Basic nutritional investigation

# Early and prolonged intake of partially hydrogenated fat alters the expression of genes in rat adipose tissue

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**Abstract**

**Objective:** Our previous study indicated that partially hydrogenated fat (PHF) diets, rich in *trans*-isomers, alter plasma lipids and increase the lipogenesis rate on adipose tissue in rats at a young age. In the present study we investigated the effects of dietary PHF on the expression of genes associated with glucose and lipid metabolism in rat adipose tissue.

**Methods:** Female Wistar rats were fed normolipidic diets containing PHF (rich in *trans*-fatty acids and poor in polyunsaturated fatty acids [PUFAs]), soy oil (rich in $\omega$-6 PUFAs), and fish oil (rich in $\omega$-3 PUFAs) during gestation and lactation; young male pups were fed the same diets from weaning until 120 d of life. The mRNA expression of peroxisome proliferator-activated receptor-$\gamma$, tumor necrosis factor-$\alpha$, resistin, adiponectin, and leptin were analyzed in retroperitoneal adipose tissue (RET) using real time polymerase chain reaction.

**Results:** The PHF group showed the highest triacylglycerol, glucose, and insulin levels and the lowest plasma adiponectin level. The RET of PHF incorporated *trans*-fatty acids, whereas fish oil and soy oil groups had increased $\omega$-3 and $\omega$-6 PUFAs, respectively. In the RET the PHF group had the highest resistin and tumor necrosis factor-$\alpha$ levels and the lowest adiponectin and peroxisome proliferator-activated receptor-$\gamma$ gene expressions, whereas the fish oil group had the highest peroxisome proliferator-activated receptor-$\gamma$ and the lowest leptin gene expression.

**Conclusion:** Prolonged intake of PHF has a negative effect on the expression of genes in RET when compared with diets with $\omega$-6 and $\omega$-3 PUFAs. These changes may be an effect of the smaller proportions of PUFAs in this fat, instead of being only caused by *trans*-fatty acids.   Published by Elsevier Inc.

*Keywords:* Trans-fatty acids; Polyunsaturated fatty acids; Adipokines; Peroxisome proliferator-activated receptor-$\gamma$; Adipose tissue; Rats; Glucose; Lipid homeostasis

## Introduction

In recent years, adipose tissue has been regarded as a multifunctional organ, which produces and secretes countless peptides and bioactive proteins, known as adipokines or adipocytokines [1]. Alterations in the mass of adipose tissue, as occurs in obesity, affect the production of most substances secreted by the adipocytes [2]. Although these alterations are frequently associated with metabolic dysfunctions and increased risk of cardiovascular disease [3],

the role of adipose tissue in the development of these alterations, considering its endocrine function, is still under investigation. The concentrations of several adipokines increase with obesity and have been related to hypertension (angiotensinogen) [4], damage to fibrinolysis (plasminogen activator inhibitor-1) [5], and resistance to insulin (acylation-stimulating protein, tumor necrosis factor-$\alpha$ [TNF-$\alpha$], interleukin-6, and resistin) [1,6]. Furthermore, insulin resistance is related to leptin resistance and decreased plasma adiponectin levels [7]. Nevertheless, leptin and adiponectin have additional physiologic functions, namely, although leptin controls food intake and energy expenditure, adiponectin has a strong antiatherogenic action [8].

The influence of high-fat consumption and the fat content of a diet on the development of non-transmissible chronic disease has been the focus of intense scientific research [9]. The amount and quality of lipid intake highly influence cellular functions by modulating the processes of differentiation, growth, and metabolism. Fatty acids may influence the expression of adipokines such as leptin, resistin, or adiponectin directly by interaction with transcription factors or indirectly by unknown mechanisms possibly linked to fatty acid oxidation synthesis, or storage [10]. Peroxisome proliferator-activated receptors (PPARs) are among the most abundant classes of nuclear receptor transcription factors and play a critical role in the regulation of fatty acid metabolism and adipose tissue function. PPAR-$\gamma$ is mainly expressed in adipocytes, activated by fatty acids, and participates in the adipogenesis process and energy homeostasis, modulating adipose tissue hormone and cytokine expression or secretion of adipokines [11].

Cardiovascular and metabolic diseases are associated with obesity and with alterations in the production of adipokines, such as leptin, resistin, adiponectin, TNF-$\alpha$, plasminogen activator inhibitor-1, and haptoglobin [12]. Because fatty acids are the main components of adipose tissue, it is of essential interest to clarify the biological effects of different types of fatty acids on the expression of relevant adipokines. Dietary *trans*-fatty acids (TFAs) increase the production of proinflammatory cytokines such as interleukin-6 and TNF-$\alpha$ [13,14]. In addition, a high intake of TFAs has been shown to promote insulin resistance [15] and to alter blood lipid profile and adiposity [16,17]. In contrast, a high intake of $\omega$-3 polyunsaturated fatty acids (PUFAs) has active anti-inflammatory effects [18,19], and it has been proposed that the $\omega$-6/$\omega$-3 fatty acid ratio increase, currently found in the occidental diet, contributes to many of the metabolic alterations present in non-transmissible chronic disease [20].

To our knowledge, there are currently no studies showing the effects of partially hydrogenated fat (PHF) ingestion in a normolipidic diet during gestation and lactation and during offspring growth on the expression of PPAR-$\gamma$ and specific genes associated with glucose and lipid metabolism. Moreover, most studies related to lipid metabolism have been designed for the postweaning period and/or high-

fat diets [21–23]. However, maternal intake of PHF can affect the metabolism of mammalian offspring [24,25].

The main objective of the present study was to investigate whether early and prolonged exposure to PHF could modify blood lipid profiles and mRNA expression of PPAR-$\gamma$ and some adipokines linked to insulin resistance and development of non-transmissible chronic disease. In addition, because the PHF diet is also poor in PUFAS, we compared the effects of these diets with the effects of diets rich in $\omega$-6 and $\omega$-3 PUFAs, soy oil (SO; control group, rich in $\omega$-6 PUFAs) and fish oil (FO; rich in $\omega$-3 long-chain [LC] PUFAs). Regarding the FO diet, there are multiple well-documented health benefits of this diet on glucose and lipid levels, with concomitant improvement in insulin signaling [9,18,19,20].

## Materials and methods

### Animals, diets, and general procedures

Adult virgin female Wistar rats weighing 180 to 220 g were obtained from the animal breeding unit of the Institute of Nutrition, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. Animals were kept in controlled light and dark (12 h each) and temperature (24 $\pm$ 1°C) conditions. After mating, pregnant rats were housed in individual cages and divided into three groups that were fed isoenergetic (4.1 kcal/g of dry diet) and normolipidic diets: the SO group received a diet containing 7% SO (rich in $\omega$-6 PUFAs), the PHF group received a diet containing 6% partially hydrogenated vegetable oil (rich in TFAs) plus 1% SO, and the FO group received 6% FO (rich in $\omega$-3 LC-PUFAs) plus 1% corn oil. The composition of each diet complied with the recommendations of the American Institute of Nutrition [26]. SO and corn oil were added to adjust each diet to the minimum requirement for essential fatty acids. Diets were prepared as pellets and stored at 4°C until use. After weaning (day 21 of life), the mothers were excluded from the study and six male pups per each dam were fed the same diets as their mothers (Table 1) up to day 120 of life. The fatty acid compositions of the diets are summarized in Table 2. The pups had free access to food and water during the entire period of the study. Food consumption was measured daily and body mass weekly. The pups were sacrificed by guillotine on day 120 of life after an overnight fast. Blood was collected in tubes containing 100 $\mu$L of Na$_2$-ethylenediaminetetra-acetic acid (1 mmol/L) and the plasma was separated by centrifugation. Plasma aliquots were frozen at $-70$°C. Samples of retroperitoneal white adipose tissue (RET) were quickly extracted and stored at $-70$°C for further determination of gene expression. All experimental protocols and procedures were approved by the university's experimental research committee.

Table 1
Composition of diets

| Constituents (g/100 g) | SO | PHF | FO |
|---|---|---|---|
| Casein (vitamin free) | 15.0 | 15.0 | 15.0 |
| Cornstarch | 67.94 | 67.94 | 67.94 |
| Soy oil* | 7.0 | 1.0 | — |
| Hydrogenated fat† | — | 6.0 | — |
| Fish oil‡ | — | — | 6.0 |
| Corn oil§ | — | — | 1.0 |
| Butyl hydroquinone | 0.0014 | 0.0014 | 0.0014 |
| Cellulose | 5.0 | 5.0 | 5.0 |
| Vitamin mix‖ | 1.0 | 1.0 | 1.0 |
| Mineral mix¶ | 3.5 | 3.5 | 3.5 |
| Choline bitartrate | 0.25 | 0.25 | 0.25 |
| L-cystine | 0.18 | 0.18 | 0.18 |

FO, 6.0% fish oil plus 1% corn oil (AIN-93); PHF, 6.0% partially hydrogenated vegetable oil plus 1% soy oil (AIN-93); SO, 7.0% soy oil (AIN-93)

* Oil from Lisa/Brazilian Industry.

† Partially hydrogenated fat was obtained from Gessy Lever, São Paulo, Brazil.

‡ Oil from Roche/Brazilian Industry.

§ Oil from Lisa/Brazilian Industry.

‖ Vitamin mix (g/kg of diet): thiamine HCl 0.6; riboflavin 0.6; pyridoxine HCl 0.7; niacin 3.0; calcium pantothenate 1.60; folic acid 0.20; biotin 0.02; vitamin B12 2.5; vitamin A palmitate 0.80 (500.00 IU/g); vitamin E acetate 15.0 (500 IU/g); vitamin D3 0.25 (400.00 IU/g); vitamin K1 0.75 (AIN-93 vitamin mix; DYETS 310025, Dyets Inc., Bethlehem, PA, USA).

¶ Mineral mix (g/kg): calcium 357.0; phosphorus 250.0; potassium 74.6; sodium 74.0; sulfur 300; magnesium 24.0; iron 5.21; copper 0.3; manganese 0.63; zinc 1.65; chromium 0.27; iodine 0.01; selenium 0.01; boron 0.08; molybdenum 0.01; silicon 1.45; nickel 0.03; lithium 0.02; vanadium 0.007 (AIN-93 mineral mix; DYETS 210025, Dyets Inc.).

*Fatty acid analysis*

Lipid extraction, saponification, and methylation of fatty acids in the experimental diets and RET were performed in duplicate using 0.5-g samples, according to the method of Lepage and Roy [27], which includes treatment with 2 mL of 4:1 (v/v) methanol:benzene solution and addition of 200 $\mu$L of acetyl chloride under light agitation. Fatty acid methyl esters were quantified by means of gas–liquid chromatography in a Perkin Elmer autosystem XL chromatograph with an ionizable flame detector and Turbochrom software (Perkin Elmer, Norwalk, CT, USA). Fatty acids were separated on a 100-m × 0.25-mm × 0.20-$\mu$m capillary column (SP2560, Supelco, Bellefonte, PA, USA). Hydrogen was used as the carrier gas. The injection and detection temperatures were 260°C and 280°C, respectively. The run temperature was programmed to start at 135°C for 5 min, followed by a 2°C/min increase up to 195°C, and then a 4°C/min increase up to 240°C, being kept at this level for 2.5 min. The carrier gas pressure was 32 psi. The split ratio was 1:70. Esters were identified by comparing their retention times with those of standard fatty acids. Results were expressed as percentages of total fatty acids.

*Plasma analysis*

Plasma metabolites were analyzed enzymatically with commercial kits: glucose (Roche, Spain), triacylglycerol, (Biolabo, Maizy, France), total cholesterol (Biolabo), high-density lipoprotein cholesterol (HDL-C; Biolabo), non-esterified fatty acids (Wako Chemicals GmbH, Neuss, Germany), and glycerol (Sigma, St. Louis, MO, USA). Serum insulin, adiponectin, and leptin levels were assessed with an enzyme-linked immunosorbent kit (Mercodia, Uppsala, Sweden).

*Real-time reverse transcriptase polymerase chain reaction*

Total RNA was extracted with Tri Reagent (Sigma-Aldrich Co., St. Louis, MO, USA), according to the manufacturer's instructions. Total RNA (2 $\mu$g) was reverse-transcribed using M-MLV RT enzyme (Promega, Madison, WI, USA). Real-time polymerase chain reaction amplification was performed with the FastStart DNA Master SYBR Green I kit (Roche Diagnostics GmbH, Mannheim, Germany) on a LightCycler and data analysis software version 4.05 (Roche Diagnostics GmbH). The amplification cycles used were 95°C for 10 s, 60°C (PPAR-$\gamma$, adiponectin, resistin, and cyclophilin B) or 64°C (leptin) or 68°C (TNF-$\alpha$ for 20 s and 72°C for 20 s). Melting curves were evaluated for each gene, and polymerase chain reaction products were separated on a 2% agarose gel and stained with ethidium bromide to confirm the presence of a single product. To obtain a calibration curve, serial dilutions of rat cDNA were used. The individual targets for each sample were quantified by determining the crossing points and by using a calibration curve. All analyses were performed in

Table 2
Fatty acid composition in experimental diets

| Fatty acids (%) | SO | PHF | FO |
|---|---|---|---|
| C14:0 | 1.34 | 0.07 | 1.44 |
| C16:0 | 14.45 | 10.24 | 6.90 |
| C18:0 | 3.71 | 12.15 | 3.07 |
| C18:1 $\omega$-9 *trans* | ND | 14.12 | ND |
| C18:1 $\omega$-9 *cis* | 19.03 | 19.92 | 9.51 |
| C18:1 other *cis*-isomers* | ND | 4.82 | 0.38 |
| C20:1 | 0.52 | 0.32 | 1.70 |
| C24:1 | ND | 0.02 | 0.60 |
| C18:2 $\omega$-6 *trans* | ND | 0.69 | ND |
| C18:2 $\omega$-6 (linoleic) | 55.87 | 20.75 | 10.85 |
| C18:3 $\omega$-3 ($\alpha$-linolenic) | 3.59 | 2.61 | 1.13 |
| C20:5 $\omega$-3 (eicosapentaenoic) | ND | ND | 35.04 |
| C22:6 $\omega$-3 (docosahexaenoic) | ND | ND | 17.24 |
| Total SFA | 19.50 | 22.46 | 11.41 |
| Total MUFA *cis* | 19.55 | 25.08 | 12.19 |
| Total PUFA *cis* | 59.46 | 23.36 | 64.26 |
| Total TFA | ND | 14.81 | ND |
| PUFA:SFA | 3.04 | 1.04 | 5.63 |

FO, fish oil; MUFA, monounsaturated fatty acid; ND, not determined; PHF, partially hydrogenated fat; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SO, soy oil; TFA, *trans*-fatty acid

* Includes all positional *cis*-isomers of 18:1 except 18:1 $\omega$-9.

duplicate, and the relative amount of the target was normalized with the housekeeping gene cyclophilin B. The primers used were 5′-AATCCTGCCCAGTCATGAAG-3′ (sense) and 5′-CATCTCCTGGGTCACCCTTA-3′ (antisense) for adiponectin, 5′-CACCAGGATCAATGACATTTCAC-3′ (sense) and 5′-CCTCTGTGGAGTAGAGCGAGG-3′ (antisense) for leptin, 5′-CTACATTGCTGGTCAGTCTCC-3′ (sense) and 5′-GCTGTCCAGTCTATGCTTCC-3′ (antisense) for resistin, 5′-CGTCAGCCGATTTGCCATTTC-3′ (sense) and 5′-TG-GGCTCATACCAGGGCTTGAG-3′ (antisense) for TNF-$\alpha$, 5′-CCCACCCAACTTCGGAATCA-3′ (sense), and 5′-TGC-GAGTGGTCTTCCATCAC-3′ (antisense) for PPAR-$\gamma$, 5′-TACAATGAGCTGCGTGTGG-3′ (sense) and 5′-TGGT-GGTGAAGCTGTAGCC-3′ (antisense) for cyclophilin B. Results are expressed as relative increases using the method of $2^{-\Delta\Delta Ct}$ described by Livak and Schmittgen [28].

### Statistical analysis

The results are expressed as means ± standard errors of the mean, and statistical comparison among groups was performed by one-way analysis of variance. Differences between means were tested for significance by Duncan's multiple-range test. Statistical significance was defined as $P < 0.05$.

## Results

The body masses of the male pups from the different groups did not differ during lactation (up to 3 wk of age; Fig. 1). However, from 5 until 13 wk of age, the body mass in the PHF group was higher than the other two groups, whose weight did not differ from each other. From 15 wk of age on, the rats in the FO group showed a lower body mass than those in the other groups. These differences in body mass did not correspond to parallel changes in food intake.
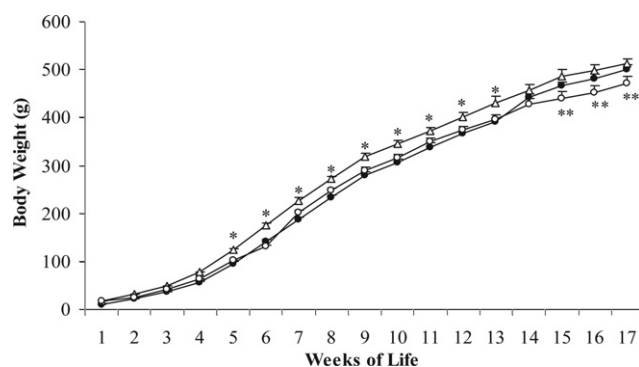


Fig. 1. Body weight (grams) of male pups during lactation and after weaning until 120 d of age (17 wk) in the SO group ($n = 6$, solid circles), PHF group ($n = 6$, triangles), and FO group ($n = 6$, open circles). Data are means ± SEMs, represented by vertical bars. *$P < 0.05$, PHF versus SO and FO; **$P < 0.05$, FO versus SO and PHF. FO, fish oil; PHF, partially hydrogenated vegetable fat; SO, soy oil.
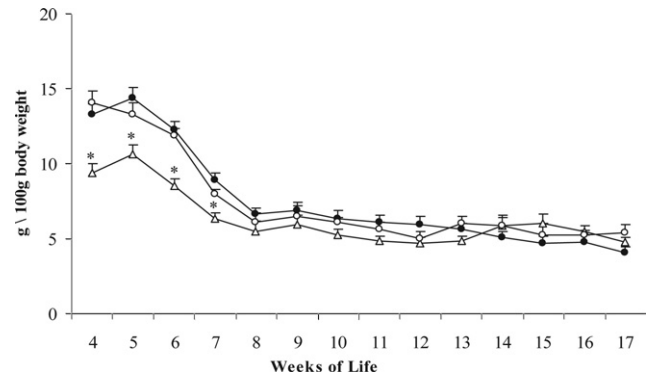


Fig. 2. Food intake (grams per 100 g of body mass) in rats fed the SO ($n = 6$, solid circles) PHF ($n = 6$, triangles), or FO ($n = 6$, open circles) diet after weaning until 120 d of age. Data are means ± SEMs, represented by vertical bars. *$P < 0.05$, PHF versus SO and FO. FO, fish oil; PHF, partially hydrogenated vegetable fat; SO, soy oil.

As shown in Figure 2, during the postweaning period, the daily food intake normalized by body mass was lower in rats in the PHF group than in the other groups up to 7 wk of age. From then on, the differences in food intake disappeared until the end of the experiment.

As presented in Table 3, the FO group presented the lowest weight of RET at the end of the experiment. The fatty acid composition of the RET varied with the type of dietary fat. The proportion of monounsaturated fatty acids was significantly higher in the PHF group, followed by the

Table 3
Weight and fatty acid composition of total lipids in retroperitoneal adipose tissue*

|  | SO | PHF | FO |
|---|---|---|---|
| Weight (g) | 5.3[a] ± 0.5 | 5.8[a] ± 0.4 | 3.4[b] ± 0.31 |
| Fatty acids (%) |  |  |  |
| C14:0 | 2.38[a] ± 0.31 | 2.84[a] ± 0.42 | 1.90[a] ± 0.55 |
| C16:0 | 36.41[a] ± 0.74 | 31.57[b] ± 0.53 | 34.33[a] ± 0.47 |
| C18:0 | 3.56[a] ± 0.29 | 7.31[b] ± 0.17 | 2.31[a] ± 0.29 |
| C18:1 $\omega$-9 *trans* | ND | 4.30 ± 0.27 | ND |
| C18:1 $\omega$-9 *cis* | 20.85[a] ± 0.53 | 39.30[c] ± 0.90 | 28.42[b] ± 0.79 |
| C18:1 other *cis*-isomers[†] | 2.49[a] ± 0.10 | 3.61[b] ± 0.30 | 4.94[c] ± 0.03 |
| C18:2 $\omega$-6 | 30.72[a] ± 1.00 | 9.58[b] ± 0.37 | 8.07[b] ± 0.50 |
| C18:3 $\omega$-3 | 0.12[a] ± 0.01 | 0.88[a] ± 0.04 | 3.39[b] ± 0.05 |
| C20:4 $\omega$-6 | 3.37[a] ± 0.03 | 0.59[c] ± 0.02 | 1.33[b] ± 0.03 |
| C20:5 $\omega$-3 | 0.02[a] ± 0.002 | 0.01[a] ± 0.004 | 6.19[b] ± 0.35 |
| C22:6 $\omega$-3 | 0.08[a] ± 0.009 | 0.01[a] ± 0.006 | 9.13[b] ± 0.43 |
| Total SFA | 42.35[a] ± 1.23 | 41.72[a] ± 2.02 | 38.54[a] ± 2.85 |
| Total MUFA *cis* | 23.34[a] ± 1.76 | 42.91[c] ± 1.98 | 33.36[b] ± 1.44 |
| Total PUFA *cis* | 34.31[a] ± 0.99 | 11.07[b] ± 0.41 | 28.11[a] ± 0.75 |
| Total TFA | ND | 4.30 | ND |

FO, fish oil; MUFA, monounsaturated fatty acid; ND, not determined; PHF, partially hydrogenated fat; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SO, soy oil; TFA, *trans*-fatty acid

* Results are expressed as mean ± SEM for six animals per group. Different superscript letters indicate significant differences from one another at $P < 0.05$ as determined by Duncan's test.

[†] Includes all positional *cis*-isomers of 18:1 except 18:1 $\omega$-9.

FO group. As would be expected, TFAs in the RET were found only in the PHF group, which received a diet rich in these isomers. Regarding the proportion of total PUFAs, the lowest value was found in the PHF group. The proportion of linoleic acid (C18:2 $\omega$-6) was much lower in the FO and PHF groups than in the SO group. The proportion of linolenic acid (C18:3 $\omega$-3) was higher in the FO group than in the other groups, whereas the lowest proportion of arachidonic acid (C20:4 $\omega$-6) was found in the PHF group. Docosahexaenoic acid (C22:6 $\omega$-3) and eicosapentaenoic acid (20:5 $\omega$-3) levels were higher in the FO group than in the other groups.

Table 4 shows that plasma triacylglycerol levels, total cholesterol/HDL-C ratio, insulin level, and insulin/glucose ratio were higher in the PHF group than in the other groups. The lowest level of plasma adiponectin was found in the PHF group. Plasma levels of triacylglycerols, total cholesterol, HDL-C, non-esterified fatty acids, glycerol, glucose, and leptin were significantly lower in the FO group compared with the other groups.

The expression (mRNA) of different adipokines and PPAR-$\gamma$ in the RET are shown in Figures 3 and 4. The PHF diet significantly increased the mRNA levels of resistin and TNF-$\alpha$ and decreased the mRNA levels of adiponectin and PPAR-$\gamma$. When compared with the SO or PHF group, the FO group showed a significant decrease in RET leptin expression and an increase in PPAR-$\gamma$ expression.

## Discussion

In the present study, food intake, body mass, levels of lipid metabolites, insulin, glucose, adiponectin, and leptin in

### Table 4
Biochemical parameters in plasma*

| Biochemical parameters | SO | PHF | FO |
|---|---|---|---|
| Triacylglycerol (mg/dL) | 56.47[a] ± 3.95 | 83.82[b] ± 6.62 | 24.90[c] ± 3.02 |
| TC (mg/dL) | 59.25[a] ± 4.66 | 64.30[b] ± 4.01 | 34.19[b] ± 2.98 |
| HDL-C (mg/dL) | 32.24[a] ± 1.91 | 30.10[a] ± 1.37 | 20.06[b] ± 1.57 |
| TC/HDL-C | 1.84[a] ± 0.11 | 2.13[b] ± 0.19 | 1.70[a] ± 0.14 |
| NEFA (mmol/L) | 0.68[a] ± 0.05 | 0.54[a] ± 0.03 | 0.29[b] ± 0.07 |
| Glycerol ($\mu$M) | 336.9[a] ± 10.12 | 373.4[a] ± 13.16 | 192.0[b] ± 8.13 |
| Glucose (mg/dL) | 98.0[a] ± 9.51 | 145.40[b] ± 3.40 | 75.69[c] ± 5.35 |
| Insulin ($\mu$g/L) | 0.56[b] ± 0.18 | 1.36[a] ± 0.27 | 0.50[b] ± 0.13 |
| Insulin/glucose | 0.005[a] ± 0.001 | 0.009[b] ± 0.0002 | 0.006[a] ± 0.001 |
| Adiponectin ($\mu$g/mL) | 14.03[a] ± 1.01 | 9.20[b] ± 0.80 | 12.95[a] ± 1.20 |
| Leptin (ng/mL) | 41.81[a] ± 0.19 | 40.70[a] ± 0.22 | 13.37[c] ± 0.38 |

FO, fish oil; HDL-C, high-density lipoprotein cholesterol; NEFA, non-esterified fatty acid; PHF, partially hydrogenated fat; SO, soy oil; TC, total cholesterol

* Results are expressed as mean ± SEM for six animals per group. Different superscript letters indicate significant differences from one another at $P < 0.05$ as determined by Duncan's test.
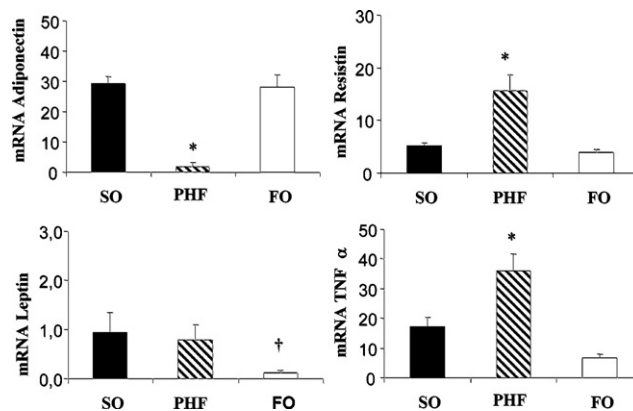


Fig. 3. Resistin, TNF-$\alpha$, adiponectin, and leptin mRNA expressions in retroperitoneal adipose tissue of rats fed the SO, PHF, or FO diet for 120 d. Results of real-time polymerase chain reaction are expressed as units relative to cyclophilin B mRNA concentrations. Cyclophilin B mRNA levels were measured as an internal standard and used as a reference gene. Results are expressed as means ± SEMs for six animals per group. *PHF versus SO and FO groups, $P < 0.05$; †FO versus SO and PHF groups, $P < 0.05$. FO, fish oil; PHF, partially hydrogenated vegetable fat; SO, soy oil; TNF-$\alpha$, tumor necrosis factor-$\alpha$.

the plasma, fatty acid profile in adipose tissue, and expression levels of adipokines linked to glucose homeostasis and PPAR-$\gamma$ in adipose tissue were evaluated in adult male rats (120 d of age) that had ingested, since the day of weaning, as did their respective mothers during pregnancy and lactation, a control diet containing SO (SO group), FO (rich in $\omega$-3 fatty acids; FO group), or PHF (rich in TFAs; PHF group). Our major focus in the present study was to investigate whether the quality, rather than quantity, of fatty acids consumed from early in life (perinatal period) could affect the gene expression of adipose tissue and its subsequent metabolism contributing to the metabolic alterations present in non-transmissible chronic disease later in life.

The FO group at the end of the experiment (17 wk) presented a lower body mass than the other groups, whereas
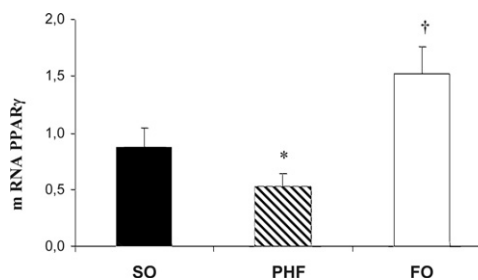


Fig. 4. PPAR-$\gamma$ mRNA expression in retroperitoneal adipose tissue of rats fed the SO, PHF, or FO diet for 120 d. Results of real-time polymerase chain reaction are expressed as units relative to cyclophilin B mRNA concentrations. Cyclophilin B mRNA levels were measured as an internal standard and used as a reference gene. Results are expressed as means ± SEMs for six animals per group. *PHF versus SO and FO groups, $P < 0.05$; †FO versus SO and PHF groups, $P < 0.05$. FO, fish oil; PHF, partially hydrogenated vegetable fat; PPAR-$\gamma$, peroxisome proliferator-activated receptor-$\gamma$; SO, soy oil.

the PHF group presented the highest body mass from 5 until 13 wk of age. From 14 to 17 wk the PHF and SO groups presented no significant difference in body mass. These results coincide with a lower amount of the RET in the FO compared with the other groups at the end of the experiment. Our previous studies indicated that normolipidic diets, rich in palmitic (palm oil) saturated fatty acid or rich in *trans*-isomers (PHF) increase the lipogenesis rate on epididymal adipose tissue in rats at a young age (45 d or 7 wk of age), leading to increased fat content in this tissue and in the carcass and to increased body mass [24]. Leyton et al. [29] demonstrated that saturated fat is oxidized to a significantly lesser degree than unsaturated fat. Subsequent studies in lean and obese rats have supported the notion that obesity is associated with greater trafficking of dietary fat for storage in the adipose tissue, whereas thinness is associated with a higher oxidation of dietary fat [30]. It is therefore reasonable to suggest that normolipidic diets containing PHF, rich in TFAs and saturated fatty acids and poor in PUFAs, provided to the rats since their perinatal period can favor fat retention in young animals and during longer periods of treatment due to increased adipose tissue lipid storage.

Studies in humans have shown that individuals consuming TFA-rich diets have a greater percentage of these fatty acids deposited in adipose tissue [31]. In the present study, the adipose tissue in the PHF group was found to incorporate TFAs, with a reduction in the percentage of PUFAs, whereas the FO and SO groups showed increases in $\omega$-3 and $\omega$-6 PUFAs, respectively. These differences agree with the proportion of these fatty acids in the diets and could also be contributing to the modulation of gene expression of adipose tissue that is linked to glucose and lipid homeostasis. In fact, the PHF group showed an increase of TNF-$\alpha$ and resistin expressions, associated with lower expressions of PPAR-$\gamma$ and adiponectin, and lower levels of PUFAs in the RET compared with the SO and FO groups.

Our data also provide evidence that ingestion of normolipidic diets containing PHF caused in the offspring an increase in the levels of glucose, insulin, triacylglycerol, cholesterol/HDL-C ratio, and hypoadiponectinemia, whereas the FO diet, rich in $\omega$-3 LC-PUFA, resulted in lower plasma concentrations of glucose, insulin, triacylglycerol, free fatty acids, cholesterol, and leptin in association with normo adiponectinemia.

Enhanced expression in the TNF-$\alpha$ and resistin genes have been associated with a lowered sensitivity to insulin [32–34], agreeing with the higher plasma glucose and insulin levels and insulin/glucose ratio found in the PHF group in the present study.

Hypoadiponectinemia, which decreases muscle and hepatic adenosine monophosphate–activated protein kinase activity, fatty acid combustion, and glucose transport, is also associated with insulin resistance [2,35,36]. Thus, the decrease in the adiponectin gene expression in adipocytes associated with lower circulating adiponectin levels could

have contributed to the higher plasma glucose and insulin levels and insulin/glucose ratio levels in the PHF group when compared with the other groups. Adiponectin also suppresses the production of proinflammatory cytokines, such as TNF-$\alpha$ [8]. Pisani et al. [25] found reduced adiponectin mRNA and increased TNF-$\alpha$ mRNA of RET in 21-d-old offspring rats fed a diet containing hydrogenated vegetable fat during gestation and lactation. These results suggested that early exposure to hydrogenated vegetable fat caused an alteration in adipose tissue adiponectin and TNF-$\alpha$ gene expressions and that this alteration remained until 120 d of age in the offspring, likely raising their predisposition to metabolic diseases such as diabetes mellitus and cardiovascular disease in later life.

Although reduced hepatic triacylglycerol synthesis and increased $\beta$-oxidation are known to contribute to the hypolipidemic effect of FO [37], the possible involvement of white adipose tissue in mediating the hypolipidemic effects of FO remains a little studied subject. Recent evidence has suggested that FO exerts its beneficial effects via adipose tissue by increasing the secretion of adiponectin by way of a PPAR-$\gamma$ mechanism [38,39]. Our present data with the animals fed a diet containing FO support this observation. Thus, $\omega$-3 PUFA may mediate its hypolipidemic effects by promoting appropriate adipokine secretion by PPAR-$\gamma$. It is also thought that adiponectin secreted from adipose tissue may provide a direct link between adipose tissue function and protection against cardiovascular disease [40].

Leptin expression and protein levels in circulation are increased during the development of obesity [41]. Thus, plasma leptin levels that reflect the sum of all peripheral leptin production correlates with body fat mass and adipocyte cell size in lean and obese mice [42]. In the present study, the leptin mRNA level of the FO group was the only one showing a reduction when compared with the SO or PHF group. This change coincides with a lower plasma leptin concentration accompanied by a smaller amount of RET in this specific group. Similarly, other studies [43] have found that FO feeding for 3 mo may reduce leptin mRNA. Thus, it is possible to suggest that the FO diet, by decreasing adipose tissue mass, appears also to improve the metabolic outcomes, which is an important determinant of circulating leptin levels in diet-induced obesity.

Peroxisome proliferator-activated receptor-$\gamma$ is mainly involved in glucose metabolism, but also exerts a potential beneficial effect on lipid metabolism. Some genes related to lipid metabolism that are activated by PPAR-$\gamma$ are lipoprotein lipase, fatty acid-binding protein, and liver X receptor-$\alpha$ [44]. The hydrolysis of triacylglycerols from circulating very low-density lipoproteins and chylomicrons is catalyzed by lipoprotein lipase [2]. We found that, whereas the FO diet produces the highest level of PPAR-$\gamma$ expression, the PHF diet produces the lowest. Thus, although more direct experiments are required, a possibility exists that the hypotriglyceridemic effect of FO versus the hypertriglyceridemic

effect of the PHF can be partly explained by PPAR-$\gamma$, via lipoprotein lipase.

There are several well-documented studies showing that $\omega$-3 LC-PUFAs are activators of PPAR-$\gamma$ in adipose tissue, suggesting that the expression of this receptor results in the remodeling of adipose tissue in adult animals, with apoptosis of hypertrophic adipocytes and an increase in the number of small new adipocytes. These favor insulin sensitivity, activating the expression of several genes of hormones, cytokines, and enzymes in the tissue [45,46]. Moreover, when the expression of PPAR-$\gamma$ is enhanced, the activation of nuclear transcription factor-$\kappa$B is inhibited, thus decreasing its proinflammatory transcription effects [47]. These interactions may explain why the reduced PPAR-$\gamma$ expression in adipose tissue found in the rats fed TFAs is associated with insulin resistance, as observed in the present study, which agrees with data from Saravanan et al. [22]. These investigators also suggested that increasing the level of PUFAs to an amount that can withstand the competition from TFAs for fatty acid desaturase activities would be beneficial in preventing the effects of TFAs on adipose tissue metabolism.

To approach the potential relation between the expression of PPAR-$\gamma$ and the different adipokines studied, Pearson's correlation coefficients ($r$) between PPAR-$\gamma$ and adipokine mRNA expression by RET were estimated in the PHF group. A negative correlation appeared between the mRNA levels of PPAR-$\gamma$ and those of TNF-$\alpha$ ($r = -0.89$, $P = 0.044$) and a positive correlation between PPAR-$\gamma$ and adiponectin ($r = 0.86$, $P = 0.031$). These findings agree with previous studies showing that the modulation of adipokines, such as TNF-$\alpha$, leptin, and adiponectin, by PPAR-$\gamma$ is dependent on agonists, such as PUFAs from the diet, possibly by modulating the activity of PPAR-$\gamma$ to improve insulin sensitivity [48,49].

In summary, our study demonstrates that the intake of PHF, rich in TFAs and saturated fatty acids and poor in PUFAs, has a negative effect on the expression of adiponectin and PPAR-$\gamma$, while enhancing the expression of resistin and TNF-$\alpha$ and causing changes in biochemical parameters in plasma. This offers a possible explanation for the mechanism by which the consumption of hydrogenated fat promotes a proinflammatory state, reduces insulin sensitivity, and increases cardiovascular disease risk. When we compare these parameters for the PHF diet with diets rich in PUFAs (SO, rich in $\omega$-6 PUFA, and FO, rich in $\omega$-3 LC-PUFA), we observe improved metabolic outcomes, mainly with diets containing $\omega$-3 LC-PUFA (FO group). In the diet rich in FO, a decrease in leptin expression and an increase in the expression of PPAR-$\gamma$ in adipose tissue, in addition to hypolipidemic effects, were observed, justifying the well-documented preventive effects of $\omega$-3 LC-PUFAs in insulin resistance and cardiovascular risk. The present data indicate that early and prolonged exposure to hydrogenated fat results in important changes in gene expression in adipose tissue, which is probably an effect of the smaller proportions of PUFAs in this fat. However, the effects of TFA and saturated fatty acid cannot be ruled out.

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