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Metabolic effects of *trans* fatty acids on an experimental dietary model

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The aim of the present study was to investigate the potential nutritional and metabolic impact of *trans* (*t*) fatty acids (FA) on an appropriate experimental dietary model. Since previously reported experimental designs have been matter of concern, we developed a dietary model to compare the effect of *t* isomers and/or the saturation of FA independently of other variables. Wistar rats were fed diets containing identical amounts of nutrients and high levels of dietary fats (200 g/kg) for 30 d. Dietary fat rich in *t*-FA was compared with fat rich in saturated (s) FA or rich in *cis* (*c*) FA, maintaining the same length of C chain of the FA. The fats were obtained through isomerization or hydrogenation of the *c*-FA present in the control fat. Apparent fat absorption, energy efficiency and triacylglycerol levels in serum and liver were different in rats fed *t*-FA or s-FA than *c*-FA. The apparent fat absorption was (%): s-FA 85·7 (SD 3·4) $<$ *t*-FA 93·1 (SD 0·4) $<$ *c*-FA 96·7 (SD 1·1) ($P<0·05$). The efficiency of energy utilization was lower in *t*-FA (11·7 %) and s-FA (18·5 %) diets, reaching statistical significance only between s-FA and *c*-FA. A striking finding was the change in the lipid profile in serum and liver. Serum and hepatic triacylglycerol levels were greater for *t*-FA and s-FA diets than in *c*-FA; however, the increases on serum triacylglycerol concentrations were greater with the s-FA diet and the increases on hepatic triacylglycerol content were greater with *t*-FA. Knowledge of the *t*-FA effects on this kind of experimental dietary model could contribute to determine the potential risk of *t*-FA intake for man.

***Trans* fatty acids: Isomerized fat: Hydrogenated fat: Dietary fat**

Trans (*t*) fatty acids (FA) are geometrical isomers of FA present in variable and important amounts in margarine, shortenings and baked products, reaching up to 50 % of total *t*-FA (Aro *et al*. 1998). The main source of *t*-FA for man's intake is through the partial hydrogenation of vegetable oils (Katan *et al*. 1995): they are undesirably produced at the same time as saturated (s) FA are generated. However, the physicochemical properties of *t*-FA are different from the natural *cis* (*c*) isomer and from the s-FA, even though from the structural view-point *t*-FA resemble s-FA. Louheranta *et al*. (1999) have suggested that *t*-FA resemble their s-FA counterparts with regard to some biochemical and metabolic effects. Studies of the influence of *t*-FA intake on plasma lipid profiles have yielded conflicting results. Most of the studies on human subjects have shown that dietary *t*-FA increased the plasma LDL-cholesterol, and contradictory effects have been observed on plasma HDL-cholesterol and on plasma lipoprotein(a) levels (Mensink & Katan, 1990; Lichtenstein *et al*. 1993; Judd *et al*. 1994; Mutanen & Aro, 1997). Information about the effect of *t*-FA intake on the human serum triacylglycerol level is also controversial. In some studies, dietary *t*-FA has increased the levels of serum triacylglycerol (Judd *et al*. 1994), whereas in other studies no effect on triacylglycerol has been reported (Lichtenstein *et al*. 1993; Mutanen & Aro, 1997). Certain differences may be due to factors such as experimental design, time of feeding or presentation of the experimental results. Similarly, conflicting results have been reported on experimental animals (Atal *et al*. 1994; Thomson *et al*. 1994; Mohamedain & Kummerow, 1999), because many studies have been carried out on animals chronically fed with fats from different sources and/or with many variables at the same time; these include the nature of dietary fat, length of C chain of the FA, presence of cholesterol, s-FA: unsaturated FA ratio or antioxidant levels. Therefore, since in most of the studies caution must be taken not to attribute differences solely to the presence of *t*-FA, the objectives of the present study were first, to develop an

Abbreviations: *c*, *cis*; FA, fatty acid; s, saturated; *t*, *trans*.
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experimental dietary model with the isomers and/or the saturation of the FA as the variables, and second, to analyse some potential nutritional and metabolic impacts of dietary *t*-FA compared with their respective s-FA and *c*-FA contents. In this regard, we specifically investigated the effect of *t*-FA on the apparent absorption of the dietary fats, energy efficiency and levels of lipids in serum and liver. Knowledge of the effects of *t*-FA in this experimental dietary model could contribute to determining the potential risk of *t*-FA intake on some human diseases caused by nutritional factors.

Materials and methods

Materials

Most nutrient compounds, including vitamins and minerals for diet preparations were chemical grade or better, with the exception of maize oil (Mazola, Escobar, Buenos Aires, Argentina), rapeseed oil (Mazola), sucrose, cellulose and maize starch, which were obtained from local sources. Maize oil was used as an unsaturated *c*-FA source or was involved in the preparation of oil enriched with *t*-FA or with s-FA. Standards were purchased from Sigma Chemical Co. (St Louis, MO, USA). Catalytic Pd in charcoal was from AlphaAesar Co. (WardHill, MA, USA). 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.

Preparation of the experimental dietary fats

In brief, oil rich in *t*-FA was obtained by isomerization of the double bonds of FA C-chain from maize oil in a reactor (Parr Instrument Co., Moline IL, USA) using Pd–C as catalyst and very low pressure H_2. In order to obtain high levels of *t*-FA and a low degree of saturation, optimal reaction conditions (temperature, agitation rate, gas pressure and Pd–C charge) were studied in pilot experiments. The catalyst was removed by filtration in a vacuum system, antioxidant butylated hydroxytoluene (200 mg/kg) was added, and the oil was kept at 4°C until the diet preparation. For the preparation of fat enriched with s-FA, the catalytic hydrogenation of the maize oil was performed in a Parr reactor. Pd–C was used as catalyst, and was removed by vacuum filtration after the end of the hydrogenation procedure with high pressure of H_2. The hydrogenation proceeded up to very low H_2 consumption (mean I value 30·01).

The *t* isomer content was analysed by Fourier transformed infrared spectroscopy using a Spectrum RX/FT-IR System (Perkin-Elmer, Norwalk, CT, USA), according to the procedure of Firestone (1999). The FA composition of each experimental oil, as methyl esters, was determined by GLC using a Hewlett/Packard 5890 chromatograph equipped with flame ionization detector. Analyses were carried out with a capillary column BPX70 (50 m, 0·25 μm film thickness) and the oven temperature was programmed with an initial temperature of 160°C with increases of 0·5°C/min up to 200°C. Fatty acid methyl esters were identified by comparison of their retention times relative to those of commercial standards.

Preparation of diets

The composition of diets is shown in Table 1, and was based on the American Institute of Nutrition ad hoc writing committee recommendation (AIN-93G diet formulated for the growth, pregnancy and lactation phases of rodents; Reeves *et al*. 1993), with the exception of replacing carbohydrate with fat to give 38·5 % energy as fat. All diets were isoenergetic, theoretically providing 19·288 MJ/kg, and contained 30 g rapeseed oil/kg to exceed the essential FA recommendations. Except for the type of fat, the three diets were identical and we could maintain the same length of FA chains, without cholesterol. The unique variables were basically the *c*: *t* isomer ratio in the *t*-FA diet when compared with *c*-FA diet, and the s-FA: unsaturated FA ratio when the s-FA diet was compared with the *t*-FA diet and with the *c*-FA diet. The FA composition of the non-treated, isomerized and hydrogenated maize oils is shown in Table 2. Each diet was freshly prepared every 3 d throughout the experimental period.

Animals and general protocol

Male Wistar rats weighing 80–100 g and provided by the Comisión Nacional de Energía Atómica (Buenos Aires, Argentina) were housed in the animal quarters under controlled conditions (23 ± 2°C and 12 h light–dark cycle) in individual stainless-steel metabolism cages. Animals were cared for in accordance with the principles of our School of Biochemistry regulations and *Guide to the Care and Use of Experimental Animals of the Laboratory*. The animals had free access to water and standard laboratory chow (Nutrimentos, Buenos Aires, Argentina) before the experimental period. After reaching 120–130 g, rats were assigned into four weight-matched groups (*n* 6 per group). One group was killed at the start of the experiment with the purpose of determining initial composition and energetic content of the carcass. The remaining animals were fed *ad libitum* for 30 d with the *c*-FA, *t*-FA or s-FA diet. Rats were weighed, food intakes were recorded, and total urine and faeces were collected daily during the whole dietary treatment. Food

Table 1. Composition of diets (g/kg diet)

Ingredient	*c*-FA diet	*t*-FA diet	s-FA diet
Maize starch	399·5	399·5	399·5
Casein	200	200	200
Sucrose	100	100	100
Rapeseed oil	30	30	30
Non-treated maize oil	170	–	–
Isomerized maize oil*	–	170	–
Hydrogenated maize oil*	–	–	170
Fibre	50	50	50
Mineral mixture†	35	35	35
Vitamin mixture†	10	10	10
L-Cystine–L-methionine	3·0	3·0	3·0
Choline	2·5	2·5	2·5
Energy (MJ/kg)	19·288	19·288	19·288

c, *cis*; *t*, *trans*; s, saturated.
* For details of isomerization and hydrogenation procedures, see p. 632.
† Vitamin and mineral mixtures were formulated according to Reeves *et al.* (1993).

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Table 2. Fatty acids composition of experimental oils (g/100 g total fatty acid methylesters)
(Mean values)

FA	Experimental maize oil		
	Non-treated	Isomerized*	Hydrogenated*
16 : 0	10·78	10·87	11·23
cis-16 : 1	0·20	0·10	0·15
18 : 0	2·24	2·61	59·33
trans-18 : 1	nd	26·47	10·47
cis-18 : 1	31·36	25·41	17·22
trans,trans-18 : 2	nd	2·09	nd
trans,cis-18 : 2 + *cis, trans*-18 : 2	0·48	1·35	nd
cis,cis-18 : 2	52·85	29·57	0·50
trans,cis,cis-18 : 3	0·06	0·03	nd
cis,cis,cis-18 : 3	0·75	0·41	nd
20 : 0	0·52	0·52	0·68
trans-20 : 1	0·12	0·07	nd
cis-20 : 1	0·24	0·13	0·22
22 : 0	0·20	0·18	0·12
24 : 0	0·20	0·19	0·08
%*trans*-FA	0·66	30·00	10·47
%Saturated-FA	13·94	14·38	71·44

FA, fatty acid; nd, not detected.
* For details of isomerization and hydrogenation procedures, see p. 632.

intakes were adjusted for waste by collecting food spillage. In order to avoid N losses, urine was collected in glass Erlenmeyer flasks containing 2·0 M-H_2SO_4 to get a final pH $<2{\cdot}00$. Faeces and urine were stored at -20°C until required.

On the morning of day 30, rats were anaesthetized with azepromazine (1 mg/kg body weight) and ketamine (100 mg/kg body weight); the body was shaved, the abdomen was cut open, blood and liver samples were taken for lipid determination and epididymal and lumbar adipose tissue were dissected. Following removal of the visceral organs, the carcass was weighed, chopped and frozen at -20°C until the compositional evaluation.

Analysis and calculations

N in samples from the carcass homogenate, urine, faeces and diets was converted to $(NH_4)_2SO_4$ by an automated Kjeldahl method (Windham, 1999) and the different contents were determined. Carcass and food protein contents were estimated by multiplying their N contents by 6·25. Dried samples of carcass, food and faeces were used to measure the fat content by extraction procedure with light petroleum ether (40–60°C; Windham, 1999). The water contents were determined by drying samples of the carcass and food to constant weight in an oven at 60°C (Windham, 1999).

The apparent absorption of dietary fat, as a bioavailability index, was assessed as the % ingested fat that was not excreted in the faeces (Nicolosi *et al.* 1998).

Carcass energy retention was estimated from protein and lipid content using the formula: carcass energy retention (kJ) = protein content (g) × 18·62 kJ/g protein + fat content (g) × 39·12 kJ/g lipid (Canolty & Koong, 1976). The results of this technique have been compared (Bernal *et al.* 2002) with those measured in carcass homogenate samples by calorimetric bomb using a Parr calorimeter (model 1341; Parr) with benzoic acid as combustion standard. The correlation coefficient (r) between both procedures was 0·9745.

The energy intake was calculated by multiplying weight of food consumed during the experimental period by the energy content of the diet (kJ/g). Carcass energy gain was assessed from the final carcass energy content and the carcass energy content at the start of the experiment in the weight-matched animals killed on day 0. Energetic efficiency was calculated as % carcass energy gain (kJ/30 d) divided by energy intake (kJ/30 d).

The cumulative N balance was estimated as the dietary N intake minus N excretion in faeces and urine during all of the experimental feeding period (Bernal *et al.* 1992).

Triacylglycerol, phospholipid and cholesterol levels were determined in serum and liver homogenates (100 g/l 0·15 M-NaCl) by the techniques of Mocchiutti & Bernal (1997) and Bernal *et al.* (1995).

Statistics

Statistical differences between mean values were established by one-way ANOVA (1×3) and significant differences between dietary groups were assessed using Scheffé's-critical range test. All statistical tests were considered significantly different at $P<0{\cdot}05$ (DeGroot, 1975). Values are expressed as means and standard deviations for six animals per group.

Results

The dietary model developed in the present study allowed us to compare the effect of *t* isomers and/or saturation of FA independently of other variables. The isomerization procedure showed a high yield of *t*-FA at moderate agitation rate, temperatures from 155 to 165°C and pressure of $7{\cdot}85\,10^4$ Pa. Nevertheless, since there was some variability in the % *t* isomers obtained, we mixed different batch preparations fixing 30 % of enrichment of *t*-FA in the fat. The mean original I value of the maize oil was 129·55 and was changed to approximately 120·00 by isomerization, and to 30·00 by hydrogenation.

Diets were very well accepted by the three dietary groups, with an average daily food intake (g/d) of 14·0 (SD 0·9) for the *c*-FA diet, 13·2 (SD 0·5) for the *t*-FA diet and 15·0 (SD 0·9) for the s-FA diet. Growth variables and tissue weights are shown in Table 3. Body weights at the start of the study were similar for the three groups, and the groups gained comparable amounts of weight during the experiment, with a slightly but not significantly lower weight gain in the *t*-FA group. Liver and epididymal adipose tissue weights were significantly influenced by the type of FA in the diet, being (*v. c*-FA diet) 25·9 and 86·9 % greater for the *t*-FA diet and 18·8 and 87·2 % greater for the s-FA diet respectively. Similarly to the epididymal adipose tissue, lumbar adipose tissue weights in the *t*-FA and s-FA groups changed, but due to the high variations in the values no statistical significance was found.

As shown in Table 4, for a similar fat intake the hydrogenation and isomerization of the dietary fat significantly

Table 3. Effect of the dietary fatty acids on growth variables and tissue weights*
(Mean values and standard deviations for six animals per group)

	c-FA diet		t-FA diet		s-FA diet		Statistical significance of effect (one-way ANOVA): P
	Mean	SD	Mean	SD	Mean	SD	
Body weight (g)							
Initial	124·6	3·1	124·3	3·0	124·8	2·9	NS
Gain	153·2	14·5	146·9	23·3	154·2	5·0	NS
Tissue weight (g)							
Carcass	222·1	7·8	218·3	13·0	213·5	3·4	NS
Liver	10·9[a]	0·8	13·8[b]	1·2	13·0[b]	1·4	0·043
Epididymal fat pads	2·9[a]	0·5	5·4[b]	1·1	5·4[b]	1·1	0·0007
Lumbar fat pads	2·8	1·2	4·6	1·5	4·3	2·1	NS

c, *cis*; *t*, *trans*; s, saturated.
[a,b]Mean values within a row with unlike superscript letters were significantly different (Scheffé's test, $P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 632–633.

modified the faecal fat excretion. Thus, the fat excretion: intake ratio was approximately twofold higher for the *t*-FA diet and fourfold greater for the s-FA diet, resulting in the apparent absorption of the dietary fat being s-FA < *t*-FA < *c*-FA. The difference in the fat excretion caused a higher mass excretion of faeces in rats fed on the s-FA diet (faecal excretion (g dry weight/d): *c*-FA 1·44 (SD 0·11), *t*-FA 1·59 (SD 0·23), s-FA 2·59 (SD 0·19)), without modification of the faecal N excretion. In addition, since no differences were found in N intake and urine N excretion, the cumulative N balance was not statistically different between the three dietary groups. Therefore, all groups had positive and similar N retention.

The effect of dietary FA on energy utilization and carcass composition is shown in Table 5. Even though the absorption was different in the *t*-FA and s-FA diets, the food energy intake, when corrected by the apparent fat absorption, was not statistically significant. Values for energy intake corrected by fat digestibility were (kJ/30 d): *c*-FA 7154 (SD 263), *t*-FA 6957 (SD 256), s-FA 7365 (SD 103). When the *t*-FA and s-FA groups were killed and the carcasses were considered, they retained slightly less total energy. These differences were caused by a lower retention of the fat into the carcass. As a consequence, the energy efficiency was modified by the dietary fats, but only reached significance between the s-FA diet and *c*-FA groups. The fat content of the carcass in animals fed on *t*-FA and s-FA diets was slightly lower; however, it did not reach statistical significance ($P=0·0542$).

The results shown in Table 6 indicate that levels of serum and liver triacylglycerol were higher due to the isomerization and hydrogenation procedure, with a slight and non-significant difference between *t*-FA and s-FA diets. However, a different pattern was followed by the dietary treatment: in the *t*-FA group the serum triacylglycerol level was 107 % greater and the liver triacylglycerol content was 126 % greater, while in the s-FA group, the main increase was observed in serum levels (225 *v*. hepatic levels 45 %). The cholesterol and phospholipid levels in serum and liver are also summarized in Table 6, and no significant differences were found between the three dietary groups.

Discussion

The nutritional and metabolic impact of dietary fats has been studied frequently. Thus, for example, in response to the very well known positive relationship between high s-FA consumption and elevated cardiovascular heart disease incidence, many recommendations have been made in order to effect a shift away from animal fats, as

Table 4. Effect of the dietary fatty acids on fat and nitrogen utilization*
(Mean values and standard deviations for six animals per group)

	c-FA diet		t-FA diet		s-FA diet		Statistical significance of effect (one-way ANOVA): P
	Mean	SD	Mean	SD	Mean	SD	
Fat intake (g/d)	2·8	0·2	2·6	0·1	3·0	0·1	NS
Faecal fat (mg/d)	85·0[a]	25·8	170·3[c]	8·1	413·9[b]	84·5	<0·0001
Faecal fat/fat intake (%)	3·3[a]	1·1	6·9[c]	0·4	14·3[b]	3·4	<0·0001
Apparent absorption (%)	96·7[a]	1·1	93·1[c]	0·4	85·7[b]	3·4	<0·0001
N intake (mg/d)	394·4	31·6	355·5	13·9	425·5	45·1	NS
N excretion							
Urine (mg/d)	109·0	28·6	116·5	42·5	133·4	57·6	NS
Faeces (mg/d)	37·6	1·8	37·7	9·2	42·2	3·5	NS
Cumulative N balance (g N/30 d)	7·5	0·2	7·0	0·4	7·4	0·5	NS

c, *cis*; *t*, *trans*; s, saturated.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different (Scheffé's test, $P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 632–633.

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Table 5. Effect of the dietary fatty acids on energy utilization and body composition*
(Mean values and standard deviations for six animals per group)

	c-FA diet		*t*-FA diet		s-FA diet		Statistical significance of effect (one-way ANOVA): *P*
	Mean	SD	Mean	SD	Mean	SD	
Energy intake (kJ/30 d)	7944	308	7815	288	8499	118	NS
Carcass energy retention (kJ)							
Total	1463[a]	47	1252[ab]	215	1229[b]	57	0·0138
Fat	912[a]	85	706[b]	87	721[b]	88	0·0014
Protein	511	39	481	82	483	31	NS
Energy efficiency (%)	18·7[a]	0·4	16·5[ab]	2·4	15·2[b]	0·8	0·0035
Carcass composition (g/kg)							
Protein	196	4	194	16	199	5	NS
Fat	138	14	119	15	119	12	NS
Water	625	7	636	10	642	5	NS

c, *cis*; *t*, *trans*; s, saturated.
[a,b]Mean values with unlike superscript letters were significantly different (Scheffé's test, $P<0·05$).
*For details of diets and procedures, see Tables 1 and 2 and p. 632–633.

well as partially hydrogenated fats, toward intake of vegetable and/or marine oils. While this recommendation in western countries led to a decrease in s-FA consumption, an elevation of the *t*-FA intake was observed as a result of the incorporation of partially hydrogenated fats. Thus, for example, Allison *et al*. (1999) have recently reported that in the US population the estimated energy ingested as fat was 35·3 %, and 7·4 % total fat ingested was *t*-FA (2·6 % total energy or 5·3 g/d).

Substantial evidence from epidemiological, clinical and experimental animal studies have linked high intake of *t*-FA with negative effects on the lipid metabolism and plasma lipid profile. However, since controversial results were reported for the experimental designs used, studies aiming at clarifying the real effects of *t*-FA isomers are needed. Therefore, we developed a dietary model with elevated fat (38·5 % energy) and *t*-FA (9·8 % energy) level, which approached the high intake of *t*-FA observed in many western countries. In brief, we obtained fat rich in *t*-FA by modification of original *c* configuration of long-chain FA, and on the other hand, we hydrogenated unsaturated FA to produce s-FA. As a consequence, the variables for comparison were *c* isomers *v*. *t* isomers and unsaturated (*c* or *t*) FA *v*. s-FA.

As long as the three groups had similar food intakes and identical levels of fat in the diet, the total fat intake was not different. However, since the apparent absorption of each type of fat was very different (*c*-FA > *t*-FA > s-FA), significant changes were observed in dietary fat utilization. Intestinal lipid absorption has many dynamic and complex phases (Ros, 2000; Phan & Tso, 2001), such as emulsification in the stomach, hydrolysis of the triacylglycerol by lipases, aqueous dispersion of lipolytic products in bile acid micelles and uptake of FA and *sn*-2 monoacylglycerols. On the basis of the spatial molecule configuration, melting points and solubility in aqueous biological fluids of FA, and taking into account that most of the lipid uptake is mediated by passive diffusion across the brush border membrane, intestinal absorption of s-FA, *t*-FA and natural *c*-FA could differ. Our present results are in agreement with this hypothesis and extend the findings of Bernard *et al*. (1987), which suggested different intestinal absorption of *t*-FA, s-FA and *c*-FA. However, the results contrast with those of Kalogeresis *et al*. (1996), who found similar lymphatic transport of glycerol trielaidate compared with glycerol trioleate, and with those of Emken *et al*. (1983), who reported that *t*-FA (elaidic acid) appears in serum chylomicrons at rates similar to oleic acid. Discrepancy of

Table 6. Effect of the dietary fatty acids on lipid levels in serum and liver*
(Mean values and standard deviations for six animals per group)

	c-FA diet		*t*-FA diet		s-FA diet		Statistical significance of effect (one-way ANOVA): *P*
	Mean	SD	Mean	SD	Mean	SD	
Serum (mM)							
Triacylglycerol	0·55[a]	0·17	1·14[b]	0·34	1·79[b]	0·78	0·0025
Cholesterol	1·83	0·29	1·79	0·24	1·51	0·17	NS
Phospholipid	1·70	0·24	1·67	0·29	1·56	0·34	NS
Liver (μmol/g wet tissue)							
Triacylglycerol	7·60[a]	1·79	17·18[b]	7·20	11·02[ab]	3·70	0·0231
Cholesterol	11·62	2·13	10·89	0·76	8·47	0·34	NS
Phospholipid	28·20	3·13	29·9	3·84	28·77	3·26	NS

c, *cis*; *t*, *trans*; s, saturated.
[a,b]Mean values within a row with unlike superscript letters were significantly different (Scheffé's test, $P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 632–633.

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the results might be attributed to the different experimental protocols used by the research groups. Thus, the most plausible explanations for the different intestinal absorption rates of FA are: (1) the aqueous solubility of FA (s-FA < *t*-FA < *c*-FA), so that s-FA and *t*-FA could become more dependent on the presence of the bile acid micelle for an efficient diffusion across the barrier exerted by the unstirred water layer; (2) the strong tendency of non-esterified s-FA, but not of the *sn*-2 monoacylglycerol, to form insoluble an Ca soap at alkaline pH of the intestine, as has been reported for palmitic acid (Ros, 2000) and behanic acid (Phan & Tso, 2001). Alterations of the lipase affinity related to the degree of saturation of FA and high rate of intestinal transit that could reduce the exposure time to digestion and/or absorption of lipids might also be involved.

Despite the high (and highest) faecal excretion of fat in *t*-FA (and s-FA) animals, dietary fats did not alter the N utilization. This was supported by the present finding of no statistical difference in the cumulative N balance and carcass N retention.

Differences in dietary FA composition can affect energy metabolism. Jones (1989) showed the efficiency of energy utilization of maize oil > coconut oil > olive oil > menhaden oil, and suggested that the partitioning of dietary fat for energy production *v*. retention within storage pools is dependent on the FA composition. Takeuchi *et al*. (1995) reported that body fat accumulation was greater in rats fed a lard diet enriched in s-FA than in those fed a high-oleic-acid safflower-oil diet, a safflower-oil diet, or a linseed-oil diet rich in monounsaturated FA, *n*-6 polyunsaturated FA or *n*-3 polyunsaturated FA. However, at least to our knowledge, there are no studies dealing with the effect of *t*-FA on energy utilization. Comparisons between s and unsaturated FA, in most studies, were made with different lengths of FA chains. In the present study, the energy efficiency in the carcasses of *t*-FA and s-FA animals was probably correlated with altered energy partition. This was supported by a high fat deposition in epididymal and lumbar adipose tissue in *t*-FA and s-FA groups, associated with either a tendency for reduced carcass energy retention in *t*-FA group or a lower carcass energy retention in s-FA group, without changes in the body-weight gains. The lower carcass energy efficiency, corrected by the apparent fat absorption coefficient, in *t*-FA and s-FA rats could be explained by the different rates of metabolism of these types of FA. In order to explain our present results, several arguments could be made. First, although the level of total fat was similar in the dietary groups, perhaps the different *c*-polyunsaturated FA level present in those groups could be involved in changes of lipolytic responses of different adipose tissue localization. Second, alterations in FA could lead to modifications in the levels of different types of membrane phospholipid and could therefore influence their lipid mobilization. Third, a lack of inhibition of FA acylation and synthesis in the epididymal and lumbar adipose tissue by the *t*-FA and s-FA groups could be present, justifying the higher fat mass gain in those tissues without any significant effect at the carcass fat level.

It has been demonstrated that dietary FA alter the body composition. For example, Takeuchi *et al*. (1995) reported that the carcass fat content was significantly greater in rats fed a s-FA (lard) diet than in those fed a high-unsaturated-FA (safflower oil rich in oleic acid, safflower oil or linseed oil) diet. In contrast to these findings, no differences in carcass composition were observed in our present study between groups of rats consuming *t*-FA, s-FA or *c*-FA diets. The discrepancy of these results may be attributed to a number of variables used when different oils are employed; the feeding period of 30 d in our present study may not have been long enough to detect these changes.

A number of investigators (Judd *et al*. 1994; Clevidence *et al*. 1997; Sundram *et al*. 1997; Roos *et al*. 2001) provided evidence that *t*-FA (and s-FA) could influence the plasma and tissue lipids profiles. An interesting result obtained with the present study was that dietary FA had a great impact on triacylglycerol levels, but the pattern of changes on plasma and liver contents in *t*-FA and s-FA rats were strikingly different. Thus, while dietary *t*-FA resulted in a higher increase in triacylglycerol hepatic levels, there was a greater increase of triacylglycerol serum concentrations by s-FA consumption. Alterations of hepatic secretion and extrahepatic removal of triacylglycerol could be involved in these changes, but we do not have results to support this hypothesis. In addition, results in human subjects have been inconsistent. For example, Sundram *et al*. (1997) did not observe any effects on serum triacylglycerol by dietary *t*-FA, whereas other authors reported that the triacylglycerol was modestly greater (Katan *et al*. 1995; Kris-Etherton, 1995). The objectives of the present study included neither the definition of the mechanism involved in the greater levels of triacylglycerol in plasma and liver of rats fed *t*-FA and s-FA diets, nor of the different profiles of the increases. However, possible explanations for the differences in their effects are emerging. One explanation of the greater levels of hepatic triacylglycerol levels in rats fed s-FA and *t*-FA might be an increased FA esterification: oxidation ratio. A number of studies (Lawson & Kummerow, 1978, 1979; Lawson & Holman, 1981) demonstrated that liver and heart mitochondria oxidize *t*-FA at a rate less than that observed with the corresponding *c* isomer. In addition, we did not measure the non-esterified FA levels, but one would expect that due to different absorption rates of fats, *t*-FA and/or s-FA rats might have elevated plasma levels of non-esterified FA all day, and therefore greater FA esterification in the liver than the *c*-FA group. The lack of effect observed in cholesterol levels in *t*-FA and s-FA groups in opposition to the well-known effect reported in human subjects by Judd *et al*. (1994), Sundram *et al*. (1997) and Ascherio *et al*. (1999) might be due to the different lipoprotein profile (high HDL-cholesterol levels) in the rats, which makes it difficult to change the cholesterol levels, and to the absence of cholesterol in the experimental diets.

In conclusion, these present results suggest that experimental animals fed for 30 d with a high levels of *t*-FA showed: (1) a lower apparent absorption of the dietary fat; (2) an altered energy partition with high deposition of fat in epididymal and lumbar adipose tissue with a slightly lower carcass energy efficiency; (3) a pronounced elevation of triacylglycerol levels in plasma and liver. Even though most of these effects were also observed in s-FA, the results

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on apparent absorption of fats and the pattern of changes on plasma and liver contents of triacylglycerol suggest that *t*-FA may produce metabolic lipid alterations by mechanisms that differ from those of s-FA.

Knowledge of the effects that *t*-FA have in this kind of experimental dietary model could contribute to determine the potential risk of *t*-FA intake for human diseases of elevated incidence on our population as hypertriacylglycerolaemia, cardiovascular heart disease and atherosclerotic disease.

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