Dietary Magnesium Prevents Fructose-Induced Insulin Insensitivity in Rats
Thomas W. Balon, Arnie Jasman, Stephen Scott, Woerner P. Meehan, Robert K. Rude, Jerry L. Nadler

Abstract Increased dietary fructose may produce insulin insensitivity and elevate blood pressure in rats. It is possible that the reduced magnesium content of the high-fructose commercial diet used in some studies may play a role in these abnormalities because it is known that magnesium deficiency can produce insulin insensitivity and increased angiotensin II action in humans. To study this, we maintained rats for 9 weeks on either a normal control diet, a standard high-fructose diet, or the same high-fructose diet supplemented with magnesium. Glucose uptake was assessed using a perfused rat hindquarter preparation sequentially with 0, 900, and 120,000 pmol/L of added insulin. Basal serum glucose, plasma insulin, and basal glucose uptake in the absence of insulin were similar among all three groups. However, insulin sensitivity, defined as glucose uptake in the presence of 900 pmol/L insulin minus basal, was depressed in the high-fructose compared with the control group (1.02±0.38 to 1.77±0.57 μmol/g per hour, P<.05). In contrast, the high-fructose group supplemented with normal magnesium had similar insulin sensitivity as the control group (2.09±0.69 μmol/g per hour). Total serum magnesium was reduced in the high-fructose group compared with control or high-fructose plus magnesium-supplemented groups. Blood pressure and fasting insulin levels were also lower in the magnesium-supplemented group. These results suggest that magnesium deficiency and not fructose ingestion per se leads to insulin insensitivity in skeletal muscle and changes in blood pressure. (Hypertension. 1994;23[part 2]:1036-1039.)

Key Words • calcium • insulin • ions • magnesium • perfusion • blood pressure

Refined sugar usage has declined over the past 20 years, whereas high-fructose corn sweetener consumption has increased more than 700%.

The ingestion of fructose, an epimer of glucose, has been implicated in the induction of diminished glucose tolerance and elevated blood pressure in rats. However, it should be noted that diminished glucose tolerance has not been noted in all studies. In conjunction with these observations it has been noted that an increased incidence of diabetes mellitus is positively correlated with a subnormal dietary intake of magnesium. Nadler and colleagues have also observed decreased insulin sensitivity and increased angiotensin II action after dietary magnesium deficiency in humans. A number of previous studies that implemented fructose interventions for evaluation of insulin action or blood pressure in rats did not control or account for simultaneously induced changes in dietary ionic composition.

For the past 20 years, assessment of both insulin sensitivity and responsiveness of metabolism by skeletal muscle has been made with perfused rat hindquarter preparations. This method has the advantage of making it possible to measure local glucose disposal without the interactive effect of other organs such as the liver or peripheral fat depots.

The purpose of this study was to define the effects of a high-fructose diet with either a normal (0.24%) or deficient (0.06%) magnesium intake on the insulin sensitivity of skeletal muscle and changes in blood pressure. The results suggest that magnesium deficiency but not fructose ingestion per se leads to insulin insensitivity in skeletal muscle and increases in blood pressure.

Methods
Animals and Diet
Thirty-one male Sprague-Dawley rats (Charles River Breeding Laboratories) initially weighing between 164 and 182 g (172±1 g) were housed under standard conditions (20°C, 50% relative humidity, 12-hour light/dark cycle). They were assigned to one of three diet treatments, which provided chow and water ad libitum for 6 weeks with the exception of fasting, which was imposed from 8 to 9 AM until 2 to 3 PM, when fasting state measurements were obtained. Control rats were maintained on Teklad Rodent Diet W 8604 (Harlan Teklad), which contained 0.24% magnesium supplied as the oxide salt. Fructose and fructose plus magnesium groups were fed Teklad custom rat chows (TD#89054 and TD#93018, respectively). The TD#89054 diet was similar to previous high-fructose diets used by other groups and contained only 0.06% magnesium. The TD#93018 diet was identical to the TD#89054 diet with the exception that the magnesium content was identical to that of the control diet (0.24%). Both diets supplied calcium as calcium carbonate at a concentration of 3.0 g/kg. All studies were conducted in accordance with the guidelines of the Research Animal Care Committee of the City of Hope National Medical Center.

Urine Collection
During the fourth week of the study, rats were placed in Nalgene metabolic cages (Fisher Scientific) without food but with access to water. Twenty-four-hour urine samples were collected during this time.

Hindquarter Perfusion
Rats were weighed and anesthetized with an intraperitoneal injection of pentobarbital (5 mg/100 g body wt). Tail blood
specimens were obtained, and rats were prepared for hindquarter perfusion as described by Ruderman et al. for female rats with modifications for male rats. Basically, this surgical procedure consisted of a midline incision from the xiphoid process to the penis, followed by a pelvic evisceration, castration, ligation of the major branches of the abdominal aorta and vena cava, and subsequent cannulation of the abdominal aorta and vena cava. The following modifications for male rats were made: The arteries and veins supplying the urogenital organs were ligated before the subsequent ablation of the cauda epididymis, corpus epididymis, vas deferens, and caput epididymis. After cannulation of the abdominal aorta and vena cava the preparation was placed into the perfusion cabinet, which was maintained at 37°C. The first 25 mL of perfusate that passed through the preparation was discarded, followed by recycling of the medium at a flow rate of 13 mL/min. The perfusion cabinet was an exact replica of that described by Goodman and coworkers with the exception of the absence of a flowmeter. The perfusion media (175 mL) consisted of Krebs-Henseleit solution, age-rejuvenated human erythrocytes (30% hematocrit), 4% bovine serum albumin (fraction V, Miles Pentex), 6 mmol/L glucose, 0.15 mmol/L pyruvate, and 1 to 2 mmol/L lactate (originating from the erythrocytes). Porcine monocomponent insulin (Eli Lilly) was added in concentrations of 900 and 120,000 pmol/L where indicated. As described by Goodyear and coworkers, the perfusion protocol consisted of three consecutive 45-minute perfusion periods. In brief, during the first 15 minutes of each period the preparation was allowed to equilibrate. During the subsequent 30 minutes glucose uptake was monitored. Perfusion samples were collected at the beginning and end of this measurement period. During the initial 45-minute period the preparation was perfused in the absence of added insulin. Glucose was added during the initial 15 minutes of the second and third 45-minute periods to restore the glucose concentration to 5.5 mmol/L. The preparation was perfused with 900 and 120,000 pmol/L insulin during the second and third periods, respectively.

**Blood Pressure**

Blood pressures in a separate group of 24 rats on the identical three diets were measured via the tail-cuff method starting at baseline and weekly until 9 weeks. This method correlates highly (r=0.94) with direct cannulation measurements.

**Assay Measurements**

Serial perfusates and blood samples were kept on ice and assayed for glucose uptake in duplicate with a YSI model 27 Analyzer (Yellow Springs Instrument Co). Samples were centrifuged at 16,000g for 4 minutes. Plasma samples were stored at −80°C for subsequent determinations. Fasting and fed insulin concentrations were determined by the method of Yalow and Berson. Serum calcium and magnesium and urinary magnesium concentrations were ascertained by atomic absorption. Ionized calcium was determined by a new ion-selective electrode (Nava 8, NOVA Biomedical).

**Statistics**

All statistics are expressed as mean±SEM. A general linear model ANOVA with a Fisher's exact post hoc comparison test was used to analyze mean blood pressure data. ANOVA was used to compare values from sequentially calculated glucose uptake measurements with a Newman-Keuls post hoc comparison. The significance level was set at a value of P<0.05.

**Results**

**Blood Glucose and Plasma Insulin Concentrations**

There were no significant differences in either blood glucose or plasma insulin concentrations among the dietary groups (Table 1) in the fed state. However, there was a significant difference (P<0.03) between fasting insulin levels in the fructose (204±45) versus the fructose plus magnesium groups (107±10) and the control group (99±16) (values are expressed as picomoles per liter; n=8 per group).

**Basal Glucose Uptake and Skeletal Muscle Insulin Sensitivity and Responsiveness**

In the absence of added insulin there were no differences in glucose uptake by the perfused muscle for the control group compared with the fructose with low magnesium or fructose with normal magnesium groups (Table 2). However, the glucose uptake in the presence of a submaximal concentration of insulin was depressed in the group fed fructose plus low magnesium compared with the two other groups (P<0.05), whereas glucose uptake in response to a maximal stimulating concentration of insulin (120,000 pmol/L) did not differ among groups.

**Serum and Urinary Ion Concentration**

Neither total serum nor urinary total calcium concentrations differed among the three groups (Table 3). However, both serum and urinary magnesium concentrations were significantly lower in the fructose group compared with the two other groups with normal magnesium intakes. Serum ionized calcium did not differ among groups. The values (expressed as millimoles per liter, n=4-6 per group) were as follows: control, 0.34±0.05; fructose, 0.35±0.05; fructose plus magnesium supplementation, 0.36±0.05.

**Blood Pressure**

Before dietary intervention all three groups had similar mean blood pressures. The values (expressed as millimeters of mercury, n=8 per group) were as follows: control, 114±4; fructose, 118±3; and fructose plus magnesium supplementation, 118±4. When the cumulative blood pressure measurements were averaged and analyzed from week 5 to the conclusion of the study at week 9, a

### Table 1. Blood Glucose and Plasma Insulin Concentrations in the Fed State

<table>
<thead>
<tr>
<th></th>
<th>Control (0.24% Mg)</th>
<th>Fructose (0.06% Mg)</th>
<th>Fructose (0.24% Mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mmol/L</td>
<td>4.5±0.5</td>
<td>4.6±0.6</td>
<td>5.2±0.83</td>
</tr>
<tr>
<td>Plasma insulin, pmol/L</td>
<td>822±18</td>
<td>810±30</td>
<td>798±54</td>
</tr>
</tbody>
</table>

Samples were obtained from anesthetized rats in the fed state immediately before preparation for perfusion. Values are mean±SEM of 10 to 11 observations per group. No significant differences were observed among groups.

### Table 2. Glucose Utilization by Perfused Rat Hindquarter

<table>
<thead>
<tr>
<th>Insulin, pmol/L</th>
<th>Control (0.24% Mg)</th>
<th>Fructose (0.06% Mg)</th>
<th>Fructose (0.24% Mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.91±0.65</td>
<td>3.07±0.76</td>
<td>2.96±0.81</td>
</tr>
<tr>
<td>900</td>
<td>4.68±0.57</td>
<td>4.09±0.38</td>
<td>5.05±0.69</td>
</tr>
<tr>
<td>120,000</td>
<td>9.06±1.30</td>
<td>8.41±0.91</td>
<td>8.47±1.06</td>
</tr>
</tbody>
</table>

Values are mean±SD of five to six observations per group. Glucose uptake is expressed in micromoles per gram per hour. *Significantly different from value of other diet groups, P<0.05.
significant ($P<.03$) difference was noted between the fructose (140±3 mm Hg) and the control (132±2 mm Hg) and fructose with normal magnesium groups (131±2 mm Hg).

**Discussion**

Several studies have indicated that magnesium plays a role in the etiology of hypertension and in glucose homeostasis.8,17-25 Likewise, Landsberg24 has proposed that hypertension, insulin resistance, and obesity may have common mediators. In support of this it has been demonstrated that the ingestion of fructose under certain conditions may contribute to an abnormal carbohydrate metabolism and hypertension.2,3,25,26

A number of studies have demonstrated that fructose induces hypertension2,26 and diminished glucose tolerance.26 However, other studies have more carefully defined the conditions under which these abnormalities may occur (for review see Reference 1), including a prolonged exposure to the diet and a period of fasting before testing as well as an increased susceptibility of certain species.

We did not demonstrate any changes in blood glucose or plasma insulin concentrations as a result of the high-fructose diet in the fed state. This is in agreement with a number of other studies.5,6 However, we did note elevated insulin concentrations in the high-fructose group that did not receive magnesium supplementation during the fasted state.

Although insulin and glucose concentrations are valuable in describing the metabolic state of the subject, the response of the organism to a glucose challenge may be more reflective of the dynamic endocrine and metabolic status.

Indexes of insulin sensitivity may be derived from a number of different procedures, including glucose tolerance test via oral, intravenous, and intraperitoneal routes. Other techniques, such as euglycemic clamps and isolated organ preparations including incubated and perfused tissues, have been used to define the sites of action. Quantification of glucose utilization by skeletal muscle, which accounts for most of the glucose utilization,27 is well established in the hind limb perfusion model.11

The major finding of the present study demonstrated that fructose ingestion per se did not affect insulin sensitivity or responsiveness when an adequate amount of dietary magnesium was present. Examination of the diets referenced in other studies did not account for magnesium deficiencies, or the studies actually used diets low in magnesium.3,4,9,26

Although the mechanism for the decrease in glucose utilization remains obscure, we hypothesized that the defect is caused by a post-insulin binding defect, resulting in decreases in glucose transport, utilization, or both. This hypothesis is supported in vitro by other studies showing a reduction of insulin-mediated glucose transport28 without changes in insulin receptor binding during magnesium deficiency.29

Neither serum nor urinary calcium was altered in the three study groups, suggesting that neither fructose alone nor changes in the levels of magnesium seen here altered extracellular calcium metabolism. Therefore, it is unlikely that changes in extracellular calcium were responsible for these changes in insulin responsiveness. However, it was clear that the high-fructose diet with 0.06% magnesium leads to serum and whole-body magnesium deficiency. In an elegant series of studies using the incubated soleus muscle as a model, Gould and Chaudry30 noted that magnesium and not calcium was required for the stimulation of glucose uptake by insulin, thus supporting our hypothesis that magnesium plays a permissive role in the homeostasis of insulin sensitivity. Further proof of this in humans was provided by a recent report showing that dietary-induced magnesium deficiency could lead to reduced insulin action.8

In a recent preliminary communication in which diets matched for trace element composition and adequate in magnesium content were used, it was noted that a 66% fructose diet did not cause hypertension or changes in plasma glucose or insulin concentrations in rats.31 The current study concurs with that report because magnesium supplementation prevented any clear effect of fructose on blood pressure or insulin responsiveness.

In conclusion, the present study clearly shows that magnesium deficiency and not fructose ingestion per se leads to insulin insensitivity in skeletal muscle and elevations in blood pressure in rats.

**Acknowledgments**

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**References**


**Table 3. Effect of Diet on Serum and Urinary Ion Concentrations**

<table>
<thead>
<tr>
<th>Control (0.24% Mg)</th>
<th>Fructose (0.06% Mg)</th>
<th>Fructose (0.24% Mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ca, mmol/L</td>
<td>0.438±0.007</td>
<td>0.476±0.012</td>
</tr>
<tr>
<td>Serum Mg, mmol/L</td>
<td>0.357±0.027</td>
<td>0.249±0.013*</td>
</tr>
<tr>
<td>Urinary Mg, mmol/24 h</td>
<td>0.160±0.020</td>
<td>0.049±0.003t</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 10 to 11 observations per group.

*P<.02, **P<.01.
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