Role of Endothelin-1 in Blood Pressure Regulation in a Rat Model of Visceral Obesity and Hypertension

Alexandre A. da Silva, Jay J. Kuo, Lakshmi S. Tallam, John E. Hall

Abstract—Endothelial dysfunction has been suggested to play an important role in the development of obesity-induced hypertension. Because endothelin release increases in response to endothelial damage, we examined whether endothelin-1 contributes to increased arterial pressure in a model of visceral obesity produced by feeding Sprague-Dawley rats a high-fat (HF) diet (40% fat w/w, n=6) for 12 months. Arterial and venous catheters were implanted for measurement of mean arterial pressure (MAP) and heart rate (HR) 24 hours per day and intravenous infusions. After a 5-day control period, rats were infused with the selective endothelin-1 type A receptor (ET-A) blocker ABT-627 (2.5 mg/kg per day, IV) for 9 days, followed by a recovery period. Rats fed a standard chow (normal fat, or NF, group: n=6) for 12 months were also infused with ET-A blocker and were used as controls. Compared with NF rats, HF rats had higher MAP (113±4 versus 98±2 mm Hg), increased visceral fat (18.7±2.0 versus 10.8±1.4 g), and 3.2-fold increase in plasma leptin despite similar total body weight gain. Long-term ET-A blockade markedly reduced MAP in HF (−14±3 mm Hg) and NF (−14±2 mm Hg), but it had no effect on HR, GFR, or PRA. These results indicate that a long-term HF diet may cause visceral obesity and increased MAP, even in the absence of major changes in total body weight. Endothelin-1 appears to play an important role in the maintenance of arterial pressure in rats fed HF and NF diets, but it does not appear to contribute to increased MAP in this model of diet-induced hypertension. (Hypertension. 2004;43[part 2]:1-5.)

Key Words: hypertension ■ renin ■ kidney ■ diet ■ obesity

Considerable evidence suggest that excess weight gain is the most common cause of human essential hypertension. Risk estimates from the Framingham Heart Study, for example, indicate that ≈78% of hypertension in men and 65% in women can be directly attributed to obesity.1 Clinical studies have also shown that most hypertensive subjects are overweight2 and that weight loss is an effective way of reducing arterial pressure in these patients.3 Although multiple factors have been suggested to contribute to obesity hypertension, including activation of the sympathetic nervous system4–6 and the renin-angiotensin system,7,8 the mechanisms by which increased adiposity raises arterial pressure have not been fully elucidated.

Another potential mechanism that could contribute to increased blood pressure in obesity is endothelial dysfunction. Endothelin is a powerful vasoconstrictor that has been demonstrated to mediate increased blood pressure in several experimental models of hypertension, especially those associated with salt-sensitivity blood pressure.9 Obese subjects with endothelial dysfunction have elevated plasma endothelin, and blockade of the endothelin-1 type A (ET-A) receptors improves endothelin-dependent vasodilation in these patients.10 Endothelin has also been reported to modulate endothelium-dependent vasoconstriction in obese mice fed a high-fat (HF) diet.11 However, there has been no study, to our knowledge, that has addressed the importance of endothelin in mediating long-term increases in arterial pressure in dietary-induced obesity. Therefore, the main objective of this study was to determine whether endothelin-1 contributes to increased arterial pressure in rat model of visceral obesity produced by a long-term HF diet.

Methods

Animals

The experimental procedures and protocol of this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Three-week-old male Sprague-Dawley rats (Harlan, Indianapolis, Ind) averaging body weight of 37±1 grams were randomly assigned to 1 of 2 dietary groups, normal-fat (NF) chow group (NF: 3.3% fat w/w, n=6) or a HF diet group (40.0% fat w/w, n=6), and were maintained on these diets for 12 months (Table 1).

After 12 months on one of the diets, the rats were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal), and atropine sulfate (0.1 mg/kg) was administered to attenuate excess airway secretions. Arterial and venous catheters were implanted according
Experimental Design

After 5 days of control measurements, the selective ET-A antagonist ABT-627 (Abbott Laboratory, Chicago, Ill) was dissolved in 20 μL of 70% alcohol and added to the saline infusion to deliver 2.5 mg/kg per day for 9 days. This dose has been shown to effectively block the vasoconstrictor effects of endothelin.14 The infusion of the ET-A antagonist was then stopped and measurements were made during a 5-day recovery period. Mean arterial pressure, heart rate, urine volume, sodium, potassium, and urea nitrogen concentration, and food and water intake were recorded daily. Blood samples (1.5 mL) were collected once during the control period, on day 9 of ET-A antagonism and 4 days after stopping ET-A antagonism for assessment of glomerular filtration rate, plasma renin activity (PRA), and plasma insulin and leptin concentrations. The blood was replaced with an equal volume of 0.9% saline. At the end of the experiment, rats were euthanized with a lethal dose of pentobarbital and the retroperitoneal, perirenal, and perirenal fat pads were excised and weighed for determination of visceral fat.

Analytical Methods

PRA, plasma insulin, and plasma leptin concentrations were determined by radioimmunoassay as previously described.15 Plasma glucose concentration was measured using the glucose oxidation method (Beckman glucose analyzer 2). Urine sodium and potassium concentrations were measured using ion-sensitive electrodes (NOVA electrolyte analyzer 1). Glomerular filtration rate was calculated from the clearance of [125I]iothalamate (Questcor Pharmaceutical, Union City, Calif) after 24-hour infusion, as previously described.12

Statistical Analysis

The data are expressed as mean ± SEM. Comparisons between rats fed a NF or HF diet were performed using unpaired t tests. Data obtained on a daily basis were analyzed by ANOVA with repeated measures. Statistical significance was accepted at a level of P<0.05.

Results

Effects of HF Diet on Food Intake, Body Weight, Visceral Fat, and Hormones

The two groups of rats started with similar weights. After 12 months in the HF or NF group, there was no significant difference in body weight between the groups, although rats in the HF group were slightly heavier than rats in the NF group (576±25 versus 538±22 grams). Despite the similar total body weight gain, HF diet caused an 80% increase in visceral adiposity and a 3.2-fold increase in plasma leptin levels (Figure 1). Fasting plasma glucose and insulin concentration did not differ significantly between HF and NF groups (Table 2). PRA was not significantly different in rats fed the HF compared with NF diet (Table 2). Although food intake was higher in NF group compared with HF group (26.6±2.2

*P<0.05 compared with NF.
versus 18.7±1.9 grams per day), the caloric intake was similar (99±6 versus 100±10 kcal per day). The same caloric intake despite different food consumption was caused by the fact that the HF diet contained more calories per gram of food compared with the NF diet (Table 1).

Effects of HF on Mean Arterial Pressure, Heart Rate, and Renal Function
As illustrated in Figure 2, despite no significant difference in total body weight, Sprague-Dawley rats fed the HF diet had significantly higher mean arterial pressure compared with NF diet rats (113±4 versus 98±2 mm Hg, respectively). Heart rate, glomerular filtration rate, and urinary sodium excretion were not significantly different in the HF compared with the NF diet group (Figure 2 and Table 2).

Effects of ET-A Antagonism on Mean Arterial Pressure, Heart Rate, Renal Function, and Hormones
Long-term selective ET-A antagonism for 9 days in rats fed the HF diet reduced mean arterial pressure from 113±4 to an average of 99±3 mm Hg. In rats fed the NF diet, arterial pressure decreased from 98±2 to an average of 84±2 mm Hg during ET-A antagonism. Thus, ET-A antagonism lowered blood pressure by ~14 mm Hg in NF and HF diet rats. ET-A blockade did not cause significant changes in heart rate (Figure 2), glomerular filtration rate, or PRA in rats fed the HF or NF diet. Although we did not observe a marked increase in body weight in rats fed the HF diet for 12 months, compared with rats fed the NF diet, the HF diet caused a greater increase in visceral adiposity. This indicates that in rats, a long-term HF diet may alter fat distribution, favoring accumulation of visceral fat, even in the absence of marked changes in total body weight. Moreover, plasma leptin concentration increased more than 3-fold in rats fed the HF diet. These results suggest that visceral obesity produced by feeding a long-term HF diet may cause hyperleptinemia and hypertension, even in the absence of greater overall weight gain.

Discussion
The most important findings of this study are that a long-term HF diet causes visceral obesity and increased arterial pressure, even in the absence of major changes in total body weight and that activation of ET-A receptors appears to be important in maintaining arterial pressure in rats fed NF as well as HF diets. However, the results from the present study provide no evidence that ET-A stimulation contributes to increased arterial pressure in this model of visceral obesity.

Visceral Obesity and Increased Arterial Pressure in Rats Fed the HF Diet
Although previous studies have reported increased blood pressure in Sprague-Dawley rats fed the HF diet, most of these studies have measured only systolic blood pressure using tail-cuff methods. In the present study, we measured arterial pressure and heart rate 24 hours per day in conscious freely moving rats fed the HF or NF diet. Although we did not observe a marked increase in body weight in rats fed the HF diet for 12 months, compared with rats fed the NF diet, the HF diet caused a greater increase in visceral adiposity. This indicates that in rats, a long-term HF diet may alter fat distribution, favoring accumulation of visceral fat, even in the absence of marked changes in total body weight. Moreover, plasma leptin concentration increased more than 3-fold in rats fed the HF diet. These results suggest that visceral obesity produced by feeding a long-term HF diet may cause hyperleptinemia and hypertension, even in the absence of greater overall weight gain.

The mechanisms by which visceral obesity causes increased arterial pressure are not fully understood. Previous studies have shown that muscle sympathetic nerve activity is elevated in men with higher visceral fat compared with peers with lower abdominal visceral fat. Whether increased visceral obesity causes elevated renal sympathetic activity remains to be determined.

We have also previously suggested that increases in plasma leptin may be important in linking obesity with sympathetic activation and hypertension. In the present study, plasma leptin was increased more than 3-fold in rats fed the HF diet compared with rats fed the NF diet. Although we have previously shown that infusion of exogenous leptin raises blood pressure in rats, the role of endogenous leptin in contributing to obesity-induced sympathetic activation and hypertension remains to be determined.

Our previous studies have also suggested that the renin-angiotensin system may contribute to obesity-induced hyper-

### Table 2. Body Weight, Food Intake, Plasma Hormones, and Urinary Sodium Excretion in Rats Fed a Normal- or High-Fat Diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal-Fat Diet</th>
<th>Control</th>
<th>ABT-627</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>538±22</td>
<td>576±25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>29±2</td>
<td>20±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caloric intake (kcal/d)</td>
<td>107±8</td>
<td>113±5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>83±12</td>
<td>69±12</td>
<td>69±13</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>111±3</td>
<td>109±5</td>
<td>107±4</td>
<td></td>
</tr>
<tr>
<td>UrineV (mmol/d)</td>
<td>2.1±0.1</td>
<td>2.2±0.1</td>
<td>2.1±0.1</td>
<td></td>
</tr>
<tr>
<td>Urinary volume (mL/d)</td>
<td>39±3</td>
<td>40±4</td>
<td>43±5</td>
<td></td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>4.4±0.2</td>
<td>4.4±0.2</td>
<td>4.5±0.3</td>
<td></td>
</tr>
<tr>
<td>PRA (ngAI/mL per hour)</td>
<td>5.8±1.2</td>
<td>5.0±0.7</td>
<td>5.1±0.7</td>
<td></td>
</tr>
</tbody>
</table>

GFR indicates glomerular filtration rate; PRA, plasma renin activity; UnaV, urinary sodium excretion.

*P<0.05 compared with normal fat.
tension. In the present study, there were no significant differences in PRA in rats fed the HF or NF diet. However, normal PRA in the face of increased arterial pressure in rats that were fed the HF diet suggests that PRA may be inappropriately elevated in rats that were fed the HF diet. Whether the renin-angiotensin system contributes to increased arterial pressure in rats that were fed the HF diet, however, will require further investigation.

Role of Endothelin in the Control of Blood Pressure in Rats Fed the HF Diet

Several previous reports have suggested that long-term ET-A blockade has no significant long-term effect on kidney function or arterial pressure regulation in normal rats. These studies, however, have measured systolic blood pressure with the tail-cuff method or have used short-term recordings of intraarterial pressure in restraining cages. As mentioned previously, in this study we measured arterial pressure 24 hours per day and demonstrated that ET-A blockade was effective in lowering arterial pressure, even under normal physiological conditions. These observations indicate that endothelin-1 plays a significant role in the normal regulation of blood pressure in rats. This finding is consistent with previous studies in non-human primates.

Endothelin-1 has been shown to decrease renal plasma flow and glomerular filtration rate through vasoconstriction of the glomerular afferent and efferent arterioles, likely via stimulation of inositol triphosphate and diacylglycerol, leading to increases in intracellular calcium concentration. In the present study, however, ET-A antagonism did not evoke major changes in glomerular filtration rate or sodium excretion. It is possible that the reduction in arterial pressure during ET-A antagonism could have counterbalanced renal vasodilation and a tendency to increase renal plasma flow.

It is important to note that the absence of significant changes in sodium excretion, despite a marked reduction in blood pressure, suggests that long-term ET-A blockade shifted the renal-pressure natriuresis relationship to lower

Figure 2. Effect of 9 days of long-term selective ET-A receptor antagonist (ABT-627 2.5 mg/kg per day IV) on mean arterial pressure and on changes in mean arterial pressure and heart rate that were measured 24 hours per day in rats fed NF (n=6) or HF diet (n=6).
blood pressures. In the absence of improved pressure natriuresis, a decrease in arterial pressure would tend to decrease renal water and sodium excretion.23

Another possible explanation for the improved pressure natriuresis and the decrease in arterial pressure observed with ET-A blockade is stimulation of ET-B receptors caused by higher endothelin-1 levels during ET-A antagonism. There is growing evidence that ET-1, acting on ET-B receptors, is involved in regulating sodium balance under normal physiological conditions. ET-B receptors are highly expressed in the renal medulla,24 and pharmacological blockade of ET-B results in salt-sensitive hypertension.25 In this study, however, the role of ET-B receptors in the regulation of arterial pressure and sodium balance was not addressed. Further studies are necessary to answer this question.

Although ET-1 appears to be important in normal blood pressure regulation, we found no evidence for a greater role in rats with visceral obesity compared with normal rats. It is possible that in this model of visceral obesity, the rats did not have severe endothelial dysfunction and that once endothelial dysfunction is established endothelin may become more important for the maintenance of hypertension. However, the rats in our studies were fed the HF diet for 12 months, which is considerably longer than most previous studies of diet-induced obesity in rats. Further studies are needed to assess the possibility that endothelin contributes to increased blood pressure in models of obesity that are associated with significant endothelial dysfunction.

Perspectives
Our results indicate that a long-term HF diet, lasting 12 months, causes visceral obesity and increased blood pressure even in the absence of overall obesity. Moreover, endothelin-1 appears to be important in regulating arterial pressure under normal physiological conditions as well as in visceral obesity. Although our study provided no evidence that endothelin plays a greater role in blood pressure regulation in this model of visceral obesity, compared with rats fed the NF diet, blockade of endothelin may still prove to be useful in reducing blood pressure and protecting against cardiovascular injury associated with obesity. However, further experimental and clinical studies are needed to test this hypothesis.

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References
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