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Dietary n-9, n-6 and n-3 fatty acids modulate the oxidative stress in brain and liver of mice. Effect of *trans* fatty acids supplementation

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Abstract: BACKGROUND:Arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acids interaction affects brain structure and function. Unsaturated fatty acids (UFAs) generate oxygenated lipid-derived eicosanoids which modulate the inflammatory response. The presence of trans fatty acids (TFA) in neuronal membranes can favor to generation of pro-oxidant metabolites. OBJECTIVE:This study evaluated the effect of supplementation with TFA to diets containing different proportions of FA, on the oxidative stress (OS) generation and the inflammatory response in mice brain and liver. METHODS:CF1 mice were fed diets (16 weeks) with olive (O), corn (C) or rapeseed (R) oils. OS parameters and gene expression of some key liver and brain enzymes involved in OS production were evaluated. RESULTS:In brain and liver, lipoperoxidation was increased and catalase activity was decreased in C. In brain, glutathione was diminished by supplementation with TFA in all diets and histological sections showed lymphocytes in O and C. In liver, decreased amount of lipid vacuoles and increased of cyclooxygenase-1 (COX-1) and PPARγ mRNA levels were observed in R and Rt. IL-1b and IL-6 in serum were augmented in O and Ot. CONCLUSIONS:Rapeseed oil could have protective effects on the development of OS and inflammation, while TFA supplementation did not showed marked effects on these parameters.

Keywords: Edible oils, inflammation; *trans* fatty acids, polyunsaturated fatty acids, cyclooxygenase, monounsaturated fatty acids

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Dietary n-9, n-6 and n-3 fatty acids modulate the oxidative stress in brain and liver of mice. Effect of *trans* fatty acids supplementation

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Abstract

BACKGROUND: Arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acids interaction affects brain structure and function. Unsaturated fatty acids (UFAs) generate oxygenated lipid-derived eicosanoids which modulate the inflammatory response. The presence of trans fatty acids (TFA) in neuronal membranes can favor to generation of pro-oxidant metabolites. **OBJECTIVE:** This study evaluated the effect of supplementation with TFA to diets containing different proportions of FA, on the oxidative stress (OS) generation and the inflammatory response in mice brain and liver.

METHODS: CF1 mice were fed diets (16 weeks) with olive (O), corn (C) or rapeseed (R) oils. OS parameters and gene expression of some key liver and brain enzymes involved in OS production were evaluated.

RESULTS: In brain and liver, lipoperoxidation was increased and catalase activity was decreased in C. In brain, glutathione was diminished by supplementation with TFA in all diets and histological sections showed lymphocytes in O and C. In liver, decreased amount of lipid vacuoles and increased of cyclooxygenase-1 (COX-1) and PPARγ mRNA levels were observed in R and Rt. IL-1b and IL-6 in serum were augmented in O and Ot.

CONCLUSIONS: Rapeseed oil could have protective effects on the development of OS and inflammation, while TFA supplementation did not showed marked effects on these parameters.

Keywords: Edible oils; Inflammation; *Trans* fatty acids; Polyunsaturated fatty acids; Cyclooxygenase; Monounsaturated fatty acids

Abbreviations:

ARA	arachidonic acid (20:4n-6)
C	corn oil
CAT	catalase
CLA	conjugated linoleic acid
COX	cyclooxygenase
Ct	corn oil + *trans* fatty acids
DHA	docosahexaenoic acid (22:6n-3)
FAME	fatty acid methyl esters
GSH	glutathione
IL-1b	interleukin-1b
IL-6	interleukin 6
LNA	linoleic acid (18:2n-6)
LC-PUFA	long chain polyunsaturated fatty acids
ALA	alpha-linolenic acid (18:3n-3)
LPO	lipoperoxidation

MDA	malondialdehyde
O	olive oil
Ot	olive oil + *trans* fatty acids
PHVO	partially hydrogenated vegetable oil
PPARγ	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acids
R	rapeseed oil
Rt	rapeseed oil + *trans* fatty acids
TFA	t*rans* fatty acids;

γ -LNA: γ-linolenic acid.

Introduction

Arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) interactions affects brain structure and function [1,2,3]. Linoleic acid (LA, 18: 2n-6) and α-linolenic acid (ALA, 18: 3n-3) are the respective precursors of these long chain polyunsaturated fatty acids (LC-PUFA). LA and ALA are nutritionally essential because they cannot be synthesized de novo in vertebrate tissues [4]. Both fatty acids (FA) (LA and ALA) and their LC-PUFA derivates, are important for biochemical integrity of the brain, playing different roles in the modulation of multiple biochemical functions, including synthesis of inflammatory mediators, cell membrane fluidity, intracellular signalling and gene expression in central nervous system [2,5]. PUFA generate oxygenated lipid-derived eicosanoids which modulate the inflammatory response. PUFA n-6 are the precursors of various pro-inflammatory eicosanoids, which are implicated in adverse inflammatory processes [6,7]. ARA can be metabolized into pro-inflammatory prostaglandins by cyclooxygenase-2 (COX-2) [8]. Although the chemical properties of COX-1 and COX-2 are similar, it has been observed that COX-2 activity can damage neurons [9,10]. The peroxisome proliferator-activated receptor gamma (PPARγ) in brain has been implicated in anti-inflammatory processes. In cortical neuron-glia cocultures, PPARγ agonists abolished the expression of COX-2 [11]. PPARγ, as a nuclear receptor, binds a number of lipids including fatty acids, eicosanoids and other natural lipid ligands [12]. In liver, in addition to the major role in lipid metabolism, PPARγ is involved in glucose metabolism and inflammatory response between others [13].

On the other hand, n-3 PUFA possess immunosuppressive and anti-inflammatory properties [14]. Also, long-chain n-3 PUFA (LC-PUFA) decrease the availability of ARA as a substrate for eicosanoid synthesis and they also inhibit ARA metabolism [15]. Changes in the composition of FA can exert important influence on the nervous system functions, as well as on development of neuronal diseases [1,16,17]. In a previous study, we showed that a diet with higher LA content increased ARA in the brain, and diet with higher ALA increased docosapentaenoic acid n-3 (DPA; 22:5n-3) and DHA in the same tissue [18]. On the other way,

the consumption of processed foods rich in saturated and trans fatty acids (TFA) [19,20] has increased in Western countries, and chronic intake has brought health concerns [21]. TFA may be incorporated into membrane phospholipids, altering membrane fluidity, biochemical properties, and also can favor to generation of pro-oxidant metabolites [22-24]. Previously, we demonstrated that TFA can be incorporated into the brain, and diets with a high proportion of n-3 FA decrease it incorporation [18]. Thus, we could hypothesize that a minimum incorporation of TFA in brain neuronal membranes may be sufficient to cause oxidative stress and to promote an inflammatory response, and these effects could be modulated by the type of unsaturated FA in the diet.

The aim of this work was to investigate the influence of TFA supplementation in diets containing different content of n-9, n-6 and n-3 FA on the oxidative stress and inflammatory response in brain and liver. The hepatic parameters were investigated considering that dietary FA are bioconverted in this tissue and then PUFA are transported to the brain.

Materials and Methods

Animals, diets preparation and experimental design

The experimental procedures were approved by the Ethics Committee of our School of Biochemistry and were financed by the research project (CAI+D - 501 201101 00166 LI). Animal handling and sacrifice was in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, National Research Council: Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, USA 1996) [25]. Post-weaning male CF1 mice (4 weeks old, 22g) were obtained from the facilities at our University. The mice were housed in animal quarters under controlled conditions (23 ± 2ºC and 12 hour light-dark cycle) in collective cages, having free access to water and standard laboratory chow. After two weeks of adaptation period, mice were randomly grouped (n=6) and fed with the corresponding diets for 16 weeks: group O was fed on a diet rich in virgin olive oil; group C was fed on a diet rich in corn oil; group R was fed on a diet rich

in rapeseed oil; groups Ot, Ct and Rt were fed with diets containing the corresponding oil (O, C or R) where 1/7 parts were replaced with partially hydrogenated vegetable oil (PHVO). Diets were freshly prepared, gassed with nitrogen and stored at 0-4°C. The FA composition of dietary fats was determined by gas chromatography (GC) (Table 1). The proportion of oleic acid/LA/ALA in O, C and R diets was: 55.2/17.2/0.7; 32.0/51.3/0.9 and 61.1/18.4/8.6, respectively. Experimental diets were based on the American Institute of Nutrition *ad hoc* writing committee recommendation (AIN-93G diet formulated for the growth, pregnancy and lactation phases of rodents) [26]. Diet composition (w/w) was: 200 g/kg casein, 5.5 g/kg cystine/methionine/choline, 529 g/kg corn starch, 100 g/kg sucrose, 50g/kg cellulose, 10 g/kg of vitamin mix and 35 g/kg of mineral mix. Diets provided the energy distributed as follows: 64.4% carbohydrates, 19.9% protein and 15,7% fat. O, C and R diets contained 70 g/kg olive, corn or rapeseed oil, respectively (15.7% of dietary energy as total fat), while Ot, Ct and Rt diets contained 50 g/kg olive, corn or rapeseed oil, respectively and 20 g/kg PHVO, which provided approximately 42 g TFA/100 g fat (15.7% of dietary energy as total fat and 1.5% of energy as TFA). TFA were composed by (*t*6+ *t*7+ *t*8)-18:1; *t*9-18:1; *t*10-18:1, *t*11-18:1 and *t*9, *t*12-18:2 isomers (Table 1). Cysteine, methionine and choline were purchased from Sigma (St. Louis, MO, USA). Oils, sucrose and corn starch were obtained from local sources and the PHVO was kindly provided by CALSA (Compañía Argentina de Levaduras S.A., Buenos Aires, Argentina).

Mice were kept under controlled conditions (23 ± 2° C and 12 hour light-dark cycle) with free access to food and water, during the experimental period.

At the end of the dietary treatments (day 120, 9.00-11.00 AM), mice were anaesthetized with azepromazine (1 mg/kg body weight) and ketamine (100 mg/kg body weight) and were sacrificed by cardiac exsanguination. Serum was obtained after blood centrifugation (1000xg for 10 min at 4ºC). Liver and brain were weighed, frozen and stored at -80°C until processed.

Diet lipid extraction and FA analysis

Total lipids of diets (Table 1) were extracted using the method described by Bligh and Dyer [27]. Briefly, samples were homogenized in trichloromethane:methanol 1:2 (vol:vol). After the extraction process, the samples solvent was evaporated under nitrogen stream and then samples were dissolved in hexane for methylation. Extracted FA were methylated using a methanolic potassium hydroxide solution (ISO 5509:2000, Point 5 IUPAC method 2.301). Fatty acid methyl esters (FAME) were analyzed by gas chromatography using a Shimadzu (GC 2014) chromatograph equipped with a flame ionization detector. Analyses were carried out with a capillary column CP Sil 88 (100 m, 0.25 μm film thickness) and the oven temperature was programmed with an initial temperature of 160°C and increases of 0.5°C/min up to 200°C. FAME were identified by comparison of their retention times relative to those of commercial standards. The chromatographic data processing was performed with Gas Chromatography Solution software. Values of FA content were expressed like percentage of total FA. The FA with low metabolic importance and concentrations lower than 0.5% were considered minor and were not shown. Other details could be obtained from previous publications [28].

Serum interleukin levels

Interleukin IL-1b and interleukin-6 levels were measured in serum by ELISA method. (ELISA Kit Invitrogen, USA). Values were expressed as pg/ml.

Oxidative status parameters

The amount of lipid peroxides (LPO) in liver and brain was determined by the reaction with thiobarbituric acid described by Ohkawa *et al*. [29]. The results were expressed as nmol of malondialdehyde (MDA)/g of tissue. Reduced glutathione (GSH) levels (expressed as μmol/g of tissue) in liver and brain were measured according to the method reported by Ellman & Lysko [30]. Catalase (CAT) activity (expressed as μmol/min/mg protein), was assessed in liver and brain by the method reported by Aebi [31]. Protein concentration was determined according Lowry *et al*. [32].

Extraction and analysis of RNA and quantification by QRT-PCR

Total RNA from liver and brain (100 mg), was isolated using Trizol (Invitrogen, Carlsbad, Calif., USA). To remove any contamination with genomic DNA, RNA samples were treated with a DNA-free kit (Applied Biosystems, Foster City, California, USA). The yield and quality of the RNA were assessed by measuring absorbance at 260, 270, 280 and 310 nm and by electrophoresis on 1.3% agarose gels. RNA of each sample (1.0 µg) was reverse-transcribed to first-strand complementary DNA (cDNA) using an iScript TM cDNA Synthesis Kit (Bio-Rad, Hercules, Calif., USA). Relative mRNA levels of COX-1, COX-2 and PPARγ were quantified using real-time PCR detection system (StepOne 18 TM, Applied Biosystems). PCR reagent (SYBR Green Master Mix, Applied Biosystems) and specific primers (900 nmol/l), were mixtured and then 5 ng of each cDNA was added. There were used three replicates per sample in each QRT-PCR reaction. Specific primers were synthesized commercially (Invitrogen Custom Primers) and the sequences were: COX-1 (NM_008969.4) 5'-CGATCTGGCTTCGTGAAC-3'(F),5'-GAGCTGCAGGAAATAGCC-3' (R); COX-2 (NM_011198.4) 5'-GGCCATGGAGTGGACTTA-3'(F), 5'-CTGCAGGTTCTCAGGGAT-3'(R), PPARγ (NM_001127330.2) 5'-CGGTTTCAGAAGTGCCTTGC-3'(F), 5'-CCGCCAACAGCTTCTCCTT3' (R) and β–actin (NM_007393.5) 5´-ACGAGGCCCAGAGCAAGAG-3´ (F), 5´-GGTGTGGTGCCAGATCTTCTC-3´ (R). The standard curves to test adequate efficiency of QRT-PCR reactions were generated on separate runs using several serial dilutions (1/10–1/1000) of pooled cDNA samples. All the efficiencies of the primers were 100 (SEM 10) %. Target genes were normalized with the housekeeping gene β-actin [33,34]. The results were expressed as fold changes of the threshold cycle (Ct) value relative to controls using the $2^{-\Delta\Delta Ct}$ method [35].

Liver and brain histology

In another set of animals, liver and brain samples were taken at the time that mice were killed. The total brain and three hepatic fragments were extracted from the right, left and middle

lobes, being divided into samples of ~ 0.125 cm3 for histological evaluation (fixation in 10% formalin in PBS). Tissues were included in paraffin and 3-5 sections (5 µm) of each sample were obtained; liver and brain sections were stained with hematoxylin-eosin (H&E) and also Masson's trichromy was used in liver [36]. Initially all the preparations were evaluated at 10x, subsequently, 20 consecutive fields were analyzed with the 40x objective. For the liver, the hepatocytes of the three zones of the hepatic accia of Rappaport were studied: periportal, intermediate and perivenous. The following histological characteristics of the liver tissue were detailed: hepatocyte (relation nucleus cytoplasm, chromatin and presence or absence of intracytoplasmic inclusions), periportal spaces and characteristics of the triad [37,38]. Finally it was evaluated in all cases presence or not of ballonizing degeneration and lipid micro- or macro-vesicles, dilatation of the centrilobular veins (qualitative observation), inflammatory infiltrate in the portal cells and increased connective tissue in the hepatic parenchyma (Masson's trichromic stain). Sections were photographed by a 10MG CMOS camera with TSView software.

Statistical analysis

Values in Tables 2 to 4 were expressed as the mean with their standard error of the mean (SEM) (n=6). The sample size was calculated taking into account a minimum test power of 0.78 (α=0.05), and a maximum difference between the response of each variable of the control group with respect to the average of the differences with the experimental groups [39]. The statistical analysis of values was performed using SPSS 17.0 (SPSS inc., Chicago, IL, USA) by one-way ANOVA. All post-hoc multiple comparisons were made using Tukey´s critical range test. In all cases, assumptions were analyzed, the normality test with the Ryan Joiner test [40] and the homogeneity of variance test with the Bartlet test. Significant differences were considered at $P<0.05$.

Results

All diets were well accepted and data about growth and did not affect development of mice negatively.

Serum interleukin levels

The levels of IL-1b and IL-6 in serum are shown in Table 2. Serum IL-1b and IL-6 levels were augmented in O vs. C and R diets. When these diets were supplemented with TFA, only IL-1b was augmented in Ot vs. O diet. TFA addition did not produce changes in Ct vs. C or in Rt vs. R diets.

Oxidative status parameters

The amount of MDA formed, CAT activity and reduced GSH levels in brain and liver are shown in Table 3. In brain, the levels of MDA were increased in animals fed corn oil compared with olive and rapeseed oil diets, being corn>olive>rapeseed oil. MDA levels were decreased in Ot vs. O diet. CAT activity, was lower in animals fed corn oil than in animals fed olive oil; TFA did not induce alterations in CAT activity in the different experimental diets. GSH levels were not affected by different oils; however, it was diminished by supplementation with TFA in Ot, Ct and Rt diets. In liver, MDA levels were augmented in animals fed corn oil (C and Ct diets) compared with olive and rapeseed oil diets, however TFA supplementation did not induce any effect. The hepatic antioxidant defenses analyzed CAT activity and reduced GSH levels, were augmented by the consumption of rapeseed oil (R and Rt diets) and the TFA supplementation did not modify these parameters.

Relative mRNA levels

The relative mRNA levels of COX-1, COX-2 and PPARγ in brain and liver are shown in Table 4. In brain, no significant differences were observed in the relative mRNA levels of COX-1 by the consumption of the different diets, while COX-2 relative mRNA levels was significantly increased in mice fed corn oil diets (C and Ct). Brain relative mRNA levels of PPARγ were no changed between O, C and R diets. In liver, the relative mRNA levels of COX-1 and PPARγ were augmented in R and Rt diets while COX-2 relative mRNA levels was

increased in C and Ct diets. TFA suplementation (Ot vs. O, Ct vs. C or Rt vs. R diet) did not modify the relative mRNA levels of COX-1, COX-2 or PPARγ in brain or liver.

Liver and brain histology

Histopathologic H&E stained sections are shown in figures 1 and 2, and possitive sections to Masson´s trichromic stain are shown in figure 3. In brain (figure 1), the sections of animals fed olive (O and Ot) and corn oil (C and Ct) showed intraparenchymal inflammatory foci and choroid plexuses with subependymal infiltrates. None of those alterations were observed in the brain histopathologic sections of animals fed rapeseed oil (R and Rt). In liver (figure 2), in O group, lipid microvacuoles were observed around the central vein and scattered in the parenchyma, while the TFA supplementation to this diet showed a process of ballooning and lipid microvacuoles without an specific distribution. In C diet was observed lipid vacuoles mainly around the central vein, and also in periportal tissue. The Ct diet showed accumulation of lipid vacuoles near blood vessels with a similar pattern to C diet. The R and Rt diets showed only a few isolated microvacuoles in the parenchyma. The presence of lipid vacuoles depended on the source of dietary fat and was more exacerbated in the livers of animals fed corn oil. In Figure 3 (Masson's trichromic stain), C and Ct diets showed increased connective tissue.

Discussion and conclusions

The present work aimed to investigate the influence of TFA supplementation in diets containing different content of n-9, n-6 and n-3 FA on the oxidative stress and inflammatory response in brain and liver. The hepatic parameters were investigated considering that dietary FA are bioconverted in this tissue and then PUFA are transported to the brain.

The type of dietary oil (O, C or R) more than the TFA supplementation (Ot, Ct or Rt) produced the greatest changes on the stress oxidative parameters, COX-2 or PPARγ relative mRNA levels and histological analysis. A posible explanation of that could be related that the effect of TFA depend to different variables like as type and level of the dietary TFA isomer, metabolization rate and release from the tissue, and possible other factors like animal model sex,

age and physiological status [23,41,42]. Literature data indicates that the intake of TFA increased plasmatic marker of inflammation and decreased the cellular defense against oxidative stress [43,44]. In a previous study, we have observed that the level of TFA in brain of mice fed Ot, Ct and Rt diets was 0.96, 0.81 and 0.56 % respectively. The lowest percentage was found in animals fed Rt diet [18]. Other studies have reported that the incorporation of TFA into brain membranes is related to changes to its fluidity and permeability [45], density and function of transmembrane receptor, as well as modifications in neurotransmitter release [43]. Our present results showed that TFA supplementation only increased the IL-1b levels in animals fed olive oil. Other authors, found that TFA may impart their effect by enhancing intrinsic signaling mechanisms leading to a chronic pro-inflammatory state [46,47].

Dietary FA composition, as one of the major factors capable of modulating oxidative stress in brain and others tissues [8,48]. Our results in brain showed that animals fed R and Rt diets presented the lowest MDA levels. On the other hand, C and Ct diets produced the highest LPO associated with a diminished CAT activity. Regarding GSH levels, the variation of this parameter can be related as a signal of oxidative damage or as protection against its production. It is possible that the decreased observed after TFA supplementation will occur as a preventive effect of LPO induced by these isomers. GSH could be acting providing an initial and rapid response to oxidative stress, by combining directly with various compounds (O_2^-, lipid peroxides, proteins) or could participates as a cofactor of antioxidant enzymes (GSH-Peroxidase, Glutathione-S-transferase and others) [49,50]. In agreement with our results, Pase *et al*. [16] found that diet female rat supplementation with fish oil, was associated with lower brain oxidative damage after acute restrain stress exposure. Also they observed that TFA supplementation was associated with higher LPO in cortex of animals unexposed to stress. Chen *et al*. [51] found that n-3 PUFA supplementation inhibited gene expression of inflammatory factors (TNF-α, IL-1β, IL-6, and IFN-γ), reduced brain edema, decreased neuronal apoptosis, and improved neurological functions after traumatic brain injury in rats. Other authors also found that diet n-3 PUFA supplementation reduced brain or spinal cord

oxidative stress, apoptosis or levels of inflammatory markers in several rodent models [52-54]. On the other hand, in liver, we have observed that LPO was increased in animals fed C and Ct diets. Nevertheless, in this tissue the CAT activity and GSH content were increased in mice fed R oil diets. These results are in concordance with Wang *et al.* [48], who observed that an increase of n-3 PUFA in the diet increased activity and gene expression of some antioxidant enzymes in liver. Also a decreased hepatic injury, inflammation and oxidative stress in mice or rat models after diet n-3 PUFA supplementation was found by others authors [55-57]. No changes were observed in our study in oxidative parameters after TFA supplementation, in both tissues.

N-3 PUFA have anti-inflammatory and antiapoptotic effects, leading to the protection of neurons in the aged and damaged brain [58]. Rapoport *et al.* [4] demonstrated that n-3 PUFA deficient diet during 15-week increased COX-2 relative mRNA levels, protein expression and enzyme activity in brain. Also Kim *et al.* [59], who studied the threshold changes in brain lipids and lipid enzymes during dietary n-3 PUFA deprivation (4,6%-0,2% α-LNA), found that, at 0,2 % dietary α-LNA, brain COX-1 relative mRNA levels were reduced, while COX-2 were increased. It has been demonstrated that changes in ARA metabolism would promote neuronal damage in experimental ischemia model, as well as neuroinflammation, and cerebral trauma [60, 61]. This could be one of the multiple factors that can explain why the deficiency of n-3 PUFA in the diet is critical in several brain diseases [62-64]. In a previous work we demonstrated that ARA levels, ∑ n-6 LC-PUFA and LC-PUFA n-6/n-3 ratio in brain were increased after O and C diets [18]. In the present study, we observed higher COX-2 relative mRNA levels in the brain of animals fed C diet compared with those animals fed R and O diet. Other authors found that, in rats fed n-6 PUFA deficiency, gene expression of enzymes related to the 20:4n-6 cascade like cytosolic phospholipase A_2 (cPLA2-IVA) and COX-2 in brain, were down- regulated, whereas expression of DHA- preferring calcium independent phospholipase A2 (iPLA2-VIA) [65-69] and of 15-lipoxygenase, were up-regulated [70]. In the same way, our results showed that the alterations observed in parameters related to oxidative stress and the

increase in COX-2 relative mRNA levels in animals fed corn oil was related to the histological study showing certain morphological alterations like intraparenchymal inflammatory foci and choroid plexuses with subependymal infiltratefoci. Other authors demonstrated that rapeseed oil had brain protective properties after different injuries, and also diets with different n-6/n-3 PUFA ratios during maternal pregnancy and lactation provoked diverse brain histological changes [7,58,71].

On the other hand, in liver, we observed an increase in the relative mRNA levels of COX-1 and PPARγ in the liver of animals fed rapeseed oil. Similarly, Du *et al*. [72] demonstrated that hepatic levels of COX-2 mRNA were down-regulated after the intake of a mixture of DHA rich fish oil and soybean oil in rats with acute hepatitis. Also, Lian *et al.* [13], found that PPARγ expression in liver tissues was significantly elevated at mRNA and protein level in animals fed low and high concentration of n-3 PUFA. In relation to the results observed in our work, the histological liver sections of animals fed C and Ct diet had a high content of lipid vesicles distributed with a defined pattern, while the animals fed R and Rt diet showed few vesicles scattered on the parenchyma. The liver of animals fed O and Ot diet showed an intermediate state between corn and rapeseed oils. Our observations are in agreement with Lian *et al*. [13], who demonstrated that mice consuming n-3 PUFA-enriched diet had less liver injury reflected by liver histology analysis. Others authors observed that rats fed corn oil presented in a dose-dependent manner, a progressive development of hepatic lipid accumulation, macrophage infiltration and oxidative stress [73,74]. Bagga *et al.* [75], proposed that the increase of LA and ARA in the liver lipids favors the production of pro-inflammatory eicosanoids. Previously, we have shown that liver of animals fed R diet had higher levels of DHA and LC-PUFA n-6/n-3 ratio was greatly decreased compared to animals fed C or O diet [18]. These previous results are aligned to the histological observations found in the liver of animals at the present work.

TFA supplementation did not modify relative mRNA levels of COX and PPARγ and the histologycal analysis in brain and liver.

In summary, the results of the present study showed evidence that rapeseed oil had protective effects on some parameters related to oxidative stress and inflammation in both liver and brain. In addition, it was shown that corn oil produced effects in the opposite directions to that produced by rapeseed oil. Nevertheless, TFA supplementation did not showed marked effects in the evaluated parameters at the level used in this study. It is important to remark, as stated earlier, that the possible effects of the incorporation of TFA from the diet in a particular tissue, would be related to both the type and the level of the isomer, as well as its absorption and metabolism.

To conclude, a higher intake of dietary oil with low FA n-6/n-3 ratio, may promote a protective effect in brain and liver. Although more studies should be done, we could suggest that diets with high n-3 FA content could be recommended in order to prevent inflammation and oxidative stress.

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Author contributions

Marcela González and Jimena Lavandera conceived and designed the study, and also were responsible of data handling, writing and interpretation. Verónica Reus participated in the collection, analysis and interpretation of the histological sections. Juliana Saín and Claudio Bernal collaborated in the interpretation of results, and in the writing and revision of the article. All authors have contributed to an approved the final version, and have agreed to submit the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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Table 1: Fatty acid composition of experimental diets

Fatty acid	O	Ot	C	Ct	R	Rt
SFA						
14:0	ND	ND	0.03	ND	0.07	ND
16:0	17.10	16.40	12.21	11.76	3.99	6.11
17:0	0.08	ND	ND	0.12	ND	ND
18:0	1.58	4.52	1.93	4.49	2.22	4.54
20:0	0.30	0.41	0.50	0.39	0.52	0.54
22:0	0.13	0.20	0.16	0.22	0.24	0.30
Total	19.18	21.54	14.84	16.98	7.04	11.49
MUFA						
*c*9-16:1	1.97	1.72	0.12	0.11	0.19	0.15
(*t*6+ *t*7+ *t*8)-18:1	ND	1.57	ND	1.57	ND	1.65
*c*6-18:1	ND	1.90	ND	1.79	ND	2.04
*t*9-18:1	ND	2.20	ND	2.45	ND	2.14
*c*9-18:1	55.18	47.17	31.95	28.97	61.11	51.55
*c*11-18:1	4.76	3.68	0.54	1.26	3.49	3.27
*t*10-18:1	ND	2.75	ND	3.06	ND	2.99
*t*11-18:1	ND	2.46	ND	2.53	ND	2.57
*c*11-20:1	0.24	0.23	0.25	0.25	0.90	0.67
Total	62.16	63.69	32.86	41.98	65.69	67.01
Total TFA	ND	9.26	ND	9.88	ND	9.62
PUFA						
*t*9, *t*12-18:2	ND	0.27	ND	0.27	ND	0.27
*c*9,*c*12-18:2	17.21	11.68	51.26	38.38	18.41	13.28
*c*9,*c*12,*c*15-18:3	0.74	0.61	0.88	0.72	8.64	6.18

*c*5,*c*8,*c*11,*c*14-20:4	0.23	0.37	ND	ND	ND	ND
*c*5,*c*8,*c*11,*c*14,c17-20:5	ND	ND	0.15	0.18	ND	ND
Total	18.19	12.92	52.30	39.55	27.05	19.73
Unidentified	0.47	1.85	ND	1.58	0.22	1.77
∑n-3 PUFA	0.74	0.61	1.03	0.90	8.64	6.18
∑n-6 PUFA	17.44	12.05	51.26	38.38	18.41	13.28

Values are expressed as mean (% of total FAME); O: olive oil diet; C: corn oil diet; R: rapeseed oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet. SFA: Saturated Fatty Acids. MUFA: Monounsaturated Fatty Acids. PUFA: Polyunsaturated Fatty Acids. TFA: trans fatty acids. ND: Undetected.

Table 2: Serum interleukin levels

	O	Ot	C	Ct	R	Rt
IL-1b (pg/ml)	21.6^{a}±1.9	33.8^{b}±3.7	11.7^{c}±1.4	18.4^{ac}±2.0	14.5^{c}±0.7	17.8^{ac}±1.7
IL-6 (pg/ml)	28.1^{a}±1.1	30.8^{a}±2.3	18.05^{b}±1.9	17.19^{b}±0.3	19.5^{b}±4.6	16.7^{b}±3.8

Data are expressed as mean±SEM of n=6 per group. Different letters in each row indicate statistical differences at P<0.05. Statistical analyses between different groups were established by one-way ANOVA, followed by Tukey's test. O: olive oil diet; C: corn oil diet; R: rapeseed oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet; IL-1b: interleukin 1b; IL-6: interleukin 6.

Table 3: Brain and liver oxidative stress and antioxidant response

	O	Ot	C	Ct	R	Rt
Brain						
LPO (nmolMDA/ g of tissue)	125.8^{a}±5.2	111.2^{b}±2.1	145.1^{c}±2.4	139.8^{c}±1.2	101.9^{d}±1.6	105.6^{bd}±2.4
CAT (µmol/min/ mg protein)	4.7 ^{a}±0.2	4.5^{a}±0.3	2.9^{b}±0.2	2.9^{b}±0.4	3.5^{ab}±0.1	3.4^{ab}±0.5
GSH (µmol/g)	0.72^{ab}±0.06	0.49^{cd}±0.03	0.81^{a}±0.02	0.42^{d}±0.08	0.79^{a}±0.01	0.59^{bc}±0.01
Liver						
LPO (nmolMDA/g of tissue)	140.0^{a}±4.2	138.2^{a}±5.1	201.5^{b}±2.6	189.7^{b}±8.3	142.2^{a}±5.6	140.2^{a}±4.1
CAT (µmol/min/ mg protein)	108.8^{a}±5.0	111.6^{a}±4.4	107^{a}.1±2.8	110.8^{a}±3.9	141.2^{b}±3.6	137.6^{b}±2.1
GSH (µmol/g of tissue)	3.30^{a}±0.28	4.40^{ab}±0.46	3.50^{a}±0.27	4.30^{ab}±0.35	4.80^{b}±0.31	4.90^{b}±0.35

Data are expressed as mean±SEM of n=6 per group. Different letters in each row indicate statistical differences at $P<0.05$. Statistical analyses between different groups were established by one-way ANOVA, followed by Tukey's test. O: olive oil diet; C: corn oil diet; R: rapeseed

oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet; LPO: lipid peroxidation; MDA: malondialdehyde; CAT: catalase; GSH: glutathione.

Table 4: Brain and liver relative mRNA levels of COX-1, COX-2 and PPARγ

	O	Ot	C	Ct	R	Rt
Brain						
COX-1	1.00±0.12	0.93±0.05	0.84±0.12	0.84±0.09	0.90±0.04	0.84±0.19
COX-2	1.00^{a}±0.13	1.20^{a}±0.28	2.23^{b}±0.08	1.91^{c}±0.19	1.32^{a}±0.03	0.98^{a}±0.17
PPARγ	1.00±0.10	1.01±0.05	1.04±0.27	0.99±0.10	1.35±0.15	1.46±0.25
Liver						
COX-1	1.00^{a}±0.16	1.32^{ab}±0.40	0.76^{a}±0.08	0.84^{a}±0.14	1.62^{b}±0.12	1.89^{b}±0.24
COX-2	1.00^{a}±0.04	1.23^{a}±0.29	3.23^{b}±01.18	2.85^{b}±0.71	1.42^{a}±0.75	1.31^{a}±0.62
PPARγ	1.00^{a}±0.08	1.30^{a}±0.23	0.97^{a}±0.04	1.25^{a}±0.18	2.85^{b}±0.15	2.76^{b}±0.49

Data are expressed as mean±SEM of n=6 per group (relative units). Different letters in each row indicate statistical differences at $P<0.05$. Statistical analyses between different groups were established by one-way ANOVA, followed by Tukey's test. O: olive oil diet; C: corn oil diet; R: rapeseed oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet; COX: cyclooxygenase; PPARγ: peroxisome proliferator-activated receptor gamma.

Figure Captions

Figure 1: Microphotographs of brain H&E stained. 10x. O: olive oil diet; C: corn oil diet; R: rapeseed oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet. The arrows indicate choroid plexuses with subependymal infiltrates (⇨) and intraparenchymal inflammatory foci (➞).

Figure 2: Microphotographs of liver H&E stained. 10x. O: olive oil diet; C: corn oil diet; R: rapeseed oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet. The arrows indicate lipid microvacuoles (➞), ballooning degeneration (⇓) and lipid vacuoles (➡).

Figure 3: Microphotographs of liver Masson´s trichromic stained. 10x. O: olive oil diet; C: corn oil diet; R: rapeseed oil diet; Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet. The arrows indicate areas with increased connective tissue (⇨).

Figure 1

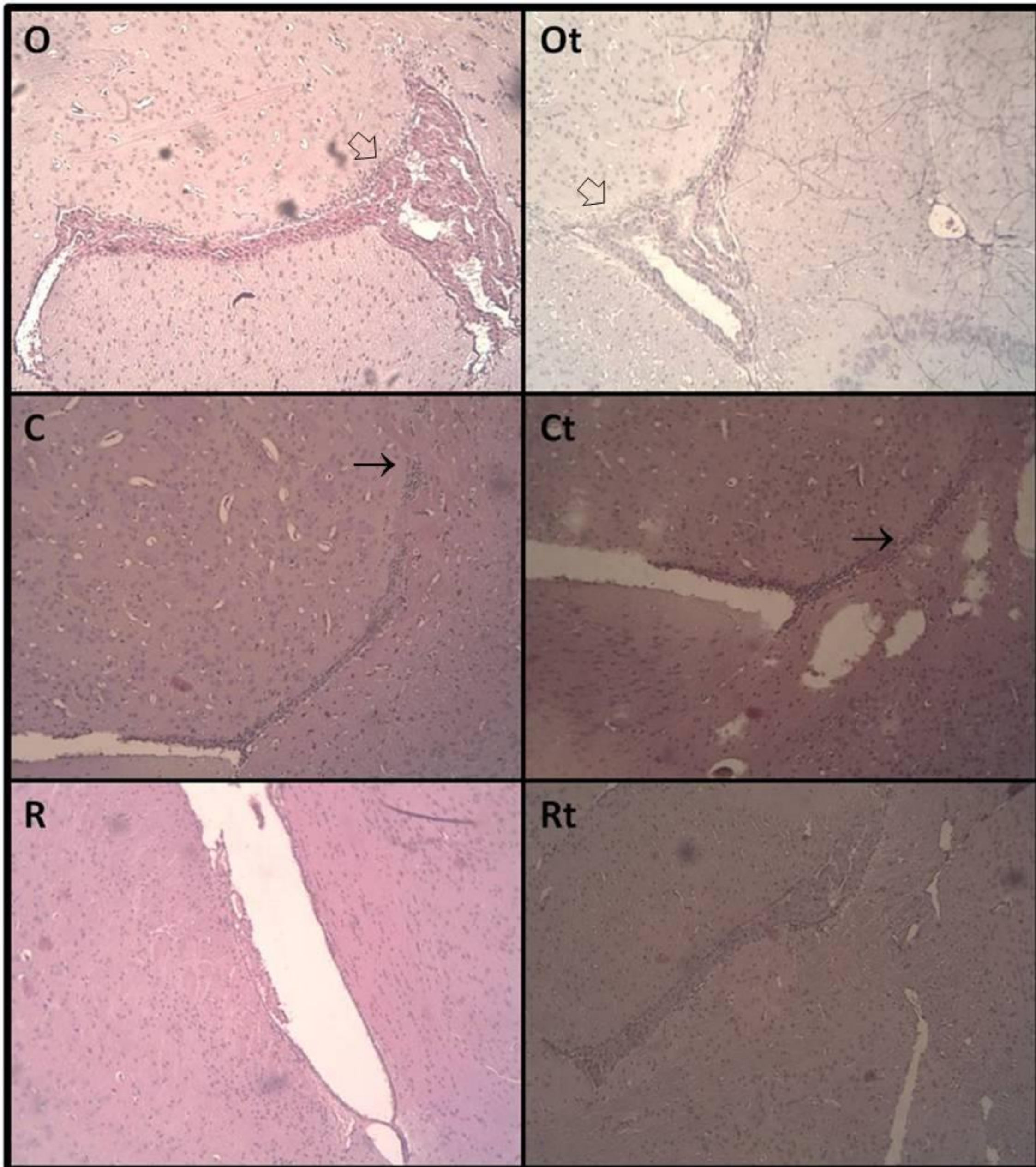

Figure 2

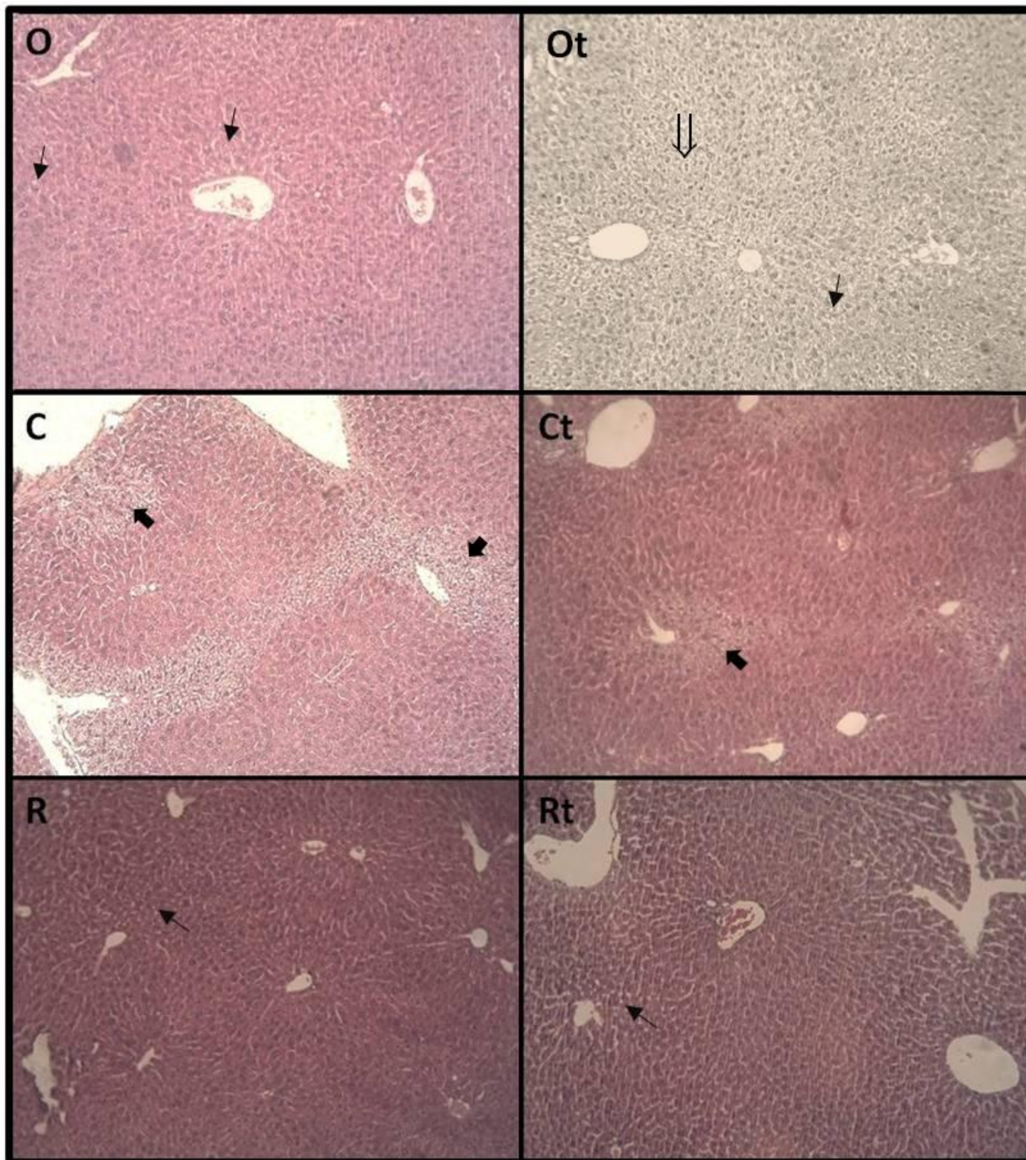

Figure 3