Research Article

Trans fatty acid retention and conversion rates of fatty acids in tissues depend on dietary fat in mice

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Given that the effects of *trans* fatty acids (TFA) may be associated with the type of TFA isomer and the proportion of n-3/n-6/n-9 fatty acids (FA), this work aimed to investigate the influence of different oils on the following parameters: content, incorporation, and retention of TFA; conversion of vaccenic acid (VA) to rumenic acid (RA) in different tissues; and hepatic output of TFA by VLDL. Another objective was to assess relative conversion rates of key FA and the potential alteration of FA composition induced by TFA in tissues. Male CF1 mice were fed (30-days) diets containing 7% olive, corn or rapeseed oils either supplemented with 0.75% of TFA or without added TFA. FA composition of liver, epididymal adipose tissue, gastrocnemius muscle, brain, and serum was assessed. With the exception of the brain, TFAs were incorporated into the analyzed tissues and serum. TFA retention and RA bioconversion from VA depended on the dietary unsaturated FA proportions. The higher levels of hepatic RA in the liver of mice fed an olive oil+TFA diet could be associated with a raised Δ9-desaturase index. The FA composition of tissues was scarcely modified by the consumption of partially hydrogenated vegetable oils containing similar proportions of *t*9-, *t*10-, and *t*11-18:1.

Practical applications: The present study evaluates the interaction of hydrogenated vegetable oils with different edible oils and the impact on the FA profile and, specifically, TFA incorporation and retention in the tissues. Since the characterization of FA present in tissues might be determining the biological effects of edible fats, this study is relevant for elucidation of existing controversial findings associated with the intake of TFA and its effects on human metabolic alterations.

Keywords: Corn oil / Fatty acid metabolism / Olive oil / Partially hydrogenated vegetable oil / Rapeseed oil

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Abbreviations: ALA, α-linolenic acid; **ARA,** arachidonic acid; **C,** corn oil; **CLA,** conjugated linoleic acids; **Ct,** corn oil + *trans* fatty acids; **DHA,** docosahexaenoic acid; **EPA,** eicosapentaenoic acid; **EWAT,** epididymal white adipose tissue; **FA,** fatty acids; **FAME,** fatty acid methyl esters; **GLA,** γ-linolenic acid; **LA,** linoleic acid; **LC-PUFA,** long chain polyunsaturated fatty acids; **O,** olive oil; **Ot,** olive oil + *trans* fatty acids; **PHVO,** partially hydrogenated vegetable oil; **PUFA,** polyunsaturated fatty acids; **R,** rapeseed oil; **RA,** rumenic acid; **Rt,** rapeseed oil + *trans* fatty acids; **TFA,** *trans* fatty acids; **VA,** vaccenic acid; **VLDL,** very low density lipoproteins

1 Introduction

The intake of dietary *trans* fatty acids (TFA) has been associated with alterations in blood lipids, endothelial dysfunction, risk of cardiovascular diseases [1, 2], and increased incidence of different types of cancer [3–5]. Most of these effects have been associated with the intake of industrial TFA containing high levels of elaidic acid (*t*9-18:1) [6]. Beside this TFA, major attention is paid to vaccenic acid (VA), a ruminant-derived TFA, which was found to have no adverse effects on biomarkers for cardiovascular diseases [7]. VA is bioconverted into *c*9, *t*11-18:2, rumenic acid (RA), a conjugated linoleic acid (CLA) isomer, through the Δ9-desaturase pathway [8]. This

bioconversion was extensively studied, due to the numerous beneficial effects of RA on human health [9–11].

TFA have been shown to be incorporated into many tissues in experimental animal models [12, 13] and the levels that can be reached in tissues are related to the amount and type of TFA in dietary fat [14]. The different biological effects of TFA could be associated with the TFA incorporation and retention into the tissue, the metabolic fate, which could depend on the rate of fatty acids (FA) oxidation, TFA bioconversion and biosynthesis of long chain polyunsaturated fatty acids (LC-PUFA) derivates as well as with the alteration of the FA metabolic pathways. Moreover, the lipid metabolism and, specifically, the elongation and desaturation of FA are regulated by the type of dietary FA [15].

In a previous work, we demonstrated that TFA induced hepatic steatosis in mice fed an olive oil diet but not in those fed a corn oil diet. In addition, differences in lipid metabolic regulation were associated with the consumption of olive oil supplemented with partially hydrogenated vegetable oil (PHVO) [16]. Nevertheless, we did not determine the relation of these metabolic alterations with TFA incorporation and retention in liver. Since the tissue TFA content may be modified by dietary FA and, on the other hand TFA interfere with the metabolic pathways of FA, this work aimed to investigate the influence of different dietary unsaturated fats on the following parameters: content, incorporation, and retention of TFA; conversion of VA to RA in different tissues; and hepatic output of TFA by VLDL in mice. Another objective was to assess relative conversion rates of key FA and the potential alterations of FA composition induced by TFA.

2 Materials and methods

2.1 Animals, diet preparation, and experimental design

The experiment was conducted with two sets of 36 male CF1 mice ($n = 6$/group) at two weeks after weaning (22 g), 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 [17]. The animals were kept under controlled conditions (23 ± 2°C and 12 h light-dark cycle) and they had free access to food and water. Each set of mice was randomly divided into six groups of six animals each fed on diets for 30-days: Group O was fed a diet rich in olive oil as source of oleic acid (*c*9-18:1), group C was fed a diet rich in corn oil as source of linoleic acid (*c*9,*c*12-18:2; LA), group R was fed a diet rich in rapeseed oil as source of α-linolenic acid (*c*9,*c*12, *c*15-18:3; ALA); Groups Ot, Ct, and Rt were fed an O, C, or R diet, respectively, supplemented with PHVO. The PHVO contained a complex mixture of TFA with three main isomers: *t*9-, *t*10- , and *t*11-18:1.

Experimental diets consisted of 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 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), while 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).

PHVO was kindly provided by CALSA (Compañía Argentina de Levaduras S.A., Buenos Aires, Argentina). O, C, and R oils, sucrose, cellulose, casein, and corn starch were obtained from local sources. Vitamin and mineral mixes were formulated according to AIN-93 guidelines [18] supplied by ICN Pharmaceuticals (Costa Mesa, CA, USA). Cysteine, methionine and choline were purchased from Sigma (St. Louis, MO, USA).

The FA profile of experimental diets was determined as explained below and it is shown in Table 1. The experimental diets were freshly prepared every 3 days, gassed with nitrogen and stored at 0–4°C.

2.2 Extraction of tissues and serum samples

After 30 days of dietary treatment, one set of animals ($n = 36$) was sacrificed (9.00–11.00 AM) under anesthesia (1 mg azepromazine + 100 mg ketamine/kg body weight) by cardiac exsanguination. Blood was collected and serum was obtained after centrifugation (1000 *g* for 10 min at 4°C). Liver, brain, gastrocnemius muscle, and epididymal white adipose tissue (EWAT) were dissected, weighed, and immediately frozen. All samples were stored at −80°C until analysis.

Another set of animals ($n = 36$) submitted to the same dietary treatment was fasted overnight, and anaesthetized as indicated above. Six-hundred milligram per kilogram of body weight of Triton WR 1339 in saline solution, an agent known to inhibit peripheral removal of triglycerides rich lipoproteins, was intravenously injected [19]. Blood samples were taken 120 min after the injection of Triton solution to obtain serum rich in very low density lipoproteins (VLDL) and analyze its FA composition.

2.3 Gas chromatography analysis of dietary, serum, and tissue fatty acid composition

The FA composition of the tissues, serum, and experimental diets was determined by gas chromatography with a Shimadzu (GC 2014) chromatograph equipped with an automatic injector (AOC-20i auto injector Shimadzu) and a flame ionization detector (SFID1). Analyses were carried out with a capillary column CP Sil 88 (100 m length, 0.25 mm i.d., 0.25 μm film thickness) (Varian, Walnut Creek, CA, USA, Part N°CP7489). The column temperature was held at

Table 1. Fatty acid composition of experimental diets (% of total FAME)

Fatty Acid	O	C	R	Ot	Ct	Rt
SFA						
14:0	ND	0.03	0.07	ND	ND	ND
16:0	17.10	12.21	3.99	16.40	11.76	6.11
17:0	0.08	ND	ND	ND	0.12	ND
18:0	1.58	1.93	2.22	4.52	4.49	4.54
20:0	0.30	0.50	0.52	0.41	0.39	0.54
22:0	0.13	0.16	0.24	0.20	0.22	0.30
Total	19.18	14.84	7.04	21.54	16.98	11.49
cis-MUFA						
*c*9-16:1	1.97	0.12	0.19	1.72	0.11	0.15
*c*6-18:1	ND	ND	ND	1.90	1.79	2.04
*c*9-18:1	55.18	31.95	61.11	47.17	28.97	51.55
*c*11-18:1	4.76	0.54	3.49	3.68	1.26	3.27
*c*11-20:1	0.24	0.25	0.90	0.23	0.25	0.67
Total	62.15	32.86	65.69	54.7	32.38	57.68
trans-MUFA						
(*t*6+ *t*7+ *t*8)-18:1	ND	ND	ND	1.57	1.57	1.65
*t*9-18:1	ND	ND	ND	2.20	2.45	2.14
*t*10-18:1	ND	ND	ND	2.75	3.06	2.99
*t*11-18:1	ND	ND	ND	2.46	2.53	2.57
Total	ND	ND	ND	8.98	9.61	9.35
cis-PUFA						
*c*9,*c*12-18:2	17.21	51.26	18.41	11.68	38.38	13.28
*c*9,*c*12,*c*15-18:3	0.74	0.88	8.64	0.61	0.72	6.18
*c*5,*c*8,*c*11,*c*14- 20:4	0.235	ND	ND	0.37	ND	ND
*c*5,*c*8,*c*11,*c*14,c17-20:5	ND	0.15	ND	ND	0.18	ND
Total	18.19	52.30	27.05	12.66	39.28	19.46
trans-PUFA						
*t*9, *t*12-18:2	ND	ND	ND	0.27	0.27	0.27
Total	ND	ND	ND	0.27	0.27	0.27
Unidentified	0.47	ND	0.22	1.85	1.58	1.77

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, not detected.

75°C for 2 min after injection, then 5°C/min to 170°C, held for 40 min, 5°C/min to 220°C and held 40 min. Nitrogen was the carrier gas with an inlet pressure set at 100 kPa and a split ratio of 1:20. The injector and detector temperatures were maintained at 220 and 250°C, respectively. Injection volume was 0.5 μL and the column flow was 0.8 mL/min. Total fat in tissues, serum, and diets were extracted using the method described by Bligh and Dyer [20]. Briefly, samples were homogenized in trichloromethane:methanol 1:2 (vol:vol). After drying under nitrogen, the samples were dissolved in 1 mL of hexane for methylation. 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 using GC Solution Postrun software (Version 2.30 00 SU6). Standards GLC-463 Reference Standard containing 52 FAME mixture (purity > 99%) and *trans*-mix GLC 481 (purity > 99%) were purchased from Nu-Chek (Nu-Chek Prep, Inc., Elysian, MN, USA). Linoleic acid methyl esters, *cis*/*trans* mix (Catalog #47791) was obtained from Supelco (Bellefonte, PA, USA). Conjugated linoleic acid, *cis*/*trans* mix (Catalog #05507) was purchased from Sigma Chemical Co. Others FAME standards were provided by the International CYTED Net (208RT0343). All solvents and reagents used for the FA quantification were of chromatography grade, and all the other chemicals used were at least American Chemical Society (ACS) degree. Values of FA content were expressed as percentage of total FAME.

2.4 Estimation of key product/precursor ratios involved in the FA metabolism

To estimate the index of Δ9- and Δ6-desaturase activities, the product/precursor ratios were calculated in liver and EWAT.

Thus, *c*9-16:1/16:0 and *c*9-18:1/18:0 ratios were considered to assess the index of Δ9-desaturase activity [21, 22] as well as the *c*9,*t*11-18:2/*t*11-18:1 (RA/VA) ratio to estimate the relative conversion rate of VA to RA. Moreover, the *c*6,*c*9,*c*12-18:3/*c*9,*c*12-18:2 (GLA/LA, where GLA = γ-linolenic acid) ratio was used to evaluate the index Δ6-desaturase activity [23]. In addition, the relative conversion rates of LC-PUFA of n-6 and n-3 series were calculated using the *c*5,*c*8,*c*11,*c*14-20:4/*c*9,*c*12-18:2 (ARA/LA, where ARA = arachidonic acid) and *c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6/*c*9,*c*12,*c*15-18:3 (DHA/ALA, where DHA = docosahexaenoic acid) ratios, respectively [23, 24].

2.5 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. The variables considered were source of fat: O, C, and R and the presence or absence of TFA in diets. All post-hoc multiple comparisons were made using Tukey's critical range test. Significant differences were considered at $p < 0.05$. F, t, and Fxt correspond to the *p* values of 2×3 ANOVA to the effect of fat, TFA, and interaction of fat x TFA, respectively.

3 Results

All diets used in this experiment were well accepted and did not affect growth and development of mice negatively.

The content of FA in EWAT (Table 2), liver (Table 3), muscle (Table 4), serum (Table 5), and VLDL-rich serum (Table 8), reflected the proportion of FA in the experimental diets. In particular, oleic acid, LA, and ALA were found in a high percentage in those animals fed with O, C, and R diets, respectively. In the brain (Table 6), results showed a different pattern of FA, where the LC-PUFA, specifically DHA and ARA, were prevalent compared with other tissues.

3.1 Incorporation and retention of TFA in tissues and serum

With the exception of the brain, which did not incorporate any TFA isomer, each dietary TFA was incorporated and retained in liver, EWAT, muscle and transported in serum (Table 7). The incorporation and retention of each TFA isomer in serum and tissues were estimated using the relation between the TFA percentages in the biological sample with respect to the TFA percentages in the diet. The highest incorporation and retention values of total TFA were found in EWAT and they were independent of the relative proportions of dietary n-9, n-6, and n-3 FA. In this tissue, *t*9-18:1 and *t*10-18:1 showed the highest incorporation and retention with respect to other *t*-18:1 isomers. Similarly to EWAT, *t*9-18:1 exhibited the highest *t*-18:1 incorporation and retention in gastrocnemius muscle, but they were dependent on the dietary fat source, reaching the lowest values in the Rt group. In contrast, the liver showed a different pattern, VA being the major *t*-18:1 isomer retained, mainly in the Ct and Rt groups. In serum the pattern of the individual *t*-18:1 resembled that of the dietary fat source. The incorporation and retentions of individual *t*-18:1 in serum were Ot > Ct > Rt, while *t*9-18:1 was the main TFA transported in VLDL-rich serum, reaching the highest values in the Ct and Rt groups.

3.2 Content of rumenic acid in serum and tissues and Δ9-desaturase index

RA was absent in the diet and was bioconverted from VA in different tissues. Similarly to *t*-18:1, the brain did not show detectable levels of RA but they could be detected either in serum as well as in liver, EWAT and muscle. The levels of RA were EWAT > gastrocnemius muscle > VLDL-rich serum > liver > serum (Tables 2, 4, 8, 3, 5, respectively). In EWAT, muscle and VLDL-rich serum the content of RA was independent of dietary fat, but in the Ot group revealed the highest content of RA in liver and the lowest in serum.

The relative conversion rate of VA to RA via Δ9-desaturase, defined by the RA/VA ratio, was higher in Ot group than in Ct and Rt groups in both liver and EWAT (Tables 2 and 3). The Δ9-desaturase index was expressed by the *c*9-18:1/18:0 and *c*9-16:1/16:0 ratios. In liver, the *c*9-18:1/18:0 ratio was higher in groups fed diets rich in oleic acid (O and R) than in groups fed diets with low content of this FA (C) and the *c*9-16:1/16:0 ratio was greater in olive oil diets and higher in C than in Ct (Table 3). In both cases, TFA supplementation did not induce any significant alteration in these ratios except in Ct in the *c*9-16:1/16:0 ratio. Similarly, in EWAT, the *c*9-16:1/16:0 ratio was greater in O fed animals compared to R fed animals and the *c*9-18:1/18:0 ratio was higher in O and R groups than in C group (Table 2). The *c*9-16:1/16:0 and *c*9-18:1/18:0 ratios in EWAT were not modified by TFA.

3.3 Relative conversion rates of n-3 and n-6 LC-PUFA in liver

The levels of LA and ARA in liver were associated with the proportion of the LA in the experimental diets, showing the highest levels in those animals fed a C diet (Table 3). The TFA supplementation in Ct animals increased even more the levels of LA in liver. Except in group Ct, the Δ6-desaturase index, expressed by the GLA/LA ratio, was decreased by TFA. Nevertheless, the levels of ARA and intermediate metabolites of their biosynthesis were not modified by TFA supplementation.

Table 2. Fatty acid composition of epididymal adipose tissue in mice fed experimental diets (% of total FAME)

							ANOVA		
Fatty acids	O	Ot	C	Ct	R	Rt	F	t	Fxt
16:0	18.85 ± 0.53^{a}	17.90 ± 0.34^{a}	17.92 ± 0.53^{a}	18.34 ± 0.25^{a}	14.99 ± 0.41^{b}	15.45 ± 0.26^{b}	0.000	0.948	0.161
18:0	1.63 ± 0.10^{a}	1.88 ± 0.06^{ab}	1.59 ± 0.02^{a}	2.12 ± 0.13^{b}	1.87 ± 0.12^{ab}	2.29 ± 0.14^{b}	0.015	0.000	0.425
*c*9-14:1	0.09 ± 0.01^{a}	0.06 ± 0.01^{a}	0.10 ± 0.02^{a}	0.00 ± 0.00^{b}	0.06 ± 0.01^{ab}	0.06 ± 0.01^{a}	0.138	0.000	0.001
*c*9-16:1	5.96 ± 0.35^{a}	5.79 ± 0.38^{a}	4.76 ± 0.12^{ab}	4.87 ± 0.38^{ab}	3.59 ± 0.30^{b}	3.81 ± 0.23 ^{b}	0.000	0.839	0.814
*c*9-18:1	44.96 ± 1.64^{a}	43.64 ± 0.87^{a}	31.39 ± 0.68^{b}	31.68 ± 0.55^{b}	47.48 ± 1.49^{a}	44.30 ± 0.37^{a}	0.000	0.111	0.269
*c*11-18:1	3.50 ± 0.30^{ab}	3.75 ± 0.11^{a}	1.62 ± 0.06^{e}	2.23 ± 0.11^{de}	2.60 ± 0.07^{cd}	3.06 ± 0.07^{bc}	0.000	0.001	0.486
(*t*6+*t*7+*t*8)-18:1	0.00 ± 0.00^{a}	0.55 ± 0.04^{b}	0.00 ± 0.00^{a}	0.52 ± 0.09^{b}	0.00 ± 0.00^{a}	0.45 ± 0.03^{b}	0.375	0.000	0.375
*t*9-18:1	0.00 ± 0.00^{a}	1.17 ± 0.09^{b}	0.00 ± 0.00^{a}	0.93 ± 0.15^{b}	0.00 ± 0.00^{a}	1.14 ± 0.12^{b}	0.184	0.000	0.184
*t*10-18:1	0.00 ± 0.00^{a}	1.15 ± 0.04^{b}	0.00 ± 0.00^{a}	1.05 ± 0.16^{b}	0.00 ± 0.00^{a}	1.18 ± 0.09^{b}	0.570	0.000	0.570
*t*11-18:1	0.00 ± 0.00^{a}	0.52 ± 0.05^{b}	0.00 ± 0.00^{a}	0.64 ± 0.13^{b}	0.00 ± 0.00^{a}	0.69 ± 0.03^{b}	0.184	0.000	0.184
*c*11-20:1	3.50 ± 0.30^{a}	3.75 ± 0.11^{bc}	1.62 ± 0.06^{ad}	2.23 ± 0.11^{d}	2.60 ± 0.07^{bc}	3.06 ± 0.07^{c}	0.000	0.219	0.069
*c*9,*c*12-18:2	19.36 ± 1.14^{a}	16.34 ± 0.28^{d}	37.18 ± 0.38^{c}	30.44 ± 0.64^{b}	21.19 ± 0.86^{a}	18.36 ± 0.34^{ad}	0.000	0.000	0.011
*c*9,*t*11-18:2	0.00 ± 0.00^{a}	0.66 ± 0.03^{b}	0.00 ± 0.00^{a}	0.57 ± 0.04^{b}	0.00 ± 0.00^{a}	0.63 ± 0.04^{b}	0.074	0.000	0.074
*t*9,*t*12-18:2	0.00 ± 0.00^{a}	0.34 ± 0.02^{b}	0.00 ± 0.00^{a}	0.29 ± 0.03^{b}	0.00 ± 0.00^{a}	0.32 ± 0.05^{b}	0.389	0.000	0.389
*c*6,*c*9,*c*12-18:3	0.09 ± 0.01^{ab}	0.06 ± 0.00^{d}	0.14 ± 0.01^{c}	0.10 ± 0.01^{b}	0.07 ± 0.01^{ad}	0.07 ± 0.01^{ad}	0.000	0.000	0.017
*c*9,*c*12,*c*15-18:3	1.03 ± 0.12^{a}	0.62 ± 0.05^{a}	0.98 ± 0.14^{a}	0.79 ± 0.05^{a}	3.57 ± 0.17^{b}	2.30 ± 0.21^{c}	0.000	0.000	0.001
*c*11,*c*14-20:2	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.19 ± 0.01^{c}	0.14 ± 0.01^{bc}	0.13 ± 0.02^{ab}	0.11 ± 0.01^{ab}	0.000	0.017	0.268
*c*8, *c*11,*c*14-20:3	0.10 ± 0.01^{ab}	0.04 ± 0.01^{a}	0.21 ± 0.02^{c}	0.11 ± 0.01^{b}	0.09 ± 0.01^{ab}	0.06 ± 0.01^{ab}	0.000	0.000	0.054
*c*5,*c*8,*c*11,*c*14-20:4	0.18 ± 0.01^{a}	0.11 ± 0.02^{a}	0.38 ± 0.07^{b}	0.15 ± 0.01^{a}	0.11 ± 0.01^{a}	0.08 ± 0.02^{a}	0.000	0.000	0.006
ΣTFA	0.00 ± 0.00^{a}	4.42 ± 0.18^{b}	0.00 ± 0.00^{a}	4.12 ± 0.65^{b}	0.00 ± 0.00^{a}	4.54 ± 0.36^{b}	0.709	0.000	0.709
ΣTotal SFA	22.03 ± 0.50^{a}	21.23 ± 0.35^{ab}	20.98 ± 0.57^{ab}	21.97 ± 0.24^{a}	18.59 ± 0.64^{c}	19.26 ± 0.34^{bc}	0.000	0.456	0.136
ΣTotal MUFA	54.91 ± 2.11^{a}	54.05 ± 0.69^{a}	38.53 ± 0.74^{b}	39.40 ± 0.99^{b}	54.61 ± 1.45^{a}	52.20 ± 0.30^{a}	0.000	0.420	0.406
ΣTotal PUFA	21.09 ± 2.19^{ab}	17.26 ± 0.35 ^{a}	39.07 ± 0.53 ^{d}	31.76 ± 0.72^{c}	25.10 ± 0.83^{b}	20.96 ± 0.17^{ab}	0.000	0.000	0.195
Σn-6 LC- PUFA	0.37 ± 0.02^{ab}	0.25 ± 0.03^{ab}	0.77 ± 0.07^{c}	0.43 ± 0.07^{b}	0.26 ± 0.02^{ab}	0.23 ± 0.02^{a}	0.000	0.000	0.007
NI	1.51 ± 0.07	2.39 ± 0.15	1.18 ± 0.05	2.21 ± 0.09	1.33 ± 0.05	2.42 ± 0.08			
RA/VA	0.00 ± 0.00 ^{a}	133.19 ± 14.25^{b}	0.00 ± 0.00^{a}	102.63 ± 7.11^{bc}	0.00 ± 0.00^{a}	91.86 ± 3.95^{c}	0.014	0.000	0.014
*c*9-16:1/16:0	32.15 ± 1.44^{a}	32.41 ± 2.15^{a}	26.67 ± 0.95^{ab}	26.50 ± 1.97^{ab}	23.93 ± 1.76^{b}	24.69 ± 1.57^{b}	0.000	0.838	0.963
*c*9-18:1/18:0	28.16 ± 2.80^{a}	23.29 ± 0.59^{ab}	19.77 ± 0.32^{c}	15.40 ± 1.20^{cb}	25.90 ± 1.86^{ab}	19.99 ± 1.31^{bc}	0.000	0.000	0.884
GLA/LA	0.49 ± 0.07^{a}	0.34 ± 0.03^{ab}	0.37 ± 0.03^{ab}	0.34 ± 0.02^{ab}	0.32 ± 0.03^{b}	0.38 ± 0.02	0.160	0.170	0.030
ARA/LA	1.06 ± 0.18^{a}	0.67 ± 0.08^{ab}	1.01 ± 0.17^{ab}	0.67 ± 0.14^{ab}	0.48 ± 0.05^{b}	0.44 ± 0.10^{b}	0.008	0.022	0.361

Values expressed as media ± SEM of $n = 6$ per group. F, t, Fxt correspond to p values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat x 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; RA/VA = *c*9,*t*11-18:2/ *t*11-18:1 ratio; GLA/LA = *c*6,*c*9,*c*12-18:3/ *c*9,*c*12-18:2 ratio; ARA/LA = *c*5,*c*8,*c*11,*c*14-20:4/ *c*9,*c*12-18:2 ratio; TFA, *trans* fatty acids; SFA, saturated fatty acids. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

In a similar manner, the ALA levels were higher in those groups fed the R diet. TFA supplementation did not modify the incorporation of ALA in the experimental dietary groups but increased the relative conversion rate to DHA (DHA/ALA ratio) in Ct versus its respective control group.

3.4 Relative conversion rates of n-3 and n-6 LC-PUFA in adipose tissue

In adipose tissue, the levels of LA and ALA were associated with the proportion of these FA in the diets (Table 2). The TFA supplementation decreased the content of LA in Ot and Ct, ARA in Ct and the ALA levels in group Rt versus their respective control groups. Neither the relative conversion rate of n-6 LC-PUFA (ARA/LA ratio) nor the Δ6-desaturase index (GLA/LA ratio) was modified by TFA. Due to the fact that EPA and DHA were undetected, we could not estimate the relative conversion rate of n-3 LC-PUFA in EWAT.

4 Discussion

The levels of TFA in a particular tissue might be related to different variables including: (1) type and level of the dietary TFA isomer, (2) uptake, metabolization, and release from the tissue, (3) interference with different dietary FA, and (4) other factors like species, sex, age, physiological status. Therefore, the aim of the present work was to investigate the influence of dietary fats containing different types of unsaturated FA on the incorporation and retention of

Table 3. Fatty acid composition of liver in mice fed experimental diets (% of total FAME)

							ANOVA		
Fatty acids	O	Ot	C	Ct	R	Rt	F	t	Fxt
16:0	23.49 ± 0.68	22.44 ± 0.18	23.20 ± 1.08	22.14 ± 0.33	22.78 ± 0.72	21.48 ± 0.36	0.423	0.036	0.976
18:0	5.35 ± 0.34^{a}	5.94 ± 0.13^{ab}	7.55 ± 0.45^{c}	9.06 ± 0.29^{d}	7.02 ± 0.38^{bc}	7.30 ± 0.31^{bc}	0.000	0.007	0.178
*c*9-16:1	2.17 ± 0.07^{a}	1.86 ± 0.11^{ab}	1.47 ± 0.23^{bc}	1.00 ± 0.07^{c}	1.35 ± 0.24^{bc}	1.07 ± 0.10^{c}	0.000	0.010	0.810
*c*9-18:1	37.99 ± 2.38^{a}	32.99 ± 0.47^{ab}	22.19 ± 1.75^{c}	15.51 ± 0.99^{d}	31.30 ± 2.19^{b}	27.08 ± 1.24^{bc}	0.000	0.000	0.663
*c*11-18:1	4.59 ± 0.32^{a}	3.89 ± 0.25^{a}	2.13 ± 0.31^{b}	1.65 ± 0.12^{b}	2.43 ± 0.27^{b}	1.95 ± 0.10^{b}	0.000	0.009	0.871
(*t*6+*t*7+*t*8)-18:1	0.00 ± 0.00^{a}	0.14 ± 0.01^{b}	0.00 ± 0.00^{a}	0.22 ± 0.02^{b}	0.00 ± 0.00^{a}	0.28 ± 0.04 ^{b}	0.006	0.000	0.006
*t*9-18:1	0.00 ± 0.00^{a}	0.46 ± 0.02^{b}	0.00 ± 0.00^{a}	0.43 ± 0.02^{b}	0.00 ± 0.00^{a}	0.44 ± 0.03^{b}	0.676	0.000	0.676
*t*10-18:1	0.00 ± 0.00^{a}	0.50 ± 0.02^{b}	0.00 ± 0.00^{a}	0.44 ± 0.03 ^{b}	0.00 ± 0.00^{a}	0.41 ± 0.06^{b}	0.282	0.000	0.282
*t*11-18:1	0.00 ± 0.00^{a}	0.48 ± 0.05^{b}	0.00 ± 0.00^{a}	0.66 ± 0.01^{c}	0.00 ± 0.00^{a}	0.61 ± 0.03^{c}	0.003	0.000	0.003
*c*11-20:1	1.25 ± 0.12^{a}	1.03 ± 0.02^{ab}	0.76 ± 0.09^{bc}	0.59 ± 0.03^{c}	1.07 ± 0.12^{ab}	0.83 ± 0.02^{bc}	0.000	0.003	0.932
*c*13-22:1	0.11 ± 0.02^{ab}	0.13 ± 0.01^{bc}	0.08 ± 0.01^{ad}	0.09 ± 0.01^{abd}	0.18 ± 0.01^{c}	0.05 ± 0.01^{d}	0.004	0.001	0.000
*c*9,*c*12-18:2	9.55 ± 0.66^{a}	11.48 ± 0.28^{ab}	19.96 ± 1.02^{d}	23.31 ± 0.50^{e}	14.77 ± 1.20^{c}	14.22 ± 0.18^{bc}	0.000	0.013	0.040
*t*9,*t*12-18:1	0.00 ± 0.00^{a}	0.18 ± 0.02^{b}	0.00 ± 0.00^{a}	0.14 ± 0.02^{bc}	0.00 ± 0.00^{a}	0.12 ± 0.01^{c}	0.038	0.000	0.038
*c*9,*t*11-18:2	0.00 ± 0.00^{a}	0.31 ± 0.01^{b}	0.00 ± 0.00^{a}	0.22 ± 0.01^{c}	0.00 ± 0.00^{a}	0.24 ± 0.03^{c}	0.019	0.000	0.019
*c*6,*c*9,*c*12-18:3	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.24 ± 0.02^{b}	0.23 ± 0.01^{b}	0.15 ± 0.01^{a}	0.10 ± 0.01^{a}	0.000	0.055	0.147
*c*9,*c*12,*c*15-18:3	0.12 ± 0.01^{a}	0.09 ± 0.01^{a}	0.18 ± 0.02^{a}	0.10 ± 0.01^{a}	1.06 ± 0.09^{b}	1.00 ± 0.05^{b}	0.000	0.109	0.795
*c*11,*c*14-20:2	0.16 ± 0.01^{a}	0.22 ± 0.02^{a}	0.38 ± 0.04^{b}	0.41 ± 0.01^{b}	0.19 ± 0.01^{a}	0.21 ± 0.01^{a}	0.000	0.045	0.593
*c*8, *c*11,*c*14-20:3	1.07 ± 0.10	1.13 ± 0.05	1.44 ± 0.24	1.25 ± 0.04	1.20 ± 0.09	1.37 ± 0.06	0.110	0.886	0.318
*c*11,*c*14, *c*17-20:3	0.03 ± 0.01^{ab}	0.00 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.00 ± 0.00^{a}	0.05 ± 0.02^{b}	0.00 ± 0.00^{a}	0.429	0.000	0.429
*c*5,*c*8,*c*11,*c*14-20:4	7.41 ± 0.70^{a}	7.96 ± 0.19^{a}	11.62 ± 0.58^{b}	13.40 ± 0.17^{b}	6.37 ± 0.59^{a}	6.23 ± 0.42^{a}	0.000	0.075	0.149
*c*5,*c*8,*c*11,*c*14,*c*17-20:5	0.12 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.89 ± 0.07^{b}	0.83 ± 0.03^{b}	0.000	0.027	0.149
*c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6	2.03 ± 0.21^{a}	2.67 ± 0.07^{a}	2.38 ± 0.06^{a}	3.19 ± 0.05^{a}	7.48 ± 0.55^{b}	9.00 ± 0.44 ^{b}	0.000	0.000	0.312
ΣTFA	0.00 ± 0.00^{a}	1.77 ± 0.11^{b}	0.00 ± 0.00^{a}	1.86 ± 0.06^{b}	0.00 ± 0.00^{a}	1.84 ± 0.08^{b}	0.747	0.000	0.747
ΣTotal SFA	30.08 ± 0.99	29.42 ± 022	31.64 ± 1.29	32.33 ± 0.04	31.59 ± 0.82	29.74 ± 0.62	0.028	0.355	0.292
ΣTotal MUFA	45.82 ± 2.69^{a}	39.90 ± 0.75^{ab}	27.00 ± 2.17^{c}	18.81 ± 0.56^{d}	34.34 ± 1.70^{bc}	30.98 ± 1.46^{c}	0.000	0.000	0.384
ΣTotal PUFA	20.87 ± 2.10^{a}	23.67 ± 0.50^{a}	38.28 ± 2.52^{bc}	41.89 ± 0.69^{c}	32.17 ± 2.20^{b}	32.97 ± 1.00^{b}	0.000	0.093	0.699
Σn-6 LC-PUFA	7.54 ± 1.22^{a}	9.32 ± 0.24^{a}	12.96 ± 0.43^{b}	15.06 ± 0.21^{b}	7.76 ± 0.52^{a}	7.81 ± 0.46^{a}	0.000	0.014	0.216
Σn-3LC-PUFA	2.47 ± 0.26^{a}	2.67 ± 0.07^{a}	2.97 ± 0.33^{a}	3.19 ± 0.05^{a}	8.43 ± 0.59^{b}	9.84 ± 0.46^{b}	0.000	0.043	0.160
NI	3.21 ± 0.31	3.73 ± 0.08	3.07 ± 0.20	3.31 ± 0.11	1.91 ± 0.16	2.92 ± 0.12			
RA/VA	0.00 ± 0.00^{a}	66.91 ± 8.05^{b}	0.00 ± 0.00^{a}	33.85 ± 3.18^{c}	0.00 ± 0.00^{a}	40.62 ± 8.89^{c}	0.010	0.000	0.010
*c*9-16:1/16:0	9.40 ± 0.23^{a}	8.31 ± 0.57^{a}	8.23 ± 0.49^{a}	4.49 ± 0.25^{b}	4.66 ± 0.21^{b}	5.05 ± 0.56^{b}	0.000	0.000	0.000
*c*9-18:1/18:0	704.3 ± 102.7^{a}	556.3 ± 11.4^{ab}	300.7 ± 32.0^{cd}	173.2 ± 9.3^{d}	464.0 ± 44.6^{bc}	382.0 ± 35.5^{bcd}	0.000	0.007	0.798
GLA/LA	1.29 ± 0.10^{a}	0.98 ± 0.02^{b}	1.22 ± 0.10^{ab}	1.00 ± 0.04^{ab}	1.07 ± 0.12^{b}	0.71 ± 0.06^{c}	0.008	0.000	0.710
ARA/LA	79.67 ± 6.43^{a}	69.52 ± 1.98^{ab}	55.46 ± 5.02^{cb}	57.62 ± 0.76^{cb}	43.34 ± 2.56^{c}	43.70 ± 2.75^{c}	0.000	0.414	0.227
DHA/ALA	2405 ± 347^{ab}	3115 ± 275^{b}	1389 ± 249^{ac}	3378 ± 341^{b}	744 ± 94^{c}	931 ± 76^{c}	0.000	0.000	0.004

Values expressed as media ± SEM of $n = 6$ per group. F, t, Fxt correspond to p values of 2×3 ANOVA to the effect of fat, TFA and interaction of fat x 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. RA/VA = *c*9,*t*11-18:2/ *t*11-18:1 ratio; GLA/LA = *c*6,*c*9,*c*12-18:3/ *c*9,*c*12-18:2 ratio; ARA/LA = *c*5,*c*8,*c*11,*c*14-20:4/ *c*9,*c*12-18:2 ratio; DHA/ALA = *c*4,*c*7,*c*10,*c*13, *c*16,*c*19-22:6/ *c*9,*c*12,*c*15-18:3; TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

individual TFA, the conversion of VA to RA, and the hepatic output of individual TFA by VLDL. In addition, the experimental design allowed us to study the effects of TFA on the relative conversion rates of key FA, as well as on the content of FA in tissues. To the best of our knowledge, there are no publications examining the interaction between TFA and different dietary unsaturated FA on the parameters mentioned above.

Most evidence demonstrated that the brain appears to be protected from the incorporation of TFA isomers into the complex lipids in many animal species and humans [25–27]. Nevertheless, studies by Teixeira et al. [28] showed an incorporation of 0.30% of TFA in the brain tissue after a prolonged intake of diets containing high levels of TFA. Under our experimental conditions, TFA were not detected, including RA, into the total lipids of the brain. With this exception, all TFA isomers were incorporated into, and therefore retained in the tissues analyzed, i.e., EWAT, gastrocnemius muscle and liver. However, the magnitude of incorporation and retention was highly dependent on the

Table 4. Fatty acid composition of gastrocnemius muscle in mice fed experimental diets (% of total FAME)

							ANOVA		
Fatty acids	O	Ot	C	Ct	R	Rt	F	t	Fxt
16:0	20.75 ± 0.14^{ac}	21.79 ± 0.42^{c}	20.22 ± 0.25^{a}	20.43 ± 0.40^{a}	18.10 ± 0.10^{b}	21.18 ± 0.37^{ac}	0.000	0.000	0.000
18:0	4.44 ± 0.16	5.14 ± 0.36	4.96 ± 0.49	5.65 ± 0.38	4.82 ± 0.40	5.09 ± 0.35	0.372	0.080	0.808
*c*9-16:1	8.20 ± 0.31^{ab}	9.64 ± 0.55^{a}	6.69 ± 0.47^{b}	6.37 ± 0.36^{b}	6.06 ± 0.63^{b}	8.58 ± 1.20^{ab}	0.004	0.032	0.113
*c*9-18:1	28.43 ± 0.50^{ab}	23.26 ± 0.92^{d}	21.61 ± 0.90^{cd}	18.63 ± 1.15^{c}	30.92 ± 1.18^{b}	24.46 ± 0.69^{ad}	0.000	0.000	0.184
*c*11-18:1	5.68 ± 0.16^{a}	5.21 ± 0.19^{ad}	3.44 ± 0.00^{b}	3.51 ± 0.11^{b}	4.76 ± 0.05^{cd}	4.43 ± 0.10^{c}	0.000	0.020	0.084
(*t*6+*t*7+*t*8)-18:1	0.00 ± 0.00^{a}	0.32 ± 0.03^{b}	0.00 ± 0.00^{a}	0.28 ± 0.07^{b}	0.00 ± 0.00^{a}	0.31 ± 0.06^{b}	0.838	0.000	0.838
*t*9-18:1	0.00 ± 0.00^{a}	0.79 ± 0.05^{bc}	0.00 ± 0.00^{a}	0.81 ± 0.03^{b}	0.00 ± 0.00^{a}	0.66 ± 0.02^{c}	0.018	0.000	0.018
*t*10-18:1	0.00 ± 0.00^{a}	0.47 ± 0.04^{b}	0.00 ± 0.00^{a}	0.55 ± 0.06^{b}	0.00 ± 0.00^{a}	0.41 ± 0.08^{b}	0.270	0.000	0.270
*t*11-18:1	0.00 ± 0.00^{a}	0.27 ± 0.03^{b}	0.00 ± 0.00^{a}	0.48 ± 0.09^{c}	0.00 ± 0.00^{a}	0.29 ± 0.04^{bc}	0.052	0.000	0.052
*c*11-20:1	0.51 ± 0.01^{a}	0.39 ± 0.03^{b}	0.50 ± 0.02^{a}	0.37 ± 0.03^{b}	0.64 ± 0.00^{b}	0.45 ± 0.01^{ab}	0.000	0.000	0.174
*c*9,*c*12-18:2	12.17 ± 0.02^{a}	10.66 ± 0.42^{a}	22.57 ± 1.31^{b}	17.76 ± 1.06^{c}	13.21 ± 0.37^{a}	10.47 ± 0.33^{a}	0.000	0.000	0.099
*t*9,*t*12-18:1	0.00 ± 0.00^{a}	0.18 ± 0.03^{b}	0.00 ± 0.00^{a}	0.17 ± 0.02^{b}	0.00 ± 0.00^{a}	0.18 ± 0.01^{b}	0.800	0.000	0.800
*c*9,*t*11-18:2	0.00 ± 0.00^{a}	0.46 ± 0.03^{b}	0.00 ± 0.00^{a}	0.45 ± 0.04^{b}	0.00 ± 0.00^{a}	0.45 ± 0.03^{b}	0.983	0.000	0.983
*c*6,*c*9,*c*12-18:3	0.09 ± 0.01^{ab}	0.09 ± 0.01^{ab}	0.12 ± 0.01^{b}	0.10 ± 0.01^{ab}	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.009	0.255	0.421
*c*9,*c*12,*c*15-18:3	0.26 ± 0.01^{a}	0.23 ± 0.02^{a}	0.34 ± 0.03^{a}	0.30 ± 0.03^{a}	1.43 ± 0.11^{b}	1.03 ± 0.06^{c}	0.000	0.001	0.003
*c*11,*c*14-20:2	0.14 ± 0.01^{ab}	0.16 ± 0.01^{b}	0.32 ± 0.02^{c}	0.28 ± 0.01^{c}	0.15 ± 0.01^{b}	0.08 ± 0.01^{a}	0.000	0.008	0.007
*c*8, *c*11,*c*14-20:3	0.63 ± 0.04^{ab}	0.82 ± 0.08^{b}	0.49 ± 0.03^{a}	0.68 ± 0.06^{ab}	0.54 ± 0.05^{a}	0.57 ± 0.03^{a}	0.007	0.003	0.204
*c*5,*c*8,*c*11,*c*14-20:4	6.17 ± 0.17^{ab}	6.29 ± 0.46^{ab}	6.07 ± 0.64^{abc}	7.66 ± 0.65^{b}	3.98 ± 0.47^{c}	4.49 ± 0.34^{ac}	0.000	0.072	0.308
*c*5,*c*8,*c*11,*c*14,*c*17-20:5	0.02 ± 0.01^{a}	0.08 ± 0.01^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.18 ± 0.02^{c}	0.20 ± 0.01^{c}	0.000	0.001	0.040
*c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6	6.76 ± 0.19^{ab}	7.46 ± 0.53^{ab}	6.13 ± 0.69^{a}	7.98 ± 0.62^{ab}	9.92 ± 1.44^{b}	10.07 ± 1.12^{b}	0.003	0.215	0.612
ΣTFA	0.00 ± 0.00^{a}	2.04 ± 0.09^{bc}	0.00 ± 0.00^{a}	2.28 ± 0.13^{b}	0.00 ± 0.00^{a}	1.85 ± 0.08^{c}	0.025	0.000	0.025
ΣTotal SFA	26.60 ± 0.36^{a}	28.34 ± 0.61^{a}	26.87 ± 0.80^{a}	27.27 ± 0.74^{a}	24.24 ± 0.14^{b}	27.97 ± 0.17^{a}	0.050	0.000	0.017
ΣTotal MUFA	42.81 ± 0.96^{a}	38.51 ± 1.42^{a}	32.24 ± 1.39^{ab}	28.88 ± 1.01^{c}	42.38 ± 1.86^{a}	37.92 ± 1.61^{ab}	0.000	0.002	0.915
ΣTotal PUFA	26.23 ± 0.34^{a}	25.78 ± 1.30^{a}	36.05 ± 0.04^{b}	34.78 ± 0.26^{b}	29.48 ± 1.51^{a}	27.00 ± 1.60^{a}	0.000	0.116	0.632
Σn-6 LC-PUFA	6.93 ± 0.15^{abc}	7.26 ± 0.51^{bc}	6.88 ± 0.69^{abc}	8.61 ± 0.69^{c}	4.67 ± 0.53^{a}	5.15 ± 0.36^{ab}	0.000	0.059	0.354
Σn-3 LC-PUFA	6.78 ± 0.19^{ab}	7.54 ± 0.54^{ab}	6.14 ± 0.69^{a}	7.99 ± 0.62^{ab}	10.09 ± 1.46^{b}	10.27 ± 1.12^{b}	0.002	0.203	0.627
NI	3.31 ± 0.19	3.99 ± 0.36	3.71 ± 0.41	5.16 ± 0.22	3.61 ± 0.26	3.75 ± 0.19			
RA/VA	0.00 ± 0.00	180.28 ± 25.91	0.00 ± 0.00	114.39 ± 27.96	0.00 ± 0.00	180.93 ± 45.85	0.220	0.000	0.220

Values expressed as media ± SEM of $n = 6$ per group. F, t, Fxt correspond to p values of 2×3 ANOVA to the effect of fat, TFA and interaction of fat x 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; RA/VA = *c*9,*t*11-18:2/ *t*11-18:1 ratio; TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

tissue and type of isomer. Specifically, the EWAT showed a higher capacity of incorporation and retention of TFA than the liver and skeletal muscle. The results found in EWAT are in agreement with other authors in experimental animal models [12]. The lack of endogenous biosynthesis of TFA by the adipose tissue and the slow turnover of FA into this tissue [29] let us claim that the levels of TFA in adipose tissue could be the best biomarker of long term TFA intake. Moreover, our results showed different patterns of the individual TFA incorporation and retention in EWAT from those observed by Baylin et al. [30]. Independently of the dietary fat, 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 levels of VA might be mainly associated with a high conversion to RA. Recently, it has been shown that VA might also be converted to another CLA isomer, specifically *t*11,*c*13-18:2, by a Δ13-desaturase that becomes important in the presence of a high level of dietary VA [31]. Probably, this is not a major metabolic pathway and the physiological role of the CLA isomer obtained is still unknown. In addition, other minor metabolic products might be obtained from the action of Δ6-desaturase on VA [31, 32], and of Δ5-desaturase on elaidic acid [33, 34]. Moreover, 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 compared with those of *t*9-18:1 could be related to a higher rate of oxidation and/or metabolization of this isomer. Despite the differences in TFA retention, it is important to note that the levels of individual *t*-18:1, *t*9,*t*12-18:2, and RA in EWAT were not affected by the relative proportions of dietary n-9, n-6, and n-3 FA. In addition, the higher levels of RA in the EWAT compared to liver or skeletal muscle are in agreement with other authors that found a high incorporation of RA in adipose tissue [35] and mammary fat pad [36], and

Table 5. Fatty acid composition of serum in mice fed experimental diets (% of total FAME)

							ANOVA		
Fatty acids	O	Ot	C	Ct	R	Rt	F	t	Fxt
16:0	24.97 ± 1.20	25.72 ± 0.32	26.94 ± 1.01	24.54 ± 0.77	23.14 ± 0.79	25.47 ± 0.47	0.213	0.740	0.024
18:0	8.66 ± 0.62	9.39 ± 0.72	9.66 ± 0.31	9.29 ± 0.30	9.22 ± 0.77	9.65 ± 0.70	0.714	0.593	0.644
*c*9-16:1	1.15 ± 0.12^{a}	0.84 ± 0.16^{ab}	0.69 ± 0.09^{b}	0.76 ± 0.03^{ab}	0.76 ± 0.05^{ab}	0.85 ± 0.04^{ab}	0.024	0.509	0.073
*c*9-18:1	22.87 ± 0.75^{a}	20.16 ± 1.64^{a}	12.39 ± 0.66^{b}	13.98 ± 1.02^{b}	23.11 ± 0.54^{a}	19.52 ± 1.16^{a}	0.000	0.075	0.043
*c*11-18:1	3.05 ± 0.24^{a}	2.81 ± 0.37^{ab}	1.20 ± 0.04^{c}	1.83 ± 0.13^{c}	2.03 ± 0.15^{bc}	1.98 ± 0.23^{bc}	0.000	0.538	0.140
(*t*6+*t*7+*t*8)-18:1	0.00 ± 0.00^{a}	0.60 ± 0.13^{b}	0.00 ± 0.00^{a}	0.41 ± 0.03^{bc}	0.00 ± 0.00^{a}	0.27 ± 0.02^{c}	0.023	0.000	0.023
*t*9-18:1	0.00 ± 0.00^{a}	0.96 ± 0.20^{b}	0.00 ± 0.00^{a}	0.65 ± 0.08^{bc}	0.00 ± 0.00^{a}	0.59 ± 0.02^{c}	0.096	0.000	0.096
*t*10-18:1	0.00 ± 0.00^{a}	0.94 ± 0.25^{b}	0.00 ± 0.00^{a}	0.58 ± 0.10^{bc}	0.00 ± 0.00^{a}	0.40 ± 0.03^{c}	0.064	0.000	0.064
*t*11-18:1	0.00 ± 0.00^{a}	1.05 ± 0.29^{b}	0.00 ± 0.00^{a}	0.77 ± 0.02^{b}	0.00 ± 0.00^{a}	0.63 ± 0.02^{b}	0.215	0.000	0.215
*c*11-20:1	0.62 ± 0.04^{ab}	0.42 ± 0.05^{ac}	0.44 ± 0.04^{ac}	0.41 ± 0.03^{c}	0.73 ± 0.04^{b}	0.49 ± 0.07^{ac}	0.002	0.000	0.069
*c*9,*c*12-18:2	18.35 ± 0.25^{a}	20.14 ± 0.77^{a}	29.84 ± 0.63^{b}	25.46 ± 1.44^{c}	20.32 ± 0.45^{a}	20.51 ± 0.40^{a}	0.000	0.211	0.001
*c*9,*t*11-18:2	0.00 ± 0.00^{a}	0.06 ± 0.02^{b}	0.00 ± 0.00^{a}	0.14 ± 0.01^{c}	0.00 ± 0.00^{a}	0.15 ± 0.02^{c}	0.001	0.000	0.001
*c*6,*c*9,*c*12-18:3	0.08 ± 0.02^{ab}	0.02 ± 0.01^{a}	0.23 ± 0.04^{c}	0.11 ± 0.01^{b}	0.10 ± 0.01^{b}	0.10 ± 0.01^{b}	0.000	0.001	0.012
*c*9,*c*12,*c*15-18:3	0.15 ± 0.03^{a}	0.18 ± 0.02^{a}	0.16 ± 0.02^{a}	0.13 ± 0.02^{a}	1.27 ± 0.09^{b}	0.79 ± 0.13^{c}	0.000	0.007	0.001
*c*11,*c*14-20:2	0.19 ± 0.02^{a}	0.26 ± 0.02^{ab}	0.28 ± 0.03^{b}	0.27 ± 0.03^{ab}	0.24 ± 0.01^{ab}	0.23 ± 0.02^{ab}	0.039	0.410	0.125
*c*8,*c*11,*c*14-20:3	1.71 ± 0.21^{ab}	1.71 ± 0.25^{ab}	1.10 ± 0.08^{ab}	1.72 ± 0.11^{ab}	1.68 ± 0.16^{ab}	1.89 ± 0.10^{b}	0.072	0.048	0.179
*c*5,*c*8,*c*11,*c*14-20:4	8.77 ± 0.49^{a}	8.42 ± 0.18^{a}	9.85 ± 0.78^{a}	11.24 ± 1.42^{a}	4.53 ± 0.48^{b}	4.99 ± 0.48^{b}	0.000	0.423	0.519
*c*5,*c*8,*c*11,*c*14,*c*17-20:5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.61 ± 0.03^{b}	0.50 ± 0.08^{b}	0.000	0.252	0.271
*c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6	2.42 ± 0.13^{a}	1.88 ± 0.25^{a}	1.81 ± 0.16^{a}	2.27 ± 0.34^{a}	5.02 ± 0.53^{b}	6.50 ± 0.49^{b}	0.000	0.117	0.028
ΣTFA	0.00 ± 0.00^{a}	3.55 ± 0.85^{b}	0.00 ± 0.00^{a}	2.42 ± 0.20^{b}	0.00 ± 0.00^{a}	2.32 ± 0.43^{b}	0.244	0.000	0.244
ΣTotal SFA	34.57 ± 1.72	34.32 ± 1.91	37.49 ± 0.79	35.16 ± 1.24	33.15 ± 1.37	36.00 ± 1.11	0.346	0.939	0.201
ΣTotal MUFA	27.42 ± 0.82^{a}	24.24 ± 1.93^{a}	15.98 ± 1.47^{b}	17.00 ± 1.17^{b}	26.52 ± 0.64^{a}	22.78 ± 1.35^{a}	0.000	0.076	0.156
ΣTotal PUFA	32.06 ± 0.46^{a}	32.40 ± 0.46^{a}	43.22 ± 1.21^{c}	41.20 ± 2.06^{bc}	36.36 ± 1.00^{ab}	35.46 ± 0.92^{a}	0.000	0.373	0.599
Σn-6 LC-PUFA	10.84 ± 0.41^{abc}	9.30 ± 0.95^{ab}	11.23 ± 0.85^{bc}	13.23 ± 1.48^{c}	7.31 ± 0.42^{a}	7.11 ± 0.51^{a}	0.000	0.903	0.137
Σn-3 LC-PUFA	2.62 ± 0.15^{a}	1.88 ± 0.25^{a}	1.81 ± 0.16^{a}	2.27 ± 0.34^{a}	6.21 ± 0.26^{b}	7.00 ± 0.41^{b}	0.000	0.460	0.027
NI	3.75 ± 0.62	3.47 ± 0.13	3.20 ± 0.44	3.16 ± 0.16	2.56 ± 0.48	2.60 ± 0.52			

Values expressed as media ± SEM of $n = 6$ per group. F, t, Fxt correspond to p values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat x 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; TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids.

it could be associated with the fact that CLA are incorporated mainly into the neutral lipid molecules [37], and approximately 99% of adipose tissue is composed of triglycerides [38]. Moreover, Kraft et. al. [39] suggested a higher conversion efficiency of VA to RA in adipose tissue than in muscle or liver and this could be due to low Δ9-desaturase activity in the last tissues as a result of deficient expression or low tissue-specific metabolic rate [39].

In the same way as in the adipose tissue, the incorporation and retention of *t*9-18:1 in gastrocnemius muscle was significantly higher than that of other *t*-18:1 isomers, and these results could be explained by the same mechanisms cited above. However, it should be noted that the levels reached were lower than those achieved in the adipose tissue and showed some dependence on dietary fats. There is no research reporting the influence of different types of dietary FA on the retention/incorporation of TFA isomers. In addition, dietary FA did not modify the relative conversion rate of VA to RA in the gastrocnemius muscle reaching similar levels of RA in the different dietary fat groups supplemented with PHVO. In agreement with other authors [35, 39], the skeletal muscle retained higher levels of RA than the liver and this could be associated with different factors, including a greater conversion efficiency of VA to RA in muscle than in liver [39] and others related to a FA mobilization, i.e. output of VLDL containing RA.

The hepatic pattern of the individual *t*-18:1 incorporation and retention was different from other tissues analyzed showing that the levels of VA were the highest. In addition, it was found that olive oil consumption reduced the incorporation and retention as well as the content of this isomer, and raised the levels of RA, showing a dependence on the type of dietary fat. The lower VA levels were not associated with a higher VA secretion by the VLDL; therefore, it seems to be related to a higher bioconversion to RA. The RA bioconversion rate depends on several factors such as species [40–42], tissue [43], experimental conditions [44, 45], dietary compounds [46], and on the type of dietary fat

Table 6. Fatty acid composition of brain in mice fed experimental diets (% of total FAME)

							ANOVA		
Fatty acids	O	Ot	C	Ct	R	Rt	F	t	Fxt
16:0	23.28 ± 0.73^{a}	23.28 ± 0.50^{a}	22.85 ± 0.44^{a}	22.16 ± 0.47^{a}	23.05 ± 0.48^{a}	23.12 ± 0.20^{a}	0.026	0.747	0.942
18:0	21.03 ± 0.10^{ab}	20.88 ± 0.08^{ab}	21.51 ± 0.52^{b}	21.22 ± 0.27^{b}	20.06 ± 0.34^{a}	21.06 ± 0.07^{ab}	0.013	0.362	0.031
*c*6-16:1	0.12 ± 0.02^{a}	0.14 ± 0.01^{a}	0.14 ± 0.02^{a}	0.10 ± 0.01^{a}	0.15 ± 0.03^{a}	0.13 ± 0.01^{a}	0.154	0.460	0.340
*c*9-16:1	0.40 ± 0.03^{a}	0.38 ± 0.03^{a}	0.48 ± 0.03^{a}	0.37 ± 0.03^{a}	0.49 ± 0.07^{a}	0.35 ± 0.02^{a}	0.592	0.008	0.311
*c*9-18:1	18.62 ± 0.25^{a}	18.09 ± 0.21^{ab}	17.30 ± 0.47^{b}	17.29 ± 0.31^{b}	18.55 ± 0.35^{a}	18.74 ± 0.12^{a}	0.000	0.625	0.439
*c*11-18:1	4.58 ± 0.12^{a}	4.56 ± 0.10^{a}	3.78 ± 0.16^{c}	4.14 ± 0.06b^{c}	4.15 ± 0.08^{bc}	4.34 ± 0.04^{ab}	0.000	0.031	0.142
*c*11-20:1	2.99 ± 0.09^{a}	2.88 ± 0.08^{a}	2.31 ± 0.17^{b}	2.71 ± 0.10^{ab}	2.59 ± 0.09^{ab}	2.94 ± 0.03^{a}	0.001	0.009	0.023
*c*9,*c*12-18:2	0.42 ± 0.04^{a}	0.34 ± 0.18^{a}	0.86 ± 0.13^{b}	0.63 ± 0.02^{c}	0.59 ± 0.10^{ac}	0.51 ± 0.02^{ac}	0.000	0.015	0.031
*c*11,*c*14-20:2	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}	0.16 ± 0.02^{b}	0.17 ± 0.01^{b}	0.12 ± 0.01^{a}	0.13 ± 0.01^{ab}	0.000	0.373	0.622
*c*8,*c*11,*c*14-20:3	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.16 ± 0.01^{ab}	0.16 ± 0.01^{ab}	0.20 ± 0.01^{bc}	0.26 ± 0.04^{c}	0.000	0.118	0.709
*c*11,*c*14,*c*17-20:3	0.05 ± 0.005^{a}	0.05 ± 0.005^{ab}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.03 ± 0.002^{b}	0.03 ± 0.002^{b}	0.000	0.299	0.871
*c*5,*c*8,*c*11,*c*14-20:4	9.48 ± 0.12^{a}	9.72 ± 0.08^{a}	10.40 ± 0.22^{b}	10.38 ± 0.09^{b}	8.78 ± 0.09^{c}	8.62 ± 0.05^{c}	0.000	0.729	0.198
*c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6	13.1 ± 0.84^{a}	13.5 ± 0.56^{ab}	15.4 ± 0.57^{bc}	15.0 ± 0.38^{abc}	16.1 ± 0.50^{c}	14.5 ± 0.24^{abc}	0.001	0.244	0.170
ΣTotal SFA	44.88 ± 0.80	44.75 ± 0.56	45.29 ± 1.13	44.03 ± 0.55	43.65 ± 0.42	44.73 ± 0.26	0.638	0.851	0.247
ΣTotal MUFA	26.71 ± 0.41^{a}	26.05 ± 0.27^{ab}	23.95 ± 0.56^{c}	24.67 ± 0.41^{bc}	25.94 ± 0.33^{ab}	26.49 ± 0.11^{a}	0.000	0.515	0.153
ΣTotal PUFA	27.11 ± 1.07^{ab}	27.84 ± 0.70^{abc}	29.81 ± 1.32^{bc}	30.25 ± 0.87^{c}	26.12 ± 0.60^{ab}	24.38 ± 0.29^{a}	0.000	0.790	0.318
Σn-6 LC-PUFA	13.51 ± 0.25^{a}	13.92 ± 0.17^{ab}	14.74 ± 0.32^{bc}	15.11 ± 0.17^{c}	9.20 ± 0.12^{d}	9.10 ± 0.06^{d}	0.000	0.186	0.377
Σn-3 LC-PUFA	13.17 ± 0.84^{a}	13.58 ± 0.56^{ab}	14.20 ± 1.06^{ab}	14.50 ± 0.72^{ab}	16.33 ± 0.51^{b}	14.77 ± 0.26^{ab}	0.018	0.627	0.307
NI	1.30 ± 0.04	1.36 ± 0.04	0.98 ± 0.06	1.11 ± 0.05	1.59 ± 0.05	1.63 ± 0.04			

Values expressed as media ± SEM of $n = 6$ per group. F, t, Fxt correspond to p values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat x 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; TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids.

according to other studies [47]. The high RA content in mice fed Ot, compared with Ct and Rt, might be due to lower dietary PUFA levels. It is very well known that Δ9-desaturase is inhibited by high dietary PUFA levels [46, 48], and to a greater extent by ARA [46], which was higher in the liver of mice fed C and Ct diets. The lower levels of RA in liver than in EWAT and gastrocnemius muscle might be related to the low hepatic bioconversion of VA to RA in comparison with other tissues [44], and additionally to the high output of RA in the VLDL particles secreted by the liver and/or to the high rate of hepatic RA oxidation [49]. Despite the differences in hepatic RA content, the RA secreted by the VLDL particles did not show dependence on dietary fats. In our experimental design it is possible to hypothesize that the extrahepatic uptake and/or utilization of RA was increased by the olive oil diet. This might be associated with lower circulating levels of RA found in the Ot group, despite the similar output of RA by liver and RA content in extrahepatic tissues.

The increased hepatic Δ9-desaturase index, expressed by the *c*9-16:1/16:0 and *c*9-18:1/18:0 ratios, in animals fed olive oil diets in both the absence and presence of TFA seems to reflect a raised enzyme activity but not an increase of flux by substrate induction. Thus, dietary olive oil might increase the hepatic relative conversion rate of VA to RA as well as amplify the increased biosynthesis of FA previously observed in animals fed Ot diets [16] and in animals fed olive oil supplemented with RA [50]. Moreover, in agreement with Hurtado de Catalfo et al. [15] animals fed olive oil showed an increase of the relative conversion rate of n-6 and n-3 LC-PUFA in liver. Although TFA lowered the hepatic Δ6-desaturase index (GLA/LA ratio), the relative conversion rate of LA to ARA and the ARA content was not modified by PHVO. The decreased Δ6-desaturase index might be in agreement with different studies that observed a reduced enzyme activity induced by TFA [13, 51–54]. However, the relative conversion rate of n-3 LC-PUFA was not reduced; it was even increased in those animals fed with low levels of ALA (olive and corn oils). Furthermore, the reduced Δ6-desaturase index in TFA animals was accompanied by an increased LC-PUFA conversion through the alternative pathways [55–58], driving to an elongation of the LA, followed by desaturation of *c*11,*c*14-20:2 reaching similar levels of ARA in the presence or absence of TFA. This metabolic pathway was not detected in the n-3 LC-PUFA biosynthesis because *c*11,*c*14,*c*17-20:3 was negligible in TFA supplemented animals. Moreover, it is important to mention that most experiments of the references reporting inhibition of LC-PUFA biosynthesis were carried out with hydrogenated fats rich in elaidic acid, and not in a mixture of several *t*-18:1.

The increased RA/VA ratio in EWAT of mice fed the Ot diet, reflecting a high Δ9-desaturase activity, correlated with

Table 7. Percentage of incorporation and retention of individual TFA in different tissues of mice fed experimental diets containing *trans* fatty acids

	Ot	Ct	Rt
Liver			
(*t*6+*t*7+*t*8)-18:1	9.05 ± 1.32	13.92 ± 2.43	17.45 ± 3.79
*t*9-18:1	20.50 ± 1.44	19.16 ± 1.06	19.58 ± 2.36
*t*10-18:1	17.01 ± 0.93	14.96 ± 1.60	14.12 ± 3.02
*t*11-18:1	19.15 ± 3.09[a]	26.23 ± 0.60[b]	24.16 ± 2.07[ab]
*t*9,*t*12-18:2	66.25 ± 9.43	52.71 ± 9.59	45.08 ± 7.35
Total TFA	18.47 ± 1.14	19.44 ± 0.65	19.16 ± 0.88
Epididymal White Adipose tissue			
(*t*6+*t*7+*t*8)-18:1	34.30 ± 3.04	32.80 ± 5.51	28.52 ± 2.57
*t*9-18:1	51.52 ± 5.21	40.88 ± 6.49	50.52 ± 6.85
*t*10-18:1	39.20 ± 1.90	35.86 ± 5.38	40.20 ± 3.98
*t*11-18:1	20.61 ± 2.57	25.26 ± 4.98	27.34 ± 1.34
*t*9,*t*12-18:2	124.57 ± 7.33	104.99 ± 10.23	118.38 ± 23.31
Total TFA	38.83 ± 1.34	35.71 ± 4.98	39.54 ± 3.00
Gastrocnemius muscle			
(*t*6+*t*7+*t*8)-18:1	20.30 ± 2.18	17.40 ± 4.22	23.75 ± 2.05
*t*9-18:1	34.84 ± 2.41[ab]	35.69 ± 1.12[a]	29.06 ± 1.39[b]
*t*10-18:1	16.10 ± 1.30	18.80 ± 2.11	17.19 ± 1.10
*t*11-18:1	10.55 ± 1.08	18.89 ± 3.76	11.63 ± 2.28
*t*9,*t*12-18:2	67.75 ± 10.31	61.00 ± 6.79	66.49 ± 5.63
Total TFA	21.23 ± 0.94[ab]	23.78 ± 1.40[a]	19.26 ± 0.86[b]
Serum			
(*t*6+*t*7+*t*8)-18:1	37.37 ± 10.34	25.76 ± 2.73	16.97 ± 1.84
*t*9-18:1	42.48 ± 11.42	28.60 ± 4.35	26.04 ± 1.15
*t*10-18:1	32.05 ± 10.91	19.88 ± 4.53	13.63 ± 1.52
*t*11-18:1	41.85 ± 14.74	30.77 ± 1.23	25.11 ± 1.24
Total TFA	37.06 ± 11.38	25.21 ± 2.75	19.74 ± 1.17
VSTG- rich Serum			
(*t*6+*t*7+*t*8)-18:1	15.42 ± 0.87	23.06 ± 4.02	17.61 ± 1.02
*t*9-18:1	36.85 ± 0.67[a]	42.22 ± 0.57[b]	42.96 ± 1.52[b]
*t*10-18:1	14.47 ± 1.90	17.87 ± 2.39	18.67 ± 0.96
*t*11-18:1	14.15 ± 2.15[a]	16.04 ± 0.28[a]	23.11 ± 0.41[b]
*t*9,*t*12-18:2	28.57 ± 16.49	40.35 ± 23.30	11.11 ± 6.41
Total TFA	23.22 ± 0.30[a]	32.35 ± 1.00[b]	31.89 ± 0.25[b]

Values expressed as media ± SEM of $n = 6$ per group. Incorporation and retention of each TFA isomer was calculated using the relation: % isomer in the biological sample/ % isomer in the diet x 100%. "Total TFA" correspond to incorporation and retention values of all *t*-18:1 and *t*,*t*-18:2 isomers. 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.

the *c*9-18:1/18:0 and *c*9-16:1/16:0 ratios, without showing an increase in fat accretion (data not shown). Further research should be carried out in order to clarify the effect of the individual TFA on FA metabolism and LC-PUFA biosynthesis in adipose tissue of animals fed different fats.

5 Conclusions

In conclusion, with the exception of the brain, TFA were highly incorporated into EWAT, liver, gastrocnemius muscle, and serum. TFA retention and RA bioconversion from VA depended on the dietary FA proportions. The higher levels of RA in liver of mice fed the Ot diet could be associated with a raised Δ9-desaturase index. We suggest that due to the dose and type of hydrogenated fat and also to the high bioconversion to RA, the FA composition of the tissues was scarcely modified by TFA.

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Table 8. Fatty acid composition of VLDL-rich serum in mice fed experimental diets (% of total FAME)

							ANOVA		
Fatty acids	O	Ot	C	Ct	R	Rt	F	t	Fxt
16:0	24.53 ± 0.52^{a}	23.43 ± 0.33^{ab}	22.79 ± 0.82^{abc}	20.91 ± 0.17^{c}	21.95 ± 0.15^{bc}	22.56 ± 0.54^{abc}	0.001	0.060	0.054
18:0	8.40 ± 0.16^{a}	5.05 ± 0.10^{d}	8.67 ± 0.27 ^{a}	6.42 ± 0.15^{c}	7.24 ± 0.17^{b}	6.83 ± 0.09^{bc}	0.000	0.000	0.000
*c*9-16:1	2.42 ± 0.01^{a}	5.31 ± 0.57^{c}	2.46 ± 0.12^{a}	4.02 ± 0.37^{b}	2.74 ± 0.08^{a}	2.83 ± 0.01^{ab}	0.005	0.000	0.000
*c*9-18:1	24.41 ± 0.39^{a}	24.08 ± 0.76^{a}	15.52 ± 0.45^{c}	16.78 ± 1.41^{c}	29.12 ± 0.83^{b}	22.64 ± 0.46^{a}	0.000	0.011	0.000
*c*11-18:1	2.17 ± 0.04^{a}	2.52 ± 0.01^{b}	0.97 ± 0.09 ^{c}	1.19 ± 0.04 ^{c}	1.92 ± 0.13^{a}	1.58 ± 0.03^{d}	0.000	0.199	0.000
(*t*6+*t*7+*t*8)-18:1	0.00 ± 0.00^{a}	0.25 ± 0.01^{b}	0.00 ± 0.00^{a}	0.37 ± 0.06^{b}	0.00 ± 0.00^{a}	0.28 ± 0.02^{b}	0.101	0.000	0.101
*t*9-18:1	0.00 ± 0.00^{a}	0.84 ± 0.01^{b}	0.00 ± 0.00^{a}	0.96 ± 0.01^{c}	0.00 ± 0.00^{a}	0.97 ± 0.03^{c}	0.001	0.000	0.001
*t*10-18:1	0.00 ± 0.00^{a}	0.43 ± 0.06^{b}	0.00 ± 0.00^{a}	0.52 ± 0.07^{b}	0.00 ± 0.00^{a}	0.55 ± 0.03^{b}	0.260	0.000	0.260
*t*11-18:1	0.00 ± 0.00^{a}	0.36 ± 0.05^{b}	0.00 ± 0.00^{a}	0.40 ± 0.01^{b}	0.00 ± 0.00^{a}	0.58 ± 0.01^{c}	0.000	0.000	0.000
*c*11-20:1	0.53 ± 0.05^{a}	0.46 ± 0.02^{ab}	0.36 ± 0.07^{a}	0.32 ± 0.06^{a}	0.62 ± 0.02^{b}	0.48 ± 0.05^{ab}	0.002	0.058	0.594
*c*13-22:1	0.00 ± 0.00	0.08 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.04	0.05 ± 0.03	0.156	0.270	0.266
*c*9,*c*12-18:2	18.00 ± 0.26^{a}	14.58 ± 0.01^{d}	31.12 ± 1.13^{c}	25.44 ± 0.26^{b}	19.29 ± 0.44^{a}	18.85 ± 1.07^{a}	0.000	0.000	0.004
*c*9,*t*11-18:2	0.00 ± 0.00^{a}	0.40 ± 0.02^{b}	0.00 ± 0.00^{a}	0.45 ± 0.01^{b}	0.00 ± 0.00^{a}	0.44 ± 0.04^{b}	0.402	0.000	0.402
*t*9,*t*12-18:2	0.00 ± 0.00	0.08 ± 0.05	0.00 ± 0.00	0.11 ± 0.06	0.00 ± 0.00	0.03 ± 0.02	0.483	0.014	0.483
*c*6,*c*9,*c*12-18:3	0.71 ± 0.00^{a}	0.41 ± 0.02^{cd}	1.10 ± 0.05^{b}	0.71 ± 0.05^{a}	0.53 ± 0.06^{d}	0.32 ± 0.01^{c}	0.000	0.000	0.120
*c*9,*c*12,*c*15-18:3	0.20 ± 0.04^{ab}	0.39 ± 0.03^{b}	0.23 ± 0.05^{ab}	0.16 ± 0.04^{a}	1.54 ± 0.05^{d}	0.97 ± 0.07^{c}	0.000	0.001	0.000
*c*11,*c*14-20:2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.21 ± 0.03 ^{b}	0.16 ± 0.09^{ab}	0.09 ± 0.03^{ab}	0.00 ± 0.00^{a}	0.001	0.185	0.597
*c*8,*c*11,*c*14-20:3	0.68 ± 0.07^{ab}	0.94 ± 0.01^{b}	0.54 ± 0.05^{a}	0.42 ± 0.06^{a}	0.62 ± 0.08^{ab}	0.71 ± 0.14^{ab}	0.002	0.252	0.072
*c*5,*c*8,*c*11,*c*14-20:4	11.44 ± 0.33^{a}	9.06 ± 0.04^{ab}	10.02 ± 0.91^{a}	10.53 ± 1.22^{a}	5.51 ± 0.61^{c}	6.06 ± 0.57^{bc}	0.000	0.465	0.095
*c*5,*c*8,*c*11,*c*14,*c*17-20:5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.40 ± 0.05^{b}	0.36 ± 0.01^{b}	0.000	0.451	0.563
*c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6	3.16 ± 0.00^{ab}	3.11 ± 0.21^{a}	2.39 ± 0.34^{a}	2.06 ± 0.08^{a}	4.63 ± 0.56^{bc}	5.34 ± 0.40^{c}	0.000	0.674	0.279
ΣTFA	0.00 ± 0.00^{a}	1.94 ± 0.05^{b}	0.00 ± 0.00^{a}	2.36 ± 0.09^{c}	0.00 ± 0.00^{a}	2.41 ± 0.02^{c}	0.000	0.000	0.000
ΣTotal SFA	33,63 ± 0,36^{a}	29,45 ± 0,43^{bc}	32,51 ± 0,68^{a}	28,21 ± 0,35^{c}	30,54 ± 0,05^{b}	30,23 ± 0,46^{b}	0.020	0.000	0.000
ΣTotal MUFA	29,52 ± 0,47^{ab}	32,45 ± 1,35^{bc}	19,30 ± 0,41^{d}	22,31 ± 1,88^{d}	34,45 ± 0,82^{c}	27,57 ± 0,51^{a}	0.000	0.719	0.000
ΣTotal PUFA	34,20 ± 0,11^{a}	28,49 ± 0,20^{d}	45,61 ± 0,52^{c}	39,47 ± 0,76^{b}	32,60 ± 0,90^{a}	32,61 ± 0,01^{a}	0.000	0.000	0.000
Σn-6 PUFA	12.12 ± 0.40^{a}	10.01 ± 0.05^{ab}	10.78 ± 0.93^{a}	11.11 ± 1.19^{a}	6.04 ± 0.68^{c}	6.77 ± 0.71 ^{bc}	0.000	0.574	0.155
Σn-3 PUFA	3.17 ± 0.01^{a}	3.11 ± 0.21^{a}	2.39 ± 0.34^{a}	2.07 ± 0.08^{a}	5.15 ± 0.62^{b}	5.70 ± 0.41^{b}	0.000	0.833	0.444
NI	2.65 ± 0.00	3.80 ± 0.16	2.48 ± 0.10	4.30 ± 0.41	2.30 ± 0.08	4.25 ± 0.23			

Values expressed as media ± SEM of $n = 6$ per group. F, t, Fxt correspond to p values of 2 × 3 ANOVA to the effect of fat, TFA and interaction of fat x 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; TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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