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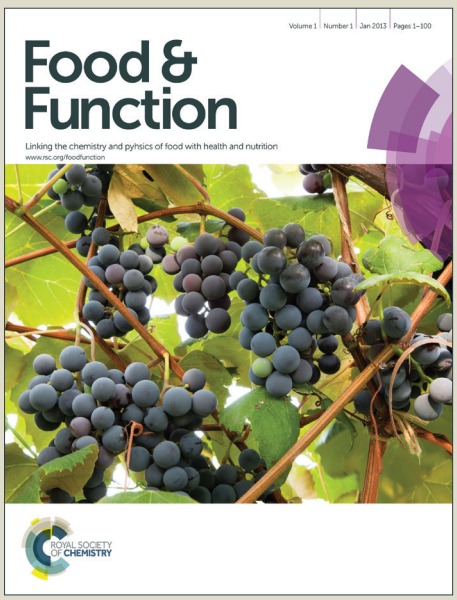

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ALPHA LINOLENIC ACID (ALA) FROM *ROSA CANINA*, *SACHA INCHI* AND *CHIA* OILS MAY INCREASE ALA ACCRETION AND ITS CONVERSION INTO N-3 LCPUFA IN DIFFERENT TISSUES OF THE RAT

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Abstract

Alpha-linolenic acid (ALA) is an essential n-3 PUFA and its n-3 LCPUFA derivatives EPA and DHA which have diverse beneficial effects are scarce in our diet. In recent years it has been developed the production of nontraditional vegetable oils rich in ALA (up to 45%) as a new alternative to increase ALA consumption. This work evaluated the accretion of ALA, EPA and DHA into the phospholipids extracted from erythrocytes, liver, kidney, small intestine, heart, quadriceps and the brain in rats fed Sunflower (SFO), Canola (CO), *Rosa canina* (RCO), *Sacha inchi* (SIO) and *Chia* (ChO) oils. Five experimental groups (n=12/group) were fed 21 days with either: a) SFO (1% ALA); b) CO (10% ALA); RCO (33% ALA); SIO (49% ALA) and ChO (64% ALA). SIO and ChO allowed higher ALA accretion in all tissues, with the exception of brain, and a reduction in the content of arachidonic acid in all tissues except brain. EPA increased in erythrocytes, liver, kidney, small intestine, heart and quadriceps, but no in the brain. DHA increased in liver, small intestine and in brain. Our results demonstrate that ALA, when provided in significant amounts, can be converted into n-3 LCPUFA, mostly DHA in the liver and brain. It is suggested that oils rich in ALA, such SIO and ChO, are good sources to obtain higher tissue levels of ALA, also allowing its selective conversion into n-3 LCPUFA in some tissues of the rat.

Keywords: alpha linolenic acid; *Rosa canina* oil; *Sacha inchi* oil; *Chia* oil; bioconversion to n-3 LCPUFA.

1. Introduction

Lipids (fats and oils) are essential for mammals, included humans[1].This essentiality was identified in the early 1930s[2] but in 1960s was established that humans could not biosynthesize two polyunsaturated fatty acid (PUFA): linoleic acid (LA, 18:2 n-6) and alpha linolenic acid (ALA,18:3 n-3), which were considered essentials[3]. LA and ALA are important for an adequate physiology in mammals[4]. These fatty acids are the precursors of the long-chain polyunsaturated fatty acid (LCPUFA) of C20 and C22. LA can be metabolically transformed into arachidonic acid (AA, 20:4 n-6) and ALA to eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). LA is frequently present in our nutrition, whereas the presence of ALA is restrictive because few foods are good sources of this n-3 PUFA[5]. EPA and DHA are found only in marine organisms and the consumption of these n-3 LCPUFA is also restrictive due the reduced consumption of marine foods especially in some western countries[5]. The low consumption of n-3 LCPUFA creates concerns because these fatty acids have been extensively associated with benefits for the cardiovascular and nervous system health[6,7]. Consumption of EPA and DHA in the next future will be more restrictive because the actual low availability of fatty fish, which are the main source of these fatty acids[8]. Traditionally the diet of western population is characterized by a high consumption of n-6 PUFA (LA + AA) which is associated with increment in the risk of the development of different chronic diseases, particularly cardiovascular disease, non-alcoholic fatty liver disease and even neurological diseases[9,10,11]. Health professionals are now looking to ALA, as a source of n-3 PUFA to be transformed in our body into n-3 LCPUFA, however it is necessary to demonstrate that this fatty acid can be efficiently transformed into EPA and DHA. These professionals are also interested in the availability of common foods which can provide ALA in metabolically adequate amounts to obtain the benefits associated to these n-3 LCPUFA. Actually a group of oils, which contain high concentration of ALA, are commercially available. These oils are extracted from seeds harvested in Central and South America and may contain up to 30-60% of ALA. Nontraditional oils extracted from ancestrally consumed seeds such as *Rosa canina*, from Chilean origin; *Sacha inchi* (*Plukenetia volubilis*) from Peruvian origin and *Chia* (*Salvia hispanica*) from Mexican and Central America origin (Honduras, Guatemala, El Salvador) are now commercially produced and may be consumed as such or used for food preparation. However, the possible future nutritional recommendation of these oils as good sources of ALA is

subjected to the demonstration that their consumption may supply ALA in enough amounts to fulfill the nutritional requirements of n-3 LCPUFA. In the present work we investigated the effect of the consumption of *Rosa can*ina oil, *Sacha inchi* oil and *Chia* oil in the accretion of ALA, EPA and DHA into the phospholipids of different tissues of rats which received these oils as the exclusive dietary fat. The metabolic effect of the oils was compared to the effect of two commonly consumed oil, such as Sunflower oil (*Helianthus annuus*) and Canola oil (*Brassica campestris*), which respectively provide a very low amount of ALA, (sunflower oil 1%) and a low amount of ALA (canola oil 10%).

2. Material and methods

2.1. Materials

The components for the elaboration of the diets were locally purchased. *Chia* oil was obtained from Benexia S.A. (Santiago, Chile), *Rosa canina* oil was obtained from COESAM S.A. (Santiago, Chile) and *Sacha inchi* oil was obtained from (Amazonia Agro - Industry S.A., Lima, Perú). Zolazepam chlorhydrate, tiletamine chlorhydrate and Zoletil 50, used for the anesthesia of animals were obtained from Virbac S.A. (Carros, France). Solvents, reagents and other materials for lipid extraction were obtained from Merck Química Chilena (Santiago, Chile). Fatty acid standards for gas-chromatography and reagents for fatty acid methyl ester derivative preparation were obtained from Sigma Chemical (St. Louis, MO, USA). The mixture of fatty acids used as standard was obtained from Nu-Check Prep. (Elysian, MN, USA).

2.2. Animals and Diets

All procedures were performed according to the institutional guidelines for the use of animals established by the Bioethics Committee about Animal Research of the Faculty of Medicine, Universidad de Chile (Protocol number CBA# 0654 FMUCH). Sixty young male Wistar rats (three weeks age) were obtained from the Bioterio of the Nutrition Department, Faculty of Medicine, Universidad de Chile. Animals were randomly assigned at one of five groups with free access to the different experimental diets (n=12/experimental group). Each group was fed an isocaloric diet, with a macronutrient distribution: 20% protein, 10% fat and 70% carbohydrates and supplemented with micronutrients according to the

nutritional requirement for these animals. The total fat in each group was exclusively provided as vegetable oil: Sunflower oil (SFO), Canola oil (CO), *Rosa canina* oil (RCO), Sacha inchi oil (SIO) and Chia oil (ChO). The same short notations were used to identify the experimental groups. The diet composition was previously published[12]. The fatty acid composition of each diet is shown in Table 1. The dietary intervention was carry-out 21 days. Animals were allowed free consumption of diets. At the end of the study, animals were fasted overnight and anaesthetized by intraperitoneal injection (1ml/kg) of zolazepam chlorhydrate (25mg/mL) and tiletamine chlorhydrate (25mg/mL) mixture (Zoletil 50). Blood samples were obtained by cardiac puncture, plasma and erythrocytes were separated by centrifugation of whole blood at 1500g for 5 min. Erythrocytes and liver, kidney, small intestine, hearth, quadriceps and brain tissues were collected from each rat, placed immediately into chilled sample vials and frozen at -80°C for later fatty acid assessing.

2.3. Extraction and separation of tissue lipids

Quantitative extraction of total lipids from erythrocytes, liver, kidney, small intestine, hearth, quadriceps and brain tissues was carried out according to Bligh and Dyer[13] containing BHT (butylated hydroxytoluene) as antioxidant. Erythrocytes and tissues samples were homogenized in ice-cold chloroform/methanol (2:1 v/v) containing 0.01% BHT in an Ultraturrax homogenized (Janke & Kunkel, Stufen, Germany). Total lipids from erythrocytes were extracted with chloroform/isopropanol (2:1 v/v). Phospholipids from erythrocytes, liver, kidney, intestine, hearth, quadriceps and brain tissues were separated from total lipid extracts by thin layer chromatography (TLC) on silica gel plates (aluminum sheets 20x20 cm, silica gel 60 F-254; Merck), using the solvent system hexane/diethyl ether/acetic acid (80:20:1 v/v). After the development of plates and solvent evaporation lipid spots were visualized by exposing the plates to a Camag UV (250 nm) lamp designed for TLC. The solvent system allows the separation of phospholipids, cholesterol, triacilglycerols and cholesterol ester according to their relative mobility. Phospholipid spots were removed from the plate with either diethyl ether or chloroform/methanol (2:1 v/v), according to Ruiz-Gutierrez et al[14].

2.4. Preparation of fatty acid methyl ester (FAME)

FAME from erythrocytes, liver, kidney, small intestine, hearth, quadriceps and brain phospholipids were prepared with boron trifluoride (12% methanolic solution) according to Morrison and Smith[15], and followed by methanolic sodium hydroxide (0.5N) solution. Phospholipids for FAME synthesis were extracted from the silica gel spots with 15 mL of chloroform/methanol/water (10:10:1) and evaporated under nitrogen stream. FAME samples were cooled and extracted with 0.5 mL of hexane.

2.5. Gas chromatographic analysis of FAME

FAME were separated and quantified by gas-liquid chromatography in an Agilent Hewlett-Packard equipment (model 7890A, CA, USA) using a capillary column (Agilent HP-88, 100m x 0.250 mm; I.D. 0.25 μm) and a flame ionization detector (FID). The injector temperature was set at 250°C and the FID temperature at 300°C. The oven temperature at injection was initially set at 140°C and was programmed to increase to 220°C at a rate of 5°C per min. Hydrogen was utilized as the carrier gas (35 cm per second flow rate) in the column and the inlet split ratio was set at 20:1. The identification and quantification of FAME were achieved by comparing the retention times and the peak area values (%) of the unknown samples with those of a commercial lipid standard (Nu-Chek Prep Inc). C23:0 was used as internal standard (Nu-Chek Prep Inc) and a Hewlett-Packard Chemstation data system was used for peak analysis.

2.6. Statistical analysis

Statistical analysis was performed with GraphPad Prism 5.1 software (GraphPad Prism Software, Inc. San Diego, USA). Values shown represent the mean ± SEM for each experimental group. Evaluations of normality data distribution was performed using the Shapiro Wilk test. Assessment of the statistical significance of differences between mean values was performed by one-way ANOVA and the Newman-Keuls test. A p-value less than 0.05 were considered as significant.

3. Results

There were not significant differences in the initial weight of animals in the five experimental protocols and feeding with the different vegetable oils not produced

significant differences in the final weight and in the food intake in all animal groups during the 21 days of intervention (data not shown). No mortality was produced during the study.

3.1 Fatty acid composition of erythrocytes phospholipids

Fatty acid composition of phospholipids extracted from erythrocyte samples is shown in table 2. Total SAFA were not modified by the oils. Total MUFA were increased in CO compared to SFO, RCO, SIO and ChO. SFO showed the lower total MUFA value compared to the other groups. Total PUFA and LCPUFA were significantly higher in SFO, SIO and ChO compared to CO and RCO. Total n-6 LCPUFA were significantly higher for SFO and RCO showing CO, SIO and ChO very low values. Total n-3 LCPUFA were higher in RCO, SIO and ChO compared to SFO and CO. When n-6 and n-3 fatty acids were individually compared, differences were also observed. LA was significantly higher in SFO compared to all other groups. ALA was higher in RCO, SIO and ChO, being significantly lower for SFO. AA was higher in SFO and RCO compared to CO, SIO and ChO. EPA increased in CO, RCO, SIO and ChO compared to SFO. DHA was not modified in all groups. n-6/n-3 Ratios were drastically reduced in CO, RCO, SIO and ChO, this reduction being most significant for SIO and ChO.

3.2. Fatty acid composition of hepatic phospholipids

Fatty acid composition of phospholipids extracted from hepatic samples is shown in table 3. Total SAFA were not modified by the oils. Total MUFA was higher for CO compared to all other groups. Total PUFA and LCPUFA were lower in CO when compared to the other groups. When total n-6 and n-3 PUFA and LCPUFA were individually compared, relevant differences were observed. Total n-6 LCPUFA for SFO and RCO were higher than CO, SIO and ChO. Total n-3 LCPUFA for ChO was higher compared to all other groups, SFO and CO showing the lowest values. LA was significantly higher in SFO when compared to the other groups. ALA was significantly higher in SIO and ChO compared to SFO, CO and RCO, SFO showing the lowest value. AA was lower in CO, SIO and ChO when compared to SFO and RCO. Compared to all other groups EPA showed the lowest value in SFO, being ChO the group showing the higher value for this fatty acid. The modification of DHA was also remarkable, higher values are observed for RCO, SIO and ChO, showing SFO

the lowest value. n-6/n-3 PUFA ratios were drastically reduced in CO, RCO, SIO and ChO compared to SFO, being this reduction most significant for SIO and ChO.

3.3. Fatty acid composition of kidney phospholipids

The fatty acid composition of kidney phospholipids is shown in table 4. Total SAFA were not modified. Total MUFA were higher in CO compared to SFO, RCO, SIO and ChO. Total PUFA, total LCPUFA, total n-6 LCPUFA and total n-3 LCPUFA were significantly lower in CO compared to the other groups. LA was significantly higher in SFO compared the other groups. ALA was significantly higher in SIO and ChO compared to SFO, CO and RCO. However, when compared to the other groups, SFO showed the lowest value. AA was higher in SFO and RCO when compared to the other groups. EPA was higher in SIO and ChO, SFO showing the lowest value. DHA was not modified in all groups. n-6/n-3 Ratios were drastically reduced in SIO and ChO.

3.4. Fatty acid composition of small intestine phospholipids

The fatty acid compositions of phospholipids extracted from small intestine is shown in table 5. Total SAFA were not modified. Total MUFA were higher in CO compared to SFO, RCO, SIO and ChO. Compared to the other groups, animals fed CO showed the lowest total PUFA, total LCPUFA, total n-6 LCPUFA and total n-3 LCPUFA. SFO showed the higher LA and the lower ALA content compared to the other groups. AA was higher in SFO and RCO compared to CO, SIO and ChO. EPA accretion was significantly higher in RCO, SIO and ChO, SFO showing the lowest value for this fatty acid. DHA content was increased in CO, RCO, SIO and ChO, compared to SFO. n-6/n-3 Ratios were similar as observed in the other preceding tissues.

3.5. Fatty acid composition of hearth phospholipids

The fatty acid composition of phospholipids extracted from heart is shown in Table 6. Total SAFA were not modified by the oils. Total MUFA in CO were higher compared to the other groups. Total PUFA and total LCPUFA were lower in CO compared to the other groups. n-6 LCPUFA were higher in SFO and RSO, compared to the other groups, ChO showing the lowest value. n-3 LCPUFA were higher in ChO and the lower value was observed in SFO.

LA was significantly higher in SFO compared to the other groups. ALA was significantly higher in ChO, SFO showing the lowest value respect to the other groups. AA was higher in SFO compared to the other groups, ChO showing a drastic reduction of this fatty acid. EPA was increased in SIO and ChO compared to SFO, CO and RCO, SFO showing the lowest value. DHA accretion not was modified. n-6/n-3 Ratios were drastically reduced in both SIO and ChO, compared to SFO, CO and RCO, this reduction being most relevant in ChO.

3.6. Fatty acid composition of quadriceps phospholipids

The fatty acid composition of phospholipids extracted from quadriceps is shown in table 7. Total SAFA were not modified in all groups. Total MUFA and PUFA were significantly lower in CO compared to SFO, RCO, SIO and ChO. Total LCPUFA was higher in SFO, ChO showing the lowest value. n-6 LCPUFA accretion was lower in RCO compared to the other groups. n-3 LCPUFA were higher in SIO and ChO compared to SFO, CO and RCO, however SFO presented the lowest value. LA was higher in SFO respect to all other groups. ALA was higher in SIO and ChO compared to SFO, CO and RCO. SFO showed the lowest value. LA was higher in SFO compared to the other groups. ALA content was higher for SIO and ChO, and SFO having the lowest value. AA was higher in SFO and RCO compared to CO, SIO and ChO, this group showing the lowest value. EPA was higher in SiO and ChO. SFO showed the lowest value compared to the other groups. DHA was not modified. n-6/n-3 Ratios were drastically reduced in ChO compared to the other groups. SFO showed the higher value for this ratio.

3.7. Fatty acid composition of brain phospholipids

The fatty acid composition of brain phospholipids is shown in table 8. Total SAFA, MUFA, PUFA, LCPUFA and n-6 LCPUFA were not modified by the oils. n-3 LCPUFA were significant lower in SFO and CO compared to RCO, SIO and ChO. LA was higher in SFO compared to SIO and ChO. ALA was lower in SFO compared to the other groups. AA and EPA were not modified in all groups. DHA was higher in RCO, SIO and ChO compared to SFO and CO. However, the accretion of DHA in ChO was higher than RCO but similar to SIO. n-6/n-3 Ratios were higher in SFO and CO compared to the other groups.

4. Discussion and Conclusions

The objective of the present study was to evaluate the effect of increasing dietary ALA, its differential accretion into the phospholipids of different tissues of the rat and its tissue-selective transformation into EPA and DHA. ALA accretion into erythrocyte phospholipids (shown in table 2) reflex a direct relationship to dietary ALA, because the accretion of the fatty acid into membrane phospholipids was increased with the amount of ALA provided by the different oils assayed (SFO, CO, RSO, SIO and ChO). This result is in concordance with previous research which demonstrated that erythrocyte membrane phospholipid fatty acid composition show a good correlation with dietary fatty acids[16]. However, in our experimental model no significant differences were observed for SIO and ChO in spite of the higher amount of ALA provided by these oils, particularly by ChO, which may suggest a maximum capacity of erythrocytes to incorporate ALA into membrane phospholipids (table 2). Accretion of AA and EPA to erythrocyte phospholipids, were incremented with the supply of LA and ALA of the diets. However, this effect is not observed for DHA, which remains unchanged in spite of the amount of ALA provided by the diets. The drastic differences in the supply of LA and ALA of the diets and the differential accretion of n-6 and n-3 fatty acids into membrane phospholipids was also reflexed by the changes in the n-6/n-3 ratios. The high accretion of EPA observed for SIO and ChO in erythrocytes, which is not emulated into DHA accretion, can be interpreted in terms that erythrocytes are not selective target for the accretion of this n-3 LCPUFA (table 2). A similar result was obtained for plasma by Gibson et al., who observed that increasing amounts of dietary ALA does not modify the amount of DHA in plasma phospholipids[17].

Hepatic phospholipid fatty acid composition also showed good association with dietary ALA and the hepatic accretion of EPA. DHA was also increased, however no significant differences were observed for SIO and ChO. This may suggest the existence of a metabolic regulation in the hepatic bioconversion of ALA into DHA, possible due to inhibition by excess of ALA of the enzymatic machinery which carries out the conversion of ALA, first to EPA and after to DHA, and/or to a negative feed-back exerted by DHA to its formation from EPA[18,19]. Previous reports of our group demonstrated that dietary ChO increases EPA and DHA content of hepatic tissue, also increasing the expression of the peroxisome proliferator-activated receptor-α (PPAR-α) and of two enzymes regulated for this transcription factor of the tissue[12]. AA was significantly reduced in CO, SIO and ChO, when compared to SFO and RSO, which should be consequence of the low amount of LA

provided by these diets (table 1). Again n-6/n-3 ratio showed a significant reduction for SIO and ChO as was also observed in erythrocytes.

Differences in LA and ALA in the kidney (table 4) may be also associated to the supply of both fatty acids, as was observed for erythrocytes and liver, increasing EPA content (SIO and ChO) and reducing AA content (CO, SIO and ChO). However, similar to erythrocytes, DHA was not modified by the increase of ALA content of diets. It can be speculated that ALA is transported to the kidney and partially transformed into EPA, being the transformation of EPA into DHA metabolically restricted. Small intestine (table 5) behaved as erythrocytes, liver and kidney when LA, ALA, AA and EPA accretion was evaluated. DHA was significant higher in RCO, SIO and ChO compared to SFO, however no significant differences were observed for CO and RCO. After the dietary intervention, hearth (table 6) and quadriceps (table 7), showed a similar response between them and also similar to the preceding tissues respect to their LA, ALA, AA and EPA content. However, it is remarkable that DHA was not modified in hearth and quadriceps. The low accretion of DHA in these tissues may be consequence of either; a low transport of the fatty acid as such to the tissue and/or to a reduced conversion from EPA, whose accretion is increased by the dietary intervention in both tissues.

Brain tissue showed a particular fatty acid composition compared to the other tissues previously evaluated, because very low levels of LA, ALA and EPA and high levels of AA and DHA were observed. However, the high levels of DHA, normally found in this tissue[20], were significantly increased after RCO, SIO and ChO compared to SFO, which is the diet which provided the lowest supply of ALA (0.1 g ALA/100 g diet). The selective accretion of AA and DHA into brain cells, may results from either: the selective transport of these fatty acids to this tissue and/or to the effective transformation of AL and ALA to their respective n-6 and n-3 LCPUFA which occurs at glial astrocytes[21]. A recent report from Domenichiello et al.,[22] shows that in rats fed ALA, brain DHA synthesis and accretion is 100-fold higher than the brain DHA accretion produced in rats fed preformed DHA. These results give good support to our proposal about the effective transformation of ALA into DHA in the brain. However the selective transport of n-6 and n-3 LCPUFA to the brain cannot be discarded. AA and DHA are transported from the liver to the brain through the plasma as lysophospholipids[23] which are highly permeable to the brain blood barrier[24].

ALA, when supplied from different vegetable oils (RCO, SIO and ChO) produced important modifications into the fatty acid composition of phospholipids extracted from the different tissues of the rat as was observed in our study. Metabolism of ALA and of its

metabolic derivatives (EPA and DHA), show remarkable differences in the studied tissues. With the exception of brain all other tissues showed higher ALA and EPA levels, on line with the amount of ALA supplied by the different diets. It has been demonstrated that excess of ALA, not converted into n-3 LCPUFA, is β-oxidized[25]. With the exception of brain, we observed ALA in all other tissues. Therefore we postulate that ALA supplied in excess, as was provided in our intervention model, not converted to n-3 LCPUFA and not β-oxidized, is incorporated into membrane phospholipids. DHA was almost exclusively accreted into liver and brain. Hepatic DHA may results from its active transformation from ALA, meanwhile brain DHA may be associated to two mechanisms: from the almost selective transport from the liver[26] after being formed from ALA and/or from an effective brain bioconversion from ALA[22,27]. It has been demonstrated that ALA may cross the brain blood barrier being metabolized to DHA by glial cells (astrocytes) and after selectively transported to neurons[28].

Our results demonstrate that when high dietary levels of ALA are provided, an enhancing of its transformation into EPA and DHA in some tissues of the rat occurs. However, remains to study the possible modification of the activity and/or expression of tissue desaturase and elongase enzymes[25], which are responsible for the conversion of ALA into EPA and DHA in the liver and almost exclusively into DHA in the brain[29]. It has been demonstrated that tissue conversion of ALA into EPA and DHA is generally low in many tissues[30] with the exception of brain, because in spite of the low conversion of ALA into DHA, the tissue allows an adequate accretion of the n-3 LCPUFA[22].

The low availability of marine foods in the next future[8] and therefore of n-3 LCPUFA, is a strong directive force to search other sources to provide the substrate for the formation of these essential fatty acids. RCO, SIO and ChO are good sources of ALA and its consumption allows in the rat the selective accretion of EPA and DHA at some tissues. These oils, which are now produced and commercially available in some Latin America countries, may represent a good solution to the nutritional requirements of n-3 PUFA and eventually of n-3 LCPUFA. However, it remains to demonstrate that humans may perform the transformation of ALA into EPA and DHA, as efficient as was observed in the rat in our experimental model. The rat is considered as an efficient converter of ALA into n-3 LCPUFA as compared to humans[30], therefore results of ALA supplementation may be eventually different to those obtained in rats. The conversion of ALA into DHA is of the order of 1% in infants and is considerably lower in adults[30] and this conversion may be drastically reduced in subjects affected by chronically hepatic diseases, such as non-

alcoholic fatty liver disease[31]. Present evidence indicated that in humans n-3 LCPUFA status can be improved by increasing their intake, or by decreasing LA intake, and a combination of the two is likely to be most effective[30]. In our intervention model SIO (LA/ALA 0.81) and ChO (LA/ALA 0.31) accomplished with these premises providing low amount of LA and high amount of ALA. However, independently of the capacity for the conversion of ALA into n-3 LCPUFA in humans, ALA consumption in women during pregnancy allow a reduction of premature parturition and increases the weight of children at delivery[32], demonstrating the nutritional importance of this essential fatty acid.

Conflict of interest

Authors don't have any conflict of interest

Acknowledgment

Authors are grateful to FONDECYT and INNOVA-CORFO the support of their research. We are also grateful to the Nutrition and Dietetics School and the Nutrition Department, Faculty of Medicine, Universidad de Chile.

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474 **Table 1**

475 Fatty acid composition of each diet: sunflower oil (SFO), canola oil (CO), rosa canina oil
476 (RCO), sacha inchi oil (SIO) and chia oil (ChO). Values are expressed as g per 100 g of
477 diet.

Content (g per 100 g of diet)	SFO	CO	RCO	SIO	ChO
SAFA	0.9	0.6	0.6	0.6	0.9
MUFA	1.3	6.4	1.6	1.0	0.6
Oleic acid	1.0	6.0	1.5	0.8	0.5
PUFA	7.4	3.0	7.8	8.5	8.5
Total n-6 PUFA	7.3	2.0	4.4	3.5	2.1
Linoleic acid	7.2	1.9	4.2	3.6	2.0
Total n-3 PUFA	0.1	1.0	3.4	4.9	6.4
Alpha linolenic acid	0.1	1.0	3.3	4.8	6.3
n6 / n-3 PUFA ratio	73	2.0	1.3	0.7	0.3

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Table 2

Fatty acid composition of erythrocyte phospholipids obtained from the different experimental groups. Sunflower oil (SFO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	19.4±2.4^{b,c,d,e}	9.80±0.8^{a}	12.3±1.2^{a}	10.2±1.1^{a}	9.81±1.0^{a}
18 : 3,n-3 (ALA)	0.24±0.1^{b,c,d,e}	1.12±0.4^{a,c,d,e}	3.87±0.7^{a,b,d,e}	14.6±1.2^{a,b,c}	16.4±1.7^{a,b,c}
20 : 4,n-6 (AA)	13.5±1.2^{b,c,d,e}	1.83±0.2^{a,c}	10.3±1.5^{b,d,e}	2.85±0.4^{a,c}	1.68±0.4^{a,c}
20 : 5,n-3 (EPA)	0.23±0.11^{b,c,d,e}	1.38±0.1^{a,d,e}	1.63±0.2^{a,d,e}	3.12±0.4^{a,b,c}	4.34±0.5^{a,b,c}
22 : 6,n-3 (DHA)	0.84±0.2	1.12±0.2	1.20±0.2	1.41±0.3	1.44±0.2
Total SAFA	39.4±3.4	38.5±2.6	37.6±2.6	36.5±2.1	36.3±1.8
Total MUFA	24.2±1.6^{b,c,d,e}	44.8±3.5^{a,c,d,e}	33.1±2.7^{a,b}	31.3±3.0^{a,b}	30.0±1.5^{a,b}
Total PUFA	36.4±2.2^{b,c}	16.7±1.4^{a,c,d,e}	29.3±2.8^{a,b}	32.2±2.8^{a}	33.7±2.1^{a}
Total LCPUFA	15.5±1.4^{b,d,e}	4.34±0.6^{a,c,d,e}	14.1±1.2^{b,d,e}	7.74±1.1^{a,b,c}	7.63±0.8^{a,b,c}
Total n-6 LCPUFA	14.1±1.6^{b,c,d,e}	1.52±0.2^{a,c}	10.9±0.7^{a,b,d,e}	2.91±0.3^{a,c}	1.71±0.2^{a,c}
Total n-3 LCPUFA	1.40±0.2^{c,d,e}	2.82±0.2^{d,e}	3.14±0.2^{a,e}	4.83±0.6^{a,b}	5.92±0.6^{a,b}
PUFA n-6/n-3 ratio	25.1±1.4^{b,c,d,e}	3.22±0.4^{a,d,e}	3.37±0.4^{a,d,e}	0.69±0.1^{a,b,c}	0.51±0.1^{a,b,c}

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).

Table 3

Fatty acid composition of hepatic phospholipids obtained from the different experimental groups. Sunflower oil (SFO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	22.1±1.3^{b,c,d,e}	11.4±1.4^{a}	15.3±1.2^{a}	13.4±2.3^{a}	12.9±2.5^{a}
18 : 3,n-3 (ALA)	0.25±0.1^{b,c,d,e}	1.12±0.8^{a,c,d,e}	4.52±0.9^{a,b,d,e}	15.6±1.4^{a,b,c}	17.1±2.8^{a,b,c}
20 : 4,n-6 (AA)	14.9±3.1^{b,d,e}	1.68±0.2^{a,c}	11.9±2.2^{b,d,e}	2.74±0.6^{a,c}	1.74±0.4^{a,c}
20 : 5,n-3 (EPA)	0.30±0.1^{b,c,d,e}	0.84±0.5^{a,c,d}	1.74±0.4^{a,b,d,e}	4.76±0.8^{a,b,c,e}	9.91±0.9^{a,b,c,d}
22 : 6,n-3 (DHA)	0.98±0.6^{b,c,d,e}	1.41±0.4^{a,c,d,e}	5.05±0.6^{a,b}	5.84±0.9^{a,b}	6.41±0.6^{a,b}
Total SAFA	34.3±4.1	32.7±2.1	32.4±2.5	33.7±1.8	31.4±2.7
Total MUFA	19.1±2.2^{b}	49.2±3.2^{a,c,d,e}	25.4±2.7^{b}	20.2±1.4^{b}	22.0±3.1^{b}
Total PUFA	46.6±4.7^{b}	18.1±1.9^{a,c,d,e}	42.2±2.9^{b}	46.1±2.5^{b}	46.6±2.7^{b}
Total LCPUFA	16.9±1.6^{b}	4.40±0.9^{a,c,d,e}	19.8±1.5^{b}	15.3±1.3^{b}	19.4±2.1^{b}
Total n-6 LCPUFA	15.1±2.1^{b,d,e}	1.94±0.5^{a,c}	12.1±1.8^{b,d,e}	2.98±0.4^{a,c}	2.31±0.5^{a,c}
Total n-3 LCPUFA	1.15±0.8^{c,d,e}	2.42±0.7^{c,d,e}	7.13±1.4^{a,b,d,e}	10.9±0.8^{a,b,c,e}	17.1±1.4^{a,b,c,d}
PUFA n-6/n-3 ratio	24.2±2.1^{b,c,d,e}	3.89±0.3^{a,c,d,e}	2.41±0.4^{a,b,d,e}	0.62±0.3^{a,b,c}	0.44±0.2^{a,b,c}

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).

Table 4

Fatty acid composition of kidney phospholipids obtained from the different experimental groups. Sunflower oil (SFO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	21.4±1.6^{b,c,d,e}	10.2±1.2^{a}	14.5±1.6^{a}	12.6±1.8^{a}	11.8±1.8^{a}
18 : 3,n-3 (ALA)	0.27±0.1^{b,c,d,e}	1.14±0.7^{a,c,d,e}	4.65±0.8^{a,b,d,e}	19.5±1.3^{a,b,c}	16.9±2.1^{a,b,c,}
20 : 4,n-6 (AA)	16.4±2.8^{b,d,e}	2.2±0.3^{a,c}	12.6±2.4^{b,d,e}	3.04±0.5^{a,c}	1.85±0.3^{a,c}
20 : 5,n-3 (EPA)	0.22±0.1^{b,c,d,e}	1.41±0.3^{a,d,e}	1.65±0.2^{a,d,e}	3.15±0.5^{a,b,c}	5.45±0.6^{a,b,c}
22 : 6,n-3 (DHA)	0.86±0.2	0.92±0.1	0.94±0.1	0.95±0.1	0.96±0.2
Total SAFA	36.5±3.8	33.4±2.3	34.6±2.9	34.5±2.1	32.5±2.7
Total MUFA	21.4±1.9^{b}	47.2±2.4^{a,c,d,e}	28.9±3.7^{b}	22.6±1.9^{b}	24.5±2.7^{b}
Total PUFA	42.1±2.7^{b,c}	19.4±1.4^{a,c,d,e}	36.5±1.9^{a,b,d,e}	42.9±2.6^{b,c}	43.0±3.1^{b,c}
Total LCPUFA	17.7±2.2^{b,d,e}	4.65±0.4^{a,c,d,e}	16.0±1.2^{b,d,e}	7.14±0.8^{a,b,c}	9.12±0.6^{a,b,c}
Total n-6 LCPUFA	16.6±1.1^{b,c,d,e}	2.41±0.3^{a,c,e}	12.9±1.8^{a,b,d,e}	3.50±0.6^{a,b,c,e}	1.94±0.2^{a,c,d}
Total n-3 LCPUFA	1.08±0.4^{b,c,d,e}	2.33±0.2^{a,c,d,e}	3.10±0.4^{a,b,e}	3.64±0.3^{a,b,e}	7.18±0.3^{a,b,c,d}
PUFA n-6/n-3 ratio	28.1±2.4^{b,c,d,e}	4,29±2.1^{a,c,d,e}	3.75±0.4^{a,b,d,e}	0.66±0.2^{a,b,c}	0.59±0.1^{a,b,c}

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).

Table 5

Fatty acid composition of small intestine phospholipids obtained from the different experimental groups. Sunflower oil (SFO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	27.7±2.2^{b,c,d,e}	13.4±1.4^{a}	15.1±1.7^{a}	13.3±2.0^{a}	11.7±1.4^{a}
18 : 3,n-3 (ALA)	0.31±0.1^{b,c,d,e}	2.57±0.6^{a,c,d,e}	6.23±1.2^{a,b,d,e}	19.8±2.2^{a,b,c}	21.4±2.3^{a,b,c,}
20 : 4,n-6 (AA)	13.7±1.7^{b,d,e}	2.11±0.2^{a,c}	10.2±2.3^{b,d,e}	2.92±0.4^{a,c}	1.78±0.2^{a,c}
20 : 5,n-3 (EPA)	0.27±0.04^{b,c,d,e}	1.16±0.3^{a,c,d,e}	2.16±0.2^{a,b}	2.34±0.3^{a,b}	2.67±0.4^{a,b}
22 : 6,n-3 (DHA)	0.91±0.2^{c,d,e}	1.42±0.4^{d,e}	1.87±0.3^{a,b}	2.27±0.4^{a,b}	2.38±0.3^{a,b}
Total SAFA	34.2±3.1	32.1±2.1	33.7±3.3	35.4±2.6	33.1±2.9
Total MUFA	22.3±2.2^{b}	45.3±4.6^{a,c,d,e}	30.4±3.1^{b}	23.7±3.4^{b}	25.2±3.2^{b}
Total PUFA	43.5±4.8^{b}	22.6±1.9^{a,c,d,e}	35.9±3.3^{b}	40.9±4.6^{b}	41.7±3.4^{b}
Total LCPUFA	15.1±2.1^{b,d,e}	4.83±0.7^{a,c,d,e}	14.8±2.4^{b,d,e}	7.82±1.4^{a,b,c}	7.1±1.1^{a,b,c}
Total n-6 LCPUFA	13.9±2.0^{b,d,e}	2.21±0.4^{a,c,d}	10.7±1.2^{b,d,e}	3.01±0.2^{a,b,c}	1.88±0.3^{a,c,d}
Total n-3 LCPUFA	1.20±0.3^{b,c,d,e}	2.62±0.6^{a,c,d,e}	4.10±0.3^{a,b}	4.81±0.4^{a,b}	5.22±0.7^{a,b}
PUFA n-6/n-3 ratio	27.8±2.8^{b,c,d,e}	3.04±0.4^{a,c,d,e}	2.47±0.3^{a,b,d,e}	0.68±0.1^{a,b,c}	0.51±0.1^{a,b,c}

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).

Table 6

Fatty acid composition of hearth phospholipids obtained from the different experimental groups. Sunflower oil (SFO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	19.5±2.3^{b,c,d,e}	9.4±1.4^{a}	16.3±1.3^{a}	12.7±1.5^{a}	11.2±1.6^{a}
18 : 3,n-3 (ALA)	0.24±0.1^{b,c,d,e}	1.12±0.5^{a,c,d,e}	9.23±0.6^{a,b,d,e}	22.8±1.2^{a,b,c}	26.5±2.0^{a,b,c,}
20 : 4,n-6 (AA)	15.1±2.4^{b,d,e}	7.90±0.7^{a,c,d,e}	11.2±1.7^{b,d,e}	4.12±0.3^{a,c}	1.54±0.2^{a,c}
20 : 5,n-3 (EPA)	0.18±0.1^{b,c,d,e}	1.23±0.2^{a,d,e}	1.31±0.2^{a,d,e}	3.04±0.3^{a,b,c}	4.31±0.4^{a,b,c}
22 : 6,n-3 (DHA)	0.80±0.1	0.84±0.1	0.88±0.1	0.90±0.1	0.91±0.1
Total SAFA	35.1±3.7	34.5±5.2	33.0±3.1	34.2±2.2	32.3±3.1
Total MUFA	25.3±2.5^{b}	44.4±4.1^{a,c,d,e}	27.0±2.3^{b}	21.2±1.7^{b}	23.2±3.5^{b}
Total PUFA	39.6±5.4^{b}	21.1±2.1^{a,c,d,e}	40.0±3.2^{b}	44.6±5.2^{b}	44.5±3.8^{b}
Total LCPUFA	16.4±2.0^{b,d,e}	10.6±0.5^{a,c,d,e}	14.1±2.1^{b,d,e}	8.21±0.4^{a,b,c,e}	6.80±0.6^{a,b,c,d}
Total n-6 LCPUFA	15.3±1.4^{b,d,e}	8.11±0.2^{a,c,d,e}	11.5±1.3^{b,d,e}	4.43±0.3^{a,b,c,e}	1.58±0.2^{a,b,c,d}
Total n-3 LCPUFA	1.10±0.2^{b,c,d,e}	2.21±0.2^{a,d,e}	2.60±0.3^{a,d,e}	3.78±0.2^{a,b,c,d}	5.22±0.3^{a,b,c,d}
PUFA n-6/n-3 ratio	28.7±2.2^{b,c,d,e}	5.5±0.7^{a,c,d}	2.42±0.4^{a,b,d,e}	0.64±0.1^{a,c,e}	0.40±0.03^{a,b,c,d}

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).

Table 7

Fatty acid composition of quadriceps phospholipids obtained from the different experimental groups. Sunflower oil (SFO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	19.1±2.0^{b,c,d,e}	9.7±1.3^{a}	15.1±1.4^{a}	12.9±1.6^{a}	10.1±1.6^{a}
18 : 3,n-3 (ALA)	0.27±0.1^{b,c,d,e}	1.17±0.6^{a,c,d,e}	9.11±0.4^{a,b,d,e}	22.2±1.0^{a,b,c}	25.8±2.2^{a,b,c}
20 : 4,n-6 (AA)	15.3±2.3^{b,d,e}	7.44±0.6^{a,c,d,e}	10.9±1.4^{b,d,e}	4.14±0.2^{a,c}	1.53±0.3^{a,c}
20 : 5,n-3 (EPA)	0.20±0.04^{b,c,d,e}	1.26±0.1^{a,d,e}	1.34±0.16^{a,d,e}	3.80±0.4^{a,b,c}	4.34±0.3^{a,b,c}
22 : 6,n-3 (DHA)	0.81±0.03	0.83±0.04	0.87±0.02	0.91±0.04	0.93±0.02
Total SAFA	36.8±3.1	33.9±4.3	32.9±3.1	32.5±2.3	33.0±2.7
Total MUFA	25.9±2.3^{b}	45.2±3.7^{a,c,d,e}	29.2±2.4^{b}	23.4±1.8^{b}	23.9±3.2^{b}
Total PUFA	37.3±4.5^{b}	20.9±1.8^{a,c,d,e}	37.9±3.3^{b}	44.1±4.3^{b}	43.1±3.2^{b}
Total LCPUFA	16.8±2.3^{b,d,e}	9.83±0.6^{a,c,d,e}	13.8±1.7^{b,d,e}	9.02±0.3^{a,b,c,e}	6.84±0.4^{a,b,c,d}
Total n-6 LCPUFA	15.4±1.1^{b,d,e}	7.70±0.1^{a,c,d,e}	11.5±1.2^{b,d,e}	4.25±0.2^{a,b,c,e}	1.53±0.1^{a,b,c,d}
Total n-3 LCPUFA	1.41±0.3^{b,c,d,e}	2.13±0.1^{a,d,e}	2.27±0.2^{a,d,e}	4.77±0.3^{a,b,c,d}	5.31±0.2^{a,b,c,d}
PUFA n-6/n-3 ratio	26.9±2.4^{b,c,d,e}	5.3±0.2^{a,c,d}	2.30±0.3^{a,b,d,e}	0.63±0.04^{a,c,e}	0.38±0.04^{a,b,c,d}

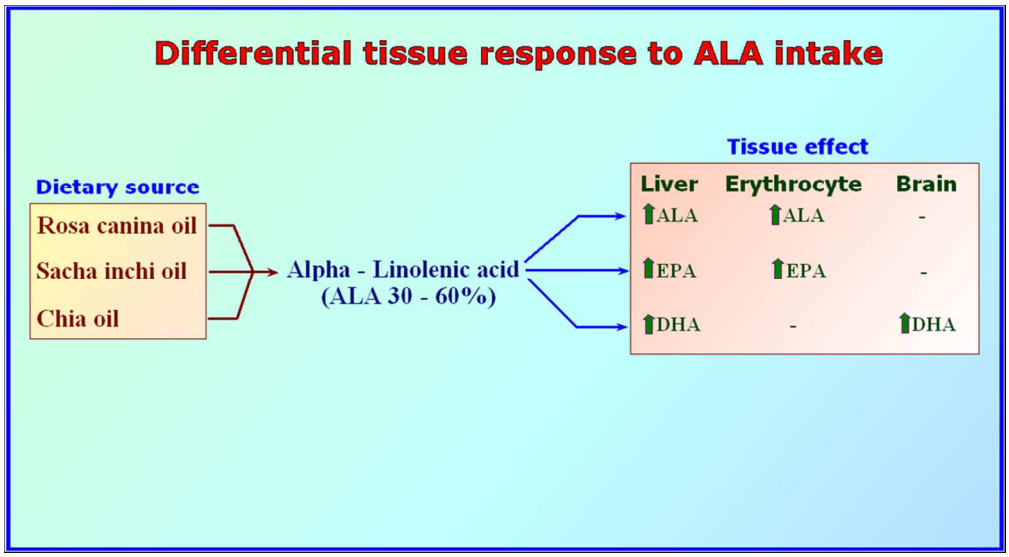
Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).

Table 8

Fatty acid composition of brain phospholipids obtained from the different experimental groups. Sunflower oil (SO), canola oil (CO), rosa canina oil (RCO), sacha inchi oil (SIO) and chia oil (ChO).

	Fatty acid composition (g per 100 g FAME)				
	Groups				
Fatty acid	(a) SFO	(b) CO	(c) RCO	(d) SIO	(e) ChO
18 : 2,n-6 (LA)	3.10±0.1^{d,e}	2.92±0.5	3.03±0.1	2.74±0.05^{a}	2.71±0.1^{a}
18 : 3,n-3 (ALA)	0.80±0.1^{b,c,d,e}	1.41±0.2^{a}	1.54±0.1^{a}	1.59±0.04^{a}	1.68±0.1^{a}
20 : 4,n-6 (AA)	17.6±2.4	17.4±1.7	17.1±1.1	16.8±1.4	16.6±1.3
20 : 5,n-3 (EPA)	0.82±0.04	0.86±0.03	0.90±0.1	0.92±0.03	0.94±0.04
22 : 6,n-3 (DHA)	10.6±0.5^{c,d,e}	10.7±0.3^{c,d,e}	12.1±0.3^{a,b,e}	12.5±0.4^{a,b}	13.2±0.3^{a,b,c}
Total SAFA	43.5±3.3	42.4±2.4	41.9±3.2	40.6±3.4	40.7±2.8
Total MUFA	23.5±2.4	24.2±2.7	23.3±2.1	24.4±2.1	22.9±3.1
Total PUFA	33.0±3.5	33.4±3.1	34.8±3.3	35.0±2.4	36.4±2.0
Total LCPUFA	29.1±2.6	29.1±2.8	30.5±3.2	30.4±2.6	31.2±3.2
Total n-6 LCPUFA	17.9±1.2	17.6±1.4	17.4±1.1	16.9±1.8	17.1±1.4
Total n-3 LCPUFA	11.2±0.4^{c,d,e}	11.5±0.5^{c,d,e}	13.1±0.7^{a,b}	13.5±0.6^{a,b}	14.1±0.5^{a,b}
PUFA n-6/n-3 ratio	1.69±0.2^{b,c,d,e}	1.57±0.3^{a,c,d,e}	1.39±0.1^{a}	1.31±0.4^{a}	1.22±0.3^{a}

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n=12 rats/experimental group. Values sharing the same letter in each row are not statistically different ($p<0.05$). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 Long chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3 + 22:5, n-3 + 22:6, n-3).



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