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Trans fatty acids modify nutritional parameters and triacylglycerol metabolism in rats: differential effects at recommended and high-fat levels

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Abstract

Introduction: **there is still little evidence on the metabolic trans fatty acids (TFA) effects at recommended fat levels.**

Objective: **to investigate the differential TFA effects on some nutritional parameters, TFA retention, and triacylglycerol (TAG) regulation in rats fed recommended and high-fat diets.**

Methods: **male Wistar rats were fed (30 days) diets containing recommended (7%,w/w) or high-fat (20%,w/w) levels, supplemented or not with TFA (C7, C20, TFA7 and TFA20).**

Results: **TFA7 (vs.C7) rats showed an increased body weight associated with higher fat pads and liver and serum TAG. The hypertriacylglyceridaemia was related to a decreased muscle LPL activity, while the higher hepatic TAG content was associated with both an increased SREBP-1c gene expression and ACC activity, and a reduced CPT-Ia gene expression. The TFA20 diet did not potentiate the higher body weight, fat pads and TAG levels induced by the C20 diet. Although the hepatic TAG-secretion rate (TAG-SR) increased by TFA20 vs. C20, the same triacylglyceridaemia was associated with a compensatory increase of the adipose tissue LPL activity. The attenuated hepatic TAG accretion in TFA20 was related to an increase of TAG-SR and to a lower increase of SREBP-1c and SCD1 mRNA expressions, paralleled to a relative decrease of SCD1 index and ACC activity.**

Discussion and conclusion: **TFA alters nutritional parameters and lipid metabolism in rats. However, different responses to the TFA on TAG levels and their regulation were observed between rats fed recommended and high-fat diets. These divergences might be related to different tissue TFA retentions and rumenic acid bioconversion.**

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Key words: *Isomer retention. Triacylglycerol-secretion rate. Lipoprotein lipase. Lipid regulation. Lipid accretion.*

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LOS ÁCIDOS GRASOS *TRANS* MODIFICAN PARÁMETROS NUTRICIONALES Y EL METABOLISMO DE TRIACILGLICÉRIDOS EN RATAS: EFECTOS DIFERENCIALES A NIVELES RECOMENDADOS Y ELEVADOS DE GRASA

Resumen

Introducción: **existen escasas evidencias sobre los efectos metabólicos de los AGT a niveles recomendados de grasa.**

Objetivo: **investigar los efectos diferenciales de los ácidos grasos *trans* (TFA) sobre parámetros nutricionales, retención de TFA y regulación de triacilglicéridos (TAG) en ratas alimentadas con niveles recomendados o elevados de grasa.**

Métodos: **ratas macho Wistar fueron alimentadas (30 días) con dietas que contenían un 7% o 20% de grasas suplementadas o no con TFA (C7-C20-TFA7-TFA20).**

Resultados: **TFA7 (*vs.* C7) incrementó el peso corporal asociado a mayores panículos adiposos y TAG. La hipertriacilgliceridemia fue relacionada con una menor actividad LPL muscular, y el incrementado TAG hepático con una elevada expresión génica de SREBP-1c y actividad ACC, y reducida expresión génica de CPT-Ia. Los TFA no potenciaron los elevados pesos corporales, los panículos adiposos y los TAG inducidos por C20. Aunque la secreción hepática de TAG (TAG-SR) incrementó en TFA20 *vs.* C20, la similar triacilgliceridemia fue asociada a un compensatorio incremento de la actividad LPL en tejido adiposo. La atenuada acumulación hepática de TAG en TFA20 estuvo relacionada con una incrementada TAG-SR y un menor incremento de la expresión génica de SREBP-1c y SCD1, paralela a un relativo descenso del índice SCD1 y de la actividad ACC.**

Discusión y conclusión: **los TFA alteran los parámetros nutricionales y lipídicos en ratas. Sin embargo, diferentes respuestas sobre los niveles y regulación de los TAG por los TFA fueron observadas entre ratas alimentadas con niveles recomendados y elevados de grasa dietaria. Estas divergencias pueden estar relacionadas con diferentes retenciones de TFA y su bioconversión a ácido ruménico.**

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Palabras clave: *Retención de isómeros. Velocidad de secreción de triacilglicéridos. Lipoproteína lipasa. Regulación de lípidos. Acumulación de lípidos.*

Abbreviations:

ACC: acetyl-CoA carboxylase.
AIN: American Institute of Nutrition.
CPT-Ia: carnitine palmitoil transferase-Ia.
EAT: epididymal adipose tissue.
FA: fatty acids.
FAME: fatty acid methyl esters.
FAS: fatty acid synthase.
LPL: lipoprotein lipase.
PHVO: partially hydrogenated vegetable oil.
PPAR-α: peroxisome proliferator-activated receptor-alpha.
PUFA: polyunsaturated fatty acids.
RA: rumenic acid.
SFA: saturated fatty acids.
SCD1: stearoyl-CoA desaturase-1.
SREBP-1c: sterol regulatory element-binding protein-1c.
TFA: trans fatty acids.
TAG: triacylglycerol.
TAG-SR: triacylglycerol secretion rate.
VA: vaccenic acid

Introduction

Epidemiological studies have shown that a high intake of industrial trans fatty acids (TFA) increases the risk of coronary heart disease and other non-communicable diseases through their adverse effects on circulating lipid and lipoprotein levels, endothelial function and inflammatory response[1,2]. However, in recent years, the human intake of industrial TFA has considerably decreased[3,4], which has been correlated with a potential beneficial influence on cardiovascular risk[5].

Several biochemic al disorders associated with high intakes of industrial TFA in humans have also been observed in experimental animal models fed TFA-rich diets, thus allowing the study of some of the mechanisms involved in these metabolic alterations[6-9]. However, in experimental animals, there are controversial results regarding the effect of a high intake of TFA on serum and liver triacylglycerol (TAG) levels, as well as on their regulation mechanisms[8,10-14]. Furthermore, there is little evidence available on moderated or low consumption of TFA on the potential metabolic alterations. Therefore, the aim of the present study was to investigate the differential effect of TFA on some nutritional parameters, serum and tissue TAG levels and the mechanisms involved in TAG regulation in rats fed diets with recommended and high-fat levels.

Methods

Animals, diets and experimental protocol

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[15]. Male Wistar rats weighing 100-120g were housed in collective stainless-steel metabolic cages (n=6/group) under controlled conditions (23±2°C and 12h light–dark cycle) and subjected *ad libitum* to different diets.

The diets (Table I) differed either in 1) fat level: 7% (recommended) or 20% (high), and/or 2) the presence or absence of TFA. The composition of the control (C7) diet was based on the American Institute of Nutrition ad hoc writing committee recommendation (AIN-93G)[16], except for the fatty acid (FA) source that was based on AIN-76[17]. The high-fat diet (C20), was enriched in fat by substituting 13g of carbohydrate/100g of diet with an equal amount of corn oil, reaching 20g of fat/100g of diet. The TFA7 diet was obtained by substitution of 1% (w/w) of corn oil of the C7 diet with 1% (w/w) of a partially hydrogenated vegetable oil (PHVO) rich in TFA (51%); while the TFA20 diet was obtained by replacing 2.86% (w/w) of corn oil of the C20 diet with the same amount of PHVO rich in TFA. The PHVO contained a complex mixture of TFA with four main isomers: 9t-, 10t-, 11t- and 12t-18:1. The energetic densities of the diets were: C7 and TFA7: 16.5 MJ/Kg and C20 and TFA20: 19.2 MJ/Kg; while the energy contributions of TFA (% of energy) were: TFA7: 1.17 and TFA20: 2.87. The relative composition of the individual TFA isomers was identical between the TFA7 and TFA20 groups. Each diet was freshly prepared every 3 days throughout the experimental period and maintained at 4°C.

In one series of 30 animals, 6 rats were killed at the start of the experiment with the purpose of determining initial composition and energetic content of the carcass. The remaining 24 animals were weighed, food intake was recorded and faeces were collected three times a week during the whole dietary treatment. Food intake was adjusted for waste by collecting food spillage. Faeces were stored at -80°C until analysis. On the morning of day 30, the rats (n=6/group) were sacrificed under anaesthesia (1 mg of acepromazine + 100 mg of ketamine/kg of body weight), the bodies were shaved and the abdomens were 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/group) was treated with the same diets 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, gastrocnemius muscle and epididymal adipose tissue (EAT) were frozen, weighed and stored at -80°C until analysis. A third series of rats (n=6/group) was employed to estimate the *in vivo* hepatic triacylglycerol-secretion rate (TAG-SR).

Table I
Composition of the experimental diets

Dietary constituent (g/kg dry diet)	*C7*	*TFA7*	*C20*	*TFA20*
Corn starch	529.5	529.5	399.5	399.5
Casein	100	100	100	100
Sucrose	200	200	200	200
Corn oil	70	60	200	171.4
TFA rich fat	-	10	-	28.6
Fibre	50	50	50	50
Vitamin mixture	10	10	10	10
Mineral mixture	35	35	35	35
L-Cystine–L-methionine	3.0	3.0	3.0	3.0
Choline	2.5	2.5	2.5	2.5
Energy (MJ/kg)	16.5	16.5	19.2	19.2
*Fatty acid composition**				
14:0	0.05	0.06	0.05	0.06
16:0	12.29	12.11	12.29	12.10
7*c*16:1	0.11	0.09	0.11	0.09
17:0	0.07	0.06	0.07	0.06
18:0	1.90	3.17	1.90	3.17
(6-8)*t*18:1	ND	0.81	ND	0.81
9*t*18:1	ND	1.04	ND	1.04
10*t*18:1	ND	1.52	ND	1.53
11*t*18:1	ND	1.38	ND	1.38
12*t*18:1	ND	1.07	ND	1.07
(6-8)*c*18:1+(13/14)*t*18:1	ND	1.18	ND	1.17
9*c*18:1	30.39	27.90	30.39	27.89
11*c*18:1	1.15	1.45	1.15	1.46
19:0	ND	0.08	ND	0.08
9*t*12*t*18:2	ND	0.19	ND	0.20
9*c*12*t*18:2	ND	0.11	ND	0.11
9*t*12*c*18:2	ND	0.10	ND	0.10
9*c*12*c*18:2	52.34	44.95	52.34	44.95
9*c*12*c*15*c*18:3	0.96	0.83	0.96	0.82
20:0	0.45	0.39	0.45	0.39
8*c*20:1	0.20	0.18	0.20	0.18
22:0	ND	0.05	ND	0.05
∑ Minors	0.10	1.37	0.10	1.37
∑ TFA	ND	7.32	ND	7.32
∑ SFA	14.76	15.92	14.76	15.92
∑ MUFA	31.85	29.62	31.85	29.62
∑ PUFA	53.30	45.78	53.30	45.77

Diets were prepared according to AIN-93G[16], except for the FA source that was based on AIN76[17]. *Values are presented as weight percentages of total FAME.
C7: control diet, corn oil at recommended fat level; TFA7: diet at recommended fat level with substitution of 1% corn oil by TFA rich fat; C20: diet corn oil at high fat level; TFA20: diet at high fat level with substitution of 2.86% of corn oil by TFA rich fat. TFA: trans fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Laboratory analyses

- *Fatty acid composition of dietary fats*

The FA composition of dietary fats was determined by gas chromatography under the conditions, standards, solvents and chemicals previously reported[13].

- *Nutritional parameters*

Nitrogen in samples from the carcass homogenate was determined by the Kjeldahl method[18]. 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 faeces was extracted with light petroleum-ether. 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. Energy efficiency was estimated from the final carcass energy content and carcass energy at the start of the experiment in the weight-matched rats killed on day 0 divided by energy intake during 30 days. The fat apparent absorption, as a bioavailability index, was assessed as the percentage of fat intake that was not excreted in the faeces[19].

- *Triacylglycerol levels in serum, liver and muscle*

TAG levels in serum were determined using a commercially available test kit (Sociedad de Bioquímicos, Santa Fe, Argentina). Portions of liver or gastrocnemius muscle were powdered and homogenized in saline solution for TAG content quantification by the Laurell's method[20].

- *Serum and tissues fatty acids analysis and estimation of TFA retention and some key product/precursor ratios involved in the FA metabolism*

The extraction of total lipids was performed by Bligh and Dyer's method[21], followed by the transesterification with a methanolic potassium hydroxide solution and the analysis of FA by gas chromatography as previously described[13]. TFA retention (%) in serum, liver and EAT was estimated by the percentage of the individual TFA isomer measured in the serum or tissue/percentage of TFA isomer in the dietary fat ratio multiplied by 100. The 9c16:1/16:0 and 9c18:1/18:0 ratios were considered to assess the stearoyl-CoA desaturase-1 (SCD1) index[22] as well as the 9c,11t18:2/11t18:1 ratio to estimate the relative conversion rate of vaccenic acid (VA) to rumenic acid (RA).

- *In vivo hepatic triacylglycerol-secretion rate*

The hepatic triacylglycerol-secretion rate (TAG-SR) was determined in another set of rats (n=6/group) submitted to the same dietary treatments by the method of Otway et al.[23]. Further details have been previously reported[24].

- *Lipoprotein lipase activity in adipose tissue and gastrocnemius muscle*

The adipose tissue lipoprotein lipase (LPL) activity was quantified in EAT acetone powder by the fluorometric method of Del Prado et al.[25]. To assess muscle LPL activity, gastrocnemius muscle samples were homogenized in a NH_4Cl/NH_4OH–Heparin buffer. Then, the quantification of the LPL activity in muscle was performed by the fluorometric method[25].

- *ACC enzyme activity in liver*

The Acetyl-CoA Carboxylase (ACC) activity was assessed in liver homogenized according to Zimmermann et al.'s technique[26].

- *Extraction and analysis of RNA and quantification by RT-PCR*

Total RNA was isolated from liver samples using Trizol (Invitrogen, Carlsbad, Calif., USA) according to the manufacturer's instructions. RNA samples were then treated with a DNA-free kit (Applied Biosystems, Foster City, Calif., USA) to remove any contamination with genomic DNA. The yield and quality of the RNA were assessed by measuring absorbance at 260, 270, 280 and 310nm and by electrophoresis on 1.3% agarose gels. 1.5mg of total RNA of each sample was reverse-transcribed to first-strand complementary DNA (cDNA) using a M-MLV Reverse Transcriptase (Invitrogen, Argentina). Relative FA synthase (FAS), sterol regulatory element-binding protein-1c (SREBP-1c), SCD1, peroxisome proliferator-activated receptor-alpha (PPAR-α) and carnitine palmitoil transferase-Ia (CPT-Ia) mRNA levels were quantified using real-time PCR with a StepOne TM Real-Time PCR Detection System (Applied Biosystems). Actin mRNA levels were similarly measured and used as the reference gene. 0.1ml of each cDNA was added to the PCR reagent mixture, SYBR_Green Master Mix (Applied Biosystems), with the upstream and downstream primers (600nmol/l for FAS and SCD1 and 900nmol/l for CPT-Ia, PPAR-α and SREBP-1c). Specific primers were synthesized commercially (Invitrogen Custom Primers) and the sequences were:

FAS: 5'-AGCCCCTCAAGTGCACAGTG-3' (forward);
5'-TGCCAATGTGTTTTCCCTGA-3' (reverse).
SREBP-1c: 5'-GGAGCCATGGATTGCACATT-3' (forward);
5'-GCTTCCAGAGAGGAGCCCAG-3' (reverse).
CPT-Ia: 5'-ACGTGAGTGACTGGTGGGAAGAAT-3' (forward);
5'-TCTCCATGGCGTAGTAGTTGCTGT-3' (reverse).
SCD1: 5'-TGGGTTGGCTGCTTGTG-3' (forward);
5'-GCGTGGGCAGGATGAAG-3' (reverse).
PPARα: 5'-CCCCACTTGAAGCAGATGACC-3' (forward);
5'-CCCTAAGTACTGGTAGTCCGC' (reverse).

All the PCR procedures were performed according to the techniques previously described[14] and the results expressed using the recommended $2^{-\Delta\Delta Ct}$ method[27].

Statistical analysis

Values were expressed as mean±standard errors of mean (SEM) of 6 animals/group. Statistical differences between mean values were established by two-way ANOVA (2x2) using the levels of dietary fat and TFA supplementation as independent variables. All post hoc multiple comparisons were made using Scheffe's critical range test. For TFA retention, statistical differences between means were established by unpaired Student's *t* test. Differences were considered statistically significant at $p<0.05$.

Results

Nutritional parameters

Energy intakes, growth variables, carcass composition and tissue weights are shown in table II. Even though the energy food intake was similar among all groups of animals, an increased body weight gain was noted in the TFA7, C20 and TFA20 groups vs. C7. The change of body weight gain in the TFA7 group was not associated with changes in energy efficiency and carcass energy retention; however an increase in these parameters was induced by the high-fat diet. In addition, the changes in body weight gain were paralleled to an increase in the epididymal fat pads. No changes in carcass composition, or liver and gastrocnemius muscle weights were induced either by TFA and high-fat diets. In contrast to the results observed at recommended levels of fat, comparing with C20, TFA20 did not modify all the mentioned nutritional parameters.

Serum and tissue trans fatty acid retention

Dietary TFA were retained into serum, liver and EAT (Table III). The total and individual TFA retentions in EAT were significantly greater than those observed in liver and serum. In addition, the average isomer retention in the TFA20 group was 291%, 66% and 34% higher than in the TFA7 group in adipose tissue, liver and serum, respectively. Comparing all the trans-18:1 isomers, the TFA retention in serum was: 9t18:1 ≈ 12t 18:1 > 11t18:1 > 10t18:1 ≈ (6-8)t18:1; in liver: 9t18:1 ≈ 12t18:1 > 11t18:1 > 10t18:1 > (6-8)t18:1 and in adipose tissue: 9t18:1 ≈ 12t18:1 > 11t18:1 > 10t18:1 > (6-8)t18:1. Compared with the trans-18:1, due to the low dietary levels of non-conjugated trans-18:2, the TFA retention of these isomers showed a wide variability. The levels of RA biosynthesized in tissues from VA were in EAT TFA7: 0.86±0.05 vs. TFA20: 1.45±0.04 ($p<0.05$); in liver TFA7: 0.30±0.03 vs. TFA20: 0.28±0.04 ($p>0.05$); and in serum were non-detected either in TFA7 as well as TFA20.

Serum and tissues triacylglycerol contents and their bioregulation

The TAG levels in serum were increased in a similar magnitude in the TFA7, C20 and TFA20 vs. C7 group (Table IV). Comparing with the C7 diet, rats fed TFA7 and TFA20 diets showed a significant increase in the TAG content in liver, but a higher increase was observed in animals fed C20 diets. Thus, the TFA20 diet, slightly but significantly attenuated the TAG accretion induced by C20 diet in liver. In the gastrocnemius muscle, TFA did not increase the TAG content at recommended levels of fats, and it attenuated the TAG accretion induced by the high-fat diet.

The hepatic TAG-SR was significantly increased in the TFA20 vs. C20 group, but was not altered in the TFA7 vs. C7 group. LPL activities in EAT were significantly increased in TFA7 vs. C7, and even greater in TFA20. In contrast, the LPL activity in the gastrocnemius muscle was reduced in TFA7, C20 and TFA20 vs. C7; but no additive effect was observed.

The liver expression of some key genes related to the synthesis (SREBP-1c, FAS and SCD1) and oxidation (PPAR-α and CPT-Ia) of FA are shown in table V. Regarding the hepatic FA biosynthesis, the SREBP-1c mRNA expression was significantly higher in TFA7, C20 and TFA20 vs. C7, without additive effects when both variables were presented. Strikingly, comparing with the C7 diet, rats fed TFA7, C20 and TFA20 diets showed a significant decrease in the FAS mRNA expression. However, TFA20 slightly but significantly attenuated the reduction in the FAS mRNA expression induced by the C20 diet. Hepatic SCD1 mRNA expression was reduced by TFA and increased by high fat diets. Additionally, the SCD1 index, estimated throughout the 9c18:1/18:0 ratio, showed the following values: C7: 112.1±3.1[a]; TFA7: 152.4±6.0[b]; C20: 161.2±3.7[b] and TFA20: 90.7±2.9[a]. Moreover, the hepatic ACC activity was measured and the values were (nmol NADH consumed/min/mg of protein): C7: 113.0±5.3[a]; TFA7: 137.3±3.4[b]; C20: 197.4±2.6[c] and TFA20: 116.4±1.0[a].

Concerning the fatty acid oxidation, PPAR-α and CPT-Ia mRNA expressions were significantly increased by the high fat diets; however the PPAR-α gene expressions was not modified by TFA either at 7% or 20% of dietary fat, while the CPT-Ia gene expression decreased by TFA at recommended fat levels.

Discussion

Taking into account that there is little information available from experimental animal models concerning TFA effects on TAG regulation at recommended fat levels and their relation with the nutritional status, the aim of this work was to investigate the differential effect of industrial TFA on some nutritional parameters, serum and tissue TAG levels; the mechanisms in-

Table II
Effect of experimental diets on nutritional parameters and body composition

	C7	*TFA7*	*C20*	*TFA20*	*ANOVA*		
					L	*TFA*	*L x TFA*
Energy intake (kJ/30 days)	7676±359	7567±131	7795±191	7574±275	NS	NS	NS
Body weight gain (g/30 days)	140.6±3.1[a]	159.7±2.3[b]	159.5±2.8[b]	160.2±3.8[b]	S	S	NS
Energy efficiency (%)	14.0±0.29[a]	16.0±0.42[a,b]	18.1±0.18[b]	19.2±0.69[b]	S	NS	NS
Fat apparent absorption (%)	90.2±0.9[a]	92.4±1.1[a,b]	95.5±0.5[a,b]	95.2±0.7[b]	S	NS	NS
Carcass energy retention (kJ)							
Total	1616±21[a]	1762±18[a,b]	1974±31[b]	2005±20[b]	S	S	NS
Fat	952±18[a]	1083±20[a,b]	1210±48[b]	1223±40[b]	S	NS	NS
Protein	664±7[a]	679±12[a]	764±20[b]	782±35[b]	S	NS	NS
Carcass composition (g/100 g)							
Protein	12.0±0.9	12.1±1.3	13.0±1.1	12.8±0.9	NS	NS	NS
Fat	18.9±0.8	17.4±0.7	17.8±0.7	18.4±0.9	NS	NS	NS
Water	63.5±1.0	63.4±1.4	61.6±1.1	62.1±1.9	NS	NS	NS
Tissues weight (g/100 g)							
Liver	3.40±0.09	3.64±0.09	3.61±0.18	3.83±0.09	NS	NS	NS
EAT	1.18±0.09[a]	1.52±0.03[b]	1.50±0.04[b]	1.47±0.03[b]	S	S	NS
Gastrocnemius muscle	0.68±0.06	0.64±0.04	0.66±0.03	0.67±0.03	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) after ANOVA (2x2). L, effect of dietary fat level; TFA, effect of absence or presence of trans fatty acids; NS, not significant.
C7: control diet, corn oil at recommended fat level; TFA7: diet at recommended fat level with substitution of 1% corn oil by TFA rich fat; C20: diet corn oil at high fat level; TFA20: diet at high fat level with substitution of 2.86% of corn oil by TFA rich fat. EAT: epididymal adipose tissue.

Table III
Effect of experimental diets on fatty acid isomers retention

Fatty acids	*Isomer retention (%)*	
	TFA7	*TFA20*
Serum		
(6-8)*t*18:1	22.4±1.5[a]	28.2±2.9[b]
9*t*18:1	82.1±3.3[a]	97.6±5.3[b]
10*t*18:1	21.1±3.6[a]	31.3±5.4[b]
11*t*18:1	42.4±3.2[a]	61.4±5.4[b]
12*t*18:1	73.1±9.4[a]	105.4±9.5[b]
∑TFA	46.8±2.8[a]	63.4±4.5[b]
Liver		
(6-8)*t*18:1	21.1±1.1[a]	41.5±5.5[b]
9*t*18:1	90.8±7.0[a]	154.7±5.3[b]
10*t*18:1	35.1±2.3[a]	53.1±4.0[b]
11*t*18:1	40.8±2.2[a]	100.1±3.6[b]
12*t*18:1	67.6±2.3[a]	168.9±2.1[b]
∑(9-12)*t*18:2	98.5±9.4	94.3±9.1
∑TFA	54.5±2.7[a]	101.5±2.8[b]
Epididymal adipose tissue		
(6-8)*t*18:1	83.9±5.7[a]	327.9±4.6[b]
9*t*18:1	142.0±6.5[a]	585.5±21.6[b]
10*t*18:1	45.7±4.1[a]	327.5±12.4[b]
11*t*18:1	99.8±9.5[a]	419.8±18.4[b]
12*t*18:1	78.0±5.0[a]	601.6±4.0[b]
∑(9-12)*t*18:2	337.5±36.9[a]	818.1±42.3[b]
∑TFA	117.1±6.0[a]	469.1±10.7[b]

Data are expressed as mean±SEM (n=6) and was calculated as percentage of the TFA isomer measured into the tissue/percentage of TFA isomer in the diet [o]multiplied by 100. Different letters in each row indicate statistical differences at $p<0.05$ (Student's *t* test).
TFA7: diet at recommended fat level with substitution of 1% corn oil by TFA rich fat and TFA20: diet at high fat level with substitution of 2.86% of corn oil by TFA rich fat. TFA: trans fatty acids

volved in the TAG regulation in rats fed with diets at recommended and high fat levels were also analyzed. It is important to note that in the FA composition of the experimental fats we combined linoleic acid-rich oil with PHVO oil containing different proportion of TFA. The purpose of using an oil containing a high n-6:n-3 ratio was to generate an unsaturated FA unbalance frequently observed in the Western diets. Because the FA proportion in the diet influences the FA retention in the tissues, and thereby the biological effects[14,28], the relative composition of the individual TFA isomers and the other FA was identical between the TFA7 and TFA20 diets. The TFA level chosen in the TFA7 diet (1.17% of energy) can be achieved in populations consuming high levels of PHVO. Finally, in comparison with other studies where the elaidic acid was the main TFA isomer in the diet, it is remarkable to note that the individual TFA levels of the PHVO used for diet preparation was 10t18:1>11t18:1>12t18:1>9t18:1>(6-8) t18:1.

It has been shown[6,13,14] that the TFA levels retained into a particular tissue depend on the specific TFA, tissue and FA composition of the diet. In the present study it is shown that by maintaining the same relative composition of the individual FA, the TFA retention in liver and more clearly in EAT, but not in serum augmented with increased amounts of fats and, consequently, the TFA consumed. These results might suggest that the TFA retention in serum was mainly associated with the relative dietary composition of the TFA, while the TFA retention in liver and more marked in EAT was associated with the total amount of

Table IV
Effect of experimental diets on serum and tissue triacylglycerol levels and parameters related to their regulation

	C7	*TFA7*	*C20*	*TFA20*	*ANOVA*		
					L	TFA	Lx TFA
TAG levels							
Serum (mmol/l)	0.44±0.03[a]	0.54±0.04[b]	0.55±0.03[b]	0.52±0.03[b]	S	S	NS
Liver (μmol/g)	13.8±1.1[a]	21.3±1.2[b]	40.4±3.0[c]	27.0±1.7[d]	S	S	NS
Gastrocnemius muscle (μmol/g)	2.7±0.1[a]	2.7±0.2[a]	4.1±0.4[b]	3.5±0.1[c]	S	S	NS
TAG-SR (ηmol/min/100 g)	186±4[a]	163 ±3[a]	178±6[a]	226±9[b]	S	S	NS
Epididymal adipose tissue LPL (U/EAT)	1.08±0.07[a]	2.41±0.09[b]	2.40±0.17[b]	3.15±0.04[c]	S	S	S
Gastrocnemius muscle LPL (U/total muscle)	3.33±0.07[a]	2.73±0.09[b]	2.86±0.08[b]	3.00±0.02[b]	S	S	NS

Data are expressed as mean±SEM (n=6). Different letters in each row indicate statistical differences at $p<0.05$ (Scheffe's test) after ANOVA (2x2). L, effect of dietary fat level; TFA, effect of absence or presence of trans fatty acids; NS, not significant.
C7: control diet, corn oil at recommended fat level; TFA7: diet at recommended fat level with substitution of 1% corn oil by TFA rich fat; C20: diet corn oil at high fat level; TFA20: diet at high fat level with substitution of 2.86% of corn oil by TFA rich fat. EAT: epididymal adipose tissue; LPL: lipoprotein lipase; TAG: triacylglycerol; TAG-SR: hepatic TAG-secretion rate. U: units (ηmol fluorescein/ min).

Table V
Effect of experimental diets on hepatic expression of genes related to de novo fatty acid synthesis and fatty acid oxidation

	C7	*TFA7*	*C20*	*TFA20*	*ANOVA*		
					L	TFA	L x TFA
SREBP1c	1.00±0.03[a]	3.09±0.46[b]	5.79±0.60[c]	3.99±0.40[b]	S	NS	NS
FAS	1.00±0.09[a]	0.21±0.03[b]	0.34±0.06[b]	0.55±0.05[c]	S	S	NS
SCD1	1.00±0.01[a]	0.35±0.04[b]	5.84±0.62[c]	1.20±0.23[a]	S	S	NS
PPAR-α	1.00±0.01[a]	1.00±0.09[a]	2.12±0.16[b]	2.27±0.06[b]	S	NS	NS
CPT-Ia	1.00±0.01[a]	0.76±0.04[b]	4.01±0.19[c]	5.17±0.50[c]	S	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) after ANOVA (2x2). L, effect of dietary fat level; TFA, effect of absence or presence of trans fatty acids; NS, not significant.
C7: control diet, corn oil at recommended fat level; TFA7: diet at recommended fat level with substitution of 1% corn oil by TFA rich fat; C20: diet corn oil at high fat level; TFA20: diet at high fat level with substitution of 2.86% of corn oil by TFA rich fat. CPT-Ia: carnitine palmitoil transferase-Ia; FAS: fatty acid synthase; PPAR-α: peroxisome proliferator-activated receptor-alpha; SCD1: stearoyl-CoA desaturase-1; SREBP-1c: sterol regulatory element-binding protein.

TFA consumed and the relative oxidative rate or metabolic fate of each TFA isomer. Likewise, few studies have reported about the relative oxidation rate and the specific biological effects of individual t18:1 isomers. A low relative retention of 10t18:1 in liver and EAT has been also previously inferred in the liver of experimental rats[13] and these results might be associated with a higher oxidative rate of this isomer. It is important to mention that Park et al.[29] did not observe changes in body fat in mice fed 10t18:1, whereas Anadón et al.[30] suggested that this isomer tended to increase serum TAG levels in rats. On the other hand and in agreement with Du et al.[31] 9t18:1 presented the highest relative retention in liver and EAT among the monoenic-TFA. This isomer has been mainly related to increased levels of circulating lipids and lipoproteins[32]. Similar to what has been previously reported for another animal model[13], in the present study VA was moderately retained in tissues. This isomer may provide some beneficial effects on health[30,33], or ameliorate some deleterious effects of the other TFA isomers, by itself or through its bioconversion to RA. It is important to note that the similar levels of RA observed in the liver of animals fed TFA7 and TFA20, might suggest that the hepatic levels of this FA depend on the proportion of VA in the diets, whereas in EAT it has been influenced by the level of dietary fat and amount of VA consumed. Nevertheless, the relative conversion of RA from VA was decreased by high levels of fats in both tissues (RA/VA; liver: TFA7: 0.54 vs. TFA20: 0.19 and EAT: TFA7: 0.62 vs. TFA20: 0.24). The results of the distinctive effects of individual TFA, including biosynthesized RA, influenced by different dietary fat levels, may explain the different effects on nutritional status and lipid regulation.

In agreement with results published elsewhere, at recommended fat levels TFA increased the body weight gain associated with higher fat pads[6], while TFA at high-fat levels did not potentiate the higher body weight gain and fat accretion induced by a high-fat diet[8,10].

In this work, different responses to TFA on TAG levels and their regulation between rats fed recommended and high fat diets were found. At recommended fat levels, the hypertriacylglyceridaemia induced by TFA might be explained by a decreased muscle TAG removal. While, despite the elevated TAG-SR, the TFA did not intensify the hypertriacylglyceridaemia produced by high-fat. The similar serum TAG levels between C20 and TFA20 could be explained by a compensatory TAG removal from adipose tissue. The differences with previous investigations showing hypertriacylglyceridaemia[8] might be attributed to the fact that the predominant TFA was elaidic acid, while in the present study the prevailing TFA were 10t18:1 and VA. In agreement with this explanation, Anadón et al.[30] showed that rats treated with VA rich fat did not increase the serum TAG levels, rather tended to decrease them. Moreover, reinforcing previous results from our laboratory in other experimental animal models with the same PHVO[13], it seems that the adipose tissue TAG removal might play a more important role than the hepatic TAG-SR in the plasma TAG regulation. This effect appears to be related to the dietary TFA composition.

A more complex metabolic state with different changes in liver TAG content and its regulation has been observed by TFA and high fat diets. The increased levels of liver TAG induced by TFA at recommended levels of dietary fats were related to an increased hepatic FA biosynthesis and a reduced β-oxidation. Even though we have measured the expression of genes related with some key transcription factors and enzyme involved in the hepatic TAG regulation, the higher SREBP-1c and the lower CPT-Ia gene expressions might explain, at least in part, the elevated liver TAG accretion. The higher SREBP-1c could induce a higher lipogenesis through the ACC enzyme, regardless of the fact that the expression of genes encoding FAS and SCD1 enzymes was not increased. The increased ACC activity observed in TFA7 could contribute to a high production of malonyl-CoA increasing the FA synthesis and inhibiting the mitochondrial fatty acid oxidation by blocking the CPT-Ia. In addition, the expression of CPT-Ia enzyme has been reduced in the TFA7 group contributing to the unbalance between FA biosynthesis/β-oxidation. Similar results were also reported under other experimental conditions[7]. However, Cho et al.[34], comparing with high TFA diets, showed that low trans structured fat decreased the hepatic steatosis by lowering the hepatic lipogenic enzyme activities but elevated the plasma lipids in an atherogenic animal model. In addition, the alterations induced by the PHVO in mice with the same TFA composition appear to be related to other mechanisms of action[14], suggesting that the animal species might also provide different results.

Different effects on hepatic TAG regulation have been observed at high fat diets. In comparison with the effect of TFA7, it is important to mention that in the absence of TFA a high fat diet may lead to a higher hepatic TAG accumulation associated with an accentuated increase of the gene expression of SREBP-1c and SCD1, together with an increase of the SCD1 index and of the ACC enzyme activity. Nevertheless, the TFA20 diet did not potentiate the high hepatic TAG accretion, but even attenuated such alteration. This effect might be supported by the lower increase of the expression of genes encoding the SREBP-1c and SCD1 in parallel with a relative decrease of the SCD1 index and of the ACC enzyme activity. However, Jeyakumar et al.[35] showed that despite higher hepatic ACC, FAS, SCD1 and SREBP-1c protein levels, steatosis was not present in TFA-fed rats. The divergence with other researchers concerning the mechanism involved in the hepatic TAG regulation might be associated with the type of TFA isomers, among other reasons. Nevertheless, the findings of this work suggest that the metabolic FA milieu generated at recommended and high fat

levels induced different TFA responses on the hepatic TAG regulation.

In brief, diets containing TFA (1.17% of energy) at recommended fat levels as well as 2.86% of energy of TFA at high-fat levels differentially alter body weight gain, fat accretion, serum and liver TAG levels and their regulation in rats. This divergence might be related to the metabolic FA milieu induced by different TFA retentions and RA bioconversion into the tissues.

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