Abstract—Acute studies suggest that leptin has pressor and depressor actions, including stimulation of sympathetic activity as well as increased release of NO from the vascular endothelium. The goal of this study was to examine the role of NO in modulating the chronic blood pressure, heart rate, and renal responses to hyperleptinemia, comparable to that found in obesity-induced hypertension. Male Sprague-Dawley rats were implanted with arterial and venous catheters, and mean arterial pressure and heart rate were monitored continuously 24 h/d. After a 4-day control period, the rats were infused with isotonic saline vehicle (n=6) or Nω-nitro-L-arginine methyl ester (L-NAME, 10 μg/kg per minute; n=9) to inhibit NO synthesis for 7 days. After 7 days of vehicle or L-NAME administration, leptin was infused intravenously for 7 days at a rate of 0.5 μg/kg per minute, followed by a leptin infusion at 1.0 μg/kg per minute for 7 days, along with vehicle or L-NAME. A 21-day infusion of L-NAME alone (n=6) served as a control for the L-NAME+leptin rats. Although the low dose of leptin alone did not significantly elevate arterial pressure, it raised the heart rate by 18±3 bpm. The higher leptin infusion rate raised arterial pressure from 96±3 to 104±3 mm Hg but did not increase the heart rate further. L-NAME+leptin increased arterial pressure by 40±6 mm Hg and heart rate by 79±19 bpm compared with pretreatment levels. In control L-NAME rats, mean arterial pressure increased by 31±4 mm Hg, whereas the heart rate was not altered significantly compared with pretreatment levels. Neither chronic leptin infusion alone nor L-NAME alone altered the glomerular filtration rate or renal plasma flow significantly, but L-NAME+leptin reduced glomerular filtration rate by 27±11% and renal plasma flow by 47±9%. These results indicate that impaired NO synthesis mildly enhances the chronic renal hemodynamic and hypertensive effects of leptin but markedly amplifies the tachycardia caused by hyperleptinemia. (Hypertension. 2001;37[part 2]:670-676.)

Key Words: hypertension • blood pressure • heart rate • nitric oxide • diet

The discovery of leptin and its effects on the sympathetic nervous system have provided possible links between obesity, sympathetic activation, and hypertension. Circulating leptin, secreted mainly from adipocytes, increases in proportion to body fat and acts on the hypothalamus to regulate energy balance not only by reducing the appetite but also by increasing energy expenditure through sympathetic stimulation.1-3 Several studies have shown that acute infusions of leptin increase sympathetic activity in the kidneys, adrenal glands, and brown adipose tissue, and these effects have been suggested to be important in blood pressure regulation.4-6 However, leptin administration for 2 to 3 hours usually has little effect on arterial pressure despite an increase in sympathetic activity.4,5 The lack of an acute pressor effect of leptin has been suggested to be due to opposing depressor effects, such as stimulation of endothelium-derived NO,7 which offset the effects of increased sympathetic activity. Supporting this possibility, Fruhbeck8 has demonstrated that the infusion of leptin increases serum NO concentrations and that after the inhibition of NO synthesis, acute leptin infusions significantly increase arterial pressure.

We have previously shown that infusions of leptin in nonobese Sprague-Dawley rats cause a slow rise in arterial pressure and heart rate (HR) after 3 to 5 days, despite a reduction in food intake.8 Moreover, the hypertensive effects of leptin were completely abolished by adrenergic blockade.10 However, the chronic hypertensive effects of leptin are modest, with mean arterial pressure (MAP) increasing by only 8 to 10 mm Hg during 7 days of leptin infusion at a rate that raised plasma concentrations to ~90 to 95 ng/mL. The relatively mild increases in blood pressure during chronic hyperleptinemia could be modulated by increased NO synthesis, which may partly attenuate the hypertensive effects of sympathetic activation. However, the importance of NO in modulating the chronic cardiovascular and renal effects of hyperleptinemia is still unclear.

The present study was therefore designed to determine whether the inhibition of NO synthesis amplifies the chronic blood pressure, HR, and renal responses to hyperleptinemia in normal Sprague-Dawley rats. In addition, because leptin markedly reduces appetite, we also determined the cardiovascular consequences of reducing food intake to the same levels
observed during chronic leptin infusions. And finally, because NO has been suggested to modulate the anorexic effects of leptin, we investigated whether the inhibition of NO synthesis alters the reduction in food intake observed with chronic hyperleptinemia.

Methods

Animal Surgery

The experimental procedures and protocols of these studies conformed to the National Institute 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. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind), weighing 300 to 350 g, were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal), and atropine sulfate (0.37 mg/kg) was administered to prevent excessive airway secretions. Under aseptic conditions, a laparotomy was performed, and a nonocclusive catheter was inserted orthograde into the abdominal aorta, distal to the kidneys, through a puncture made in the aortic wall with an 18-gauge needle tip. The catheter was made from medical grade polyvinyl tubing (BoLab, size V/4, 20 g) with one end formed into an S shape. Into this end was inserted a 5- to 6-mm segment of PE-90 tubing tapered over heat to approximately PE-30 size. The insertion point was sealed with a cyanoacrylic adhesive, and the catheter was exteriorized through the lateral abdominal wall. A femoral vein catheter (BoLab, size V/4, 20 g) was implanted through a separate incision, and the tip was placed into the inferior vena cava distal to the kidneys. All incisions were infiltrated with penicillin G procaine and bupivacaine HCl, and both catheters were routed subcutaneously to the scapular region and exteriorized through a stainless-steel button that was implanted subcutaneously.

After recovery from surgery, the rats were housed in individual metabolic cages in a quiet air-conditioned room with a 12/12 hour light-dark cycle. The arterial and venous catheters were connected to a dual-channel infusion swivel (Instech) mounted above the cage and protected by a stainless-steel spring. The venous catheter was connected, via the swivel, to a syringe pump for continuous infusions, and the arterial catheter was filled with heparin (1000 U/mL) and connected, via the swivel, to a pressure transducer (Maxxim). Pulsatile arterial pressure signals were sent to an analog-digital converter and analyzed by computer with customized software. The analog signal was sampled at 500 samples per second for 4 seconds every minute, 24 h/d, throughout the experiment.

All rats received food and water ad libitum throughout the study, except for 1 group that was used for pair-feeding experiments. Total sodium intake was maintained constant at ~4.8 mmol/d by a continuous infusion of 31 mL/d of 0.9% saline combined with a sodium-deficient rat chow (0.006 mmol sodium/g food, Teklad). All solutions were infused via a sterile filter (22 μm, Millipore), and the saline infusion was started immediately after placement of the rats into their metabolic cages. An acclimation period of 4 to 7 days was allowed before control measurements were recorded.

Experimental Protocols

In group 1 (leptin group, n=6), the rats were infused with 0.9% saline vehicle during a 4-day control period, followed by 7 more days of vehicle infusion and then 7 days of infusion of murine leptin (Ampgen) at a low rate (0.5 μg/kg per minute), 7 days of leptin infusion at a moderate rate (1.0 μg/kg per minute), and then a 5-day vehicle infusion recovery period.

In group 2 (L-NAME+leptin group, n=9) were infused with saline vehicle during a 4-day control period followed by 7 days of L-NAME (10 μg/kg per minute, Sigma Chemical Co) infusion. With the L-NAME infusion continuing, leptin was then infused at 0.5 μg/kg per day for 7 days and then at 1.0 μg/kg per minute for 7 more days; after 14 days of leptin+L-NAME infusion, saline vehicle was infused, and recovery measurements were made for 5 days.

In group 3 (L-NAME group, n=6), the 4-day vehicle infusion control period was followed by 21 days of L-NAME infusion (10 μg/kg per minute); this group served as a time control for the effects of L-NAME in the leptin+L-NAME–treated rats.

In group 4, (pair-fed group, n=5), the protocol was the same as in group 1, except that instead of infusing the rats with leptin, food intake was reduced to the same levels as observed during leptin infusion in group 1. This group served as a control for the effects of decreased food intake caused by the leptin infusion.

MAP, HR, urine volume, urinary sodium excretion, and food and water intake were recorded daily. Blood samples (1.5 mL) were collected on the sixth day of each experimental period during vehicle, leptin, L-NAME+leptin, and L-NAME infusions for measurements of plasma insulin and glucose concentrations, glomerular filtration rate (GFR), and renal plasma flow (RPF). The blood samples were replaced with an equal volume of saline.

Analytical Methods

GFR and RPF were calculated from the clearances of [125I]iothalamate and [131I]iodohippuran, respectively, after a 24-hour infusion of the isotopes as previously described. After 24 hours of isotope infusion, a steady state is reached, and the urinary excretion rate is equal to the infusion rate of the isotopes. Therefore, the infusion rates of the isotopes were substituted for the urinary excretion rates to calculate clearances. Plasma insulin was determined by radioimmunoassay (Diagnostic Products), and plasma glucose was measured with an automatic analyzer by the glucose oxidase method (Beckman). Urinary sodium concentration was determined by use of ion-sensitive electrodes (NOVA).

Statistical Methods

The data are expressed as mean±SE. The data were analyzed by using 2-factor ANOVA with repeated measures and the Scheffé F test for comparison between groups and the Dunnett test for multiple comparisons within groups, when appropriate. Statistical significance was accepted at P<0.05.

Results

Effects of Leptin and L-NAME on Food Intake, Arterial Pressure, and HR

The low rate of leptin infusion (0.5 μg/kg per minute) reduced food intake from 22.0±1.6 to 9.9±0.4 g/d (Figure 1). Increasing the leptin infusion rate to 1.0 μg/kg per minute did not lower food intake further. L-NAME infusion (10 μg/kg per minute) tended to decrease food intake, but the changes were not significant. Leptin administration during L-NAME infusion did not reduce food intake to a greater extent than it did during leptin infusion alone, either at low or moderate infusion rates (Figure 1).

Leptin infusion for 7 days at the low rate did not significantly alter MAP (Figure 2). However, MAP increased from 96±3 to 104±3 mm Hg by day 5 of the moderate leptin infusion rate. Administration of L-NAME alone for 7 days elevated MAP from 95±2 to 126±7 mm Hg. After 21 days of L-NAME infusion, MAP increased further to 157±8 mm Hg. During L-NAME infusion, leptin increased MAP from 118±4 to 159±6 mm Hg by day 5 of the moderate leptin dose. Compared with baseline (7 days of L-NAME), L-NAME infusion for 14 days raised MAP by 31±3 mm Hg, whereas L-NAME+leptin increased MAP by 40±6 mm Hg. In 3 of the rats treated with L-NAME+leptin, MAP increased to 170 to 180 mm Hg, and the rats were unable to complete the protocol. Inspection of the kidneys of these rats suggested severe renal injury, consistent with malignant hypertension. Thus, the data shown in Figure 2 on days 6 and 7 of the higher leptin dose+L-NAME do not
include the very high blood pressures for rats that developed malignant hypertension. Therefore, the effects of L-NAME to amplify the hypertensive effect of leptin may be underestimated on Figure 2. None of the rats treated with L-NAME alone or leptin alone developed malignant hypertension.

Leptin infusion at 0.5 μg/kg per minute elevated the HR from 400±6 to 414±6 bpm, and increasing the leptin dose to 1.0 μg/kg per minute did not increase the HR further (Figure 2). In rats of groups 2 and 3, L-NAME infusion for 7 days decreased the HR by 25 to 40 bpm, but the HR returned to the control value with continued L-NAME treatment for 21 days in group 3. Leptin administration during L-NAME treatment (group 2) raised the HR from 386±7 bpm to a peak of 471±20 bpm by day 4 of the higher leptin infusion rate. Thus, L-NAME treatment markedly amplified the tachycardia caused by leptin infusion, with HR increasing by 65±9 bpm after 7 days of L-NAME+leptin infusion compared with 23±7 bpm after 7 days of leptin infusion alone (Figure 2).

Effects of Leptin and L-NAME on Renal Function
Leptin infusion alone had no significant effects on water drinking or on the urinary excretion of sodium and water. However, urinary potassium excretion decreased from 3.5±0.2 to 2.8±0.2 mmol/d during leptin infusion at the low dose, paralleling the decrease in food intake and, therefore, potassium intake (Table). Doubling the leptin infusion rate did not cause further reductions in urinary potassium excretion. L-NAME infusion alone did not significantly alter drinking or the urinary excretion of sodium, potassium, or water. Leptin administration at the moderate dose during L-NAME treatment significantly increased water intake as well as urine volume and sodium excretion. However, potassium excretion decreased significantly during L-NAME+leptin infusion, paralleling the reduction in food intake and potassium intake.

Leptin had no significant effects on GFR or RPF, either at the low or moderate rates of infusion (Table). L-NAME treatment alone slightly reduced GFR from 2.7±0.3 to 2.2±0.4 mL/min and RPF from 5.9±0.9 to 4.7±1.2 mL/min and increased renal vascular resistance from 14.0±4.6 to 27.0±8.8 mm Hg/mL per minute (Table). In rats infused with L-NAME+leptin, GFR and RPF decreased from 2.6±0.1 to 1.8±0.3 mL/min and from 6.0±0.3 to 3.1±0.6 mL/min, respectively, whereas renal vascular resistance increased from 12.6±1.0 to 32.6±8.6 mm Hg/mL per minute.

Effects of Leptin Infusion on Circulating Hormones
Chronic leptin infusion lowered the plasma insulin concentration from 27.7±5.1 to 3.1±0.9 μU/mL but did not significantly alter the plasma glucose concentration (Table). L-NAME alone did not alter plasma insulin or glucose levels, and L-NAME did not attenuate leptin-induced decreases in plasma insulin levels, which fell from 28.7±5.4 to 4.1±0.9 μU/mL. Neither leptin nor L-NAME alone significantly altered plasma glucose levels. In addition, leptin administration during L-NAME infusion caused no significant changes in the plasma glucose concentration.

Effect of Food Restriction on Arterial Pressure and HR
Sprague-Dawley (pair-fed, group 4) rats were given the same amount of food that the leptin-treated rats had eaten daily. The pair-fed rats showed no significant changes in MAP (Figure 3). However, decreasing the food intake lowered the...
HR from 400±5 to 354±6 bpm (Figure 3). Food restriction also decreased potassium excretion from 3.6±0.3 to 2.6±0.1 mmol/d, in proportion to the decreased intake of potassium. However, food restriction had no significant effect on GFR or urinary sodium excretion; sodium intake did not change significantly because almost all of the sodium intake was provided in the saline vehicle infusion, which was maintained constant in all groups of rats.

**Discussion**

The present study demonstrates that chronic increases in plasma leptin to levels comparable to those found in severe human obesity not only reduce food intake but also increase arterial pressure and HR in normal rats and that these responses are exacerbated by the inhibition of NO synthesis. However, inhibition of NO synthesis did not significantly modify the appetite-suppressing effects of leptin.

**Metabolic and Hormonal Effects of Leptin and Inhibition of NO Synthesis**

Although plasma leptin levels were not measured in these experiments, a previous study from our laboratory indicated that chronic intravenous leptin infusion at a rate of 1.0 μg/kg per minute increases plasma concentrations to ≈90 to 95 ng/mL, levels comparable to those observed in severe human obesity. However, the maximal appetite-suppressing effects in our studies were observed at a leptin infusion rate of 0.5 μg/kg per minute, which would be expected to produce plasma concentrations of 45 to 50 ng/mL.

Decreased neuropeptide Y in the hypothalamus was initially believed to be the primary mediator of the effects of leptin on satiety. However, recent studies indicate that the effects of leptin on food intake are complex and involve multiple mechanisms, including the pro-opiomelanocortin/α-melanocyte-stimulating hormone pathway and NO synthesis in the hypothalamus. For example, injections of leptin decreased NO synthase (NOS) in the hypothalamus while decreasing food intake and body weight. Moreover, the effect of leptin to suppress the appetite was markedly attenuated in neuronal NOS knockout mice. In contrast, decreases in neuronal NOS mRNA in the paraventricular and supraoptic nuclei of rats have also been reported to be associated with fasting, which reduces leptin levels. Therefore, at least part of the decrease in the hypothalamic NOS activity that is associated with increased leptin may be related to decreased food intake rather than direct effects of leptin on the hypothalamus.

In the present study, chronic inhibition of NO synthesis with L-NAME did not markedly alter the appetite and did not significantly influence the reduction in food intake during chronic leptin administration. The dose of L-NAME used in the present study, 10 μg/kg per minute, has been shown to effectively inhibit NO synthesis in multiple tissues throughout the body. In addition, this dose markedly increased arterial pressure. Therefore, it is unlikely that our results are due to ineffective inhibition of NO synthesis. Instead, our results suggest that changes in NO synthesis may not play a key role in mediating the chronic effects of leptin on food intake.

Leptin infusions in these studies markedly reduced plasma insulin concentrations, whereas glucose levels remained relatively constant. This finding is consistent with previous reports that leptin suppresses insulin release from pancreatic β cells. However, the fact that plasma glucose remains constant despite reduced insulin levels suggests that leptin also increases insulin sensitivity in peripheral tissues. In the present study, the inhibition of NO synthesis with L-NAME did not alter plasma insulin or glucose and had no significant effect on the responses to leptin. These observations suggest that increased NO synthesis does not play a major role in mediating the chronic effects of leptin on insulin secretion or insulin sensitivity.

**Hemodynamic Effects of Leptin During Inhibition of NO Synthesis**

Previous studies have shown that intravenous or intracerebroventricular infusions of leptin increase sympathetic activity but usually have minimal effects on arterial pressure. Stimulation of endothelium-derived NO has been suggested to play an important role in attenuating the effects of sympathetic activity on arterial pressure. Supporting this possibility is the finding that leptin infusion increases serum NO concentrations and that after NO synthesis, acute leptin infusion significantly raises arterial pressure. However, there have been no previous studies, to our knowledge, that have examined the importance of NO synthesis in modulating the chronic cardiovascular actions of leptin.

We have previously shown in nonobese rats with intact NO synthesis that chronic infusion of leptin at rates that raise plasma concentrations to ≈94 ng/mL caused a slow but steady rise in arterial pressure of 8 to 10 mm Hg and increased HR. These effects appear to be mediated primarily by activation of the sympathetic nervous system, as much as they are completely abolished by adrenergic blockade. In the present study, we observed a similar increase in HR and blood pressure during chronic infusion of leptin at a rate of 1.0 μg/kg per minute, the same rate of infusion used in our previous studies. Reducing the rate of infusion by 50% caused similar increases in HR but no significant change in arterial pressure. To determine whether stimulation of NO synthesis plays an important role in attenuating the hypertensive actions of leptin, we also investigated the effects of inhibition of NO synthesis with L-NAME. Our results indicate that L-NAME markedly amplified the HR response to leptin but had only a modest effect on the rise in blood pressure in most of the rats. However, in 3 of the rats studied, leptin infusion during impairment of NO synthesis appeared to cause severe malignant hypertension. These findings suggest that NO inhibition may attenuate some of the chronic increases in arterial pressure that are associated with hyperleptinemia. However, in most rats, the effects of inhibition of NO synthesis and leptin on arterial pressure appear to be additive rather than synergistic. One possible explanation for why we did not observe a greater effect of inhibition of NO synthesis on the chronic blood pressure responses to leptin is that we did not effectively inhibit NO synthesis. As discussed above, this seems unlikely, especially because the dose of L-NAME used in the present study markedly raised arterial pressure.
pressure in time-controlled studies. Also, the inhibition of NOS with L-NAME in the present study markedly amplified the tachycardia caused by chronic hyperleptinemia. These data suggest that the dose of L-NAME used in the present study, as in previous studies, was effective in inhibiting NOS.

The mechanisms by which NOS inhibition markedly increased the tachycardia associated with hyperleptinemia were not tested in the present study. However, our previous studies have shown that the increased HR caused by chronic leptin infusion was completely abolished after \( \alpha \)- and \( \beta \)-adrenergic blockade. In fact, after adrenergic blockade, leptin infusion reduced HR as well arterial pressure. These findings suggest that the tachycardia caused by hyperleptinemia is due mainly to increased adrenergic activity. Therefore, it seems likely that the effects of L-NAME to enhance the HR response to leptin may also be mediated by neurogenic mechanisms, although this hypothesis remains to be tested.

Our finding (ie, that increases in plasma leptin to levels similar to those found in obesity raised arterial pressure in nonobese rats) is consistent with the hypothesis that leptin might be an important link between obesity, sympathetic activity, and hypertension. Likewise, a recent study\(^{21}\) demonstrating that transgenic rats with ectopic oversecretion of leptin from the liver have increases in arterial pressure similar to those that we observed with chronic leptin infusion is also consistent with a potential role for leptin in causing hypertension. On the other hand, if obesity is associated with resistance to the effects of leptin on the hypothalamus and therefore with resistance to the effects of leptin on satiety and sympathetic activity, elevated leptin concentrations might cause minimal stimulation of sympathetic activity in obese subjects. In support of this possibility, acute leptin administration has been reported to increase lumbar sympathetic activity in nonobese rats but to have minimal effects in obese rats fed a high fat diet.\(^{4}\) Although this observation is consistent with the concept that obesity induces resistance to the acute effects of leptin on sympathetic activity, other explanations are also possible. For example, basal sympathetic activity is already elevated in obese rats, possibly because of high circulating leptin; therefore, further increases in leptin above physiological levels may not cause greater sympathetic stimulation. This explanation is consistent with the observation that leptin is transported through the blood-brain barrier via a saturable transport system\(^{22}\) and that the infusion of leptin in obese animals may exceed the transport maximum for leptin across this barrier. Therefore, it is still unclear

<table>
<thead>
<tr>
<th>Group</th>
<th>GFR, mL/min</th>
<th>RPF, mL/min</th>
<th>RVR, mm Hg - mL (^{-1} \cdot \text{min}^{-1} )</th>
<th>( \text{U}_N\text{aV}, \text{mmol/d} )</th>
<th>( \text{U}_V, \text{mmol/d} )</th>
<th>Urine Volume, mL/d</th>
</tr>
</thead>
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<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.3±0.5</td>
<td>7.1±0.9</td>
<td>9.4±1.4</td>
<td>3.9±0.2</td>
<td>3.5±0.2</td>
<td>27±2</td>
</tr>
<tr>
<td>Leptin (0.5 ( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} ))</td>
<td>3.3±0.4</td>
<td>7.2±0.7</td>
<td>8.6±0.9</td>
<td>3.5±0.4</td>
<td>2.8±0.2</td>
<td>28±3</td>
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<tr>
<td>Leptin (1.0 ( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} ))</td>
<td>3.4±0.3</td>
<td>7.4±0.7</td>
<td>8.3±0.9</td>
<td>3.9±0.3</td>
<td>2.6±0.2</td>
<td>32±4</td>
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<td></td>
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<td></td>
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<tr>
<td>L-NAME</td>
<td>2.6±0.1</td>
<td>5.9±0.3</td>
<td>12.8±1.2</td>
<td>4.0±0.2</td>
<td>3.2±0.2</td>
<td>32±2</td>
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<tr>
<td>L-NAME+leptin (0.5 ( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} ))</td>
<td>2.5±0.2</td>
<td>4.5±0.5</td>
<td>18.4±2.4</td>
<td>4.0±0.2</td>
<td>2.4±0.1</td>
<td>35±3</td>
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<tr>
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<td>1.8±0.3</td>
<td>3.1±0.6(^*)</td>
<td>32.6±8.6(^*)</td>
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<td>2.6±0.2</td>
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<td>27.0±8.8</td>
<td>3.6±0.4</td>
<td>2.8±0.3</td>
<td>33±4</td>
</tr>
</tbody>
</table>

Values are mean±SE. RVR indicates renal vascular resistance; \( \text{U}_N\text{aV} \), urinary sodium excretion; and \( \text{U}_V \), urinary potassium excretion. \(^*P<0.05\) compared with control.

Figure 3. Effect of food restriction (pair feeding) on MAP and HR in conscious normal Sprague-Dawley rats (n=5).
whether diet-induced obesity attenuates the sympathetic responses to endogenous leptin.

To the extent that obesity causes endothelial dysfunction and impaired NO release, one might expect greater blood pressure responses to hyperleptinemia in obese than in lean persons, particularly if obesity does not induce resistance to the sympathetic effects of leptin. Thus, the net effect of leptin on blood pressure and obesity may depend on the degree of endothelial dysfunction as well as the degree of resistance in the hypothalamus to the sympathoexcitatory effects of leptin. Additional studies will be required to test the interaction among these factors in linking obesity with hyperleptinemia and hypertension.

**Effects of Food Restriction on Hemodynamics**

Because leptin suppresses appetite and causes weight loss, we also investigated the effects of reducing food intake, per se, in the absence of hyperleptinemia. Our studies demonstrate that lean Sprague-Dawley rats that were pair-fed the same amount of food eaten by the leptin-treated rats had a significant reduction in HR but no changes in arterial blood pressure. These observations indicate that the tachycardia and hypertension induced by leptin cannot be attributed to changes in food intake. In fact, reduction in food intake may, in part, offset some of the tachycardia associated with hyperleptinemia. This observation may have implications for the quantitative importance of leptin in stimulating sympathetic activity and tachycardia in obesity, in which hyperleptinemia is associated with increased appetite and weight gain instead of decreased appetite and weight loss. Thus, the importance of leptin in causing tachycardia and increased blood pressure may be underestimated by our studies in lean rats.

**Renal Effects of Leptin During Inhibition of NO Synthesis**

Previous acute studies have shown that the injection or infusion of large amounts of leptin may cause natriuresis and diuresis. However, in the present study, we found no significant changes in sodium excretion or urine volume during chronic infusions of leptin at rates that elevated plasma concentrations to levels similar to those found in severe obesity. Leptin infusions did significantly reduce the urinary excretion of potassium, but it seems likely that this was mainly due to a reduction in the potassium intake associated with decreased appetite. Sodium intake in the present study was held relatively constant by continuous intravenous infusion of most of the daily intake of sodium, whereas all of the potassium ingested was derived from the food.

The absence of a significant change in sodium excretion in the present study does not necessarily indicate that leptin has no effect on renal sodium handling. In fact, leptin infusion caused an increase in renal vascular resistance, especially after the inhibition of NO synthesis. Moreover, the observation that sodium excretion remained unchanged in spite of an increase in arterial pressure indicates that leptin also shifted the renal pressure–natriuresis relationship to higher blood pressures. In the absence of altered pressure natriuresis, increased arterial pressure would tend to raise urinary sodium and water excretion. This effect of leptin to shift pressure natriuresis appears to be mainly due to sympathetic stimulation, inasmuch as it is completely abolished by adrenergic blockade.

The effects of leptin on the kidney appear to be modulated, in part, by NO synthesis. After blockade of NO synthesis, leptin caused a greater decrease in RPF and a further shift of pressure natriuresis, as evidenced by the fact that sodium excretion remained constant in spite of a greater increase in arterial pressure. Thus, impairment of NO synthesis appears to exacerbate the effects of leptin to shift renal pressure natriuresis toward higher blood pressures.

In summary, our results indicate that pathophysiological increases in plasma leptin to levels similar to those found in severe human obesity causes relatively small increases in arterial pressure, HR, and renal vascular resistance as long as NO synthesis remains intact. However, after inhibition of NO synthesis with L-NAME, these effects are exacerbated, although L-NAME was observed to have no significant effects on the appetite-suppressing effect of leptin. These findings may have important implications for obesity hypertension, which is often associated with endothelial dysfunction and impaired NO synthesis.

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