Abnormalities of Carbohydrate and Lipid Metabolism in Dahl Rats

Gerald M. Reaven, Jack Twersky, and Helen Chang

Plasma glucose, insulin, and triglyceride concentration, blood pressure, and insulin action on isolated adipocytes were determined in weight-matched Sprague-Dawley, Dahl salt-resistant, and Dahl salt-sensitive rats. Blood pressure and plasma glucose concentrations were not significantly different in the three groups. However, Dahl salt-sensitive rats had significantly higher plasma insulin (39±2 microunits/ml) and triglyceride (213±11 mg/dl) concentrations than did Sprague-Dawley rats (27±2 microunits/ml and 101±6 mg/dl, respectively). Values for insulin (34±4 microunits/ml) and triglyceride (159±11 mg/dl) were intermediate in Dahl salt-resistant rats. In contrast, maximal insulin-stimulated glucose transport was significantly lower in adipocytes isolated from Dahl salt-sensitive as compared with Sprague-Dawley rats (400±16 versus 523±14 ft/cell/sec), with Dahl salt-resistant rats again having intermediate values. However, the ability of insulin to maximally inhibit catecholamine-stimulated lipolysis was similar in all three groups, averaging ~20% of the activity present in the absence of insulin. All of these differences were seen when the rats were eating conventional chow and did not change in Dahl rats after 2 weeks of an 8% NaCl diet. On the other hand, the predicted rise in blood pressure took place in Dahl salt-sensitive rats, increasing from 147±4 to 181±6 mm Hg. These data indicate that Dahl rats have higher values for plasma triglyceride and insulin concentration than control Sprague-Dawley rats, associated with a defect in insulin-stimulated glucose uptake by isolated adipocytes. These metabolic changes are not dependent on Dahl rats eating a high salt diet and do not vary as a function of salt intake. (Hypertension 1991;18:630–635)

It has recently become apparent that patients with high blood pressure tend to be hyperinsulinemic, hypertriglyceridemic, and resistant to insulin-stimulated glucose uptake.1–5 Similar changes have also been demonstrated in rats with spontaneous hypertension (SHR),6–8 as well as in normal Sprague-Dawley (S-D) rats when the cornstarch in their diet is replaced with fructose.9–12 Furthermore, it has been possible to show that adipocytes isolated from SHR are resistant to insulin-stimulated glucose uptake,7 and the magnitude of this cellular defect is significantly related to the associated increases in blood pressure and plasma insulin and triglyceride concentrations.8 Because plasma triglyceride concentrations also seem to be increased in Dahl salt-sensitive (S) rats,13 the current study was initiated to see if changes in insulin action and concentration accompany the previously noted abnormality in lipid metabolism in this genetic form of rodent hypertension.

Methods

Male Dahl S and salt-resistant (R) Brookhaven-derived rats were obtained from Harlan Sprague Dawley, Inc., Indianapolis, Ind., as were male control S-D rats. They were fed ad libitum with conventional Sprague-Dawley chow (Ralston Purina Co., St. Louis, Mo.) containing 0.6% NaCl, which in some experiments was replaced with the same basic diet containing 8% NaCl. Water was given ad libitum, and rats were maintained on a 12-hr light/dark cycle (from 6:00 AM to 6:00 PM). Plasma insulin14 and triglyceride15 concentration was measured on blood drawn from the tail vein of unanesthetized rats at 2:00 PM, 6 hours after removal of all food. Awake systolic blood pressure (IITC Co., Woodland Hills, Calif.) was measured at the same time in quietly restrained rats as described previously.7–11 In one series of experiments all measurements were made before and at weekly intervals for 2 weeks after increasing the dietary content of the chow from 0.6% to 8% NaCl. Isolated adipocytes were prepared from epididymal fat pads according to the method of Rodbell16 with minor modifications. The fat pads were minced

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with scissors and placed in plastic flasks in Krebs bicarbonate buffer with 4% bovine serum albumin (BSA), 3 mM glucose, and 1 mg/ml collagenase. Collagenase digestion was carried out at 37°C in a gyratory water bath shaker for 75 minutes. Cells were washed three times in fresh Krebs buffer with 4% albumin and 2.5 mM glucose and were allowed to separate from the infranatant by flotation. A 100 µl aliquot of diluted cells was fixed in a solution of 2% osmium tetroxide in collidine buffer and counted in a Coulter counter (Hialeah, Fla.) to determine cell number. Lipocrit was also determined, and cell size (µg lipid/cell) was calculated from lipocrit and cell number. Aliquots of cells were taken for measurement of glucose transport and catecholamine-stimulated lipolysis.

Glucose transport by isolated adipocytes was determined by a recently validated method based on the observation that glucose uptake is a measurement of glucose transport when studies are carried out at trace glucose concentration.17-18 Briefly, isolated adipocytes (2% lipocrit) were incubated in 500 µl 3.5% albumin buffer in the absence and presence of different concentrations of insulin (50–8,000 pM) and tracer (300 nM) amounts of D-[U-14C]-glucose. Cell suspension was incubated at 37°C for 1 hour with continuous shaking at 40 rpm. The incubation was terminated by centrifuging a 400 µl aliquot in a 500 µl microfuge tube, and the amount of activity associated with the adipocytes (and the total radioactivity in the incubation medium) was determined by liquid scintillation counting. Values for EC50 (concentration of insulin required for half-maximal effect) were calculated from an insulin dose-response curve as described previously.17,18 Earlier studies have demonstrated that values for glucose transport are similar when either the conventional 3-O-methylglucose method or the one in this study was used.17,18

The ability of insulin to inhibit catecholamine-induced lipolysis was estimated as follows. Adipocytes were diluted in Krebs buffer with 4% albumin and 2.5 mM glucose buffer, pH 7.4; aliquots of diluted cells placed in plastic vials (1 x 10^7 cells/ml) and incubated for 1 hour at 37°C in the presence of adenosine deaminase (1 unit/ml) and isoproterenol (10^-7 M) in a 95% O2–5% CO2 atmosphere. At the end of incubation, an aliquot (0.2 ml) of infranatant was removed from each incubation mixture for measurement of glycerol concentration by an enzymatic method.19 Measurements were made in the absence and presence of increasing concentrations of insulin in the incubation medium.

Results

Table 1 lists the weight, blood pressure, and plasma glucose, insulin, and triglyceride concentrations of Dahl S, Dahl R, and S-D rats when they were given conventional rat chow to eat. The weight of the three groups was similar. Although mean blood pressure varied somewhat in the three groups, the differences were not statistically significant. Plasma glucose concentrations were also the same in all three groups. However, the insulin (F=6.3, p<0.004) and the triglyceride (F=40.0, p<0.001) concentrations of the three groups were significantly different (one-way ANOVA). Specifically, Dahl S rats had significantly higher plasma insulin (p<0.01) and triglyceride (p<0.001) concentrations than did S-D rats. Plasma insulin and triglyceride concentrations were also higher, on average, in Dahl S as compared with Dahl R rats, but only the difference in triglyceride level was statistically significant (p<0.002). Dahl R rats had higher average plasma insulin and triglyceride concentration than did S-D rats, and the difference in plasma triglyceride level was statistically significant (p<0.001).

Glucose uptake by isolated adipocytes in the absence of insulin (basal), in response to an insulin concentration of 8,000 pM (maximal), and the incremental increase in glucose uptake to insulin (maximal-basal) is illustrated in Figure 1. When analyzed by three-way

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Table 1. Weight, Blood Pressure, and Plasma Glucose, Insulin, and Triglyceride Concentrations in Dahl Salt-Sensitive, Dahl Salt-Resistant, and Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt (g)</th>
<th>Blood pressure (mm Hg)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (microunits/ml)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl S (n=20)</td>
<td>258±6</td>
<td>142±3</td>
<td>135±3</td>
<td>39±2</td>
<td>213±10</td>
</tr>
<tr>
<td>Dahl R (n=10)</td>
<td>253±4</td>
<td>135±5</td>
<td>138±3</td>
<td>34±4</td>
<td>158±11</td>
</tr>
<tr>
<td>S-D (n=20)</td>
<td>252±7</td>
<td>125±7</td>
<td>133±4</td>
<td>27±2</td>
<td>102±6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Wt, weight; TG, triglyceride; Dahl S and Dahl R, Dahl salt-sensitive and salt-resistant rats; S-D, Sprague-Dawley rats.
FIGURE 1. Bar graph shows glucose transport by adipocytes isolated from Sprague-Dawley (S-D, n=10), Dahl salt-resistant (Dahl R, n=10), and Dahl salt-sensitive (Dahl S, n=10) rats. Measurements were made in the absence of insulin (Basal) and at a maximal insulin concentration of 8,000 pM (Max). The incremental response in glucose uptake (Δ) due to insulin was calculated by subtracting the basal from the maximal value.

ANOVA, it was clear that glucose transport was different among the three groups (F=5.0, p<0.05). Since there was statistically significant interaction between "rat group" and "insulin dose" (F=16.1, p<0.001), the differences among the three groups in terms of basal glucose uptake were not the same as the differences in maximal glucose uptake. Using Bonferroni's multiple comparison approach, no differences were found in the basal uptake of all three groups. However, using the same approach, maximal insulin-stimulated glucose uptake was greatest in adipocytes from S-D rats, lowest in adipocytes from Dahl S rats, and intermediate in adipocytes from Dahl R rats. All of these differences were significant at the p<0.001 level. The incremental effect of insulin on glucose transport in the three groups was similar to the results of maximal transport.

The results shown in Figure 1 demonstrated that maximal insulin-stimulated glucose transport by adipocytes isolated from Dahl S rats was lower than that of S-D rats. To compare insulin sensitivity, complete insulin dose-response curves were defined in Dahl S and S-D rats. The results of these studies appear in Figure 2 and indicate that insulin-stimulated glucose transport by adipocytes isolated from Dahl S rats was higher than that of S-D rats (F=10.1, p<0.005).

However, neither the EC50 of adipocytes from Dahl S and S-D rats (108±12 versus 110±10 pM) nor adipocyte cell size (0.232±0.004 versus 0.238±0.006 μg lipid/cell) were different in the two groups.

The effect of 2 weeks of a high salt diet on weight, blood pressure, and plasma glucose, insulin, and triglyceride concentrations of Dahl S and Dahl R rats is shown in Table 2. It can be seen that the weight of the two groups was the same at baseline and increased to a similar degree in response to 2 weeks of an 8% NaCl diet. However, the blood pressure response of the two groups was significantly different (F=23.7, p<0.001). In Dahl S rats, the blood pressure rose from 147±4 to 181±6 mm Hg (p<0.002), whereas it did not change in Dahl R rats (133±5 versus 141±4 mm Hg). There was no significant change in plasma glucose, insulin, or triglyceride concentration.
concentrations in response to the high salt diet in either Dahl S or Dahl R rats.

Figure 3 shows glucose uptake by isolated fat cells from Dahl S and Dahl R rats after 2 weeks of an 8% NaCl diet. It is apparent that the values in the two groups were quite similar. In addition, by comparing Figures 2 and 3 it can be seen that glucose uptake by adipocytes isolated from Dahl S rats was similar when the rats were eating conventional rat chow or an 8% NaCl diet.

The results in Figure 1 show that maximal insulin-stimulated glucose uptake by adipocytes isolated from Dahl S rats was lower than that of fat cells from S-D rats. Insulin also acts on the fat cell to inhibit lipolysis, and experiments were performed to evaluate this aspect of insulin action on adipocytes isolated from Dahl S, Dahl R, and S-D rats. The data in Figure 4 illustrate the ability of $10^{-7}$ isoproterenol to stimulate lipolysis in adipocytes isolated from the three groups of rats in the absence of insulin (basal) and in response to a maximal dose of insulin (4,000 pM). These studies were performed while the rats were eating conventional chow, and it can be seen that catecholamine-induced lipolysis was the same in all three groups in the absence of insulin. In addition, the response to the maximal inhibitory dose of insulin was not different. The ability of insulin to inhibit catecholamine-induced lipolysis was also evaluated in adipocytes obtained from Dahl S and Dahl R rats after 2 weeks of eating an 8% NaCl diet and indicated that lipolytic activity of isolated adipocytes was also similar in the two groups of Dahl rats after the high salt diet (data not shown).

**Discussion**

The results of the current study have shown that Dahl rats have higher plasma insulin and triglyceride concentration than do S-D rats: the strain from which Dahl rats were originally derived. Furthermore, although the differences between S-D and Dahl rats were of greater magnitude in Dahl S rats, Dahl R rats were always intermediate between S-D and Dahl R rats. In addition to showing that Dahl rats had higher plasma insulin and triglyceride concentrations than S-D rats, a defect in the ability of insulin to stimulate glucose uptake could also be demonstrated in adipocytes isolated from Dahl rats. As with the other variables measured, glucose uptake by adipocytes from Dahl R rats was intermediate between that of S-D and Dahl S rats. It should be emphasized that these changes were seen in both Dahl S and R rats when they were eating a normal sodium intake. Thus, the insulin resistance, hyperinsulinemia, and hypertriglyceridemia are not sodium dependent. Indeed, very little if any of the measures, with the exception of blood pressure in the Dahl S rats, changed with the increase in dietary sodium intake.

The metabolic abnormalities observed in the Dahl rats in this study resemble those previously described in patients with high blood pressure,\textsuperscript{1}–\textsuperscript{5} SHR,\textsuperscript{7,8} and fructose-fed rats.\textsuperscript{9}–\textsuperscript{12} The presence of insulin resistance, hyperinsulinemia, and hypertriglyceridemia in multiple models of hypertension obviously focuses attention on the relation between these metabolic variables and the change in blood pressure. For example, it could be speculated from the data in Table 1 that Dahl S and Dahl R rats have higher blood pressures than do S-D rats, along with their somewhat higher values for plasma insulin concentration, implying a relation between these two vari-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Line graph shows glucose transport by adipocytes isolated from Dahl salt-resistant (Dahl R, n=10) and salt-sensitive rats (Dahl S, n=10) 2 weeks after consuming an 8% NaCl diet. Values depict glucose uptake in the absence of insulin and in response to increasing doses of insulin.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Bar graph shows lipolytic activity (glycerol release) by adipocytes isolated from Sprague-Dawley (S-D, n=10), Dahl salt-resistant (Dahl R, n=10), and Dahl salt-sensitive (Dahl S, n=10) rats. Values depict the lipolytic response to $10^{-7}$ isoproterenol in the absence of insulin (Basal) and at a maximal insulin dose of 4,000 pM (Max).}
\end{figure}
ables. On the other hand, the obvious increase in blood pressure after consumption of an 8% NaCl diet in Dahl S rats was not associated with any change in plasma insulin concentration (Table 2). Thus, at the most, one might argue that insulin resistance and hyperinsulinemia predispose an organism to develop high blood pressure. There is evidence that acute hyperinsulinemia can increase sodium and water reabsorption. Perhaps Dahl R rats are less sensitive to the anti-nature effects of hyperinsulinemia, and they are, therefore, more resistant to salt-induced hypertension. Thus, insulin resistance and hyperinsulinemia could be viewed as permissive, representing changes that predispose an individual to high blood pressure, but do not necessarily cause hypertension by themselves. Such a view might also apply to humans and help explain why the relation between insulin levels and hypertension seems to vary as a function of ethnic groups.

Attention to this point has focused on the relation between insulin resistance, hyperinsulinemia, and high blood pressure. It is apparent that Dahl rats are also hyperterglyceridemic, as are rats with fructose-induced hypertension. and patients with high blood pressure. Although the relation between insulin resistance, hyperinsulinemia, and hypertension is obviously complex and not well understood, that between insulin resistance, hyperinsulinemia, and hypertriglyceridemia is more straightforward. Indeed, there is substantial evidence in both humans and animals that an increase in plasma triglyceride concentration is the expected consequence of insulin resistance and hyperinsulinemia. It is unlikely that the increase in plasma triglyceride concentration associated with insulin resistance and hyperinsulinemia in individuals with high blood pressure plays a role in the regulation of blood pressure. On the other hand, hypertriglyceridemia does increase risk of coronary heart disease and its presence in patients with hypertension may contribute to morbidity and mortality from coronary heart disease in this clinical syndrome.

In summary, Dahl S rats have higher plasma insulin and triglyceride concentration than do control S-D rats. In addition, isolated adipocytes from Dahl S rats are insulin resistant as compared with those from S-D rats. These changes are seen when Dahl S rats are given conventional chow to eat, and only the blood pressure goes up when they consume an 8% NaCl diet. Changes similar to those described for the Dahl S rats are seen in the Dahl R rat when eating chow but are intermediate in magnitude between the S-D and the Dahl S rat. Although plasma insulin and triglyceride concentrations are similar in Dahl S and Dahl R rats when they are given a high salt diet to eat, Dahl R rats do not have an increase in blood pressure. These data show that Dahl rats share the defect in insulin and triglyceride metabolism previously noted in patients with high blood pressure, in SHR, and in rats with fructose-induced hypertension.

References


**KEY WORDS**  • carbohydrate metabolism  • lipid metabolism  • metabolism  • insulin  • Dahl rats
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