High-Fructose Feeding Elicits Insulin Resistance, Hyperinsulinism, and Hypertension in Normal Mongrel Dogs

F. Javier Martinez, Robert A. Rizza, J. Carlos Romero

Abstract To determine whether chronic high-fructose feeding causes insulin resistance and hypertension in normal dogs, we fed 10 male dogs a normosodic diet containing 60% of the calories as fructose for 20 to 28 days; a control group of 8 dogs was fed a similar diet containing dextrose instead of fructose. In the fructose-fed group, (1) fasting triglyceridemia increased from 35.3±0.63 to 91.9±11.55 mg/dL after 25 days (P<.001); (2) fasting insulinemia increased from 19.0±1.9 to 58.9±7.22 μU/mL after 25 days (P<.001); (3) insulin resistance, which was estimated by steady-state glycemia during an insulin suppression test, increased from 105.8±21.5 to 187.8±32.6 mg/dL after 15 days (P<.001), whereas steady-state insulinemia did not change; (4) mean arterial pressure increased from 100.4±1.6 to 122.6±2.3 mm Hg after 28 days (P<.01); and (5) cumulative sodium balance was increased on days 7 through 11 (111.60±4.44 mEq on day 8, P<.01), returning to normal for the rest of the experiment. All these parameters were similar between the fructose-fed and dextrose-fed groups before the diets were started and remained constant in the dextrose-fed group. Neither group showed any change in body weight, fasting plasma glucose, atrial natriuretic factor, or endothelin-1 levels. We conclude that chronic high-fructose feeding elicits hypertriglyceridemia, insulin resistance, hyperinsulinemia, hypertension, and a transient sodium retention in dogs without fostering fasting hyperglycemia or weight gain. Endothelin and atrial natriuretic factor do not appear to play a role in the development of hypertension in this model. (Hypertension. 1994;23:456-463.)

Key Words • fructose • insulin • triglycerides • hypertension, experimental • atrial natriuretic factor • endothelins • dogs

There is overwhelming epidemiologic evidence that insulin resistance and hyperinsulinemia are strongly associated with hypertension. A constellation of cardiovascular risk factors fostered by insulin resistance, including alterations in the circulating lipidic profile (such as hypertriglyceridemia and hypercholesterolemia with high low-density lipoprotein and low-high-density lipoprotein cholesterol), atherosclerosis, and hypertension, has been described and conventionally called "syndrome X." The frequent association between non-insulin-dependent diabetes mellitus, obesity, and hypertension has been tentatively ascribed to the presence of insulin resistance. However, a marked impairment in insulin action has also been demonstrated in nonobese, nondiabetic essential hypertensive subjects.

Insulin resistance and hypertension have been induced in rodents by chronic high-sucrose or high-fructose feeding. These models have provided important information regarding the biochemical mechanisms responsible for the impairment of insulin activity.

However, despite these advances, the pathogenic mechanisms that relate insulin resistance to hypertension remain undefined. Most of our knowledge about the homeostatic mechanisms responsible for coupling the renal excretion of sodium to the control of blood volume and blood pressure has been obtained in dogs; however, to date there have been no available nonobese, nonhyperglycemic canine models for insulin resistance and hypertension. Although a high-fat feeding model for insulin resistance and hypertension has been developed in dogs, the concomitant development of obesity in this model may be a confounding factor and has yielded results that conflict with those obtained in rodent models. It is well recognized that obesity per se can impair insulin sensitivity; nonetheless, the nature, causes, and consequences of this impairment are not necessarily identical to those of the impairment in insulin sensitivity found in essential hypertension. For this reason, we considered that a nonobese insulin-resistant model would be preferable for studying the development of hypertension.

Information regarding the effects of chronic high-fructose feeding in dogs is scanty, although such feeding has been reported to cause an impairment in glucose disposal, suggesting that defects in insulin action similar to those fostered in rats or humans by high-fructose or high-sucrose diets may also be induced in dogs. Thus, we undertook the present study to determine whether high-fructose feeding in dogs induces insulin resistance (specifically, an impairment in insulin-stimulated glucose uptake) and to ascertain whether the development of insulin resistance is accompanied by an increase in blood pressure. During the study, we monitored
changes in body weight, sodium balance, arterial pressure, insulin-stimulated glucose uptake, and fasting levels of glucose, insulin, triglycerides, atrial natriuretic peptide (ANP), and endothelin-1 (ET-1).

Methods

Experimental Procedures

Experiments were performed in 18 male mongrel dogs whose initial body weight averaged 19.94±0.57 kg. All experiments were performed in accordance to the guidelines of Health Care and Use Committee. Animals were trained to stand quietly on an examination table while suspended by a sling. Polyvinyl chloride catheters (Tygon S-54-HL; 0.05-inch internal diameter, 0.09-inch external diameter) were implanted in the femoral artery and vein of the dogs under aseptic conditions. Surgery was performed with dogs under 30 mg/kg IV pentobarbital sodium anesthesia. All catheters were tunneled subcutaneously and exteriorized on the back between the scapulae. A dose of 300,000 U penicillin G procaine plus 200,000 U penicillin G benzathine was administered intramuscularly immediately after surgery as well as 48 and 96 hours postoperatively to prevent or treat postoperative infections. The catheters were filled with heparin (1000 U/mL) and flushed daily to maintain patency. The dogs were fed 700 g standard pelleted dog chow daily (Lab Canine Diet, Purina Mills) and had free access to water. Six to 8 days after catheter implantation, standard dog chow feeding was interrupted; 10 of the animals were fed every afternoon (3 to 5 p.m.) with a high-fructose diet. This diet was prepared daily for each dog by mixing 225 g fructose with a can of Prescription Diet E2A (Merck Animal Products) and 2 g sodium chloride. The remaining 8 dogs were fed a diet in which 225 g dextrose was added instead of fructose. The caloric content of both diets was 1563 kcal/d (60% as fructose or dextrose, 15% as fat, 13% as starch, and 12% as protein). For each dog by mixing 225 g fructose with a can of Prescription Diet E2A (Merck Animal Products) and 2 g sodium chloride. The remaining 8 dogs were fed a diet in which 225 g dextrose was added instead of fructose. The caloric content of both diets was 1563 kcal/d (60% as fructose or dextrose, 15% as fat, 13% as starch, and 12% as protein). The daily sodium intake of both diets was similar to what would have been consumed with standard dog chow feeding—approximately 67 mEq/d. The fructose, dextrose, and sodium chloride were obtained from Sigma Chemical Co.

After catheter implantation and before dietary intervention, arterial blood pressure measurements were performed for 30 minutes every morning (between 8 and 10 a.m.) by attaching the arterial catheter to a pressure transducer (TXX-R, Viggo Spectramed) connected to a recorder while the dog, suspended by a sling, was standing quietly on an examination table. The mean of the last three average daily values for arterial pressure obtained before dietary intervention was taken as the basal blood pressure for each dog. After the fructose or dextrose diet was started, blood pressure was recorded every other day for the rest of the experiment.

Arterial blood samples were withdrawn from 8 to 10 a.m. on the last 3 days before dietary intervention for measurement of fasting glucose, triglyceride, and insulin levels. The mean value for these 3 days was considered as the basal glucose, triglyceride, and insulin values for each dog. After the fructose or dextrose diet was started, arterial samples for determination of these three parameters were withdrawn every fifth day until the end of the experiment. Also, arterial blood samples for ET-1 and ANP were withdrawn the day before the dietary intervention began and 15 days afterwards in 10 dogs (6 fructose-fed and 4 dextrose-fed).

Resistance to insulin-stimulated peripheral glucose uptake was estimated by the insulin suppression test. This was accomplished by infusing intravenously for 3 hours (from 10 a.m. to 1 p.m. in the fasting state) 12 mg/kg per minute dextrose as a 20% solution in distilled water, 1 mU/kg per minute porcine insulin as a 100 mU/mL solution in 0.9% saline, and 1 μg/min somatostatin (added to the dextrose solution). Dextrose, insulin, and somatostatin were obtained from Sigma. During the last hour of the test, arterial blood samples for glucose and insulin were withdrawn every 10 minutes. The purpose of the insulin suppression test is to reach in all animals comparable levels of insulinemia in the high physiological range by infusing a fixed insulin dose while endogenous insulin secretion is inhibited by a somatostatin infusion. Once a steady state is reached, and assuming that the hepatic glucose output is largely suppressed by the high circulating levels of insulin, the rate of whole-body glucose uptake equals the exogenous glucose infusion rate. Under these circumstances, a higher steady-state glucose level indicates greater suppression to insulin-stimulated glucose uptake. Different modifications of this technique have been used successfully in dogs and also in rats and humans.

Analytic Procedures

Blood samples for glucose were collected in Venoject sodium fluoride/potassium oxalate heparin tubes (Terumo); blood samples for insulin and triglycerides were collected in Vacutainer serum separator tubes (Becton Dickinson) for measurement of ANP were collected in Venoject potassium-EDTA tubes (Terumo) in which phenylmethylsulfonyl fluoride (1.75 μg/mL plasma) and pepstatin (3.5 μg/mL plasma) had been added; ANP was measured by radioimmunoassay as previously described. Plasma samples for measurement of ANP were collected in Venoject potassium-EDTA tubes, and ET-1 was extracted and measured by radioimmunoassay with specific antibodies as previously described. Body weight was measured in a Shor-Line scale (Northern Balance and Scale) the day before dietary intervention began and every fifth day afterwards until the end of the experiment. Eight dogs (four fructose-fed and four dextrose-fed) were killed in metabolic cages that allowed for 24-hour urine collection. Urine was collected daily at 9 a.m. and the total volume measured in a 2000-mL graduate cylinder; a 3-mL aliquot was kept for determination of urinary sodium concentration with a flame photometer (Beckman system E2A).

Statistical Analysis

Statistical calculations were performed with an NCSS software package (Pacific Ease). All values are given as mean±SEM. Except where otherwise specified, two-dimensional ANOVA tests were performed for comparisons, with type of diet and time on diet as variables, followed by a post hoc Tukey test where appropriate; for comparison between samples of different sizes, the Kram er correction was used. Probability at a value of .05 was considered as the significance threshold.

As four of the dogs (three fructose-fed and one dextrose-fed) were killed on day 21, the body weight, fasting glucose, fasting insulin, and fasting triglyceride values for day 25 were compared by a single nonpaired Student’s t test. Mean arterial pressure values for days 22, 24, 26, and 28 were compared by multiple nonpaired Student’s t tests; the Bonferroni correction for multiple runs was applied, which reduced the probability threshold to .0125.

Cumulative sodium balance was calculated as Δ(sodium intake−natriuresis)=(basal sodium intake−basal natriure sis), as published elsewhere; the subtracted term compensates for nonurinary sodium losses, which are assumed to be constant. The values for this parameter between the fructose-fed and dextrose-fed groups were compared by a two-dimensional ANOVA followed by a post hoc Dunnett’s test.

For the purposes of linear correlation analysis, as mean arterial pressure values were not available for odd-numbered
Values for body weight and fasting plasma glucose, insulin, and triglyceride levels measured during the experiment are presented in Table 1. The development of insulin resistance in the fructose-fed animals was characterized by a progressive increase in the fasting levels of insulin, which reached statistical significance on day 15 after the special diet was started. On days 20 and 25 insulin levels were 2.3- and 3.1-fold higher than the values recorded during the basal period (*P<.001). These increments of insulinemia contrast with the maintenance of remarkably constant levels of fasting plasma glucose.

Fructose feeding was accompanied by a progressive increase in fasting levels of triglycerides, which became significant (*P<.05) by day 10. On day 25 triglyceride levels were 2.6-fold higher (*P<.001) than basal values.

As shown in Table 1, none of the alterations induced by fructose feeding were reproduced in the dextrose-fed animals. In fact, the levels of fasting glucose and insulin and body weight remained constant. However, in the dextrose-fed dogs triglyceride levels increased 19.4% by day 25, but this change failed to achieve statistical significance.

The alterations in mean arterial pressure induced by both (fructose and dextrose) diets are illustrated in Fig 2. The average value recorded in the fructose-fed group during the basal period was 100.4±2 mm Hg; this value did not vary more than ±2 mm Hg during the first 10 days of fructose feeding. From then on, mean arterial pressure steadily increased, achieving statistical significance (*P<.05) on day 18 (116±3 mm Hg). On the last day of the experiment, blood pressure values were 123±3 mm Hg on day 26 and 123±2 mm Hg on day 28. The total increment of blood pressure recorded from the basal period was 22.3±2.3 mm Hg.

Fig 2 also shows that in the dextrose-fed group the average value of mean arterial pressure recorded before the dextrose diet was begun was 102±2 mm Hg and that this value did not change significantly during the 28 days of the experiment. The average values of mean arterial pressure recorded during the last 2 days of the experiment were 99±2 mm Hg on day 26 and 97±3 on day 28.

### Table 1. Body Weight and Fasting Glucose, Insulin, and Triglyceride Levels During the Experiment

<table>
<thead>
<tr>
<th>Parameter and Group</th>
<th>Basal</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>19.92±0.66</td>
<td>19.90±0.64</td>
<td>19.70±0.57</td>
<td>19.69±0.59</td>
<td>19.62±0.55</td>
<td>19.20±0.69</td>
</tr>
<tr>
<td>Dextrose</td>
<td>18.35±1.19</td>
<td>19.75±1.20</td>
<td>19.75±1.17</td>
<td>19.77±1.13</td>
<td>19.82±1.16</td>
<td>19.60±1.29</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>103.9±1.6</td>
<td>105.1±1.9</td>
<td>103.0±1.2</td>
<td>102.9±1.1</td>
<td>102.5±1.9</td>
<td>103.7±1.4</td>
</tr>
<tr>
<td>Dextrose</td>
<td>102.0±1.2</td>
<td>103.1±1.6</td>
<td>105.0±1.1</td>
<td>102.0±1.9</td>
<td>104.3±1.9</td>
<td>104.2±1.8</td>
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<tr>
<td>Fasting insulin, μU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>19.0±1.8</td>
<td>20.3±1.3</td>
<td>21.3±1.3</td>
<td>37.1±7.8*</td>
<td>43.9±6.2†</td>
<td>56.9±6.0‡</td>
</tr>
<tr>
<td>Dextrose</td>
<td>18.8±2.2</td>
<td>19.9±2.3</td>
<td>21.0±2.1</td>
<td>19.6±2.4</td>
<td>17.3±1.6</td>
<td>18.2±1.2</td>
</tr>
<tr>
<td>Fasting triglycerides, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>35.3±0.6</td>
<td>41.6±1.3</td>
<td>47.1±2.2§</td>
<td>62.7±1.6†</td>
<td>72.1±1.7†</td>
<td>91.9±11.5‡</td>
</tr>
<tr>
<td>Dextrose</td>
<td>36.6±1.9</td>
<td>40.4±0.6</td>
<td>38.7±2.0</td>
<td>42.7±3.1</td>
<td>42.5±2.1</td>
<td>43.7±1.9</td>
</tr>
</tbody>
</table>

In the fructose-fed group, n=10 (n=7 on day 25); dextrose-fed, n=8 (n=7 on day 25). Values are mean±SEM.

*P<.05, †P<.01, ‡P<.001 vs basal value in the same group and vs control (dextrose) group at the same time.

§P<.05 vs basal value in the same group.
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Figure 2. Line graph shows mean arterial pressure (MAP) during the experiment in dextrose-fed (n=10; n=7 on days 22, 24, and 26; n=6 on day 28) and fructose-fed (n=8; n=6 on days 22, 24, and 26; n=5 on day 28) groups. Values are mean±SEM.  *P<0.05, **P<0.005 vs basal in the same group and vs dextrose in the same period.

Fasting plasma ANP and ET-1 values are shown in Table 2. No changes were apparent between the groups at baseline or after 15 days on either diet.

Daily urine collections were successfully completed in four fructose-fed and four dextrose-fed dogs for a basal period of 6 days before the fructose or dextrose diet was started and 28 days after the diet was started. Daily natriuresis values are shown in Fig 3. The mean daily values for 24-hour sodium excretion before the dietary intervention were 60.78±0.18 mEq in the fructose-fed group and 60.57±0.93 mEq in the dextrose-fed group.

In the fructose-fed group, the daily natriuresis dropped significantly on days 5 (34.9±11.3 mEq, P<0.05), 6 (28.9±2.6 mEq, P<0.01), and 7 (32.7±3.3 mEq, P<0.01), showing on days 10 to 13 a tendency to increase that peaked on day 11 (89.8±22.2 mEq, P=NS). In the dextrose-fed group, the daily natriuresis remained unchanged during the experiment. Total urinary sodium excretion for 28 days after the dietary intervention was 1685.70±10.73 mEq in the fructose-fed group and 1702.58±17.91 mEq in the dextrose-fed group, resulting in an average of 60.20±1.92 and 60.8±0.72 mEq/24 h, respectively (differences between groups and versus baseline within each group were not significant). Thus, average daily natriuresis for the whole experiment was not modified by any of the diets. The daily values of sodium excretion were somewhat inferior to the dietary sodium intake (approximately 67 mEq); this was probably due to fecal losses, which were not measured.

Cumulative data for sodium balances are presented in Fig 4. Initially, a progressive sodium retention was observed in the fructose-fed group, which reached significance on day 6 (72.1±2.6 mEq, P<0.05) and peaked at 111.6±4.4 mEq on day 8 (P<0.01); afterwards, cumulative sodium balance decreased, reaching 33.4±7.1 mEq on day 13 (P=NS) and then remaining stable. In contrast, the dextrose-fed group exhibited a normal sodium balance, with mild negative values (minimum at −28.6±2.6 mEq on day 13, P=NS).

The results of simple linear correlation studies for fasting insulinemia, steady-state plasma glucose, triglycerides, and mean arterial pressure are presented in Table 3. All these variables were significantly correlated with each other (P<0.05 for MAP/SSPG; P<0.005 for INS/SSPG; P<0.0001 for MAP/INS, SSPG/TG, MAP/TG, and INS/TG [where INS is insulinemia, SSPG is steady-state plasma glucose, TC is triglycerides, and MAP is mean arterial pressure]). Exponential, potential, logarithmic, or hyperbolic correlations did not significantly improve the correlation coefficient in any case. The remaining variables (body weight, fasting glycemia, fasting ANP, fasting ET-1, steady-state plasma insulin, and cumulative sodium balance) did not show any significant correlation with any other variable.

A stepwise multiple linear correlation analysis yields the following equation:

\[
\text{MAP} = 89.006 + (0.384 \times \text{TG}) + (0.191 \times \text{INS}) - (0.062 \times \text{SSPG})
\]

Table 2. Fasting Atrial Natriuretic Peptide and Endothelin-1 Levels Before and After 15 Days of Fructose or Dextrose Feeding

<table>
<thead>
<tr>
<th>Parameter and Group</th>
<th>Basal</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting ANP, fmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose (n=6)</td>
<td>17.75±4.54</td>
<td>14.23±3.30</td>
</tr>
<tr>
<td>Dextrose (n=4)</td>
<td>15.91±5.78</td>
<td>24.22±6.25</td>
</tr>
<tr>
<td>Fasting ET-1, fmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose (n=6)</td>
<td>0.58±0.07</td>
<td>0.56±0.12</td>
</tr>
<tr>
<td>Dextrose (n=4)</td>
<td>0.56±0.12</td>
<td>0.58±0.03</td>
</tr>
</tbody>
</table>

ANP indicates atrial natriuretic peptide; ET-1, endothelin-1. Values are mean±SEM. *P<0.05.
as the predictor for mean arterial pressure ($r = .7019$; $P = .0145$ for steady-state plasma glucose, $P = .0016$ for fasting insulinemia, and $P < .0001$ for fasting triglycerides).

The predictor for fasting insulin is

$$\text{INS} = -60.720 + (0.827 \times \text{MAP})$$

with $r = .545$ and $P < .0001$ for mean arterial pressure.

The predictor for steady-state plasma glucose is

$$\text{SSPG} = 24.4317 + (2.372 \times \text{TG})$$

with $r = .698$ and $P < .0001$ for fasting triglycerides.

No adequate predictors could be found for the remaining parameters (body weight, fasting glycemia, steady-state plasma insulin, fasting ANP, fasting ET-1, and sodium balance).

**Discussion**

It has been recognized recently that insulin resistance may play a role in the development of essential hypertension in humans, at least in a large proportion of cases, which could be close to 60% in Western populations. Several mechanisms that could lead from insulin resistance to hypertension have been proposed, but the factual pathophysiology of insulin resistance-associated hypertension remains unknown. It is known that rats fed fructose or sucrose develop insulin resistance and hypertension; however, dogs are better suited for the analysis of the renal mechanisms involved in long-term blood pressure regulation, so most studies on this matter have been conducted in dogs. For this reason, we developed a high-fructose-fed canine model, with the immediate goal of determining whether insulin resistance and hypertension are reliably elicited by this dietary intervention and the distant goal of characterizing the physiopathology of the development of hypertension in this model.

The ability of a high-fructose diet to cause systemic metabolic changes in dogs could be challenged on the basis that the canine small intestine can metabolize fructose to glucose, thus preventing further effects of fructose. In fact, Bollman and Mann reported that hepatectomized dogs convert intravenously given fructose to glucose, losing this ability if their stomach and bowels are also removed. However, dogs seem to be able to metabolize to glucose in their bowel only a fraction of an orally or intrajejunally given fructose load. Thus, a large proportion (roughly half) of the administered fructose can be recovered from the mesenteric circulation of a dog, but no fructose can be recovered from the systemic circulation, indicating that the canine liver is involved in the metabolism of oral fructose. In fact, it has been shown that high-fructose feeding impairs glucose disposal in dogs in a reversible fashion, thus demonstrating the ability of fructose to foster systemic metabolic changes in dogs.

**Fructose Feeding, Fuel Metabolism, and Insulin Action**

Information about the precise biochemical and metabolic alterations induced in the dog by high-fructose feeding and leading to insulin resistance is scanty, although significant advances have been obtained in rat studies. Fructose or sucrose feeding has been shown to modify multiple aspects of carbohydrate metabolism, causing a decrease in glucose oxidation in hepatic tissue, adipose tissue, and striated muscle tissue. Moreover, hepatic gluconeogenesis is enhanced, and hepatic glycogen synthesis is impaired. Moreover, there are important alterations in hepatic lipid metabolism, with increased hepatic lipogenesis and triglyceride output.

These parallel effects of fructose or sucrose feeding on carbohydrate and lipid metabolism seem to overlap, as the aforementioned alterations in lipid metabolism may further impair glucose disposal. Concurrently, insulin resistance and compensatory hyperinsulinemia are developed, as the ability of insulin to suppress hepatic glucose output and stimulate glucose uptake in striated muscle and adipose tissue is impaired. This impairment has been shown to be quite specific, as adipocytes from insulin-resistant hypertensive rats, which showed a significant decrease in their insulin-stimulated glucose transport, had no alterations in either the total number of insulin receptors or their affinity for insulin. Moreover, certain responses mediated by these receptors — namely, insulin-stimulated autophosphorylation, tyrosine kinase activity, and also their ability to suppress catecholamine-induced lipoly-

![Graph showing cumulative sodium balance during the experiment in dextrose-fed (n=4) and fructose-fed (n=4) groups.](image)

**TABLE 3. Simple Linear Correlation Analyses**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept</th>
<th>Slope</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP/INS</td>
<td>95.226</td>
<td>0.358</td>
<td>.545</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>MAP/SSPG</td>
<td>95.477</td>
<td>0.067</td>
<td>.340</td>
<td>.0424</td>
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<tr>
<td>MAP/TG</td>
<td>86.207</td>
<td>0.375</td>
<td>.644</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>INS/SSPG</td>
<td>1.667</td>
<td>0.172</td>
<td>.486</td>
<td>.0004</td>
</tr>
<tr>
<td>INS/TG</td>
<td>-1.083</td>
<td>0.555</td>
<td>.573</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SSPG/TG</td>
<td>24.4317</td>
<td>2.372</td>
<td>.698</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**MAP** indicates mean arterial pressure; **INS**, fasting insulin; **SSPG**, steady-state plasma glucose; and **TG**, fasting triglycerides. Intercept, slope, correlation coefficient, and probability are shown for each pair of variables (dependent/independent). No significant correlation was found for variable pairs that are not listed.
sis—were unaffected. Insulin secretion is largely regulated by circulating levels of glucose in the blood, and thus, these specific defects in insulin action, which impair glucose disposal, are adequately compensated by the ensuing hyperinsulinemia. However, other effects of insulin for which there is not a specific resistance will likely be exaggerated in this situation.

Although detailed metabolic studies in the dog were beyond the scope of our work, we consider it highly likely that most of the information obtained by others in rats is valid for dogs and humans. In fact, our results resemble those obtained in rodent models in which fructose or sucrose feeding caused comparable degrees of hypertriglyceridemia, hyperinsulinemia, resistance to insulin-stimulated glucose uptake, and hypertension without causing body weight gain or hyperglycemia, although a slightly longer time was required to elicit these changes in our model. In our study fasting glycemia was remarkably well controlled despite resistance to insulin-stimulated glucose uptake; this is adequately explained by the presence of secondary insulin resistance. Also, in healthy human volunteers, sucrose feeding also causes hypertriglyceridemia, decreases insulin-stimulated glucose uptake, and increases insulinemia and blood pressure.

**Fructose-Feeding and Hypertension Mechanisms**

Several investigators have reported that hypertension develops in fructose- or sucrose-fed rats; however, little is known about the underlying mechanisms. Sympathetic activity, as measured by direct recordings of hypothalamic and ganglionic activity, is enhanced, which in turn may cause further impairment of insulin sensitivity. However, it is doubtful that sustained hypertension can be explained by this mechanism alone. Hyperinsulinism has also been associated with renal sodium retention and interestingly, is not affected in subjects whose insulin-stimulated glucose uptake is markedly impaired. Also, vascular smooth muscle hyperreactivity and hypertrophy have been described in connection with hyperinsulinism, but these latter phenomena have not been studied in these models.

Dogs fed a high-fat diet develop hypertension only as they gain body weight, but in our model there was no such gain, nor did body weight correlate with blood pressure (or any other parameter). This suggests that the mechanisms involved in the development of insulin resistance and hypertension must be different in the obese and nonobese canine models. In fact, it is known that obese subjects have a general impairment of insulin sensitivity caused by a decreased density of insulin receptors in the cell membranes of target tissues, and only a minority of these subjects show a specific postreceptor defect for insulin-stimulated glucose uptake similar to the defect found in essential hypertensive patients. Moreover, in a study by Beck-Nielsen et al on human volunteers, high-sucrose and high-fat feeding caused divergent effects on insulin action: Sucrose feeding impaired both insulin binding and insulin-stimulated glucose uptake; fat feeding also impaired insulin binding but did not affect insulin-stimulated glucose uptake. Also, sodium balance studies show a striking contrast between obese and nonobese canine models. In the fat-fed model, large amounts of sodium were retained, and cumulative sodium balance was strongly correlated with blood pressure; in our model, sodium balance did not correlate with blood pressure or any other parameter. Only a moderate amount of sodium was transiently retained, peaking at 111.6±4.44 mEq by day 8; by days 11 to 12, sodium balance had returned to normal. We have not identified the mechanisms implied by these sodium shifts. Of the remaining studied parameters, only fasting triglycerides were increased by the time sodium was retained, suggesting that fructose-induced metabolic disturbances could play a role in sodium retention, as has been empirically described by others. However, we found no long-term correlation between fasting triglycerides and sodium balance. One possible explanation for these results is that as sodium accumulation begins, compensatory mechanisms come into play, preventing further sodium or volume retention. In fact, a decrease in aldosterone has been reported in fructose-fed rats as well as in sucrose-fed humans. In contrast, and once again showing important differences between fructose- or sucrose-fed models, in obese fat-fed dogs aldosterone is progressively increased in parallel with sodium accumulation. To explain this inadequate increase, it has been argued that the non-insulin-dependent stimulation of angiotensin-stimulated aldosterone secretion; however, it is unclear why in the fructose- or sucrose-fed models, which are comparably hyperinsulinemic, aldosterone is not similarly increased.

No significant changes in circulating levels of ANP or ET-1 were found in our study, suggesting that they are not involved in the development of high blood pressure in this model. However, as these hormones are diffusely produced, we cannot rule out the possibility that they play a local role without their systemic levels being altered. Hwang et al have described an increase in ANP in fructose-fed hypertensive rats, explaining the increase as secondary and compensatory to an implied volume retention. No data for ET-1 are available in fructose- or sucrose-fed models, although it has been shown in vitro that insulin increases the release of ET-1 from vascular endothelium. Clearly, further research is needed to clarify the possible role of these mediators.

No definitive evidence shows that insulin resistance and hyperinsulinism cause high blood pressure; however, there is enough evidence to show that insulin resistance is not a secondary consequence of hypertension. In our model, insulin resistance and hyperinsulinism were established before blood pressure increased. In fructose-fed rats, the addition of clonidine to the drinking water prevents the development of hypertension but not of insulin resistance, hyperinsulinemia, and hypertriglyceridemia; on the other hand, blockade of insulin secretion with octreotide prevents hypertension. 43 Adipocytes from young normotensive spontaneously hypertensive rats show resistance to insulin-stimulated glucose uptake, which is not increased with the age of the rat as blood pressure is. Normotensive human subjects with a family history of hypertension show insulin resistance, hyperinsulinemia, and hypertriglyceridemia. Renovascular hypertension in humans or rats is not associated with insulin resistance. These facts strongly suggest that insulin resistance is not a mere consequence of hypertension; however, they do not necessarily prove that the opposite (i.e., that hypertension is a consequence of insulin resistance) is true. In fact, both of these alterations could be secondary to...
other factors. One such factor may be hypertriglyceridemia: it is known to be directly caused by high-fructose feeding and in our experience preceded both hyperinsulinism and hypertension. Also, our results show a strong correlation between fasting triglycerides and insulinism and hypertension. Also, our results show a strong correlation between fasting triglycerides and insulinism and hypertension.

However, the possible mechanisms linking hypertriglyceridemia to insulin resistance and hypertension remain undefined. It has been postulated that changes in circulating lipids may alter the composition of cell membranes, thus altering their permeability to divalent cations. Reduced permeability to magnesium and its subsequent decreased intracellular concentration have been related to insulin resistance. 

Also, alterations in sodium and calcium membrane transport may cause vascular hyperreactivity and increased vascular resistance. In addition, alterations in the circulating lipid profile may affect the synthesis of prostanoids, with possible repercussions in vascular tone and renal tubular sodium handling.

Conclusion

We have developed a new canine model in which hypertriglyceridemia, insulin resistance, hyperinsulinism, and hypertension are induced by a normosodic and otherwise balanced high-fructose diet. Body weight, fasting glycemia, and peripheral levels of ANP and ET-1 remain unchanged in this model. There is a modest transient sodium retention, but long-term sodium balance is not affected. The description of this model adds to the ever-growing evidence that insulin resistance and hyperinsulinemia are involved in the development of essential hypertension and will allow further studies on its pathogenesis and pathophysiology.

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