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Opposite lipemic response of Wistar rats and C57BL/6 mice to dietary glucose or fructose supplementation

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Abstract

The metabolic effects of carbohydrate supplementation in mice have not been extensively studied. In rats, glucose- and fructose-rich diets induce hypertriacylglycerolemia. In the present study, we compared the metabolic responses to two monosaccharide supplementations in two murine models. Adult male Wistar rats (N = 80) and C57BL/6 mice (N = 60), after 3 weeks on a standardized diet, were submitted to dietary supplementation by gavage with glucose (G) or fructose (F) solutions (500 g/L), 8 g/kg body weight for 21 days. Glycemia was significantly higher in rats after fructose treatment (F: 7.9 *vs* 9.3 mM) and in mice (G: 6.5 *vs* 10 and F: 6.6 *vs* 8.9 mM) after both carbohydrate treatments. Triacylglycerolemia increased significantly 1.5 times in rats after G or F supplementation. Total cholesterol did not change with G treatment in rats, but did decrease after F supplementation (1.5 *vs* 1.4 mM, $P < 0.05$). Both supplementations in rats induced insulin resistance, as suggested by the higher Homeostasis Model Assessment Index. In contrast, mice showed significant decreases in triacylglycerol (G: 1.8 *vs* 1.4 and F: 1.9 *vs* 1.4 mM, $P < 0.01$) and total cholesterol levels (G and F: 2.7 *vs* 2.5 mM, $P < 0.05$) after both monosaccharide supplementations. Wistar rats and C57BL/6 mice, although belonging to the same family (Muridae), presented opposite responses to glucose and fructose supplementation regarding serum triacylglycerol, free fatty acids, and insulin levels after monosaccharide treatment. Thus, while Wistar rats developed features of plurimetabolic syndrome, C57BL/6 mice presented changes in serum biochemical profile considered to be healthier for the cardiovascular system.

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- Triacylglycerolemia
- Insulin resistance
- C57BL/6 mice
- Wistar rats

Introduction

In the 1950's, the energy from dietary fat was replaced by carbohydrates (1,2) in an attempt to reduce serum cholesterol concentration. However, high carbohydrate diets, particularly diets containing sucrose and fructose, increase serum triacylglycerol (TG) and decrease high-density lipoprotein (HDL) cholesterol, contributing to an increased risk of cardiovascular disease (3-6).

Before industrialization, starch was the principal carbohydrate in regular diets, while fructose was a minor diet component. During the past two decades, fructose intake has increased steadily due to its use as a sweetener in pharmaceutical and food products such as carbonated beverages, canned fruits, jams, jellies, and dairy products.

Several studies have shown that high fructose or glucose consumption by humans (5-8) and laboratory animals (7,9-17) can affect carbohydrate and lipid metabolism. Rats fed a high-sucrose and -fructose diet presented metabolic changes observed in the human X syndrome, a disorder characterized by insulin resistance, hypertension and dyslipidemia (10,18-22). However, in mice, the effect of a high-carbohydrate diet on lipid and carbohydrate metabolism has not been studied extensively. Surwit et al. (23) showed that C57BL/6J mice carry a genetic predisposition to the development of non-insulin-dependent diabetes mellitus and obesity, hyperglycemia, hyperinsulinemia, insulin resistance, and hypertension when fed a high-fat, high-simple carbohydrate diet. They described different effects of dietary fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. For instance, plasma glucose levels were not affected by dietary sucrose in A/J and C57BL/6J mice. However, different types of diet did not affect the plasma insulin levels in the A/J mice, whereas in C57BL/6J mice a low-sugar diet resulted in higher levels of insulin when compared to A/J mice on a low-fat diet with either low or high sugar content (15,16). Shafrir et al. (24) showed a significant increase in triacylglycerol levels in spiny mice (*Acomys cahirinus*) after long-term high sucrose consumption. Also, Ostos et al. (25) showed that long-term fructose consumption had a strong adverse effect in hypertriacylglycerolemic atherosclerosis-protected transgenic mice that over-expressed the human apolipoprotein AI-CIII-AIV gene cluster.

Since rats and mice are frequently used as experimental models for several metabolic disturbances and for the study of diet manipulations, the objective of the present study was to compare the effects of two simple carbohydrate diet supplementations (fructose or glucose) on the glucose and lipid profile of two members of the Muridae family: Wistar rats (*Rattus norvegicus*) and C57BL/6 mice (*Mus musculus*).

Material and Methods

Animals and experimental design

The experimental protocol was approved by the Ethics Committee for Animal Experimentation (Biology Institute, State University of Campinas, Campinas, SP, Brazil). Forty male Wistar rats and 30 C57BL/6 male mice obtained from the Multidisciplinary Center of Biological Investigation (CEMIB), State University of Campinas (UNICAMP), 3-5 weeks old, weighing on average 240 ± 32 and 21 ± 2.5 g, respectively, were used in this study. Wistar rats were housed (4 animals per cage) in a temperature-controlled room (22ºC) with 12-h light-dark cycles and received for 3 weeks a standard rodent diet (Nuvital, Curitiba, PR, Brazil) and water *ad libitum*. After 12 h of fasting, tail blood was obtained at 7 am.

The basal biochemical serum parameters considered were: cholesterol, HDL cholesterol, TG, free fatty acids, glucose, and insulin concentrations. The rats received fruc-

tose or glucose solutions (500 g/L) at 8 g/kg body weight/day by gavage, twice a day at 8 am and 2 pm for 21 days. Free water and chow diet (composition: 220 g protein, 45 g fat, 408 g carbohydrate, 122 g mineral mix, 80 g fiber, 125 g humidity per 1000 g of diet) consumptions as well as body weight were measured weekly. At the end of the 21-day period, 12-h fasted rats were lightly anesthetized with ether for tail blood collection to measure the same biochemical parameters. C57BL/6 mice were housed in cages for 6 animals kept under the same conditions and submitted to the same protocol as described above.

At the end of the experiment, all animals were anesthetized intraperitoneally with ketamine (50 mg/kg, Ketalar, Parke-Davis, Guarulhos, SP, Brazil) and xylazine (16 mg/kg, Rompum, Bayer S.A., São Paulo, SP, Brazil) and blood was obtained through the retro-orbital plexus. Rat and mouse serum pools (3 animals in each condition) were submitted to fast protein liquid chromatography (FPLC), as described in Methods, to determine the triacylglycerol and cholesterol distribution in plasma lipoproteins.

Methods

Serum total cholesterol, HDL cholesterol, TG, and glucose were determined by enzymatic colorimetric methods using an automated Hitachi 917 system (Boehringer, Mannheim, Germany). Free fatty acids (FFA) were measured with an enzymatic colorimetric kit (Wako Chemicals, Richmond, VA, USA) and insulin was determined by ELISA (Ultra Sensitive Rat Insulin ELISA, Crystal Chem. Inc., Dowers Grove, IL, USA). Interassay coefficients of variation were 1.4, 5.8, 1.4, 1.5, 2.4, and 3.8%, respectively, for glucose, insulin, cholesterol, TG, HDL cholesterol, and FFA determinations. Lipoprotein was isolated by FPLC (Pharmacia, Uppsala, Sweden) using an HR 10/30 Superose 6 column (Amersham Pharmacia Biotech, Uppsala, Sweden) with Tris-buffered saline, pH 7.4, in a 0.5 mL/min flow rate (26). The cholesterol and TG present in the fractions were determined with an automatic Cobas system (Roche, Basel, Switzerland). Insulin resistance was estimated by the homeostasis model assessment (HOMA) index as follows (27): fasting insulin (mU/L) x fasting glucose (mmol/L)/22.5.

Statistical analysis

The paired *t*-test and ANOVA followed by the *post hoc* Tukey test were used to compare the effects of carbohydrates (before and after) and the effects of the monosaccharides fructose and glucose administered to rats and mice, respectively. Some variables were log transformed due to the absence of normal distributions. The Pearson coefficient was used to test the correlation of diet, water consumption, and weight gain (areas under the weight *vs* time curves, AUC) with the percent variation of the biochemical parameters. Comparison between animal genera (*Rattus x Mus*) was made by the Student *t*-test (28). All comparisons where made using SAS System for Windows (Statistical Analyses System Institute Inc., 1999-2000, Cary, NC, USA), version 8.01. Statistical significance was set at $P < 0.05$.

Results

Body weight, food and water consumption of Wistar rats and C57BL/6 mice

In rats, the total AUCs for body weight, food and water consumption were significantly larger during glucose than during fructose supplementation (Figure 1A). The incremental AUCs (IAUC) of body weight were similar in the two dietary programs (Figure 1B: 40 ± 3.3 and 48 ± 4.3 g for 3 weeks for glucose- and fructose-treated rats, respectively). However, the IAUC (Figure 1B) of food intake was significantly smaller

for the fructose group than for the glucose group. This suggests that food efficiency (food consumption/weight gain) was higher when fructose was used as a dietary supplement compared to glucose. Interestingly, glucose-supplemented rats drank approximately twice as much water as fructose-supplemented rats ($P < 0.05$, Tukey test).

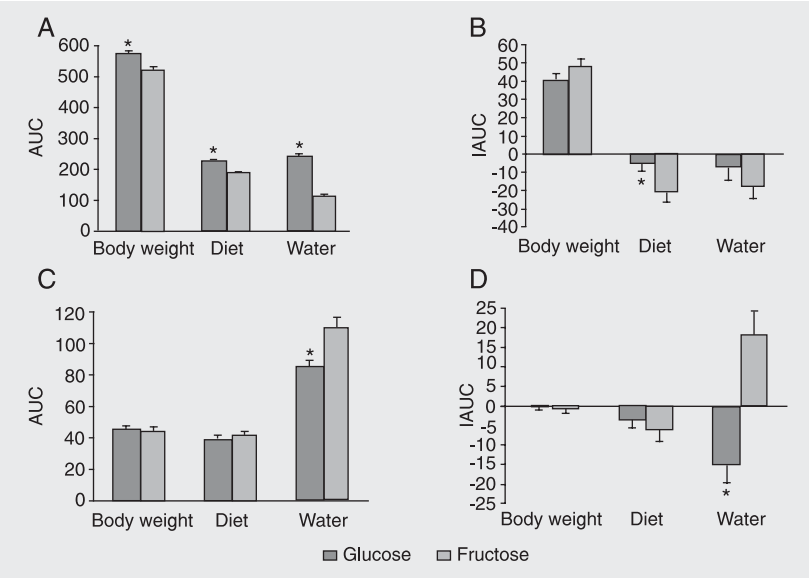

Figure 1. Total and incremental areas under the curves (AUC and IAUC) of body weight, diet and water consumption for Wistar rats (N = 40, panels A and B) and C57BL/6 mice (N = 30, panels C and D) treated with 8 g/kg body weight of glucose/day or fructose/day for 21 days. Data are reported as means ± SEM. *P < 0.01 compared to fructose (one-way ANOVA).

Table 1. Fasting serum biochemical parameters of Wistar rats before and after supplementation with glucose or fructose for 21 days.

	Glucose		Fructose	
	Before	After	Before	After
Glucose (mM)	8.9 ± 0.2	9.3 ± 0.2	7.9 ± 0.23	9.3 ± 0.1*
Insulin (mU/L)	26.9 ± 12.9	34.1 ± 12.3	23.9 ± 6.7	37.1 ± 15.9
Cholesterol (mM)	1.6 ± 0.03	1.6 ± 0.03	1.5 ± 0.03	1.4 ± 0.04*
Triacylglycerol (mM)	0.9 ± 0.06	1.5 ± 0.08*	1.4 ± 0.1	2.1 ± 0.1*
HDL cholesterol (mM)	0.8 ± 0.06	0.9 ± 0.04*	1.1 ± 0.02	1.0 ± 0.03*
nHDL cholesterol (mM)	0.8 ± 0.06	0.7 ± 0.05*	0.4 ± 0.02	0.4 ± 0.03
Free fatty acids (mEq/L)	0.6 ± 0.06	0.5 ± 0.05	0.4 ± 0.03	0.4 ± 0.01

Rats received 8 g/kg body weight per day of glucose and fructose. Data are reported as means ± SEM. N = 40 for each treatment except for insulin data (glucose, N = 5; fructose, N = 7). HDL cholesterol = cholesterol in high-density lipoproteins; nHDL cholesterol = cholesterol in non-HDL particles.
*P < 0.05 before *vs* after treatment (ANOVA).

In mice, the AUC and IAUC of body weight and food intake were similar for the two dietary supplements. Contrary to the rats' response, water consumption was significantly larger in fructose- than in glucose-supplemented mice (Figure 1C and D).

Biochemical serum parameters of Wistar rats before and after carbohydrate supplementation

A comparison of the effects of glucose and fructose supplementation on the fasting lipid profile and on insulin and glucose concentrations in Wistar rats is presented in Table 1. Glycemia and cholesterolemia were significantly higher and lower, respectively, when fructose was administered. An increase in insulin levels was observed in glucose- (26%) and fructose-supplemented (54%) rats. However, no statistical differences was demonstrable, probably due to the small sample number available for this assay (N = 5 and 7 animals in each group, respectively). Also, the homeostasis model assessment-insulin resistance ($HOMA_{IR}$) parameter was increased after glucose and fructose supplementation (9.5 ± 3.4 *vs* 12.7 ± 5.3, and 7.8 ± 2.2 *vs* 8.9 ± 1.4, before *vs* after glucose and fructose supplementation, respectively). No statistical differences were demonstrable.

The triacylglycerolemia was significantly increased 1.5-fold after supplementation with both carbohydrates (glucose or fructose) and this effect was also demonstrated on very low-density lipoprotein (VLDL)-TG content (Figure 2, panels A and B, respectively, for the glucose and fructose supplementation). In contrast, FFA levels did not change after supplementation with glucose or fructose (Table 1).

Serum total cholesterol was not modified by glucose supplementation. However, cholesterol distribution in plasma lipoproteins was significantly altered: HDL cholesterol increased by 13% and non-HDL cholesterol decreased by 15%. Total serum cholesterol

level decreased by 7% after fructose supplementation in rats ($P < 0.05$). The percentage of cholesterol content in LDL particles also decreased (Figure 2, panels C and D) after both glucose and fructose supplementation, and HDL cholesterol decreased by 10% in rats treated with fructose.

When comparing the effects of glucose and fructose (expressed as the percentage of basal levels) in promoting serum biochemical variations, the fructose supplementation promoted a higher increase of fasting glycemia (16.7 *vs* 4.7%, $P = 0.004$), a milder increase in triacylglycerolemia (41 *vs* 62%, $P = 0.08$) and a significant decrease in cholesterolemia (-6.7 *vs* 0%, $P = 0.01$, respectively) when compared with glucose supplementation.

Biochemical serum parameters of C57BL/6 mice before and after carbohydrate supplementation

A comparison of the effects of carbohydrate supplementation on the fasting lipid profile and on insulin and glucose concentrations in C57BL/6 mice is presented in Table 2.

Mice presented a significant increase in fasting glycemia, 45% on average after both carbohydrate diet supplementations. The glucose treatment was responsible for a significant decrease in insulin levels (33%), but the $HOMA_{IR}$ was not modified by either carbohydrate treatment (1.8 ± 0.5 *vs* 1.8 ± 0.5, and 1.2 ± 0.3 *vs* 1.3 ± 0.2, before *vs* after glucose and fructose treatments, respectively).

The lipid profile was significantly altered by glucose and fructose supplementations, with decreases in cholesterol, TG, non-HDL cholesterol and decreases in FFA levels only in fructose-treated mice (Table 2).

The decrease in triacylglycerolemia (24%) was also observed in the TG content of VLDL particles (Figure 3, panels A and B). The decrease in cholesterol in non-HDL particles was caused by a decrease mainly in VLDL cholesterol content (Figure 3, panels C and D).

Comparison between the effects of glucose and fructose, as percentage of basal levels, shows that fructose supplementation

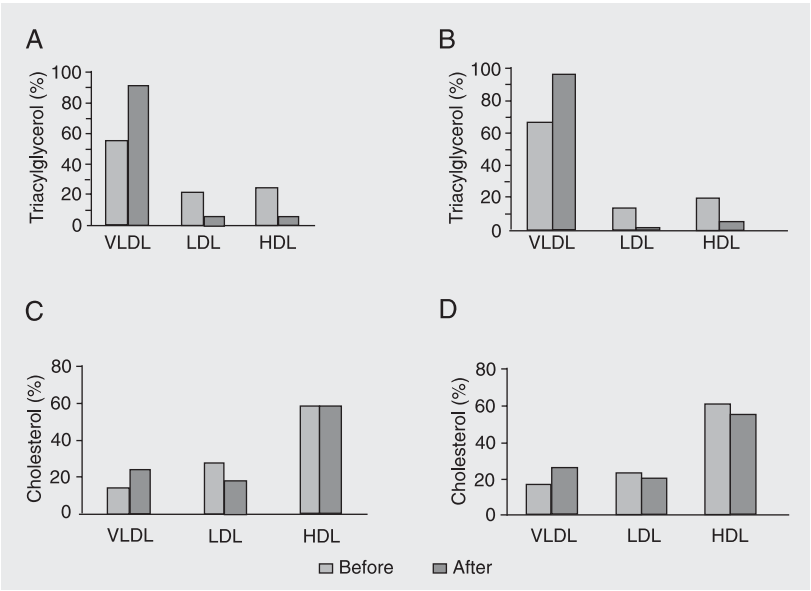

Figure 2. Distribution of triacylglycerol (A and B) and cholesterol (C and D) in Wistar rat serum lipoproteins isolated by FPLC before and after dietary supplementation with 8 g/kg body weight of glucose/day (A, C) or fructose/day (B, D) for 21 days. The data were obtained by FPLC analyses of pooled serum samples from 3 rats in each condition. The percentage of triacylglycerol and cholesterol in lipoprotein classes was calculated as the areas under the peaks of each lipoprotein class using the sum of all values of triacylglycerol or cholesterol determined in each fraction as 100% (total area under the three peaks of very low- (VLDL), low- (LDL) and high-density lipoprotein (HDL).

Table 2. Fasting serum biochemical parameters of C57BL/6 mice before and after supplementation with glucose or fructose for 21 days.

	Glucose		Fructose	
	Before	After	Before	After
Glucose (mM)	6.5 ± 0.2	10.0 ± 0.5*	6.6 ± 0.2	8.9 ± 0.47*
Insulin (mU/L)	6.7 ± 2.2	4.4 ± 0.9*	5.4 ± 1.0	3.5 ± 0.6
Cholesterol (mM)	2.7 ± 0.08	2.5 ± 0.03*	2.7 ± 0.05	2.5 ± 0.03*
Triacylglycerol (mM)	1.8 ± 0.11	1.4 ± 0.05*	1.9 ± 0.1	1.4 ± 0.05*
HDL cholesterol (mM)	2.0 ± 0.03	1.9 ± 0.04	2.1 ± 0.03	1.9 ± 0.04*
nHDL cholesterol (mM)	0.7 ± 0.08	0.6 ± 0.04*	0.6 ± 0.04	0.5 ± 0.03*
Free fatty acids (mEq/L)	2.4 ± 0.3	1.8 ± 0.1	2.2 ± 0.2	1.9 ± 0.2*

Mice received 8 g/kg body weight per day of glucose and fructose. Data are reported as means ± SEM. N = 30 for each treatment except for insulin data (N = 9 for each treatment). HDL cholesterol = cholesterol in high density lipoproteins; nHDL cholesterol = cholesterol in non-HDL particles.
*$P < 0.05$ before *vs* after treatment (ANOVA).

was more potent for lowering free fatty acid concentrations than glucose treatment in C57BL/6 mice ($P < 0.01$).

Correlation analyses

A significant positive correlation was found between the variation of glycemia and body weight gain (AUC) in rats treated with glucose and fructose ($r = 0.36$, $P = 0.014$). The variation in serum HDL cholesterol ($r = -0.42$, $P = 0.0033$) and TG ($r = -0.49$, $P = 0.0006$) correlated negatively with body weight gain (AUC) in glucose-treated but not in fructose-treated rats.

Comparison of glucose and fructose dietary supplementation in rats and mice

Wistar rats and C57BL/6 mice presented different metabolic responses when submitted to a relatively short period of dietary supplementation with glucose and fructose. They responded in opposite ways in terms of variations in triacylglycerolemia, FFA and insulinemia after both monosaccharide treatments. In addition, C57BL/6 mice presented a greater decrease in cholesterol, HDL cholesterol and non-HDL cholesterol concentrations ($P = 0.04$; $P = 0.01$; $P = 0.03$, respectively) when compared with glucose- and fructose-supplemented Wistar rats.

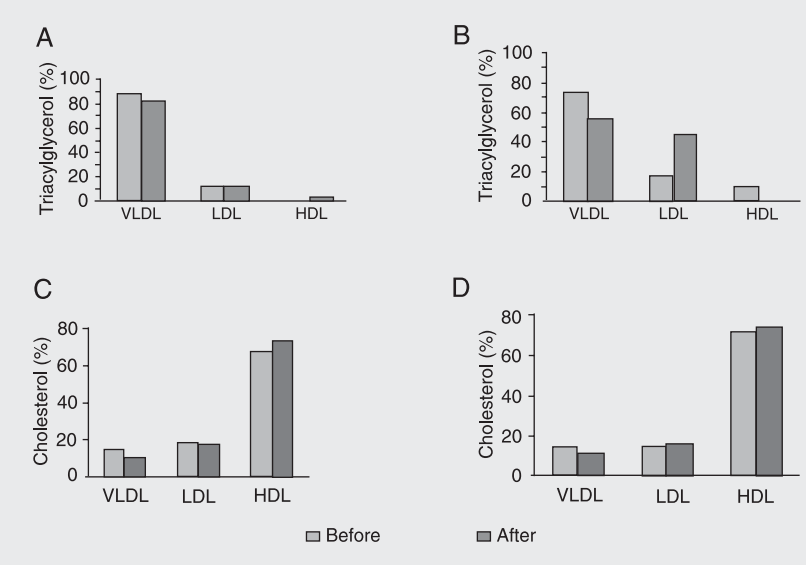

Figure 3. Distribution of triacylglycerol (A and B) and cholesterol (C and D) in C57BL/6 mouse serum lipoproteins isolated by FPLC before and after dietary supplementation with glucose (A and C) or fructose (B and D) for 21 days. The data were obtained by FPLC analyses of pooled serum samples from 3 mice in each condition. The percentage of triacylglycerol and cholesterol in lipoprotein classes was calculated as the areas under the peaks of each lipoprotein class using the sum of all values of triacylglycerol or cholesterol determined in each fraction as 100% (total area under the three peaks of very low- (VLDL), low- (LDL) and high-density lipoprotein (HDL).

Discussion

Genetic and environmental factors are considered to be responsible for metabolic disturbances such as plurimetabolic syndrome, diabetes mellitus, dyslipidemia, and obesity. Among environmental factors, the refined carbohydrates, extensively used in industrialized countries, are among the most important causes of these disturbances.

Several investigators have demonstrated that high-glucose or -fructose diets are associated with the development of insulin resistance, and increased serum TG and insulin levels (1,2,4,6,13,29-34).

The present results show that carbohydrate supplementations were responsible for the increase in fasting glycemia in both rats and mice, although this increase was not statistically significant in glucose-supplemented rats. In rats, the increases in insulin levels after carbohydrate treatments were 26 and 54%, respectively, for glucose- and fructose-treated rats, in agreement with the results of Huang et al. (34) and Kelley et al. (13). Higher insulin, glucose and TG levels in the glucose- and fructose-treated rats indicate that the animals probably developed insulin resistance. Surprisingly, the carbohydrate-supplemented C57BL/6 mice showed significant reductions in fasting insulin levels. Similar results were obtained by Ostos et al. (25) with fructose-supplemented control C57BL/6 mice. Differently, Rebuffe-Scrive et al. (17) showed hyperglycemia, hyperinsulinemia, hypercholesterolemia, and increased visceral fat depot in C57BL/6J

mice when compared with the control A/J mouse strain after 5 months of high-fat, high-simple (primarily disaccharides) carbohydrate diets. Thus, the differences between Wistar rats and C57BL/6 mice described here may be related to the specific mouse strain rather than to the animal genus.

High-carbohydrate diets are recommended for humans to lower the risk of coronary heart diseases because they decrease LDL cholesterol concentrations (35-37). In the present study, we found significant decreases in total cholesterol in both rodent genera (Wistar rats and C57BL/6 mice) after both glucose and fructose supplementation. Although glucose-treated rats did not show a significant decrease in total cholesterol, they presented lower non-HDL cholesterol levels.

The increase in triacylglycerolemia found in glucose-supplemented rats was more pronounced than that produced by fructose (62 *vs* 41% for glucose and fructose, respectively), although the absorbed fructose is preferably taken up by the liver independently of the rate-limiting glycolytic enzymes (8). The concomitant increases in the TG and cholesterol content of VLDL in carbohydrate-supplemented rats suggest an increased hepatic secretion rate of VLDL particles and impaired plasma VLDL clearance, which are frequently observed in diabetes and insulin resistance states.

In C57BL/6 mice, on the other hand, the significant decrease of approximately 20% in triacylglycerolemia, observed after supplementation with both monosaccharides, was probably due to a decrease in hepatic VLDL-TG secretion. Our results differ from those reported by Shafrir et al. (24) in a study on spiny mice treated with a sucrose-rich diet for a long time (7 months). These investigators found a significant increase in TG when compared to controls treated with a commercial diet. On the other hand, Ostos et al. (25) did not find any statistical difference in TG concentration in C57BL/6 mice supplemented with fructose for 9 months. However, transgenic C57BL/6 mice over-expressing the human gene cluster AI-CIII-AIV, that present basal hypertriacylglycerolemia, responded with an additional increase in TG levels when submitted to fructose supplementation.

Menahan and Sobocinski (38) described a differential response to regular feeding in Sprague-Dawley rats and Swiss mice. In the fed state, Swiss mice presented higher glycemia than Sprague-Dawley rats, but this difference disappeared after 48 h of fasting. Serum concentrations of TG and insulin were similar in these rats and mice in the fed as well as in the fasting state.

Wistar rats and C57BL/6 mice belonging to the same Muridae family are regularly used as experimental models for metabolic studies in an indistinct way, but they are very different metabolically. This study presents for the first time in the literature strikingly different responses between these two genera after challenge with both carbohydrate oral supplementations for 21 days: Wistar rats acquired insulin resistance and hyperinsulinemia whereas C57BL/6 mice presented carbohydrate intolerance with low-plasma insulin levels; rats increased triglycerides and had no changes in FFA and C57BL/6 mice reduced both triglycerides and FFA (significantly only with fructose treatment).

Glucose supplementation caused an elevation in HDL cholesterol in Wistar rats but no changes were observed in C57BL/6 mice, and, in contrast, fructose significantly reduced HDL cholesterol in both rats and mice. Glucose, but not fructose, supplementation reduced non-HDL cholesterol in Wistar rats, while in C57BL/6 mice both carbohydrates reduced non-HDL cholesterol.

Since the mainly used rodent reference for metabolic studies in the literature is the Wistar rat, this study points to important differences in C57BL/6 mice that should be highlighted and emphasized in the scientific community.

The new contribution of this research is that similar metabolic responses to humans, after sub-acute carbohydrate intake, were detected only in Wistar rats and were mainly the features of the metabolic syndrome. Surprisingly, the response of C57BL/6 mice under the same treatment conditions pointed to an anti-atherogenic lipid plasma profile. Future studies of Murine models should aim at understanding the mechanisms of these striking metabolic differences and of the suggestive diverse atherogenic patterns.

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