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ORIGINAL CONTRIBUTION

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Impact of fish intake on oxidative stress when included into a moderate energy-restricted program to treat obesity

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Abstract *Background* The role of some nutritional factors and hypocaloric diets on oxidative balance is a matter of debate, especially related to the prevention and treatment of obesity and co-morbidities. *Aim of the study* The aim was to investigate the antioxidant capacity of different energy restricted diets in the treatment of obesity, paying emphasis to the effect of incorporating omega-3 fatty acids with or without other seafood components. *Methods* The study was a randomized 8-weeks parallel intervention trial prescribed to lose weight, which was implemented in 276 subjects aged 31.4 ± 5.4 y.o. following four different balanced hypocaloric diets (TEE-30%): fish-restricted (control), cod and salmon based diets and DHA+EPA supplemented administration. At baseline (day 0) and at the end of the trial (day 56), anthropometry, dietary intake, erythrocyte membrane fatty acid content, circulating malondialdehyde (MDA) and plasma antioxidant status (AOP) were determined. *Results* Overall, percent weight loss was −5.8 ± 3.2% ($P < 0.001$) and the AOP statistically increased after the energy restriction period ($P = 0.015$), basically due to the seafood-based diets. In contrast, MDA statistically only decreased ($P = 0.026$) after the cod-based diet intake with no changes after the other nutritional treatments. In fact, the cod-based intervention statistically decreased oxidative stress when expressed as the MDA/AOP ratio ($P = 0.006$). *Conclusions* A moderate calorie-restricted cod-based diet was found as a useful strategy to lose weight, which was accompanied by a specific improvement on oxidative stress markers. The low saturated fat content and the seafood protein source of this diet may be important factors involved in these findings.

Key words obesity – oxidative stress – weight loss – PUFA – MDA

Introduction

Cell oxidative stress is stimulated when a shift between excessive free radicals and counteracting antioxidant substances occurs [26]. Nowadays, a number of scientific evidences are linking the oxidative-stress injury with an excess in body weight-for-height [3, 10–12, 26]. In fact, weight loss mediated by a restriction in caloric intake has been related with a decrease in free radicals production, especially affecting lipid peroxidation [9, 19, 29, 32]. Furthermore, dietary intake is one of the most important factors involved in the modulation of oxidative stress, since several foods and nutrients have been classically

considered as antioxidant such as fruit components [9], weight lowering diets [8], and specific amino acids [19, 22, 27], among others. Seafood has been described as a competent antioxidant source [14], since its composition offers lower amount of saturated fat than many other food items, and it is rich in antioxidant substances, especially in some amino acids such as taurine [16]. However, the composition of seafood often includes representative PUFA amounts [31], such as omega-3 fatty acids, whose chemical structure makes them suitable for peroxidation [34], although it may be claimed that this prooxidant effect could be ameliorated by antioxidant components occurring in the fish flesh.

Therefore, the aim of the present study was to evaluate the antioxidant effect of four energy restricted diets with different seafood content, searching specifically for the effect of incorporating omega-3 fatty acids with or without other seafood components in the nutritional treatment of young adults with excessive body weight.

Subjects and methods

Subjects

The current study named *SEAFOODplus YOUNG* was carried out in 276 subjects (118 men and 158 women) as part of the multicenter study *SEAFOODplus: A better life with seafood* (http://www.seafoodplus.org). Volunteers were recruited through advertisements from October 2004 until April 2005 from three SEAFOODplus YOUNG country partners: Iceland (43 men and 71 women), Spain (53 men and 52 women), and Ireland (22 men and 35 women). Inclusion criteria were body mass index (BMI) between 27.5 and 32.5 kg/m^2, with an age range of 20–40 years. Exclusion criteria were weight change (±3 kg) within three months before the start of the study, use of supplements containing n-3 fatty acids, drug treatment of diabetes mellitus, hypertension or hyperlipidemia and women's pregnancy or lactation. All potential subjects that answered the call were screened for inclusion and exclusion criteria, first over the phone and later in person. A total of 324 subjects were included at start, while participation rate was 85% (n = 276) with no specific dropout effect (P = 0.729) on any dietary group.

The study was approved by the National Bioethical Committee in Iceland (04/031), the Research Ethics Committee of University of Navarra in Spain (24/2004) and the Clinical Research Ethics Committee of the Cork University Hospital in Ireland. The study followed the Helsinki guidelines and all volunteers gave their written informed consent to participate before they started the intervention.

Protocol

The study was a randomized 8 weeks parallel intervention trial devised to weight loss and developed in the three European countries, Iceland, Spain and Ireland, by using four diets that were different depending on the fatty acid and protein source, but with the same energy-restriction approach and dietary macronutrient distribution. Thus, subjects were randomly assigned to one of the four dietary treatments: control, cod, salmon or fish oil. Information on physical activity pattern during the last year, smoking habits and alcohol consumption was collected using a questionnaire filled in at the baseline interview [20]. The subjects were instructed not to change their physical activity level during the 8 weeks intervention period and to keep their alcohol consumption to a minimum (max 1 drink of wine or beer per week). The foreseen experimental measurements were carried out at baseline (day 0) and at endpoint (day 56).

Randomization and control group (placebo)

After baseline clinical investigation, each volunteer was randomly assigned to one of the four experimental diets (1: control, 2: cod, 3: salmon, 4: fish-oil). There was only one randomization list, which was computer generated (SPSS program) by the Icelander partner with no stratification for gender or age. In each country, the research dietitian assigned the randomization number indicated by Iceland to each subject enrolled into the trial. People following the control and fish oil diets diet were single blind supplemented every day with six sunflower oil capsules as placebo which was used by others [25], and six fish-oil capsules, respectively. The fish (cod or salmon) as well as the capsules were freely given to the participants.

Nutritional intervention

The energy restriction of the hypocaloric diets was −30% with respect to the individual energy expenditure of each participant calculated by Harris–Benedict equation applying the WHO's correction factors on physical activity [21]. The physical activity pattern was evaluated assigning to each activity the metabolic equivalent, MET [20] and the physical activity level factor was chosen based on the calculated METs during the last year.

The four diets were matched for total fat (30–35% of total energy), carbohydrate (50–55% of total energy), protein (16–20% of total energy) and dietary fiber (20–25 g/day). Each subject got a detailed diet plan to follow for 8 weeks based on a food exchange system used by our group (9–10) and met with a nutritionist or

dietician at baseline, when the research diets were explained. The experimental diets were designed to vary in seafood content, both omega-3 fatty acids and fish proteins, so instructions were given to minimize differences with respect to macronutrient and dietary fiber contents. Thus, lean meat was the main protein source in the control and in the fish-oil supplemented diets while cod and salmon were the main protein sources for cod-based and salmon-based diets, respectively. The daily omega-3 fatty acid intake for each diet was 5.6 ± 0.2 mg/day for control, 227 ± 29 mg/d for cod-based, 1,418 ± 34 mg/day for salmon-based and 3,003 ± 128 mg/day for fish-oil supplemented. To follow up the prescribed diets with regard to macronutrient distribution, dietary intake was followed by two days-weighed food records (one week day and one weekend day) performed at baseline and during the last two weeks of the intervention. Dietary records were analyzed using the food-nutrient database in each country.

Specific adherence to each diet with regard to fatty acid contribution was tested by measuring changes in fatty acid composition of erythrocyte membrane related to intervention [5].

Experimental measurements

Determinations were performed at baseline (day 0) and at the end of the nutritional trial (day 56) using standard procedures as outlined in a research protocol used by all countries participating in the study (http://www.seafoodplus.org). The experimental protocol included anthropometric (weight, height, body mass index, waist perimeter) and bioelectrical impedance analysis (Bodystat 1500, Bodystat Ltd, UK), which were standardized for all participating centers [30, 36]. Blood pressure was measured by standard procedures and blood samples were extracted to obtain serum, plasma and erythrocytes that were frozen at −80°C-until assay.

Total cholesterol, HDL-cholesterol and triacylglycerol were analysed using commercially available enzymatic colorimetric assays (Hitachi 911, Roche, Switzerland). The LDL-cholesterol was calculated using the Friedewald formula. Plasma insulin was assessed by an electrochemiluminescence immunoassay (Elecsys Modular Analytics E170, Roche Diagnostics, Switzerland), and the homeostatic model assessment insulin resistance index (HOMA) was calculated [29]. The fatty acids composition of extracted erythrocytes membrane phospholipids was measured by gas chromatography following the conditions previously described by Bandarra et al. [5].

Total plasma antioxidant capacity (AOP) was evaluated by means of a colorimetric assay kit (AOP-450, OXIS International, USA). This method measures the antioxidant activity of the system constituted by uric acid, ascorbic acid, vitamin E, glutathione, albumin and bilirubin that directly correlates with lipid resistance to oxidation.

Plasma malondialdehyde (MDA) was colorimetrically determined as marker of lipid peroxidation using a commercial kit that measured free and total malondialdehyde compounds (LPO, OXIS International, USA). Both colorimetric assays were read using a Multiskan Spectrum (Thermo Electron Corporation, Finland).

Statistical analysis

Sample size was calculated as described elsewhere [23] by considering weight loss as the main variable and applying published values for the standard deviation (SD) of this marker [8, 9] and 2 kg as the potential difference between means of the interventions to be statistically different. The statistical power was calculated at 80%. Therefore, and by applying a P-value < 0.05, the sample size required was a minimum of 62 volunteers per group. The Kolmogorov–Smirnov and the Shapiro–Wilk tests were used to determine the observed and theoretical frequency of occurrence of the variables that were adjusted to the parametric distribution. Accordingly, the nonparametric Wilcoxon-paired or the parametric Student t-tests were applied to detect differences before and after nutritional intervention in each group. When appropriate, percent changes were calculated as the difference between endpoint and baseline measurements, and differences in diet-related changes between groups were compared by using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Multivariate linear regression analyses were fitted to detect potential predictors for the observed changes in the oxidative stress markers by applying a normalized model, since volunteers were young and healthy, except for their overweightness.

Results are given as the mean ± standard deviation and multivariate coefficients are expressed by using the 95% confidence intervals. The P-value < 0.05 was considered as statistically significant and all the statistical analyses were performed by SPSS 13.0 software (SPSS Inc, USA) for Windows XP (Microsoft, USA).

Results

Overall outcome of the nutritional intervention

The recruitment process produced a homogeneous group of volunteers giving similar baseline values in

Table 1 Anthropometric, biochemical and clinical biomarkers (mean ± SD) before and after the four nutritional intervention by the experimental diets

Biomarkers	Control (24 men/42 women)		Cod (30 men/39 women)		Salmon (37 men/37 women)		Fish-oil (27 men/40 women)	
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint
Age (y.o.)	30 ± 5		31 ± 6		31 ± 5		31 ± 5	
Body weight (kg)	86.7 ± 9.3	82.3 ± 8.8[a]	89.4 ± 9.7	84.0 ± 9.3[a]	91.1 ± 11.8	85.6 ± 10.8[a]	84.9 ± 9.9	79.6 ± 8.9[a]
Fat mass (kg)	29.5 ± 5.9	26.6 ± 6.1[a]	29.4 ± 5.8	26.0 ± 5.8[a]	29.1 ± 5.2	25.6 ± 5.8[a]	27.9 ± 5.5	24.3 ± 5.9[a]
Waist (cm)	94.5 ± 7.0	90.5 ± 7.4[a]	96.7 ± 6.8	91.8 ± 6.9[a]	97.2 ± 7.9	91.8 ± 7.5[a]	94.3 ± 6.5	89.2 ± 5.9[a]
SBP (mmHg)	126 ± 10	121 ± 10[a]	125 ± 12	122 ± 11[a]	127 ± 13	122 ± 11[a]	123 ± 13	119 ± 10[a]
DBP (mmHg)	73 ± 8	69 ± 7[a]	73 ± 8	69 ± 6[a]	73 ± 8	68 ± 6[a]	71 ± 7	67 ± 6[a]
Cholesterol (mM)	5.2 ± 0.9	4.9 ± 0.8[a]	5.2 ± 1.1	4.7 ± 0.9[a]	5.1 ± 0.9	4.6 ± 0.9[a]	5.0 ± 1.0	4.7 ± 0.9[a]
c-LDL (mM)	3.2 ± 0.8	3.0 ± 0.7[a]	3.3 ± 1.0	3.0 ± 0.8[a]	3.2 ± 0.8	2.9 ± 0.8[a]	3.1 ± 0.9	2.9 ± 0.9[a]
c-HDL (mM)	1.4 ± 0.4	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.3[a]	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.3	1.3 ± 0.3[a]
Triacylglycerol (mM)	1.1 ± 0.6	1.1 ± 0.5	1.3 ± 0.7	1.0 ± 0.5[a]	1.2 ± 0.5	0.9 ± 0.3[a]	1.2 ± 0.8	1.0 ± 0.6[a]
Glucose (mM)	4.9 ± 0.4	4.8 ± 0.5	4.9 ± 0.5	4.8 ± 0.5	5.0 ± 0.5	4.7 ± 0.4[a]	4.9 ± 0.5	4.8 ± 0.4
Insulin (microU/ml)	10.4 ± 5.6	8.6 ± 4.3[a]	10.0 ± 3.9	8.9 ± 4.0[a]	11.1 ± 5.2	8.4 ± 3.9[a]	10.1 ± 4.7	7.7 ± 3.8[a]
HOMA-IR	2.29 ± 1.38	1.86 ± 0.96[a]	2.15 ± 0.92	1.86 ± 0.79[a]	2.51 ± 1.33	1.72 ± 0.88[a]	2.17 ± 1.05	1.59 ± 0.81[a]
Malondialdehyde, MDA (nM)	1.99 ± 0.68	2.01 ± 0.68	1.81 ± 0.72	1.72 ± 0.72[a]	2.06 ± 0.81	2.12 ± 0.84	1.84 ± 0.63	1.89 ± 0.61
Antioxidant capacity, AOP (nM)	0.61 ± 0.17	0.59 ± 0.18	0.62 ± 0.22	0.71 ± 0.41[b]	0.62 ± 0.16	0.65 ± 0.17	0.63 ± 0.15	0.65 ± 0.17[b]

[a] $P < 0.05$ for differences when comparing before and after the nutritional intervention
[b] $P < 0.10$ for differences when comparing before and after the nutritional intervention

all groups after randomization with regard to overweight status, age, and baseline markers of obesity comorbidities, such as blood pressure, insulin function and circulating lipid profile, with the exception of triacylglycerol after the control diet (Table 1). Results of the caloric restriction in the diets induced an average weigh loss of −5.8 ± 3.2% ($P < 0.001$), which was statistically significant in all treatment groups and slightly higher in the dietary groups including seafood or fish-oil capsules (Table 1). Globally, the decrease in body weight promoted fat mass and waist perimeter reduction (Table 1), as well as improvement in blood pressure, lipid markers and insulin (Table 1).

The macronutrient distribution of experimental diets agreed with the initial design, being nutritionally balanced with no differences between diets. Only, protein intake was statistically higher but with no nutritional relevance in people following the fish-based diets, due to the dietary adjustment to get similar fat intake in the no-fish (control and supplemented) and the fish (cod and salmon-based) interventions (Fig. 1).

The erythrocyte membrane composition assessment (Table 2) showed that omega-3 fatty acids statistically decreased in the control group subjects (−1.5 ± 3.2%; $P < 0.001$), while people that followed salmon (2.0 ± 2.4%; $P < 0.001$) and fish-oil (2.5 ± 3.2%; $P < 0.001$) based diets statistically increased the omega-3 fatty acid content. Total monounsaturated fatty acid (MUFA) content in the erythrocyte membranes tended to decrease after control diet (−0.6 ± 5.8%; $P = 0.072$) with no changes after salmon ($P = 0.590$) and fish-oil supplementation ($P = 0.398$). Also, no changes were detected for the saturated fatty acid (SFA) membrane content after the intervention by control diet ($P = 0.823$), salmon-based ($P = 0.851$) and fish-oil diet ($P = 0.920$). In contrast, after the cod-based diet, total omega-3 fatty acids did not statistically change ($P = 0.105$), total SFA decreased (−1.1 ± 2.9%; $P = 0.003$) and total MUFA slightly increased (0.8 ± 2.9%; $P = 0.024$).

Effect of nutritional intervention on oxidative stress

At baseline, circulating malondialdehyde and plasma antioxidant capacity did not differ ($P > 0.050$) among volunteers randomized to each experimental group.

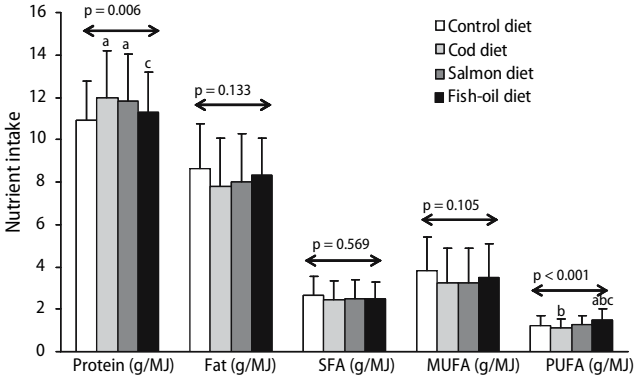

Fig. 1 Protein and fat intake depending on experimental diets reported by means of a 48-h weighted food record. Carbohydrate intake is not showed since no inter-group differences were observed ($P = 0.359$). Figure includes the ANOVA P-values between groups and (a) indicates statistical differences ($P < 0.005$) with respect to control diet; (b) indicates statistical differences ($P < 0.005$) with respect to cod-based diet and (c) indicates statistical differences ($P < 0.005$) with respect to salmon-based diet

Table 2 Fatty acid composition (mean ± SD) of erythrocyte membrane before and after the nutritional intervention by the four experimental diets

Erythrocyte membrane fatty acid composition (%)	Control (24 men/42 women)		Cod (30 men/39 women)		Salmon (37 men/37 women)		Fish oil (27 men/40 women)	
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint
Saturated	36.0 ± 2.4	36.1 ± 2.9	37.3 ± 2.4	36.1 ± 2.1[a]	37.3 ± 2.8	37.2 ± 2.9	36.9 ± 1.7	36.9 ± 1.9
Monounsaturated	19.9 ± 4.4	18.9 ± 2.8[b]	17.7 ± 1.9	18.5 ± 2.5[a]	18.4 ± 3.5	18.6 ± 2.1	17.9 ± 2.4	18.3 ± 2.9
Polyunsaturated	39.9 ± 5.1	40.5 ± 3.5	41.7 ± 2.9	41.9 ± 2.6	40.9 ± 3.9	40.7 ± 3.7	41.9 ± 2.4	41.4 ± 3.1
Omega-3 fatty acid	11.4 ± 3.4	9.9 ± 2.5[a]	10.2 ± 2.2	10.8 ± 2.4	10.1 ± 1.7	12.2 ± 2.3[a]	10.2 ± 2.5	12.7 ± 2.9[a]
Omega-6 fatty acid	28.1 ± 6.9	29.5 ± 4.1[b]	30.9 ± 3.2	29.7 ± 3.8[a]	30.2 ± 3.9	28.3 ± 3.6[a]	31.4 ± 3.6	29.5 ± 3.2[a]
Omega-3/Omega-6 ratio	0.48 ± 0.33	0.35 ± 0.12[a]	0.34 ± 0.10	0.38 ± 0.13[a]	0.34 ± 0.09	0.44 ± 0.12[a]	0.34 ± 0.14	0.44 ± 0.14[a]
EPA	1.64 ± 1.27	1.19 ± 0.84[a]	1.11 ± 0.50	1.05 ± 0.51	1.06 ± 0.51	1.76 ± 0.62[a]	1.14 ± 0.71	1.76 ± 0.73[a]
DPA	5.98 ± 1.57	5.62 ± 1.34	5.54 ± 1.22	6.06 ± 1.25[a]	5.72 ± 1.27	7.13 ± 1.34[a]	5.87 ± 1.60	6.55 ± 0.99[a]

[a] $P < 0.05$ for differences when comparing before and after the nutritional intervention
[b] $P < 0.10$ for differences when comparing before and after the nutritional intervention

The comparison between oxidative stress markers before and after each nutritional intervention is shown in Table 1.

Overall, the AOP statistically increased after the energy restriction period ($P = 0.015$), but in a different way (ANOVA, $P = 0.005$) depending on the seafood-based diets effect (Table 1). Indeed, the Tukey post hoc test evidenced that the cod-based diet was the most effective strategy rising AOP as compared with control ($P = 0.005$) and fish-oil diet ($P = 0.034$) but no reaching statistical significance ($P = 0.139$) when compared with the salmon-based diet. In contrast, MDA only statistically decreased ($P = 0.026$) after the cod-based diet with no statistical changes after the other nutritional treatments. In fact, the inclusion of cod in the calorie-restricted intervention was able to markedly decrease ($P = 0.006$) oxidative stress expressed as the MDA/AOP ratio (Fig. 2).

Considering all diets together, circulating MDA status statistically correlated with waist circumference ($r = 0.204$; $P = 0.001$), circulating cholesterol ($r = 0.338$; $P < 0.001$), HOMA-IR ($r = 0.152$; $P = 0.012$), SFA intake ($r = 0.223$; $P < 0.001$) and PUFA intake ($r = 0.156$; $P = 0.012$) after the nutritional intervention. Similarly, statistical relationships were found between AOP and fat free mass ($r = 0.120$; $P = 0.001$), SFA ($r = -0.127$ $P = 0.041$) and protein intake ($r = 0.173$; $P = 0.005$) at the end of the trial. Considering these factors as potential predictors for circulating oxidative stress markers, multiple regression analyses (Table 3) finally revealed that weight loss, serum cholesterol and SFA and PUFA intake were involved in the explanation of MDA (corrected $R^2 = 0.56$; $P < 0.001$) while weight loss, SFA and protein intake partially explained AOP at the end of the trial (corrected $R^2 = 0.24$; $P < 0.001$).

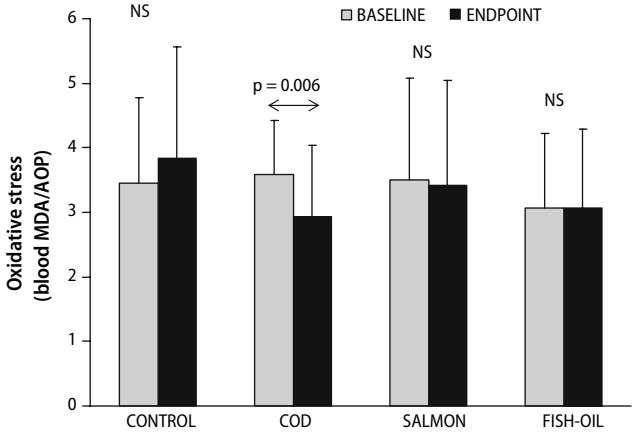

Fig. 2 Diet-related changes (Mean and SD) in oxidative stress measured as the ratio between circulating malondialdehyde and antioxidant plasma capacity (MDA/AOP)

Discussion

The current research was devised to investigate the conjoint impact on the antioxidant capacity of a

Table 3 Multivariate regression models to explain circulating malodialdehyde and antioxidant capacity at the end of the nutritional intervention, taking as references control diet and Iceland, adjusting for oxidative stress marker at baseline (MDA-b and AOP-b respectively)

Multiple regression model	B (95% CI)	*P*-value
Circulating MDA (mM) after the nutritional intervention		
Weight loss (kg)	−0.003 (−0.018:0.024)	0.804
SFA (g/MJ/day)	0.108 (0.037:0.179)	0.003
PUFA (g/MJ/day)	0.165 (0.037:0.294)	0.012
srm-Cholesterol (mM)	0.108 (0.034:0.183)	0.005
MDA-b (mM)	0.682 (0.594:0.770)	<0.001
Corrected R^2: 0.56		<0.001
Circulating AOP (mM) after the nutritional intervention		
Weight loss (kg)	0.003 (−0.006:0.013)	0.492
SFA (g/MJ/day)	−0.027 (−0.059:0.005)	0.101
Protein intake (g/MJ/day)	0.018 (0.004:0.032)	0.011
AOP-b (mM)	0.696 (0.540:0.852)	<0.001
Corrected R^2: 0.24		<0.001

caloric restriction together with fish intake in the treatment of obesity. Since fish contains some lipids susceptible to suffer peroxidation, we compared the effects of fish-oil supplementation (EPA + DHA) versus the intake of fish, the food containing PUFA, but also potential antioxidant compounds. Overall, the outcome of the caloric restriction program was acceptable, according the design, which reached a 5% weight loss in most of the participants and produced healthy changes in metabolic biomarkers related with the slimming process, as previously reported [3, 8, 9, 12].

The experimental diets were also designed to induce differences in the source of seafood fatty acids and the compliance was explored by searching for changes in erythrocyte membrane composition [33]. The analysis of these data confirmed the volunteeŕs adherence to the assigned treatment because the increment in both EPA and DHA content was specifically linked with salmon intake and fatty acid supplementation, as previously described [31].

Based on previous trials in which the relationship between nutrition and free radical production was studied, the effect of the current experimental diets on oxidative stress was evaluated by using MDA and AOP as oxidative stress markers [8, 9]. The regression analysis confirmed that weight loss was related to the improvement in antioxidant capacity and the decrease in lipid peroxidation, as earlier described [3, 12, 26]. The calorie-restricted cod-based diet resulted as the most effective strategy to improve oxidative stress. Indeed, volunteers fed on this diet evidenced a specific protective effect against lipid peroxidation as the decrease in MDA showed [8, 9], while plasma antioxidant capacity increased.

On the other hand, we found that circulating MDA directly correlated with different markers in which obesity was a common feature, in agreement with previous works [15, 24, 35]. Thus, abdominal fat, circulating cholesterol and insulin resistance [9, 36] were parameters related to MDA that improved after the caloric restriction period, although MDA only statistically decreased after the cod-based diet. With respect to nutrients, MDA was related not only with saturated, but also with poly-unsaturated dietary fats, suggesting the dietary lipids involvement in peroxidation [34]. In agreement with these findings, the direct relationship between lipid peroxidation and the body fat and the PUFA intake has been previously demonstrated [35]. Taking into account this observation and the outcome of the current work, the low PUFA and SFA intake that cod-based diet appeared as the most important factor in decreasing the blood concentration of MDA in spite of the overall improvement in the obesity- and MDA-related features that occurred after the four experimental diets tested in the current trial.

Detrimental effects attributable to saturated fat consumption has been widely described [6, 24], and decrease in this type of lipids could improve oxidative stress [1, 17, 35]. As mentioned, the nutritional interventions offered a similar percentage of fat, but with differences in the proportion between saturated and polyunsaturated lipids. Therefore, a decrease in saturated fat could be one of the mechanisms involved regarding antioxidant data. In agreement with previous studies [14], slimming by the inclusion of cod in the hypocaloric diet induced the improvement in plasma antioxidant capacity, while weight loss was the main factor decreasing lipid per oxidation, as the fall in circulating MDA showed. Therefore, the cod-based was the best nutritional strategy against oxidative stress.

Considering these dietary features, MUFA increased after the cod based diet and this fact could be related with the improvement in oxidative stress observed [7]. However, this change was not nutritionally relevant in spite of statistical significance, as multiple regression analysis showed. On the other hand, plasma antioxidant status was proportionally related to decrease in fat mass and saturated fat intake, in agreement with previous works [19, 37] and, interestingly, with dietary protein. The possible explanation for this relationship could be due to the role of some the amino acids on oxidative stress rather than the difference in protein intake, which was no nutritionally relevant (about 1%) in the current research. Thus, L-arginine, an amino acid that fish contains [4], is indirectly related to vasodilatation due to its participation on nitric oxide synthesis [19, 27] and by controlling the reactive nitrogen species produced by vascular endothelial cells [2, 38]. Taurine, also abundant in fish protein [4], is a potent antioxidant [28]. Previous works have described the role of taurine to inhibit cytotoxicity mediated by free radicals in insulin dysfunction status [22, 27] and to ameliorate ischemia-reperfusion injury [13, 28]. Therefore, these amino acids that are abundant in cod proteins could be involved in the observed process, although other substances related to fat-free composition of fish, such as selenium [8], probably exerted antioxidant effects after fish consumption.

In summary, the outcome of this work shows that cod intake within and energy-restricted diet increases plasma antioxidant capacity and is able to decrease lipid peroxidation together with the benefits related to weight loss. Therefore, the moderate calorie-restricted cod-based experimental diet was found as a useful strategy to lose weight together with the improvement

on oxidative stress markers in adults with excess in body weight. The low fat content and the protein characteristics of this seafood seems to be major factors involved in these observations, although more studies are needed to further elucidate whether other fish components could actively protect against free radical damage.

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