A High Sucrose, High Linoleic Acid Diet Potentiates Hypertension in the Dahl Salt Sensitive Rat
Hong Yen Zhang, Sreenivas Reddy, and Theodore A. Kotchen

Insulin resistance can be induced by diets high in simple carbohydrates or fatty acids. To determine whether these nutrients also affect arterial pressure in genetic models of salt sensitive and salt resistant hypertension, Dahl salt sensitive (S) and salt resistant (R) rats were each fed the following isocaloric diets containing 3% NaCl for 4 weeks (10 rats/group): 1) control; 2) high sucrose (60%); 3) high linoleic acid (LA, provided as 10% safflower oil); and 4) high sucrose plus high LA. Tail systolic blood pressures (SBP) were measured weekly, and at 4 weeks, direct mean arterial pressures (MBP) were measured in conscious animals. Insulin sensitivity was assessed by in vitro uptake of tritiated glucose by adipocytes in response to graded doses of insulin.

Weight gain did not differ among groups. High sucrose alone and high LA alone did not affect blood pressure in either strain. However, SBP and MBP were increased (P < .05) by the high sucrose plus high LA diet in Dahl-S but not in Dahl-R rats. Sucrose alone and LA alone decreased (P < .05) insulin sensitivity in Dahl-S and Dahl-R rats. In both strains, sucrose plus LA decreased insulin sensitivity to a greater extent (P < .05) than sucrose alone or LA alone.

Thus, the sucrose plus LA diet decreased insulin sensitivity in both Dahl-S and Dahl-R rats, whereas blood pressure was increased only in Dahl-S rats. The phenotype of elevated arterial pressure is influenced both by a genetic-nutrient interaction and by an interaction among specific nutrients resulting in insulin resistance. Am J Hypertens 1999;12:183–187 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Hypertension, Dahl rat, insulin resistance, linoleic acid, sucrose.

Hypertension is associated with resistance to insulin stimulated glucose uptake in the human and in several experimental models of hypertension.1,2 Both heritable and environmental factors contribute to insulin resistance and to hypertension. Clinically, insulin resistance has been described in normotensive offspring of hypertensive families.3,4 Similarly, the Dahl-salt sensitive (S) rat is insulin resistant in comparison with the Dahl-salt resistant (R) rat, even before the onset of hypertension.5,6 In addition, insulin sensitivity may be affected by diet. Both sucrose and fructose feeding cause a defect in insulin-stimulated glucose use in the rat, as determined by the euglycemic insulin clamp technique.7,8 Unsaturated, monounsaturated, and polyunsaturated fatty acids (including linoleic acid) also lead to impairment of insulin action in peripheral tissues in the rat,9 and a high dietary intake of linoleic acid decreases insulin sensitivity in humans.10

Arterial pressure may also be modified by varying dietary carbohydrate and lipid contents. In the rat,
diets high in simple carbohydrate content have generally, although not invariably been reported to increase arterial pressure.\(^{11,12}\) We have previously reported that a high sucrose diet increases blood pressure in normotensive Sprague Dawley rats fed a high, but not a low, NaCl diet.\(^{13}\) In contrast, diets high in linoleic acid content lower blood pressure, more convincingly in high renin than in low renin models of hypertension.\(^{14}\) Blood pressure is not affected by a high linoleic acid diet in the Dahl-S rat, which is a low renin model of hypertension.

The purpose of this study is to determine whether rats that are genetically protected against NaCl induced hypertension are also protected against increases of blood pressure associated with diet induced insulin resistance. Specifically, we evaluated the effects of diets high in simple carbohydrate and high in linoleic acid content, alone and in combination, on blood pressure and insulin sensitivity in both Dahl-S and Dahl-R rats.

### METHODS

Male Dahl-salt sensitive (Dahl-S) and salt resistant (Dahl-R) rats were purchased from Harlan Sprague Dawley (Indianapolis, IN) and were housed in individual metabolic cages in a temperature controlled (22°C) and light controlled (12 h on, 12 h off) small animal facility. Salt sensitive and salt resistant rats were placed on one of the following four diets (Harlan Teklad, Madison, WI): a) control (Teklad diet #96184); b) high sucrose, provided as 60% sucrose (Teklad diet #96182); c) high linoleic acid, provided as 10% safflower oil (Teklad diet #96183); d) high sucrose and high linoleic acid (Teklad diet #96181). All diets contained 3% NaCl and were isocaloric. Table 1 lists the composition of the four diets.

Rats were maintained on these diets for 28 days. Body weights and tail systolic blood pressures were measured weekly. For the measurement of systolic blood pressures by plethysmography, rats were placed in a heated rodent restrainer (Harvard Apparatus, South Natick, MA) and were maintained at a constant temperature of 39°C, beginning 15 to 20 min before the measurement. For each data point, nine tail-cuff measurements were obtained with a pneumatic pulse transducer (Narco BioSystems, Austin, TX), a pressure transducer (P23-ID, Gould Electronics, Cleveland, OH), and a polygraph (model 7D, Grass Instrument, Quincy, MA). The first two readings and the highest and lowest remaining readings were discarded. The remaining five readings were averaged to obtain each data point.

On day 24 to 25, Tygon microprobe catheters (0.015 internal diameter, 0.030 outside diameter) were placed in the femoral artery with rats under methohexital anesthesia (50 mg/kg, intraperitoneally). Catheters were tunneled subcutaneously to exit at the base of the skull. Three days after catheter placement, baseline mean arterial pressures were recorded in the resting state over 30 min, after a 60-min stabilization period. Arterial pressures were measured with a Statham pressure transducer (Gould, Oxnard, CA) and a model 7 Grass polygraph. Rats remained on their respective diets for an additional 1 to 3 days and were then killed by decapitation to obtain epididymal adipocytes to evaluate in vitro insulin stimulated glucose uptake.

As previously described,\(^{15}\) adipocytes were isolated from epididymal fat pads by shaking finely minced tissue at 37°C for 1 h in KRB buffer containing 4% bovine serum albumin and 3% collagenase. Cells were filtered through nylon mesh and washed twice with BSA-KRB buffer. The adipocytes were incubated in 0.5% KRB-albumin buffer with graded concentrations of insulin (1 to 100 ng/mL) for 30 min.\(^{16}\) Fifty microliters of H\(^2\)-2-deoxyglucose (6.4 μCi/mL) were added to the cell suspensions and incubated for 3 min. The incubation was terminated by centrifuging, and uptake of radioactivity by the cells was determined by liquid scintillation counting.

Statistical significance of group differences was determined by analysis of variance. When overall differences were observed, the significance of differences between specific groups was determined with two factor, repeated measures analysis of variance. Group differences were considered statistically significant at a value of \(P < .05\). Results are presented as means ± SE.

### RESULTS

At the initiation of the diets, mean body weights among the four groups of Dahl-S and Dahl-R rats did not differ (Table 2). On the different diets, all groups gained weight at equivalent rates, and body weights at the termination of the experiment did not differ among groups.

<table>
<thead>
<tr>
<th>TABLE 1. COMPOSITION OF THE FOUR STUDY DIETS (g/kg)</th>
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</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>α-Methionine</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Corn starch</td>
</tr>
<tr>
<td>Safflower oil</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>Mineral mix</td>
</tr>
<tr>
<td>CaCO(_3)</td>
</tr>
<tr>
<td>Vitamin mix</td>
</tr>
</tbody>
</table>

LA, linoleic acid.
During the 4 weeks on the diets, the increases of systolic blood pressure did not differ in Dahl-S rats fed the control diet, the high sucrose diet, or the high linoleic acid diet (Figure 1A). However, in Dahl-S rats fed the diet containing both high sucrose plus high linoleic acid, systolic blood pressures were significantly higher \((P < .01)\) than in any of the other groups. In contrast, among Dahl-R rats, systolic blood pressures did not differ on any of the diets (Figure 1B).

Similarly, among Dahl-S rats, after 4 weeks on the diets, direct mean arterial pressure of rats fed the high sucrose plus high linoleic acid diet was significantly higher \((P < .05)\) than that of controls, or of Dahl-S rats fed sucrose alone or linoleic acid alone (Table 2). Mean arterial pressure of Dahl-S rats fed sucrose alone or linoleic acid alone did not differ from that of Dahl-S rats fed the control diet. In animals fed the control diet, mean arterial pressure of Dahl-R rats was lower \((P < .01)\) than that of Dahl-S rats. In contrast to Dahl-S rats, mean arterial pressures did not differ among Dahl-R rats on any of the four diets.

In rats fed the control diets, in vitro insulin stimulated glucose uptake by adipocytes was less \((P < .05)\) in Dahl-S than in Dahl-R rats. Among Dahl-S rats, glucose uptake in rats fed sucrose alone and in rats fed linoleic acid alone was less \((P < .05)\) than that in rats fed the control diet (Figure 2A). Further, glucose uptake by adipocytes of Dahl-S rats fed the sucrose plus linoleic acid diet was less \((P < .05)\) than that of Dahl-S rats fed either sucrose alone or linoleic acid alone. Among Dahl-R rats, compared with that in rats fed the control diet, insulin stimulated glucose uptake was suppressed \((P < .01)\) in rats fed sucrose alone, linoleic acid alone, and the sucrose plus linoleic acid diet (Figure 2B). There were no statistically significant differences among Dahl-R rats fed sucrose or

**TABLE 2. BODY WEIGHTS AND MEAN ARTERIAL PRESSURE ON THE FOUR STUDY DIETS IN DAHL-S AND DAHL-R RATS**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>High Sucrose</th>
<th>High LA</th>
<th>High Sucrose + High LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl-S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>145 ± 9</td>
<td>136 ± 10</td>
<td>144 ± 10</td>
<td>139 ± 10</td>
</tr>
<tr>
<td>End body weight (g)</td>
<td>311 ± 8</td>
<td>312 ± 9</td>
<td>319 ± 11</td>
<td>320 ± 10</td>
</tr>
<tr>
<td>End mean arterial pressure (mm Hg)</td>
<td>130 ± 2</td>
<td>132 ± 4</td>
<td>132 ± 4</td>
<td>148 ± 6†</td>
</tr>
<tr>
<td>Dahl-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>146 ± 7</td>
<td>150 ± 12</td>
<td>146 ± 7</td>
<td>147 ± 10</td>
</tr>
<tr>
<td>End body weight (g)</td>
<td>293 ± 11</td>
<td>300 ± 11</td>
<td>304 ± 7</td>
<td>306 ± 9</td>
</tr>
<tr>
<td>End mean arterial pressure (mm Hg)</td>
<td>116 ± 3</td>
<td>116 ± 3</td>
<td>113 ± 4</td>
<td>119 ± 3</td>
</tr>
</tbody>
</table>

LA, linoleic acid.
†\(P < .05\) versus all other Dahl-S groups.

**FIGURE 1.** Systolic blood pressures of Dahl-S and Dahl-R rats on the different diets. Different subscripts indicate statistically significant differences within strains among groups on the different diets. LA, linoleic acid.
Glucose uptake on the sucrose plus linoleic acid diet did not differ in Dahl-S and Dahl-R rats.

**DISCUSSION**

Previous reports of the capacity of high simple carbohydrate feeding to increase blood pressure in the rat are conflicting. Variable results among studies have been attributed to differences in the contents of the diets, to different blood pressure responses among different ages and strains of rats, and to the blood pressure measurement itself. In the present study, in rats fed high NaCl, arterial pressure was not affected by a high sucrose diet alone or a high linoleic acid diet alone, either in the Dahl-S or the Dahl-R rat. However, arterial pressure was increased by a high sucrose diet when accompanied by a concomitant high intake of linoleic acid, only in the Dahl-S, but not in the Dahl-R rat.

The purpose of this study was to evaluate the interaction of a high NaCl diet with sucrose or linoleic acid on blood pressure in a genetic model of salt sensitive hypertension. Rats were not studied on lower NaCl intakes. Although it is possible that a 3% NaCl intake would affect the overall balance of other electrolytes, we have previously reported that serum potassium and serum calcium concentrations do not differ in Dahl-S rats fed 1% NaCl or 4% NaCl for 11 weeks.

Results of the present study confirm that the Dahl-S rat is insulin resistant, and that diets high in sucrose or linoleic acid content induce insulin resistance in both strains. Although the phenotypic expression of insulin resistance in response to the high sucrose plus high linoleic acid diet was similar in both Dahl-S and Dahl-R rats, arterial pressure was increased by the diet only in Dahl-S rats. Similarly, in Fisher 344 rats, a 2-year exposure to a high sucrose, high fat diet results in hyperinsulemia, hypertension, and obesity. The present study extends this observation by demonstrating that insulin resistance induced by a high sucrose, high linoleic acid diet is associated with hypertension in the absence of obesity and that the blood pressure of genetically salt resistant rats is also resistant to this high carbohydrate, high fat intake.

Consistent with our results, Preuss et al reported that blood pressures of Dahl-S rats are increased by a high sucrose-low protein diet within 3 weeks, whereas blood pressures of Dahl-R rats consuming this diet did not increase until 2 to 3 months. Additionally, chronic euglycemic insulin infusion has also been reported to increase blood pressure in Dahl-S rats on a high NaCl, but not on a low NaCl intake, and not in Dahl-R rats. Taken together, these observations indicate that blood pressure of genetically salt sensitive rats is more responsive to other dietary interventions resulting in insulin resistance than blood pressure of genetically salt resistant rats.

Although a number of putative mechanisms have been proposed, it is unclear if hypertension is causally related either to insulin resistance or to related hyperinsulinemia. In the present study, the absence of a rise of blood pressure in Dahl-R rats, despite a diet-induced increase in insulin resistance, indicates that induced insulin resistance by itself is not sufficient to cause hypertension. The association between diet-induced insulin resistance and hypertension is modified by the genetic background of the host. Future studies are planned to dissect out the heritable mechanisms that lead to or protect against the development of hypertension in the Dahl-S and Dahl-R rat, respectively.

**REFERENCES**

3. Donnelly R, Connell JMC: Insulin resistance: possible