

Healthy effect of different proportions of marine ω -3 PUFAs EPA and DHA supplementation in Wistar rats: lipidomic biomarkers of oxidative stress and inflammation

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Abstract

Dietary intervention with ω -3 marine fatty acids may potentially modulate inflammation and oxidative stress markers related with CVD, metabolic syndrome, and cancer. The aim of this study was to evaluate whether different proportions of ω -3 EPA and DHA intake provoke a modulation of the production of lipid mediators and then, an influence on different indexes of inflammation and oxidative stress in a controlled dietary animal experiment using Wistar rats. For such scope, a lipidomic SPE-LC-ESI-MS/MS approach previously developed was applied to determine lipid mediators profile in plasma samples. The effect of ω -3 fatty acids associated to different ratios EPA:DHA was compared with the effect exerted by ω -3 ALA supplementation from linseed oil and ω -6 LA from soybean oil. CRP showed a tendency to greater inflammatory status in all ω -3 fed animals. Interestingly, ratios 1:1 and 2:1 EPA:DHA evidenced a noteworthy healthy effect generating a less oxidative environment and modulating LOX and COX activities towards a decrease in the production of pro-inflammatory ARA eicosanoids and oxidative stress biomarkers from EPA and DHA. In addition, the ability of 1:1 and 2:1 fish oil diets to reduce lipid mediator levels was in concurrence with the protective effect exerted by decreasing inflammatory markers as ω -6/ ω -3 ratio in plasma and membranes. It was also highlighted the effect of a higher DHA amount in the diet reducing the healthy benefits described in terms of inflammation and oxidative stress. Results support the anti-inflammatory and anti-oxidative role of fish oils, and particularly the effect of adequate proportions EPA:DHA.

Keywords

Lipid mediators; fish oil; EPA; DHA; oxidative stress; inflammation.

Abbreviations

ALA (linolenic acid), ARA (arachidonic acid), CAT (catalase), COX (cyclooxygenase), CRP (C-reactive protein), CVD (cardiovascular disease), DHA (docosahexaenoic acid), DPA (docosapentaenoic acid), EPA (eicosapentaenoic acid), FA (fatty acid), FFA (free fatty acid), GPX (glutathione peroxidase), HbA1c (Glycated hemoglobin), HDL (high-density lipoprotein), LA (linoleic acid), LDL (low-density lipoprotein), LOX (lipooxygenase), MUFA (monosaturated fatty acid), ORAC (oxygen radical absorbance capacity), PUFA (polyunsaturated fatty acid), ROS (reactive oxygen specie), SFA

(saturated fatty acid), SOD (superoxide dismutase), TC (total cholesterol), TG (triglyceride), TFA (total fatty acid).

1. Introduction

Oxidative stress is known to trigger oxidative damage of cellular biomolecules (i.e., lipids, proteins, DNA) and has been linked to the development of inflammation and metabolic diseases [1]. Moreover, exhaustion or lack of activation of necessary inflammatory resolution mechanisms could result in subsequent organ damage, obesity, metabolic syndrome, diabetes, CVD or even cancer [2,3]. Anti-inflammatory and pro-inflammatory targets can alter hormonal signaling cascades to the modulation of innate immune system, via toll-like receptors and gene transcription factors resulting in the inhibition or activation of inflammatory pathways [2]. Accordingly, one of the most selective markers of cellular inflammation is the blood ratio ω -6/ ω -3, the higher ratio the greater pro-inflammatory conditions [4]. In addition, fatty acid desaturases (FAD) are important regulators of the endogenous metabolism of ω -6 and ω -3 PUFAs, and also regulate the desaturation of SFAs to corresponding MUFAs. High FADs activity related with the formation of oleic, palmitoleic, and arachidonic acids has been associated with obesity, hypertriglycerolemia, metabolic syndrome, and the risk of developing insulin resistance. In contrast, FADs associated with ω -3 EPA and DHA pathways indicate insulin sensitivity, and the decrease of metabolic syndrome and CVD [5].

Moreover, ω -6 and ω -3 PUFAs are the main substrate of COXs, LOXs and CYP450 which produce a wide range of oxygenated lipid mediators that have been suggested as signaling molecules that may influence inflammation in a highly coordinated active process [6,7]. Specific examples include ARA eicosanoids (PGs, TXs, and LTs) [6] and IsoPs [8] which have been suggested as strong pro-inflammatory substances. The most studied: PGE₂, PGF_{1 α} , PGF_{2 α} , TXA₂ and 8isoPGF_{2 α} , have been associated with inflammatory stages in studies related to hypercholesterolemia, liver cirrhosis, myocardial reperfusion, type-2 diabetes, obesity, atherosclerosis, cancer, and CVD [2,3,9,10]. On the other hand, many EPA and DHA derivatives have been suggested as signaling molecules and less harmful compounds than the corresponding ω -6 metabolites [11,12]. Finally, the strong anti-inflammatory and cellular protective activity of EPA and DHA resolvins and protectins has been described in studies related to CVD, dry eyes or Alzheimer [13,14]. The formation cascade of ARA, EPA and DHA metabolites is shown in the Fig. 1.

Dietary interventions have shown to modulate the specific markers of cellular inflammation and oxidative stress. Regular consumption of marine ω -3 PUFAs, principally EPA and DHA, has been associated with the reduction of CVD risk factors like plasma TG, LDL, and platelet aggregation, and with the improvement of endothelial functions, and prevention of inflammatory pathways [15]. Brahmabhatt et al. have found protective effects over the intestinal inflammation of rats fed EPA:DHA (3:2) diet. The reduction of the intestinal injury was associated with the decline of oxidative stress, the up-regulation of 8isoPGF_{3 α} (anti-inflammatory marker derived from EPA), and down-regulation of ARA pro-inflammatory metabolites (i.e. PGE₂, LTB₄, PGD₂, Trioxilin A₃ and B₃) [16]. In a similar way, McDaniel et al. have suggested that EPA:DHA (1.3:1) supplements may alter inflammation indexes in human plasma and blister fluids. They found a decrease of ω -6/ ω -3 ratio and ARA pro-inflammatory metabolites (i.e., LTB₄, 15HETE, TXB₂) in a microenvironment of acute

human wounds; meanwhile an increase of EPA anti-inflammatory derivatives (i.e., 5-12-15HEPE, PGD₃, PGE₃, TXB₃) and 18HEPE, precursor of anti-inflammatory RvE₁ [17]. Similar results have been achieved by Neilson et al. in dietary experiments in rats [18].

This investigation was aimed to delve deeper into the potential anti-inflammatory and anti-oxidant effects of ω -3 marine fatty acids in a controlled fed animal experiment in Wistar rats. Despite the growing evidence of health benefits associated to marine ω -3 lipids, there is no agreement about a recommended daily intake in the form of DHA and EPA [19]. Therefore, we evaluated the influence that marine oils having different proportions of EPA and DHA exerted on different lipid biomarkers of oxidative stress and inflammation. Wistar female rats were fed standard diets enriched with fish oil containing three different EPA:DHA ratios (1:1, 2:1, and 1:2), and were compared with animals fed soybean and linseed oils. Soybean oil is a rich source of the ω -6 LA, the dietary precursor of ARA biosynthesis; whereas, linseed oil has an elevated content of ω -3 ALA, which slightly derives to EPA and DHA. The synthesis of ω -3 and ω -6 eicosanoid and docosanoids, the regulation of plasma and membrane FA composition, and the FADs activity were correlated with biochemical measurements and parameters of oxidative stress and inflammation (i.e. ORAC, antioxidant enzymes activity, CRP and protein carbonylation level in plasma, kidney, muscle, and liver).

2. Materials and Methods

2.1. Animals and diets

Thirteen-week old female Wistar (n=35, Janvier, France) rats were used as animal models. Body weight was recorded on arrival and weekly thereafter (data not shown). All the specimens were kept in an isolated room with a constantly regulated temperature and controlled humidity (22±2 °C, 50±10 % humidity) on a 12h light/dark cycle with *ad libitum* access to water and standard pelleted A04 chow for rodents (16% protein, 60% carbohydrate, 3% fat, 4% fiber and 5% ash; Harlan Iberica, Barcelona, Spain). Animals were randomized in five dietary groups: soybean (n=7), linseed (n=7), EPA:DHA 1:1 (n=7), EPA:DHA 2:1 (n=7), and EPA:DHA 1:2 group (n=7). Each group was fed with a single weekly dose of 0,8 ml/kg of the assigned oil supplement as listed in Supplementary Material S1. Oil supplements were prepared as previously described [20]. All diets had a similar fat and energy content. The molar percentage of SFA, MUFA and PUFA was kept constant. Nonetheless, they significantly differed in the proportion of individual fatty acids. Because PUFA are easily oxidized and the oxidation products are potentially toxic, the peroxide value of the oils administered was checked periodically by the ferric thiocyanate method [21]. It was below 5 meq O₂/Kg of oil throughout the interventional study. After a 2-week adaptation period, the oils were administered for 13 weeks and the experiment ended when the rats were 28 weeks old. Then, the rats were fasted overnight, anesthetized and killed by exsanguinations. Handling and killing of the animals were in full accordance with the European Union guidelines for the care and management of laboratory animals and the pertinent permission was obtained from the CSIC Subcommittee of Bioethical Issues (ref. AGL2009-12 374-C03-03).

2.2. Fatty acid analysis of the oil supplements

To determine the fatty acid composition of the oil supplements, 0.6 mg of lipid were methylated according to Lepage and Roy [22]. The fatty acid nonadecanoic acid was

used as an internal standard. The FAMES were analyzed by gas chromatography GC-FID. Results are shown in Supplementary Material S1.

2.3. Plasma and erythrocyte sampling for FA analysis

Blood samples were collected via cardiac puncture into polypropylene tubes containing EDTA (1 mg/ml) to remove erythrocytes, and centrifuged for 15 min, at 4 °C and 850 g. After that, the buffy coat was removed and the packed erythrocyte cells were washed according to the protocol developed by Sonenberg [23]. Erythrocytes were collected into clean polypropylene tubes, frozen and kept at -80 °C until required. Then, erythrocyte free plasma was supplemented with 5 mM PMSF (protease inhibitor) and samples were immediately stored at -80 °C until required.

2.4. Extraction and analysis of plasma TFA and FFA

Plasma samples for the analysis of TFA (30 µL) and FFA (100 µL) were first spiked with an internal standard of nonadecanoic acid (Larodan Fine Chemicals, Malmö-Sweden), and then, extracted with a dichloromethane:methanol:water mixture (2:2:1, v/v) according to Bligh and Dyer [24]. TFA were directly analyzed in the organic phase after dryness under a stream of nitrogen gas. Then, samples were transesterified and analyzed by gas chromatography (GC-FID, Clarus 500, Perkin–Elmer) following the method of Lepage and Roy [22]. To isolate the FFA fraction, the resulting lipid mixture obtained from the Bligh and Dyer extraction was subjected to SPE on aminopropyl cartridges (500mg, 6mL, Biotage, Uppsala-Sweden) as Kaluzny et al. described [25]. The solvent was removed under a stream of nitrogen and then subjected to transesterification and GC-FID analysis. Results are shown in Tab. 1.

2.5. Extraction and analysis of FA from erythrocyte membranes

To study the profile of FA from the erythrocyte membranes, lyophilized erythrocyte samples (50 mg) were extracted with the Bligh and Dyer modified procedure [26]. The organic layer was dried under nitrogen and the lipid content was gravimetrically quantified. Finally, an aliquot of organic phase containing 0.15 mg of lipids was dried, the internal standard was spiked and transesterification and GC analysis were done as before indicated to plasma FA (Tab. 1).

2.6. Fatty acid desaturase (FAD) indexes measurement

Desaturase activities of dietary supplemented groups were measured using a validated methodology from the TFA data [27]. FAD indexes were calculated as product/precursor ratio for: SCD (SCD-16 or SCD-18) = [palmitoleic (16:1 ω-7)/palmitic (16:0)] or [oleic (18:1 ω-9)/stearic (18:0)]; Δ5D = [ARA (20:4 ω-6)/DGLA (20:3 ω-6)] and [DHA (22:6 ω-3)/DPA (22:5 ω-3)]; Δ6D = [DGLA (20:3 ω-6)/LA (18:2 ω-6)]; and Δ5/6D = [EPA (20:5 ω-3)/ALA (18:3 ω-3)]. Results are shown in Tab. 2.

2.7. Solid-phase Extraction method for lipid metabolites isolation from plasma samples

ARA, EPA and DHA oxidized lipid mediators were extracted from plasma samples by SPE following the method previously developed [28]. Briefly, samples (90 µL) were diluted with 30% cold methanol (v/v), to a final volume of 1 mL and spiked with the internal standard 11HETE-d₈. After centrifugation (1800g, 10 min, 4° C), samples were loaded into conditioned Oasis-HLB cartridges (60mg, 3mL, Waters, MA-USA). Then, cartridges were washed with 5mL 15% methanol (v/v), 5 mL Milli-Q water and 2.5 mL hexane in succession. After sorbent dryness, analytes were eluted with 2 mL methyl formate. Extracts were evaporated to dryness, re-dissolved in 30 µL ethanol, and

analyzed by LC-ESI-MS/MS. Standard solutions of lipid mediators were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Methanol and Water, Optima LC-MS, were purchased from Fisher Scientific (New Jersey, USA).

2.8. Analysis of lipid metabolites by LC-ESI-MS/MS

Lipid mediators were quantified according to the methodology previously developed [28] and results are shown in Tab. 3. Briefly, analyses of SPE extracts were carried out on an Agilent 1260 Series (Agilent, Palo Alto, CA) coupled to a linear ion trap mass spectrometer LTQ Velos Pro with ESI (Thermo Fisher, Rockford, IL, USA). A Waters C18-Symmetry column, 150×2.1 mm, 3.5 μm (Milford, MA, USA) protected with a 4×2 mm C18 guard cartridge provided by Phenomenex (Torrance, CA, USA) was used. A binary eluent system of water (A) and methanol (B), both with 0.02% (v/v) of formic acid, was used as mobile phase. The injection volume was set to 10 μL, ESI source operated in negative ion mode, and MS/MS conditions has been reported in detail elsewhere [28]. The quantification of target compounds was made using the most intense, or selective, ion in their product ion scan MS/MS spectra. Retention times for target compounds and individual MS/MS parameters are summarized in Supplementary Material S2.

2.9. Statistical analysis

Data presented are expressed as mean ± SD. Statistical analyses were performed by one-way analysis of variance (ANOVA) with R free software (version 386 3.1.0). The means were further compared by the post-hoc test Fisher least square difference (Fisher LSD) and significant differences were set at $p < 0.05$. Pearson test was used in order to determine the statistical correlation between compounds and PUFAs intake.

3. Results

The effect of dietary interventions on eicosanoid and docosanoids synthesis, plasma and membrane fatty acid composition, and desaturases activity is described. Data obtained were correlated with previous parameters determined in the same animals and summarized in Tab. 2 [20,29]. In detail, different CVD related parameters were measured: TFA, TG, TC, HDL, LDL, HbA1c. General inflammation was measured by the concentration of CRP. Oxidative stress of animals was determined through the plasma antioxidant capacity, activity of major erythrocyte antioxidant enzymes (SOD, CAT, GPX), and by carbonylation of proteins of liver, muscle, kidney and plasma.

3.1. Effect of dietary interventions on plasma FFA profile, and FA incorporation into tissues. Ratio ω -6/ ω -3.

It has been observed that animals fed with enriched ω -3 PUFAs (fish and linseed oils) exhibited significant higher levels of plasma ω -3 FFA compared to those fed with the control ω -6 soybean diet. Meanwhile, the content in plasma ω -6 FFA was not modified by dietary intervention; therefore, the ratio ω -6/ ω -3 became lower with all ω -3 supplements and higher by soybean treatment (Tab. 1). In addition, it is important to highlight the different fatty acid species that contributed to this ω -3 enhancement. The content of EPA and DHA increased in the three fish oil diets (without significant differences between them) when compared with soybean group. Whereas, animals fed linseed diet enhanced the content of ω -3 ALA when compared with soybean animals. Data were in agreement with the elevated content of EPA and DHA in fish supplements,

while ALA was the main ω -3 fatty acid in the linseed diet. The profile level of individual PUFAs showed the same tendency between the five groups: LA \gg ARA > DHA > ALA > EPA (Supplementary Material S3).

The PUFAs incorporation from dietary intake into tissues is reflected in the erythrocyte membrane fatty acid composition [30]. Experimental groups under fish oil supplementation showed a significant enrichment of EPA, DPA, and DHA in the membranes, and thus, they increased the total ω -3 composition when compared with control ω -6 and ω -3 linseed group. Moreover, fish diets revealed the lowest ARA incorporation and lowest total ω -6 levels into tissues. Therefore, the inflammatory index ω -6/ ω -3 was significantly reduced by fish oil diets (without differences between 1:1, 2:1 and 1:2 groups) in comparison with linseed and soybean treatments. In addition, modulation of PUFA ratios in erythrocyte membrane by linseed supplementation was more moderated than fish diets. EPA and DPA also exhibited a significant enrichment by linseed supplementation when compared with soybean group, whereas DHA was poorly incorporated in linseed diet and it remained in the same level as soybean fed animals, significantly lower than fish ones. The ARA content was slightly reduced by linseed diet, and interestingly, the main ω -3 PUFA of linseed supplement, ALA, was not significantly incorporated into the erythrocyte membranes. As a consequence, the ω -6/ ω -3 ratio of linseed fed animals showed a highest value compared with fish oil groups. Results are shown in Tab. 1, and the comparison of the FA erythrocyte membrane profiles between the five groups is shown in the Supplementary Material S4: ARA \gg LA > DHA > EPA > ALA.

3.2. Effect of dietary interventions on FAD activity

Results outlined that the studied supplements did not exert a different influence on SCD-16 [palmitoleic/palmitic], SCD-18 [oleic/stearic] and Δ 6D [DGLA/LA] activities. Nevertheless, ω -3 intake from fish and linseed diets was effective in down regulating Δ 5D-(ARA/DGLA) activity when compared with soybean diet. Results highlighted the ability of fish EPA and DHA intake in harnessing Δ 5/6D [EPA/ALA] and Δ 5D [DHA/DPA] activities in comparison with linseed and soybean groups. No differences were found between diets with different EPA:DHA ratios (Tab. 2).

3.3. Determination of lipid mediators in plasma depending on the ingested amount of ARA, EPA and DHA

Plasma collected from dietary groups was analyzed to determine a wide range of lipid mediators from the family of prostaglandins, leukotrienes, thromboxanes, isoprostanes, resolvins, protectins, hydroxy and hydroperoxy acids. The correlation between the weekly dose of ARA, EPA and DHA given to rats and the further production of eicosanoids and docosanoids was also statistically analyzed.

Several hydroxy and hydroperoxy derivatives, thromboxane and prostaglandin metabolites from ARA, EPA and DHA have been identified and levels are shown in Tab. 3. In detail, five EPA eicosanoids from the family of hydroxides (15HEPE and 12HEPE), hydroperoxides (15HpEPE and 12HpEPE), and a thromboxane (TXB₃) have been identified. From DHA, the 17HDoHE (hydroxide) and 17HpDoHE (hydroperoxide) were identified. Finally, a hydroxide and a prostaglandin derived from ARA, 11HETE and PGE₂ respectively, have also been found in plasma samples from dietary interventions. After quantification and statistic comparison between groups, data revealed that dietary interventions with 1:1 and 2:1 EPA:DHA fish oils generally

decreased levels of lipid mediators when compared with 1:2 fish oil, linseed and soybean groups.

The general comparison between dietary groups showed that levels of hydroxides and hydroperoxides from EPA and DHA were lower by 1:1 and 2:1 fish diets, while higher by 1:2 fish, linseed and soybean treatments. A similar trend was observed for ARA strong-inflammatory derivatives. According to precursor PUFAs, they followed an opposite tendency in comparison with their derivatives: ARA, EPA and DHA levels were higher in 1:1 and 2:1 animals. Particularly, TXB₃ was only observed in linseed group and isoprostanes, leukotrienes, resolvins, and protectins were not detected. Interestingly, these results suggest that dietary 1:1 and 2:1 fish oil interventions were the best EPA:DHA proportions in order to reduce the production of lipid derived hydroxides and hydroperoxides and further strong pro-inflammatory substances.

In detail, levels of 15 and 12HEPEs (EPA hydroxides) significantly increased in linseed diets compared with soybean, 1:1, and 2:1 groups. Animals fed with 1:2 fish diet produced similar average levels as linseed group, but not statistically different when compared with other diets. In addition levels of 15 and 12HpEPE (EPA hydroperoxides) decreased in 1:1 and 2:1 fish oil supplementations compared with 1:2 and linseed groups, while soybean diet produced intermediate levels. A similar pattern was found for docosanoids derived from DHA: the level of 17HpDoHE was found to be higher in 1:2, soybean and linseed groups and lower in 1:1 fish diet; and the level of 17HDoHE significantly increased in linseed group compared with 1:1, 2:1 and soybean diets. Levels of 17HDoHE produced in 1:2 diet were similar to linseed one, but not statistically different when compared with other diets.

Moreover, the strong pro-inflammatory lipid mediators derived from ARA, 11HETE and PGE₂, were significantly less-produced by dietary 1:1 and 2:1 interventions while linseed diet produced the highest concentrations for these compounds. Control soybean diet showed intermediate values but significantly higher than 1:1 fish oil treatment for 11HETE. Diet 1:2 produced similar values as linseed one, higher than other diets but not statistically different.

In addition, the analysis of the ω -3 precursors, EPA and DHA, showed the highest levels by 1:1 and 2:1 fish diets when compared with 1:2, linseed and soybean groups. Accordingly, the level of ω -6 ARA showed the same tendency as EPA and DHA, although only the comparison between 2:1, 1:2 and linseed groups was statistically different.

Finally, TXB₃ was found in all animals (n=7) fed with linseed supplement but it was barely identified in the rest of the groups (n=1-3). Other searched compounds as 5HEPE, 8iso-PGF_{3 α} , 8iso-PGF_{2 α} , PGD₃, PGE₃, 11HDoHE, 4HDoHE, RvD₁, PD_x, and LTB₄ were not produced by any of the diets or were under the detection limits of the method.

Interestingly, the correlation analysis between the levels of lipid mediators in plasma and the dietary intakes of EPA and DHA, showed that the intake of 20C fatty acids (EPA and ARA) exerted a different effect over the production of metabolites when compared with 22C DHA. In detail, an increase in the ingested amount of EPA or ARA produced an enhancement in plasma levels of ARA, EPA and DHA (positive correlation

indexes over 88-97%), however it produced a decrease in hydroperoxide levels (primary oxidation products) with negative correlation indexes over 87-96%, and a tendency was observed towards the decrease of secondary metabolites production (hydroxides, prostaglandins), but not statistically correlated. On the other hand, an increase in the ingested amount of DHA was weakly correlated with a higher level of ARA, EPA and DHA in plasma and a lower level of hydroperoxides. Moreover, DHA intake produced an inversion in the tendency of secondary metabolites production compared with EPA and ARA intakes towards an enhancement. Calculated Pearson indexes are shown in Tab. 4. Therefore, results suggested a decrease of the oxidation of ARA, EPA, and DHA and thus, a minor production of their metabolites when the dietary intake of EPA is higher; meanwhile, the increase of DHA in the diet reduced this antioxidant effect and even enhanced the production of secondary oxidative metabolites. Interestingly, these findings are in agreement with the stronger antioxidant effect exerted by 1:1 EPA:DHA and 2:1 EPA:DHA diets when compared with 1:2 EPA:DHA as it was previously described [20].

4. Discussion

Data of this study bring evidence that the consumption of EPA/DHA provokes a more anti-inflammatory than pro-inflammatory effect in animals fed with standard diets. In particular, fish oil supplements with 1:1 and 2:1 EPA:DHA proportions demonstrated to be the most effective treatments to produce an anti-inflammatory response compared with 1:2 EPA:DHA, linseed and soybean supplements. Such affirmation is based on the identification and quantitation of several lipid local mediators. In detail, ARA ω -6 eicosanoids like PGE₂ and 11HETE, have been widely studied and identified as a key pro-inflammatory signaling molecules related with pro-aggregating, vasoconstrictive and immunosuppressive processes [9]; and they have also been associated with promotion of different human cancers including colon, lung, breast, head or neck [3]. On the other hand, derived eicosanoid and docosanoids from EPA and DHA have been related with protective effects on inflammation diseases [16], the regulation of tumor factors [18], or the reduction of CVD risk [10]. However, hydroxides and hydroperoxides derived from EPA and DHA were also considered as biomarkers of oxidative stress and weaker inflammatory substances than the corresponding ARA derivatives [2,31]. In the same way, TXB₃ derived from EPA is related with the inflammatory response, and a weaker pro-inflammatory product than corresponding series-2 thromboxanes from ARA [17].

One of the mechanisms by which EPA and DHA are thought to act is through substrate competition with ARA for enzymes that generate several inflammatory mediators [2,10]. As a consequence, when increasing amounts of ω -3 substrate are included in the diet, LOXs and COXs would preferably regulate the production of ω -3 derivatives instead of ω -6 ones. Therefore, strong-inflammation targets derived from ω -6 PUFAs would be less produced and replaced by less harmful ω -3 eicosanoids. Accordingly, it was observed a significant reduction of related pro-inflammatory ω -6 eicosanoids after 1:1 and 2:1 diets compared with other groups; although levels of searched ω -3 were not equally enhanced. On the other hand, 1:2 diet showed similar results as soybean (source of ω -6 LA), and linseed (source of ω -3 ALA). Therefore, not only a substrate competition for LOX and COX but other mechanisms may be implicated in the minor anti-inflammatory response observed in 1:2, linseed and soybean fed animals.

It is known that LOXs regulate the synthesis of hydroperoxides (HpEPEs and HpDoHEs), which are further reduced into hydroxides (HEPEs and HDoHEs) through GPX activity. In addition, COXs regulate the synthesis of strong pro-inflammatory ω -6 eicosanoids like 11HETE and PGE₂, and less harmful ω -3 ones like TXB₃ [6]. Soybean, linseed and 1:2 groups showed significant lower GPX activities and higher hydroperoxides levels than 1:1 and 2:1 ones. Therefore, the down-regulation of GPX in soybean, linseed and 1:2 groups may be correlated with a decrease of hydroperoxides detoxification and consequently, with the highest values found for 12HpEPE, 15HpEPE, and 17HpDoHE. Moreover, a decrease of LOX activity after 1:1 and 2:1 interventions could explain the lowest hydroxide levels found in these diets despite the higher GPX activity observed. A similar trend was observed for COX products: soybean, linseed and 1:2 groups showed higher values than 1:1 and 2:1 ones. Specifically, TXB₃ was preferably found after linseed treatments; and thus, it seemed that this diet had a direct influence on the pathway of this eicosanoid. In concordance with these findings, the statistical correlation analysis between weekly dose of EPA and DHA in the diet and the production of lipid metabolites showed that the increase of DHA amount in the diet lead to higher eicosanoid and docosanoids production. Meanwhile, the increase of EPA amount in the diet lead to lower metabolites production as it was observed when 1:2 EPA:DHA supplement was compared with 2:1 one. Therefore, in addition to the substrate competition for LOXs and COXs, it seemed that specific PUFAs profile of the diet may modulate enzymes activity towards different eicosanoid and docosanoids production.

In addition, not only enzymatic pathways but free radical oxidation mechanisms initiated by ROS also lead to the formation of described eicosanoid and docosanoids [32]. Accordingly, the susceptibility of fatty acids to oxidation is thought to be directly dependent on their degree of unsaturation, and subsequently, supplementations with highly unsaturated ω -3 PUFA have been reported to increase oxidative damage [30]. Therefore, the higher unsaturation level of DHA may increase the susceptibility of the molecule to be oxidized compared to EPA, rendering to a higher level of free radicals. Then, the supplement with higher DHA proportion (1:2) would produce a higher concentration of ROS and be more prone to suffer free radical oxidation processes than supplements with higher EPA or balanced ratios.

Richard et al. investigated the free radical-scavenging potential of ω -3 and ω -6 supplements in cell cultures studies [33]. They observed that supplemented cells with ω -3 PUFAs produced lower amounts of ROS than cells fed with ω -6 ARA and LA despite the lower unsaturation level of the molecules. It was shown that ω -6 series were more susceptible to oxidize than ω -3 ones. Therefore, it was suggested that the susceptibility to oxidation of PUFA molecules is not as straightforward as hypothesized. Not only the lower degree of unsaturation leads to higher antioxidant activity, but the chemical structure may play an important role in the different antioxidant aptitude. Regarding to our results, ω -3 ALA from linseed and ω -6 LA from soybean seemed to be easier oxidized than ω -3 EPA and DHA in mixtures 1:1 and 2:1, but the same results were found when compared with 1:2 mixture. In addition, the higher oxidizability suggested for soybean, linseed and 1:2 supplements was in concurrence with the lower PUFA levels found in plasma. Accordingly, PUFAs would be oxidized into the corresponding eicosanoid and docosanoids due to free radical reactions and enzymes pathways. The opposite trend was achieved for 1:1 and 2:1 where PUFA levels were higher and lipid derivates lower than the other diets. Other studies have also described the activation of

the antioxidant response after ω -3 EPA and DHA supplementation: enhance of SOD [34], and CAT activities [35], and genes related to endogenous antioxidant system [36]; although the different aptitude of EPA vs DHA have not been described yet.

As a conclusion for eicosanoid and docosanoids profiles, 1:1 and 2:1 interventions were the most effective treatments to reduce the synthesis of inflammatory lipid mediators. Different mechanisms were thought to modulate the activity of crucial enzymes involved in the generation of these compounds. In detail, it was suggested a competition for the same enzymes between substrates, and the different oxidizability between PUFAs due to their chemical structure and unsaturation degree. As a result, enriched diets with ALA, LA or 1:2 EPA:DHA exhibited lower anti-oxidative and anti-inflammatory properties than 1:1 and 2:1 EPA:DHA.

In addition to the lipidomic analysis, the influence of dietary supplements on metabolic parameters like FAD, and FFA in plasma and tissues was measured. Results were finally correlated with previous data about inflammation, biochemical measurements, and oxidative stress. Fatty acid desaturases are one of the main targets for the treatment of metabolic related disorders, and the rate-limiting step in the biosynthesis of different PUFAs [5]. According to their activities, diets enriched with ω -3 PUFAs from fish and linseed provoked a down-regulation of FAD involved in the transformation of LA into ARA. Therefore, intervention on the diet with ω -3 PUFAs seemed to reduce ω -6 ARA levels, the main substrate of pro-inflammatory ω -6 eicosanoids. Interestingly, the high intake of ALA due to linseed diet did not address an increase of FADs activity involved in the synthesis of EPA and DHA from ALA. This finding is in agreement with Fu et al. who demonstrated ALA to be more prone to β -oxidation or excretion rather than elongate to EPA and DHA [37]. Our results suggested that linseed supplements were not effective to potentiate EPA and DHA endogenous synthesis. Therefore, some benefits directly attributed to fish PUFAs like production of specific anti-inflammatory metabolites could not be obtained by vegetal ω -3 supplements.

A huge number of lipid species in plasma are triacylglycerol, glycerophospholipids and cholesterol esters. Increasing levels of these substances have been associated with chronic inflammation and CVD conditions [38]. In addition, a small fraction of total lipids, the FFA released from adipose tissue; are thought to exert relevant functions as signaling molecules participating in inflammation processes and oxidative stress [39]. Results showed that ω -6 PUFA levels in plasma remained unaltered with the different dietary interventions; however, ω -3 EPA and DHA increased after fish oil diets and ω -3 ALA after linseed one as expected. The capacity of fish oil interventions to enrich the plasma FFA in DHA and EPA, and the anti-inflammatory effect attributed was already investigated [40]. As a consequence, animals fed with fish and linseed oils exhibited significantly lower ω -6/ ω -3 plasma ratios than the soybean group. It has been reported that the higher ω -6/ ω -3 ratio the greater pro-inflammatory conditions [4,17]. Furthermore, fish supplements were also effective to increase EPA and DHA incorporation into erythrocyte membranes, meanwhile ARA incorporation was reduced. Results were in agreement with those obtained by Massaro et al. [10]. Therefore, ω -6/ ω -3 ratios in tissues were also reduced due to fish oil diets. Nevertheless, although linseed fed animals showed similar ω -6/ ω -3 plasma ratios than fish groups, linseed diet was not effective to incorporate ALA into tissues. And thus, membrane ω -6/ ω -3 ratios showed lower values than obtained after fish oils.

The decrease of the inflammation index ω -6/ ω -3 due to ω -3 supplements was in concordance with the tendency to decrease CRP marker under fish and linseed diets observed in previous studies in the same animals [29]. Lower values of CRP indicate a reduction in general inflammation conditions linked to CVD [41]. Although CRP is regarded a potential inflammatory index, additional biomarkers like IL-6, IL-1 or TNF α would be desirable to strongly conclude the differences in the inflammatory status of dietary groups [42]. In any case, the general anti-inflammatory effect described by lipidomic and CRP data after ω -3 fish diets may be also correlated with the general decrease observed in other parameters like TFA, TG, TC, LDL, and HbA1c when compared with soybean group [29]. Results for EPA:DHA interventions are in agreement with other studies that have suggested a protective role of fish PUFAs, essentially by improving the lipid profile and cardiovascular indexes [43], and with Reinders et al. who reported CRP levels to be inversely correlated to circulating ω -3 PUFA concentrations [44]. To finish, the ability of fish diets to ameliorate general inflammatory conditions, specially 1:1 and 2:1 EPA:DHA, was in agreement with the effectiveness to reduce ROS concentration (higher ORAC), enhance the antioxidant endogenous system (SOD, and CAT), and reduce the oxidative damage in proteins [20,29]. The general decrease of oxidative stress and inflammatory markers may be correlated with the lower production of eicosanoid and docosanoids after 1:1 and 2:1 supplements as it was discussed above.

5. Conclusion

The investigation shed light on how PUFA composition of the diet may influence the synthesis of eicosanoid and docosanoids, and evidenced that 1:1 and 2:1 EPA:DHA supplements were the most effective treatments to reduce inflammation and oxidative stress when compared with 1:2 EPA:DHA, linseed and soybean ones. It was suggested a higher anti-oxidant and anti-inflammatory capacity of EPA vs DHA, and fish ω -3 vs vegetal ω -3 and ω -6 PUFAs. It was also studied the influence of ω -6 and ω -3 supplements in FADs and thus, in the composition and ω -6/ ω -3 ratios in plasma and tissues. Results suggested greater anti-inflammatory conditions generated after ω -3 interventions. These findings were in agreement with previous results that had shown the effect of 1:1 and 2:1 diets ameliorating inflammation and activating the antioxidant response of the organism. Therefore, this research evidenced that in addition to an adequate dietary proportion between ω -6 and ω -3 PUFA, the proportion between marine ω -3 type lipids is also important and its intake could contribute towards the prevention of chronic inflammation and oxidative stress processes that have been correlated with metabolic syndrome, CVD, obesity, diabetes or even cancer [2,3]. Previous studies also supported the evidence of the protective effect of EPA and DHA intakes [16,31].

Acknowledgements

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Table captions:

Tab. 1: Plasma composition of FFA and FA of erythrocyte membranes (EM) from Wistar rats supplemented with different fish oils diets (EPA:DHA 1:1, 2:1 and 1:2), linseed oil or soybean oil. Results are expressed as percentage of total fatty acids (mg/100mg). Values are shown as means \pm SD. Values with different superscript letters in the same row indicate significant difference at $p < 0.05$ between dietary groups. 16:0 (palmitic acid), 16:1 ω 7 (palmitoleic acid, PA), 18:0 (stearic acid), 18:1 ω 9 (oleic acid, OA), 18:1 ω 7 (vaccenic acid), 18:2 ω 6 (LA), 18:3 ω 3 (ALA), 20:3 ω 6 (DGLA), 20:4 ω 6 (ARA), 20:5 ω 3 (EPA), 22:5 ω 3 (DPA), 22:6 ω 3 (DHA)

Tab. 2: Biochemical measurements (TFA, TG, TC, LDL, HDL, CRP, HbA1c), Antioxidant indexes (SOD, GPX and CAT activities, and ORAC), and FAD activity measurements obtained from the supplemented groups with different fish oils diets (EPA:DHA 1:1, 2:1 and 1:2), linseed oil or soybean oil. Values are shown as means \pm SD. Values with different superscript letters in the same row indicate significant difference at $p < 0.05$ between dietary groups.

Tab. 3: Levels of lipid mediators in plasma derived from ARA, EPA and DHA obtained from the dietary interventions. Results are expressed as ng/mL for derived eicosanoids and docosanoids, and μ g/mL for PUFAs. Values are shown as means \pm SD. For the ANOVA analysis, data from 1:2 diet have not been considered for most of compounds due to the high variability observed. Values with different superscript letters in the same row indicate significant difference at $p < 0.05$ between dietary groups.

Tab. 4: Correlation Pearson indexes between lipid mediator levels in plasma and the weekly dietary dose of ARA, EPA and DHA.

Figure captions:

Fig.1: Cascade of formation to ARA, EPA and DHA oxidized derived compounds from enzymatic and non enzymatic pathways [8,45].

Supplementary electronic material captions:

S1: Fatty acid composition of soybean oil, linseed oil and fish oil mixtures (EPA:DHA 1:1, 2:1 and 1:2) supplemented to Wistar rats. Results are expressed as a percentage of total fatty acids (mg/100mg of Total FA). Weekly dose of EPA, DHA, ALA, ARA and LA expressed as mg/kg rat. Values are shown as means \pm SD. 16:0 (palmitic acid), 16:1 ω 7 (palmitoleic acid, PA), 18:0 (stearic acid), 18:1 ω 9 (oleic acid, OA), 18:1 ω 7 (vaccenic acid), 18:2 ω 6 (LA), 18:3 ω 3 (ALA), 20:3 ω 6 (DGLA), 20:4 ω 6 (ARA), 20:5 ω 3 (EPA), 22:5 ω 3 (DPA), 22:6 ω 3 (DHA)

S2: Retention times, collision energies and MS/MS transitions for ESI-LC-LIT/MS/MS

S3: Comparison of the plasma FFA profile from Wistar rats supplemented with different fish oils diets (EPA:DHA 1:1, 2:1 and 1:2), linseed oil or soybean oil. Results are expressed as percentage of total fatty acids (mg/100mg)

S4: Comparison of the FA erythrocyte membrane profile from Wistar rats supplemented with different fish oils diets (EPA:DHA 1:1, 2:1 and 1:2), linseed oil or soybean oil. Results are expressed as percentage of total fatty acids (mg/100mg)

Table 1

FA	RATIO 1:1 (EPA:DHA)				RATIO 2:1 (EPA:DHA)				RATIO 1:2 (EPA:DHA)				Soybean				Linseed			
	FFA (Plasma)		FA (EM)		FFA (Plasma)		FA (EM)		FFA (Plasma)		FA (EM)		FFA (Plasma)		FA (EM)		FFA (Plasma)		FA (EM)	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
16:0 (palmitic)	32.72 ^a	1.1	22.61 ^a	1.5	31.69 ^a	1.4	22.55 ^a	0.6	31.63 ^a	1.1	22.58 ^a	0.7	32.12 ^a	0.8	22.48 ^a	0.8	31.03 ^a	1.1	23.76 ^a	2
16:1 ω7 (PA)	5.27 ^{ab}	0.8	0.53 ^a	0.1	5.43 ^{ab}	0.6	0.50 ^a	0.04	5.26 ^{ab}	0.6	0.60 ^a	0.1	6.09 ^a	0.5	0.63 ^a	0.1	4.86 ^b	0.6	0.56 ^a	0.1
18:0 (stearic)	10.71 ^a	2.5	14.09 ^a	1.5	9.51 ^a	3.5	15.09 ^a	0.8	8.86 ^a	1.2	15.22 ^a	0.7	9.12 ^a	0.6	14.60 ^a	0.3	8.73 ^a	1.2	12.83 ^a	2.2
18:1 ω9 (OA)	21.54 ^a	0.9	6.55 ^a	0.6	21.37 ^a	1.5	6.12 ^a	0.2	22.89 ^a	1.2	6.80 ^a	0.8	22.89 ^a	1.4	6.62 ^a	0.4	23.84 ^a	1.3	6.82 ^a	0.5
18:1 ω7 (vaccenic)	2.45 ^a	0.2	2.28 ^a	0.1	2.37 ^a	0.3	2.22 ^a	0.2	2.76 ^a	0.3	2.23 ^a	0.1	2.78 ^a	0.1	2.51 ^a	0.1	2.56 ^a	0.2	2.32 ^a	0.1
18:2 ω6 (LA)	17.31 ^a	1.9	7.63 ^a	0.7	17.50 ^a	2.2	7.64 ^a	0.3	17.72 ^a	1.7	7.44 ^a	0.7	17.31 ^a	1	6.95 ^a	0.3	18.11 ^a	1.3	7.45 ^a	0.6
18:3 ω3 (ALA)	0.94 ^a	0.4	n.d.	n.d.	1.01 ^a	0.1	n.d.	n.d.	0.84 ^a	0.4	n.d.	n.d.	0.96 ^a	0.04	n.d.	n.d.	1.73 ^a	0.3	n.d.	n.d.
20:3 ω6 (DGLA)	n.d.	n.d.	0.51 ^a	0.03	n.d.	n.d.	0.53 ^a	0.04	n.d.	n.d.	0.53 ^a	0.1	n.d.	n.d.	0.47 ^a	0.01	n.d.	n.d.	0.51 ^a	0.04
20:4 ω6 (ARA)	4.10 ^a	0.1	21.51 ^{ab}	1.3	4.55 ^a	0.7	21.63 ^{ab}	1.3	4.29 ^a	1.1	21.42 ^a	0.8	4.61 ^a	0.7	23.32 ^b	0.9	4.50 ^a	1	22.72 ^{ab}	1
20:5 ω3 (EPA)	0.49 ^a	0.1	1.03 ^a	0.2	0.66 ^a	0.2	1.09 ^a	0.2	0.44 ^a	0.1	0.89 ^a	0.1	0.20 ^b	0.02	0.56 ^b	0.1	0.36 ^a	0.1	0.82 ^a	0.1
22:5 ω3 (DPA)	0.67 ^a	0.2	2.77 ^a	0.2	0.83 ^a	0.3	3.02 ^a	0.2	0.76 ^a	0.2	2.53 ^a	0.1	0.31 ^a	0.2	2.09 ^a	0.1	0.76 ^a	0.2	2.73 ^a	0.2
22:6 ω3 (DHA)	2.06 ^a	0.3	7.11 ^a	0.8	2.83 ^a	0.7	6.82 ^a	0.5	2.54 ^a	0.4	6.92 ^a	0.4	1.36 ^b	0.2	5.70 ^b	0.2	1.84 ^{ab}	0.3	5.84 ^b	0.5
Σ ω3	4.16 ^a	0.3	10.91 ^a	0.4	5.33 ^b	0.3	10.94 ^a	0.3	4.58 ^a	0.3	10.34 ^a	0.2	2.83 ^c	0.1	8.35 ^b	0.1	4.69 ^a	0.2	9.39 ^c	0.3
Σ ω6	21.41 ^a	1	29.64 ^{ab}	0.7	22.05 ^a	1.4	29.79 ^{ab}	0.5	22.01 ^a	1.4	29.39 ^a	0.5	21.92 ^a	0.9	30.74 ^b	0.4	22.61 ^a	1.2	30.68 ^b	0.5
Σ SFA	43.43 ^a	1.8	36.69 ^a	1.5	41.20 ^a	2.5	37.64 ^a	0.7	40.49 ^a	1.2	37.80 ^a	0.7	41.24 ^a	0.7	37.08 ^a	0.5	39.76 ^a	1.1	36.60 ^a	2.1
Σ MUFA	29.26 ^a	0.7	9.37 ^a	0.3	29.17 ^a	0.8	8.84 ^b	0.1	30.91 ^b	0.7	9.63 ^a	0.3	31.76 ^b	0.7	9.76 ^a	0.2	31.26 ^b	0.7	9.70 ^a	0.2
Σ PUFA	25.57 ^a	0.5	40.56 ^{ab}	0.5	27.38 ^b	0.7	40.73 ^b	0.4	26.59 ^{ab}	0.7	39.73 ^a	0.4	24.75 ^a	0.4	39.09 ^a	0.3	27.30 ^b	0.5	40.07 ^{ab}	0.4
ω6/ω3	5.15		2.72		4.14		2.72		4.81		2.84		7.75		3.68		4.82		3.27	

Table 2

Group	RATIO 1:1 (EPA:DHA)		RATIO 2:1 (EPA:DHA)		RATIO 1:2 (EPA:DHA)		Soybean		Linseed	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Biochemical measurements										
TFA (mg/dL)	267.7 ^{ab}	38.4	233.8 ^a	24.2	280.8 ^{ab}	30.3	355.6 ^c	87.7	316.9 ^{abc}	61
TG (mg/dL)	93.8 ^a	67.8	64 ^a	23.4	83.1 ^a	10.4	108.5 ^a	51.9	73.5 ^a	25.2
TC (mg/dL)	102 ^a	26.5	98 ^a	14.8	112.3 ^a	20.8	125.8 ^a	16.5	133.4 ^a	41.2
LDL (mg/dL)	8.6 ^a	3.3	7.4 ^a	0.8	8.3 ^a	1.3	10.4 ^a	2.2	10.1 ^a	7.1
HDL (mg/dL)	41.2 ^a	11.1	42.3 ^a	7.9	47.3 ^a	8.1	51.3 ^a	7.8	49.7 ^a	9.1
HDL/LDL	4.8 ^a		5.7 ^b		5.7 ^b		4.9 ^a		4.9 ^a	
CRP (ug/mL)	147 ^a	20.5	147.1 ^a	22.4	140.1 ^a	52.7	172.9 ^a	38.2	142.9 ^a	52
HbA1c (%)	4.1 ^a	0.2	4.4 ^a	0.7	4.17 ^a	0.3	6.4 ^b	1	6.3 ^b	1.8
Antioxidant indexes										
ORAC (mg/dL)	48.2 ^a	16.9	23.6 ^b	6	26.4 ^b	9	30.3 ^b	9.6	24.7 ^b	9.7
SOD (U/gHb)	2129.2 ^a	586.5	1880.9 ^{ab}	341.2	1226.1 ^b	517.4	1443.8 ^{ab}	426.2	1230.6	283.9 ^b
GPX (U/gHb)	107.1 ^a	15	116.7 ^b	30.7	67.7 ^c	34.5	101.5 ^{abc}	38.2	71.1 ^c	9.6
CAT (mmol/min/gHb)	57.4 ^a	11.1	76.7 ^a	28.2	61.4 ^a	13.2	41.5 ^a	34.6	37.2 ^a	20.9
FAD activity										
SCD-16 [palmitoleic/palmitic]	0.1		0.1		0.1		0.1		0.1	
SCD-18 [oleic/stearic]	0.9		0.9		0.8		0.9		1	
Δ 5D [ARA/DGLA]	48.4		49		45.7		68.1		48.4	
Δ 6D [DGLA/LA]	0.03		0.03		0.04		0.03		0.04	
Δ 5/6D [EPA/ALA]	3.6		4.4		4.2		2.6		2.5	
Δ 5D [DHA/DPA]	8.2		7.5		8.1		7.8		6.4	

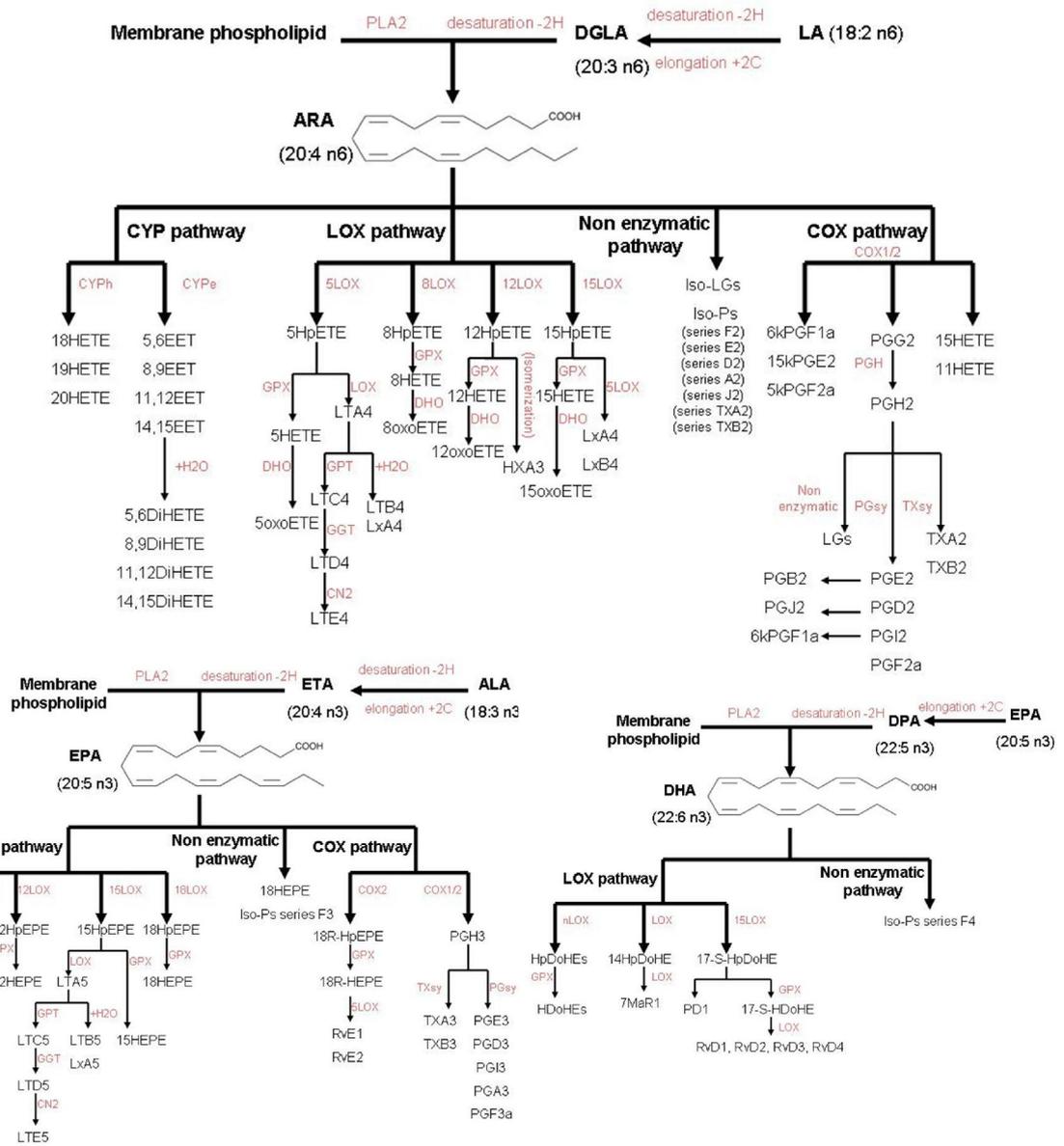
Table 3

Group	RATIO 1:1 (EPA:DHA)		RATIO 2:1 (EPA:DHA)		RATIO 1:2 (EPA:DHA)		Soybean		Linseed	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Eicosanoids from EPA (ng/mL)										
12HEPE	59 ^a	46	65 ^a	23	299	356	65 ^a	40	250 ^b	62
15HEPE	3.3 ^a	1.1	3.7 ^a	1.3	6.2	3.7	3.2 ^a	0.7	7.1 ^b	1.3
12HpEPE	387 ^a	300	411 ^a	279	1269 ^b	524	640 ^{ab}	376	1462 ^b	1043
15HpEPE	388 ^a	128	403 ^{ab}	164	898	574	691 ^{bc}	280	968 ^c	156
TXB ₃	3.3 ^a	0.004	n.d.	n.d.	4.4	1.0	3.5 ^a	0.2	4.2 ^b	0.3
Docosanoids from DHA (ng/mL)										
17HDoHE	40 ^a	20	41 ^a	11	173	175	64 ^a	56	134 ^b	23
17HpDoHE	1482 ^a	691	1596 ^a	700	1853 ^a	553	2175 ^a	475	2159 ^a	927
Eicosanoids from ARA (ng/ml)										
11HETE	3.9 ^a	2.1	4.4 ^{ab}	1.5	9.9	9.6	6.7 ^b	2.2	13.6 ^c	1.8
PGE ₂	8.2 ^a	1.8	9.9 ^a	1.4	40.1	42	18.7 ^a	12.4	38.8 ^b	8.7
PUFA precursors (ng/uL)										
EPA	2.6 ^a	1.4	2.5 ^a	0.6	1.4 ^b	0.8	1.1 ^b	0.3	1.1 ^b	0.3
DHA	13.1 ^a	5.9	12.6 ^{ab}	3.9	9.2 ^{abc}	3.5	7.4 ^{bc}	2.8	6 ^c	1.7
ARA	48 ^{ab}	8	51 ^a	9	36 ^b	12	43 ^{ab}	11	37 ^b	6

Table 4

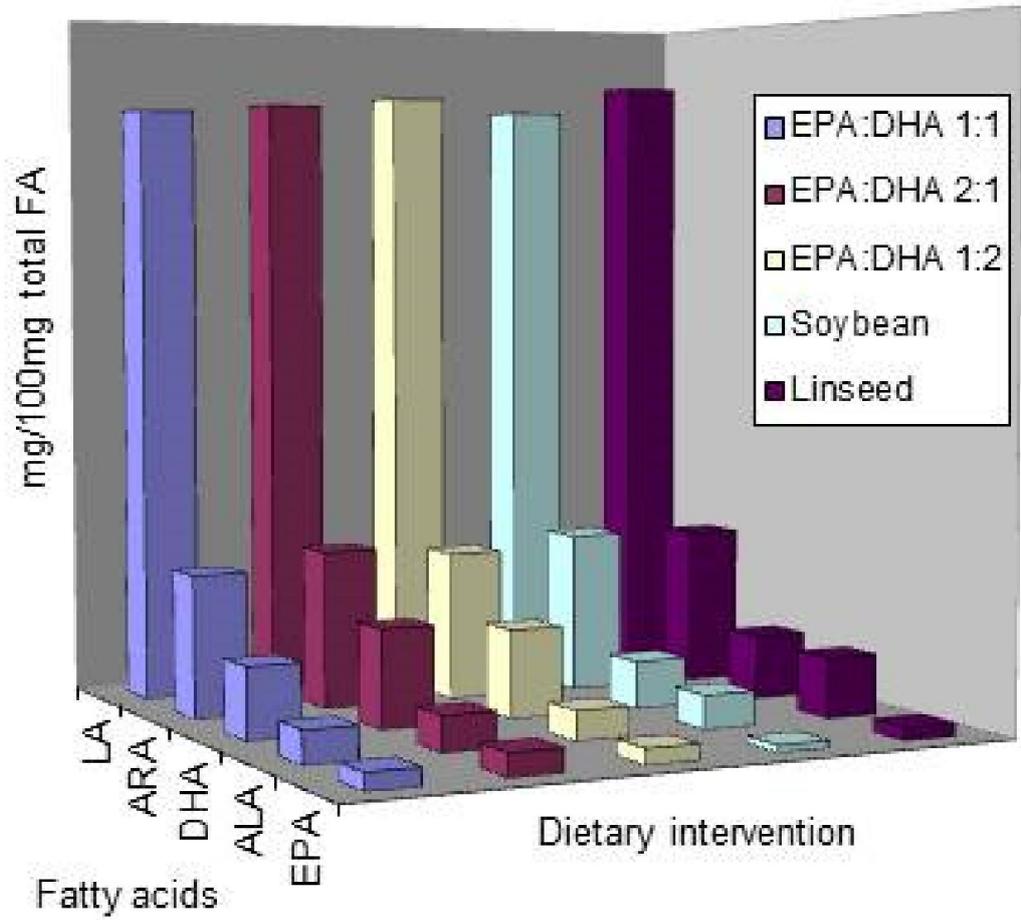
Compounds	Weekly intake of EPA	Weekly intake of DHA	Weekly intake of ARA
	<i>Correlation Pearson indexes (-1 to 1)</i>		
12HEPE	0,1781	0,6898	0,1066
15HEPE	0,0486	0,7375	-0,0169
12HpEPE	-0,9122	-0,7924	-0,9120
15HpEPE	-0,9621	-0,7272	-0,9609
TXB3	0,5295	0,2605	0,4746
17HDoHE	-0,1928	0,4941	-0,2623
17HpDoHE	-0,8662	-0,6991	-0,8820
11HETE	-0,2209	0,3144	-0,2934
PGE2	-0,4604	0,2172	-0,5247
EPA	0,9417	0,4363	0,9639
DHA	0,9519	0,5521	0,9683
ARA	0,8803	0,2146	0,9121

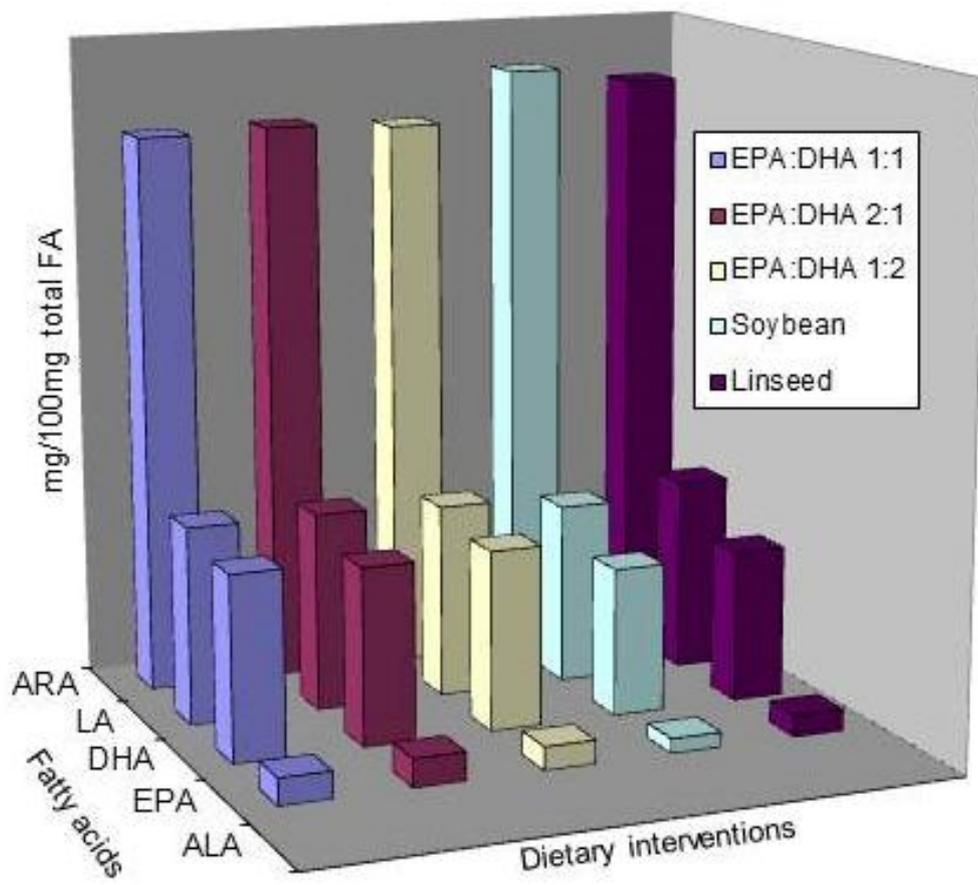
Figure 1



<i>FATTY ACID</i>	RATIO 1:1 (EPA:DHA)		RATIO 2:1 (EPA:DHA)		RATIO 1:2 (EPA:DHA)		SOYBEAN		LINSEED	
	<i>MEAN</i>	<i>SD</i>	<i>MEAN</i>	<i>SD</i>	<i>MEAN</i>	<i>SD</i>	<i>MEAN</i>	<i>SD</i>	<i>MEAN</i>	<i>SD</i>
16:0 (palmitic)	10.15	0.2	9.09	0.03	10.98	0.1	17.78	0.1	5.73	0.03
16:1 ω7 (PA)	4.99	0.04	4.57	0.02	5.39	0.03	0.90	0.03	0.11	0.01
18:0 (stearic)	2.94	0.03	2.95	0	2.97	0.01	2.07	0.01	4.75	0.02
18:1 ω9 (OA)	6.41	0.06	6.18	0	6.61	0.04	18.75	0.03	21.37	0.06
18:1 ω7 (vaccenic)	1.91	0.03	1.93	0.02	1.95	0.02	1.52	0.02	1.11	0.01
18:2 ω6 (LA)	0.65	0.01	0.61	0	0.65	0.02	47.55	0.01	16.76	0.03
20:0	0.32	0.01	0.39	0.01	0.20	0	0.00	0	0.00	0
18:3 ω3 (ALA)	0.36	0.01	0.32	0	0.33	0.02	4.00	0.04	50.02	0.1
20:1 ω9	0.98	0.03	1.39	0.01	0.63	0.02	1.43	0.09	n.d.	n.d.
18:4 ω3	1.51	0.02	1.56	0.02	1.64	0	0.15	0	n.d.	n.d.
20:2 ω6	0.21	0	0.28	0.01	0.17	0.01	0.20	0.05	n.d.	n.d.
20:3 ω6 (DGLA)	0.22	0.01	0.27	0.01	0.15	0	0.00	0	n.d.	n.d.
20:4 ω6 (ARA)	1.68	0.04	1.98	0.03	1.16	0.02	0.40	0.02	n.d.	n.d.
22:1 ω11	1.14	0.01	1.58	0.02	0.45	0.01	1.08	0	n.d.	n.d.
22:1 ω9	0.28	0.03	0.37	0.03	0.19	0.02	0.25	0.02	n.d.	n.d.
20:4 ω3	1.02	0.02	1.31	0.02	0.75	0.01	0.20	0.03	n.d.	n.d.
20:5 ω3 (EPA)	25.09	0.1	32.43	0.06	17.33	0.03	0.70	0.02	n.d.	n.d.
24:1 ω9	0.38	0	0.55	0.02	0.25	0.01	0.28	0.05	n.d.	n.d.
22:5 ω3 (DPA)	4.30	0.05	5.24	0.02	2.60	0.1	0.26	0.01	n.d.	n.d.
22:6 ω3 (DHA)	25.70	0.2	17.98	0.03	34.85	0.1	1.15	0.03	n.d.	n.d.
Total ω3 PUFA	57.97	0.07	58.84	0.03	57.51	0.05	6.47	0.02	50.02	0.1
Total ω6 PUFA	2.76	0.01	3.14	0.01	2.14	0.01	48.15	0.02	16.76	0.03
Total SFA	18.52	0.2	17.05	0.02	19.68	0.1	21.17	0.1	10.63	0.06
Total MUFA	17.22	0.1	17.14	0.07	17.43	0.1	24.21	0.1	22.59	0.06
Total PUFA	64.26	0.3	65.81	0.08	62.90	0.2	54.62	0.03	66.78	0.1
weekly dose of EPA	159.30		205.84		110.03		4.45			
weekly dose of DHA	163.12		114.15		221.24		7.31			
weekly dose of ARA	10.65		12.55		7.37		2.54			
weekly dose of ALA	2.29		2.06		2.09		25.39		317.56	
weekly dose of LA	4.13		3.88		4.15		301.86		106.39	

Compound	Retention Time (min)	LIT parameters	
		Collision energy (eV)	Quantification transition (<i>m/z</i>)
8iso-PGF_{3α}	6.79	30	351>253
TXB₃	6.97	19	367>195
PGD₃/PGE₃	7.67	19	349>313
8iso-PGF_{2α}	8.28	28	353>299
PGE₂	9.09	20	351>315
RvD₁	9.80	25	375>141
PD_x	12.89	30	359>153
LTB₄	13.79	27	335>195
15HpEPE	17.36	20	333>315
12HpEPE	17.80	25	333>315
15HEPE	17.92	27	317>219
12HEPE	18.72	27	317>179
5HEPE	20.47	25	317>255
17HpDoHE	21.90	26	359>341
17HDoHE	21.94	27	343>245
11HETE	22.09	30	319>167
12HETEd₈	22.66	30	325>307
11HDoHE	23.20	27	343>149
4HDoHE	23.64	27	343>281
EPA	24.43	27	301>257
DHA	24.93	30	327>283
ARA	25.10	30	303>259





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