Research Article

Nutritional and metabolic effects of dietary *trans* fats depend on the intake of linoleic acid

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This study aimed to investigate the differential effects of *trans* fatty acids (TFA) on the nutritional parameters, serum, and tissue triacylglycerol (TAG) levels, as well as the mechanisms involved in their regulation, in male Wistar rats fed linoleic acid-enriched ($^{+}$LA) or LA-deficient ($^{-}$LA) diets. The TFA effects on nutritional parameters and TAG metabolism differed depending on the dietary LA status. In the $^{+}$LA + TFA diet, compared to the $^{+}$LA diet, TFA did not alter the serum TAG levels despite the increased epididymal adipose tissue (EAT) lipoprotein lipase (LPL) activity and the higher hepatic TAG content associated with a lower CPT-Ia activity. Otherwise, in $^{-}$LA + TFA rats versus $^{-}$LA, TFA increased the serum, liver, and adipose tissue TAG levels associated with higher FAS, G6PDH, and ME enzyme activities in both liver and adipose tissue, and a reduced TAG clearance by the adipose tissue LPL enzyme. Although TFA supplementation in both $^{-}$LA and $^{+}$LA-rats tended to decrease the CPT-Ib activity, the muscle TAG levels were not modified. We conclude that the nutritional and metabolic TFA effects depended mainly on the changes in the FA profile induced by dietary LA and, in a lesser extent, on the specific type of isomer retained in the tissues.

Practical applications: Our approach involved growing animals in healthy physiological conditions with recommended levels of dietary fats, moderate intake of industrial TFA, and an unbalanced dietary LA:ALA ratio. These variables constitute a feasible situation to be observed in the human population. The present study might contribute to the understanding of how nutritional and lipid parameters are affected in the metabolic disorders induced by TFA.

Keywords: Isomer retention / Lipogenic enzymes / Lipoprotein lipase / Nutrition / Triacylglycerol secretion rat

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1 Introduction

In the last century, the human population, especially in Western countries, has drastically increased the consumption of vegetable oils rich in linoleic acid (LA; 9*c*12*c*18:2) with a parallel decrease of those rich in α-linolenic acid (ALA; 9*c*12*c*15*c*18:3), shifting the n-6:n-3 fatty acid (FA) ratio to 15–50:1 [1]. Some studies [2, 3] have related a high n-6:n-3 FA ratio to increased levels of arachidonic acid (AA)-derived eicosanoids, which could contribute to both a proinflammatory state plus endothelial dysfunction and a gradual fat mass enhancement. However, compared with saturated FA intake, n-6 PUFA reduce liver fat and modestly improve the metabolic status, without weight loss [4]. In experimental animal models, high n-6 PUFA have been related to impaired serum lipid levels, eicosanoid production and other

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Abbreviations: ALA, α-linolenic acid; **EAT,** epididymal adipose tissue; **EFA,** essential fatty acids; **FA,** fatty acids; **FAME,** fatty acid methyl esters; **FAS,** fatty acid synthase; **G6PDH,** glucose-6-phosphate dehydrogenase; **LA,** linoleic acid; **LC-PUFA,** long-chain polyunsaturated fatty acids; **LPL,** lipoprotein lipase; **ME,** malic enzyme; **MUFA,** monounsaturated fatty acids; **PHVO,** partially hydrogenated vegetable oil; **PUFA,** polyunsaturated fatty acids; **RPAT,** retroperitoneal adipose tissue; **SFA,** saturated fatty acids; **TAG,** triacylglycerol; **TAG-SR,** triacylglycerol secretion rate; **TFA,** *trans* fatty acids; **VLDL,** very low density lipoprotein

metabolic alterations [5, 6]. On the other hand, LA-deficient animals have shown several lipid alterations including lower serum concentrations of triacylglycerol (TAG), cholesterol, and phospholipids [7] as well as changes in FA composition and fat accretion in liver [8].

In humans, it has been demonstrated that a high intake of industrial *trans* fatty acids (TFA) increases the risk of certain diseases, such as cardiovascular heart disease, among others, by changes of lipid levels and lipoprotein profiles as well as by alterations of the endothelial function and cytokine production [9]. In experimental animal models with high TFA intake, many metabolic dysfunctions have been observed by several research groups including ours, such as hepatomegaly, hypertriacylglyceridaemia, body fat accretion [10–12], and other lipid metabolism alterations [13, 14]. Some of these metabolic dysfunctions are associated [15, 16] with alterations of the long-chain polyunsaturated fatty-acid (LC-PUFA) biosynthesis that could be impaired in essential fatty acid (EFA) deficiency or unbalances of the n-6:n-3 FA ratio [17]. Therefore, this study aimed to investigate the differential effect of TFA on the nutritional parameters, serum and tissue TAG levels and the mechanisms involved in the TAG regulation in rats fed LA enriched and deficient diets.

2 Materials and methods

2.1 Materials

Most nutrient compounds, including vitamins and minerals for diet preparations, were chemical grade or food grade, with the exception of corn oil (Arcor, Córdoba, Argentina), hydrogenated coconut fat without TFA (Castoroil, Buenos Aires, Argentina), partially hydrogenated soybean oil (50% of total FA—Calsa, Buenos Aires, Argentina), sucrose, cellulose, and corn starch, which were obtained from local sources. Corn oil was used as an unsaturated *cis*-FA source rich in LA (51% of total FA). Coconut fat was used to produce a LA deficiency status and TFA rich fat was used as TFA source. Internal standard (IS) glyceryl tritridecanoate [13:0-triacylglycerol (TAG)], external 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). Linoleic acid methyl esters, cis/trans mix (Catalog #47791) and 12t-18:1 methyl ester (Catalog #46907-U) were obtained from Supelco (Bellefonte, PA). Conjugated linoleic acid, cis/trans mix (Catalog #05507) and the standard glyceryl trioleate (Catalog #T7140) for TAG quantification were purchased from Sigma Chemical Co. (St Louis, MO). 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. The TAG commercial test kit was obtained from Sociedad de Bioquímicos (Santa Fe, Argentina).

2.2 Animals, diets, and experimental design

The experimental procedures were approved by the Ethics Committee of our School of Biochemistry and compiled according to the Guide for the Care and Use of Laboratory Animals [18]. Seventy two male Wistar rats, divided into three series of 24 animals weighing 70–80 g were housed in the animal quarter under controlled conditions (23 ± 2°C and 12 h light–dark cycle). The animals had free access to water and standard laboratory chow before the experimental period. After reaching 100–120 g, rats were assigned to four weight-matched groups ($n = 6$ per group) and housed in individual stainless-steel metabolic cages; they were fed ad libitum for 60 days with one of the following diets: enriched with LA ($^{+}$LA), deficient of LA ($^{-}$LA), $^{+}$LA supplemented with TFA fat ($^{+}$LA + TFA), or $^{-}$LA supplemented with TFA fat ($^{-}$LA + TFA).

The composition of the diets is presented in Table 1 and was based on the American Institute of Nutrition ad hoc writing committee recommendation (AIN-93G) [19], except for the FA source that was based on AIN-76 [20]. All diets were isoenergetic, theoretically providing 16.6 kJ/g. The $^{+}$LA diet contained 7% of corn oil (15.89% of energy) as a dietary fat source, LA providing 8.13% of energy in the diet. The $^{-}$LA diet contained 7% of coconut fat (0.002% of energy as LA). TFA supplementation was achieved by replacing 2% of corn oil ($^{+}$LA + TFA) or 2% of coconut fat ($^{-}$LA + TFA) by TFA rich fat (50% of TFA), LA providing 5.84% of energy in $^{+}$LA + TFA and 0.023% in $^{-}$LA + TFA. TFA provided 2.27% of the energy in both TFA supplemented diets. Except for the type of fats, all diets were identical. Each diet was freshly home-made; meals were prepared every 3 days throughout the experimental period and conserved at 4°C.

2.3 Experimental protocol

Rats were weighed, food intake was recorded, and feces were collected three times a week during the whole dietary treatment. Food intake was adjusted for waste by collecting food spillage. Feces were stored at −80°C until analysis. On the morning of day 60, one series of 24 rats ($n = 6$ per group) was sacrificed under anesthesia (1 mg of acepromazine + 100 mg of ketamine/kg of body weight), the body was shaved and the abdomen was cut open to remove visceral organs. The carcasses were weighed, chopped, and frozen at −80°C until compositional evaluation. A second series of 24 animals ($n = 6$ per experimental group) was treated under the same conditions and sacrificed as described above, with the purpose of collecting blood and dissected tissues according to the intended purpose. Serum was obtained by centrifugation immediately after blood collection. Liver,

Table 1. Composition of the experimental diets

	$^{+}$LA	$^{-}$LA	$^{+}$LA + TFA	$^{-}$LA + TFA
Dietary constituent (g/kg dry diet)				
Corn starch	529.5	529.5	529.5	529.5
Casein	200	200	200	200
Sucrose	100	100	100	100
Corn oil	70	0	50	0
Coconut fat	0	70	0	50
TFA rich fat	0	0	20	20
Fiber	50	50	50	50
Vitamin mixture	35	35	35	35
Mineral mixture	10	10	10	10
L-Cystine–L-methionine	3.0	3.0	3.0	3.0
Choline	2.5	2.5	2.5	2.5
Energy (kJ/g)	16.6	16.6	16.6	16.6
Fatty acid composition[a]				
6:0	ND	0.49	ND	0.35
8:0	ND	6.76	ND	4.83
10:0	ND	5.64	ND	4.03
11:0	ND	0.02	ND	0.01
12:0	ND	47.67	ND	34.05
13:0	ND	0.02	ND	0.01
14:0	0.03	17.46	0.05	12.50
16:0	12.21	9.21	11.88	9.74
16:1	0.12	ND	0.09	ND
17:0	ND	ND	0.02	0.02
18:0	1.93	12.53	4.46	12.03
(6–8)*t*18:1	ND	ND	1.62	1.62
9*t*18:1	ND	ND	2.08	2.08
10*t*18:1	ND	ND	3.05	3.05
11*t*18:1	ND	ND	2.76	2.76
12*t*18:1	ND	ND	2.14	2.14
(6–8)*c*18:1 + (13/14)*t*18:1	ND	ND	2.36	2.36
9*c*18:1	31.95	0.05	25.40	2.62
11*c*18:1	0.54	ND	1.31	0.92
19:0	ND	ND	0.16	0.16
9*t*12*t*18:2	ND	ND	0.39	0.39
9*c*12*t*18:2	ND	ND	0.11	0.11
9*t*12*c*18:2	ND	ND	0.13	0.13
9*c*12*c*18:2	51.26	0.01	36.79	0.19
20:0	0.50	0.14	0.37	0.11
8*c*20:1	ND	ND	0.02	0.02
11*c*20:1	0.25	ND	0.18	0.01
9*c*12*c*15*c*18:3	0.88	ND	0.63	ND
22:0	0.16	ND	0.21	0.10
24:0	0.15	ND	0.11	ND
Σ NI	ND	ND	3.68	3.68
Σ SFA	14.98	99.94	17.26	77.94
Σ MUFA	32.86	0.05	29.36	5.93
Σ PUFA	52.14	0.01	37.42	0.19

Diets were prepared according to AIN-93G [19], except for the fatty acid source that was based on AIN-76 [20].
$^{+}$LA, enriched with linoleic acid; $^{-}$LA, deficient of linoleic acid; TFA, *trans* fatty acids; NI, other fatty acids non identified; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.
[a]All values are presented as weight percentages of total fatty acid methyl esters and were estimated based on the fatty acid composition of dietary fats.

gastrocnemius muscle, epididymal adipose tissue (EAT), and retroperitoneal adipose tissue (RPAT) were frozen, weighed, and stored at −80°C until analysis. A third series of rats ($n = 6$ per group) was employed to estimate the in vivo hepatic triacylglycerol-secretion rate (TAG-SR) according to the procedure explained in item 3.8.

2.4 Fatty acid composition of dietary fats

The FA composition of dietary fats was determined by gas chromatography with a Shimadzu chromatograph (GC 2014) equipped with a flame ionization detector. The FA methyl esters (FAME) were obtained by transesterification with a methanolic potassium hydroxide solution [21]. FAME were separated on a capillary column CP Sil 88 (100 m, 0.25 μm film thickness), according to the AOCS Official Method Ce 1j-07 [22]. FAME were identified by comparison of their retention times relative to those of commercial standards and to those provided by the International CYTED Net (208RT0343). Values were expressed as percentage of total FAME. From the FA composition of the dietary fats, the FA composition of diets was estimated (Table 1).

2.5 Nutritional parameters

Nitrogen in samples from the carcass homogenate was converted to $(NH_4)_2SO_4$ by the Kjeldahl method [23] and its content was determined. Carcass protein levels were estimated by multiplying their nitrogen contents by 6.25. Water content was determined by drying aliquots of the carcass and food to a constant weight in an oven at 60°C. Total fat in the dried samples of carcasses, foods, and feces was extracted with light petroleum-ether [23]. The fat extract was evaporated in a vacuum system and the total fat was gravimetrically measured. The energy intake was calculated by multiplying the weight of the dry food consumed daily by the number of kJ/g of dry diet. Body weight gain efficiency was estimated as percentage of body weight gained (g) divided by energy intake (kJ/experimental period) during 60 days. The apparent absorption of dietary fat, as a bioavailability index, was assessed as the percentage of fat intake that was not excreted in the feces [24].

2.6 Serum and tissue fatty acids analysis

The extraction of total lipids was performed by the method described by Bligh and Dyer [25], followed by the transesterification with a methanolic potassium hydroxide solution [21] and the analysis of FA by gas chromatography as described above. From the serum, liver, and EAT FA composition, the following parameters were evaluated: (i) biomarkers of EFA status: LA, ALA, mead Acid levels, and Triene/tetraene ratio; (ii) the sum of SFA (ΣSFA), MUFA (ΣMUFA), and PUFA (ΣPUFA); and (iii) the isomeric FA incorporation and retention. TFA retention (%) was estimated by the percentage of the individual TFA isomer measured in the tissue/percentage of TFA isomer in the diet ratio multiplied by 100.

2.7 Triacylglycerol levels in serum, liver, and muscle

TAG levels in serum were determined by spectrophotometric methods using a commercially available test kit. Liver and muscle TAG levels were determined by the method of Laurell [26]. Portions of frozen tissues (0.2 g) were powdered and homogenized in distilled water 1:10 w/v for TAG content quantification.

2.8 Hepatic triacylglycerol secretion rate

Twenty four rats ($n = 6$ per experimental group), fasted overnight, were anesthetized as indicated above. Then, 600 mg/kg of body weight of triton WR 1339 in saline solution, an agent known to inhibit the peripheral removal of TAG-rich lipoproteins, was injected intravenously [27]. Blood samples were taken immediately before and 120 min after the injection of the Triton solution for the estimation of TAG accumulation in serum. Hepatic TAG-SR was estimated based on serum TAG concentration at 0 and 120 min, plasma volume and body weight. Further details have been previously reported [28].

2.9 Lipoprotein lipase activity in adipose tissue and gastrocnemius muscle

The removal capability of TAG-rich lipoproteins was evaluated by lipoprotein lipase (LPL) activities in the main tissues responsible for the uptake of TAG: adipose tissue and muscle. The enzymatic activity of adipose tissue LPL was quantified in EAT acetone powder by the fluorometric method of Del Prado et al. [29]. Briefly, EAT samples were delipidated by a double extraction with cold acetone followed by a double extraction with diethyl ether. The powders obtained were resuspended and incubated in a buffer (25 mM NH_4Cl, pH 8.1 containing 1 UI/mL of heparin). The enzymatic reaction was carried out in a medium containing dibutiryl fluorescein (DBF) as enzyme substrate. The quantification of LPL activity was performed measuring the increase in fluorescence ($\lambda_{excitation} = 490$ ηm; $\lambda_{emission} = 530$ ηm). In parallel, an identical assay was carried out in the same samples but in the presence of NaCl during incubation to inhibit specific enzyme activity. LPL activity was estimated as the difference between non-specific lipolitic and the total lipolitic activity. Values were expressed as picomol fluorescein/min/g of tissue and as ηmol fluorescein/min/total EAT. To assess muscle LPL activity, gastrocnemius muscle samples were homogenized in a NH_4Cl/NH_4OH—Heparin buffer. Then, the quantification of LPL activity in muscle was performed as previously described for adipose tissue. The measured activity was expressed as ηmol fluorescein/min/total muscle.

2.10 Lipogenic enzyme activities

Liver and EAT samples were homogenized in a buffer solution (pH 7.6 containing 150 mM KCl, 1 mM $MgCl_2$, 10 mM N-acetyl-cysteine, and 0.5 mM dithiothreitol). After centrifugation at 100 000 *g* for 40 min at 4°C, the supernatant fraction was used for the quantification of enzyme activities. Fatty acid synthase (FAS; EC 2.3.1.85), malic enzyme (ME; EC 1.1.1.40), and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) activities were measured by the methods of Lynen [30], Hsu and Lardy [31], and Kuby and Noltmann [32], respectively. Enzyme activities were expressed either as ηmol NADPH consumed (FAS) or as ηmol NADPH produced (G6PDH and ME)/min/mg of protein (1 mU = 1 ηmol NADPH/min). Protein content was determined by Lowry et al.'s technique [33] using bovine serum albumin as standard.

2.11 Carnitine palmitoyl transferase-I activity

Carnitine palmitoyl transferase-Ia (CPT-Ia) and carnitine palmitoyl transferase-Ib (CPT-Ib) (EC 1.3.99.3) activities were assessed in the mitochondrial fraction by the method of Bieber et al. [34]. Liver and muscle samples were homogenized in a buffer (pH 7.4 containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl). Homogenates were centrifuged at 700 *g* for 10 min at 4°C and supernatant fluid was again centrifuged at 12 000 *g* for 15 min at 4°C. Pellets were resuspended in a buffer (pH 7.4 containing 70 mM sucrose, 220 mM mannitol, 1 mM EDTA, 2 mM HEPES). The pellet protein content was determined as described above. The CPT-I activities were expressed as mU/mg of protein (1 mU = 1 ηmol CoA/min).

2.12 Statistical analysis

Values were expressed as mean ± standard errors of mean (SEM) of six animals per group. Statistical differences between mean values were established by two-way ANOVA (2×2) using LA status and TFA supplementation as independent variables. All post hoc multiple comparisons were made using Scheffe's critical range test. For TFA profile, statistical differences between means were established by unpaired Student's *t* test. Differences were considered statistically significant at $p < 0.05$ [35].

3 Results

3.1 Physiological status and nutritional parameters

During the experimental period, all the animals showed a healthy status without any physiological manifestation of EFA deficiency or pathological signs due to the treatments received. In addition, no symptoms of intestinal alterations or hepatomegaly were observed due to either treatment. However, even though the fat intake was similar among the groups, a reduced fecal fat excretion was observed in both $^{-}$LA and $^{-}$LA + TFA groups as a consequence of the increased apparent absorption of dietary fat (Table 2). The average daily energy intake did not differ among the groups. Nevertheless, there was an increase in body weight gain and body weight gain efficiency in the two groups supplemented with TFA. These alterations were accompanied by changes in EAT and retroperitoneal adipose tissue (RPAT) weights, reaching statistical significance in the $^{-}$LA + TFA group versus $^{-}$LA, without alterations in liver weights.

3.2 Serum and tissue fatty acid composition

Under our experimental conditions, the sum of SFA levels was significantly higher in both $^{-}$LA and $^{-}$LA + TFA groups in serum, liver, and adipose tissue, and total MUFA showed greater levels in serum due to the FA composition of the LA deficient diets and in the epididymal adipose tissue as a result of the FA composition of the LA deficient diets and TFA supplementation. The sum of PUFA levels was reduced differently because of both dietary treatments in the serum, liver, and adipose tissue (Table 3). LA levels showed a significant reduction in both, $^{-}$LA and $^{-}$LA + TFA groups, independently of the TFA supplementation and tissue considered. Nevertheless, the LA deficiency also produced alterations in the ALA levels and in the well-known EFA deficiency biomarker, mead acid (5*c*8*c*11*c*20:3). ALA levels showed a differential response depending on the tissue; specifically, serum ALA levels were not modified by the $^{-}$LA diet, but were reduced by the $^{-}$LA + TFA diet. The content of ALA in liver and EAT was reduced by LA deficiency. However, it did not reach statistical significance in the liver of $^{-}$LA + TFA versus $^{-}$LA group. Mead acid, a FA derived from the exacerbated synthesis of LC-PUFA of n-9 FA in EFA deficiency, was detected only in serum and liver of animals fed with $^{-}$LA and $^{-}$LA + TFA diets. The hepatic levels of mead acid were lower in the $^{-}$LA + TFA than in the $^{-}$LA diet. As a consequence of the detected levels of mead acid, the triene/tetraene ratio could be estimated in serum and liver of animals fed both LA-deficient diets.

All dietary *trans*-18:1 were incorporated into serum and tissues, and the levels were related to the tissue considered, the LA status and the type of isomer (Table 4). In general terms, adipose tissue best reflected the dietary TFA profile. Independently of the tissue, $^{-}$LA + TFA rats showed lower, or equivalent, levels of the individual *trans*-18:1 than those of $^{+}$LA + TFA animals. Comparing all the *trans*-18:1 isomers, 9*t*18:1 showed the highest TFA retention. On the other hand, compared with the *trans*-18:1, the levels of non-conjugated *trans*-18:2 isomers showed a different pattern and were not correlated with the dietary *trans*-18:2 intake. Due to the low dietary levels of these isomers, the retention values showed a wide variability. The levels of 9c11*t*-18:2, produced by

Table 2. Effect of TFA on nutritional and body composition parameters in rats fed LA-enriched and LA-deficient diets

					ANOVA		
	$^{+}$LA	$^{-}$LA	$^{+}$LA + TFA	$^{-}$LA + TFA	LA	TFA	LA × TFA
Energy intake (kJ/d)	293 ± 8	310 ± 6	294 ± 8	310 ± 18	NS	NS	NS
Body weight gain (g)	209 ± 7^{a}	226 ± 7^{ab}	251 ± 7^{bc}	268 ± 10^{c}	NS	0.001	NS
Body weight gain efficiency (g/100 kJ)	1.20 ± 0.06^{a}	1.22 ± 0.05^{a}	1.42 ± 0.05^{b}	1.46 ± 0.11^{b}	NS	0.001	NS
Fat utilization							
Fat intake (g/d)	1.27 ± 0.04	1.38 ± 0.05	1.30 ± 0.03	1.38 ± 0.10	NS	NS	NS
Fecal fat (mg/d)	27.12 ± 0.80^{a}	18.80 ± 1.22^{b}	26.04 ± 2.31^{ab}	18.74 ± 2.59^{b}	0.001	NS	NS
Fecal fat/fat intake (%)	2.24 ± 0.05^{a}	1.38 ± 0.10^{b}	2.04 ± 0.18^{ab}	1.44 ± 0.27^{b}	0.001	NS	NS
Fat apparent absorption (%)	97.76 ± 0.04^{a}	98.62 ± 0.10^{b}	97.96 ± 0.18^{ab}	98.56 ± 0.27^{b}	0.001	NS	NS
Carcass composition (g/100 g)							
Protein	20.85 ± 0.83	21.75 ± 0.28	20.44 ± 0.66	20.91 ± 0.47	NS	NS	NS
Fat	16.08 ± 0.60	17.29 ± 0.55	17.73 ± 0.43	19.04 ± 0.54	NS	NS	NS
Water	58.52 ± 0.65	56.88 ± 0.73	58.46 ± 0.61	56.05 ± 0.52	NS	NS	NS
Tissues weight (g/100 g)							
Liver	3.62 ± 0.04	3.16 ± 0.07	3.54 ± 0.08	3.34 ± 0.10	NS	NS	NS
Gastrocnemius muscle	0.35 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	NS	NS	NS
EAT	2.32 ± 0.10^{a}	2.69 ± 0.13^{a}	2.81 ± 0.19^{ab}	3.43 ± 0.16^{b}	0.003	0.001	NS
RPAT	2.31 ± 0.08^{ab}	2.14 ± 0.18^{a}	2.64 ± 0.19^{ab}	3.08 ± 0.25^{b}	NS	0.002	NS

Data are expressed as mean ± SEM ($n = 6$).
Different letters in each row indicate statistical differences at $p < 0.05$ (Scheffe's test).
$^{+}$LA, enriched with linoleic acid; $^{-}$LA, deficient of linoleic acid; TFA, *trans* fatty acids; EAT, epididimal adipose tissue; RPAT, retroperitoneal adipose tissue.

bioconversion from 11*t*-18:1, were higher in adipose tissue than in liver and serum; and with the exception of the serum sample, they showed similar levels independently of the LA status.

3.3 Serum and tissue triacylglycerol contents and their bioregulation

Comparing with the $^{+}$LA diet, serum TAG levels were decreased by the $^{-}$LA diet but were not modified in $^{+}$LA + TFA diet. Moreover, this parameter was higher in the $^{-}$LA + TFA than in the $^{-}$LA diet. The liver TAG content was reduced in the $^{-}$LA group and was increased by TFA supplementation either in LA-deficient and enriched diets. However, the levels of this parameter reached in $^{-}$LA + TFA were similar to those found in the $^{+}$LA group. Hepatic TAG-SR was significantly increased by LA deficiency, but was not altered by TFA supplementation (Table 5). LPL activity in EAT expressed as total tissue weight were significantly increased in the $^{-}$LA and $^{+}$LA + TFA versus $^{+}$LA group, and was reduced in the $^{-}$LA + TFA versus either the $^{-}$LA or $^{+}$LA + TFA groups, reaching values comparable with those found in the $^{+}$LA diet. Even EAT weights were dissimilar among the groups; the same effect was observed on EAT LPL activities when results were expressed per g of adipose tissue. The results were (picomol fluorescein/min/g): $^{+}$LA: 61.1 ± 8.1^{a}; $^{-}$LA: 170.9 ± 13.1^{b}; $^{+}$LA + TFA: 114.3 ± 16.7^{c}; $^{-}$LA + TFA: 58.7 ± 5.7^{a}.

The hepatic FAS, ME, and G6PDH activities were raised by the $^{-}$LA diet, and this effect was exacerbated by TFA supplementation. However, in the LA-enriched diet these enzyme activities were not modified by TFA supplementation. The hepatic CPT-Ia activity was decreased by TFA in the LA-enriched but not in the LA-deficient diet (Table 6). In EAT, ME, and G6PDH activities were increased by the $^{-}$LA versus $^{+}$LA diets, and TFA markedly exacerbated all the measured lipogenic enzyme activities. In addition, TFA supplementation did not change these enzyme activities in the EAT of animals fed LA-enriched diet. The activity of the muscular key enzyme on β-oxidation, CPT-Ib, did not show differences between the comparisons: $^{+}$LA + TFA versus $^{+}$LA and $^{-}$LA + TFA versus $^{-}$LA. Only in the $^{-}$LA + TFA versus $^{+}$LA group did the reduction reach statistical significance.

4 Discussion

This study provides evidence of some unknown effects of TFA on the nutritional parameters, serum and tissue TAG levels, and their regulation, in rats fed LA-enriched or deficient diets. It is important to note that our interest was focused on the effects of dietary TFA supplementation at two different LA levels aiming to gain mechanistic insight at two different nutritional situations: enriching or limiting the LA availability. In this regard, diets were based on Reeves' [19]

Table 3. Effect of TFA on serum and tissue fatty acids on rats fed LA-enriched and LA-deficient diets

	Composition in tissues (%)				ANOVA		
	$^{+}$LA	$^{-}$LA	$^{+}$LA + TFA	$^{-}$LA + TFA	LA	TFA	LA × TFA
Serum							
9*c*12*c*18:2	20.20 ± 0.59^{a}	7.89 ± 0.53^{b}	16.16 ± 0.53^{c}	8.47 ± 0.34^{b}	0.001	0.004	0.001
9*c*12*c*15*c*18:3	0.22 ± 0.01^{a}	0.20 ± 0.01^{a}	0.19 ± 0.01^{a}	0.14 ± 0.01^{b}	0.009	0.001	NS
5*c*8*c*11*c*20:3	ND^{a}	0.36 ± 0.05^{b}	ND^{a}	0.29 ± 0.04^{b}	0.001	NS	NS
Triene/tetraene ratio	ND^{a}	0.04 ± 0.00^{b}	ND^{a}	0.03 ± 0.00^{b}	0.001	NS	NS
Σ SFA	35.46 ± 1.22^{a}	44.97 ± 1.10^{b}	36.40 ± 0.66^{a}	43.91 ± 1.25^{b}	0.001	NS	NS
Σ MUFA	13.32 ± 1.01^{ac}	26.70 ± 1.78^{b}	16.75 ± 1.10^{c}	26.09 ± 1.44^{b}	0.001	NS	NS
Σ PUFA	48.30 ± 0.93^{a}	21.62 ± 0.35^{b}	43.52 ± 1.26^{c}	24.56 ± 0.31^{b}	0.001	NS	0.001
Liver							
9*c*12*c*18:2	21.19 ± 0.63^{a}	5.13 ± 0.20^{b}	12.47 ± 0.75^{c}	7.49 ± 0.95^{b}	0.001	0.001	0.001
9*c*12*c*15*c*18:3	0.29 ± 0.03^{a}	0.07 ± 0.03^{b}	0.22 ± 0.00^{ab}	0.15 ± 0.0^{b}	0.001	NS	0.004
5*c*8*c*11*c*20:3	ND^{a}	0.27 ± 0.04^{b}	ND^{a}	0.12 ± 0.01^{c}	0.001	0.006	0.006
Triene/tetraene ratio	ND^{a}	0.03 ± 0.00^{b}	ND^{a}	0.01 ± 0.00^{c}	0.001	0.005	0.005
Σ SFA	34.39 ± 0.44^{a}	49.94 ± 0.92^{b}	35.84 ± 0.96^{a}	45.05 ± 0.53^{b}	0.001	NS	0.002
Σ MUFA	25.31 ± 1.45	29.12 ± 1.07	30.14 ± 1.10	30.04 ± 1.91	NS	0.040	NS
Σ PUFA	38.13 ± 1.42^{a}	21.25 ± 0.99^{b}	30.06 ± 1.88^{b}	23.95 ± 1.61^{b}	0.001	0.112	0.003
Epididymal adipose tissue							
9*c*12*c*18:2	34.30 ± 1.00^{a}	1.86 ± 0.08^{b}	24.07 ± 0.75^{c}	1.71 ± 0.10^{b}	0.001	0.001	0.001
9*c*12*c*15*c*18:3	0.67 ± 0.05^{a}	ND^{b}	0.50 ± 0.02^{c}	ND^{b}	0,001	0.007	0.007
5*c*8*c*11*c*20:3	ND	ND	ND	ND	NS	NS	NS
Σ SFA	25.07 ± 0.87^{a}	42.82 ± 0.47^{b}	29.26 ± 0.77^{c}	48.24 ± 0.40^{d}	0.001	NS	0.001
Σ MUFA	38.16 ± 0.32^{a}	43.84 ± 0.22^{bc}	43.12 ± 0.80^{c}	46.32 ± 0.86^{d}	0.001	0.001	0.021
Σ PUFA	35.85 ± 1.06^{a}	1.86 ± 0.08^{b}	25.80 ± 0.90^{c}	2.67 ± 0.40^{b}	0.001	0.001	0.001

All values are presented as weight percentages of total fatty acid methyl esters, expressed as mean ± SEM ($n = 6$).
Different letters in each row indicate statistical differences at $p < 0.05$ (Scheffe's test).
$^{+}$LA, enriched with linoleic acid; $^{-}$LA, deficient of linoleic acid; TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND; not detected.

recommendations with the modification of the oil source, as previously recommended in the AIN-76 diet [20]. We did not find toxicological manifestations due to any of the dietary treatments. It is important to note that despite the low ALA level and the unbalance of the n-6:n-3 FA ratio in corn oil, we did not detect mead acid, a well-known biomarker of EFA deficiency in animals fed $^{+}$LA and $^{+}$LA + TFA diets. In contrast, animals fed LA deficient diets showed significant levels of mead acid.

TFA were incorporated into serum, liver, and adipose tissue lipids showing a different pattern depending on the trans double bound position of TFA isomer, tissue, and experimental conditions [12]. It is very well known that the main TFA isomers present in the diet might have a specific effect on fat mobilization and lipid metabolism, and this effect could be related to the levels achieved in the tissue. The potential physiological effects and the relative oxidation rate of the individual 10*t*18:1 isomers are unclear. However, the lower retention of 10*t*18:1 in liver and serum in the present study might be associated with a preferential incorporation of this isomer into adipose tissue. Otherwise, 9*t*18:1 has been highly retained in all evaluated tissues and is associated with negative effects on cholesterol levels and, therefore, increases the risk of cardiovascular heart disease [36]. On the other hand, under our experimental conditions vaccenic acid (11*t*18:1) was moderately retained and, in contrast to another *trans*-18:1 isomers, it may impart health benefits due to its own functionality and its conversion to rumenic acid (9*c*11*t*18:2) [37]. The 9*c*11*t*18:2 isomer reached significant levels (approximately 50% of its precursor) in liver and EAT and both 11*t*18:1 and 9*c*11*t*18:2 could ameliorate the deleterious effects of the other *trans*-18:1 isomers. Moreover, when statistical significance was observed, LA deficiency decreased the individual *trans*-18:1 levels in serum, liver, and adipose tissue. We do not have any referential support to explain this effect; however, it could be associated with an increased incorporation of dietary saturated FA that competes with TFA in the $^{-}$LA + TFA rats.

Since the clinical severe manifestation of EFA deficiency or unbalances of the n-6:n-3 FA ratio might hide alterations induced by TFA, in our experimental animal model we avoided inducing an extreme LA deficiency, feeding the animals during a relative short period of time with the LA-

Table 4. Effect of TFA on fatty acid isomers composition and retention on rats fed LA-enriched and LA-deficient diets

	Isomer levels (%)		Isomer retention (%)	
Fatty acids	$^{+}$LA + TFA	$^{-}$LA + TFA	$^{+}$LA + TFA	$^{-}$LA + TFA
Serum				
(6–8)*t*18:1	0.39 ± 0.01[a]	0.29 ± 0.02[b]	24	18
9*t*18:1	0.75 ± 0.10	0.80 ± 0.05	36	38
10*t*18:1	0.29 ± 0.04	0.36 ± 0.05	9	12
11*t*18:1	0.71 ± 0.08	0.52 ± 0.07	26	19
12*t*18:1	0.77 ± 0.03	0.61 ± 0.07	36	28
9*t*12*t*18:2	0.07 ± 0.01[a]	0.16 ± 0.02[b]	18	42
9*c*12*t*18:2	ND[a]	0.31 ± 0.04[b]	0	273
9*t*12*c*18:2	ND[a]	0.49 ± 0.06[b]	0	389
9*c*11*t*18:2	ND[a]	0.22 ± 0.03[b]	–	–
Liver				
(6–8)*t*18:1	0.32 ± 0.03[a]	0.20 ± 0.02[b]	20	12
9*t*18:1	0.89 ± 0.01	0.75 ± 0.06	43	36
10*t*18:1	0.50 ± 0.03	0.46 ± 0.02	17	15
11*t*18:1	0.60 ± 0.05[a]	0.35 ± 0.06[b]	22	13
12*t*18:1	0.59 ± 0.06[a]	0.35 ± 0.05[b]	27	16
9*t*12*t*18:2	0.14 ± 0.02	0.19 ± 0.03	36	48
9*c*12*t*18:2	0.10 ± 0.07[a]	ND[b]	86	0
9*t*12*c*18:2	0.02 ± 0.01[a]	0.20 ± 0.04[b]	19	159
9*c*11*t*18:2	0.26 ± 0.01[a]	0.22 ± 0.01[b]	–	–
Epididymal adipose tissue				
(6–8)*t*18:1	0.41 ± 0.06	0.48 ± 0.06	25	39
9*t*18:1	1.07 ± 0.07	0.99 ± 0.15	51	48
10*t*18:1	1.27 ± 0.09[a]	0.87 ± 0.14[b]	42	29
11*t*18:1	1.06 ± 0.05[a]	0.74 ± 0.13[b]	38	27
12*t*18:1	0.28 ± 0.07	0.31 ± 0.06	13	14
9*t*12*t*18:2	0.33 ± 0.04	0.34 ± 0.04	85	89
9*c*12*t*18:2	0.17 ± 0.01[a]	0.42 ± 0.03[b]	148	369
9*t*12*c*18:2	0.11 ± 0.01[b]	ND[b]	87	0
9*c*11*t*18:2	0.60 ± 0.04	0.54 ± 0.06	–	–

For isomer levels in tissue, all values are presented as weight percentages of total fatty acid methyl esters, expressed as mean ± SEM ($n = 6$). Different letters in each row indicate statistical differences at $p < 0.05$ (Student's *t* test).
Isomer retention in tissue is expressed as mean value and was calculated as percentage of the individual TFA isomer measured into the tissue/percentage of TFA isomer in the diet ratio multiplied by 100.
$^{+}$LA, enriched with linoleic acid; $^{-}$LA deficient of linoleic acid; TFA, *trans* fatty acids; ND, not detected.

deficient diets. Nevertheless, lower levels of LA in serum, liver, and adipose tissue and increased levels of mead acid in serum and liver clearly showed that LA deficiency was induced. The triene/tetraene ratio has proven to be an adequate indicator of EFA status in different animal tissues [38]. Since the liver and serum levels of this ratio were in the range 0–0.4, a moderate LA-deficiency was confirmed.

In the present study, we did not find substantial changes in nutritional parameters by TFA, and there are controversial experimental results about the effect of TFA on fat accretion and body weight gain [10, 12, 39]. Our results show that the two groups supplemented with TFA increased the body weight gain, and that the tendency of higher fat depots observed by TFA reached statistical significance in the $^{-}$LA + TFA diet. This could be in part related to the increased fat absorption observed in LA-deficient animals, probably as a compensatory mechanism tending to increase the LA content.

Compared with their respective control groups, TFA supplemented animals increased the hepatic TAG contents; however, different TAG regulatory mechanisms were involved. In agreement with our results in animals fed the $^{+}$LA + TFA diet, Giudetti et al. [13] showed that rats fed hydrogenated soybean oil rich in TFA increased the liver TAG content by lowering the hepatic oxidation of fatty acids without increases in the de novo synthesis of FA measured by enzymes ACC and FAS. To the best of our knowledge, there are no studies dealing with the regulatory mechanism involved in hepatic TAG content when LA deficiency and

Table 5. Effect of TFA on triacylglycerol contents and related parameters in rats fed LA-enriched or LA-deficient diets

					ANOVA		
	$^{+}$LA	$^{-}$LA	$^{+}$LA + TFA	$^{-}$LA + TFA	LA	TFA	LA × TFA
TAG levels							
Serum (mmol/L)	1.43 ± 0.19^{ac}	0.93 ± 0.07^{b}	1.21 ± 0.08^{abc}	1.60 ± 0.30^{c}	NS	NS	NS
Liver (μmol/g)	19.85 ± 1.06^{a}	15.99 ± 1.17^{b}	30.59 ± 1.16^{c}	20.39 ± 1.03^{a}	0.001	0.001	0.001
Gastrocnemius muscle (μmol/g)	4.63 ± 0.44	3.64 ± 0.32	3.45 ± 0.30	3.42 ± 0.11	NS	0.041	NS
TAG-SR (ηmol/min/100 g)	161 ± 5^{ac}	240 ± 11^{b}	183 ± 7^{c}	231 ± 13^{b}	0.001	NS	NS
Epididymal adipose tissue LPL (ηmol fluorescein/min/total EAT)	0.53 ± 0.09^{a}	1.73 ± 0.09^{b}	1.06 ± 0.22^{c}	0.68 ± 0.04^{a}	0.045	0.040	0.001
Gastrocnemius muscle LPL (ηmol fluorescein/min/total tissue)	3.56 ± 0.39	3.99 ± 0.51	4.50 ± 0.42	3.03 ± 0.12	NS	NS	NS

Data are expressed as mean ± SEM ($n = 6$).
Different letters in each row indicate statistical differences at $p < 0.05$ (Scheffe's test).
$^{+}$LA, enriched with linoleic acid; $^{-}$LA, deficient of linoleic acid; TFA, *trans* fatty acid; TAG, triacylglycerol; TAG-SR, triacylglycerol secretion rate; LPL, lipoprotein lipase; EAT, epididymal adipose tissue.

TFA supplementation are combined. Some studies [15, 17] have suggested that TFA impair other lipid parameters intensifying the EFA deficiency in rats. In $^{-}$LA + TFA rats the increase of hepatic TAG levels induced by TFA was associated with higher lipogenic enzyme activities, as observed by Cho et al. [40] in mice fed high *trans* fat diets during 12 weeks. Different from our results, these authors additionally observed a lower hepatic CPT activity, but Jeyakumar et al. [41] found that FA oxidation in Fischer rats fed long-term TFA diets was not adversely affected, explaining in this manner the absence of hepatic steatosis and hypertriglyceridaemia. The divergences in β-oxidation reported could be related, among other reasons, to the liver FA composition and to the type of TFA isomers present in the diet that are not clearly shown by these authors. Even though there are few data on the effects of the individual TFA on FA oxidation/synthesis, recently Du et al. [42] reported that elaidic acid (9*t*18:1) has a lower oxidation rate than vaccenic acid in rat liver cells and these results might explain the higher retention of elaidic acid found in the present study.

In the serum of $^{-}$LA rats, we found lower TAG levels, associated with higher serum TAG removal by the adipose tissue LPL enzyme. This mechanism has been previously inferred by Werner et al. [43]. Surprisingly, in our

Table 6. Effect of TFA on lipogenic and oxidative enzyme activities in rats fed LA-enriched and LA-deficient diets

					ANOVA		
	$^{+}$LA	$^{-}$LA	$^{+}$LA + TFA	$^{-}$LA + TFA	LA	TFA	LA × TFA
Liver (mU/mg protein)							
FAS	9.79 ± 1.53^{a}	14.15 ± 0.93^{b}	12.03 ± 1.47^{a}	23.24 ± 3.09^{c}	NS	NS	0.021
ME	36 ± 1^{a}	67 ± 2^{b}	34 ± 1^{a}	132 ± 1^{c}	0.001	0.001	0.001
G6PDH	41 ± 2^{a}	126 ± 8^{b}	70 ± 7^{a}	270 ± 11^{c}	0.001	0.001	0.001
CPT-Ia	1.23 ± 0.12^{a}	0.89 ± 0.11^{ab}	0.61 ± 0.00^{b}	0.86 ± 0.01^{a}	NS	0.002	0.003
EAT (mU/mg protein)							
FAS	9.31 ± 3.04^{a}	13.52 ± 2.01^{a}	12.91 ± 1.05^{a}	20.16 ± 0.23^{b}	0.001	0.001	NS
ME	165 ± 9^{a}	228 ± 7^{b}	146 ± 3^{a}	272 ± 6^{c}	0.001	NS	0.001
G6PDH	141 ± 9^{a}	176 ± 5^{b}	169 ± 7^{ab}	266 ± 10^{c}	0.001	0.001	0.001
Muscle-CPT-Ib (mU/mg protein)	1.35 ± 0.21^{a}	1.28 ± 0.01^{ab}	0.81 ± 0.12^{ab}	0.77 ± 0.06^{b}	NS	0.001	NS

Data are expressed as mean ± SEM ($n = 6$).
Different letters in each row indicate statistical differences at $p < 0.05$ (Scheffe's test).
$^{+}$LA, enriched with linoleic acid; $^{-}$LA, deficient of linoleic acid, TFA, *trans* fatty acid; FAS, fatty acid synthase; ME malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; EAT, epididymal adipose tissue; CPT-I, carnitine palmitoyltranferase-I.

experiments, hepatic TAG-SR was increased by LA deficiency, suggesting that VLDL output tended to compensate the reduction of circulating TAG. On the other hand, serum TAG levels were not modified by TFA in the $^{+}$LA diet, despite the significant increase in EAT LPL activity associated with a normal TAG-SR. These data are neither in agreement with results reported by Machado et al. [39] nor with previous results from our laboratory [10]. In our previous studies, we used isomerized fats rich in elaidic acid at high levels of dietary fats, and in the present protocol, TFA were obtained from commercial PHVO where the prevailing TFA isomers were 10*t*18:1 and 11*t*18:1. Additionally, recommended levels of dietary fats were used in the current study. There are controversial and scarce reports about the effect of the individual TFA isomers on lipid regulation, and only a few results on plasma TAG levels [37]. Nevertheless, it seems clear that vaccenic acid does not have the deleterious effects that are reported for elaidic acid, and in fact, 11*t*18:1 might ameliorate the negative effect of 9*t*18:1. Therefore, we could infer that the deleterious effects of elaidic acid are attenuated by the influence of vaccenic acid and, possibly, by the rumenic acid synthesized from this fatty acid. In contrast to $^{+}$LA animals, in the serum of $^{-}$LA rats, TFA supplementation increased the serum TAG levels, associated with a lower TAG clearance by the adipose tissue without changes in the hepatic TAG-SR. Therefore, in LA deficiency, it seems that the adipose tissue LPL enzyme might play a more important role in the circulating TAG regulation than the TAG-SR. Although a lower EAT LPL activity was observed in the $^{-}$LA + TFA rats, the significant increase in the EAT lipogenic enzymes expanded the epididymal fat pads. There are no studies showing that TFA might potentiate the lipogenesis in the white adipose tissue of LA-deficient animals, but it has been demonstrated that both individual variables, that is, EFA deficiency or unbalances of n-6:n-3 FA ratio and TFA supplementation, increase the FA and TAG synthesis. It is known that LA and ALA and their long-chain FA derivates suppress lipogenic enzymes gene expression in white adipose tissue [44]. Moreover, Demeyer et al. [45] and Du et al. [46] showed that lipid synthesis is increased in animals with EFA deficiency. These data support our findings of higher lipogenic enzyme activities in the LA-deficient, but not in the LA-enriched diet.

Finally, despite the fact that the TFA supplementation to both LA-enriched or deficient diets tended to decrease the CPT-Ib activity, TAG levels were not modified in muscle. To the best of our knowledge, there is no evidence that LA deficiency might modify muscle TAG levels, and there is scarce information about the effect of TFA on this parameter. In a different experimental protocol, with elevated levels of dietary fats we found that rats fed high levels of TFA resulted in normal gastrocnemius muscle TAG content without changes in glucose metabolites [11]. In contrast, Natarajan et al. [47] observed that TFA increased intramyocellular TAG content, associated with decreased insulin-stimulated glucose transport in the diaphragm of male weanling WNIN rats.

5 Conclusions

The effect of TFA on nutritional parameters and TAG metabolism differed depending on the dietary LA status. In the $^{+}$LA + TFA diet, compared to the $^{+}$LA diet, TFA did not alter the serum TAG levels, despite the higher hepatic TAG accretion due to a lower β-oxidation. Differently, in $^{-}$LA + TFA rats versus $^{-}$LA, TFA increased the serum, liver, and adipose tissue TAG levels associated with a higher lipogenesis in both liver and adipose tissue, and a reduced TAG clearance by the adipose tissue LPL enzyme. We conclude that the nutritional and metabolic TFA effects depended mainly on the changes in the FA profile induced by dietary LA and, in a lesser extent, on the specific type of isomer retained in the tissues.

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References

[1] Fedacko, J., Vargova, V., Singh, R. B., Anjum, B., et al., Association of high w-6 / w-3 fatty acid ratio diet with causes of death due to noncommunicable diseases among urban decedents in north India. *Open Nutraceuticals J.* 2012, *5*, 113–123.

[2] Simopoulos, A. P., Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomed. Pharmacother.* 2006, *60*, 502–507.

[3] Massiera, F., Barbry, P., Guesnet, P., Joly, A., et al., A Western-like fat diet is sufficient to induce a gradual enhancement in fat mass over generations. *J. Lipid Res.* 2010, *51*, 2352–2361.

[4] Bjermo, H., Iggman, D., Kullberg, J., Dahlman, I., et al., Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: A randomized controlled trial. *Am. J. Clin. Nutr.* 2012, *95*, 1003–1012.

[5] Lee, J. H., Fukumoto, M., Nishida, H., Ikeda, I., Sugano, M., The interrelated effects of n-6/n-3 and polyunsaturated/saturated ratios of dietary fats on the regulation of lipid metabolism in rats. *J. Nutr.* 1989, *119*, 1893–1899.

[6] Broughton, K. S., Wade, J. W., Total fat and (n-3):(n-6) fat ratios influence eicosanoid production in mice. *J. Nutr.* 2002, *132*, 88–94.

[7] Igarashi, M., Gao, F., Kim, H. W., Ma, K., et al., Dietary n-6 PUFA deprivation for 15 weeks reduces arachidonic acid concentrations while increasing n-3 PUFA concentrations in organs of post-weaning male rats. *Biochim. Biophys. Acta* 2009, *1791*, 132–139.

[8] Allmann, D. W., Gibson, D. M., Fatty acid synthesis during early linoleic acid deficiency in the mouse. *J. Lipid Res.* 1965, *6*, 51–62.

[9] Mozaffarian, D., Katan, M. B., Ascherio, A., Stampfer, M. J., et al., Trans fatty acids and cardiovascular disease. *New Engl. J. Med.* 2006, 1601–1613.

[10] Colandré, M. E., Diez, R. S., Bernal, C. A., Metabolic effects of trans fatty acids on an experimental dietary model. *Br. J. Nutr.* 2003, *89*, 631–639.

[11] Bernal, C. A., Rovira, J., Colandré, M. E., Cussó, R., Cadefau, J. A., Effects of dietary cis and trans unsaturated and saturated fatty acids on the glucose metabolites and enzymes of rats. *Br. J. Nutr.* 2006, *95*, 947–954.

[12] Dorfman, S. E., Laurent, D., Gounarides, J. S., Li, X., et al., Metabolic implications of dietary trans-fatty acids. *Obesity* 2009, *17*, 1200–1207.

[13] Giudetti, A. M., Beynen, A. C., Lemmens, A. G., Gnoni, G. V., Geelen, M. J. H., Hepatic fatty acid metabolism in rats fed diets with different contents of C18: 0, C18: 1 cis and C18: 1 trans isomers. *Br. J. Nutr.* 2007, *90*, 887–893.

[14] Guzmán, M., Klein, W., Gómez del Pulgar, T., Geelen, M. J., Metabolism of trans fatty acids by hepatocytes. *Lipids* 1999, *34*, 381–386.

[15] Hill, E. G., Johnson, S. B., Holman, A. T., Intensification of essential fatty acid deficiency in the rat by dietary trans fatty acids. *J. Nutr.* 1979, *109*, 1759–1765.

[16] Hill, E. G., Johnson, S. B., Lawson, L. D., Mahfouz, M. M., Holman, R. T., Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. *Proc. Natl. Acad. Sci. USA* 1982, *79*, 953–957.

[17] Privett, O. S., Phillips, F., Shimasaki, H., Nozawa, T., Nickell, E. C., Studies of effects of trans fatty acids in the diet on lipid metabolism in essential fatty acid deficient rats. *Am. J. Clin. Nutr.* 1977, *30*, 1009–1017.

[18] Bayne, K., Revised guide for the care and use of laboratory animals available. American Physiological Society. *Physiologist* 1996, *39*, 208–211.

[19] Reeves, P. G., Nielsen, F. H., Fahey, G. C., AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 1993, *123*, 1939–1951.

[20] Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J. Nutr. 1973, 1340–1348.

[21] ISO 5509(E). Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids, 2000.

[22] AOCS Official Method Ce 1j-07. Official methods and recommended practices of the AOCS, 2007, 6th edition, AOCS, Champaign, IL, USA.

[23] Windham, W. R., in: Cunniff, P. (Ed.), *Official Methods of Analysis*, 16th edition, 5th revision of AOAC. AOAC International, Gaithersburg, MD, USA 1999, pp. 4.1–4.45.

[24] Nicolosi, R. J., Wilson, T. A., Rogers, E. J., Kritchevsky, D., Effects of specific fatty acids (8:0, 14: 0, cis-18:1, trans-18:1) on plasma lipoproteins, early atherogenic potential, and LDL oxidative properties in the hamster. *J. Lipid Res.* 1998, 1972–1980.

[25] Bligh, E. G., Dyer, W. J., A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 1959, *37*, 911–917.

[26] Laurell, S., A method for routine determination of plasma triglycerides. *Scand. J. Clin. Lab. Invest.* 1966, *18*, 668–672.

[27] Otway, B. S., The effect of a non-ionic detergent (Triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rat. *J. Physiol.* 1967, *1339*, 309–319.

[28] Mocchiutti, N. O., Bernal, C. A., Effects of chronic di(2-ethylhexyl) phthalate intake on the secretion and removal rate of triglyceride-rich lipoproteins in rats. *Food Chem. Toxicol.* 1997, *35*, 1017–1021.

[29] Del Prado, M., Hernández-Montes, H., Villalpando, S., Characterization of a fluorometric method for lipoprotein lipase. *Arch. Med. Res.* 1994, *25*, 331–335.

[30] Lynen ,F., Yeast fatty acid synthase. *Methods Enzym.* 1969, *14*, 14–17.

[31] Hsu, R. Y., Lardy, H. A., Malic enzyme. *Methods Enzym.* 1969, *17*, 230–235.

[32] Kuby, S. A., Noltmann, E. A., Glucose 6-phosphate dehydrogenase from brewers yeast. *Methods Enzym.* 1966, *9*, 116–117.

[33] Lowry, O. H., Rosebrough, N. J., Farr Lewis, A., Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951, *193*, 265–275.

[34] Bieber, L. L., A rapid spectrophotometric assay for carnitine palmitoyltransferase. *Anal. Biochem.* 1972, *50*, 509–518.

[35] DeGroot, M. H., *Probability and Statistics*, Addison-Wesley, Reading, MA 1986.

[36] Stender, S., Astrup, A., Dyerberg, J., Ruminant and industrially produced trans fatty acids: Health aspects. *Food Nutr. Res.* 2008, *52*, 1–8.

[37] Wang, Y., Lu, J., Ruth, M. R., Goruk, S. D., et al., Trans-11 vaccenic acid dietary supplementation induces hypolipidemic effects in JCR:LA-cp rats. *J. Nutr.* 2008, 2117–2122.

[38] Holman, R. T., The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J. Nutr.* 1960, *70*, 405–410.

[39] Machado, R. M., Stefano, T., Oliveira, C. P. M. S., Mello, E. S., et al., Intake of trans fatty acids causes nonalcoholic steatohepatitis and reduces adipose tissue fat content. *J. Nutr.* 2010, *140*, 1127–1132.

[40] Cho, Y. Y., Kwon, E. Y., Kim, H. J., Jeon, S. M., et al., Differential effect of corn oil-based low trans structured fat on the plasma and hepatic lipid profile in an atherogenic mouse model: Comparison to hydrogenated trans fat. *Lipids Heal. Dis.* 2011, *10*, 15.

[41] Jeyakumar, S. M., Prashant, A., Rani, K. S., Laxmi, R., et al., Chronic consumption of trans-fat-rich diet increases hepatic cholesterol levels and impairs muscle insulin sensitivity without leading to hepatic steatosis and hypertriglyceridemia in female Fischer rats. *Ann. Nutr. Metab.* 2011, *58*, 272–280.

[42] Du, Z. Y., Degrace, P., Gresti, J., Loreau, O., Clouet, P., Dissimilar properties of vaccenic versus elaidic acid in beta-oxidation activities and gene regulation in rat liver cells. *Lipids* 2010, *45*, 581–591.

[43] Werner, A., Havinga, R., Bos, T., Bloks, V. W., et al., Essential fatty acid deficiency in mice is associated with hepatic steatosis

and secretion of large VLDL particles. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2005, *288*, G1150–G1158.

[44] Sessler, A. M., Ntambi, J. M., Polyunsaturated fatty acid regulation of gene expression. *J. Nutr.* 1998, *128*, 923–926.

[45] Demeyer, D. I., Tan, W. C., Privett, O. S., Effect of essential fatty acid deficiency on lipid metabolism in isolated fat cells of epididymal fat pads of rats. *Lipids* 1974, *9*, 1–7.

[46] Du, J. T., Kruger, F. A., Effect of essential fatty acids on the fatty acid synthesis in epididymal fat cells of the rat. *J. Nutr.* 1972, *102*, 1033–1038.

[47] Natarajan, S., Ibrahim, A., Dietary trans fatty acids alter diaphragm phospholipid fatty acid composition, triacylglycerol content and glucose transport in rats. *Br. J. Nutr.* 2005, *93*, 829–833.

