Endothelial dysfunction precedes hypertension in diet-induced insulin resistance

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Katakam, Prasad V. G., Michael R. Ujhelyi, Margarethe E. Hoenig, and Allison Winecoff Miller. Endothelial dysfunction precedes hypertension in diet-induced insulin resistance. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R788–R792, 1998.—The insulin-resistant (IR) syndrome may be an impetus for the development of hypertension (HTN). Unfortunately, the mechanism by which this could occur is unclear. Our laboratory and others have described impaired endothelium-mediated relaxation in IR, mildly hypertensive rats. The purpose of the current study is to determine if HTN is most likely a cause or result of impaired endothelial function. Sprague-Dawley rats were randomized to receive a fructose-rich diet for 3, 7, 10, 14, 18, or 28 days or were placed in a control group. The control group received rat chow. After diet treatment, animals were instrumented with arterial cannulas, and while awake and unrestrained, their blood pressure (BP) was measured. Subsequently, endothelium-mediated relaxation to acetylcholine was determined (in vitro) by measuring intraluminal diameter of phenylephrine-preconstricted mesenteric arteries (—250 μM). Serum insulin levels were significantly elevated in all groups receiving fructose feeding compared with control, whereas there were no differences in serum glucose levels between groups. Impairment of endothelial-mediated relaxation starts by day 14 (mean percent maximal relaxation (Emax): 69 ± 10% of baseline) and becomes significant by day 18 (Emax: 52 ± 11% of baseline; P < 0.01). However, the mean BP (mmHg) does not become significantly elevated until day 28 [BP: 132 ± 1 (day 28) vs. 116 ± 3 (control); P < 0.05]. These findings demonstrate that both IR and endothelial dysfunction occur before HTN in this model and suggest that endothelial dysfunction may be a mechanism linking insulin resistance and essential HTN.

vasodilation; fructose; Sprague-Dawley

INSULIN RESISTANCE and compensatory hyperinsulinemia have been associated with hypertension in a number of cross-sectional and population-based studies (3, 10, 21). Strong evidence exists to suggest that this relationship is causal. First, prospective studies have established that fasting insulin concentrations predict the incidence of hypertension (21). In addition, a direct correlation exists between plasma insulin concentrations and severity of hypertension (3). This relationship is independent of other confounding factors, such as obesity or lipid abnormalities (21). Finally, insulin resistance is present in genetic hypertensive strains such as the spontaneously hypertensive rats (14), the Dahl salt-sensitive rats (19), and the Milan hypertensive rats (2), suggesting that insulin resistance is a cause of primary hypertension in both animals and humans. In contrast, insulin resistance does not exist in human and animal models of secondary hypertension. Although the majority of these data is correlative, taken together they implicate insulin resistance in the etiology of essential hypertension.

Unfortunately, the exact mechanism(s) by which insulin resistance may induce hypertension is less clear. One proposed mechanism is impaired endothelium-mediated relaxation (13, 24, 26). This was demonstrated in both lean and obese patients with insulin resistance who exhibited impaired endothelium-mediated relaxation to escalating doses of methacholine (24). Moreover, animal studies in our laboratory (13) and others (26) have demonstrated an impaired endothelium-mediated relaxation response to acetylcholine in the fructose-fed rat model of insulin resistance. Specifically, our laboratory has demonstrated impaired endothelium-mediated relaxation in mesenteric resistance arteries (13). These findings led us to hypothesize that impaired endothelial function results in elevated systemic vascular resistance and thus, hypertension. Unfortunately, hypertension has also been shown to impair endothelium-mediated relaxation (7, 11, 12, 15, 27). Because hypertension was present in the previous studies, it is not clear if insulin resistance or hypertension impairs endothelial function. The purpose of the present investigation was to determine when endothelial dysfunction occurs in relation to the development of hypertension in the fructose-fed, insulin-resistant rat.

MATERIALS AND METHODS

The Animal Care Committees at the Medical College of Georgia and the Augusta Veterans Affairs Medical Center approved the current protocol. Male Sprague-Dawley rats were obtained between 6 and 10 wk of age and were assigned to one of seven groups based on the number of days they were to receive a fructose-rich diet. Animals were assigned to groups based on their age such that all animals would be between 10 and 12 wk of age at the time of experimentation. The groups were as follows: 3 days (n = 8), 7 days (n = 6), 10 days (n = 8), 14 days (n = 11), 18 days (n = 6), 28 days (n = 8), and control (n = 9). Because the 28-day animals were the oldest group, the control group was kept for the same amount of time to control for age-related changes. All animals except the control group were fed a fructose-rich diet (contains as percentage of total calorie intake: 66% fructose, 22% casein, and 12% lard, plus essential vitamins and minerals; Teklad...
Laboratories, Madison, WI). Animals in the control group received normal rat chow. Glucose clamp experiments and glucose tolerance tests have been performed, establishing that this model does develop insulin resistance at the level of the skeletal muscle (1, 6, 9, 28). This was shown to correlate with fasting hyperinsulinemia in this model (1, 6, 9, 28). For the current experiment, fasting hyperinsulinemia was used as a marker for the development of insulin resistance.

Measurement of blood pressure. One day before ending diet treatment, rats were sedated with pentobarbital sodium (30 mg/kg ip) and ketamine (1 mg/kg ip). With use of an aseptic technique, an arterial cannula (PE-10 tubing coupled to PE-50 tubing) was placed into the femoral artery for measurement of arterial pressure. The external portion of the cannula (PE-50) was tunneled under the skin, sutured between the scalpula, and filled with heparinized saline solution. Animals were allowed to recover from this procedure for 24 h. During the first 18 h of the recovery period, all animals had free access to food and water. This period was followed by a 6-h fast. After the recovery period, the arterial cannula was aligned to a fluid-filled transducer (CPXL-23; Statham, Costa Mesa, CA), and the signal was conditioned, amplified, and digitized for measurement of conscious, unrestrained blood pressure. Animals were allowed to acclimate to their new environment for 30 min before recording of blood pressure. All data were fed online to an IBM personal computer and an analog-to-digital converter. Acquisitions of blood pressure were taken every 20 s for a period of 30 min. Software (DataQ; Windaq) stored the blood pressure for each experiment. These data were averaged for each animal to determine the mean resting systolic blood pressure.

Measurement of mesenteric diameter in vitro. After blood pressure measurements, rats (after 6-h fasting period) were anticoagulated (heparin, 500 units ip) and anesthetized (pentobarbital sodium, 50 mg/kg ip). A midline incision was made, and the abdominal and chest cavities were opened. A subcutaneous injection (pentobarbital sodium, 30 mg/kg ip) and ketamine (1 mg/kg ip). With use of an aseptic technique, an arterial cannula (PE-10 tubing coupled to PE-50 tubing) was placed into the femoral artery for measurement of arterial pressure. The external portion of the cannula (PE-50) was tunneled under the skin, sutured between the scalpula, and filled with heparinized saline solution. Animals were allowed to recover from this procedure for 24 h. During the first 18 h of the recovery period, all animals had free access to food and water. This period was followed by a 6-h fast. After the recovery period, the arterial cannula was aligned to a fluid-filled transducer (CPXL-23; Statham, Costa Mesa, CA), and the signal was conditioned, amplified, and digitized for measurement of conscious, unrestrained blood pressure. Animals were allowed to acclimate to their new environment for 30 min before recording of blood pressure. All data were fed online to an IBM personal computer and an analog-to-digital converter. Acquisitions of blood pressure were taken every 20 s for a period of 30 min. Software (DataQ; Windaq) stored the blood pressure for each experiment. These data were averaged for each animal to determine the mean resting systolic blood pressure.

RESULTS

Mean body weight, fasting glucose, resting arterial diameter, and percent constriction are shown in Table 1. Mean body weights were similar across groups with the exception of rats in 28-day and control groups, which were significantly heavier. The rats in these two groups were 10 days older than the rest of the groups, thus explaining their difference in weight. Because these animals would still be considered young adults, we do not believe that differences in endothelial function or blood pressure are age related. Data from the control group advocate this supposition.

There was no difference in mean fasting plasma glucose levels between rats in various diet groups or control. This is consistent with our previous data illustrating that these animals were not diabetic (13). In contrast, mean fasting plasma insulin levels of all rat groups receiving the fructose-rich diet were markedly elevated compared with the control group. However, these levels did not differ between groups receiving the fructose diet (Fig. 1). Thus insulin levels were significantly elevated from control after 3 days of

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Day 18</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>303 ± 8</td>
<td>270 ± 6</td>
<td>278 ± 5</td>
<td>272 ± 6</td>
<td>273 ± 6</td>
<td>276 ± 4</td>
<td>310 ± 6</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>149 ± 11</td>
<td>142 ± 16</td>
<td>149 ± 7</td>
<td>160 ± 7</td>
<td>151 ± 5</td>
<td>143 ± 10</td>
<td>142 ± 8</td>
</tr>
<tr>
<td>Arterial diameter, µm</td>
<td>268 ± 10</td>
<td>252 ± 8</td>
<td>255 ± 12</td>
<td>254 ± 8</td>
<td>255 ± 7</td>
<td>257 ± 8</td>
<td>256 ± 9</td>
</tr>
<tr>
<td>Preconstriction, %</td>
<td>47 ± 3</td>
<td>41 ± 4</td>
<td>39 ± 4</td>
<td>38 ± 5</td>
<td>42 ± 3</td>
<td>42 ± 2</td>
<td>46 ± 2</td>
</tr>
</tbody>
</table>

Data are means ± SE.
fructose diet and remained consistently elevated through day 28.

Resting intraluminal diameter and percent preconstriction values for isolated mesenteric arteries did not differ between groups (Table 1). The dose-response curves to acetylcholine for control, 14-day, 18-day, and 28-day groups are shown in Fig. 2. Clearly, relaxation to acetylcholine in the dose-response curves for 14-day, 18-day, and 28-day groups is significantly inhibited compared with the control group, illustrating impaired endothelial-mediated relaxation. Dose-response curves for 3-day, 7-day, and 10-day groups were identical to control and therefore are not included in the graph. The E_max for each group is represented in Fig. 3. The E_max for control, 3-day, 7-day, 10-day, and 14-day groups did not differ significantly. At day 14, endothelial function appears to decline (E_max: 69 ± 10%), becomes significantly impaired at day 18 (E_max: 52 ± 11%; P < 0.01), and remains depressed through day 28 (E_max: 44 ± 4%; P < 0.01).

Mean systolic blood pressure measurements for 3-day, 7-day, 10-day, 14-day, and 18-day groups were not different from control (Fig. 4). However, mean systolic blood pressure became significantly elevated from control by day 28 (132 ± 1 mmHg; P < 0.05).

Although vascular dysfunction became evident by day 14 and statistically significant at day 18, there was no change in systolic blood pressure until day 18, which became significant at day 28. These data clearly show that the development of hypertension lags behind the onset of endothelial dysfunction.

DISCUSSION

Insulin resistance, hypertension, and impaired endothelium-mediated relaxation occur concurrently in both human and animal models; however, how these problems occur in relation to one another is not clear (8, 24, 27). We hypothesized that, in the fructose-fed, insulin-
resistant rat, impaired endothelial-mediated relaxation develops before, as opposed to as the result of, hypertension. Insulin resistance, in the fructose-fed model, has been shown to develop within 7–10 days of beginning the diet (1, 6, 9, 28). This is consistent with the current findings that hyperinsulinemia, a marker for insulin resistance, develops after only 3 days of fructose feeding. Subsequently, endothelium-mediated relaxation becomes significantly impaired after 18 days of fructose feeding. However, blood pressure does not significantly elevate until animals receive 28 days of fructose feeding, 10 days after endothelial dysfunction develops. Although we cannot prove cause and effect, these data suggest an interrelationship between the occurrence of insulin resistance, endothelial dysfunction, and hypertension, based on the order in which they develop. Endothelial-dependent relaxation was shown to be impaired before the development of hypertension and did not significantly worsen after the hypertension developed. Hence, endothelial dysfunction is not the result of hypertension but may be a contributor to its development.

Endothelium-dependent relaxation has been shown to be depressed in animal models and humans with hypertension (7, 11, 12, 15, 27). Unquestionably, long-standing hypertension can damage the endothelium; however, the current data and others suggest that hypertension may also occur as a result of endothelial dysfunction. Evidence to advocate this supposition can be found in both epidemiological and experimental data. First, normotensive children of hypertensive patients have impaired responses to endothelium-mediated vasodilators (25). Interestingly, this same population also has increased fasting insulin levels, implying insulin resistance may be the common genetic link (4). Second, treatment of hypertension does not consistently improve endothelial function (16), suggesting that endothelial dysfunction is either not reversible or not caused by hypertension. Finally, the eNOS (endothelial constitutive nitric oxide synthase) knockout mouse develops hypertension due to endothelial dysfunction and increased total peripheral resistance. These data strongly suggest that hypertension can result from endothelial dysfunction.

Endothelial dysfunction in resistance arteries, such as the mesenteric bed, may lead to increased total peripheral resistance and thus an elevation of blood pressure. The current data assume that the mesenteric arterial bed is representative of the majority of resistance arteries in order for this to be the operative mechanism in this model. However, the mechanism relating insulin resistance to endothelial dysfunction is less clear. Currently, there are two theories regarding the relationship between endothelial dysfunction and insulin resistance. One theory suggests that endothelial dysfunction within the skeletal muscle vasculature causes insulin resistance by decreasing blood flow to insulin-sensitive tissues and delivery of insulin to the interstitium (18), while the second theory suggests that insulin resistance at the endothelium results in impaired endothelial function (22, 29). The current data cannot determine which of these theories is correct. It is possible that both mechanisms are operative such that endothelial dysfunction within skeletal muscle vasculature induces insulin resistance, which then progresses to induce endothelial dysfunction in the mesentery vascular bed. On the other hand, indirect evidence shows that the use of either pharmacological agents or exercise to improve insulin sensitivity normalizes endothelial function, thus suggesting that the second theory is more plausible (8, 20). Insulin is known to enhance endothelial-mediated vasodilation, and this effect is related to insulin sensitivity such that as insulin sensitivity declines, so does endothelial function (17, 23). Therefore, we speculate that endothelial dysfunction may be caused by insulin resistance.

In summary, we have clearly demonstrated that impaired endothelium-mediated relaxation precedes hypertension in the fructose-fed rat model of insulin resistance. These findings provide further evidence that insulin resistance is related to the etiology of hypertension and suggest that the mechanism that links these two pathophysiological states is endothelial dysfunction.

**Perspectives**

The insulin-resistant syndrome is linked to many independent risk factors for ischemic heart disease. However, a cause and effect relationship has not been clearly delineated. The current study was designed to determine if, after the development of insulin resistance, endothelial dysfunction occurs before or after the development of hypertension. This study shows that, in the fructose-fed, insulin-resistant rat, impaired endothelium-mediated relaxation is present in the mesenteric resistance arterial bed and precedes the development of hypertension. These findings illustrate that hypertension is not the cause of endothelial dysfunction in this model and in fact may be the mechanism by which hypertension develops. Future studies need to be performed to further address the mechanism of endothelial dysfunction in this model and its relationship to the development of hypertension.

We are grateful for the laboratory support provided by the Augusta Veterans Affairs Medical Center, Augusta, Georgia.

This work was supported by a Grant-in-Aid from the American Heart Association Georgia Affiliate and the National Pharmacy Cholesterol Council.

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Received 11 March 1998; accepted in final form 20 May 1998.

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