory cytokines.[4-6,21] Results of the present study corroborate those reported by Seierstad *et al* [12] regarding the beneficial effect derived from the intake of fish fed with fish oil on the lipid and inflammatory parameters of consumers, in comparison to that following the intake of fish fed with

a partial or total replacement of fish oil with vegetable oil. Most importantly, the present intervention study highlights the importance of the PUFA n-6/n-3 ratio, assuring suggested intake of EPA+DHA by itself. The intake of a more favourable n-6/n-3 PUFA ratio has become a frequently discussed aspect of human nutrition.[7] The high intake of n-6 PUFA is considered harmful because arachidonic acid may give rise to inflammation via production of pro-inflammatory leukotriens and prostaglandins, whereas intake of n-3 PUFA modulates the production of inflammatory mediators.[21,22]

Nevertheless, intervention studies in healthy humans confirmed that when it comes to enriching blood lipids with n-3 PUFA, consumption of EPA and DHA from fish is more effective than supplementing the diet with fish oil.[23,24] Indeed, consuming n-3 PUFA from fish and not from capsules results in other beneficial effects. An increased intake of seafood entails a substitution of other food items and some of the health benefits may be explained by the effect of substitution, i.e. a decreased consumption of unhealthy foods.[24]

In addition, our study shows a beneficial effect of fish fed with fish meal on blood rheology, as seen from the significant improvement in the erythrocyte filtration index after the 10-week period of dietary intervention. Epidemiological studies showed that increased blood viscosity is associated with several cardiovascular risk factors, as well as with both prevalence and incidence cardiovascular disease. Mechanisms by which elevations in rheologic factors may promote cardiovascular events are different and include increases in blood pressure, shear stress, ischemia, and blood vessel wall interactions. A relevant feature of the rheologic flow is the erythrocyte morphology, since deformability of circulating cells greatly influences the rheologic properties of the blood, thereby playing a key role in maintaining and regulating microcirculation. Under pathologic conditions, erythrocyte deformability is altered, thus affecting the rheologic environment at the level of microcirculation.
We previously found that an altered deformability index is significantly associated with fish intake but in the present study we emphasise the importance of n-3 PUFA in ameliorating the erythrocyte deformability index.

Certain limitations can be identified in the present study. First of all, the sample population is limited. Secondly, the consumption of fish may displace other foods, such as meat and dairy products, in the diet. Although, this may contribute in part to the beneficial effects observed in the study, the overall effect is likely to account for little of the observed health benefits, because the replacement foods selected would have been be highly variable among individuals. Moreover, it is also possible that other dietary nutrients could have influenced the results observed in the study group, even if the two groups of subjects did not show significant differences in term of diet quality.

In conclusion, we observed, through a single-blinded, randomized, controlled trial, the need to modulate the diet of farmed fish in terms of both a proper EPA+DHA content and a favourable n-6/n-3 ratio, in order to maintain the significant beneficial effect of fish intake on the atherosclerotic risk profile of a group of healthy subjects. This evidence is highly relevant, not only for the primary prevention of cardiovascular disease, but also to provide the feed industry with a deeper awareness of healthy eating aspects in using plant sources as fish meal/oil alternatives.

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AUTHOR DISCLOSURES

All authors declare that this work is original and has not been submitted in total or in part to other journals; the authors declare that they had no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous year; no other relationships or activities that could appear to have influenced the submitted work.

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Original Article

The atherosclerotic risk profile is affected differently by fish flesh with a similar EPA and DHA content but different n-6/n-3 ratio

Francesco Sofi MD, PhD[1-3], Gianluca Giorgi MSc, PhD[4], Francesca Cesari MSc, PhD[1], Anna Maria Gori MSc[1,3], Lucia Mannini MD[1], Giuliana Parisi MSc, PhD[4], Alessandro Casini MD[2], Rosanna Abbate MD[1], Gian Franco Gensini MD[1], Bianca Maria Poli MSc[4]

[1]*Department of Clinical and Experimental Medicine, Thrombosis Centre*
[2]*Agency of Nutrition, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy*
[3] *Don Carlo Gnocchi Foundation Italy, Florence, Italy*
[4]*Department of Agricultural Biotechnology, Section of Animal Sciences, University of Florence, Italy*

攝取 EPA 及 DHA 含量相似但 n-6 與 n-3 脂肪酸比例相異的魚肉對於動脈粥狀硬化危險因子之不同效應

本研究目的，在於評估攝取 n-6 及 n-3 脂肪酸比例不同之黑鯛魚片對於動脈粥狀硬化生化指標的影響。共有 20 位健康的受試者參與此隨機單盲交叉試驗。研究初始，隨機將受試者分為 A、B 兩組，A 組先給予每週大約 630 克黑鯛魚片(FM-以全魚飼料餵養)，連續攝食 10 週後，更換為以混合 50%植物性蛋白飼料餵養的黑鯛魚(PP)，B 組則相反。A 組在給予 FM 黑鯛魚的介入後，總膽固醇濃度顯著地減少 29.3%(-26 mg/dL)，然而換成給予 PP 黑鯛魚後，又回升至介入前的濃度。且低密度脂蛋白膽固醇(減少 21.6%; -19 mg/dL)及三酸甘油酯(減少 11.7%; -10.7 mg/dL)也具有相似的現象。FM 黑鯛魚的攝取，同時改善發炎細胞激素、介白素-6 及介白素-8 的濃度。此外，紅血球濾過率顯著提高 7.59%(+4.59 mPA)，顯示 A 組參與者的全血黏度有明顯的改善。總結而論，攝取 EPA 及 DHA 含量相似，但不同比例 n-6 與 n-3 脂肪酸之魚肉，對於健康受試者的脂質、發炎及血液動力學參數，具有不同的效應。

關鍵字：魚類攝取、心血管疾病、發炎、飲食、ω-3 脂肪酸