Impaired in Vivo Adrenergic Responses in Diet-Induced Hypertensive Rats

Pongamorn Bunnag, Mark T. Hori, Bernard Ormsby, Morris E. Berger, Michael S. Golub, and Michael L. Tuck

This study was conducted to investigate whether altered vascular responsiveness to vasoactive compounds contributes to the development of hypertension in diet-induced hyperinsulinemic rats. Male Sprague-Dawley rats were randomly assigned to receive high fructose, high sucrose, or standard rat chow for 13-18 wk. Blood pressure was monitored by indirect (tail-cuff) measurements at regular intervals during the diet treatment. Vascular responses to various vasoactive agents were studied both in vivo and in vitro. Blood pressure response, as assessed by direct (intra-arterial) measurement, to graded dose infusions of norepinephrine or angiotensin II or bolus infusion of acetylcholine were determined. In vitro vascular responses of the tail arteries to exogenous norepinephrine were also studied. The fructose- and the sucrose-fed rats had significantly higher blood pressure than controls. Serum insulin levels were also significantly higher in fructose- and sucrose-fed rats than in controls. The blood pressure responses to graded infusions of norepinephrine were significantly less in the fructose-fed rats than in controls. The blood pressure responses to angiotensin II and acetylcholine infusion were not significantly different among the three groups of rats. In vitro studies of vascular reactivity in the tail arteries revealed than the concentration of norepinephrine that produced half-maximal contraction (NE EC₅₀) was significantly higher in the fructose group than control. Thus, impaired vascular responses to exogenous norepinephrine were observed in fructose-fed rats both in vivo and in vitro. This may be due to an adaptation to increased sympathetic nervous activity, or may be a compensatory response to other structural or functional changes that produce hypertension in this model. (Hypertens Res 1997; 20: 17-21)

Key Words: diet-induced hyperinsulinemic rats, adrenergic responses, hyperinsulinemia, vascular reactivity

High fructose or high sucrose diets have been documented to increase blood pressure in rats (1, 2). Hypertension develops in Sprague-Dawley rats fed a high fructose diet as early as 2 wk after initiation of the diet (3). The hypertension is accompanied by the metabolic abnormalities of hyperinsulinemia, insulin resistance, and hypertriglyceridemia, without the development of obesity (3, 4). High sucrose feeding produces similar results (5). Several lines of evidence, both in epidemiologic studies and experimental studies, point to a relationship between hyperinsulinemia and arterial hypertension. Many biological actions of insulin on the cardiovascular, renal, and sympathetic nervous systems may be linked to the development of hypertension. However, some of these actions exert opposite effects in terms of vascular responses. For example, although insulin has been shown to increase sympathetic nervous system activity, it also has direct vasodilatory action on blood vessels. It is possible that some effects of insulin that regulate blood pressure may be attenuated during the development of insulin resistance, whereas other effects are retained or augmented. In this study, we examined the in vivo blood pressure responses to various vasoactive agents in diet-induced hyperinsulinemic, hypertensive rats. We also studied in vitro vascular reactivity to exogenously applied norepinephrine in blood vessels harvested from these rats.

Materials and Methods

Study Design
Male Sprague-Dawley rats, initially weighing between 200-230 g, were used for the experiment. The rats were kept in a vivarium with a 12-h light/dark cycle. The temperature was maintained at...
24°C. Prior to dietary manipulation, all rats were fed standard rat chow (Purina Laboratory). The rats were trained for the first 2 wk to become acclimated to the procedure of indirect blood pressure measurement. After that, the experimental rats were randomly assigned to receive high-fructose, high-sucrose (60% of total calories), or standard rat chow (Teklad Labs, Madison, WI). There were 19 rats in the fructose-fed group, 20 in the sucrose-fed group, and 19 rats were used as controls. All rats had free access to water and their specified diets. Indirect (tail-cuff) blood pressure measurement confirmed that all animals were normotensive before dietary manipulation. Direct blood pressure measurement was performed in each rat 13-18 wk after starting the diet. Each rat was randomly selected to receive an infusion of one of the following drugs: norepinephrine (NE), angiotensin II (Ang II), or acetylcholine (Ach). Continuous blood pressure responses to these agents were recorded via an indwelling carotid catheter. After that, the rats were killed, and the tail arteries were used for in vitro vascular studies.

Blood samples were collected before initiation of the diet and at the end of the study for assay of plasma insulin and glucose levels.

Statistics
Statistical analysis was performed using paired t-test or analysis of variance (ANOVA), with multiple individual group comparisons by Tukey or Dunken's method as appropriate.

Experimental Procedure

Blood Pressure Measurement
a) Indirect blood pressure measurement
The systolic blood pressure was measured by the tail-cuff method with the use of a programmed electro-sphygmomanometer (PE-300, Narco Biosystems, Inc., Houston, Texas). The rats were prewarmed for about 10-15 min to promote vasodilation. The mean of four consecutive readings was used as the value of the systolic blood pressure for each rat.

b) Direct blood pressure measurement
Intra-arterial pressure was measured directly via a polyethylene catheter (PE-50) surgically implanted in the common carotid artery 24 h prior to the experiments. The rats were first anesthetized using a combination of Ketamine (90 mg/kg) and Xylazine (10 mg/kg). Direct intra-arterial pressure was measured the next day when the rats were fully awake and freely moving in their cages. The catheter was connected to a pressure transducer (Statham, P23Db) and the arterial pressure was monitored on a Beckman 411 recording system.

Infusion Studies
After baseline blood pressure measurements, each rat received one of the vasoactive drugs through the arterial catheter. Norepinephrine (NE bitartrate, Levophed, Winthrop) was infused at graded doses of 2, 4, and 8 µg/kg/min for 3 min using an infusion pump (Harvard model 975). Ang II (Hypertensin, Ciba) was given at 2, 20, and 100 ng/kg/min for 3 min in a similar fashion. Ach (Sigma Chemical Co.) was given as a bolus dose of 1, 10, and 100 µg/kg. The interval between each dose for all of the drugs was at least 5-10 min, and the blood pressure was allowed to establish a new baseline. In each rat, the subsequent dose-related alterations in blood pressure were calculated on the basis of this newly established baseline. The total volume of all infusions did not exceed 1 ml in each rat.

In Vitro Vascular Studies
The day after the infusion study, the rats were sedated by carbon dioxide narcosis and killed by decapitation. The tail arteries were removed and dissected free of connective tissue and fat. Vascular segments, 4 mm in length, were prepared and placed in oxygenated Krebs-bicarbonate solution containing 144.2 mM Na, 4.9 mM K, 1.3 mM Ca, 1.2 mM Mg, 126.7 mM Cl, 25 mM HCO3, 1.2 mM SO4, 1.2 mM PO4, 11.1 mM glucose, and 0.025 mM EDTA. Stainless steel wires were inserted through the vascular lumen, and the vascular segment was then mounted in a specially constructed water-jacketed (37.5°C) tissue bath. Each bath was oxygenated (5% CO2, 95% O2) and equipped with an isometric force transducer (UC-2, Gould-Statham, Oxnard, CA) coupled to an eight-channel recording system (8800, Gould, Cleveland, OH). The arteries were equilibrated for 2 h at an optimum passive tension of 0.6 g. After that, norepinephrine was added to the organ bath in graded cumulative concentrations from 1 X 10^-9 M to 3 X 10^-5 M to generate a dose-response curve. From this curve, the concentration of NE that produced half-maximal contraction (NE EC50) was calculated.

Biochemical Analysis
Plasma glucose concentrations were measured using a Beckman Glucose Analyzer II with the glucose oxidase method. Plasma insulin concentration was measured by radioimmunoassay using 125I insulin kit (ICN Biochemicals, Inc.).

Results

Body Weight and Blood Pressure
After about 14 wk of dietary manipulation, the body weights of the rats in each group were not significantly different. The mean arterial pressure, however, was significantly higher in fructose- and sucrose-fed rats as compared with the control group (Table 1).

Infusion Studies
NE infusion: Graded infusions of NE at 2, 4, and 8 µg/kg/min produced significant increases in mean arterial pressure in all three groups of rats. However, the increments were significantly less in the fructose-fed rats as compared with control at all doses of NE infused (p < 0.05). The increments in mean arterial pressure did not differ significantly between fructose-fed and sucrose-fed rats after NE in-
fusion.

**Ang II and Ach administration:** Graded infusions of Ang II at 2, 20, and 100 ng/kg/min for 3 min produced no significant differences in incremental blood pressure among the three groups of rats (Fig. 1b). Similarly, there were no differences among the groups with respect to the response to administration of Ach at 1, 10, and 100 mg/kg (Fig. 1c).

**In Vitro Vascular Response to Norepinephrine**
The EC50 for norepinephrine was significantly greater in the fructose-fed rats than in the controls. The mean ± SEM NE-EC50 in the control, the fructose, and the sucrose groups were 1.64 ± 0.27, 2.55 ± 0.4, and 2.68 ± 0.62 x 10^-6 M, respectively (p < 0.05 between fructose and control). This indicated that the vessels from the fructose-fed rats were less sensitive to exogenous norepinephrine (Fig. 2). No differences were observed in the maximal responses to NE (4.71 ± 0.51, 5.58 ± 0.49, and 4.99 ± 0.65 g for control, fructose and sucrose, respectively).

**Biochemical Analysis**
Plasma glucose and insulin concentrations were not statistically different before initiation of the experimental diets. At the end of the experiment, there were also no significant differences in plasma glucose levels among the three groups of rats (Table 1).

Plasma insulin levels increased significantly in both the fructose- and sucrose-fed rats after dietary manipulation. At the end of the study, plasma insulin levels in both the fructose and sucrose groups were significantly higher than those in the control group (Table 1). The plasma insulin/plasma glucose ratios, an index of insulin sensitivity, were also higher in the fructose- and sucrose-fed groups than in the controls.

**Discussion**
The results of our study confirm that high fructose and high sucrose diets can induce hypertension in Sprague-Dawley rats. Plasma insulin levels were higher in these rats while plasma glucose levels were not significantly different, suggesting that the rats were insulin-resistant. The frequent concurrence of hyperinsulinemia and increases in blood pressure suggests that there may be a cause-and-effect relationship. A number of studies favor the hypothesis that hyperinsulinemia, insulin resistance, or both contribute to the development of hypertension in these rats. Moreover, other reports have shown that reducing insulin levels in these rats leads to a reduction in blood pressure and correction of other metabolic abnormalities. Thus, somatostatin, an agent that reduces plasma insulin levels, also reduces blood pressure in fructose-fed rats (6). Exercise training, which improves insulin sensitivity and reduces plasma insulin levels, also normalizes blood pressure in this model (7). Hypertension is also accompanied by hyperinsulinemia in spontaneously hypertensive rats and vanadate, an agent that improves insulin sensitivity and decreases plasma insulin levels, also lessens the severity of hypertension in these rats (8).

The mechanism(s) by which hyperinsulinemia or insulin resistance contributes to hypertension in these rats remains controversial, but has been linked in many studies to increased sympathetic nervous system activity (9, 10). Insulin infusion has been shown to increase plasma norepinephrine levels during euglycemic clamp studies in normal man (11). Hyperinsulinemia increases sympathetic activity in healthy human subjects, as measured by electroencephalography (12). Studies in premenopausal women revealed that hypertensive subjects had increased sympathetic activity as assessed by plasma catecholamines, in response to infused insulin (13). In this study, differences in insulin sensitivity could not be demonstrated by glucose disposal rates or by glucose/insulin ratios, suggesting an independent effect of acute insulin exposure on the sympathetic nervous system. Increased muscle sympathetic nerve activity is associated with insulin resistance in normotensive obese human subjects (14) and positively correlates with increased body mass index and plasma insulin concentration (15). Rats fed high sucrose showed increased sympathetic nervous system activity, as measured by increased norepinephrine turnover in the heart, brown adipose tissue, and liver (16). Other refined carbohydrates have also been documented to increase catecholamine excretion in rats (17, 18). It is postulated that the consumption of diets high in carbohydrates increases the activity of the sympathetic nervous system, a phenomenon probably mediated by the central effects of insulin, *i.e.*, at the hypothalamic level (19). Other studies in humans and rats have demonstrated that chronic stimulation of the sympathetic nervous system could blunt the effect of exogenously administered norepinephrine, most likely because of down-regulation of adrenergic receptors (20, 21). In our study, we tested the post-ganglionic neuroreceptor activity by

**Table 1. Physiological and Biochemical Characteristics of Sprague-Dawley Rats after Dietary Manipulation**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fructose</th>
<th>Sucrose</th>
</tr>
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<tbody>
<tr>
<td>BW (g)</td>
<td>498.1 ± 9.4</td>
<td>479.5 ± 5.2</td>
<td>499.8 ± 7.8</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>113.5 ± 2.5</td>
<td>125.9 ± 2.2**</td>
<td>122.5 ± 2.3*</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>118.1 ± 2.0</td>
<td>126.0 ± 5.2</td>
<td>123.8 ± 2.5</td>
</tr>
<tr>
<td>Plasma insulin (μU/ml)</td>
<td>36.8 ± 1.9</td>
<td>63.7 ± 4.5**</td>
<td>58.3 ± 4.5**</td>
</tr>
<tr>
<td>insulin/glucose ratio</td>
<td>0.32 ± 0.02</td>
<td>0.53 ± 0.04**</td>
<td>0.48 ± 0.04**</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. control, **p < 0.01 vs. control.
Our in vivo results showed that the vascular responsiveness to norepinephrine was decreased in fructose-fed rats as compared with control. The in vitro data further revealed that the norepinephrine EC50 was also greater in the vessels harvested from these rats. These data suggest that these vessels were less sensitive to the effect of norepinephrine, without altering efficacy for the adrenergic receptor. Thus, the decreased vascular responses seen in the fructose-fed animals in vivo can be predicted by the decreased sensitivity to NE noted in vitro, although other cardiac responses associated with blood pressure elevation cannot be totally eliminated.

No significant differences in blood pressure responses to infused Ang II or Ach were observed in our experiments, suggesting that no increase in medial thickness or endothelial dysfunction was produced by the mild blood pressure elevation in these animals. Therefore, our study demonstrated only altered adrenergic responses in fructose-fed rats. The precise mechanism underlying this finding remains to be elucidated but is consistent with a down-regulation of the adrenergic receptors or a counter-regulatory response to other pro-hypertensive changes in the vasculature in this model. Acute treatment of rat mesenteric arteries with insulin has been shown to augment norepinephrine-induced contraction (22). The mechanism for this response was considered to involve the a receptor itself or an amplification of the post-receptor signal transduction. It is not unreasonable then to argue that desensitization to this response contributes to our observations in a chronic hyperinsulinemia setting. In contrast, studies by Lembo et al. in human subjects (23) showed that insulin acts through the a2-adrenergic pathway to blunt sympathetic vasoconstriction. Insulin can hyperpolarize vascular smooth
muscle cell membranes by stimulation of Na\(^+\), K\(^+\)-ATPase activity (24). This may be another mechanism that contributes to the attenuation of vascular responses to exogenous norepinephrine.

In conclusion, our study confirmed that dietary-induced hyperinsulinemic Sprague-Dawley rats have significantly increased blood pressure. Alterations in sympathetic nervous system activity may be one of the mechanisms that links hyperinsulinemia to hypertension in these rats. Our study does not support the role of the renin-angiotensin system or endothelium-derived relaxing factor as a major contributor to hypertension in these rats.

References