High Fat and Highly Thermolyzed Fat Diets Promote Insulin Resistance and Increase DNA Damage in Rats

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Many studies have demonstrated that DNA damage may be associated with type 2 diabetes mellitus (T2DM) and its complications. The goal of this study was to evaluate the effects of the potential relationship between fat (thermolyzed) intake, glucose dyshomeostasis and DNA injury in rats. Biochemical parameters related to glucose metabolism (i.e., blood glucose levels, insulin tolerance tests, glucose tolerance tests and fat cell glucose oxidation) and general health parameters (i.e., body weight, retroperitoneal and epididymal adipose tissue) were evaluated in rats after a 12-month treatment with either a high fat or a high thermolyzed fat diet. The high fat diet (HFD) and high fat thermolyzed diet (HFTD) showed increased body weight and impaired insulin sensitivity at the studied time-points in insulin tolerance test (ITT) and glucose tolerance test (GTT). Interestingly, only animals subjected to the HFTD diet showed decreased epididymal fat cell glucose oxidation. We show which high fat diets have the capacity to reduce glycogen synthesis by direct and indirect pathways. HFTD promoted an increase in lipid peroxidation in the liver, demonstrating significant damage in lipids in relation to other groups. Blood and hippocampus DNA damage was significantly higher in animals subjected to HFDs, and the highest damage was observed in animals from the HFTD group. Striatum DNA damage was significantly higher in animals subjected to HFDs, compared with the control group. These results show a positive correlation between high fat diet, glucose dyshomeostasis, oxidative stress and DNA damage. Exp Biol Med 234:1296–1304, 2009

Key words: high fat diet; thermolyzed fat diet; type 2 diabetes mellitus; insulin resistance; DNA damage

Introduction

The tendency to overeat is spreading rapidly, and about 1 billion people worldwide are overweight or obese. In particular, around 60% of all cases of type 2 diabetes mellitus (T2DM), which is a pathological condition associated with insulin resistance, hyperglycemia and oxidative stress, can be attributed to obesity. Unhealthy and abundant ("junk") food, sugar-rich drinks and physical inactivity appear to be the main reasons for this epidemic (1).

Fat and energy intake have been shown to be important dietary factors that modulate insulin resistance and the risk of T2DM (2, 3). In this regard, diets that provide low carbohydrate, low fiber and high saturated fat contribute to disease complications in patients with T2DM and are not

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recommended. Particularly, high fat diets containing high levels of advanced glycation/lipoxidation end products (AGEs/ALEs), which are originated during the heat processing of food, are linked to the development of insulin resistance and T2DM (4, 5). High levels of fat have been reported to increase fat-mediated oxidative stress and decrease antioxidative enzyme activity (6, 7). The liver plays a central role in the maintenance of systemic lipid homeostasis and is especially susceptible to damage from reactive oxygen species (ROSs) (8). This organ supplies energy substrates to peripheral tissues via the Cori cycle and glycogen catabolism, and it is important for detoxification. Factors related to oxidative stress could be implicated in the functional impairment of the liver, associated with exacerbated nutrient oxidation (9, 10).

DNA damage may be associated with T2DM and its complications (11). In this context, the accumulation of oxidative stress-related products appears to be responsible, at least in part, for DNA damage in diabetes patients (12).

As noted above, the excessive intake of high (mainly thermolyzed) fat diets is linked to the occurrence of T2DM (4). On the other hand, DNA damage may be associated with T2DM and its complications (6). Fried foods are an important source of fat in developed countries (13), allowing for the consumption of oxidized fats containing a large amount of AGEs/ALEs, which affect mammalian metabolism. Despite, there are no studies on the potential link between the thermal oxidation of dietary fat, glucose dyshomeostasis and DNA damage. This study took into account that (i) high fat diets and obesity are linked to an increased susceptibility to T2DM, (ii) fried (thermally oxidized) foods represent an important source of fat in developed countries, (iii) DNA damage may be associated with T2DM and its complications, and (iv) there are no studies underlying thermolyzed fat diet intake, glucose dyshomeostasis and DNA damage. Thus, this work aimed at investigating the effects of high fat and high thermolyzed fat diets on biochemical parameters related to glucose metabolism (i.e., blood glucose levels, insulin tolerance tests, glucose tolerance tests and fat cell glucose oxidation), general health (i.e., body weight, retroperitoneal and epididymal adipose tissue) and markers of DNA injury (i.e., blood, hippocampus and striatum DNA damage) in rats. Lam *et al*. (14) showed that glucose homeostasis depends on lactate metabolism in the hypothalamus. Pierre *et al*. (15) showed that mice fed with a high fat diet had a significant increase in the number of the hippocampal monocarboxylic acid transporters MCT1 and MCT2.

Glucose homeostasis depends on lactate metabolization (14) and on a high fat diet to induce an increase in transporters of monocarboxylic acids MCT1 and MCT2 in the hippocampus. Therefore, we opted to determine the effects of high fat diets (HFD and HFTD) on DNA damage in this structure. The striatum was chosen since its structure is submitted to a larger oxidative stress, due to dopamine catabolism by monoamine oxidase (MAO) (16).

Material and Methods

Chemicals. D-[U-^{14}C]glucose (297 mCi/mmol) and [U-^{14}C]glycerol (142 mCi/mmol) were purchased from Amersham International (Little Chalfont, Bucks, UK). Low and normal melting point agarose (Gibco, USA), ethylenediamine-tetraacetic acid (EDTA), Triton X-100 and dimethyl sulfoxide (DMSO) (Labsynth Produtos de Laboratório Ltda, SP, Brazil), RMPI 1640 medium (Nutricell, Campinas-SP, Brazil), ethidium bromide, cytochalasin B, bovine albumin (essentially fatty acid free) and hyamine hydroxide (J. T. Baker Chemical Company, Phillipsburg, NJ, USA) were all analytical grade. Optiphase Hi-Safe 3 was purchased from PerkinElmer (RJ, Brazil).

Animals and Diets. Thirty adult Wistar rats (male, 60 days old) from the Central Animal House of the Department of Biochemistry were maintained under a standard dark–light cycle (lights on between 7:00 a.m. and 7:00 p.m.) at a room temperature of 22 ± 2°C. The protocol used for this research was employed according to 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.

Rats were divided into three groups: (i) the control group (C, $n = 10$), which received standard laboratory rat chow (50% carbohydrates from starch, 22% protein and 4% fat); (ii) the high fat diet group (HFD, $n = 10$), which received an enriched fat diet (60% fat, composed of 59% lard and 1% soy bean oil, 20% protein and 15% carbohydrates); and (iii) the high fat thermolyzed diet group (HFTD, $n = 10$), which received the same diet as for group 2, but the food was heated for 30 min at 130°C, and a vitamin mixture was added after heating. Diet thermolyzation was based on the study by Sandu *et al*. (4), which indicated a high AGE content (around 1 unit/μg) in the high fat diet after thermolyzation. During the treatment period (12 months), animals had free access to food and water.

Glucose Tolerance Test (GTT). A glucose tolerance test was carried out after six months of diet treatment. A 50% glucose solution was injected into the animals (2 mg/kg IP) after 6 h of starvation. Blood was collected by 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 by a glucometer (AccuChek Active, Roche Diagnostics, USA).

Insulin Tolerance Test (ITT). An insulin tolerance test was performed after six months of diet treatment. Insulin (1 U insulin/kg) was injected intraperitoneally after 6 h of starvation, and blood was collected by a small puncture on the tail immediately prior to the injection, as well as 30, 60, and 120 min afterward. Each time, glucose was measured by a glucometer (AccuChek Active, Roche Diagnostics, USA).

Tissue Preparation. Rats were killed by decapitation. Retroperitoneal and epididymal fat tissues were dissected and weighted. The liver was dissected, weighed

for glycogen synthesis and stored at –70°C for future analyses of thiobarbituric acid reactive species (TBARS) and carbonyl. Blood was collected immediately after decapitation into heparinized tubes, and 5 μl was immediately separated out for a comet assay. The remaining whole blood was centrifuged at 2500 × *g* for 10 min to yield the plasma fraction, which was used for subsequent biochemical analyses.

The brains of the rats were removed in no more than 1 min. These were placed on a Petri plate dish with filter paper and over ice. The brains were washed with Krebs-Ringer bicarbonate buffer solution, pH 7.4, at 3°C. Hippocampus and striatum tissues were dissected and stored at –70°C for posterior analyses of DNA damage (comet assay).

Glucose Oxidation by Epididymal Adipose Tissue. For the measurement of glucose oxidation, epididymal fat was dissected and cut into small pieces (15–25 mg). This was incubated in closed bottles in a medium containing Krebs-Ringer bicarbonate buffer (pH 7.4) with 1% of fatty acid free albumin, 5 mM glucose and 0.2 μCi D-[U-^{14}C]glucose. Incubations were carried out in flasks after contents were gassed with a 95% O_2:5% CO_2 mixture for 1 min. Flasks were subsequently sealed with rubber caps. The small pieces (between 15–20 mg) of epididymal tissue were incubated at 37°C for 1 h in a metabolic shaker (60 cycles/min), according to the method of Dolnikoff *et al.* (17). Incubation was stopped by adding 0.25 ml 50% TCA through the rubber cap. Subsequently, 0.2 ml of 1 M hyamine hydroxide was injected into central wells. The flasks were shaken further for 30 min at 37°C to trap CO_2. Next, the contents of the central well were transferred to vials and assayed for CO_2 radioactivity in a liquid-scintillation counter. All results were expressed considering the initial specific activity of the incubation medium. The CO_2 production rate was constant during 30, 60 and 90 min of incubation.

Thiobarbituric Acid Reactive Species (TBARS). As an index of lipid peroxidation, we used the formation of TBARS during an acid-heating reaction. This has been widely adopted for the measurement of lipid redox states, as previously described (18). In brief, the samples were mixed with 0.6 ml of 10% trichloroacetic acid (TCA) and 0.5 ml of 0.67% thiobarbituric acid and then heated in a boiling water bath for 25 min. TBARS were determined by absorbance in a spectrophotometer at 532 nm. Results are expressed as TBARS/mg protein.

Measurement of Protein Carbonyls. Oxidative damage to proteins was measured by the quantification of carbonyl groups based on their reaction with dinitrophenylhydrazine (DNPH), as previously described (19). In brief, proteins were precipitated by the addition of 20% TCA and dissolved in DNPH. Absorbance was measured at 370 nm using a spectrophotometer. Results are expressed as nmol carbonyl/mg protein.

Comet Assay. DNA damage was evaluated in blood, hippocampus and striatum through alkaline single gel electrophoresis (comet assay), according to previously described work (20).

The alkaline comet assay was carried out as described by Tice *et al.* (21), with minor modifications. Each piece of brain was placed in 0.5 ml of cold phosphate-buffered saline (PBS) and minced into fine pieces to obtain a cellular suspension. Cell suspensions from brain and peripheral blood (5 μl) were embedded in 95 μl of 0.75% low melting point agarose (Gibco BRL) and spread on agarose-precoated microscope slides. After solidification, the cover slip was gently removed, and the slides were placed in lysing solution (2.5 M NaCl, 100 mM disodium EDTA and 10 mM Tris, pH 10.0, with freshly added 1% Triton X-100 and 10% dimethyl sulfoxide) for up to 24 h. Subsequently, the slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 12.6) for 10 min and electrophoresed. Following electrophoresis, slides were immersed in neutralizing buffer (0.4 M Tris-HCl, pH 7.5, 4°C) for 5 min. Subsequently, 50 ml of 5 mg/ml ethidium bromide was applied to the slides, and they were left in the dark for 20 min for DNA staining. Negative and positive controls were used for each electrophoresis assay to ensure the reliability of the procedure. Images of 100 randomly selected nuclei (50 nuclei from two replicated slides) were analyzed for each treatment. Nuclei were scored visually for comet tail size, based on an arbitrary scale of 0–4 (i.e., ranging from no DNA damage to extensive DNA damage). Thus, a group damage index could range from 0 (all nuclei without tail, 100 cells × 0) to 400 (all nuclei with maximally long tails, 100 cells × 4) (22). During electrophoresis, any relaxed or broken DNA fragments migrated farther than supercoiled, undamaged DNA. Slides were viewed on a Nikon inverted microscope using a TE-FM Epi-Fluorescence accessory. Images were transferred to a computer with a digital camera (Sound Vision Inc., Wayland, MA, USA).

Blood Biochemical Parameters. Plasma glucose, triacylglycerol (TAG), total cholesterol, HDL cholesterol levels, as well as aspartate and alanine aminotransferases (AST and ALT, respectively) and alkaline phosphatase activities, were measured using commercial kits (Labtest, MG, Brazil). Reactions were performed using the Labmax apparatus (Labtest, MG, Brazil). Plasma free fatty acids and insulin activities were measured using commercial kits (Roche Diagnostics, Germany and MP Biomedicals, NY, USA, respectively).

Hepatic Glycogen Synthesis. For the measurement of hepatic glycogen synthesis, the liver was dissected and cut into 300 μm slices using a McIlwain tissue chopper (100–120 mg). It was incubated in a beaker with a medium containing Krebs-Ringer bicarbonate buffer (pH 7.4), 5 mM glucose and 0.2 μCi D-[U-^{14}C]glucose, or 1 mM glycerol and 0.5 μCi [U-^{14}C]glycerol, for glycogen synthesis from glycerol. Incubations were carried out in ambient content that was gassed with a 95% O_2:5% CO_2 mixture for 1 h. Liver slices (between 100–120 mg) were incubated at 37°C for 1 h in a metabolic shaker (60 cycles/min), according to

Table 1. Body Parameters in Rats After 12 Months of Different Nutritional Treatment[a]

Body parameters	C	HFD	HFTD
Initial body weight (g)	305 ± 14.7	309 ± 10.8	302 ± 13.2
Final body weight (g)	475 ± 46[a]	610 ± 47[b]	575 ± 51[b]
Body weight gain (g)	170 ± 32[a]	301 ± 52[b]	273 ± 41[b]
Retroperitoneal adipose tissue (g)	10.7 ± 5.2[a]	39 ± 15[b]	37.7 ± 10[b]
Retroperitoneal adipose tissue (%)	2.1 ± 0.9[a]	6.1 ± 1.9[b]	6.5 ± 1.2[b]
Epididymal adipose tissue (g)	9.2 ± 3.6[a]	17.9 ± 8.7[b]	16 ± 2[b]
Epididymal adipose tissue (%)	1.8 ± 0.6[a]	2.8 ± 1.2[b]	2.8 ± 0.2[b]

[a] For treatment details, see Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD). Retroperitoneal and epididymal adipose tissue are expressed in % of total body weight. Additional data are expressed in grams (g), and represented as mean ± SEM ($n = 10$ per group). Different letters at the same row indicate significant difference ($P < 0.05$) by one-way analysis of variance followed by Duncan multiple range test.

the method of Dolnikoff *et al*. (17). Incubation was stopped by placing the bottles in ice. Afterward, 1 ml of 60% KOH was added to each beaker. After 15 min in a boiling water bath, 3 ml of 96% ethanol was added to the tubes to precipitate glycogen. After precipitation, glycogen was suspended in 0.2 ml of water, and the scintillation liquid (Opti-Phase HiSafe3 from PerkinElmer-USA) was added. The samples were assessed in a scintillation liquid counter.

Statistical Analysis. Comparisons between different groups were performed, employing a two-way analysis of variance (ANOVA) followed by the post hoc Duncan test, whenever necessary. The level of significance adopted was $P < 0.05$.

Results

Table 1 shows the body parameters of rats after 12 months of nutritional treatment. Animals from both groups supplemented with the high fat diets showed higher weight gains (77% for HFD and 60% for HFTD) when compared to animals from the control group. The amounts of retroperitoneal and epididymal adipose tissues were significantly higher in animals subjected to the high fat diets when compared to controls (Table 1).

Table 2 shows the biochemical parameters of rats after 12 months of nutritional treatment. Plasma glucose levels were higher in animals from both HFD and HFTD groups, when compared to controls. Plasma triglycerides, cholesterol and HDL levels, as well as AST and ALT activities, were not different between the groups. However, plasma alkaline phosphatase activity was higher in animals submitted to both high fat diets. Only the HFTD group showed increased levels of free fatty acids in relation to other groups. Insulin levels were higher in animals from both HFD and HFTD groups when compared to controls. A homeostasis model assessment of insulin resistance (HOMA-IR) was used to demonstrate which high fat diet protocol was efficient to induce insulin resistance.

The Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) were performed to verify if high fat diets were able to induce insulin resistance in our experimental/nutritional protocol. In the GTT (Fig. 1A), animals fed with high fat diets showed higher glycemia after glucose administration when compared to control rats and independently of the time-point evaluated. Indeed, two-way ANOVA showed a significant main effect of diets ($F_{2,72} = 7.12$; $P = 0.0037$) and time ($F_{3,72} = 190.24$; $P < 0.0001$) for GTT. Moreover, two-way ANOVA showed significant ($F_{6,72} = 3.17$; $P = 0.0081$) time versus diet interactions for the GTT.

In Figure 1B, animals fed with high fat diets showed

Table 2. Biochemical Parameters in Rats After 12 Months of Different Nutritional Treatments[a]

Biochemical parameters	C	HFD	HFTD
Glucose (mg/dL)	93.6 ± 10.7[a]	116.5 ± 14.7[b]	111.83 ± 19.7[b]
Cholesterol (mg/dL)	71.1 ± 10.8	74 ± 9.5	84.5 ± 16.3
HDL (mg/dL)	35 ± 2.8	35.5 ± 5	41.3 ± 6.2
AST (U/L)	308 ± 87	315.8 ± 100	267 ± 97
ALT (U/L)	80.2 ± 8.5	79 ± 37	89.2 ± 25.2
Alkaline phosphatase (U/L)	108.8 ± 31[a]	165 ± 57.9[b]	261.3 ± 110[b]
Free fatty acids (μM/ml)	435.6 ± 82[a]	445.7 ± 96.5[a]	642.6 ± 152.1[b]
Insulin (μUI/ml)	33.8 ± 12[a]	47.1 ± 8[b]	46.4 ± 9.3[b]
HOMA-IR	9.1 ± 3.7[a]	15.1 ± 3.6[b]	18.8 ± 4[b]

[a] For treatment details, see Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD). Glucose, cholesterol and high density lipoprotein (HDL) levels are expressed as mg/dL. Free fatty acids was expressed as μM/ml. Insulin was expressed as μUI/ml. Aspartate (AST) and alanine (ALT) aminotransferases, as well as alkaline phosphatase activities are expressed as U/L and represented as mean ± SEM ($n = 10$ per group). Different letters at the same row indicate significant difference ($P < 0.05$) by one-way analysis of variance followed by Duncan multiple range test.

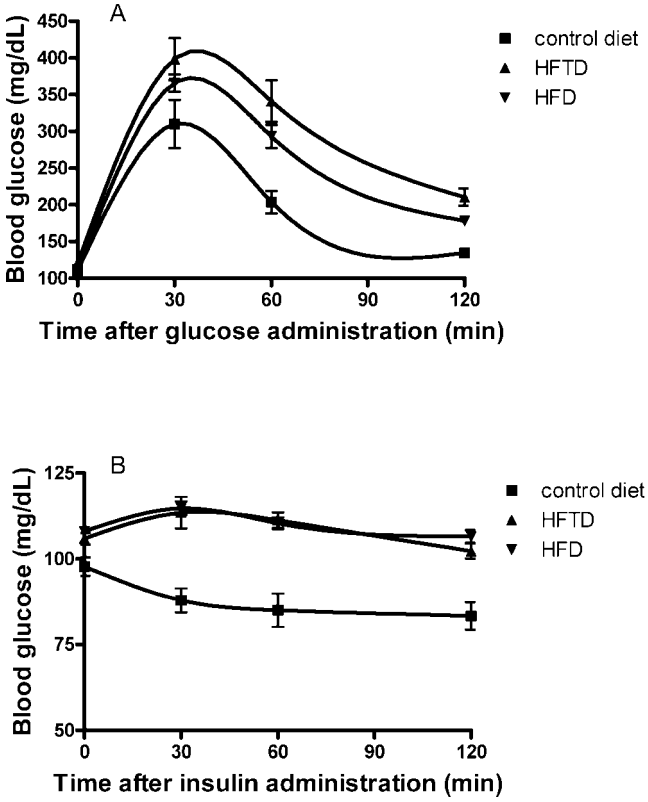

Figure 1. The Glucose Tolerance Test (A) and Insulin Tolerance Test (B) in rats after six months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD). Blood glucose levels are expressed as mg/dL and represented as the mean ± SEM ($n = 6$ per group). Two-way analysis of variance showed significant time vs. diet interactions for Glucose ($F_{6,72} = 3.17$; $P = 0.0081$) and Insulin ($F_{6,87} = 4.46$; $P = 0.0006$) Tolerance Tests.

higher glycemia after insulin administration when compared to control rats and independently of the time-point evaluated (30, 60 or 120 min). In fact, two-way ANOVA showed a significant main effect of diets ($F_{2,87} = 30.96$; $P < 0.0001$) and time ($F_{3,87} = 7.47$; $P = 0.0002$) for ITT. Two-way ANOVA showed significant ($F_{6,87} = 4.46$; $P = 0.0006$) time versus diet interactions for the ITT.

Figure 2 shows decreased glucose oxidation by the epididymal tissue in animals fed a high fat thermolyzed diet when compared to animals of the control and HFD groups.

DNA damage is related to T2DM, including its complications (11), and high fat diets may contribute to the development of T2DM (4). Therefore, we sought out the effects of high fat diets on DNA damage levels in rat blood (Fig. 3). Both high fat diets induced marked DNA damage, but this phenomenon was higher in animals of the HFTD group. As neuropathy (23, 24) and cognitive dysfunction (25, 26) are usual events in diabetes patients, DNA damage was also evaluated in the hippocampus of rats fed with high fat diets. Both high fat diets induced significant hippocampus DNA damage, but this phenomenon was much higher in animals from the HFTD group (Fig. 4). In the

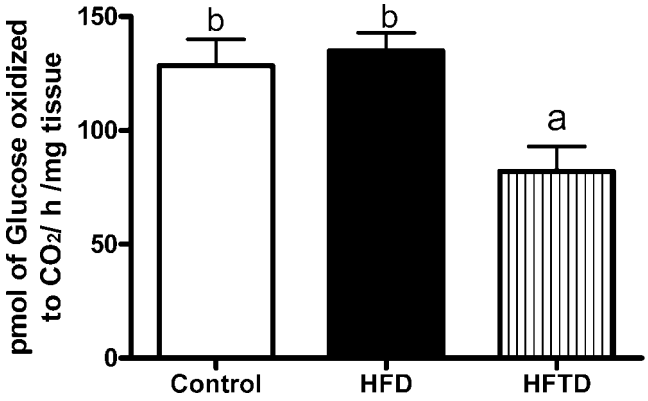

Figure 2. Glucose oxidation by epididymal adipose tissue in rats after 12 months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD). Data are expressed as pmol of glucose oxidized per milligram per hour of tissue and represented as the mean ± SEM ($n = 8$ per group). Different letters indicate the significant difference ($P < 0.05$) by one-way analysis of variance followed by a Duncan multiple range test.

striatum (Fig. 5), both high fat diets induced significant DNA damage, compared to the control group.

HFTD promotes an increase of lipid peroxidation in the liver (Fig. 6A), demonstrating significant lipid damage in relation to the HFD and control group. In the protein carbonylation (Fig. 6B) assay, we did not observe any differences between all of the groups studied ($P < 0.05$).

Figure 7 shows the effect of diets on hepatic glycogen synthesis, both for the direct (Fig. 7A) and indirect (Fig. 7B) pathways. For glycogen synthesis from glucose (Fig. 7A), we found that both high fat diets reduced glycogen synthesis in relation to the control group. We found that both the high fat diets reduced significantly the glycogen synthesis by direct (Fig. 7A) and indirect (Fig. 7B) pathways.

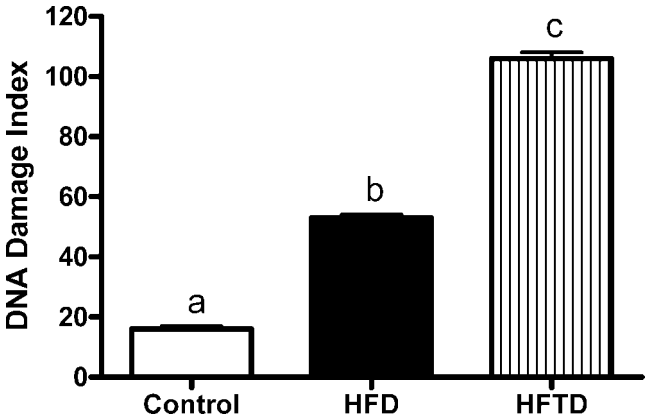

Figure 3. DNA damage in total blood of rats after 12 months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD) ($n = 8$ per group). Different letters indicate the significant difference ($P < 0.05$) by one-way analysis of variance followed by a Duncan multiple range test.

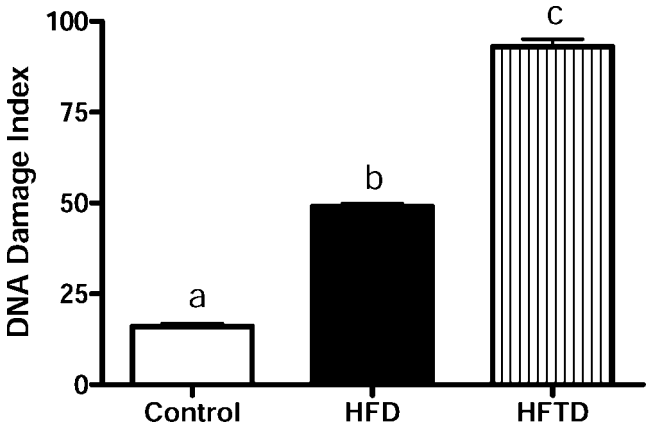

Figure 4. DNA damage in the hippocampus of rats after 12 months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD) ($n = 8$ per group). Different letters indicate the significant difference ($P < 0.05$) by one-way analysis of variance followed by a Duncan multiple range test.

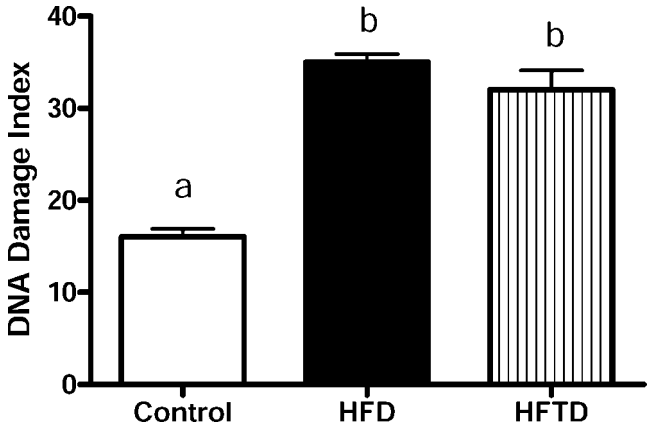

Figure 5. DNA damage in the striatum of rats after 12 months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD) ($n = 8$ per group). Different letters indicate the significant difference ($P < 0.05$) by one-way analysis of variance followed by a Duncan multiple range test.

Discussion

This study found that normal rats exposed to high fat diets over 12 months exhibited metabolic changes that led to the appearance of T2DM signals. These included increased body weight, increased blood glucose levels, hyperinsulinemia and impaired responses in insulin and glucose tolerance tests. However, only animals subjected to the HFTD diet showed decreased epididymal fat cell glucose oxidation, pointing to a decreased capability to uptake and/or metabolize glucose.

Metabolic stress caused by an HFD results in activation of the regulatory protein JNK 1 (27). JNK is activated, in part, by increased serum free fatty acids that induce a stress signaling pathway in target tissues. This increase of free fatty acids, as observed in the HFTD group (Table 2), can justify lipid peroxidation in the liver (Fig. 6A). In a previous study by Matsuzawa-Nagata *et al*. (28), protein carbonyl levels were found to be elevated by 35% in the liver, but they were not altered in the adipose tissue of mice fed a high fat diet compared with control mice at 6 weeks. In protein carbonylation (Fig. 6B), we did not observe differences between the groups. Diamanti-Kandarakis *et al*. (5) submitted female rats during a six-month period to a warm diet that was heated to 100°C for 20 sec (L-AGE) and to a diet with the same composition of L-AGE, though this diet was heated to 125°C for 30 min (H-AGE).

The female rats fed with the high AGE-diet (H-AGE diet) presented an increased glycemia and fast insulinemia in relationship to the female rats fed with L-AGE diet (low AGE-diet). Additionally, the H-AGE showed an increase in AGE localization in the cells of the ovarian tissue, compared to the L-AGE group. Furthermore, fasting serum AGE levels were significantly higher in the H-AGE-D group compared with the L-AGE-fed group. Diamond-Kandarakis *et al*. (5) found an increase in the glycemia and fast insulinemia in rats submitted to HFD and HFTD in relation to diet controls (L-fat-diet no warm). The results found in the present work are due to the effects of the hyperlipid diet and to the heating of the hyperlipid diet.

Previous studies have shown that fat and energy intake represent important dietary factors, modulating insulin resistance and increasing the risk of T2DM (2, 3). Decreased expression of type 2 glucose transporters (GLUT-2) and glucokinase in pancreatic beta-cells has been proposed as an important mechanism by which high fat diet leads to an impaired glucose-stimulated insulin secretion (6). In this regard, a high fat diet induces oxidative stress and apoptosis, which reduces beta-cell mass and compromises beta-cell function (6). Individuals with impaired glucose tolerance are maximally or near-maximally insulin resistant, they have lost 80% of their cell function, and they have an approximate 10% incidence of diabetic retinopathy. By both pathophysiological and clinical standpoints, these pre-diabetic individuals with impaired glucose tolerance should be considered to have type 2 diabetes (29). Although epidemiological (30) and experimental (31) studies point to the involvement of a high fat diet on insulin resistance and increased risk of T2DM, knowledge regarding the effects of dietary fat thermolyzation on these phenomena is scarce. Indeed, the relationship between thermolyzed fats and T2DM is only recently becoming clarified.

Hepatic glycogen storage is impaired in all major forms of diabetes, contributing to the development of hyperglycemia (32). Regulation of carbohydrate metabolism in the liver is perturbed in type 2 diabetes and insulin resistance, resulting in increased hepatic glucose production. Factors contributing to this imbalance include increased gluconeogenesis and impaired hepatic glycogen storage. In Figures 7A and 7B, we show the high fat diets that have the capacity to reduce glycogen synthesis by the direct pathway (glucose → glucose-6-P → glucose-1-P; glucose-1-P + UTP → UDPG + PPi; UDPG glucose transporter to glycogen

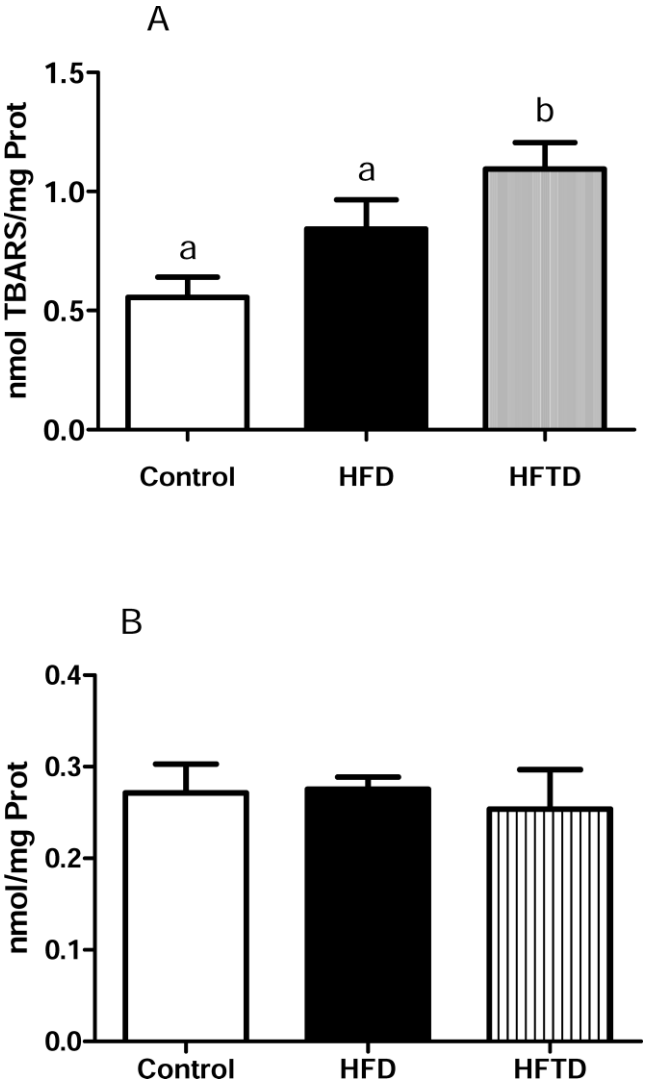

Figure 6. Lipid peroxidation (A) and protein carbonylation (B) in the liver of rats after 12 months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD) ($n = 8$ per group). Different letters indicate the significant difference ($P < 0.05$) by one-way analysis of variance followed by a Duncan multiple range test.

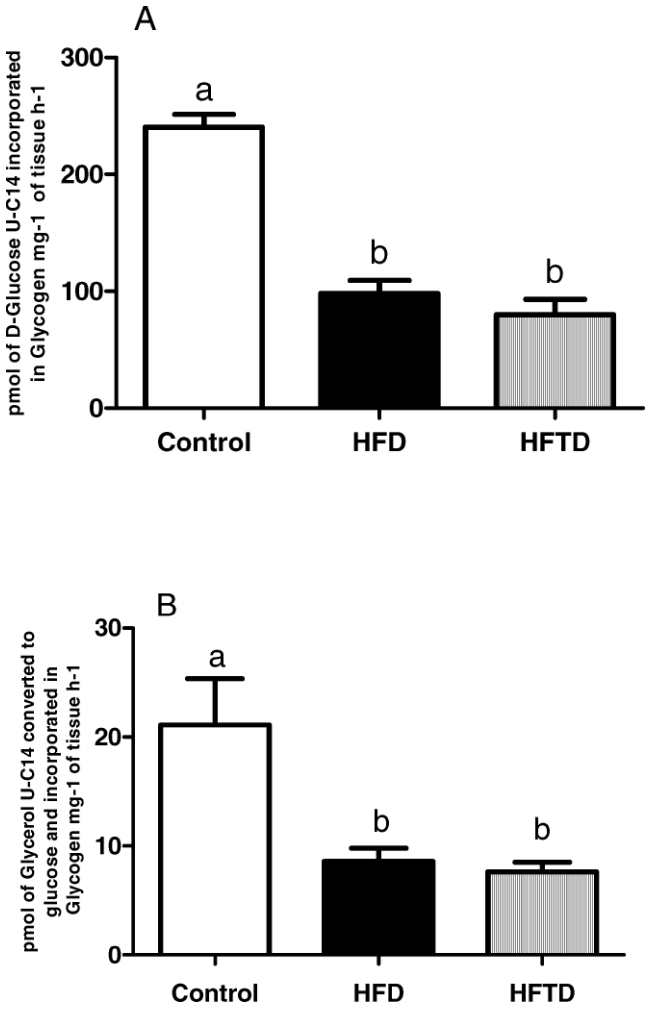

Figure 7. Hepatic glycogen synthesis from D-[U-^{14}C]Glucose (A) and hepatic glycogen synthesis from [U-^{14}C]Glycerol (B) in rats after 12 months of different nutritional treatments. For treatment details, see the Materials and Methods section: Control (C), High Fat Diet (HFD) and High Fat Thermolyzed Diet (HFTD) ($n = 8$ per group). Different letters indicate the significant difference ($P < 0.05$) by one-way analysis of variance followed by a Duncan multiple range test.

synthesis) and indirect pathway (the indirect pathway uses gluconeogenetic substrates toward glucose-6-P synthesis, for example: glycerol + ATP → glycerol-P + ADP → by gluconeogenesis: glucose-6-P → glucose-1-P; glucose-1-P + UTP → UDPG + PPi; UDPG glucose transporter to glycogen synthesis) (33).

In this respect, Sandu *et al*. (4) have elegantly shown that HFTD induces T2DM symptoms to a higher extent when compared with HFD group. These symptoms were related to high dietary levels of toxins [advanced glycation/lipoxidation end products (AGEs/ALEs)], which possess pro-oxidant and pro-inflammatory properties (34). In addition, Sauter *et al*. (35) showed that the interleukin-1 receptor antagonist (IL-1Ra) protected mouse islets from HFD-induced beta-cell apoptosis and improved glucose-stimulated insulin secretion, suggesting the involvement of pro-inflammatory events in HFD-induced glucose dyshomeostasis. In agreement with these studies, we also observed that HFTD elicited the most hazardous effects toward glucose metabolism, when compared to HFD. This manifested as a higher impairment in controlling glycemia after glucose administration during the GTT. Interestingly, animals fed HFTD showed a decrease in glucose oxidation capabilities by epididymal adipose tissue when compared to controls. This phenomenon was not observed in animals of the HFD group. These are novel results, pointing to deficient glucose metabolism by the adipose tissue as a potential phenomenon involved in the T2DM symptoms induced by thermolyzed dietary fat. Taking into account that thermolyzation-derived toxins (AGEs/ALEs) possess pro-oxidant and pro-inflammatory properties (34), one could suggest the involvement of these molecules on the metabolic deficit

impairment observed in epididymal adipose tissues of HFTD-fed animals. However, this hypothesis has yet to be proven.

Another interesting and important finding of our study was the increased blood, hippocampus and striatum DNA damage induced by high fat diets. To the best of our knowledge, there are no prior results in the literature showing a link between high fat diets, T2DM symptoms and DNA damage. It is noteworthy that HFTD aggravates such conditions. In fact, a significantly higher degree of DNA damage was observed in the blood (leucocytes), hippocampus and striatum of animals fed a high fat diet that was subjected to thermolyzation.

It is known that AGEs cause retinal vascular leakage (36). Vlassara *et al*. (37) showed that EV injection of AGE-RSA (AGE rat serum albumin) caused an increase in the vascular permeability of several rat tissues, as well as in the cerebral tissue. Certainly, AGEs propagators, such as carboxymethyllysine, fructoselysine, furosine and others, could cross the blood-brain barrier and act on the CNS cell.

From a nutritional point of view, these results appear to be relevant because they are derived from an experimental protocol that simulates a nutritional condition that is spreading rapidly. Fried (thermolyzed) foods represent a significant source of fat in developed countries (13). Therefore, the extrapolation of our results to humans may suggest that an important percentage of the population is exposed to a dietary condition that greatly increases the susceptibility of developing T2DM, DNA damage and genomic instability.

Conclusion

This study indicates that a high fat diet induces T2DM symptoms, oxidative stress and DNA damage. Moreover, the fat thermolyzation process aggravates such a condition, decreases epididymal fat cell glucose oxidation and oxidative stress in the liver, and increases free fatty acids in the plasma. These results are the first to show a positive correlation between high fat diets, glucose dyshomeostasis, oxidative stress and DNA damage, being in agreement with epidemiological studies reporting that diabetes patients are more prone to the development of different types of cancer. Our findings support the need for rigorous nutritional attention toward fried (thermolyzed) foods, which represent an important source of fat in developed countries.

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