The effects of hypercaloric diets on glucose homeostasis in the rat: Influence of saturated and monounsaturated dietary lipids

ARTICLE *in* CELL BIOCHEMISTRY AND FUNCTION · OCTOBER 2011

Impact Factor: 2.01 · DOI: 10.1002/cbf.1789 · Source: PubMed

CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct 2011; **29**: 569–576.
Published online 12 August 2011 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/cbf.1789

The effects of hypercaloric diets on glucose homeostasis in the rat: influence of saturated and monounsaturated dietary lipids

A. L. Hoefel[1], F. Hansen[1], P. D. Rosa[1], A. M. de Assis[1], S. L. da Silveira[1], C. C. Denardin[1], L. Pettenuzzo[1], P. R. Augusti[2], S. Somacal[2], T. Emanuelli[2], M. L. S. Perry[1,†] and C. M. D. Wannmacher[1]*

[1]*Departamento de Bioquímica, Instituto de Ciências Básicas e da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[2]*Departamento de Ciência e Tecnologia dos Alimentos, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Porto Alegre, RS, Brazil*

Consumption of energy-dense/high-fat diets is strongly and positively associated with overweight and obesity, which are associated with increase in the prevalence of certain chronic diseases. We evaluated the effect of hypercaloric/fat or normocaloric diets on some biochemical parameters in rats. Seventy-two rats were divided into four groups that were fed for 16 weeks with diets: normocaloric [9.12% soy oil, normocaloric soy oil (NSO)], hypercaloric olive oil [43.8% olive oil, hypercaloric olive oil (HOO)], hypercaloric saturated fat [43.8% saturated fat, hypercaloric saturated fat (HSF)] and normocaloric saturated fat [43.8% saturated fat, normocaloric saturated fat (NSF)]. HSF rats consumed more calories daily than the others and gained more retroperitoneal fat, although HSF and HOO rats had higher body weight. In liver, glycogen synthesis and concentration were higher in rats HSF and NSF. In plasma, total cholesterol (TC) levels were higher in HSF rats than in the others, and triacylglycerol (TAG) levels were lower in HOO and higher in HSF rats in relation to the others. In liver, TC and TAG were elevated in HSF, NSF and HOO rats. Paraoxonase 1 activity, which is related to high-density lipoprotein cholesterol and has anti-atherogenic role was lower in rats HSF. In HOO rats, glucose tolerance test was altered, but insulin tolerance test was normal. These results suggest that consumption of energy-dense/high-fat diets, both saturated or monounsaturated, causes damaging effects. However, more studies are necessary to understand the mechanisms by which these diets cause the metabolic alterations observed. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS—hypercaloric diet; glucose homeostasis; saturated fat; monounsaturated fat; rats

INTRODUCTION

Diet and nutrition are important factors in promoting and maintaining health.[1] High-fat/caloric diet lead to overweight and obesity, which are strongly associated with increase in the prevalence of others chronic diseases; these chronic disease overwhelm health services generating high costs. In 2001, they contributed to 60% of the 56·5 million deaths, and this represented 46% of the total cost of illness. It is estimated that by 2020, these values will reach a plateau of 57%.[2] Nearly half of all deaths from chronic diseases are attributed to cardiovascular diseases (CVDs), obesity and diabetes, and these are not just problems of developing regions.[2]

The prevalence of overweight and obesity is increasing exponentially in many countries.[3,4] It is estimated that worldwide, more than 1 billion people are overweight, and 300 million are obese.[2,5,6]

Obesity is characterized by the accumulation of body fat. It is a complex and multifactorial disease where genetic predisposition and environmental factors are involved.[7,8] In simple terms, obesity occurs when energy intake exceeds expenditure.[9] Obesity is the major risk factor for development of the insulin resistance (IR) and diabetes mellitus type II. Among diabetics, the major causes of death are CVD and renal failure.[10]

In obesity states occurs increased circulating fatty acids that, in turn, causes the increase of intracellular acyl-CoA long chain and diacylglycerols (DAGs). This increase in intracellular DAG activates Protein kinase Cs (PKCs), which phosphorylate insulin receptor substrate (IRS1) on serine residue, reducing this action in insulin signalling.[11]

The central nervous system (CNS) has mechanisms to maintain homeostasis of body weight, which is tightly regulated for centrals and peripheral signals reflecting the availability of body energy.[9] In spite of the existence of these mechanisms, when humans or rodents are exposed to hypercaloric diets, they lose this mechanism and become obese.[12]

Fat and oils are one important source of energy for the body. Evidences suggest that the Paleolitic Age humans evolved eating a diet that contained smaller amounts of saturated fat and *trans* fatty acids in relation to the current diet.[13]

*Correspondence to: Clóvis Milton Duval Wannmacher; Rua Ramiro Barcelos, 2600 anexo, Santana, Porto Alegre, RS, Brazil, CEP 90035–003. E-mail: clovisdw@ufrgs.br
†In memoriam

Received 21 February 2011
Revised 13 May 2011
Accepted 23 June 2011

Diets rich in calories, especially highly processed, and in saturated fat, as observed in the usual occidental diet, are considered the main cause of atherosclerosis and CVD, particularly coronary heart disease.[13] Besides elevating total cholesterol (TC), triacylglycerol (TAG), and low-density lipoprotein (LDL) levels,[9,14] fat saturated is related with decreased insulin signalling at the level of CNS, causing IR in the brain and hyperphagia and leading to obesity.[9]

The so-called Mediterranean diet, in which the main source of dietary lipid is the olive oil, rich in monounsaturated fat, is associated with low rates of obesity, CVD, neurodegenerative diseases and the incidence of cancers.[15] Laboratory studies shown that oleic acid mimics the action of insulin in the hypothalamus, decreasing food intake and hepatic glucose production and thus regulating body weight.[16]

Considering the following: (1) the consumption of diets rich in calories and fats is in part responsible for the epidemic of obesity and its related problems in industrialized countries and (2) the costs generated by problems associated with chronic diseases is high and has a strong impact on the economy, the objective of this study was to assess the effects of hypercaloric or normocaloric diets with saturated or monoinsaturated fats in some metabolic parameters in rats.

MATERIALS AND METHODS

Chemicals

D-[U-^{14}C]glucose (297 mCi·mmol^{-1}) and [U-^{14}C]alanine (157 mCi·mmol^{-1}) were purchased from Amersham International (Little Chalfont, Bucks, UK). Triton X-100 was purchased from Labsynth Produtos de Laboratório Ltda, SP, Brazil, and bovine albumin was purchased from J. T. Baker Chemical Company, Phillipsburg, NJ, USA. Optiphase Hi-Safe 3 was purchased from Perkin-Elmer (RJ, Brazil), TCA from Merck S.A. (RS, Brazil) and D-Glucose from Quimbrás (RJ, Brazil). POP and POPOP were purchased from Sigma (St. Louis/EUA), kits for dosage NEFAs from Roche (Germany) and kits for dosage TAG and cholesterol from Diagnostica SA (MG, Brazil).

Animals and diets

Seventy-two adult male Wistar rats 60 days old from the Central Animal House of the Department of Biochemistry, which were divided into two experiments (40 and 32 rats), were maintained under a standard dark–light cycle (lights on between 07:00 h and 19:00 h) at room temperature of 22 ± 2 °C. The protocol used for this research was employed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of Veterinary Medicine and Animal Science from the University of São Paulo, Brazil.

The hypercaloric diets had 43·2% of total calories as fat, varying only in fat quality; in normocaloric soy oil (NSO) group, 9·12% of total caloric intake was from soy oil. Although in the hypercaloric diet with olive oil (HOO) group, 43·2% of total fats came from virgin olive oil and in hypercaloric with saturated fat (HSF) group, 15·84% came from virgin olive oil, 14·04% from palmitic acid (16:0), 6·48% myristic acid (14:0) and 6·84% stearic acid (18:0). The normocaloric saturated fat (NSF) group consisted of the same diet with saturated fat (HSF) but received the same calories of the NSO group. During the treatment period (16 weeks), the animals had free access to water.

Fat determination in the feces

The fat determination in the feces was accomplished by the method Bligh-Dyer.[17] Shortly, samples of feces (2·5 g) were placed in *Falcon* tubes, and chloroform and methanol (1:2) and distilled water were added. After agitation for 30 min, chloroform and Na_2SO_4 1·5% (1:1) were added, and the samples were agitated for 2 min. Soon after the samples were centrifuged and the layer containing the fat was removed and placed in test tubes containing 0·8 g of Na_2SO_4 and immediately vortexed vigorously and filtered. Five milliliters of the filtrate were collected and placed in beaker previously weighed, solvent and humidity removed and weighed again.

Glucose tolerance test and insulin tolerance test

When we verified the glycogen metabolism (synthesis and concentration), the results intrigued us, and we checked the glucose tolerance test (GTT) and insulin tolerance test (ITT) in the second group of rats after 6 h of starvation. In GTT, a 50% glucose solution was injected into the animals (2 mg·kg^{-1} ip). ITT was accomplished 3 days after GTT. Insulin (1 U·kg^{-1}) was injected intraperitoneally.

Blood was collected through a small puncture on the tail immediately prior to the injection, as well as 30, 60 and 120 min afterward. At each time point, glucose was measured using a glucometer (AccuChek Active, Roche Diagnostics, USA).

Tissue preparation

Rats were killed by decapitation without anesthesia. Blood was collected immediately, and the plasma was stored at −70 °C until analysed. Fat tissues, liver and soleus muscle were immediately removed and weighted.

Paraoxonase I activity

Paraoxonase I (PON1) activity was assessed by measuring the rate of paraoxon hydrolysis to yield p-nitrophenol, at 412 nm and 25 °C as previously described.[18]

Protein quantification

Protein was measured according to Lowry *et al.*[19] using bovine serum albumin as the standard.

Blood Biochemical Parameters

Plasma TAG and TC levels were measured using commercial kits (Labtest, MG, Brazil). Reactions were performed

using the Labmax apparatus (Labtest, MG, Brazil). Plasma free fatty acid activities were measured using commercial kits (Roche Diagnostics, Germany, and MP Biomedicals, NY, USA, respectively).

Glycogen synthesis in liver and muscle

For the measurement of glycogen synthesis, 100- to 120-mg fragments of liver or soleus were dissected and cut into 300-μm slices using a McIlwain tissue chopper. Slices were incubated in a beaker with a medium containing Dulbbecco buffer 2·7 mmol·l^{-1} (pH 7.3), 5 mmol·l^{-1} of glucose and 0·3 μCi D[U-^{14}C]glucose or 0·2 mmol·l^{-1} alanine and 0·3 μCi [U-^{14}C]alanine. Incubations were carried out in ambient content that was gassed with a 95% O_2:5% CO_2 and incubated at 37°C for 1 h in a metabolic shaker (60 cycles per minute), according to the method of Dolnikoff *et al*.[20] Incubation was stopped by placing the beaker in ice. KOH 60% (1 ml) was added to each beaker and immediately in boiling water bath for 15 min. After this, ethanol was added to the tubes for glycogen precipitation. After precipitation, glycogen was resuspended in water, and the scintillation liquid (Opti-Phase HiSafe3 from PerkinElmer-USA) was added. The samples were assessed in a scintillation liquid counter.

Hepatic glycogen concentration

The glycogen concentration was measured from slices of liver maintained in KOH 30%, placed in a water bath at 100°C and subjected to other subsequent steps as described above. After obtaining the glycogen pellet that was dissolved in Milli-Q water and an aliquot was removed for estimation of glycogen content by the colorimetric method by Krisman,[21] with color reagent produced from a solution of KI, I_2 and saturated solution $CaCl_2$. The reading was held on Beckman DU 640 spectrophotometer provided by the Department of Biochemistry.

Statistical analysis

Comparisons between different groups were performed employing analysis of variance or repeated measure analysis (RMA) or area under the curve (AUC) followed by the *post hoc* Tukey test when *F* values were significant. The level of significance adopted was $p < 0.05$.

RESULTS

Parameters of caloric intake and body weight

Figure 1 show the average caloric intake of rats. The RMA showed that the groups had a small decline in consumption from 4 weeks, and this decrease was similar for all groups. The RMA also showed that the caloric intake of the rats from the HSF group was always larger than the intake of the rats from the other groups (days × groups interaction, $F_{(45,720)} = 1.8$; $p < 0.05$).

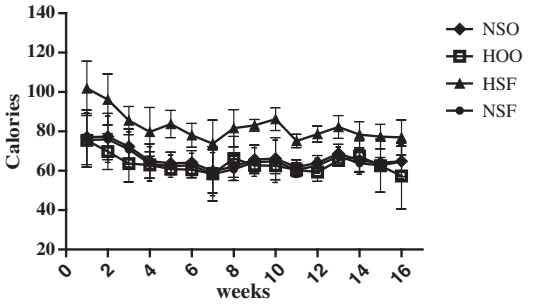

Figure 1. Average weekly caloric consumption by adult male rats after 16 weeks under different nutritional treatments. Data are mean ± SD for 17–18 animals per group and are expressed in kilocalories. Consumption of animals from the saturated fat group was significantly higher than that of the other groups (repeated measure analysis of variance, days × groups interaction ($F_{(45,720)} = 1.78$; $p < 0.05$). NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF: normocaloric saturated fat=saturated diet with normocaloric ingestion

Rats from the HSF and HOO groups showed higher weight gains (57·3% for HSF and 37·2% for HOO) when compared with rats from the NSO group ($F_{(3,58)} = 16·32$, $p < 0.01$) (Figure 2). The RMA also revealed that the rats from the NSF group gained less weight than the rats from the other groups ($F_{(45,870)} = 4·79$; $p < 0·01$) (Table 1).

Parameters of the weights are presented in Table 1. No difference was found between the weights of epididymal adipose tissue between groups. However, the rats from the HSF group had a greater accumulation of abdominal adipose tissue than the rats from the other groups ($F_{(3,58)} = 13·59$; $p < 0·01$).

In evaluating the caloric intake, we expected a different effect on weight gain observed in the rats from the HSF group. We then evaluated the loss of fat in the feces, which

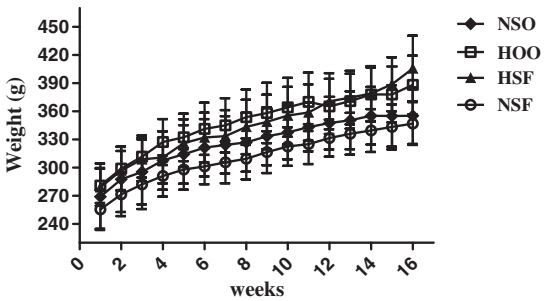

Figure 2. Average weekly body weight of adult male rats after 16 weeks under different nutritional treatments. Data are mean ± SD for 17–18 animals per group. Body weight of animals from saturated fat and olive oil groups are significantly higher than that of the other groups (repeated measure analysis of variance, week × groups interaction ($F_{(3,\ 58)} = 16.32$; $p < 0·01$). NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion

Table 1. Body weight, adipose tissue weight and soleus muscle weight of adult male rats under different nutritional treatments

Measures	NSO	HOO	HSF	NSF
Initial body weight (g) 2 months old	254·5±29.3	249.1±18.5	248.8±19.0	248·1±20·5
Final body weight (g) 6 months old	355·9±29·0	388·1±31·4	408·1*±32·7	344·8±21·3
Body weight gain (g)	101·3±18·2	139·0*±31·5	159·3*±21·4	96·7±21·5
Retroperitoneal adipose tissue (g)	6·6±1·6	8·9±2·9	11·5*±2·4	7·4±2·2
Epididymal adipose tissue (g)	8·4±2·7	9·3±2·4	8·2±2·6	7·2±2·0
Total adipose (g)	15·0±3·7	18·2*±4·9	19·7*±4·2	14·6±4·1
Soleus muscle (mg)	120·0±4·1	130·4±8·2	131·3±2·6	112·8±5·1

Data are mean±SD for 17–18 animals per group. NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil = high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion.
*$p<0·05$ compared with the other groups (Tukey test).

could explain a lower weight gain than expected in HSF rats because their calorie intake was higher than that of the other. We observed a greater loss of fat in the feces of the rats from the HSF and NSF groups ($F_{(3,36)}=6·49$; $p<0·001$) (Figure 3).

Glycogen synthesis in liver and muscle

Figure 4 shows the effect of diets on hepatic glycogen synthesis, for the direct and indirect pathways. Glycogen synthesis from glucose ($F_{(3,30)}=5·75$; $p<0·003$) (Figure 4A) and from alanine ($F_{(3,30)}=17·23$; $p<0·001$) (Figure 4B) were higher in the rats from the HSF and NSF groups. In the soleus muscle, glycogen synthesis (Figure 4C) was increased in the rats from the NSF in relation to the NSO group ($F_{(3,\ 26)}=3·79$; $p<0·02$).

Glycogen concentration in the liver

To evaluate the effect of treatments on liver glycogen content, we measured the glycogen concentration. As shown in Figure 5, the rats from the HSF and NSF groups had the highest concentrations of glycogen ($F_{(3,23)}=6·12$; $p<0·003$). Glucose uptake in the soleus muscle showed no differences between the groups (data not shown).

Glucose tolerance test and insulin tolerance test

Glucose tolerance test (Figure 6A) was altered in the rats from the HOO group, which achieved higher glucose levels after glucose administration when compared with the rats from the other groups. Analysis of the values of AUC showed that the rats from the HOO group were different from the rats from the other groups ($F_{(3,\ 29)}=5·38$, $p<0·005$).

The analysis of the ITT did not show any difference between the groups (30, 60 or 120 min) (Figure 6B).

Biochemical parameters

Biochemical parameters are presented in Table 2. We evaluated the effects of diets in TC and TAG in the plasma and the liver. In the plasma, the rats from the HSF group had higher levels of TC, and the rats from the NSF group had lower levels than the rats from the other groups ($F_{(3,28)}=20·24$; $p<0·001$). The TAG levels were higher in the rats from the HSF group and lower in the rats from the HOO group than in the rats from the other groups ($F_{(3,28)}=22·39$; $p<0·001$).

In the liver, TC content was elevated in all groups receiving hypercaloric diets, and the rats from the HOO group had the highest values ($F_{(3,28)}=40·99$; $p<0·001$). TAG content was higher in the rats from the HOO group than in the rats from the other groups ($F_{(3,28)}=7·73$; $p<0·001$). PON1 activity, which is related to high-density lipoprotein (HDL) cholesterol, was lower in the rats from the HSF group than in the rats from the other groups ($F_{(3,23)}=3·98$; $p<0·02$).

Table 2. Biochemical parameters of the animals fed different diets

Measures	NSO	HOO	HSF	NSF
Plasma total cholesterol (mg·dl^{-1})	90·3±9·8	100·1±11·9	115·9*±11·5	78·6±7·0
Liver cholesterol (mg/100 mg)	0·29±0·03	0·43*±0·04	0·34*±0·02	0·32*±0·02
Plasma triacylglycerol (mg·dl^{-1})	153·7±31·7	97·9±18·2	210·2*±30·0	126·0±32·2
Liver triacylglycerol (mg/100 mg)	2·4±0·36	3·9*±1·0	2·7±0·6	2·4±0·70
Plasma non-esterified fatty acids (mg·dl^{-1})	591·6±205·0	454·7±53·6	621·09±156·6	485·0±67·6
Plasma glucose after 6h fasting (mg·dl^{-1})	111·0±2·7	104·0±4·4	104·0±0·9	110·0±3·6
Plasma paroxonase I activity (U·ml^{-1})	228·0±21·4	230·5±56·9	166·4*±35·3	219·5±27·3

Data are mean±SD for nine animals per group. NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion.
*$p<0·05$ compared with the other groups (Tukey test).

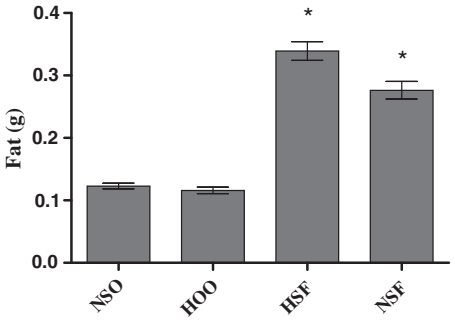

Figure 3. Loss of lipids in the feces by adult male rats after 16 weeks under different nutritional treatments. Data are mean ± SD for eight animals per group. NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion. *$p < 0{\cdot}05$ compared with the other groups (Tukey test)

DISCUSSION

In our study, the rats exposed to hypercaloric diets for 16 weeks had alterations in caloric intake, body fat deposition, and some biochemical parameters. Rats under hypercaloric diet with saturated fat increased the daily caloric intake (Figure 1) and deposition of retroperitoneal fat (Table 2). This does not happen with the animals under hypercaloric diet rich in monounsaturated fat. These results agree with other studies comparing the effects of both fats, showing that they have opposite effects on food intake,[9,16] body weight,[9,16,22] fat deposition[9,16,22] and metabolic parameters.[9,16] Control of metabolism and food intake occurs partly at the level of the hypothalamus.[22,23] Saturated and monounsaturated fatty acids triggers responses opposing signalling[24] in the hypothalamus. In rats, administration of oleic acid in the third ventricle decreased food intake and inhibited hepatic glucose production.[16] The use of inhibitors of K^+ channels sensitive to ATP (K^+_{ATP}) blocked the effect of oleic acid, suggesting that its action occurs via this mechanism.[16] Palmitic acid administered in the hypothalamus caused IR in the CNS, compromising the ability of this hormone in regulating food intake and energy expenditure.[7,9] Palmitic acid activates a PKC serine-threonine kinases (PKC-θ), which migrates from the intracellular pool to the surface of the membrane where it interacts with receptors and alter cell signalling, including metabolic alterations and response to food intake.[9]

In the present investigation, rats that consumed hypercaloric diet '*ad libitum*' (HSF and HOO groups) gained more weight (Figure 2), whereas only the rats from the HSF group

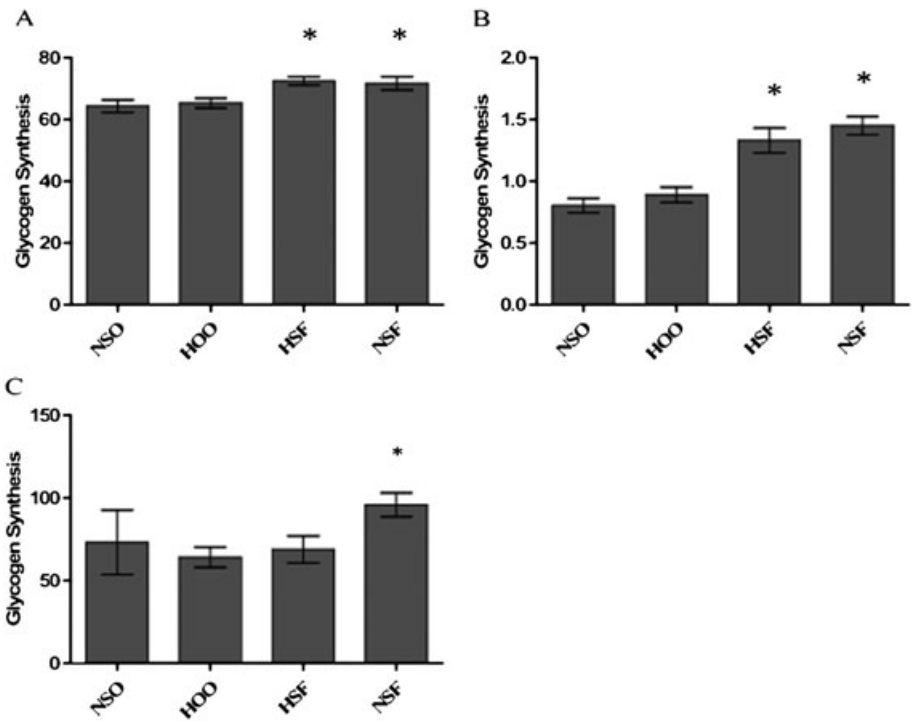

Figure 4. Glycogen synthesis in the liver from D[U-^{14}C]glucose (A), in the liver from [U-^{14}C]alanine (B) and in the soleus muscle from D[U-^{14}C]glucose (C) of adult male rats after 16 weeks under different nutritional treatments. Data are mean ± SD for nine animals per group and are expressed as picomole of glucose (A and C) or alanine (B) incorporated into glycogen per milligram of tissue per hour. NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion. *$p < 0{\cdot}05$ compared with the other groups (Tukey test)

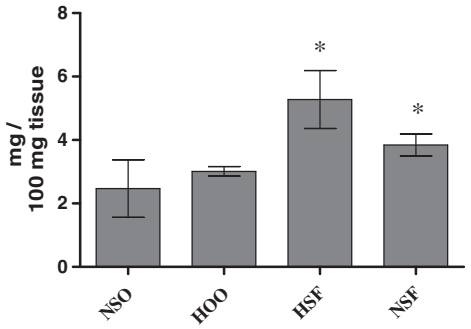

Figure 5. Hepatic glycogen concentration of adult male rats after 16 weeks under different nutritional treatments. Data are mean ± SD for nine to ten animals per group and are expressed as milligrams of glycogen per 100 mg of tissue. NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion. $*p < 0{\cdot}05$ compared with the other groups (Tukey test)

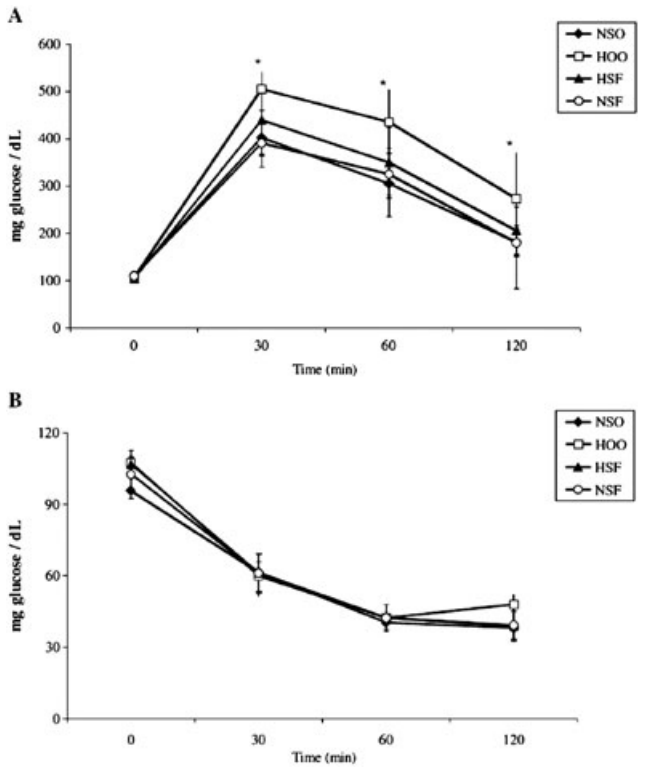

Figure 6. Glucose tolerance test (A) and insulin tolerance test (B) in adult male rats after 16 weeks under different nutritional treatments. Data are mean ± SD for eight animals per group and are expressed as milligrams per deciliter of glucose. NSO, normocaloric soy oil=normocaloric diet predominantly with soy oil; HOO, hypercaloric olive oil=high-fat hypercaloric diet predominantly with olive oil; HSF, hypercaloric saturated fat=high-fat hypercaloric diet predominantly with palmitic, myristic and stearic acids; NSF, normocaloric saturated fat=saturated diet with normocaloric ingestion

had higher retroperitoneal fat deposition (Table 1). In humans, ingestion of monounsaturated fat was associated with a lower deposition of fat in the abdominal region.[25] Rats from the NSF and HSF groups did not gain excess of weight, in agreement with Woods *et al.*[26] that demonstrated that the association of obesity with high calorie/fat diet is caused by excessive consumption and not by the fat composition of the diet. Because of the higher caloric intake of the rats from the HSF group, a higher body weight gain could be expected compared with the other groups.[9,12,26] In our experiment, rats from the HSF and NSF groups had greater loss of lipids in the feces (Figure 3), which prevented the excessive weight gain of rats from the two groups.

Epidemiologic[27] and experimental[9,28,29] studies indicated that saturated fats are related to changes in parameters of insulin response. To investigate the effects of hypercaloric or normocaloric diets with different types of fats on glycogen metabolism, we evaluated glycogen synthesis from glucose or alanine and glycogen concentration in the liver and glucose uptake and glycogen synthesis in the soleus muscle. In the liver, glycogen synthesis was increased from the glucose and alanine (indirect gluconeogenesis) in the rats from the HSF and NSF groups (Figure 4A and 4B). Duarte *et al.*[30], using glucose as substrate, found similar results in rats under high calorie/fat diet. Moreover, the glycogen concentration in the liver (Figure 5) also was increased in rats from the HSF and NSF groups. A study using high-fat diet (HFD) for a period of 12 months found a decrease in hepatic glycogen synthesis from glucose.[29] These animals were insulin resistants, different from those of Duarte *et al.*[30] In these states of IR, the hepatic concentration and synthesis of glycogen were reduced.[31] In diabetic patients and in states of IR occur difficulty in reduce gluconeogenesis and glycogen synthesis from alanine.[32] Skeletal muscle represents the major site of peripheral glucose metabolism. In this tissue, IR manifests primarily as a reduction in glycogen synthesis stimulated by insulin. The investigation of glycogen synthesis in the soleus muscle from glucose showed that only rats from the NSF group had an increased synthesis as compared with the rats from the other groups (Figure 4C). We did not observe differences of glucose uptake in all the treated groups (data not shown). Studies using HFD with saturated fat have found a decrease in glucose uptake in the muscle.[33] Addition of 20 $\mu mol \cdot l^{-1}$ palmitic acid to primary cultures of rat skeletal muscle cells for 24 h caused a decrease both on glucose uptake and glycogen synthesis stimulated by insulin.[28]

We found that rats from the HOO group had alteration in GTT with an AUC greater than that observed in the rats from the other groups (Figure 6A and 6B), but ITT did not differ between the groups. Data from other experimental protocols show that oleic acid improves insulin response.[16,34] These results may indicate changes in insulin secretion involving B cell. In these cells, chronic exposure to high levels of oleic acid alters insulin secretion in response to stimulation with glucose.[35] Fatty acids promote mitochondrial uncoupling,[36] both through uncoupling protein (UCP)-dependent and UCP-independent uncoupling. Increased expression of UCP decreases the ratio of Adenosine Triphosphate (ATP):Adenosine Diphosphate (ADP) by uncoupling mitochondrial oxidative phosphorylation, thereby

decreasing insulin secretion stimulated by glucose without changes in glucose metabolism.[37] If this increased AUC in GTT in the rats from the HOO group is caused by decreased insulin secretion UCP-mediated or decreased expression of gene associated with stimulated insulin secretion, as verified by Pinnich *et al*.[38] is actually under investigation.

Important clinical markers for hypertension, cardiovascular risk and atherosclerosis are hypertriglyceridemia and reduced concentrations of HDL cholesterol. There is a relationship between high intake of saturated fat with CVD[39]; increased plasma levels of TC, TAG and LDL; and decreased HDL levels,[40] as also is a well-established beneficial effect of a diet rich in olive oil in reducing CVD[15] and positive changes in lipid profile.[34] On the other hand, it seems that excessive caloric intake is always related to the usual occidental diet, which is rich in saturated fats, unlike the Mediterranean diet that has significant levels of monounsaturated fat.[9,12,14–16] Our results indicate that quality and quantity of fat influence the plasma lipid profile.

Rats from the HSF group had higher plasma levels of TC and TAG compared with rats from the other groups. In liver, TC concentrations were higher in the rats from the HSF, the HOO and the NSF groups compared with those in the rats from the NSO group. The TAG content in the liver of the rats from the HOO group was higher than that of the rats from the other groups. The effects of olive oil in reducing plasma lipid levels are well documented in the literature,[34] but studies involving measurements of concentrations in tissues are rare in scientific literature. HepG2 cells treated for 6 h with oleate or palmitate showed higher TAG accumulation when treated with oleate.[41] This could explain the increased TAG in the liver of the rats from the HOO group.

Paraoxonase 1 is an antioxidant enzyme associated with HDL, and its activity is inversely related to the risk of CVD and atherosclerosis.[42] In the presence of systemic inflammation, as in cases of obesity, antioxidant enzymes associated with HDL, including PON1, dissociates from lipoprotein, resulting in the generation of oxidized and peroxidized lipids that are atherogenic.[43] In PON1 knockout mice, an atherogenic diet causes significantly greater atherosclerosis than in wild-type mice.[44] The rats from the HSF group had decreased plasma PON 1 activity, hypercholesterolemia and increased retroperitoneal fat deposition, compared with rats from the other groups. In humans, these data would point to increased cardiovascular risk for the consumption of high calorie diet with saturated fat.

In summary, the present study suggest that consumption of hypercaloric diets with moderate levels of fatty acids, both saturated or monounsaturated, provokes negative effects on metabolism. Hypercaloric diet with saturated fat significantly changed caloric intake, fat deposition, hepatic glycogen synthesis and plasma lipid profile. However, hypercaloric diet with monounsaturated fat also showed negative effects in TC and TAG content in the liver and in AUC of the TTG. Further studies to understand the mechanisms by which this occurs, are currently under investigation in our research group.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

ACKNOWLEDGEMENT

This study was supported by grants from the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Pró-Reitoria de Pesquisa–Universidade Federal do Rio Grande do Sul (PROPESQ/UFRGS), IBN-NET.

REFERENCES

1. Perichart-Perera O, Balas-Nakash M, Rodriguez-Cano A, *et al*. Correlates of Dietary Energy Sources with Cardiovascular Disease Risk Markers in Mexican School-Age Children. *J Am Diet Assoc* 2010; **110**(2): 253–260.
2. WHO - World Health Organization: Diet, Nutrition, and the Prevention of Chronic Diseases. FAO/WHO Technical Report. 2003; 916.
3. Allison DB, Fontaine KR, Manson JA, *et al*. Annual deaths attributable to obesity in the US. *JAMA* 1999; **282**(16): 1530–1538.
4. Must A, Spadano J, Coakley EH, *et al*. The disease burden associated with overweight and obesity. *JAMA* 1999; **282**(16): 1523–1529.
5. WHO - World Health Organization: Obesity – preventing and managing the global epidemic. Geneva: FAO/WHO Technical Report. 1999; 894.
6. Smyth S, Heron A. Diabetes and obesity: the twin epidemics. *Nat Med* 2006; **12**(1): 75–80.
7. Morentin PBM, Varela L, Ferno J, *et al*. Hypothalamic lipotoxicity and the metabolic syndrome. *Biochim Biophys Acta* 2010; **1801**(3): 350–361.
8. Stein CJ, Colditz GA. The Epidemic of Obesity. *J Clin Endocrinol Metab* 2004; **89**(6): 2522–2525.
9. Benoit SC, Kemp CJ, Elias CF, *et al*. Palmitic acid mediates hypothalamic insulin resistance by altering PKC-θ subcellular localization in rodents. *J Clin Invest* 2009; **119**(9): 2577–2589.
10. ADA - American Diabetes Association. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997; **27**(suppl 1): S5–S10.
11. McGarry JD. Dysregulation of fatty acid metabolism in the etiology of type diabetes. *Diabetes* 2002; **51**(1): 7–18.
12. Woods SC, D´Alessio DA, Tso P, *et al*. Consumption of a high-fat diet alters the homeostatic regulation of energy balance. *Physiol Behav* 2004; **83**(4): 573–578.
13. Simopoulos A. Essential fatty acids in health and chronic disease. *Am J Clin Nutr* 1999; **70**(suppl3): 560–569.
14. Tremblay AJ, Després JP, Piché ME, *et al*. Associations between the fatty acid content of triglyceride, visceral adipose tissue accumulation and components of the insulin resistance syndrome. *Metabolism* 2004; **53**(3): 310–317.
15. Perez-Gimenez F, Alvarez de CF, Badimon L, *et al*. International conference on the healthy effect of virgin olive oil. *Eur J Clin Invest* 2004; **35**(7): 421–424.
16. Obici S, Feng Z, Morgan K, *et al*. Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* 2002; **51**(2): 271–275.
17. Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. *Can J Biochem Physiol* 1959; **37**(8): 911–917.
18. Bolayirli IM, Aslan M, Balci H, *et al*. Effects of atorvastatin therapy on hypercholesterolemic rabbits with respect to oxidative stress, nitric oxide pathway and homocysteine. *Life Sci* 2007; **81**(2): 121–127.
19. Lowry OH, Rosebrough NJ, Farr AL, *et al*. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.

20. Dolnikoff M, Martín-Hidalgo A, Machado UF, *et al*. Decreased lipolysis and enhanced glycerol and glucose utilization by adipose tissue prior to development of obesity in monosodium glutamate (MSG) treated-rats. *Int J Obes Rel Metab Dis* 2001; **25**(3): 426–433.
21. Krisman CR. A method for the colorimetric estimation of glycogen with iodine. *Anal Biochem* 1962; **4**(1): 17–23.
22. Woods SC, Seeley RJ, Porte D, Jr, *et al*. Signals that regulate food intake and energy homeostasis. *Science* 1998; **280**(5368): 1378–1383.
23. Schwartz MW, Woods SC, Porte D, JR, *et al*. *Central nervous system control of food intake. Nature* 2000; **404**(6778): 661–671.
24. Loftus TM, Jaworsky DE, Frehywot GL, *et al*. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 2000; **288**(5475): 2379–2381.
25. Panigua JA, de La Sacristana AG, Romero I, *et al*. Monounsaturated Fat Rich Diet Prevents Central Body Fat Distribution and Decreases Postprandial Adiponectin Expression Induced by a Carbohydrate-Rich Diet in Insulin-Resistant Subjects. *Diabets Care* 2007; **30**(7): 1717–1723.
26. Woods SC, Seeley RJ, Rushing PA, *et al*. A controlled high-fat diet induces an obese syndrome in rats. *J Nutrition* 2003; **133**(4): 1081–1087.
27. Marshall JA, Hamman RF, Baxter J. High-fat, low-carbohydrate diet and the etiology of non-insulin-dependent diabetes mellitus: the San Luis Valley Diabetes Study. *Am J Epidemiol* 1991; **134**(6): 590–603.
28. Hirabara SM, Curi R, Maechler P. Saturated fatty acid-induced insulin resistance is associated with motochondrial dysfunction in skeletal muscle cells. *J Cel Physiol* 2010; **222**(1): 187–194.
29. De Assis AM, Rieger DK, Longoni A. High Fat and Highly Thermolyzed Fat Diets Promote Insulin Resistance and Increase DNA Damage in Rats. *Ex Biol Med* 2009; **234**(11): 1296–1304.
30. Duarte ACGO, Fonseca DF, Manzoni MSJ, *et al*. Dieta hiperlipídica e capacidade secretória de insulina em ratos. *Rev Nutr* 2006; **19**(3): 341–348.
31. Gannon MC, Nuttall FQ. Effect of feeding, fasting, and diabetes on liver glycogen synthase activity, protein and mRNA in rats. *Diabetologia* 1997; **40**(7): 758–763.
32. DeFronzo RA. Banting Lecture. From triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009; **58**(4): 773–795.
33. Oakes ND, Cooney GJ, Camilleri S, *et al*. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes* 1997; **46**(11): 1768–1774.
34. Cicerale S, Lucas L, Keast R. Biological Activities of Phenolic Compounds Present in Virgin Olive Oil. *Int. J. Mol. Sci.* 2010; **11**(2): 458–479.
35. Lameloise N, Muzzin P, Prentki M, Assimacopoulos-Jeannet F. Uncoupling Protein 2: A Possible Link Between Fatty Acid Excess and Impaired Glucose-Induced Insulin Secretion? *Diabetes* 2001; **50**(4): 803–809.
36. Skulachev VP. Uncoupling: New approaches to an old problem of bioenergetics. *Biochim Biophys Acta* 1998; **1363**(2): 100–124.
37. Klingenberg M, Huang SG. Structure and function of the uncoupling protein from brown adipose tissue. *Biochim Biophys Acta* 1999; **1415**(2): 271–296.
38. Pinnick K, Neville M, Clark A, Fielding B. Reversibility of Metabolic and Morphological Changes Associated With Chronic Exposure of Pancreatic Islet b-Cells to Fatty Acids. *J Cell Biochem* 2010; **109**(4): 683–692.
39. Artaud-Wild SM, Connor SL, Sexton G, *et al*. Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. *A paradox. Circulation* 1993; **88**(6): 2771–2779.
40. LaRosa JC, Hunninghake D, Bush D, *et al*. The cholesterol facts. A summary of the evidence relating dietary fats, serum cholesterol, and coronary heart disease. A joint statement by the American Heart Association and the National Heart, Lung, and Blood Institute. *Circulation* 1990; **81**(5): 1721–1733.
41. Lee J, Cho HK, Kwon YH. Palmitate induces insulin resistance without significant intracellular triglyceride accumulation in HepG2 cells. *Metabolism* 2010; **59**(7): 927–934.
42. Getz GS, Reardon CA. Paraoxonase, a cardioprotective enzyme: continuing issues. *Curr Opin Lipidol* 2004; **15**(3): 261–267.
43. Soran H, Younis NN, Charlton-Menys V, Durrington P. Variation in paraoxonase-1 activity and atherosclerosis. *Curr Opin Lipidol* 2009; **20**(4): 265–274.
44. Shih DM, Gu L, Xia YR, Navab M, *et al*. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 1998; **394**: 284–287.

