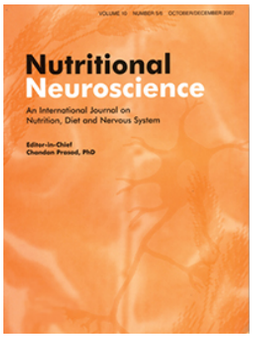

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N-3 fatty acids reduced trans fatty acids retention and increased docosahexaenoic acid levels in the brain

Jimena Verónica Lavandera[1,2], Juliana Saín[1,2], Ana Clara Fariña[1], Claudio Adrián Bernal[1,2], Marcela Aída González[1]

[1]Cátedra de Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina

Introduction: The levels of docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are critical for the normal structure and function of the brain. Trans fatty acids (TFA) and the source of the dietary fatty acids (FA) interfere with long-chain polyunsaturated fatty acids (LC-PUFA) biosynthesis.
Objectives: The aim of this study was to investigate the effect of TFA supplementation in diets containing different proportions of n-9, n-6, and n-3 FA on the brain FA profile, including the retention of TFA, LC-PUFA levels, and n-6/n-3 PUFA ratios. These parameters were also investigated in the liver, considering that LC-PUFA are mainly bioconverted from their dietary precursors in this tissue and transported by serum to the brain. Also, stearoyl-CoA desaturase-1 (SCD1) and sterol regulatory element-binding protein-1c (SREBP-1c) gene expressions were evaluated.
Methods: Male CF1 mice were fed (16 weeks) diets containing different oils (olive, corn, and rapeseed) with distinct proportions of n-9, n-6, and n-3 FA (55.2/17.2/0.7, 32.0/51.3/0.9, and 61.1/18.4/8.6), respectively, substituted or not with 0.75% of TFA. FA composition of the brain, liver, and serum was assessed by gas chromatography.
Results: TFA were incorporated into, and therefore retained in the brain, liver, and serum. However, the magnitude of retention was dependent on the tissue and type of isomer. In the brain, total TFA retention was lower than 1% in all diets.
Discussion: Dietary n-3 PUFA decreased TFA retention and increased DHA accretion in the brain. The results underscore the importance of the type of dietary FA on the retention of TFA in the brain and also on the changes of the FA profile.

Keywords: Trans fatty acids, Brain, Fatty acids profile, Olive oil, Rapeseed oil, Corn oil, Long-chain polyunsaturated fatty acids

Introduction

Lipids are mainly found in nerve cell membranes as complex lipids and their fatty acids (FA) play a key role. The brain is rich in docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6), which are critical for its normal structure and function.[1,2] These long-chain polyunsaturated fatty acids (LC-PUFA) regulate multiple processes, including cell signaling and gene transcription, and also alter membrane fluidity.[3–6] LC-PUFA must be obtained directly through the diet or be converted from their respective dietary precursors, linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3),[7,8] because they and their precursors cannot be synthesized *de novo* in vertebrate tissues.[9] In the brain, the conversion coefficients of ALA to DHA are very low with only 1% of plasma ALA entering the brain converted to DHA. Therefore, circulating plasma DHA derived from diet and biosynthesized from ALA in the liver are the sources for brain accretion of DHA.[9–11]

On the other hand, in some countries, the high intake of trans fatty acids (TFA) coming from bakery or processed food is a matter of concern due to its negative effects on human health.[12,13] Nowadays, several efforts have been made to reduce and/or eliminate TFA from food,[14,15] since their considerable influence in the development of cardiovascular disease[16] significantly impairs human health.[17,18] It is important to know that TFA, even consumed at small amounts, can be incorporated in neuronal membranes. TFA are

Correspondence to: Marcela Aida González, Bromatología y Nutrición, Departamento de Ciencias Biológicas, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, C.C. 242. (3000) Santa Fe, Argentina.
Email: maidagon@fbcb.unl.edu.ar

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able to exert long-term deleterious influence affecting the functioning of the central nervous system.[18–21]

In the diets of Western industrialized countries, unbalanced proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and n-6 and n-3 PUFA are very common. Thus, as a consequence of the ubiquitous supply of various n-6-rich oils (corn, sunflower, safflower, and cottonseed),[12] these diets are characterized to be rich in LA and poor in ALA, resulting in a higher and not recommended n-6/n-3 ratio. The impact of the amount of dietary ALA on its own tissue accumulation and conversion to n-3 LC-PUFA remains controversial and may depend on the other dietary FA mixed with ALA. Conventionally, the nutraceutical potential of n-3 PUFA has been restricted to eicosapentaenoic acid (EPA) and DHA and much less so to ALA. This happens because there is a very low conversion rate from ALA to n-3 LC-PUFA due to the competition between n-3 and n-6 biosynthesis. In some vegetable oils, like rapeseed (R), which contain 9% of ALA and 20% of LA (n-6/n-3 ratio close to 3), this competition is low.[22,23] Some studies have validated the ALA supplementation to the diets as an effective brain protective dietary measure.[22] Results have also shown that ALA intake is an important approach in inducing neuronal protection against ischemia, suggesting that R oil should be considered as an interesting alternative to fish oil.[23]

A proper SFA/MUFA ratio affects the FA composition of phospholipids and triglycerides and has been shown to contribute to membrane fluidity.[24] A key enzyme involved in the lipid composition of cellular membranes is the membrane-bound stearoyl-CoA desaturase-1 (SCD1), which is the rate-limiting enzyme in the cellular synthesis of MUFA from SFA.[25] This enzyme also catalyzes the conversion of vaccenic acid (t11-18:1, VA) to rumenic acid (c9,t11-18:2, RA),[26] which has been extensively studied due to the numerous beneficial effects of RA on human health.[27] The expression of SCD1 is regulated by a sterol regulatory element-binding protein-1c (SREBP-1c), a transcription factor that stimulates the expression of genes involved in fatty acid metabolism.[28]

Taking into account the above, we hypothesize that TFA interfere with LC-PUFA biosynthesis, which is also influenced by the source of the dietary FA and the time period of consumption. Thus, the aim of this study was to investigate the effect of TFA supplementation in diets containing different proportions of n-9, n-6, and n-3 FA on the FA profile in the brain, including the retention of TFA, LC-PUFA levels, and n-6/n-3 PUFA ratios. Furthermore, these parameters were also investigated in the liver, considering that LC-PUFA are mainly bioconverted from their dietary precursors in this tissue and transported to the brain by serum. In order to study some key regulators of FA desaturation, the SCD1 and SREBP-1c gene expressions were evaluated in the brain and the liver.

Methods

Animals, diets preparation, and experimental design

The experiments were conducted in male CF1 mice 4 weeks old, 22 g ($n = 6$/group) provided from the facilities at our University according to the regulations of the School of Biochemistry, *Guide to the Care and Use of Experimental Animals of Laboratory*.[29] After 15 days of adaptation period, mice were randomly divided into groups of six animals each and fed on different diets for 16 weeks: groups O, C, and R were fed diets containing olive, corn, or rapeseed oils, respectively. Groups Ot, Ct, and Rt were fed an O, C, or R diet, respectively, substituted in 1/7 part with partially hydrogenated vegetable oil (PHVO). The experimental diets were freshly prepared, gassed with nitrogen and stored at 0–4°C. The FA composition of dietary fats was determined by gas chromatography and is shown in Table 1. The O, C, and R oils provided O/LA/ALA in the proportions of: 55.2/17.2/0.7, 32.0/51.3/0.9, and 61.1/18.4/8.6, respectively. The diets were based on the American Institute of Nutrition Ad Hoc Committee recommendation (AIN-93G) formulated for the growth, pregnancy and lactation phases of rodents,[30] containing: (w/w): 200 g/kg casein, 5.5 g/kg cystine/methionine/choline, 529 g/kg corn starch, 100 g/kg sucrose, 50 g/kg cellulose, 10 g/kg of vitamin mix and 35 g/kg of mineral mix. Diets provided 64.4% of energy as carbohydrates and 19.9% of energy as protein. O, C, and R diets contained 70 g/kg olive oil, corn oil, or rapeseed oil, respectively (15.7% of dietary energy as total fat), whereas Ot, Ct, and Rt diets contained 50 g/kg olive oil, corn oil, 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). During the entire experimental dietary period, the mice were weighed and food intake was evaluated.

The PHVO was kindly provided by CALSA (Compañía Argentina de Levaduras S.A., Buenos Aires, Argentina). Olive oil, corn oil, rapeseed oil, sucrose, and corn starch were obtained from local sources. Cysteine, methionine, and choline were purchased from Sigma (St Louis, MO, USA). During the experimental period, the mice were kept under controlled conditions (23 ± 2°C and 12 hour light–dark cycle) with free access to food and water.

Extraction of tissues and serum samples

After 16 weeks of dietary treatment, animals were sacrificed (9:00–11:00 AM) under anesthesia (1 mg acepromazine + 100 mg ketamine/kg body weight)

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	0.235	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	1.854	ND	1.578	0.22	1.773

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.

by cardiac exsanguination. Blood was collected and serum was obtained after centrifugation (1000 × g for 10 minutes at 4°C). Triacylglycerol (TAG) and cholesterol levels were determined by spectrophotometric methods using a commercially available test kit (Sociedad de Bioquímicos, Santa Fe, Argentina). Liver and brain were dissected, weighed, and immediately frozen. All samples were stored at −80°C until analysis.

Gas chromatography analysis of dietary and fatty acid composition of tissues

The FA composition of the tissues, serum, and experimental diets was determined by gas chromatography using a Shimadzu (GC 2014) chromatograph equipped with flame ionization detector. Analyses were carried out with a capillary column CP Sil 88 (100 m, 0.25-μm-film thickness). The carrier gas was hydrogen with a split ratio of 1:20. The column temperature was held at 75°C for 2 minutes after injection, then 5°C/min to 170°C, held for 40 minutes, 5°C/min to 220°C and held 40 minutes. Injection volume was 0.5 μl and the column flow was 0.8 ml/min. Total fat in tissues, serum, and diets was extracted using the method described by Bligh and Dyer.[31] The fatty acids methyl esters (FAME) were formed by transesterification with methanolic potassium hydroxide solution as an interim stage before saponification (ISO 5509:2000, Point 5 IUPAC method 2.301). FAME were identified by comparison of their retention times relative to those of commercial standards. Values of FA content are expressed as percentage of total FA. Further details could be obtained from previous publications.[32]

Extraction and analysis of RNA and quantification by RT-PCR

Total RNA was isolated from 100 mg of the liver and the brain using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA samples were then treated with a DNA-free kit (Applied Biosystems, Foster City, CA, USA) to remove any contamination with genomic DNA. 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; 1.5 μg of total RNA of each sample was reverse-transcribed to first-strand complementary DNA (cDNA) using an iScript TM cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA).

Relative SCD1 and SREBP-1c mRNA levels were quantified using real-time PCR with a StepOne 18 TM Real-Time PCR Detection System (Applied Biosystems). β-Actin mRNA levels were similarly measured and used as the reference gene; 0.1 μl of each cDNA was added to the PCR reagent mixture, SYBR Green, Master Mix (Applied Biosystems),

with the upstream and downstream primers (900 nmol/l for SCD1 and SREBP-1c). Specific primers were designed (Genbank: NM_009127.4 SCD1, NM_011480.4 SREBP-1c, and NM_007393.5 β-actin) synthesized commercially (Invitrogen Custom Primers) and the sequences were SCD1: 5′-TGGG TTGGCTGCTTGTG-3′ (forward), 5′-GCGTGGG CAGGATGAAG-3′ (reverse). SREBP-1c: 3′-GGA GCCATGGATTGCACATT-5′ (forward), 3′-GC TTCCAGAGAGGAGCCCAG-5′ (Reverse), β-actin: 5′-ACGAGGCCCAGAGCAAGAG-3′ (forward), 5′-GGTGTGGTGCCAGATCTTCTC-3′ (reverse). The PCR parameters were as follows: initial 2 minutes at 50°C, denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 15 seconds and combined annealing and extension at 60°C for 1 minute. All sample mRNA levels were normalized to the values of β-actin and the results expressed as fold changes of the threshold cycle (Ct) value relative to controls using the $2^{-\Delta\Delta Ct}$ method.[33]

Statistical analysis

The statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Values were expressed as mean ± the standard error of the mean (SEM), and were statistically analyzed by 2 × 3 ANOVA. All *post hoc* multiple comparisons were made using Tukey's critical range test. Significant differences were considered at $P < 0.05$.

Results

Food intake, body and tissues weight, and biochemical parameters

Food consumption, body, liver and brain weights, serum TAG and cholesterol levels are shown in Table 2. There was no difference in food intake between the dietary groups. The body, brain, and liver weights were altered neither by the source of dietary fats nor by the TFA supplementation. Regarding the biochemical parameters, serum TAG levels were lower in animals fed corn oil than in those fed olive and canola oils. However, TFA supplementation did not affect this parameter. Serum cholesterol levels were not augmented in any group. In addition, there were no differences in this parameter by the supplementation with TFA in any diet.

Retention of TFA in brain, liver, and serum

The retention of TFA in the brain, liver, and serum is shown in Table 3. The retention of each TFA isomer was estimated using the relationship between the TFA percentages in the biological sample with respect to the TFA percentages in the respective diet. In the brain, the total amount of TFA retained was lower than 1% independently of the dietary fat. The isomer retention in this tissue was *t*9-18:1 > *t*10-18:1 > VA, and *t*9,*t*12-18:2 was not found. Interestingly, the content of the total TFA in the brain was lower in Rt than in the Ct and Ot groups. In the liver and serum, *t*9-18:1 was the major *t*-18:1 isomer retained. The VA showed the lower retention in the liver, while in the serum, the lower isomer retained was *t*10-18:1. Isomer *t*9,*t*12-18:2 was incorporated and retained in the liver and serum, being the largest TFA retention found in these tissues. In the liver, there was no significant difference between the total content of TFA incorporated and retained according to the type of fat administered, but the total TFA content in serum was higher in Ot than in Ct and Rt. Similarly to what happened in the brain, in the liver and serum the content of the main TFA retained, *t*9-18:1, *t*10-18:1 and VA was lower in the Rt group.

Fatty acid profiles in brain, liver, and serum

The type of dietary fat (O, C, or R) rather than the TFA supplementation affected the FA profiles in the brain, liver, and serum (Tables 4–6). Nevertheless, the content of FA reflected the proportion of FA in the experimental diets only in the liver and serum. In this regard, oleic acid, LA, and ALA were found in a high percentage in tissues of animals fed O, C, and R diets.

In the absence of TFA and compared with the serum and liver, a different pattern of FA was observed in the brain; specifically, oleic acid was found in similar percentages in all groups, and ALA was not detected (ND) in any diet. On the other hand, the content of LA in the brain of group C was the highest. This would be associated with elevated levels of LA in the C diet. The level of AA was similar in all groups, but the docosapentaenoic acid n-6 (DPAn-6; 22:5n-6) level was increased in the C and O diets. EPA (20:5n-3) was only detected in the R group and docosapentaenoic acid n-3 (DPAn-3; 22:5n-3) and DHA were higher in R than in the O and C diets. In the liver and serum, the content of AA and DPAn-6 was increased in animals fed the C and O diets. EPA was only detected in the animals of the R group, whereas DHA and DPA n-3 were increased in R vs. O and C groups.

The LC-PUFA n-6/n-3 ratio in the brain showed similar values between the O and C diets, whereas in the R diet this ratio decreased. The liver and serum showed a similar pattern in this ratio, but the decrease in the R diet was markedly pronounced.

In the brain, TFA supplementation did not produce any changes in the main FA previously analyzed, except DPAn-3. This FA was reduced in the presence of TFA in the C diet. In the liver, TFA decreased the content of LA in the C and R groups, and decreased the AA content in the O and C groups. Nevertheless,

Table 2 Food intake, weights of animals and tissues, and biochemical parameters

		O	Ot	C	Ct	R	Rt	ANOVA		
								F	*t*	*F* × *t*
Food intake (g/d)		3.81 ± 0.23[a]	3.73 ± 0.22[a]	3.74 ± 0.41[a]	3.25 ± 0.13[a]	3.82 ± 0.11[a]	3.44 ± 0.12[a]	0.467	0.075	0.562
Weight	Body (g)	39.75 ± 0.89[a]	37.37 ± 1.12[a]	34.12 ± 1.21[a]	36.65 ± 1.05[a]	36.90 ± 1.11[a]	35.67 ± 0.77[a]	0.003	0.321	0.653
	Brain	0.48 ± 0.01[a]	0.49 ± 0.03[a]	0.50 ± 0.01[a]	0.48 ± 0.01[a]	0.51 ± 0.01[a]	0.48 ± 0.01[a]	0.754	0.143	0.242
	Liver	1.67 ± 0.09[a]	1.77 ± 0.07[a]	1.53 ± 0.09[a]	1.76 ± 0.11[a]	1.81 ± 0.05[a]	1.60 ± 0.05[a]	0.691	0.112	0.254
Biochemical parameters	TAG mmol/l)	0.49 ± 0.06[a]	0.44 ± 0.02[ab]	0.29 ± 0.02[c]	0.29 ± 0.01[c]	0.46 ± 0.09[ac]	0.50 ± 0.03[a]	0.000	0.310	0.013
	Cholesterol (g/l)	1.08 ± 0.04[a]	1.03 ± 0.03[a]	1.03 ± 0.04[a]	1.01 ± 0.04[a]	1.02 ± 0.08[a]	1.07 ± 0.07[a]	0.217	0.678	0.557

Values are expressed as mean ± SEM of $n = 6$ per group. *F*, *t*, *F* × *t* correspond to *P* values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat × TFA. Statistical differences were indicated with different letters ($P < 0.05$). 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.

no changes were observed in the serum in those FA. On the other hand, TFA decreased the hepatic levels of ALA in R and DHA in the O groups. The content of ALA in the serum was decreased in all groups while DHA was not affected by TFA supplementation. The LC-PUFA n-6/n-3 ratio was increased by TFA supplementation in the liver and serum of the C group. The total content of PUFA in the liver was decreased by TFA in all groups.

Relative conversion rate of VA to RA

The RA was absent in the diet and was bioconverted from VA in the liver. The relative conversion rate of VA to RA via SCD1 was estimated by the *c*9,*t*11-18:2/*t*11-18:1 ratio. Animals fed TFA showed RA incorporation in the liver and serum, the content of RA being highest in the liver than in the serum (Tables 5 and 6, respectively). The brain did not show detectable levels of RA in any group. In the liver, the RA/VA ratio was higher in Ot than in the Rt and Ct groups. Nevertheless, the levels of RA in this tissue did not show significant differences between groups, while in the serum the circulating RA was higher in Ot than in the Ct and Rt groups.

Expression of SCD1 and SREBP-1c in the brain and liver

The brain and liver expression of some key genes related to the synthesis of FA, SCD1 and SREBP-1c are shown in Table 7. The gene expression of SCD1 and SREBP-1c in the brain was significantly lower in O vs. the C and R groups. In both tissues, TFA supplementation increased SCD1 and SREBP-1c mRNA expression in the animals fed olive oil. No differences were found in these expression in Ct and Rt vs. the C and R groups, respectively.

Discussion

The present study aimed to investigate the effect of TFA supplementation in diets containing different proportions of n-9, n-6, and n-3 FA on the FA profile in the brain, including the retention of TFA, LC-PUFA levels, and n-6/n-3 ratios. These parameters were also investigated in the liver, considering that LC-PUFA are mainly bioconverted from their dietary precursors in this tissue and transported by the serum to the brain. In order to study some key regulators of FA desaturation, the SCD1 and SREBP-1c gene expressions were evaluated in the brain and liver.

The addition of TFA to the diets during 4 months led to different effects on the parameters mentioned above depending on the tissue evaluated and the dietary FA composition. TFA were incorporated into, and therefore retained in the brain, liver, and serum. However, the magnitude of retention was highly dependent on the tissue and type of isomer.

Table 3 Retention of individual TFA in different tissues

	Ot	Ct	Rt
Brain			
(*t*6 + *t*7 + *t*8)-18:1	0.170 ± 0.024[a]	0.218 ± 0.034[a]	0.135 ± 0.000[a]
*t*9-18:1	2.512 ± 0.308[a]	1.916 ± 0.094[a]	1.730 ± 0.053[a]
*t*10-18:1	0.558 ± 0.058[a]	0.667 ± 0.055[a]	0.505 ± 0.003[a]
*t*11-18:1	0.524 ± 0.061[a]	0.407 ± 0.060[a]	0.096 ± 0.004[b]
Total TFA	0.960 ± 0.043[a]	0.814 ± 0.025[b]	0.567 ± 0.036[c]
Liver			
(*t*6 + *t*7 + *t*8)-18:1	5.489 ± 0.533[a]	5.265 ± 0.091[a]	5.872 ± 0.977[a]
*t*9-18:1	44.787 ± 3.719[a]	34.007 ± 5.722[a]	32.395 ± 3.447[a]
*t*10-18:1	25.816 ± 2.075[a]	21.845 ± 2.096[ab]	20.654 ± 2.986[b]
*t*11-18:1	12.957 ± 1.120[a]	18.143 ± 3.603[a]	12.03 ± 0.964[a]
*t*9,*t*12-18:2	48.133 ± 5.957[a]	32.946 ± 5.015[b]	37.450 ± 1.084[b]
Total TFA	24.813 ± 2.211[a]	21.707 ± 3.237[a]	18.428 ± 1.709[a]
Serum			
(*t*6 + *t*7 + *t*8)-18:1	7.308 ± 0.935[a]	6.193 ± 0.808[a]	5.341 ± 0.811[a]
*t*9-18:1	34.425 ± 2.226[a]	25.90 ± 0.675[ab]	24.418 ± 2.950[b]
*t*10-18:1	18.927 ± 0.858[a]	13.378 ± 0.901[b]	12.319 ± 0.722[b]
*t*11-18:1	21.057 ± 1.732[a]	23.161 ± 0.372[a]	17.704 ± 3.223[a]
*t*9,*t*12-18:2	72.941 ± 2.038[a]	50.059 ± 3.709[a]	55.472 ± 0.334[a]
Total TFA	23.844 ± 0.729[a]	19.379 ± 0.338[ab]	17.278 ± 2.160[b]

Values are expressed as mean ± SEM of $n = 6$ per group. The retention of each TFA isomer was calculated using the relation: % of isomer in the biological sample/% of isomer in the diet × 100%. Statistical differences between groups were indicated with different letters ($P < 0.05$). Ot: olive oil + TFA diet; Ct: corn oil + TFA diet; Rt: rapeseed oil + TFA diet. TFA: trans fatty acids.

In the brain, the amount of total TFA retained was lower than 1%, independently of the dietary fat. The type of dietary fat (O, C, or R) rather than the TFA supplementation affected the FA profiles in the brain, liver, and serum. Diets rich in n-3 FA decreased the TFA retention and increased the DHA accretion in the brain. The results from this study underscore the importance of the type of dietary FA on the retention of TFA in the brain and also on the changes of the FA profile. It is important to consider that the levels of TFA in a particular tissue might be related to different variables including type and level of the dietary TFA isomer, uptake, metabolization and release from the tissue, interference with different dietary FA and other factors like species, sex, age, and physiological status.

TFA may be incorporated into membrane phospholipids, altering membrane fluidity, biochemical properties, and cell function.[34] In effect, the incorporation of PUFA increases its fluidity, while the incorporation of SFA and TFA increases its rigidity.[35,36] In our previous work, a feeding period of 30 days seemed to be short to allow a significant incorporation of TFA into the brain.[32] This could be related to the fact that the brain appears to be protected from the incorporation of TFA isomers into the complex lipids in many animal species and humans.[37,38] Nevertheless, in the present study, using a longer period of feeding, TFA showed an incorporation of 0.08%. Teixeira *et al.*[18] showed an incorporation of 0.30% of TFA into the brain tissue after a 16-week intake of diets containing high levels of TFA. In this regard, the incorporation of TFA into the brain would be linked to the feeding period and quantity of the isomers administered. In agreement with our results, other authors found similar percentages of TFA incorporation into the brain.[37,39] Teixeira *et al.*[17,18] reported high anxiety-like symptoms, impairment of memory, and higher susceptibility to develop movement disorders in aging rats, associated with the incorporation of TFA in brain membranes. In the brain, TFA showed a pattern of retention in which *t*9-18:1>*t*10-18:1>VA. In this tissue, RA was ND. It could be related to the low bioconversion from VA to RA in the liver and, therefore, the low levels of RA transported in the serum. In agreement with our results, Alasnier *et al.*[40] found that conjugated linoleic acid (CLA) incorporation into the brain was detected only in few cases and at very low concentrations in an animal model fed with a single dose of a CLA mixture consisting of equimolecular amounts of *c*9,*t*11-18:2+ *t*10,*c*12-18:2 isomers. Nevertheless, Faa *et al.*[41] showed a greater incorporation of CLA in the brain of animals fed with high bolus of CLA, concluding that the amount of CLA incorporated into the brain could be linked to the amount supplied in the diet.

The liver showed a higher capacity of retention of TFA than the brain. Independently of the dietary fat, in the liver, the levels were *t*9-18:1>*t*10-18:1>VA and might be explained by their relative metabolization rate. In this regard, the reduced hepatic levels of VA might be mainly associated with a high conversion to RA. Even though there is no evidence about the comparative oxidative rate of the individual *t*-18:1, the lower levels of *t*10-18:1 in the liver compared with those of *t*9-18:1 could be related to a higher rate of oxidation and/or metabolization of this isomer.[32]

Table 4 Fatty acid composition in the brain

Fatty acids	O	Ot	C	Ct	R	Rt	ANOVA		
							F	***t***	***F*** × ***t***
16:0	24.291 ± 1.467	24.194 ± 0.827	23.628 ± 0.158	23.441 ± 0.177	23.405 ± 0.368	22.815 ± 0.155	0.312	0.625	0.935
17:0	0.122 ± 0.014^{ab}	0.106 ± 0.005^{ab}	0.104 ± 0.010^{ab}	0.130 ± 0.003^{ab}	0.132 ± 0.007^{b}	0.086 ± 0.014^{a}	0.709	0.149	0.009
18:0	20.943 ± 0.106^{ab}	21.006 ± 0.098^{ab}	20.217 ± 0.377^{a}	21.096 ± 0.063^{b}	20.915 ± 0.06^{ab}	20.209 ± 0.091^{a}	0.081	0.585	0.002
20:0	0.239 ± 0.02	0.216 ± 0.02	0.280 ± 0.030	0.260 ± 0.020	0.280 ± 0.030	0.26 ± 0.010	0.117	0.239	0.989
22:0	0.184 ± 0.01	0.188 ± 0.01	0.180 ± 0.010	0.170 ± 0.010	0.210 ± 0.010	0.19 ± 0.010	0.087	0.454	0.566
24:0	0.093 ± 0.014	0.087 ± 0.009	0.073 ± 0.002	0.080 ± 0.011	0.102 ± 0.004	0.088 ± 0.009	0.145	0.566	0.531
*c*9-16:1	0.498 ± 0.069^{ab}	0.474 ± 0.040^{ab}	0.538 ± 0.008b^{ab}	0.448 ± 0.013^{a}	0.403 ± 0.015^{a}	0.685 ± 0.022^{c}	0.316	0.320	0.000
(*t*6 + *t*7 + *t*8)-18:1	0.000 ± 0.000^{a}	0.003 ± 0.000^{b}	0.000 ± 0.000^{a}	0.003 ± 0.001^{b}	0.000 ± 0.000^{a}	0.002 ± 0.000^{b}	0.170	0.000	0.170
*t*9-18:1	0.000 ± 0.000^{a}	0.055 ± 0.007^{b}	0.000 ± 0.000^{a}	0.047 ± 0.002^{bc}	0.000 ± 0.000^{a}	0.038 ± 0.001^{c}	0.035	0.000	0.035
*t*10-18:1	0.000 ± 0.000^{a}	0.015 ± 0.002^{b}	0.000 ± 0.000^{a}	0.018 ± 0.003^{b}	0.000 ± 0.000^{a}	0.015 ± 0.006^{b}	0.891	0.000	0.891
*t*11-18:1	0.000 ± 0.000^{a}	0.013 ± 0.001^{b}	0.000 ± 0.000^{a}	0.010 ± 0.002^{b}	0.000 ± 0.000^{a}	0.002 ± 0.000^{c}	0.889	0.001	0.889
*c*9-18:1	17.890 ± 0.727	17.569 ± 0.356	19.232 ± 1.041	17.274 ± 0.153	18.726 ± 0.169	19.359 ± 0.172	0.095	0.246	0.098
*c*11-18:1	5.626 ± 0.198	5.593 ± 0.099	5.312 ± 0.104	5.484 ± 0.043	5.331 ± 0.130	5.462 ± 0.119	0.184	0.394	0.690
*c*11-20:1	2.552 ± 0.03^{ab}	2.425 ± 0.08^{a}	2.49 ± 0.11^{a}	2.70 ± 0.03^{ab}	2.83 ± 0.02^{b}	2.64 ± 0.07^{ab}	0.007	0.482	0.020
*c*13-22:1	0.226 ± 0.019	0.225 ± 0.012	0.203 ± 0.004	0.221 ± 0.025	0.152 ± 0.017	0.223 ± 0.020	0.127	0.062	0.140
*c*9,*c*12-18:2	0.428 ± 0.018^{a}	0.463 ± 0.073^{ab}	0.785 ± 0.020^{b}	0.704 ± 0.136^{ab}	0.553 ± 0.038^{ab}	0.591 ± 0.035^{ab}	0.004	0.961	0.634
*c*11, *c*14-20:2	0.079 ± 0.000^{a}	0.087 ± 0.000^{a}	0.210 ± 0.000^{c}	0.15 ± 0.010^{b}	0.11 ± 0.010^{a}	0.100 ± 0.01^{a}	0.00	0.008	0.001
*c*8, *c*11, *c*14-20:3	0.202 ± 0.010^{a}	0.222 ± 0.02^{a}	0.250 ± 0.01^{a}	0.22 ± 0.010^{a}	0.37 ± 0.020^{b}	0.370 ± 0.020^{b}	0.00	0.748	0.201
*c*11, *c*14, *c*17-20:3	0.022 ± 0.010	0.016 ± 0.00	0.020 ± 0.000	0.02 ± 0.00	0.01 ± 0.000	0.020 ± 0.000	0.883	0.527	0.067
*c*5, *c*8, *c*11,*c*14-20:4	9.230 ± 0.340^{ab}	9.562 ± 0.16^{b}	9.120 ± 0.280^{abc}	9.76 ± 0.08^{b}	8.41 ± 0.090^{ac}	8.230 ± 0.130^{c}	0.00	0.142	0.164
c7,c10,c13,c16-22:4	3.110 ± 0.222^{ab}	3.098 ± 0.097^{ab}	3.173 ± 0.124^{b}	3.453 ± 0.077^{b}	2.557 ± 0.043^{ac}	2.439 ± 0.027^{c}	0.000	0.611	0.252
*c*5, *c*8, *c*11,c14,c17-20:5	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.034 ± 0.003^{b}	0.037 ± 0.002^{b}	0.000	0.475	0.594
*c*4,*c*7,*c*10,*c*13,*c*16-22:5	0.910 ± 0.134^{a}	0.995 ± 0.048^{a}	1.166 ± 0.131^{ab}	1.436 ± 0.043^{b}	0.093 ± 0.06^{c}	0.101 ± 0.013^{c}	0.000	0.094	0.289
c7,*c*10,*c*13,*c*16,*c*19-22:5	0.059 ± 0.001^{a}	0.061 ± 0.002^{ab}	0.080 ± 0.006^{b}	0.051 ± 0.003^{a}	0.190 ± 0.002^{c}	0.192 ± 0.007^{c}	0.000	0.029	0.004
*c*4, *c*7, *c*13,*c*16,*c*19-22:6	12.258 ± 0.136^{a}	12.349 ± 0.176^{a}	11.699 ± 0.504^{a}	11.899 ± 0.360^{a}	14.121 ± 0.198^{b}	14.303 ± 0.157^{b}	0.000	0.913	0.751
Σ NI	0.833 ± 0.017	0.834 ± 0.030	0.740 ± 0.070	0.830 ± 0.007	0.831 ± 0.024	0.860 ± 0.062			
Σ Total SFA	46.076 ± 1.443	45.941 ± 0.743	44.611 ± 0.364	45.278 ± 0.140	45.278 ± 0.235	43.759 ± 0.281	0.131	0.575	0.320
Σ Total MUFA	26.793 ± 0.972	26.286 ± 0.417	28.043 ± 1.205	26.124 ± 0.182	27.443 ± 0.234	28.372 ± 0.326	0.171	0.386	0.153
Σ Total PUFA	26.298 ± 2.430	26.852 ± 1.139	26.598 ± 1.019	27.689 ± 0.311	26.449 ± 0.486	26.941 ± 0.650	0.906	0.471	0.952
Σ n-6 LC-PUFA	10.421 ± 0.473^{a}	10.866 ± 0.215^{a}	10.744 ± 0.410^{a}	11.564 ± 0.076^{a}	8.985 ± 0.080^{b}	8.802 ± 0.087^{b}	0.000	0.136	0.227
Σ n-3 LC PUFA	12.338 ± 1.747^{a}	12.425 ± 0.912^{a}	11.802 ± 0.496^{a}	11.967 ± 0.359^{a}	14.698 ± 0.116^{a}	15.072 ± 0.547^{a}	0.011	0.775	0.986
LC-PUFA n-6/n-3	0.872 ± 0.095^{a}	0.882 ± 0.052^{a}	0.911 ± 0.005^{a}	0.968 ± 0.030^{a}	0.628 ± 0.014^{b}	0.585 ± 0.015^{b}	0.000	0.830	0.578
Σ TFA	0.000 ± 0.000a	0.086 ± 0.004b	0.000 ± 0.000^{a}	0.078 ± 0.002^{b}	0.000 ± 0.000^{a}	0.068 ± 0.015^{b}	0.398	0.00	0.398

Values are expressed as mean ± SEM of $n = 6$ per group (% of total FAME). *F*, *t*, *F* × *t* correspond to *P* values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat × TFA. Statistical differences were indicated with different letters ($P < 0.05$).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; NI: non-identified; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: trans fatty acids.

Table 5 Fatty acid composition in the liver

Fatty acids	O	Ot	C	Ct	R	Rt	ANOVA		
							F	*t*	*F* × *t*
14:0	0.431 ± 0.070^{a}	0.492 ± 0.012^{a}	0.462 ± 0.058^{a}	0.576 ± 0.070^{ab}	0.497 ± 0.041^{a}	0.684 ± 0.010^{b}	0.011	0.001	0.274
16:0	20.827 ± 0.525^{a}	20.521 ± 0.132^{a}	17.483 ± 0.438^{c}	20.046 ± 0.673^{bc}	18.222 ± 0.368^{bc}	20.767 ± 0.714^{a}	0.003	0.001	0.009
17:0	0.110 ± 0.010^{a}	0.104 ± 0.006^{a}	0.108 ± 0.007^{a}	0.082 ± 0.010^{a}	0.117 ± 0.013^{a}	0.091 ± 0.004^{a}	0.374	0.014	0.468
18:0	5.768 ± 0.346^{a}	3.894 ± 0.283^{c}	5.288 ± 0.705^{ab}	3.761 ± 0.403^{c}	4.184 ± 0.204^{bc}	3.816 ± 0.142^{c}	0.041	0.000	0.057
19:0	0.061 ± 0.008^{a}	0.028 ± 0.001^{d}	0.069 ± 0.007^{ac}	0.070 ± 0.001^{ac}	0.049 ± 0.005^{ab}	0.084 ± 0.009^{c}	0.000	0.875	0.000
20:0	0.071 ± 0.004^{a}	0.099 ± 0.016^{a}	0.393 ± 0.081^{c}	0.328 ± 0.070^{bc}	0.184 ± 0.045^{ab}	0.158 ± 0.043^{ab}	0.000	0.539	0.534
*c*9-16:1	4.032 ± 0.363^{ab}	4.855 ± 0.340^{b}	1.749 ± 0.352^{d}	3.543 ± 0.232^{ac}	2.853 ± 0.201^{cd}	5.057 ± 0.458^{b}	0.000	0.000	0.041
*t*9-16:1	0.000 ± 0.000^{a}	0.152 ± 0.006^{b}	0.000 ± 0.000^{a}	0.145 ± 0.013^{a}	0.000 ± 0.000^{a}	0.138 ± 0.022^{b}	0.745	0.000	0.745
*c*9-18:1	32.525 ± 1.291^{a}	32.556 ± 0.365^{ab}	19.958 ± 0.466^{c}	21.559 ± 1.102^{c}	35.978 ± 0.800^{b}	32.996 ± 1.248^{ab}	0.000	0.688	0.008
*c*11-18:1	4.681 ± 0.306^{abc}	5.114 ± 0.265^{bcd}	3.553 ± 0.217^{a}	4.021 ± 0.325^{ab}	5.931 ± 0.417^{cd}	6.197 ± 0.203^{d}	0.000	0.120	0.934
(*t*6 + *t*7 + *t*8)-18:1	0.000 ± 0.000^{a}	0.078 ± 0.010^{b}	0.000 ± 0.000^{a}	0.101 ± 0.018^{b}	0.000 ± 0.000^{a}	0.097 ± 0.016^{b}	0.461	0.000	0.461
*t*9-18:1	0.000 ± 0.000^{a}	0.985 ± 0.071^{b}	0.000 ± 0.000^{a}	0.833 ± 0.121^{bc}	0.000 ± 0.000^{a}	0.693 ± 0.074^{c}	0.085	0.000	0.085
*t*10-18:1	0.000 ± 0.000^{a}	0.603 ± 0.114^{b}	0.000 ± 0.000^{a}	0.596 ± 0.085^{b}	0.000 ± 0.000^{a}	0.523 ± 0.114^{b}	0.802	0.000	0.802
*t*11-18:1	0.000 ± 0.000^{a}	0.319 ± 0.024^{b}	0.000 ± 0.000^{a}	0.459 ± 0.079^{b}	0.000 ± 0.000^{a}	0.309 ± 0.025^{b}	0.077	0.000	0.077
*c*7-19:1	0.437 ± 0.046^{ab}	0.417 ± 0.014^{ab}	0.548 ± 0.058^{bc}	0.573 ± 0.045^{bc}	0.714 ± 0.123^{c}	0.309 ± 0.038^{a}	0.065	0.008	0.002
*c*11-20:1	0.554 ± 0.047^{ab}	0.495 ± 0.041^{ab}	0.688 ± 0.111^{bc}	0.402 ± 0.058^{a}	0.851 ± 0.032^{c}	0.837 ± 0.108^{c}	0.000	0.014	0.044
*c*13-22:1	0.172 ± 0.017^{a}	0.160 ± 0.014^{a}	0.174 ± 0.025^{a}	0.160 ± 0.027^{a}	0.075 ± 0.009^{b}	0.078 ± 0.013^{b}	0.00	0.616	0.859
*c*9,*c*12-18:2	15.190 ± 0.291^{ab}	14.009 ± 0.346^{ab}	34.427 ± 1.190^{c}	27.402 ± 1.064^{d}	15.676 ± 0.166^{a}	11.976 ± 0.054^{b}	0.00	0.000	0.003
t9,t12-18:2	0.000 ± 0.000^{a}	0.130 ± 0.014^{b}	0.000 ± 0.000^{a}	0.089 ± 0.012^{c}	0.000 ± 0.000^{a}	0.101 ± 0.003^{c}	0.001	0.000	0.001
*c*9,*t*11-18:2	0.000 ± 0.000^{a}	0.797 ± 0.091^{b}	0.000 ± 0.000^{a}	0.596 ± 0.146^{b}	0.000 ± 0.000^{a}	0.749 ± 0.020^{b}	0.363	0.000	0.363
*c*11,*c*14-20:2	0.067 ± 0.037^{a}	0.062 ± 0.008^{a}	0.298 ± 0.020^{b}	0.167 ± 0.011^{c}	0.065 ± 0.001^{a}	0.044 ± 0.005^{a}	0.000	0.000	0.002
*c*6,*c*9,*c*12-18:3	0.465 ± 0.064^{a}	0.272 ± 0.018^{bc}	0.447 ± 0.111^{a}	0.390 ± 0.051^{ab}	0.325 ± 0.039^{abc}	0.190 ± 0.008^{c}	0.001	0.000	0.194
*c*9,*c*12,*c*15-18:3	0.147 ± 0.015^{a}	0.179 ± 0.038^{a}	0.199 ± 0.029^{a}	0.169 ± 0.029^{a}	2.160 ± 0.127^{b}	1.797 ± 0.097^{c}	0.000	0.031	0.012
*c*8,*c*11,*c*14-20:3	0.417 ± 0.047^{a}	0.388 ± 0.042^{a}	0.335 ± 0.140^{a}	0.311 ± 0.053^{a}	0.072 ± 0.012^{b}	0.116 ± 0.022^{b}	0.000	0.759	0.523
*c*5,*c*8,*c*11,*c*14-20:4	8.666 ± 0.461^{a}	7.249 ± 0.182^{bc}	7.684 ± 0.595^{ab}	5.831 ± 0.222^{c}	3.360 ± 0.112^{d}	3.186 ± 0.341^{d}	0.000	0.00	0.046
*c*7,*c*10,13,*c*16-22:4	0.194 ± 0.031^{a}	0.205 ± 0.014^{a}	0.515 ± 0.046^{b}	0.424 ± 0.028^{b}	0.064 ± 0.008^{c}	0.062 ± 0.003^{c}	0.000	0.191	0.098
*c*5,*c*8,*c*11,*c*14,*c*17-20:5	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.462 ± 0.006^{b}	0.484 ± 0.011^{c}	0.000	0.045	0.024
*c*4,*c*7,*c*10,*c*13,*c*16-22:5	1.018 ± 0.098^{b}	0.764 ± 0.031^{a}	1.197 ± 0.127^{b}	1.114 ± 0.051^{b}	0.023 ± 0.003^{c}	0.031 ± 0.006^{c}	0.000	0.262	0.112
c7,*c*10,*c*13,*c*16,*c*19-22:5	0.120 ± 0.010^{a}	0.101 ± 0.010^{a}	0.141 ± 0.014^{a}	0.109 ± 0.014^{a}	0.457 ± 0.024^{b}	0.487 ± 0.010^{b}	0.000	0.584	0.095
c4,c7,c10,c13,c16,c19-22:6	3.493 ± 0.291^{a}	2.314 ± 0.268^{b}	2.225 ± 0.371^{b}	1.545 ± 0.205^{b}	4.972 ± 0.183^{c}	5.145 ± 0.197^{c}	0.000	0.013	0.045
Σ NI	2.260 ± 0.192	2.878 ± 0.129	1.937 ± 0.400	2.189 ± 0.198	2.939 ± 0.355	3.486 ± 0.318			
Σ Total SFA	27.655 ± 0.948^{a}	25.382 ± 0.323^{ab}	24.299 ± 1.233^{b}	24.936 ± 0.733^{ab}	23.336 ± 0.506^{b}	25.541 ± 0.791^{ab}	0.016	0.748	0.018
Σ Total MUFA	39.735 ± 1.842^{a}	43.804 ± 0.836^{ab}	26.482 ± 0.880^{c}	31.542 ± 1.023^{d}	46.159 ± 0.507^{b}	45.323 ± 0.755^{b}	0.00	0.003	0.018
Σ Total PUFA	29.906 ± 1.208^{a}	25.023 ± 0.770^{bc}	47.202 ± 0.383^{d}	38.726 ± 1.211^{e}	26.974 ± 0.555^{ab}	23.301 ± 0.445^{c}	0.000	0.000	0.016
Σn-6 LC-PUFA	10.649 ± 0.660^{a}	8.419 ± 0.257^{a}	8.955 ± 1.234^{a}	8.576 ± 1.408^{a}	3.520 ± 0.138^{b}	3.378 ± 0.372^{b}	0.000	0.142	0.319
Σn-3 LC-PUFA	3.761 ± 0.282^{a}	2.594 ± 0.236^{ab}	2.564 ± 0.428^{b}	1.802 ± 0.232^{b}	7.390 ± 0.327^{c}	7.429 ± 0.228^{c}	0.000	0.009	0.095
LC PUFA n-6/n-3	2.847 ± 0.128^{a}	3.318 ± 0.286^{a}	3.548 ± 0.195^{a}	4.765 ± 0.418^{b}	0.476 ± 0.004^{c}	0.453 ± 0.039^{c}	0.000	0.004	0.026
Σ TFA 16:1-18:1-18:2	0.000 ± 0.000^{a}	2.380 ± 0.177^{b}	0.000 ± 0.000^{a}	2.335 ± 0.392^{b}	0.000 ± 0.000^{a}	1.931 ± 0.251^{b}	0.376	0.00	0.376
RA/VA	0.000 ± 0.000^{a}	3.010 ± 0.117^{b}	0.000 ± 0.000^{a}	1.563 ± 0.049^{c}	0.000 ± 0.000^{a}	2.450 ± 0.186^{d}	0.000	0.000	0.000

Values are expressed as mean ± SEM of $n = 6$ per group (% of total FAME). *F*, *t*, *F* × *t* correspond to *P* values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat × TFA. Statistical differences were indicated with different letters ($P < 0.05$).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; NI: non-identified fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: trans fatty acids; RA/VA = c9,t11-18:2/t11-18:1 ratio.

Table 6 Fatty acid composition in serum

Fatty acids	O	Ot	C	Ct	R	Rt	ANOVA		
							F	*t*	*F* × *t*
14:0	0.223 ± 0.013[a]	0.241 ± 0.038[a]	0.174 ± 0.035[a]	0.126 ± 0.016[a]	0.188 ± 0.043[a]	0.233 ± 0.022[a]	0.016	0.800	0.195
15:0	0.081 ± 0.008[a]	0.103 ± 0.008[a]	0.087 ± 0.017[a]	0.109 ± 0.011[a]	0.129 ± 0.022[a]	0.126 ± 0.011[a]	0.041	0.224	0.571
16:0	26.338 ± 0.732[a]	24.693 ± 0.198[ab]	26.185 ± 0.062[a]	24.952 ± 0.193[ab]	25.181 ± 0.511[a]	22.589 ± 0.943[b]	0.314	0.003	0.200
17:0	0.239 ± 0.008	0.261 ± 0.015	0.258 ± 0.010	0.251 ± 0.026	0.225 ± 0.011	0.185 ± 0.022	0.020	0.538	0.203
18:0	8.902 ± 0.172[ab]	7.667 ± 0.354[ac]	10.804 ± 0.416[d]	9.854 ± 0.151[bd]	8.878 ± 0.142[ab]	7.135 ± 0.639[c]	0.000	0.000	0.514
19:0	0.202 ± 0.026[a]	0.083 ± 0.006[b]	0.174 ± 0.000[a]	0.174 ± 0.010[a]	0.126 ± 0.004[b]	0.084 ± 0.007[b]	0.000	0.000	0.000
20:0	0.149 ± 0.011[a]	0.119 ± 0.014[a]	0.120 ± 0.001[a]	0.118 ± 0.024[a]	0.135 ± 0.008[a]	0.119 ± 0.015[a]	0.550	0.164	0.578
*c*9-16:1	1.558 ± 0.164[ab]	1.772 ± 0.097[b]	0.868 ± 0.077[c]	0.829 ± 0.051[c]	1.115 ± 0.154[ac]	1.575 ± 0.224[ab]	0.000	0.029	0.098
*t*9-16:1	0.000 ± 0.000[a]	0.111 ± 0.011[b]	0.000 ± 0.000[a]	0.094 ± 0.003[b]	0.000 ± 0.000[a]	0.110 ± 0.004[b]	0.189	0.000	0.189
(*t*6 + *t*7 + *t*8)-18:1	0.000 ± 0.000[a]	0.115 ± 0.015[b]	0.000 ± 0.000[a]	0.097 ± 0.013[b]	0.000 ± 0.000[a]	0.088 ± 0.013[b]	0.400	0.000	0.400
*t*9-18:1	0.000 ± 0.000[a]	0.757 ± 0.049[b]	0.000 ± 0.000[a]	0.635 ± 0.017[bc]	0.000 ± 0.000[a]	0.523 ± 0.063[c]	0.014	0.000	0.014
*t*10-18:1	0.000 ± 0.000[a]	0.521 ± 0.024[b]	0.000 ± 0.000[a]	0.409 ± 0.028[c]	0.000 ± 0.000[a]	0.368 ± 0.022[c]	0.002	0.000	0.002
*t*11-18:1	0.000 ± 0.000[a]	0.518 ± 0.043[b]	0.000 ± 0.000[a]	0.586 ± 0.009[b]	0.000 ± 0.000[a]	0.455 ± 0.083[b]	0.267	0.000	0.267
*c*9-18:1	18.057 ± 1.005[a]	19.418 ± 0.693[a]	10.146 ± 0.166[b]	11.282 ± 0.786[b]	18.627 ± 0.904[a]	18.712 ± 1.032[a]	0.000	0.193	0.680
*c*11-18:1	5.437 ± 0.088[a]	5.288 ± 0.006[a]	1.988 ± 0.043[b]	2.282 ± 0.115[b]	4.030 ± 0.457[c]	3.563 ± 0.317[c]	0.000	0.822	0.324
*c*7-19:1	0.395 ± 0.052[a]	0.619 ± 0.030[a]	2.087 ± 0.163[b]	1.620 ± 0.167[c]	0.487 ± 0.085[a]	0.420 ± 0.024[a]	0.000	0.184	0.008
*c*11-20:1	0.372 ± 0.015[a]	0.320 ± 0.036[a]	0.262 ± 0.014[a]	0.249 ± 0.010[a]	0.360 ± 0.056[a]	0.361 ± 0.060[a]	0.018	0.449	0.719
*c*15-24:1	0.326 ± 0.023[ab]	0.254 ± 0.026[a]	0.425 ± 0.072[b]	0.390 ± 0.009[b]	0.036 ± 0.005[c]	0.058 ± 0.016[c]	0.000	0.209	0.235
*c*9,*c*12-18:2	14.952 ± 0.123[a]	15.033 ± 0.284[a]	24.247 ± 1.161[b]	22.525 ± 0.475[b]	18.083 ± 0.379[c]	19.209 ± 1.487[c]	0.000	0.730	0.096
t9,t12-18:2	0.000 ± 0.000[a]	0.231 ± 0.034[b]	0.000 ± 0.000[a]	0.135 ± 0.010[c]	0.000 ± 0.000[a]	0.181 ± 0.032[bc]	0.086	0.000	0.086
c9,t11-18:2	0.000 ± 0.000a	0.360 ± 0.004[b]	0.000 ± 0.000[a]	0.219 ± 0.009[c]	0.000 ± 0.000[a]	0.212 ± 0.044[c]	0.002	0.000	0.002
*c*11,*c*14-20:2	0.122 ± 0.008[a]	0.131 ± 0.002[a]	0.225 ± 0.003[c]	0.140 ± 0.009[a]	0.113 ± 0.012[ab]	0.078 ± 0.012[b]	0.000	0.000	0.001
*c*13,*c*16-22:2	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.021 ± 0.008[b]	0.019 ± 0.001[b]	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.000	0.736	0.889
*c*6,*c*9,*c*12-18:3	0.221 ± 0.011[a]	0.189 ± 0.017[ab]	0.337 ± 0.026[d]	0.210 ± 0.009[a]	0.130 ± 0.019[c]	0.140 ± 0.006[bc]	0.000	0.000	0.000
*c*9,*c*12,*c*15-18:3	0.078 ± 0.002[a]	0.043 ± 0.006[b]	0.072 ± 0.008[a]	0.029 ± 0.001[b]	0.780 ± 0.122[c]	0.367 ± 0.043[d]	0.000	0.000	0.001
*c*8,*c*11,*c*14-20:3	0.936 ± 0.030[ab]	1.062 ± 0.035[b]	0.700 ± 0.011[c]	0.639 ± 0.030[c]	1.121 ± 0.093[b]	0.804 ± 0.032[ab]	0.000	0.046	0.002
*c*5,*c*8,*c*11,*c*14-20:4	15.093 ± 0.020[a]	14.509 ± 0.496[a]	14.440 ± 0.525[a]	15.621 ± 0.169[a]	8.626 ± 0.097[b]	9.946 ± 1.351[b]	0.000	0.070	0.076
*c*5,*c*8,*c*11,*c*14,*c*17-20:5	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.844 ± 0.122[b]	0.971 ± 0.048[b]	0.000	0.337	0.396
*c*4,*c*7,*c*10,*c*13,*c*16-22:5	1.384 ± 0.051[a]	1.391 ± 0.058[a]	1.693 ± 0.234[ab]	2.044 ± 0.107[b]	0.035 ± 0.006[c]	0.042 ± 0.006[c]	0.000	0.110	0.112
*c*7,*c*10,*c*13,*c*16,*c*19-22:5	0.113 ± 0.027[a]	0.111 ± 0.028[a]	0.097 ± 0.004[a]	0.087 ± 0.008[a]	0.534 ± 0.027[b]	0.361 ± 0.036[c]	0.000	0.010	0.007
*c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6	3.902 ± 0.202[a]	3.619 ± 0.242[a]	3.415 ± 0.303[a]	3.018 ± 0.189[a]	7.601 ± 0.053[b]	6.315 ± 0.648[b]	0.000	0.026	0.263
ΣNI	1.540 ± 0.190	1.725 ± 0.301	1.170 ± 0.005	1.180 ± 0.067	1.586 ± 0.273	1.858 ± 0.144			
Σ Total SFA	36.132 ± 0.727[ab]	33.167 ± 0.492[ab]	37.802 ± 0.504[b]	35.739 ± 0.228[ab]	37.201 ± 2.350[b]	30.452 ± 1.635[a]	0.086	0.002	0.174
Σ Total MUFA	27.120 ± 1.250[a]	28.564 ± 0.941[a]	17.041 ± 0.221[a]	18.352 ± 0.969[a]	24.585 ± 1.595[b]	25.125 ± 1.310[b]	0.000	0.143	0.655
Σ Total PUFA	38.160 ± 1.209[ab]	35.561 ± 1.441[a]	45.670 ± 0.035[c]	45.272 ± 0.958[bc]	36.534 ± 0.858[a]	40.959 ± 2.902[abc]	0.000	0.706	0.097
Σn-6 LC-PUFA	17.567 ± 0.126[a]	17.120 ± 0.492[a]	17.057 ± 0.773[a]	18.982 ± 0.665[a]	9.504 ± 0.386[b]	11.807 ± 1.310[b]	0.000	0.040	0.134
Σn-3 LC-PUFA	4.015 ± 0.210[a]	3.730 ± 0.266[a]	3.511 ± 0.307[a]	3.105 ± 0.196[a]	8.979 ± 0.162[b]	7.457 ± 0.493[c]	0.000	0.007	0.090
LC PUFA n-6/n-3	4.637 ± 0.208[a]	4.385 ± 0.083[a]	4.885 ± 0.207[a]	6.139 ± 0.228[b]	1.058 ± 0.038[c]	1.620 ± 0.276[c]	0.000	0.005	0.005
Σ TFA 16:1-18:1-18:2	0.000 ± 0.000[a]	2.261 ± 0.026[b]	0.000 ± 0.000[a]	1.951 ± 0.039[bc]	0.000 ± 0.000[a]	1.705 ± 0.175[c]	0.009	0.000	0.009

Values are expressed as mean ± SEM of $n = 6$ per group (% of total FAME). *F*, *t*, *F* × *t* correspond to *P* values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat × TFA. Statistical differences were indicated with different letters ($P < 0.05$). 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; NI: non-identified fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: trans fatty acids.

Table 7 Brain and liver expression of genes of SCD1 and SREBP-1c

Tissue	mRNA	O	Ot	C	Ct	R	Rt	ANOVA		
								F	*t*	*F* × *t*
Brain	SCD1	1.00 ± 0.05^{a}	2.12 ± 0.20^{b}	2.37 ± 0.08^{b}	1.94 ± 0.13^{b}	2.03 ± 0.025^{b}	1.84 ± 0.39^{b}	0.003	0.186	0.000
	SREBP-1c	1.00 ± 0.27^{a}	2.88 ± 0.03^{b}	2.97 ± 0.05^{b}	2.45 ± 0.23^{b}	2.81 ± 0.16^{b}	2.32 ± 0.37^{b}	0.006	0.048	0.000
Liver	SCD1	1.00 ± 0.29^{a}	4.175 ± 0.04^{b}	0.65 ± 0.12^{a}	0.84 ± 0.20^{a}	0.51 ± 0.16^{a}	0.43 ± 0.02^{a}	0.000	0.069	0.014
	SREBP-1c	1.00 ± 0.3^{a}	2.26 ± 0.38^{d}	0.45 ± 0.19^{ab}	0.66 ± 0.11^{ab}	0.28 ± 0.04^{c}	0.40 ± 0.06^{bc}	0.000	0.020	0.025

Data are expressed as mean ± SEM of $n = 6$ per group (relative units). Different letters in each row indicate statistical differences at $P < 0.05$ (Scheffe's test) after ANOVA (2 × 3). *F*, *t*, *F* × *t* correspond to the effect of fat, TFA and interaction of fat × TFA. 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; SCD1: stearoyl-CoA desaturase-1; SREBP-1c: sterol regulatory element-binding protein.

Furthermore, it is important to consider that the hepatic SCD1 is the major enzyme involved not only in the bioconversion of VA to RA, but also in the introduction of the first double bond between carbon 9 and 10 of palmitoyl (16:0) or stearoyl (18:0) CoA to produce palmitoleic (16:1) or oleic (18:1) acids.[42] The oleic acid (C18:1 n-9), derived from the desaturation of stearic acid (C18:0) through the SCD1 enzyme, is the primary FA in the white matter of the mammalian brain.[1] Our results show that in the liver the TFA supplementation increased the ratio 16:1/16:0 and 18:1/18:0 in O and C diets. Many mechanisms may regulate the activity of SCD1 by FA in different diseases.[43] However, studies of the brain, liver and adipocytes indicate that the effects of FA on the SCD1 activity are regulated at gene expression level.[42] Our results showed that the Ot diet increased the SCD1 and SREBP-1c gene expressions in the brain and liver. The SCD1 and SREBP-1c expressions were related only to the increase in the 16:1/16:0 and 18:1/18:0 ratios observed in the liver of animals fed O diet. These gene expressions were not linked to the results of the FA profile in the brain. It is feasible to assume that the brain MUFA/SFA ratios correlated neither with the enzyme activities nor with the gene expression of the SCD1. As previously indicated, SREBPs regulate the expression of genes required for the synthesis of fatty acids, such as SCD1.[28] Therefore, the increase in the SCD1 mRNA expression could be related to the changes observed in this transcription factor. The expression of SCD1 needs to be tightly controlled within a normal range to sustain cellular function.[44]

The lipid profile of the central nervous system has been recognized for decades. In this regard, DHA and AA are present in the highest concentration in the brain.[45] In the same way, in our study a similar profile was found. Mammals obtain the DHA either by the diet or through the precursors ALA and EPA (20:5n-3).[10,36] Brain DHA content and metabolism depend not only on the diet, but also on the ability of the liver to synthesize DHA from circulating ALA, making it critical to assess liver synthesis under different dietary conditions.[1] In this study, the fat sources used with different proportions of n-9, n-6, and n-3 FA only affect the levels of DHA in the brain. In this sense, the animals fed with rapeseed oil increased the DHA levels. Other authors reported that diets with deprivation of n-3 PUFA produced reduced brain DHA content[46] and the consumption of an essential FA adequate diet in the form of precursors resulted in higher DHA levels in the cortical phospholipids of mice.[47] We also found that DPA n-6 was augmented in groups O and C vs. the R group. Accordingly, Igarashi *et al.*[46] found that dietary n-3 PUFA deprivation for 15 weeks increased plasma and brain DPA n-6 concentration. It is known that several mechanisms contribute to maintaining the brain PUFA composition. Adequate concentrations of DHA and AA are required to maintain normal brain function and structure.[36] Igarashi *et al.*[9] reported that in diets with low ALA levels, the brain DHA loss was slowed due to down regulation of its DHA-metabolizing enzymes. In the brain, no changes in DHA levels were found with TFA supplementation. In addition to our results, Phivilary *et al.*[48] reported that a 16% TFA intake had no effect on the DHA levels in the brain; however, they observed that a high TFA intake (43%) induced a decrease in this PUFA. On the other hand, we observed that, in the liver, TFA supplementation decreased DHA levels only in the Ot group. In a previous work, we demonstrated that TFA induced hepatic alterations in mice fed olive oil. In addition, differences in lipid metabolic regulation were associated with the consumption of olive oil supplemented with TFA.[49]

In the biochemical integrity of the brain, the n-6/n-3 ratio is important for the modulation of the central nervous system.[5,50] The brain and hepatic n-6/n-3 PUFA ratio was lower in R than in O and C groups. An appropriate ratio of n-6 and n-3 PUFA in diet is an important determinant of human health. In modern Western diets, the ratio of n-6/n-3 PUFA increases to 15:1 to 25:1, which may have contributed to the prevalence of many chronic diseases including depression and Alzheimer's disease.[51,52]

These results show that while TFA decreased AA and DHA levels in the liver of animals fed olive oil, the brain was reticent to changes in the content of these LC-PUFA. However, diets high in n-3 PUFA produced less incorporation of TFA and increased levels of DHA in the brain. In brief, TFA was incorporated in all tissues; nevertheless, the type of diet rather than enough TFA supplementation affected the FA profiles in the brain, liver, and serum. Even though the experimental results in animal models cannot be directly extrapolated to humans, knowledge of the mechanisms involved in the effects of moderate or low consumption of TFA when ingested with different edible oils may be useful to prevent some metabolic disorders observed in human non-communicable chronic diseases. Nevertheless, further long-term feeding studies should be carried out in order to clarify the physiological and pathological consequences of TFA in Central Nervous System.

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Disclaimer statements

Contributor Marcela Aida González and Claudio Bernal were responsible for the overall conception and design of the study, data handling, and interpretation. Jimena Lavandera collaborated in the generation, collection, analysis, and interpretation of data. These three authors were responsible for writing the article in whole. Juliana Saín and Ana Clara Fariña participated in the analysis and interpretation of data related to fatty acid profile and in the revising the article.

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Conflict of interest The authors have no conflicts of interest to declare.

Ethics approval The animals used in these studies were maintained humanely in compliance with the guidelines established by the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC). Moreover, Institution Ethic Committee has approved the corresponding research project.

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