Effects of Diets Containing Different Types of Carbohydrates on Hepatic Metabolism

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Summary

The study of diets rich in different quantities of simple and complex carbohydrates is essential as an aid in the prevention of several types of organic damage. *Objectives:* To assess the effects of hyperglycidic, isocaloric diets with variations in carbohydrate type (simple or complex) on the metabolism of rats. *Methodology:* Forty Wistar rats were divided into 4 groups: control group (57.96% starch and 12.04% saccharose - CG), carbohydrate mixture group (35% saccharose, 35% starch - MG), simple-carbohydrate-group (70% saccharose - SG), and complex-carbohydrate-group (70% starch - CCG). The animals were allowed to habituate to the diets and then received them for 28 days, with free access to water. *Results:* Ration weight and consumption did not differ between groups. The amount of hepatic fat was found to be greater in SG compared to CG. Hepatic malondialdehyde (MDA) determination revealed that SG presented the lowest value, although this result was accompanied by the lowest vitamin E value, demonstrating that the consumption of this antioxidant was higher in SG. Reduced glutathione values did not differ between groups, raising the hypothesis that in this case vitamin E was the first antioxidant barrier to be utilized. Blood glucose levels differed between CG, MG and SG in relation to CCG only on day 14; however, the oral glucose tolerance test performed at the end of the experiment did not demonstrate a difference between groups. Serum fructosamine differed significantly between MG and CG and CCG and also between SG and CG and CCG, with the values for MG and SG being higher than those for the other groups. **Conclusion**: The consumption of simple carbohydrates led to hepatic steatosis and altered the antioxidant system even within a short period of time, in addition to modifying the fructosamine values, revealing an increase in glycated serum proteins that may cause damage over a longer period of time.

Introduction

The growing increase in obesity elevates the incidence of cardiovascular diseases, diabetes mellitus, dyslipidemia, hypertension and non-alcoholic–fatty liver disease (NAFLD) (*Zivikovic et al, 2007*). The last occurrence is due to the fact that obese subjects present an abnormal accumulation of triglycerides in the liver (*Valtueña et al, 2006*). This intracellular lipid accumulation in the cytoplasmic vacuoles characterizes NAFLD (*Grattagliano et al, 2000*), a common liver disease considered to be of public health importance (*Zou et al, 2006*).

Zivikovic et al (*2007*) defined NAFLD more precisely as a greater lipid concentration in the liver primarily in the form of triacylglycerols without a significant ingestion of alcohol and with the exclusion of other known causes of steatosis, such as some drugs and toxins.

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This disease has attracted great research interest since its incidence is also related to obesity, diabetes, jejunoileal bypass, dyslipidemia, drugs, and parenteral nutrition (*Oliveira et al, 2003*). NAFLD and nonalcoholic steatohepatitis (NASH) are increasingly considered to be due to the increase in liver diseases and to be related to the mortality and morbidity related to these conditions (*Ackerman et al, 2005*). The prevalence of NAFLD in developed countries is 24% of the population (*Delarue et al, 2004*), while the worldwide prevalence of NASH is estimated at 2 to 3% of the general population (*Rivera et al, 2006*).

NAFLD can be characterized as: type 1 – steatosis only; type 2 – steatosis plus inflammation; type 3 – steatosis plus hepatocellular injury; and type 4 – steatosis plus sinusoidal fibrosis or Mallory corpuscles, or both (*Zivikovic et al, 2007*). NASH is considered to be the most severe form of NAFLD (types 3 and 4) and is also associated with cirrhosis, hepatocellular carcinoma and advanced liver disease, leading to hepatic death (*Zivikovic et al, 2007*). Progression of NAFLD to NASH occurs due to the damage provoked by lipid peroxidation and free radical production (*Bradbury et al, 2006*). Lipid peroxidation is accompanied by an inflammatory response and by activation of stellate cells, inducing fibrogenesis (*Oliveira et al, 2006*).

Insulin resistance leads to the loss of the inhibitory effect of insulin on β-oxidation, a mechanism that induces intracellular oxidative stress. Patients with NASH have increased β-oxidation and hepatic oxidative stress. These changes are also present in patients with hepatic fat accumulation resulting from other diseases such as obesity, malnutrition, intestinal malabsorption, endocrine metabolic diseases, and thyroid disorders, but defects of mitochondrial structure are also present in NASH (*Portincasa et al, 2004*).

The mitochondria are responsible for oxidative phosphorylation and fatty acid β-oxidation. Since these two processes are the major sources of free radicals, mitochondrial disorders play a central role in the development of NASH. (*Oliveira et al, 2006*). Excess glucose causes an increased synthesis of fatty acids, which are stored in the liver, leading to NAFLD (*Bradbury et al, 2006*).

The worldwide increase in the prevalence of insulin resistance and associated syndromes has led to great interest in the development of dietary strategies in order to prevent NAFLD and its progression to NASH (*Valtueña et al, 2006*). It has been extensively reported that a diet rich in antioxidants and weight loss are the measures most indicated for the prevention of NASH and NAFLD. However, not enough attention has been paid to the quality and the type of carbohydrate consumed in relation to the accumulation of hepatic fat (*Zivikovic et al, 2007*).

In a study conducted on 241 persons with a 24 hour recall analysis and a semi-quantitative food frequency questionnaire, Valtueña et al (*2006*) reported that diets with a high glycemic rate are associated with moderate or severe hepatic steatosis, especially among insulin-resistant individuals.

Rats fed a diet rich in simple carbohydrates (SC) developed dyslipidemia, weight gain, visceral adiposity, and reduction of insulin sensitivity (*Fortino et al, 2007*). There are also reports of models of induction of metabolic alterations resembling type 2 diabetes in rats fed an SC-rich diet for several months (*Lian et al, 2007*). Axen et al (*2006*) also reported higher triglyceride levels in obese rats fed SC compared to rats fed a fat-rich diet; similarly, another study indicated that a diet in which fat was replaced with carbohydrate contributed to weight gain (*Sánchez-Lozada et al, 2007*). Still another study demonstrated that saccharose supplementation caused weight gain and a significant increase in serum triglycerides and in systolic blood pressure (*Johnson et al, 2007*).

However, few data have been obtained by the evaluation of the composition of diets containing different CHO percentages and of their effect on the liver and on hepatic metabolism. Thus, the objective of the present study was to assess the effects of the modification of the major component of the diet and its relation to diseases that are currently of extreme importance in terms of Public Health.

Materials and Methods

Diet

The diet offered was based on the American Institute of Nutrition (AIN-93) (*Reeves et al, 1993*) for laboratory studies on growing rodents. For the formulation of a hyperglycidic diet, the amount of carbohydrate was increased from 62.9 g/100 g diet, recommended by AIN-93, to 70 g/100 g diet and the amount of casein was reduced from 20 g to 12.94 g/100 g diet. The remaining components such as lipids, fibres, vitamins and minerals, were those recommended by AIN-93.

The experimental diets were divided into four groups: Control Group (CG) containing 82.8% complex carbohydrate and 12.8% simple carbohydrate, Mixture Group (MG) containing 50% of each carbohydrate type, Simple Group containing 100% simple carbohydrate consisting of saccharose, and Complex Group (CCG) containing 100% starch as the source of complex carbohydrate.

Animals and Collections

Newly weaned male Wistar rats from the Central Animal House of the Ribeirão Preto Campus, University of São Paulo, were housed in individual cages on a 12-hour light-/12-hour dark cycle at a controlled temperature of 24±2ºC. The diets and water were supplied *ad libitum*. The cages were cleaned weekly, the diet was weighed three times a week and the amount consumed was replaced. The animals were divided into four groups and allowed to adapt to the diet for 10 days, with decreasing variations of the standard diet and increasing amounts of the experimental diet for each group until they consumed only the hyperglycidic diet, which was offered for a period of 28 days. The animals were fasted for two periods of 8 hours each for the determination of glycemia in one drop of blood obtained by sectioning the tail. After the 28 days of the experimental period the animals were sacrificed by decapitation, blood samples were obtained and the liver was removed.

Determination of Glycemia

Glycemia was determined on the 14th day after the beginning of the experiment in one blood drop obtained by sectioning the tail, using a glycosimeter (Precision QID, Abbott).

Oral Glucose Tolerance Test (OGTT)

Glycemia was measured with a glycosimeter on four occasions all under fasting conditions and 30, 60 and 120 min after glucose administration by gavage. Animals from each group were selected at random for the test.

Determination and Quantitation of Total Hepatic Fat

Total fat was measured by the method proposed by Bligh and Dyer (*1959*).

Hepatic tissue (0.5 g) was first homogenized with 0.8 ml distilled water in 10 ml tubes. Next, 1 ml chloroform and 2 ml methanol were added and the tubes were shaken in a vortex mixer for 10 min. After centrifugation, 1 ml chloroform and 1 ml 1.5% sodium sulphate solution were added. The material was again shaken for about 2 min and the tubes were centrifuged at 1000 rpm for 2 min for the separation of the chloroform (lower) and aqueous (upper) layers. After layer separation, approximately 1.3 ml chloroform layer was removed and placed in a test tube containing 1 g anhydrous sodium sulphate for the removal of water traces. A 0.5 ml aliquot of the water-free chloroform phase was transferred to a previously weighed beaker, which was placed in an oven with forced air circulation at 75°C. After total chloroform evaporation, the beaker was again weighed and the amount of fat was calculated as the difference of the beaker weights divided by the initial tissue mass used.

Determination of Lipid Peroxidation

Lipid peroxidation in the liver was measured by the determination of thiobarbituric acid reactive substances (TBARS) by the method of Buege & Aust (*1978*).

Hepatic tissue (100 mg) was homogenized in 1 ml 1.15% KCl and 1.0 ml of the homogenate was

immediately combined with 2.0 of the TCA-TBA-HCL reagent and shaken in a vortex mixer. The solution was heated in boiling water (100°C) for 15 min and, after cooling, was centrifuged at 3,000 rpm for 10 min and the supernatant was collected. Absorbance of the sample was determined at 535 nm against a blank. TBARS concentration in the sample was calculated using a malondialdehyde (MDA) calibration curve.

Determination of Reduced Glutathione

Hepatic reduced glutathione (GSH) was determined by the method of Sedlack & Lindsay (*1968*). A 200 mg liver fragment was collected and homogenized in a Potter tissue grinder with 4.0 ml of 0.02 M EDTA buffer on ice. A 5.0 ml aliquot of the homogenate was removed and added to a tube containing 4.0 ml deionised water and 1.0 ml 50% trichloroacetic acid (TCA); after 15 min with occasional shaking, the tube was centrifuged at 3000 *g* for 15 min at room temperature. A 2.0 ml amount of the supernatant was separated and 4.0 ml 0.4 M TRIS buffer, pH 8.9, and 0.1 ml 0.01 M DTNB in methanol were added to it. A reading was taken with a spectrophotometer at 412 nm wavelength 5 min after the addition of DTNB against a blank with 0.02 M EDTA in place of the supernatant. Concentration was calculated using a standard GSH curve in EDTA (0.02 M).

Table 1. Animal weight on the last day of the experiment, diet consumption during the last week, and glycemia value half-way through the experiment.

	CG	MG	SG	CCG
Weight (g)	250.3 ± 27.7	241.4 ± 27.1	237.0 ± 25.8	257.6 ± 45.1
Diet consumption (g)	122.9 ± 42.2	122.7 ± 21.5	131.7 ± 26.8	118.9 ± 33.5
Glycemia (mg/dL)	105.1 ± 10.9	104.9 ± 9.3	106.4 ± 6.3	92.0 ± 10.4*

* $p<0.05$; compared to other groups.

Table 2. Values of hepatic oxidative stress parameters, hepatic fat and serum fructosamine.

	CG	MG	SG	CCG	Differences
MDA (nmol/g protein)	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01*	0.13 ± 0.01	* $p<0.05$ from CG
GSH (µmol/mg tissue)	1.16 ± 0.39	1.08 ± 0.17	1.14 ± 0.17	1.31 ± 0.16	-
Vit. E (µmol/g tissue)	68.2 ± 14.5	74.4 ± 13.7	65.7 ± 12.3	89.0 ± 13.9*	* $p<0.05$ from SG
Vit E (µmol/mg fat tissue)	1.01 ± 0.25	0.97 ± 0.25	0.82 ± 0.13	1.32 ± 0.27*	* $p<0.05$ from SG
Hepatic fat (mg/g tissue)	66.6 ± 12.5	79.5 ± 10.5	84.3 ± 16.0*	72.3 ± 12.3	* $p<0.05$ from CG
Fructosamine (mmol/L)	1.61 ± 0.10	1.93 ± 0.12	2.20 ± 0.17*	1.67 ±0.22	* $p<0.05$ from CG, MG, CG

Protein determination was performed by the method of Lowry et al (*1951*) in order to express the hepatic values of GSH.

Determination of Vitamin E Concentration

Vitamin E concentration in liver samples was determined by HPLC according to the method of Arnaud et al (*1991*) using a C-18 column (Shimpack CLC-ODS, 4.6 x 25 cm), a 4 mm x 1 cm precolumn and a flow of 2.0 L/min. A liver sample (0.5 g) was macerated in 2.0 ml absolute ethanol in a Potter tissue grinder and 1.0 ml hexane was added to the liver macerate. After shaking for 2 min, the homogenate was centrifuged at 3000 rpm for 15 min. A 1.0 ml aliquot of the supernatant was removed and dried under a nitrogen flow. The dry residue was resuspended in the methanol/dichloromethane/acetonitrile mobile phase and submitted to chromatography with reading at 292 nm wavelength. Concentration was calculated using an external α-tocopherol standard .

Determination of Plasma Fructosamine Concentration

Fructosamine was determined using a Labtest commercial kit (Labtest Diagnóstica S.A., Brazil).

Statistical Analysis

Data were compared among groups by one-way analysis of variance (Tukey test), with the level of significance set at $p<0.05$, using the GraphPad Instat software, version 3.05.

Results

As expected, there was no statistically significant difference in weight among groups on the last day of the experiment. The glycemia values determined on the 14th day of the experiment demonstrated a significant difference between CCG, which presented the lowest mean, and the remaining groups ($p<0.05$). The results are listed in Table 1.

Fasting glycemia values and the values obtained after the oral glucose dose showed no significant differences between groups (Figure 2).

Figure 2. Mean glycemia values (mg/dL) obtained with the OGTT.

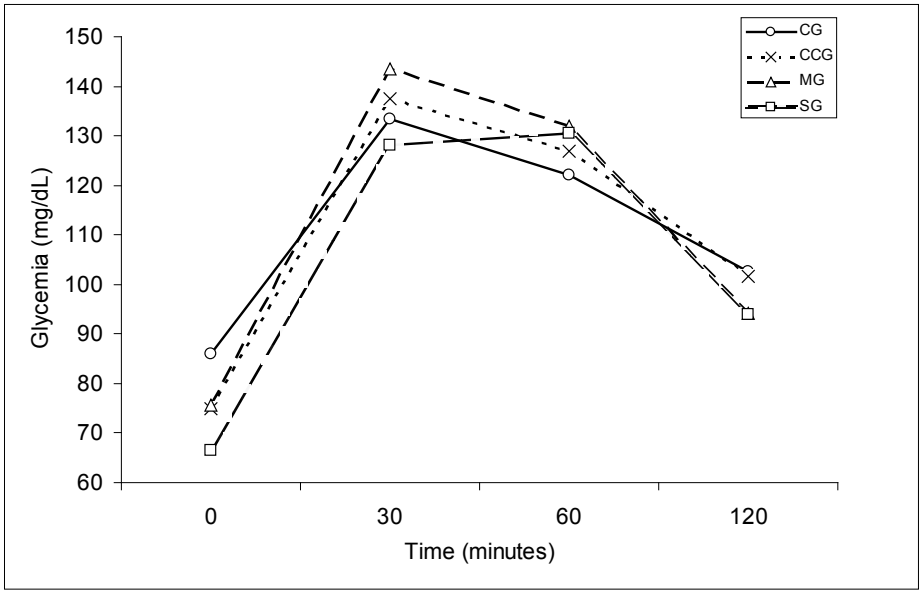

Table 2 contains the results of MDA, GSH, vitamin E, hepatic fat, and fructosamine.

Hepatic fat was found to differ significantly between CG and SG ($p<0.05$), with a larger quantity in the latter, as can be seen in Table 2.

The mean values of fructosamine are listed in Table 2. The SG data gave the highest value and significant differences from all the other groups ($p<0.05$).

Discussion

The relative weight between the groups agrees with diet consumption, since animal growth was similar for all groups and agreed with diet consumption. It is interesting to note that even in the presence of a normal body weight, there may be important hepatic and glycemic changes that should be investigated.

The results showed complex carbohydrate have a protective factor for the maintenance of glycemia even with a hyperglycidic diet. In their study, Valtueña et al (*2006*) concluded that the type of carbohydrate is a triggering factor for the control of glycemia, with an important role regarding the total quantity of carbohydrate in the diet or fibbers ingestion.

The OGTT (see heading above) indicated that, despite the higher glycemia compared to the control observed during the experiment, there was an organic process of adjustment that led to similar glycemia values in all groups. The difference in carbohydrate type was a determinant factor regarding the modification of glycemia, although adaptation seemed to occur during the study. The amount of energy, the percentage of carbohydrates and the quantity of fibres were the same for the diets of all groups. Thus, it is clear that the type of *carbohydrate consumed* is a determinant factor regarding changes in glycidic profile and the establishment of diet-induced hepatic steatosis.

The group that received simple carbohydrate presented the lowest MDA value. However, in this group this result was accompanied by the lowest vitamin E value, demonstrating that a greater consumption of this antioxidant occurred in the simple carbohydrate group. GSH values did not differ between groups, raising the hypothesis that in this case vitamin E was the first antioxidant barrier to be utilized.

We may conclude that the defence against lipid peroxidation was preserved in all groups except the one receiving simple carbohydrate. Thus, a greater progression and severity of hepatic steatosis might have occurred if the animals had received this diet for a longer period of time.

It is possible to conclude that the type of carbohydrate was the factor that triggered the differences in the amount of hepatic fat, since all groups received isocaloric diets containing the same percentage of carbohydrates. The consumption of large amounts of simple carbohydrates caused hepatic steatosis even within a short period of time, indicating that this is a good method for the induction of steatosis in rats. The induction of hepatic steatosis by a diet rich in simple carbohydrate has been extensively reported in the literature and the present study agreed with previous investigations.

Fructosamine results from the binding of a serum protein, usually albumin, to a sugar, usually glucose, and reflects glycemia 3 weeks prior to its appearance (*Mascagni et al, 2004*).

Misciagna et al (*2004*) demonstrated a strong correlation between fructosamine and dietary sugar and an inverse relation between the former and the energy value of the diet. This fact agrees with the results obtained here, since even MG, which had not shown a considerable change in glycemia, had higher fructosamine values. It is interesting to note that, since all diets were isocaloric, despite the inverse relation between fructosamine and calories, the amount of simple sugar present in the diet caused a change in the amount of blood sugar, which was not perceptible with the tests more routinely used such as plasma glycemia and OGTT. Fructosamine analysis proved to be a more sensitive method and a good indicator for the detection of changes in serum glucose concentration.

The present study clearly shows that the investigation of the type of carbohydrate consumed is very important in order to prevent

metabolic alterations and diseases; and the choice of carbohydrate type should be the main factor directed at the prevention of these diseases, even preceding the increase in dietary fibres or the reduction of the total amount of carbohydrate.

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