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Effect of a Diet Rich in Interesterified, Non-Interesterified and Trans Fats on Biochemical Parameters and Oxidative Status of Balb-c Mice

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Abstract

The aim of this study was to investigate the effects of different modified fats on the body weight, biochemical profile, and biomarkers of hepatic oxidative status in Balb-*c* mice. The animals were divided into four groups and fed for 75 days with a normolipidic (Control Group, CG) or hiperlipidic diets (40% kcal) containing a commercial interesterified fat (IFG) rich in palmitic acid (39%); a blend of non-interesterified fat (NIFG), with 2-fold less saturated fatty acids at the *sn*-2 position of triacylglycerols; or a partially hydrogenated vegetable oil (PHFG), source of trans fatty acid (20%) and of linolenic acid (6%). The mice of the IFG and NIFG presented similar results in all evaluated parameters. The serum biochemical profile and hepatic oxidative stress markers in mice of the PHFG were similar to CG, except for total cholesterol (TC) which was significantly higher ($p < 0.05$) for the mice of the PHFG. The mice feed with interesterified fat (IFG) showed serum TC ($p <$

0.01), non-HDL-C ($p < 0.05$), glucose ($p < 0.05$) and hepatic reduced glutathione values (2.7 fold, $p < 0.05$) and glutathione reductase activity (2.4 fold, $p < 0.001$) significantly higher when compared to the mice fed with partially hydrogenated vegetable oil (PHFG). The hydrogenated fat source of trans fatty acid (20%) had less important metabolic effects than fats containing amounts of palmitic acid (interesterified or non-interesterified). Our results suggest that the replacement of hydrogenated fats by interesterified fats may not be such a simple solution to reduce or eliminate *trans* fatty acids in foods.

Keywords

Interesterified Fat, *Trans* Fat, Palmitic Acid, Cholesterol, Oxidative Stress

1. Introduction

Modified fats are used in the food industry for improving oxidative stability and achieving adequate sensory characteristics in the final product. The plasticity of fats and oils is changed by partial hydrogenation and interesterification, making them suitable for food applications [1]. In the last 100 years, most of the processed food such as crisps, biscuits, confectionery, cereal bars, snacks, ice creams, and bread, among others, have been formulated with partially hydrogenated fat. The partial hydrogenation process of oils alters the configuration of unsaturated fatty acids from *cis* to *trans*. The consumption of *trans* fatty acids (TFAs) has been associated with the increase of the risk of diseases such as cardiovascular disease [2] [3], type 2 diabetes mellitus [4] [5] and cancer [6] [7]. For this reason, the intake of TFAs has been discouraged in the last years, and several countries have adopted actions to reduce or eliminate *trans* fatty acids in processed foods. Recently, the program "Replace", an initiative of the World Health Organization (WHO) to make the world free of *trans* fat until 2023, has been announced [8] [9].

The interesterification has been primarily used to replace the hydrogenation by the food industry as an economically viable alternative. The process allows the production of zero-*trans* fats with desirable functional properties. The triacylglycerol composition of blends of fats and oil is modified by rearranging the fatty acids within and between triglyceride molecules, using chemical or enzymatic catalysis [1] [10].

The interesterified fats commonly used in the food industry contain fats that are rich in long-chain SFA, such as palmitic acid (16:0) and stearic acid (18:0) [10] [11]. A proper melt profile and solid fat content can be achieved using palm oil, which presents 50% of saturated fat in its composition [12]. In addition, unlike unmodified vegetable oils, interesterified fats have a larger amount of saturated fatty acids (SFA) at the sn-2 position of glycerol. The effect of the specific arrangement of the fatty acids on the glycerol backbone on health is not understood. There is a possibility that positional composition may affect dietary fat

metabolism, digestibility and consequent effects on cardiovascular health [10] [11].

The role of interesterified fat on metabolism has been reported in previous studies [1] [10]. In a recent systematic review, the interesterification of palmitic or stearic acid-rich fats does not seem to affect fasting serum lipids and (apo) lipoproteins [13]. Other research suggests effects of the consumption of interesterified fat on lipoprotein metabolism, glycemic control, and serum liver enzymes [1] [14]. The nutritional and health implications of the long-term consumption of interesterified fat need to be better understood [1] [10]. In addition, most studies used fats that are not commercially relevant [10] [11]. For instance, interesterified oils containing groundnut oil with linseed oil [15], and palm oil with rice bran oil [16]. Therefore, there is need for studies that investigate the nutritional and health effects of long-term intake of commercially available interesterified fats.

The objective of this study was to investigate the effects of supplementation of 1) a commercial interesterified fat rich in SFA, mainly located at the sn-2 position of the TAG backbone; 2) a blend with the same FA composition as the Interesfied Fat (IF), but with a lower amount of SFA at position sn-2 and; 3) a partially hydrogenated vegetable oil source of TFA, on the nutritional and biochemical profile and hepatic oxidative stress markers in Balb-c mice.

2. Materials and Methods

2.1. Fats

Commercial interesterified (IF) and non-interesterified fats (NIF) were obtained from the Indústria Triângulo Alimentos Ltda (São Paulo, Brazil). The IF was produced by chemical interesterification for the bakery application. The IF and NIF presented the same fatty acid profile since they were composed of the same oils and fats (palm oil, palm stearin, and fully hydrogenated soybean oil). Partially hydrogenated fat (PHF) was purchased in a local supermarket, and it was formulated with a mixture of soybean and palm oil.

2.2. Fatty Acid Profile of Fats

The Fatty Acids Methyl Esters (FAMEs) were obtained using the methodology described by O'Fallon *et al.* [17]. Analyses were carried out by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) using a capillary column RTX® 2330 (105 m × 0.25 mm ID; 0.20 μm film thickness) containing 90% biscyanopropyl and 10% phenylcyanopropyl polysiloxane (Restek®, Bellefont, USA). The column flow was 1.0 mL·min^{-1} with injected volume (1.0 μL) using a split ratio of 1:40. Synthetic air was the carrier gas, and nitrogen was the makeup gas. The temperature of the column injector was 250˚C. The column heating ramp started at 130˚C and was kept for 5 min, and then increased to 180˚C, with an increased rate of 5˚C min^{-1}. Afterward, the column was held at 180˚C during 10 min. Then, after increasing temperature from 180˚C to 240˚C (increase rate of

3˚C·min^{-1}), the column temperature was held at 240˚C for 13 min. The flame ionization detector (FID) temperature was kept at 260˚C. The FAMEs were identified and quantified as percentages (%) based on their retention times and peak areas compared to the fatty acid's standards.

2.3. Regiospecific Distribution of Fatty Acids in Triacylglycerols

The regiospecific distribution of fatty acids was carried out according to Vlahov [18] using a Bruker Advance DPX 300 spectrometer (São Paulo, Brazil), performed with multinuclear probe (5 mm) operating at 30˚C at a constant frequency (75.8 MHz).

2.4. Triacylglycerol Composition of Fat

The triacylglycerol composition of the fats was determined according to Segura *et al.* [19] using an HPLC Shimadzu Prominence 20A (Kyoto, Japan) coupled with an evaporative light scattering detector (ELSD-LTII Shimadzu) with two serial columns (Supelcosil™ C18; 25 cm × 4.6 mm × 5 μm) operating at 20˚C. Each sample (5 mg) was firstly dissolved in acetone (1 mL) and then injected (1 μL) using acetone/acetonitrile (1:1; v/v) as mobile phase with a constant flow rate (1 mL·min^{-1}). Additionally, a chloroform gradient (0% to 20%) was applied for 60 min. Then, this condition was kept for 20 min. The order of elution of triacylglycerols standards and its corresponding equivalent carbon number (ECN) was used to identify each triacylglycerols peak in the sample.

2.5. *In Vivo* Study

2.5.1. Animals and Experimental Design

Twenty four female Balb-c mice (60 days old; 20.0 ± 1.0 g body weight) were household under controlled environmental conditions (photoperiod 12 h; temperature 21˚C ± 1˚C and air humidity 53% ± 2%). Mice were kept in plastic cages (maximum 6 animals/cage) receiving water and commercial or formulated pelleted chow *ad libidum.* Animal maintenance and treatments followed the protocol approved by the Ethics Committee for Animals Use (CEUA/UFSC PPOO784) and in agreement with the National Institutes of Health (NIH) Guidelines.

Animals were randomly assigned to one of four experimental groups (n = 6 for each group): control group (CG); interesterified fat group (IFG); non-interesterified fat group (NIFG), and partially hydrogenated fat group (PHFG). The CG received commercial chow Bio Tec Rats and Mice, manufactured by Bio Base® Company (Águas Frias, Brazil), composed of ground corn, soy bran, wheat bran, degummed soy oil, mixture of vitamins, mixture of mineral salts, adsorbent additive, antifungal additive and antioxidant additive. The other groups received the same feed enriched with the specific fats (interesterified fat, non-interesterified fat, or partially hydrogenated fat). The hyperlipids and isocaloric formulations were prepared by adding proper amounts of specific fats in the standard chow. Table 1 presents the ingredients, composition (Kcal%) and energy value of the

Table 1. Ingredients, composition and energy value (Kcal %) of the experimental diets: Control Group (CG); Interesterified Fat Group (IFG); Non-interesterified Fat Group (NIFG), and Partially Hydrogenated Fat Group (PHFG).

Ingredients (g/kg of diet)	**Normolipid diet**	**Hyperlipids diets**		
	CG	**IFG**	**NIFG**	**PHFG**
Commercial chow Bio Tec Rats and Mice[1]	1000	833	833	833
Interesterified fat[2]	0	167	0	0
Non-interesterified fat[3]	0	0	167	0
Partially hydrogenated vegetable fat[4]	0	0	0	167
Composition (Kcal %)/ **Energy value** (kJ/g and kcal/g)				
Lipids	10	40	40	40
Proteins	24	15	15	15
Carbohydrates	66	45	45	45
Energy	15.06 kJ (3.6 kcal)	22.6 kJ (5.4 kcal)	22.6 kJ (5.4 kcal)	22.6 kJ (5.4 kcal)

[1]Manufactured by Bio Base® Company (Águas Frias, Brazil), composed of ground corn, soy bean, wheat bran, degummed soy oil, mixture of vitamins, mixture of mineral salts, adsorbent additive, antifungal additive and antioxidant additive. [2]Commercial interesterified fat obtained from the Indústria Triângulo Alimentos Ltda (São Paulo, Brazil), produced by chemical interesterification, for the bakery application, and composed of palm oil, palm stearin, and fully hydrogenated soybean oil. [3]Blend composed of palm oil, palm stearin, and fully hydrogenated soybean oil, obtained from the Indústria Triângulo Alimentos Ltda (São Paulo, Brazil). [4]Partially hydrogenated vegetable fat formulated with a mixture of soybean and palm oil. Purchased in a local supermarket.

experimental diets. The formulations were pelletized according to a previous report [20], and the pellets were frozen at −18˚C. Small amounts of the chows were periodically thawed before feeding the mice during 75 days. Mice were weighed weekly, and food intake was estimated as the difference between the food offered and the residual food in the cages. The nutrition efficiency was expressed as a result of the rate between weight gain (g) and energy consumption (Kcal).

2.5.2. Serum Biochemical Profile

After 75 days of treatment, all mice received anesthesia, and blood samples were collected through a cardiac puncture after 8-h fasting. Recovered serum (3000 rpm/10 min) was used for quantifying the lipids, lipoproteins, glucose, and transaminases. Triglycerides and total cholesterol were measured using the Tinder's reaction [21] [22]. HDL-C was determined by the homogeneous direct method [23] with a commercial colorimetric kit (LABTEST®, Lagoa Santa City, Brazil). The non-HDL-C levels were estimated by the difference between total and HDL-C. Serum glucose was measured with a colorimetric, enzymatic method [24] (LABTEST®). All measurements were performed using multiwell plate readings with TECAN Infinity M200®. The activities of serum transaminases enzymes, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured by kinetic method at 340 nm, using commercial kits LABTEST® [25].

2.5.3. Hepatic Status Oxidative

At the end of the treatment, the animals were euthanized by cervical dislocation. Livers were rapidly removed, kept on ice, and perfused with ice saline solution (0.9% NaCl) for 5 min. A portion of 100 mg of liver was removed and immediately homogenized (1:9 w/v) in 0.1% Triton X-100, 0.12 M NaCl, 30 mM Na-phosphate buffer, pH 7.4. For the measurement of GSH content, TCA 12% was used. Homogenization was carried out at 4˚C in a Teflon piston homogenizer Tecnal® (Campinas, Brazil), followed by centrifugation at 10,000 g for 10 min. The supernatant was used for the measurement of antioxidant defense (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase activities, and reduced glutathione, GSH content) and also oxidative stress markers lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) and protein carbonyl content.

Superoxide dismutase (SOD) activity was measured by monitoring the oxidation of adrenaline to adrenochrome, as described by Misra and Fridovich [26]. Catalase (CAT) activity was measured indirectly through the decomposition of hydrogen peroxide monitored at 240 nm, according to Aebi [27]. Glutathione peroxidase (GPx) activity was measured at 340 nm, through the glutathione/NADPH/glutathione reductase system, by the reduction of *terc*-butylhydroperoxide, as described by Flohé and Günzler [28]. Glutathione reductase (GR) activity was determined through the NADPH oxidation rate measured at 340 nm, according to Carberg and Mannervik [29]. Reduced glutathione (GSH) content was directly proportional to the reaction between dithiobisnitrobenzoic acid (DTNB) and GSH thiol groups in the liver samples. The yellowish thiolate formed was determined spectrophotometrically at 412 nm, as described by Beutler *et al.* [30]. Enzyme activities and reduced GSH content results were normalized by the protein content using the Lowry method [31].

Evaluation of oxidative damage to lipids was determined through measurements of TBARS, according to Bird and Draper [32]. The oxidative damages related to protein carbonylation levels were determined spectrophotometrically at 340 nm, as described by Levine *et al.* [33]. All measurements were done using multiwell plate reader (TECAN Infinity M200®).

2.6. Statistical Analysis

All assays were done in triplicate, and results were expressed as mean ± standard deviation. Data were analyzed by the one-way ANOVA test, followed by the Bonferroni test. Comparisons were made using GraphPad Prism software version 6.0 (San Diego, USA). Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Fatty acid Composition, Regiospecific Distribution of Fats, and Triacylglycerol Composition of Fat

Table 2 shows the fatty acid composition and the regiospecific distribution of

Table 2. Fatty acid composition of fats (g/100) and regiospecific distribution of fatty acids in sn-1,3 and sn-2 positions in triacylglycerols of Interesterified Fat (IF), Non-interesterified Fat (NIF) and Partially Hydrogenated Fat (PHF).

Fatty Acids	IF	NIF	PHF
Lauric acid (C 12:0)	2.05 ± 0.21[a]	1.95 ± 0.21[a]	0.20 ± 0.00[b]
Myristic acid (C 14:0)	1.45 ± 0.07[a]	1.25 ± 0.07[a]	0.20 ± 0.00[b]
Palmitic acid (C 16:0)	39.10 ± 0.42[a]	38.90 ± 0.14[a]	11.65 ± 0.21[b]
Stearic acid (C 18:0)	15.10 ± 0.14[a]	15.25 ± 0.35[a]	13.25 ± 0.49[b]
Oleic acid (C18:1 n9c)	33.2 ± 0.71[a]	33.55 ± 0.64[a]	40.70 ± 0.14[b]
Linoleic acid (C 18:2 n6c)	8.15 ± 0.21[a]	8.00 ± 0.00[a]	8.05 ± 0.07[a]
Linolenic acid (C 18:3 n3c)	0.20 ± 0.28[a]	0.20 ± 0.28[a]	5.60 ± 0.85[b]
Elaidic acid (C18:1 n9t)	0.10 ± 0.14[a]	0.10 ± 0.14[a]	17.00 ± 0.57[b]
Linolelaidic acid (C 18:2 n6t)	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.89 ± 0.69[b]
Others	0.65± 0.78[a]	0.80± 0.00[a]	0.46± 0.08[a]
Total SFA	57.70 ± 0.0[a]	57.35 ± 0.49[a]	25.30 ± 0.28[b]
Total MUFA	33.20 ± 0.71[a]	33.55 ± 0.64[a]	40.70 ± 0.14[b]
Total PUFA	8.35 ± 0.07[a]	8.20 ± 0.28[a]	13.65 ± 0.92[b]
Total TFA	0.10 ± 0.14[a]	0.10 ± 0.14[a]	19.89 ± 1.26[b]
Fatty acids position	**IF**	**NIF**	**PHF**
SFA (sn-1.3)	59.90	72.60	41.20
SFA (sn-2)	61.20	28.10	0.00
MUFA (sn-1.3)	31.20	20.40	52.00
MUFA (sn-2)	21.30	50.60	49.10
PUFA (sn-1.3)	8.90	7.00	6.80
PUFA (sn-2)	17.50	21.30	50.90

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids TFA: trans fatty acids. Mean ± S.D. (n = 2). Different letters at the same row mean statistical difference between experimental groups ($p < 0.05$).

fatty acids in the *sn*-1,3 and *sn*-2 positions in triacylglycerols of IF, NIF, and PHF. The IF and NIF fats presented the same fatty acid profile, which was rich in SFA (especially palmitic acid) and TFA free. IF presented a higher amount of SFA at the *sn*-2 position of triacylglycerol (2-fold) when compared to NIF. On the other hand, PHF presented a higher concentration of oleic fatty acid and 20% of TFA (mainly elaidic acid), 6% of linolenic acid, and absence of SFA at *sn*-2 position. Table 3 shows the effect of the interesterification on the triacylglycerols composition of IF and NIF fats. IF presented a lower amount of trisaturated (especially SSS) and disaturated triacylglycerols (especially PPO) and a higher amount (4-fold) of tri-unsaturated triacylglycerols (especially OOO), when compared to NIF.

3.2. Food Intake, the Gain of Body Weight and Energy Consumption

A significant decrease in food consumption ($p < 0.001$; Figure 1(A)) was observed

Table 3. Triacylglycerols (TAGs) composition of the interesterified fat (IF) and non-interesterified fat (NIF).

TAGs Structure	ECN	IF (%)	NIF (%)
PPP	48	4.72	4.30
PSS	52	1.64	2.90
SSS	54	0.00	15.20
Trisaturated		**6.36**	**22.40**
PPL	46	8.01	6.10
PPO	48	5.73	31.00
POS	50	13.70	1.60
SOS	52	1.60	0.00
Disaturated		**29.04**	**38.7**
PLL	44	1.58	0.00
PLO	46	1.51	0.00
POO	48	19.19	22.80
SOO	50	7.35	4.20
Monosaturated		**29.63**	**27.00**
LLO	44	2.21	0.00
LOO	46	9.73	6.70
OOO	48	15.32	0.00
Triunsaturated		**27.26**	**6.70**
Others		7.71	5.20

ECN = equivalent carbon number; O: oleic acid; L: linoleic acid; P: palmitic acid; S: stearic acid.

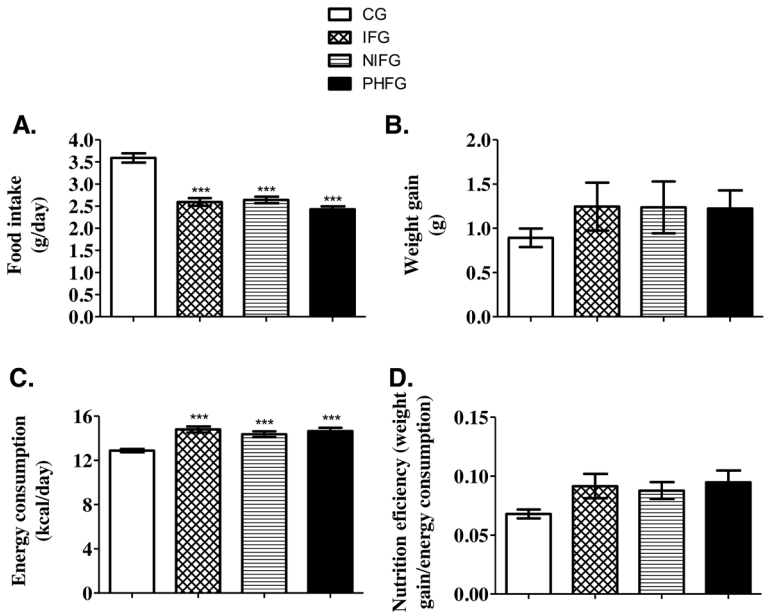

Figure 1. Effect of the consumption of interesterified fat (IFG), non-interesterified fat (NIFG), and partially hydrogenated vegetable fat (PHFG) in female Balb-c mice after 75 days. (A) Food intake (g/day); (B) Weight gain (g); (C) Energy consumption (kcal/day); and D. Nutrition efficiency (final weight − initial weight)/total energy consumption). Values are expressed as Mean ± S.D. (n = 6). ***denote statistical difference ($p < 0.001$) compared to the control group (CG), fed with a normolipidic diet.

for the animals fed with the hyperlipid diets (IFG, NIFG, and PHFG) when compared to the control group (CG). The energy consumption was significantly higher for animals from the IFG, NIFG and PHFG ($p < 0.001$; Figure 1(C)). On the other hand, it was not observed a significant difference in the body weight gain between all experimental groups (Figure 1(B)). Figure 2 shows the weight gain for the animals of the experimental groups during 75 days. The nutrition efficiency (Figure 1(D)) also did not present differences between the groups. However, the animals of the hyperlipidic groups (IFG, NIFG and PHFG) presented fat deposition in the abdomen (Figure 3).

3.3. Serum Biochemical Profile

The serum triglycerides were not affected by the fat present in the diet of the animals (Figure 4(A)). On the other hand, the TC (Figure 4(B)) increased in the Balb-c mice fed with the hyperlipidic diets, IFG and NIFG ($p < 0.001$), and PHFG ($p < 0.05$) when compared to CG. The increase for IFG and NIFG was

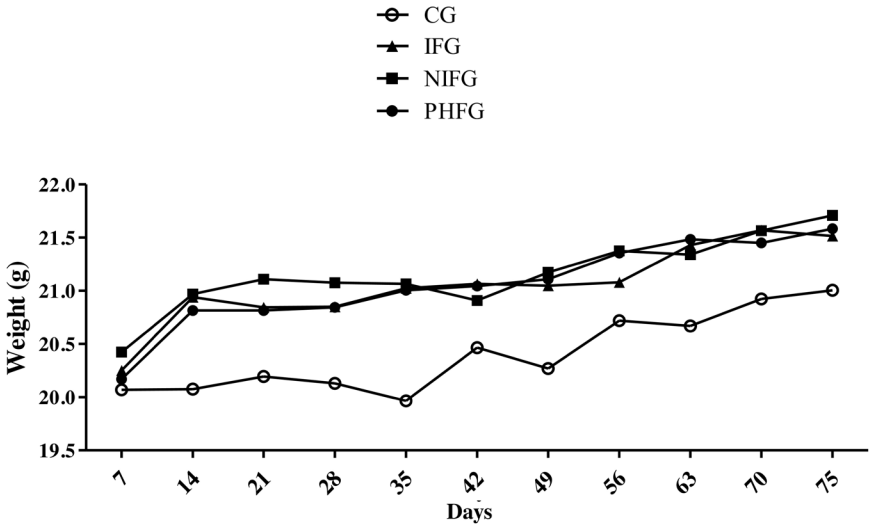

Figure 2. Progression of body weight of the experimental groups during the 75 days of treatment. Groups: Control (CG), Interesterified fat (IFG), Non-interesterified fat (NIFG) and partially hydrogenated vegetable fat (PHFG). Values are expressed as mean (n = 6).

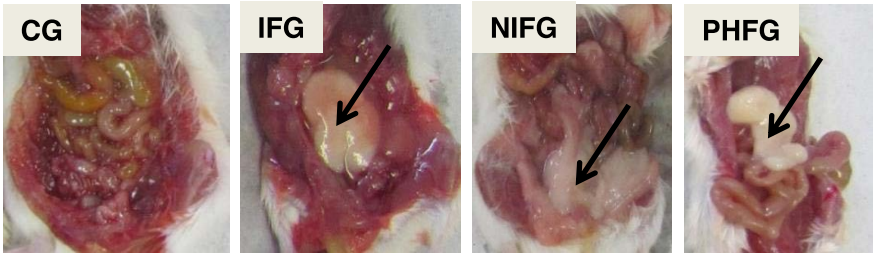

Figure 3. Abdominal fat deposition. Fat deposit (indicated by black arrow) was not observed in the Control Group (CG) fed with a normolipidic diet, but it was found in the groups which were fed with the hyperlipidic diets: Interesterified fat (IFG), Non-interesterified fat (NIFG) and partially hydrogenated vegetable fat (PHFG).

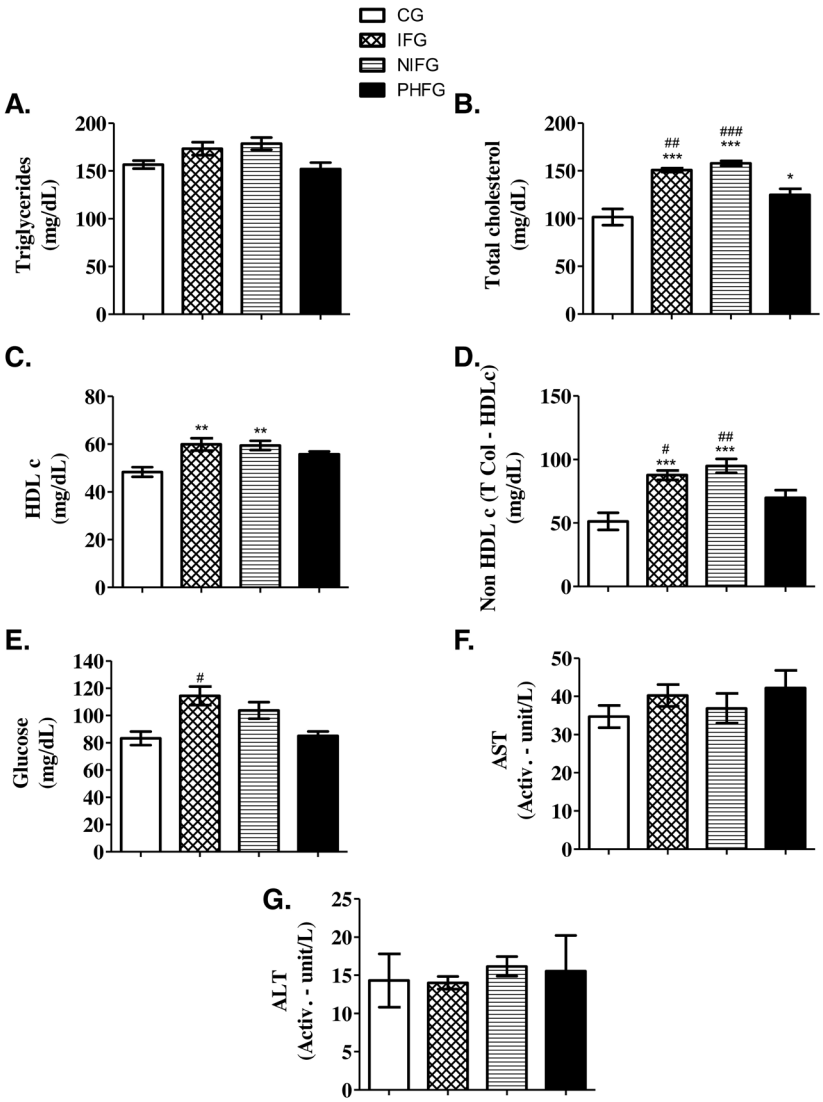

Figure 4. Effect of the consumption of interesterified fat (IFG), non-interesterified fat (NIFG), and partially hydrogenated vegetable fat (PHFG) in female Balb-c mice after 75 days, about evaluation of serum biochemical profile. Analysis of (A) Triglycerides, (B) Total cholesterol, (C) HDL-C (D) Non-HDL-C, (E) Glucose, (F) AST: aspartate amine transferase and (G) ALT: alanine amine transferase. Values are expressed as Mean ± S.D. (n = 6). *, ** and *** denote statistical difference compared to control group (CG), when $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$ denote statistical difference among groups interesterified fat (IFG), non-interesterified fat (NIFG) and partially hydrogenated vegetable fat (PHFG), when $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

more expressive and significant ($p < 0.01$ and $p < 0.001$, respectively) when compared to PHFG. The HDL-C levels were significantly higher ($p < 0.01$) in the serum of the animals fed with IF and NIF when compared with CG (Figure 4(C)). On the other hand, no significant differences were observed between IFG, NIFG, and PHFG. Figure 4(D) shows that IFG and NIFG presented a significant increase for non-HDL-C when compared to CG ($p < 0.001$) and to PHFG ($p < 0.05$, $p < 0.001$, respectively). Figure 4(E) shows that the serum levels of glucose

were statistically higher for IFG when compared to PHFG ($p < 0.05$). The serum enzymes of hepatic function AST and ALT (Figure 4(F) and Figure 4(G), respectively), did not change between the treatments.

3.4. Oxidative Stress and Antioxidant Defense

The hepatic GSH content detected in the animals fed with IF and NIF was approximately 2.3 and 2.7 times higher when compared to CG and PHFG ($p < 0.05$). On the other hand, the results for hepatic GSH content for the animals fed with PHF were similar to the animals of the CG (Figure 5(A)).

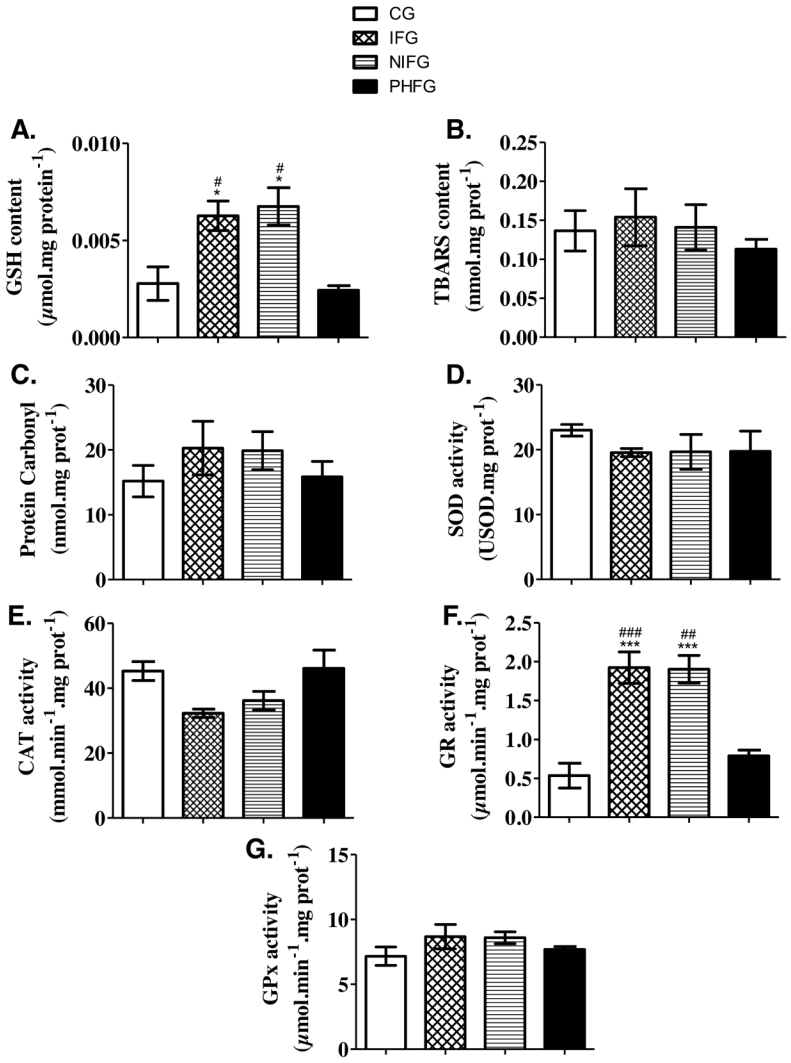

Figure 5. Evaluation of hepatic oxidative stress modulation in female Balb-*c* mice feed with different diets, for 75 days. Analysis of (A) GSH content, (B) TBARS content, (C) Protein Carbonyl, (D) SOD activity, (E) CAT activity, (F) GR activity and, (G) GPx activity. Values are expressed as Mean ± S.D. (n = 6). * and *** denote statistical difference compared to control group (CG), when $p < 0.05$ and $p < 0.001$, respectively.(#) and (###) denote statistical difference among groups interesterified fat (IFG), non-interesterified fat (NIFG) and partially hydrogenated vegetable fat (PHFG), when $p < 0.05$ and $p < 0.001$, respectively.

No differences were observed between the animals of the CG and those who received the diets studied for the oxidative markers, TBARS (**Figure 5(B)**), carbonyl protein values (**Figure 5(C)**) and for the antioxidant enzymes SOD (**Figure 5(D)**), CAT (**Figure 5(E)**), and GPx (**Figure 5(G)**). In contrast, an increase of approximately 3.5-fold in the GR activity (**Figure 5(F)**) was observed in the animals fed with IF and NIF compared to the CG. On the other hand, the GR activity in the animals fed with PHF was like those animals in the CG.

4. Discussion

The FA analysis indicated that IF and NIF presented around 40% of palmitic acid (C16:0). These results were expected since one of the main raw materials used in interesterification processes is palm oil and palm stearin, which presents between 40% - 48% and 48% - 74% of palmitic acid, respectively [34]. On the other hand, the process of interesterification does not change the profile of FA of the interesterified fats [1] [10]. Vegetable oils that have not been modified typically exhibit the saturated fatty acid at the 1,3-position of the triacylglycerol [10]. In this study, was observed an increase of 100% of the amount of SFA at the sn-2 position. Soares *et al.* (2009) [35] reported similar results for a 60:40 mixture of stearin and palm olein.

The amount of TFAs found in the PHF (20%) was lower than the content reported for partially hydrogenated fats produced in the 1990s and early 2000 (from 30% to 60%) [36] [37]. Mensink and Katan [2], in their classic study, reported an amount of 46% of TFAs in the margarine used in the experiments. The PHF also presented 6% of linolenic acid, which is an essential fatty acid. The presence of linolenic acid in the PHF indicates that this fat was produced with soybean oil, which contains 5.5% - 9.5% of this FA in its composition [34].

During the 75 days of treatments, the animals receiving the hyperlipidic diets (IFG, NIFG, and PHFG) presented lower food intake ($p < 0.001$) and higher energy consumption ($p < 0.001$). However, despite the lower dietary intake, the hyperlipidic diets increased the weight gain by about 40% (although not significant), similar to previously described, for mice Balb c [38].

The weight gain and visceral adipose tissue observed for the animals fed with the interesterified fat was not significant when compared to these parameters found in the animals fed with non-interesterified and *trans* diets. These results may be possibly related to the time of the experiment with the hyperlipidic diet. On the other hand, Gouk *et al.* [39] and Afonso *et al.* [40] reported greater weight gain in animals fed with interesterified fat after 15 and 16 weeks of treatment, respectively. Lavrador *et al.* [41] reported triggered adipocytes hypertrophy in LDLr-KO mice after 16 weeks of treatment com acid palmitic interesterified. Magri *et al.* and Velasco *et al.* [42] [43] evaluated the weight gain and adiposity in C57BL/6 mice whose mothers received interesterified fat and partially hydrogenated fat during gestation and lactation. The authors concluded that the intake of interesterified and/or palm fats in this period might predispose the

descendants to obesity in adult life. On the other hand, it was not observed for the group that received partially hydrogenated fat in the diet. Bispo *et al.* for C57BL-6 mice also reported that interesterified fat consumption led to deregulation in the expression of proteins involved in food intake [44].

The hyperlipidic diets used in this study did not change the serum levels of triglycerides. Controversial results have been reported in the literature for the levels of serum triglycerides after the consumption of interesterified fat. Ponnampalam *et al.* [45], in a study with piglets, reported an increase in the serum triglycerides after the consumption of enzymatically interesterified palm olein during 12 weeks. On the other hand, Reena; Lokeski (2007) and Sharma; Lokeski (2013) reported for Wistar rats a reduction of serum triglycerides after the consumption of interesterified fats composed of unsaturated oils during 60 days [15] [46].

The IF and NIF diets increased significantly, the serum levels of total cholesterol, HDL-C, and non-HDL-C. These results indicate that the positional distribution of the palmitic acid in positions 1 and 3 does not alter the serum lipoproteins response. Similar results were reported in experiments with humans [1] [11]. Berry [11] reported that triacylglycerols structure has no effect on either digestibility or fasting lipids. On the other hand, it has been reported for several authors that 20% of the energy in the diet as interesterified fat administered to Wistar rats, for 60 days, reduced total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels [15] [16] [47] [48]. However, in these studies, blends composed of unsaturated oils, such as flaxseed, rice bran, sesame, peanut, and olive oil, were used. The fatty acid and triacylglycerol composition of these blends are very different from commercially available interesterified fats, which are rich in saturated FA, especially palmitic acid. The plasma lipid concentration of LDLr-KO mice fed for 16 weeks with interesterified fat (based on palm oil or totally hydrogenated canola oil and high-oleic sunflower oil); as 40% of the energy, was not changed [40]. However, the authors reported that interesterified fat rich in palmitic acid accelerated the development of atherosclerosis by promoting cholesterol accumulation in LDL particles and macrophage cells, activating the inflammatory process. The LDL, as well as other non-HDL-C (very low-density lipoprotein, VLDL; intermediate-density lipoprotein, IDL; lipoprotein (a), Lp(a); and chylomicron remnants), are related with the development of atherosclerosis [49]. Our results indicated that the increase in non-HDL-C in the serum of the animals who received fats rich in palmitic acid (IFG and NIFG) was higher than in animals fed with PHFG, a source of *trans* fat and linolenic acid. Although the *trans*-fat diet did not increase the non-HDL-C in the serum of the Balb-c mice, it is well-known that TFA consumption raises the non-HDL-C levels in the serum of humans [2] [3]. Other studies have indicated that palmitic acid also increased total and LDL-cholesterol concentration [50] [51], while linolenic acid may also contribute to the reduction of LDL-c [52]. Overall, these findings indicate that serum lipid response to IF, NIF, and trans-fat diet might be specie specific. Therefore, the results obtained using laboratory animals should be in-

terpreted with caution.

In our study, no difference in serum glucose between the groups IFG and NIFG was observed. It has been reported for humans an increase the fasting plasma glucose and a decrease in plasma insulin [14] and in postprandial glucose-dependent insulinotropic polypeptide (GIP) concentrations after consumption of interesterified palm olein [53]. An increase in the levels of fasting glucose and impaired glucose tolerance was observed in Wistar rats after the consumption of interesterified soybean oil for 16 weeks. The same effect was not observed after 8 weeks [54]. In our study, a significant increase in serum glucose was observed in the animals fed with IF, compared to those fed with PHF. As reported by Miyamoto [54], the time of exposure to the diet with interesterified fat might be determinant for the increase of the plasma glucose. Lavrador *et al.* [41] also found no differences in glucose and insulin plasma concentrations in LDLr-KO mice fed a high-fat diet containing palmitic or interesterified palmitic fats for 16 weeks.

Hyperlipidic diets can lead to liver damage, including oxidative-based injuries [55]. In this study, after 75 days, the activities of the AST and ALT, main markers of hepatic function, were not affected. In LDLr-KO mice fed for 16 weeks which Interesterified fats enriched with palmitic or stearic acids was induced hepatic fibrosis, even without raising ALT levels [41]. Although the relationship between oxidative stress and the consumption of diets rich in TFAs is well-known [56] [57], the *trans*-fat diet did not change the biomarkers values of oxidative stress in the Balb-c mice. Nagaraju; Lokeski [47] reported similar results for Wistar rats fed with IF and NIF diets.

A significant increase in the GSH and GR values was observed for the diets rich in palmitic acid. It has been shown that palmitic acid presented lipotoxicity in the liver cells [58] [59]. Therefore, the increased GR activity associated with an absence of oxidative stress markers induction might be responsible for the elevation of GSH levels in IFG and NIFG, indicating a potential protection against free radical-mediated damage in the liver. It is well known that the GSH redox system is important for decreasing oxidative stress. The GSH, a radical scavenger, is converted to oxidized glutathione (GSSG), and GR continuously converts GSSG back to GSH [60].

In addition, female mice are protected against metabolic changes caused by hyperlipidic diets, while maintaining an anti-inflammatory environment in the intra-abdominal adipose tissue with expanded Treg cell population, whereas male mice can develop adipose tissue inflammation, glucose intolerance, hyperinsulinemia, and islet hypertrophy [61]. Therefore, the metabolic changes caused by hyperlipidic diets could be even more visible if the study was conducted using male mice.

5. Conclusion

In conclusion, the modification of the triacylglycerol structure, caused by interesterification, did not lead to different metabolic alterations. The mice that received

the diet with interesterified fat presented similar results, as for the body weight gain, biochemical profile and biomarkers of hepatic oxidative stress, to the mice that received fat with the same fatty acid profile but with a lower (2-fold) amount of saturated fatty acids in the *sn*-2 position of triacylglycerol. However, the mice which received interesterified fat had higher serum total cholesterol, non-HDL-C and glucose concentrations, and different hepatic antioxidant response, than the group that received partially hydrogenated vegetable fat. Thus, in this study, a high-fat diet containing partially hydrogenated vegetable fat caused less important metabolic effects than fats rich in SFA and palmitic acid, interesterified, or non-interesterified. Our results suggest that the replacement of hydrogenated fats by interesterified fats may not be such a simple solution to reduce or eliminate *trans* fatty acids in foods, and it is necessary for further investigation in humans.

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Conflicts of Interest

None of the authors had a financial or personal conflict of interest.

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Abbreviations

CG, control group;
IFG, interesterified fat group;
NIFG, non-interesterified group;
PHFG, partially hydrogenated vegetable fat group;
TC, total cholesterol;
Non-HDL-C, non-HDL cholesterol;
HDL, high density lipoproteins;
TFAs, *trans* fatty acids;
WHO, World Health Organization;
SFA, saturated fatty acids;
FA, fatty acids;
IF, interesfied fat;
NIF, non-interesterified;
PHF, partially hydrogenated vegetable fat;
FID, flame ionization detector;
FAMEs, fatty acids methyl esters;
ECN, equivalent carbon number;
NIH, National Institutes of Health;
AST, aspartate aminotransferase;
ALT, alanine aminotransferase;
SOD, superoxide dismutase;
CAT, catalase;
GPx, glutathione peroxidase;
GR, glutathione reductase;
GSH, reduced glutathione;
DTNB, dithiobisnitrobenzoic acid;
TBARS, thiobarbituric acid-reactive substances
TAGs, triacylglycerols;
SSS, trisaturated triacylglycerols (S, stearic acid);
PPO, disaturated triacylglycerols (P, palmitic acid and O, oleic acid)
OOO, tri-unsaturated triacylglycerols (O, oleic acid);
LDL-C, low-density lipoprotein cholesterol;
VLDL, very low-density lipoprotein;
IDL, intermediate-density lipoprotein;
Lp(a), lipoprotein (a);
GIP, insulinotropic polypeptide;
GSSG, oxidized glutathione.