Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols

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In search of an adequate model for the human metabolic syndrome, the metabolic characteristics of Wistar rats were analysed after being submitted to different protocols of high fructose ingestion. First, two adult rat groups (aged 90 d) were studied: a control group (C1; n 6) received regular rodent chow (Labina, Purina) and a fructose group (F1; n 6) was fed on regular rodent chow. Fructose was administered as a 10% solution in drinking water. Second, two adult rat groups (aged 90 d) were evaluated: a control group (C2; n 6) was fed on a balanced diet (AIN-93G) and a fructose group (F2; n 6) was fed on a purified 60% fructose diet. Finally, two young rat groups (aged 28 d) were analysed: a control group (C3; n 6) was fed on the AIN-93G diet and a fructose group (F3; n 6) was fed on a 60% fructose diet. After 4–8 weeks, the animals were evaluated. Glucose tolerance, peripheral insulin sensitivity, blood lipid profile and body fat were analysed. In the fructose groups F2 and F3 glucose tolerance and insulin sensitivity were lower, while triacylglycerolaemia was higher than the respective controls C2 and C3 (P<0.05). Blood total cholesterol, HDL and LDL as well as body fat showed change only in the second protocol. In conclusion, high fructose intake is more effective at producing the signs of the metabolic syndrome in adult than in young Wistar rats. Additionally, diet seems to be a more effective way of fructose administration than drinking water.

Fructose: Metabolic syndrome: Insulin sensitivity: Body fat

Clinically, the metabolic syndrome involves a cluster of disturbances in which glucose intolerance represents an important symptom. Metabolic syndrome diagnosis implies in positive results to at least three metabolic alterations including insulin resistance, hypertension, obesity, endothelial dysfunction and blood lipid profile alterations. These multiple risk factors accelerate the incidence of CVD in a cooperative way. Obesity prevalence has quadrupled in the past 25 years in the USA; 16% of children and 30% of adults are now affected and many of these obese individuals suffer from the metabolic syndrome. Projections estimate that, in the year 2010, there will exist 50–75 million individuals with manifestation of this syndrome in this country alone.

The increased fructose consumption in contemporaneous Western society has been associated with the high prevalence of non-alcoholic fatty liver disease (NAFLD), obesity, type 2 diabetes and metabolic syndrome so far. In fact, laboratory animals fed on fructose-rich diets show glucose intolerance, insulin resistance, hyperinsulinemia and dyslipidaemia. A high flux of fructose to the liver perturbs glucose metabolism and glucose uptake pathways, and leads to a significantly enhanced rate of lipogenesis and TAG synthesis, driven by the high flux of glyceral and acyl portions of TAG molecules from fructose catabolism. These metabolic disturbances maybe underlie the induction of insulin resistance, commonly observed with high fructose feeding in both human subjects and animal models. Since fructose-fed rats reveal signs of the metabolic syndrome, they are used as a experimental model of the human condition.

The metabolic alterations observed in fructose-fed rats are quite divergent among the studies, probably due to study design. Differences between studies include: the strain of rat used, such as Wistar and Sprague–Dawley; the amount and route of fructose administration – diet or drinking water; the age of the animals at the beginning of the experiment – young or adults. Also, the period of fructose administration applied has been non-standard: from 4, 6 or 8 weeks or during several months. Natural variation of the control diet is another potential problem of animal studies. It can be a particular problem with non-purified diets, but the use of synthetic diets is largely described in the literature. Therefore, different experimental designs for fructose administration in rats induce variable degrees of physiological responses.

Abbreviations: C1, experiment 1 control group; C2, experiment 2 control group; C3, experiment 3 control group; F1, experiment 1 fructose group; F2, experiment 2 fructose group; F3, experiment 3 fructose group; NAFLD, non-alcoholic fatty liver disease.

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Methods

Animal care and experimental design

Male Wistar rats were obtained from the São Paulo State University (UNESP Central Bioterium, Botucatu Campus, SP, Brazil). The rats were kept in a room with the temperature set to 25 ± 1°C and with a photoperiod of 12 h–12 h at the Physical Education Department Biodynamic laboratory of UNESP (Rio Claro campus, São Paulo, Brazil). Free access to water and food was provided. All experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe no. 123, Strasbourg 1985).

The animals were separated at random and the studies were carried out in three separated series of experiments. In the first, two groups of adult rats were studied: the control group (C1; n = 6) received regular rodent chow (57.3 % carbohydrate, 41.2 % as starch, Labina; Purina, São Paulo, Brazil) and the fructose group (F1; n = 6) was fed on a regular rodent chow and 10 % of drinking water was composed of fructose solution. Both groups started the experiment aged 90 d and the follow-up was done for 4 weeks.

In the second experiment, two groups of adult rats were also evaluated: the control group (C2; n = 6) was fed on a purified balanced diet (AIN-93G)(21) and a fructose group (F2; n = 6) was fed on a purified 60 % fructose diet (Table 1). Both groups started the experiment at age 90 d and the follow-up was done for 8 weeks.

In the second experiment, two groups of adult rats were also evaluated: the control group (C3; n = 6) was fed on a balanced AIN-93G diet and the fructose group (F3; n = 6) was fed on a 60 % fructose diet. Both groups started the experiment at age 90 d and the follow-up was done for 4 weeks.

Two groups of young rats were analysed in the third experiment: the control group (C4; n = 6) was fed on a regular rodent chow (AIN-93G), and the fructose group (C5; n = 6) was fed on a 60 % fructose diet. Both groups started the experiment at age 28 d and the follow-up was done for 8 weeks.

All animals were weighed and measured (nose to anus length) once per week. At the end of the experimental period, the Lee index was calculated (22) (by dividing the length) once per week. At the end of the experimental period, the Lee index was calculated (22) (by dividing the length by the final body weight). Blood samples for serum glucose determination were obtained from a cut at the tip tail at 0, 30, 60 and 120 min. Serum glucose determination was made by the glucose oxidase method (Laborlab Kit; Guarulhos, SP, Brazil)(23). The glycaemic response during the oral glucose tolerance test was evaluated by the total area under the serum glucose curve using the trapezoidal method(24).

Subcutaneous insulin tolerance test

At the end of all experimental series, subcutaneous insulin tolerance tests were performed for peripheral insulin sensitivity evaluation. The insulin tolerance tests consisted of a bolus injection of regular insulin at the dorsal region (30 mU/g body weight). Blood samples were obtained from a cut at the tip tail at 0, 30, 60 and 120 min for serum glucose determination by the glucose oxidase method (Laborlab Kit)(23). A constant for serum glucose disappearance (Kitt) was calculated from the formula 0.693/t1/2. The serum glucose t1/2 was calculated from the slope of the least square analysis of serum glucose concentration from 0–30 min after insulin injection, when serum glucose concentration decreased linearly(25).

Blood chemistry and body fat

Because of multiple aspects involving insulin sensitivity evaluation, the fasting insulin concentration was also assessed.

All animals were killed by decapitation and trunk blood was collected for serum TAG, total cholesterol, HDL-cholesterol, LDL-cholesterol and glucose determinations by colorimetric procedures(26). Retroperitoneal, mesenteric (visceral) and subcutaneous posterior fat depots were excised and weighed according to Cinti(27). Liver total lipid concentration(26) was also determined.

Statistics

In each experiment, the values of weight and body length throughout the period of dietary intake were analysed by two-way ANOVA. All the other results were analysed statistically by the unpaired Student’s t test. A level of 5 % was taken for statistical significance.

Results

The weight and body-length values in the first experiment are shown in Fig. 1. No difference was revealed.
Data from the first series are displayed in Table 2. No metabolic difference was observed between control (C1) and fructose-fed (F1) rats in any of the parameters evaluated. The average amount of fructose ingested corresponded to 1·74 g/100 g body weight per d.

No differences were observed in weight or body-length values in the second experiment (Fig. 2).

The data of the second experiment are shown in Table 3. The fructose group (F2) presented higher values for the area under the serum glucose curve during glucose tolerance tests when compared with the control group (C2). Rats of the F2 group developed insulin resistance when compared with the C2 group, indicated by a lower constant for serum glucose disappearance (Kitt) value and a higher fasting insulin concentration. No difference was observed in the basal serum glucose values between the two groups. Serum total cholesterol, HDL, LDL and TAG were higher in the F2 group than in the C2 group. In this experiment higher retroperitoneal, mesenteric and subcutaneous fat depot weights were observed in the F2 group in comparison with the C2 group. The concentration of liver total lipids was also higher in the F2 group compared with the C2 group. No difference was found between the groups in the results related to the Lee index. The average amount of fructose ingested in this second experiment was 2·70 g/100 g body weight per d.

No difference was observed in weight or body-length values in the third experiment (Fig. 3).

Table 4 shows data from the third series of experiments. The area under the serum glucose curve during glucose tolerance tests and TAG concentration were higher for the F3 group when compared with the C3 group. No differences were observed between the groups in insulin resistance, serum total cholesterol, basal glucose, HDL, LDL, retroperitoneal, mesenteric and subcutaneous fat depot weights and the Lee index. In this third experiment, the average amount of fructose ingested was 3·75 g/100 g body weight per d.

### Discussion

There has been clinical and epidemiological evidence suggesting a progressive association of metabolic syndrome development and high fructose consumption\(^{28}\). Indeed, a marked increase in obesity and metabolic syndrome

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**Table 2. Effects of 8 weeks of fructose feeding as 10% solution in drinking water in adult male Wistar rats (experiment 1)**

(Mean values and standard deviations of six animals per group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (C1)</th>
<th>Fructose (F1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under serum glucose curve during GTT (mm × 120 min)</td>
<td>1084±0</td>
<td>1124±9</td>
</tr>
<tr>
<td>Kitt (%/min)</td>
<td>0.60±0.27</td>
<td>0.61±0.48</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>4.09±0.54</td>
<td>4.16±0.63</td>
</tr>
<tr>
<td>Serum glucose (mm)</td>
<td>5.7±0.6</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td>Serum total cholesterol (mm)</td>
<td>2.19±0.48</td>
<td>2.38±0.44</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mm)</td>
<td>1.64±0.26</td>
<td>1.82±0.16</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mm)</td>
<td>0.49±0.23</td>
<td>0.63±0.37</td>
</tr>
<tr>
<td>Serum TAG (mm)</td>
<td>1.90±0.64</td>
<td>1.82±0.67</td>
</tr>
<tr>
<td>Retroperitoneal fat depot weight (mg/100 g bw)</td>
<td>533±7</td>
<td>572±5</td>
</tr>
<tr>
<td>Mesenteric fat depot weight (mg/100 g bw)</td>
<td>651±5</td>
<td>697±8</td>
</tr>
<tr>
<td>Subcutaneous posterior fat depot weight (mg/100 g bw)</td>
<td>514±4</td>
<td>521±2</td>
</tr>
<tr>
<td>Liver total lipids (mg/100 mg)</td>
<td>5.35±0.94</td>
<td>5.23±0.57</td>
</tr>
<tr>
<td>Lee index</td>
<td>318±6</td>
<td>316±6</td>
</tr>
</tbody>
</table>

GTT, glucose tolerance test; Kitt, constant for serum glucose disappearance; bw, body weight.

* See Methods for details of the diets.
prevalence has been linked to a 30% overall increase in fructose ingestion in the last 20 years within the USA. It has been associated with the introduction of high-fructose maize syrup as a sweetener in soft drinks and other foods\(^5\). In the present study, we examined the effect of three different protocols of fructose administration on the metabolic characteristics of Wistar rats.

In the first protocol, fructose (10%) was administered to adult (90 d) male Wistar rats in drinking water during 8 weeks. No metabolic differences were observed between the control and fructose-fed rats. Using the same procedure, Sanchez-Lozada \textit{et al.} \(^{19}\) reported that fructose administration was able to induce systemic hypertension, hyperuricaemia and hypertriacylglycerolaemia in adult male Sprague–Dawley rats. Roglans \textit{et al.} \(^{29}\) reported hypertriacylglycerolaemia and hepatic steatosis in Sprague–Dawley rats (the authors neither mention the age nor the weight of the animals at the beginning of the experiment) that had received fructose (10%) in drinking water for 2 weeks. Additionally, while adult Sprague–Dawley rats fed fructose through drinking water developed features of the metabolic syndrome, adult Wistar rats presented a serum biochemical profile considered to be healthier for the cardiovascular system. Thus, the reported differences may be associated with the rat lineage used. In fact, Wistar rats seem to be less affected by the deleterious effects of fructose when administered in drinking water than Sprague–Dawley ones.

Sanchez-Lozada \textit{et al.} \(^{19}\) compared the metabolic effects of fructose (10%) in drinking water and a high-fructose (60%) diet in Sprague–Dawley rats. Both procedures induced hyperuricaemia and hypertriacylglycerolaemia. However, the 60% fructose diet resulted in a higher fructose energy intake, which was directly associated with worsening metabolic syndrome parameters. Other studies have also reported hyperinsulinaemia, hypertriacylglycerolaemia and glucose intolerance in Sprague–Dawley rats fed on 60% fructose diets from 4 to 7 weeks\(^{11,12,17,18,30–32}\). Thus, in the second experiment series, a fructose diet was administered to adult male Wistar rats (age 90 d) for 4 weeks.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Weight (a) and length (b) of fructose-fed (– –) and control (– –) animals in the second experiment (\(P > 0.05\)).}
\end{figure}

\begin{table}[h]
\centering
\caption{Effects of 4 weeks of feeding a 60% fructose diet in adult male Wistar rats (experiment 2)†}
\begin{tabular}{lccccc}
\hline
Group & Control (C2) & Fructose (F2) \\
\hline
Parameter & Mean & SD & Mean & SD \\
\hline
Area under serum glucose curve during GTT (mm \(\times\) 120 min) & 862.5 & 80.4 & 951.4* & 106.8 \\
Kitt (%/min) & 0.73 & 0.16 & 0.47* & 0.16 \\
Fasting insulin (\(\mu\text{U/ml}\)) & 4.04 & 0.93 & 4.91* & 1.42 \\
Serum glucose (mm) & 6.83 & 0.93 & 6.33 & 1.3 \\
Serum total cholesterol (mm) & 2.54 & 0.16 & 5.82* & 1.92 \\
Serum HDL-cholesterol (mm) & 0.78 & 0.17 & 1.08* & 0.16 \\
Serum LDL-cholesterol (mm) & 0.51 & 0.19 & 0.74* & 0.20 \\
Serum TAG (mm) & 1.28 & 0.44 & 2.55* & 0.84 \\
Retroperitoneal fat depot weight (mg/100 g bw) & 598.2 & 173.9 & 873.9* & 347.9 \\
Mesenteric fat depot weight (mg/100 g bw) & 659.9 & 192.1 & 999.9* & 263.8 \\
Subcutaneous posterior fat depot weight (mg/100 g bw) & 429.5 & 197.9 & 640.4* & 203.6 \\
Liver total lipids (mg/100 mg) & 5.4 & 0.3 & 9.70* & 4.0 \\
Lee index & 294.3 & 6.3 & 296.8 & 7.8 \\
\hline
\end{tabular}
\footnotesize{GTT, glucose tolerance test; Kitt, constant for serum glucose disappearance; bw, body weight.}
\footnotesize{* Mean value was significantly different from that of the control group \((P < 0.05); \text{unpaired } t\text{-test})}.
\footnotesize{† For details of the diets, see Methods.}
\end{table}
Using the fructose-rich diet protocol, we also succeeded in inducing signs of the metabolic syndrome in fructose-fed Wistar rats, such as glucose intolerance, insulin resistance and high serum TAG, total cholesterol and LDL as well as a high concentration of total liver lipids.

The elevated liver lipid accumulation is the sign of NAFLD that may contribute to the development of non-alcoholic steatohepatitis\(^{33}\) in individuals who do not consume significant amounts of ethanol\(^{34}\). This result reinforces the assumption that not only high-fat dietary consumption plays a role in NAFLD, but also high fructose ingestion. In this way, Ouyang et al.\(^{35}\) showed that fructose ingestion in patients with NAFLD was 2- to 3-fold higher than in control groups. It has been reported that adult Sprague–Dawley rats given fructose solution with standard diets gained more weight and had significantly more fat tissue weight than control rats given only a standard diet\(^{36}\). Alterations in body fat were also observed in the fructose-fed rats of the second experiment, which showed retroperitoneal, mesenteric and subcutaneous fat depot weight increase.

Lau et al.\(^{37}\), in a prospective study with non-diabetic patients, found no association between the glycaemic index or glycaemic load of the diet with the probability of developing insulin resistance. Barbosa et al.\(^{17}\) compared the effects of glucose and fructose supplementation (8 g/kg) in Wistar rats during 3 weeks. The authors reported insulin resistance and hypertriacylglycerolaemia in response to both treatments, but the glycaemia was higher in the fructose group. The fructose glycaemic index is about 3-fold lower than glucose and therefore the alterations observed may not be related to this index or even to glycaemic load (glycaemic index multiplied by amount in grams). In addition, the second experiment, which revealed more damage to the rats, presented just an intermediate value of daily fructose ingested in comparison with the first and third experiments.

The third experiment series was designed to analyse the metabolic alterations caused in young (28 d) Wistar rats by administration of a high-fructose diet for 8 weeks. The young animals fed on the high-fructose diet showed hypertriacylglycerolaemia and glucose intolerance in the absence of alterations in insulin sensitivity, serum total cholesterol, LDL and HDL concentration as well as mesenteric, retroperitoneal and subcutaneous fat depot weights when compared with the control group. In contrast, insulin sensitivity

**Table 4.** Effects of 8 weeks of feeding a 60% fructose diet in young male Wistar rats (experiment 3)†

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (C3)</th>
<th>Fructose (F3)</th>
<th>Control (C3)</th>
<th>Fructose (F3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under serum glucose curve during GTT (μM × 120 min)</td>
<td>1128±1</td>
<td>1246±8*</td>
<td>92.6</td>
<td>69.1</td>
</tr>
<tr>
<td>Kitt (%/min)</td>
<td>0.62±0.23</td>
<td>0.65±0.37</td>
<td>0.62±0.23</td>
<td>0.65±0.37</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>4.18±0.55</td>
<td>4.07±0.61</td>
<td>4.18±0.55</td>
<td>4.07±0.61</td>
</tr>
<tr>
<td>Serum glucose (μM)</td>
<td>7.12±0.83</td>
<td>6.75±0.89</td>
<td>7.12±0.83</td>
<td>6.75±0.89</td>
</tr>
<tr>
<td>Serum total cholesterol (μM)</td>
<td>2.47±0.58</td>
<td>3.01±0.50</td>
<td>2.47±0.58</td>
<td>3.01±0.50</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (μM)</td>
<td>0.98±0.27</td>
<td>1.09±0.3</td>
<td>0.98±0.27</td>
<td>1.09±0.3</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (μM)</td>
<td>0.79±0.33</td>
<td>0.83±0.43</td>
<td>0.79±0.33</td>
<td>0.83±0.43</td>
</tr>
<tr>
<td>Serum TAG (μM)</td>
<td>0.68±0.19</td>
<td>1.09±0.75</td>
<td>0.68±0.19</td>
<td>1.09±0.75</td>
</tr>
<tr>
<td>Retroperitoneal fat depot weight (mg/100 g bw)</td>
<td>713±7</td>
<td>764±8</td>
<td>290±4</td>
<td>228±1</td>
</tr>
<tr>
<td>Mesenteric fat depot weight (mg/100 g bw)</td>
<td>762±6</td>
<td>782±0</td>
<td>237±7</td>
<td>143±5</td>
</tr>
<tr>
<td>Subcutaneous posterior fat depot weight (mg/100 g bw)</td>
<td>772±0</td>
<td>696±4</td>
<td>356±6</td>
<td>249±5</td>
</tr>
<tr>
<td>Liver total lipids (mg/100 mg)</td>
<td>6±48</td>
<td>6±80</td>
<td>1±34</td>
<td>2.5±5</td>
</tr>
<tr>
<td>Lee index</td>
<td>298±8</td>
<td>307±9</td>
<td>7±6</td>
<td>10±7</td>
</tr>
</tbody>
</table>

GTT, glucose tolerance test; Kitt, constant for serum glucose disappearance; bw, body weight.

* Mean value was significantly different from that of the control group (\(p < 0.05\); unpaired t-test).
† For details of the diets, see Methods.
impairment has been described in 5-week-old Wistar–Han
over rats, after 28 d of high-fructose diet ingestion associated
with hypertriglyceridaemia and unchanged body-weight
gain, basal serum glucose and serum total cholesterol (38).
Interestingly, the third experiment showed that the average
amount of daily fructose ingestion was almost 40% higher
than in the second experiment, but did not induce a complete
metabolic syndrome picture in these animals. A reasonable
hypothesis could be the influence of protective mechanisms
linked to youth in Wistar rats. However, further investigation
will be necessary.

It is important to note that the AIN-93G provided in both
series of experiments is recommended for the growth phase
and is composed of 75% more fat and 42.9% more casein
than an adult maintenance diet. However, the C2 rats pre-
sented fat depot values very similar to the C1 rats that ingested
the regular rodent chow.

The dietary ingestion of fructose in humans is basically
associated with soft drinks (whose sugars are composed of
45% of glucose and 55% of fructose), which account for
just 8% of total energy intake (39). However, Montonen
et al. (40) in a cohort-designed study reported that the risk
of type 2 diabetes occurrence is 1.67 higher with this dietary
pattern than drinking water.

It seems to provide a better effective route for fructose adminis-
tration in Wistar rats, despite a shorter feeding period. Also, diet
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tose-fed rat hearts are protected against ischemia-reperfusion

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O. conducted study 1. E. S. was responsible for designing
and planning study 1. M. A. R. M. designed, planned and
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