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Research paper

Increase in liver cytosolic lipases activities and VLDL-TAG secretion rate do not prevent the non-alcoholic fatty liver disease in cafeteria diet-fed rats

Anderson Fernandes de Melo [a], Carolina Campos Lima Moreira [b], Camila Ferreira Sales [c], Thiago Rentz [d], Helena Fonseca Raposo [d], Maria Antonieta Rissato Garófalo [e], Leida Maria Botion [b], Isis do Carmo Kettelhut [f], Helena Coutinho Franco de Oliveira [d], Valéria Ernestânia Chaves [a, *]

[a] Laboratory of Physiology, Federal University of São João del-Rei, Divinópolis, Minas Gerais, Brazil
[b] Department of Physiology and Biophysics, Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Tissue Processing Laboratory, Federal University of São João del-Rei, Divinópolis, Minas Gerais, Brazil
[d] Department of Structural and Functional Biology, Institute of Biology, State University of Campinas, Campinas, São Paulo, Brazil
[e] Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[f] Department of Biochemistry-Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

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ABSTRACT

We have previously shown that the cafeteria diet increases body fat mass, plasma triacylglycerol (TAG) and insulin levels, glucose uptake by white and brown adipose tissues, as well as the sympathetic activity to both adipose tissues in Wistar rats. The metabolic pathways responsible for the development of non-alcoholic fatty liver disease (NAFLD) were examined in cafeteria diet-fed rats. After 3 weeks offering cafeteria diet, we evaluated: *(i)* activity of the sympathetic nervous system by norepinephrine turnover rates; *(ii) de novo* fatty acid synthesis *in vivo* using $^{3}H_2O$; *(iii)* secretion of very low density lipoprotein (VLDL)-TAG secretion measuring serum TAG levels after administration of lipase lipoprotein inhibitor, *(iv)* liver cytosolic lipases activities and *(v)* liver mRNA expression of enzymes involved in lipids secretion and oxidation by RT-PCR. The cafeteria diet induced an increase in TAG (120%) and cholesterol (30%) liver contents. Cafeteria diet did not change the sympathetic nervous system activity to liver, but induced a marked increase in the lipogenesis (approximately four-fold) and significant increase in cytosolic lipases activities (46%) and VLDL-TAG secretion (22%) compared to control diet-fed rats. The cafeteria diet also increased the microsomal triglyceride transfer protein (30%) and carnitine palmitoyltransferase I (130%) mRNA expression but decreased the apolipoprotein B100 (26%) mRNA expression. Our findings demonstrate that the increase in the cytosolic lipases activities and VLDL-TAG secretion rates were not able to compensate for the increased lipogenesis rates induced by the cafeteria diet, resulting in NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is now considered to be one of the most common forms of chronic liver disease in the Western world. NAFLD refers to a clinical-pathological spectrum of conditions ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), involving inflammation and some evidence of liver cell damage, and in some cases, cirrhosis [1]. In the United States, the prevalence of NAFLD is approximately 30%, while the incidence of NASH is about 3% [1,2]. The NAFLD prevalence increases among overweight (58%) and non-diabetic obese (98%) individuals [3,4].

There is considerable traffic of both non-esterified fatty acids (NEFAs) and triacylglycerols (TAGs) in the liver, independent of food state [5]. In the postprandial state, the liver synthesizes fatty acids

* Corresponding author. Avenida Sebastião Gonçalves Coelho, 400, Chanadour, 35.501-296, Divinópolis, Minas Gerais, Brazil.
E-mail addresses: valeria.chaves@gmail.com, valeriachaves@ufsj.edu.br (V.E. Chaves).

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(FAs) and takes up chylomicron remnants from the bloodstream [5]. NEFAs may be converted into other lipid species, such as glycerolipids, glycerophospholipids, and sterols, which can be packaged into very low density lipoprotein (VLDL) particles and secreted from the liver into the plasma [5]. Under fasting conditions, NEFAs are released from adipose tissue and returned to the liver, where they are oxidized in the mitochondria to provide energy or converted into TAGs and packaged in VLDL particles that are secreted into the plasma [5]. Thus, the TAG content in the cytoplasm of hepatocytes is influenced by the balance of all these metabolic pathways.

Nutritional, hormonal, and neural mechanisms regulate the lipid metabolism. Fructose increases the generation of acetyl-CoA and glycerol-3-phosphate because fructokinase is not regulated [6]. Insulin stimulates lipogenic enzymes and inhibits FA oxidation [7], while the sympathetic nervous system increases the rate of VLDL-TAG secretion [8] and mobilization of FAs from adipose tissue [9]. Several studies have described an increase in VLDL secretion in models of insulin resistance, characterized by hyperinsulinemia and hyperglycemia, such as type 2 diabetics individuals [10], high-fructose diet-fed hamsters [11], high-fat diet-fed mice [12], tumor necrosis factor-α-treated hamsters [13], and high-fat diet-fed and LDL receptor-null mice [14]. However, *ob/ob* mice, despite hyperinsulinemia and hyperglycemia, have similar VLDL-TAG secretion to lean mice [15]. Also, acute hyperinsulinemia reduces the VLDL-TAG production in these groups, showing a more pronounced effect in lean mice than *ob/ob* mice [15]. Thus, insulin effects on VLDL secretion are not fully clarified. The regulation of VLDL secretion is frequently associated with apolipoprotein B (ApoB) synthesis and secretion. It has been suggested that either the inhibitory effects of insulin signaling on ApoB secretion is very short-lived or the hepatic TAGs are the dominant regulator of ApoB secretion [16].

Furthermore, studies using adipose triglyceride lipase (ATGL)- and hormone-sensitive lipase (HSL)-deficient mice have suggested that these enzymes can contribute to the development of hepatic steatosis [17,18], although HSL and ATGL are responsible for only approximately 40% of the total hydrolases activity in the hepatic tissue [18,19]. Recently, we demonstrated that the high-fructose diet induces severe liver steatosis accompanied by a decrease in cytosolic lipases activities [20]. Here, our main objective was to investigate the biochemical pathways involved in the development of NAFLD induced by a cafeteria diet. Thus, we evaluated the effect of chronic treatment with a cafeteria diet on the liver lipid accumulation, rates of *de novo* FA synthesis, cytosolic lipases activities, VLDL-TAG secretion rates, and mRNA expression of genes involved in lipid oxidation and secretion, as well as the possible involvement of the sympathetic nervous system activity.

2. Material and methods

2.1. Animals and treatment

Male Wistar rats with a body weight (BW) of 60–80 g, were obtained from the Breeding Centre of the Federal University of São João Del-Rei (Brazil) and kept in cages in an environmentally controlled room with a 12/12 h light/dark cycle and 23 ± 2 °C. The rats were fed a cafeteria diet for 3 weeks that consisted of a standard balanced diet [Nuvilab CR1, Nuvital, Brazil (55% carbohydrate, 22% protein, and 4.5% lipid)] supplemented each day with 4 different lipid-rich palatable food items, selected from a list of 12 (bacon, caramel candies, cashew nuts, cookies, cornstarch biscuits, cheese biscuits, chocolate rolls, chocolate wafers, nougat, peanut candies, potato chips, and toasts). In addition, the water offered to these rats contained 20% sucrose. Control rats were fed the commercial diet only and consumed water *ad libitum*.

The daily energy intake of the cafeteria diet-fed rats was ~40% higher (293 ± 13 versus 209 ± 4 kJ 100 g BW^{-1} d^{-1}) than in controls [21,22]. In cafeteria diet-fed rats, protein contributed 15 ± 1%, carbohydrate 65 ± 1%, and lipid 20 ± 1% of the energy intake, compared to 25, 63, and 12%, respectively, in rats fed the control diet [21,22]. BWs were carefully monitored at regular intervals. The rats weighed 210–230 g when used for the experiments that were performed in the fed state or, when specified, fasting (12 h) state, always between 08:00 and 10:00 h. For tissue removal, rats were killed by cervical dislocation. Animal protocols received prior institutional approval from the Ethical Committee of the Federal University of São João Del Rei (protocol no. 016/2014).

2.2. Liver norepinephrine (NE) turnover rates and NE content

Liver NE turnover rates were estimated from the decline in tissue NE levels, after inhibition of catecholamine synthesis with DL-α-methyl-*p*-tyrosine methyl ester (Sigma-Aldrich, MO, USA). The NE measurement has been described in detail previously [23].

2.3. In vivo rates of de novo FA synthesis

Rats were injected *i. p*. with ^3H_2O (3 mCi in 0.5 mL saline). After 1 h, they were killed by decapitation, blood samples were collected for plasma water specific radioactivity determination, and the livers were rapidly removed for measurement of label incorporation into TAGs. The procedures used for lipid extraction, isolation of the TAG-FAs, radioactivity counting, and plasma water specific radioactivity determination have been previously described [24].

2.4. VLDL-TAG secretion

In 12-h-fasted rats, blood samples were taken before and 2 h after *i. v.* administration of Triton WR 1339 (400 mg kg^{-1}, Sigma-Aldrich), a lipoprotein lipase inhibitor. Serum TAG concentration was assayed by an enzymatic procedure with a commercial kit (Bioclin®, Belo Horizonte, Brazil). The rate of VLDL-TAG secretion was calculated by subtracting the TAG concentration at 0 h time point from the TAG concentration at the 2 h time point and was expressed as mg·dL^{-1}· h^{-1}.

2.5. Cytosolic lipases activities

Each liver was homogenized in ice-cold buffer containing 50 mM Tris-HCl, pH 7.4, 250 mM sucrose and 1 mM EDTA. The homogenate was briefly centrifuged, and the intermediate layer was centrifuged at 70,000 rpm (T880 rotor) at 4 °C for 2 h, to yield cytosolic lipid droplets, cytosol, and membrane pellets [25]. The intermediate layer, which contained the cytosolic fraction, was used in the assay. Cytosolic lipases activities were assessed by adding an aliquot of the sample to the reaction buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA and 300 μM taurodeoxycholate) containing 50 μM of 4-methylumbelliferyl heptanoate [26]. The assay was conducted in a 96-well black plate at room temperature for 30 min with shaking. The fluorescence of the reaction product was measured at excitation/emission wavelengths of 355/460 nm. Data were expressed as mU per mg of protein. Protein concentration in the tissue homogenate was measured using the Bradford reagent [27].

2.6. Real-time PCR (RT-PCR)

Carbohydrate-responsive element-binding protein (ChREBP), sterol regulatory element-binding protein (SREBP)-1c, ATGL, HSL, peroxisome proliferator-activated receptor (PPAR)-α, carnitine

palmitoyltransferase I (CPT 1), acyl CoA oxidase (ACO), ApoB100 and microsomal triglyceride transfer protein (MTTP) mRNA levels were determined by RT-PCR in the liver from rats fed the cafeteria or control diet. The livers were immediately frozen in liquid nitrogen, and RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The RNA integrity was evaluated using Tris-borate 1.2% agarose gels stained with ethidium bromide. The amount and purity of the RNA were determined by optical density readings at 260 and 280 nm (NanoVue Plus Spectrophotometer, GE Healthcare). Genomic DNA contamination was ruled out by running a polymerase chain reaction (PCR) on the RNA samples. Total RNA (2 μg) was reverse transcribed into cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. RT-PCR was performed by a sequence detection system (7500 Step One Real-time PCR System, Applied Biosystems) using a SybrGreen PCR master mix and specific primers sequences for the following: ACO (forward: 5′-GGC CGC TAT GAT GGA AAT GTG-3'; reverse: 5′-GGG CTT CAA GTG CTT GTG GTA A-3′), ApoB (forward: 5′-CAT GCT CCA TTC TCA CAT GTT TA-3'; reverse: 5′-AGA GAA CAA AGC AGA GAT TGT GG-3′), ATGL (forward: 5′-CTG TGG CCT CAT TCC TAC C-3'; reverse: 5′-GTG GCA AGT TGT CTG AAA TGC CG-3′), β-actin (forward: 5′-CAT GAA GAT CAA GAT CAT TGC TCC T-3'; reverse: 5′-CTG CTT GCT GAT CCA CAT CTG-3′), ChREBP (forward: 5′-AAA GGC CTC AAG TTG CTA TG-3'; reverse: 5′-AGA CAA CAG CCT CAG GTT TC-3′), CPT 1 (forward: 5′-ACG TGA GTG ACT GGT GGG AAG AAT-3'; reverse: 5′-TCT CCA TGG CGT AGT AGT TGC TGT-3′), HSL (forward: 5′-CAG GAG TGC TCT TTG AG-3'; reverse: 5′-CAG CCT TTA TGT AGC GTG AC-3′), MTTP (forward: 5′-AAC CAG AAA TGT CAA TGG CTA GA-3'; reverse: 5′-AGT GAT TTG ATG TCC AAA ATG CT-3′), PPAR-α (forward: 5′-TAC CAC TAT GGA GTC CAC GCA TGT-3'; reverse: 5′-TTG CAG CTT CGA TCA CAC TTG TCG-3′), and SREBP-1c (forward: 5′-GCC CAC AAT GCC ATT GAG A-3'; reverse: 5′-GCA GAT TTA TTC AGC TTT GCC TCA-3′). The mRNA was quantified by measuring the threshold cycle method (ΔΔCT), normalized to β-actin and expressed relative to the control group.

2.7. Other methods of chemical analysis

Serum glucose, TAG, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration were determined enzymatically, using commercial kits (Bioclin®). Serum insulin levels were measured by radioimmunoassay using commercial kit (Insulin Coat-a-Count®). After lipid extraction with chloroform:methanol [28], followed by chloroform evaporation and lipid resuspension in isopropyl alcohol, the liver TAG and cholesterol concentrations were determined enzymatically, using commercial kits (Bioclin®). Lipid content was also qualitatively evaluated after hematoxylin & eosin staining of fixed liver sections and oil red O (0.24%) and hematoxylin (0.2%) staining of frozen liver sections. Images were captured using a digital camera, coupled to an optical microscope.

2.8. Statistical methods

Except for calculated turnover rates, which were compared using 95% confidential intervals (CIs), as described by Taubin et al. [29], data were expressed as mean ± SEM. Differences between groups were analyzed using Student's *t*-test, with $P < 0.05$ as the significance criterion.

3. Results

Cafeteria diet feeding did not induce changes in the BW gain of rats (Table 1). Nonetheless, it triggered significant increases in the body adiposity, evaluated by the weight of retroperitoneal (225%) and epididymal (68%) white adipose tissues, in addition to the liver TAG (120%) and cholesterol (29%) content, as well as serum insulin (56%) and TAG (171%) levels (Tables 1 and 2). The increase in liver lipid content induced by cafeteria diet is also qualitatively demonstrated by histological analysis (Fig. 1). The cafeteria diet did not induce changes in serum glucose levels and the AST and ALT liver enzymes compared to the control diet (Table 2).

Table 1
Biometric data and liver concentrations of triacylglycerol and cholesterol from rats fed the control and cafeteria diets.

Biometric parameter	Control diet	Cafeteria diet
Initial body weight (g)	75 ± 5	76 ± 4
Final body weight (g)	219 ± 9	216 ± 10
Epididymal adipose tissue (g 100 g^{-1})	0.60 ± 0.04	1.01 ± 0.09*
Retroperitoneal adipose tissue (g 100 g^{-1})	0.27 ± 0.03	0.88 ± 0.10*
Liver (g 100 g^{-1})	4.75 ± 0.12	4.50 ± 0.08
Liver triacylglycerol (mg g^{-1})	4.50 ± 0.27	9.90 ± 0.94*
Liver cholesterol (mg g^{-1})	1.90 ± 0.04	2.45 ± 0.20*

Data represent mean ± SEM of 7–8 rats. *$P < 0.05$ versus control diet.

The NE turnover rate, an indicator of the sympathetic activity, did not differ between cafeteria diet-fed and control rats (Fig. 2 and Table 3). *De novo* FA synthesis, estimated with ^3H_2O, was markedly higher (~4-fold) in liver from rats fed the cafeteria diet than control diet (Fig. 3). However, the levels of mRNA expression of lipogenesis related transcription factors, SREBP-1c and ChREBP, were similar between the groups (Fig. 4). The cafeteria diet also prompted an increase (46%) in liver cytosolic lipases activities (Fig. 5), without changing the mRNA expression levels of ATGL and HSL (Fig. 4). Compared to the control rats, the expression of CPT 1, key enzyme regulating mitochondrial internalization of activated FAs, was higher (130%) in the liver of cafeteria diet-fed rats, but PPAR-α and ACO mRNA expressions, regulators of lipid oxidation, did not differ between the groups (Fig. 4). VLDL-TAG secretion rate was higher (22%) in cafeteria diet-fed rats relative to control diet-fed rats (Fig. 6). This effect is associated with an increase (30%) in MTTP and decrease (26%) in ApoB expression levels (Fig. 4), proteins both involved to VLDL assembly.

4. Discussion

In order to explain the effects of the cafeteria diet on liver lipid homeostasis, we evaluated the routes of lipid synthesis, catabolism, and exportation. Our data showed that the chronic cafeteria diet feeding induced a liver lipid accumulation, the main feature of NAFLD (Table 1, Fig. 1). This effect resulted from a marked increase in liver FA synthesis (Fig. 3), despite increased cytosolic lipases activities (Fig. 5) and VLDL-TAG secretion rate (Fig. 6). These changes were independent of the sympathetic nervous system activity in the liver (Fig. 2, Table 3) and likely reflect nutritional and hormonal regulation.

Table 2
Serum concentrations of insulin, glucose, triacylglycerol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) from rats fed the control and cafeteria diet.

Serum concentration	Control diet	Cafeteria diet
Insulin (μU mL^{-1})	32 ± 3	50 ± 5*
Glucose (mg dL^{-1})	116 ± 5	113 ± 8
Triacylglycerol (mg dL^{-1})	42 ± 4	114 ± 10*
AST (U L^{-1})	180 ± 12	194 ± 14
ALT (U L^{-1})	286 ± 54	205 ± 22

Data represent mean ± SEM of 5–8 rats. *$P < 0.05$ versus control diet.

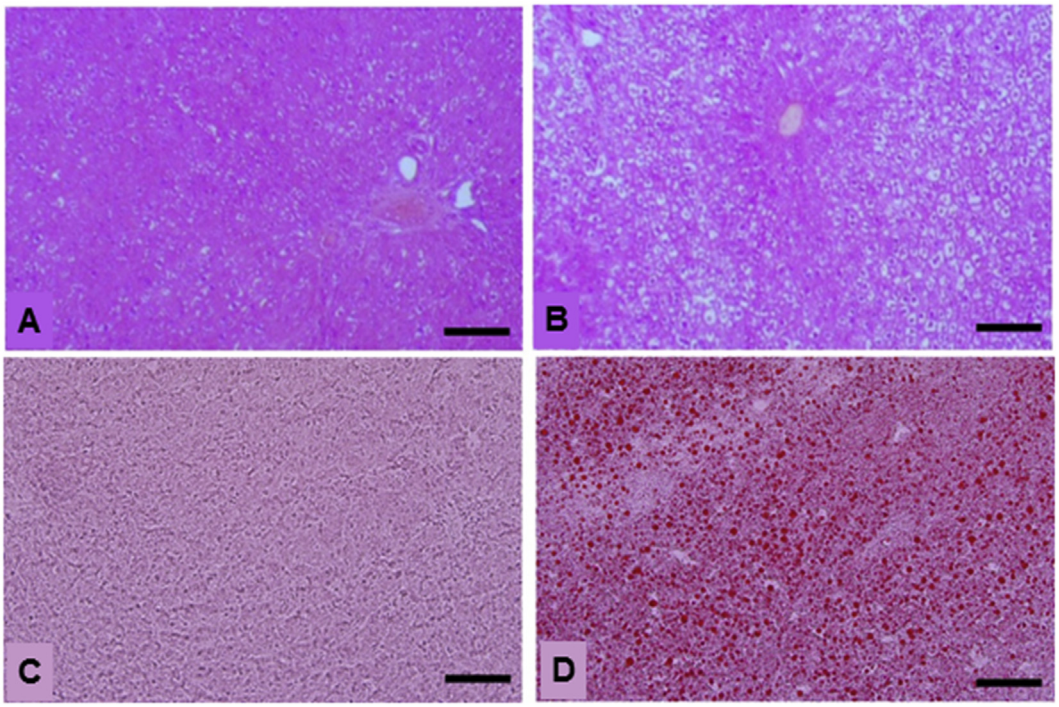

Fig. 1. Effect of cafeteria diet feeding on the liver lipids content. Hematoxylin & eosin (**A** and **B**) and Oil red O and hematoxylin staining (**C** and **D**) of liver sections from rats fed the control (**A** and **C**) and cafeteria (**B** and **D**) diets. n = 18 histological sections from 6 rats per group. Bars represent 100 μM.

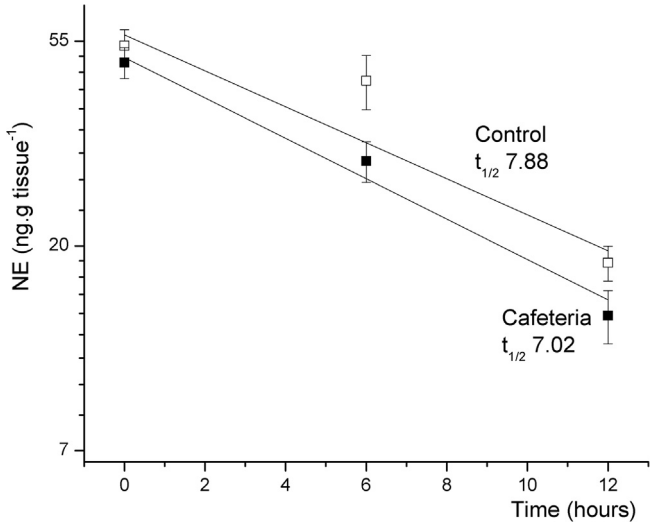

Fig. 2. Effect of cafeteria diet feeding on the disappearance of endogenous norepinephrine (NE) in rat liver after α-methyltyrosine administration. Values are mean ± SEM, n = 9–10 at each time. Half-life of NE (t1/2) = 0.693/fractional turnover rate (see Table 3).

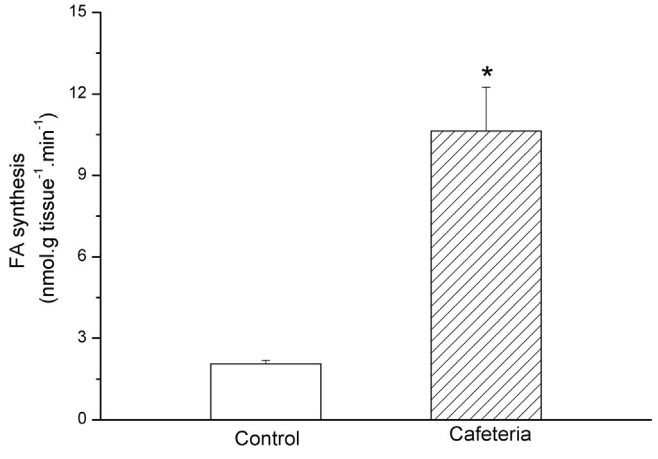

Fig. 3. Effect of cafeteria diet feeding on rates of *de novo* fatty acid synthesis in rat liver. Bars represent mean ± SEM, n = 6 rats. *$P < 0.05$ versus control diet.

Table 3
Fractional (k) and calculated turnover rates (TR) of norepinephrine (NE) and NE concentration of livers from rats fed the control and cafeteria diet.

Diet	k; %h	TR; ng g tissue^{-1} h^{-1}	[NE]; ng g tissue^{-1}
Control	8.79 ± 2.40	4.70 (3.1–6.47)	53.4 ± 4.34
Cafeteria	9.87 ± 1.30	4.86 (3.89–5.91)	49.2 ± 3.75

Values are mean ± SEM and 95% CI for TR, n = 9–10 at each time point of the TR experiments (Fig. 2).

Activation of *de novo* FA synthesis in the liver from cafeteria diet-fed rats is probably due to the increase in the plasma insulin levels (Table 2), which activates lipogenesis enzymes. Several studies have demonstrated that insulin also induces an increase in the expression of SREBP-1c and ChREBP mRNA, two transcription factors involved in the expression of genes encoding lipogenic enzymes. Fasting decreases the SREBP-1c expression in the liver of rodents, while the acute insulin administration increases the expression of this transcriptional factor in liver from fasted or streptozotocin-diabetic rodents [30–32]. Recently, it was observed that high-fat diet feeding for 18 weeks increases insulin levels without affecting the liver transcription of SREBP-1c [33]. However, high-fat diet increases the active SREBP1 in liver, suggesting that insulin regulates the processing and nuclear activity of this transcriptional factor [33,34]. In hepG2 cells, the effect of insulin is dose-dependent, i.e., 10 or 100 nM insulin induces ChREBP mRNA, whereas, this outcome does not occur with 1 nM insulin [35].

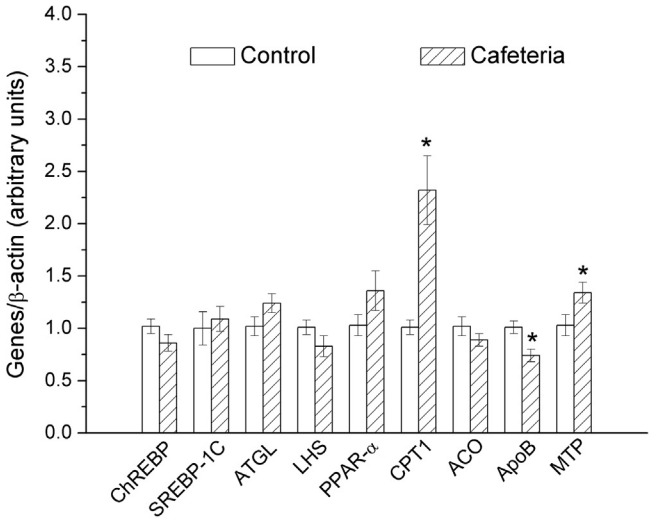

Fig. 4. Effect of cafeteria diet feeding on the liver mRNA expression of genes related to lipogenesis (ChREBP, SREBP-1c), lipolysis (ATGL, HSL), oxidation (PPAR-α, CPT 1, ACO), and very low density lipoprotein (VLDL) secretion (ApoB and MTTP). Bars represent mean ± SEM, n = 8 rats. Normalized to β-actin (internal control) and expressed relative to the control diet group. $^{*}P < 0.05$ versus control diet.

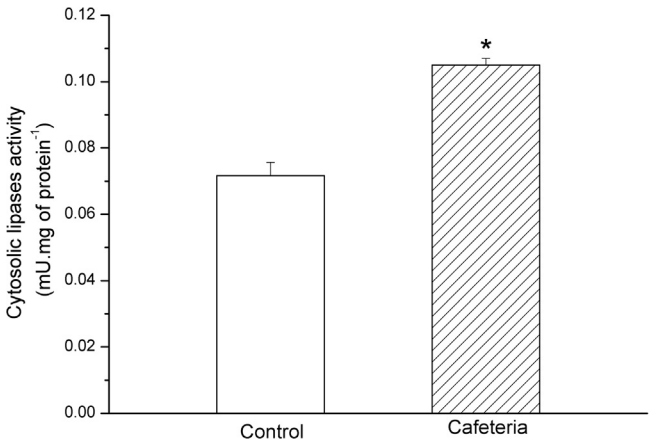

Fig. 5. Effect of cafeteria diet feeding on the liver cytosolic lipases activities. Bars represent mean ± SEM, n = 7 rats. $^{*}P < 0.05$ versus control diet.

Insulin concentrations in cafeteria diet-fed rats are higher than the control diet-fed rats, but lower than 1 nM. Thus, in this condition, similar liver levels of SREBP-1c and ChREBP mRNA are expected (Fig. 4).

Recently, we reported that rats fed a high-fructose (60%) diet for 8 weeks had decreased cytosolic lipases activities, accompanied by the development of NAFLD when compared with rats fed the AIN-93 M diet [20]. Cafeteria diet feeding also induced the development of NAFLD, but it was accompanied by an increase in the cytosolic lipases activities (Fig. 5) and similar ATGL and HSL expressions (Fig. 4). Previously, a high-fructose diet decreases the insulin levels [20], while the cafeteria diet increased the levels of this hormone (Table 2), suggesting that insulin regulates the cytosolic lipase activities. It has been documented that mice liver content of a selective inhibitor of ATGL, encoded by G0/G1 switch gene 2, is increased by fasting and decreased by refeeding [36,37]. Thus, it is possible that an increase in ATGL activity in liver from cafeteria diet-fed rats is part of the observed elevation in cytosolic lipases activities. NEFA release from endogenous TAG hydrolysis in hepatic

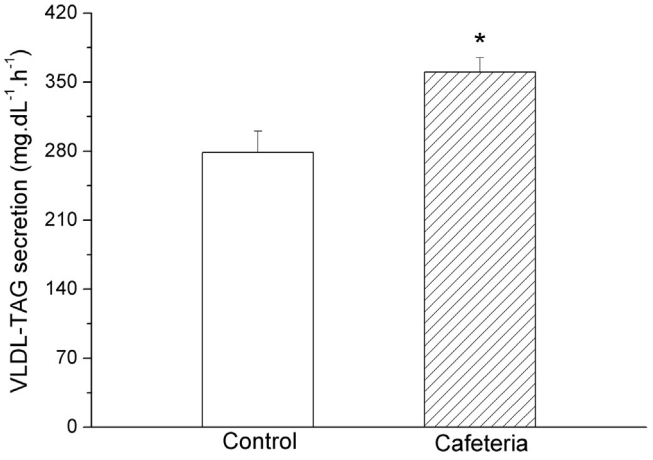

Fig. 6. Effect of cafeteria diet feeding on the rate of very low density lipoprotein (VLDL)-triacylglycerol (TAG) secretion. Bars represent mean ± SEM, n = 7–8 rats. $^{*}P < 0.05$ versus control diet.

tissue may regulate gene expression [38]. An earlier study revealed that the presence of long-chain NEFAs, in the incubation medium of hepatocytes, increases the CPT 1 expression, but not the CPT 2 [39]. More recently, it was demonstrated that long-chain NEFAs induces CPT 1 expression through a PPAR-α-independent mechanism in rat hepatoma cells [40]. These findings are in agreement with our *in vivo* findings, suggesting that there is an activation of catabolic pathways in response to the cafeteria diet. Experiments are needed to directly evaluate the fatty acid oxidation and firmly establish this hypothesis.

Different mechanisms regulate the process of VLDL secretion, such as insulin, NEFAs, and the sympathetic nervous system. An increase in VLDL secretion has been associated with insulin resistance [10–14]. However, some studies showed that VLDL secretion was similar regardless of insulin resistance [15,41–43]. Our previous studies have shown that cafeteria diet feeding increases the glucose uptake by white and brown adipose tissues [21,22,44]. The cafeteria diet also increases the hepatic glycolysis, as evaluated by $^{3}H_2O$ production from ^{3}H-glucose, and decreases the gluconeogenesis, evaluate by ^{14}C-glucose production from 2-^{14}C-pyruvate both *in vitro* using liver slices (Martins-Santos, Kettelhut, and Migliorini, unpublished data). These findings suggest that the liver and adipose tissue insulin sensitivity are not impaired in cafeteria diet-fed rats, but these rats still have an increased rate of VLDL-TAG secretion (Fig. 6). Furthermore, the plasma NEFA levels [21] and NE turnover rates (Fig. 2, Table 3) were similar between groups. The increase in the cytosolic lipases activities and *de novo* FA synthesis in liver from cafeteria diet-fed rats suggest an increased NEFA content in the hepatocytes. NEFAs are activators of hepatocyte nuclear factor 4-α, a transcriptional factor responsible for MTTP expression [45,46], overcoming a possible inhibitory effect exerted by insulin. Some studies have suggested that this hormone is not the main regulator of MTTP expression [47–49]. Insulin has a dose dependent effect (0.4–400 ng mL^{-1}) on ApoB mRNA editing, while oleic acid (1 mM) has no effect [50]. Insulin increases the ApoB48 mRNA expression and decreases ApoB100 mRNA expression in rat primary hepatocytes, inducing an increase in the total ApoB secretion without changing the ApoB100 secretion [50]. However, the regulation of ApoB at the transcriptional level is of minor importance. Rather, ApoB is regulated by the availability of TAG for VLDL assembly [16] and by insulin that decreases ApoB secretion by promoting ApoB degradation in the hepatocyte [51,52].

In summary, the cafeteria diet induces NAFLD. This effect may be

explained by a marked increase in the liver lipogenesis rate that could not be counteracted by the elevated cytosolic lipases activities and VLDL-TAG secretion rate. Our findings suggest the sympathetic nervous system plays no relevant role in cafeteria diet-induced changes in liver lipid homeostasis, which may represent responses to nutritional and hormonal regulation. Thus, increases in the cytosolic lipases activities and VLDL-TAG secretion rates were not sufficient to prevent the development of NAFLD.

Compliance with ethical standards

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Conflicts of interest

The authors report no conflicts of interest.

Ethical approval

The care and treatment of the rats received prior institutional approval by the Ethical Committee of Federal University of São João Del Rei, Minas Gerais, Brazil (#016/2014).

Author contributions

All authors contributed to the development, analysis and drafting of this article.

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