Increased circulating leptin, the peptide product of the "obese gene," has been suggested to contribute to increased blood pressure in obesity by activation of the sympathetic nervous system. Leptin production is increased in obese animals and humans and correlates positively with body mass index. Because leptin binding sites have been found in regions of the brain that are also important in cardiovascular control, there is reason to suspect that leptin could affect cardiovascular function through its CNS effects. Supporting this possibility is the observation by Haynes and colleagues, who recently demonstrated that acute intravenous infusion of leptin increased sympathetic nerve activity to the kidney, the adrenal, and the brown adipose tissue in anesthetized Sprague-Dawley rats. However, leptin has also been shown to cause nausea and diuresis following bolus IV infusion. Thus, leptin may have multiple actions on the SNS and the kidney that, in some circumstances, could have opposite effects on arterial pressure regulation. Moreover, it is not known which of these effects, if any, can be sustained and exert long-term effects on cardiovascular and renal function.

The aim of this study, therefore, was to determine whether increases in circulating leptin, to levels similar to those found in obesity, cause sustained changes in cardiovascular, renal, and neurohumoral function. Furthermore, we investigated the role of CNS versus systemic mechanisms by comparing the chronic effects of carotid artery and IV leptin infusion on control of arterial pressure and renal function.

**Methods**

**Animal Surgery**

Male Sprague-Dawley rats (n=10) weighing approximately 350 to 400 g were used in this study. The protocol was approved by the Institutional Animal Care and Use Committee of University of Mississippi Medical Center and conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Under pentobarbital sodium (30 mg/kg, intraperitoneally), anesthesia and aseptic conditions, a laparotomy was performed, and a nonocclusive polyvinyl catheter was inserted into the abdominal aorta, distal to the kidneys, through a puncture made with a 20-gauge needle tip. The insertion point was sealed with cyanoacrylate adhesive, and the catheter was exteriorized through the lateral abdominal wall. In the carotid artery infusion group (n=5), nonocclusive polyvinyl catheters were placed orthograde in both common carotid arteries via a midventral neck incision for continuous intracarotid infusion of vehicle or leptin into the cerebral circulation. In the rat, the carotid arteries provide most of the blood supply to the hypothalamus and
vasomotor centers of the medulla? The insertion points were sealed
closed with cyanoacrylate adhesive. In the systemic infusion group (n=5), a
venous catheter was inserted into the vena cava via the left femoral
vein for continuous IV infusion of vehicle or leptin. All incisions were
infiltrated with penicillin G procaine and Senosacaine, and all catheters
were routed subcutaneously to the scapular region and exteriorized
through a polypropylene button that was implanted subcutaneously.

Rats were allowed to recover from the surgery and were housed in
individual metabolic cages in a quiet, air-conditioned room with a
12:12 hour light/dark cycle. In the CNS infusion group, the carotid
artery catheters were cannulated to a syringe pump (Harvard Appara-
tus) via a dual-channel hydraulic swivel (Instech) mounted above the
cage for continuous 24-hour infusion. The aortic catheter was filled
with heparin solution (1000 USP U/mL) and plugged. For arterial
pressure measurement, the aortic catheter was flushed and connected
to a pressure transducer (Cobe) mounted on the cage exterior at the
level of the rat. Pulatile arterial pressure signals were sent to an
analog-to-digital converter and analyzed by computer using custom-
ized software. The analog signal was sampled for 4 seconds every 15
seconds from 7 AM to 1 PM daily. In the IV-infused group, the
venous catheter was connected to a syringe pump via one port of the
hydraulic swivel for continuous 24-hour infusion, and the aortic
atherosclerosis was connected to the transducer via the other port.

The rats received food and water ad libitum throughout the study.
Sodium intake was maintained constant at approximately 3.0 mEq/d
by continuous infusion of 20 mL of 0.9% saline/d, combined with
feeding a sodium-deficient rat chow. Additionally, 21 mL of sterile
water/d was infused as a vehicle for the leptin infusion during the
experimental period, yielding a total volume of 41 mL/d. This
infusion began immediately after placement of rats in their metabolic
cages. A minimum of 5 to 7 days were allowed for acclimatization before
control measurements were made. All solutions contained antibiotic
(penicillin G potassium 30,000 U/d and metilcillin 27 mg/d) and
were infused through a Millipore filter (22 μm, Cathwex, Millipore).

### Experimental Protocol

After 7 days of control measurements, murine leptin (Amgen Inc.) was
infused into the carotid arteries or femoral vein at 0.1 μg/kg/min for
5 days and at 1 μg/kg/min for 7 days, followed by a 7-day recovery
period. Mean arterial pressure (MAP), heart rate (HR), urine volume,
natrium excretion, and food and water intake were recorded daily.
Blood samples (2 mL) were collected on the fifth day of the
control, leptin infusion, and recovery periods for the measurement of
plasma insulin, leptin, aldosterone, corticosterone and protein con-
centration, blood glucose, hematocrit, PRA, GFR, and RPF. The
blood sample was replaced with an equal volume of saline.

### Analytical Methods

PRA was measured by radioimmunoassay using 125I-angiotensin I
from New England Nuclear and antibody from Axiol. Plasma insulin,
alderosterone, and corticosterone concentrations were measured by
radioimmunoassay (Diagnostic Products). Plasma leptin concentration
was measured by enzyme immunoassay (Amgen Inc.). Plasma protein
concentration was measured by refractometry (American Optical).
Urine sodium concentration was determined by using ion-sensitive
electrodes (Nova). Blood glucose was measured using an Accu-
Check III blood glucose monitor (Boehringer Mannheim Corpora-
tion). GFR and RPF were measured by using a 4-hour fasted plasma
sample following a 24-hour IV infusion of 125I-thalamosite (Glofil) and
131I-iodobenzonitrile, respectively. Steady state is achieved after 24 hours
of IV isotope infusion in this protocol, therefore, urinary excretion
rate is equal to infusion rate of the isotope, and the infusion rate of
isotope can be substituted for urinary excretion rate to calculate clearance.

### Statistical Analysis

All data are expressed as mean±SE. Data were analyzed by a
two-factor ANOVA with repeated measures and Dunnett’s test for
multiple comparisons, when appropriate. Statistical significance was
accepted at P<.05.

### Results

#### Effects of Leptin Infusion on Food Intake, Arterial Pressure, and Heart Rate

Food intake did not change significantly during leptin infusion
at 0.1 μg/kg/min. However, at the higher infusion rate (1
μg/kg/min), food intake decreased markedly from 204±0.7
g/d to 71±0.5 g/d (Fig 1). Similar to the effect of carotid
artery infusion, chronic IV infusion of leptin did not affect
food intake at the low dose but markedly decreased food intake
from 20.4±0.2 g/d to 6.2±0.5 g/d at 1 μg/kg/min (Fig 1).
Food intake in both carotid artery and IV infusion groups
returned to control 2 days after leptin infusion was stopped.

MAP increased slightly by day 5 of carotid artery leptin
infusion at 0.1 μg/kg/min, but the increase was not statistically
significant (Fig 2). At the 0.1 μg/kg/min dose, however, MAP
increased significantly, to 94±2 mm Hg by the fifth day.
Leptin infusion at 0.1 μg/kg/min IV did not change MAP but,
at the higher dose, raised MAP significantly from
87±1 mm Hg to 93±1 mm Hg (Fig 2). Arterial pressure

<table>
<thead>
<tr>
<th>Selected Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS = central nervous system</td>
</tr>
<tr>
<td>GFR = glomerular filtration rate</td>
</tr>
<tr>
<td>HR = heart rate</td>
</tr>
<tr>
<td>MAP = mean arterial pressure</td>
</tr>
<tr>
<td>PRA = plasma renin activity</td>
</tr>
<tr>
<td>RPF = renal plasma flow</td>
</tr>
<tr>
<td>RVR = renal vascular resistance</td>
</tr>
<tr>
<td>SNS = sympathetic nervous system</td>
</tr>
</tbody>
</table>

Rats were fed a standard diet throughout the study.
Shek et al. 411

MAP (mmHg)

- Carotid artery infusion
- Intravenous infusion

Figure 2. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 μg/kg/min (5 days) and 1.0 μg/kg/min (7 days) on mean arterial pressure in conscious normal SD rats (n=5 per group). *P<.05 compared to control.

decreased rapidly to levels below control levels after leptin infusion was stopped in both carotid artery and IV infusion groups.

Chronic carotid artery infusion of leptin at 0.1 μg/kg/min did not change heart rate significantly, but at the 1 μg/kg/min dose, HR increased from 323±4 beats/min to 355±5 beats/min by day 7 of the infusion (Fig 3). Similar to the effect of carotid artery infusion, chronic IV infusion of leptin did not affect HR at the low dose, but heart rate increased significantly at the high dose, from 305±9 beats/min during the control period to 322±10 beats/min by day 7 of the leptin infusion period (Fig 3). HR decreased rapidly in both carotid artery and IV infusion groups after leptin infusion was stopped and was significantly below control (302±5 beats/min and 284±5 beats/min, respectively) by day 3 of the recovery period.

Effects of Leptin Infusion on Renal Function

Although there was a tendency for urine volume to increase during leptin infusion at 1 μg/kg/min in both carotid artery and IV infusion groups, the increase was not statistically significant (Fig 4). There was also no significant change in urinary sodium excretion in either group during leptin infusion at 0.1 or 1 μg/kg/min (Fig 5). GFR, RPF, and RVR did not change during

Figure 3. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 μg/kg/min (6 days) and 1.0 μg/kg/min (7 days) on heart rate in conscious normal SD rats (n=5 per group). *P<.05 compared to control.

Figure 4. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 μg/kg/min (5 days) and 1.0 μg/kg/min (7 days) on urine volume in conscious normal SD rats (n=5 per group). *P<.05 compared to control.

Figure 5. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 μg/kg/min (6 days) and 1.0 μg/kg/min (7 days) on urinary sodium excretion in conscious normal SD rats (n=5 per group). *P<.05 compared to control.
Leptin-Induced Hypertension

Figure 6. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 μg/kg/min (5 days) and 1.0 μg/kg/min (7 days) on glomerular filtration rate (GFR), renal plasma flow (RPF), and renal vascular resistance (RVR) in conscious normal SD rats (n=5 per group). Data are expressed as percent control.*P < 0.05 compared to control.

Table 1. Effects of Chronic Bilateral Carotid Artery Infusion of Leptin at 0.1 and 1.0 μg/kg/min on Plasma Leptin, Fasting Insulin (Ins), Fasting Glucose (Glu), Plasma Renin Activity (PRA), Plasma Aldosterone (Aldo), and Plasma Corticosterone (Cort).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leptin (ng/mL)</th>
<th>Ins (μU/mL)</th>
<th>Glu (mg/dL)</th>
<th>PRA (ng AI/mL/h)</th>
<th>Aldo (ng/dL)</th>
<th>Cort (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.2±0.4</td>
<td>41.2±10.5</td>
<td>104.4±22.9</td>
<td>31±0.4</td>
<td>40.7±10.8</td>
<td>131±43.1</td>
</tr>
<tr>
<td>Leptin 0.1</td>
<td>1.4±0.5</td>
<td>40.2±6.7</td>
<td>106.2±22.2</td>
<td>36±0.5</td>
<td>35.5±43</td>
<td>94.5±12.6</td>
</tr>
<tr>
<td>Leptin 1.0</td>
<td>91.0±4.8*</td>
<td>72±1.3*</td>
<td>93±4.4*</td>
<td>37±0.3</td>
<td>25.1±49</td>
<td>87.3±28.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.8±0.2</td>
<td>42.2±10.4</td>
<td>109.2±3.5</td>
<td>32±0.6</td>
<td>31.4±5.9</td>
<td>105.8±33.6</td>
</tr>
</tbody>
</table>

Values are mean±SE, n=5 rats.
*P<0.05 compared with control, leptin (0.1 μg/kg/min), and recovery.

Discussion

The most significant finding of this study is that chronic leptin infusion, at rates that increased plasma leptin concentrations to levels comparable to those found in obese humans, markedly reduced food intake and elevated arterial pressure and heart rate in normal rats. The tendency for arterial pressure to increase only in the carotid artery infusion group at the low dose is consistent with the possibility that the pressor effect of leptin may have a CNS component. However, the higher infusion rate produced similar increases in both plasma levels of leptin and arterial pressure.

Humoral and Metabolic Actions of Leptin

Food intake did not change during carotid artery or IV leptin infusion at 0.1 μg/kg/min but was decreased significantly at 1 μg/kg/min in both groups. These observations are consistent with previous studies that chronic injection of leptin in ob/ob mice, animals with deficient leptin production, significantly reduces food intake. The mechanism for leptin's effect to reduce food intake has been suggested to be suppression of hypothalamic NPY mRNA expression and NPY release in the present study, increased circulating leptin concentration, to levels comparable to those found in obese humans, markedly reduced food intake. These observations provide further support for the concept that leptin is an important physiological regulator of energy balance.

Increased circulating leptin also markedly decreased plasma insulin concentration and modestly reduced blood glucose. Although the mechanisms responsible for leptin's effects on plasma insulin and glucose levels are not clear, a recent study by Kieffer et al suggests that there are leptin receptors located on the pancreatic β-cells, although the function of these receptors...
is not known. Leptin may also decrease insulin release by stimulating $\alpha$-adrenergic receptors in the pancreas via its effect on sympathetic activity. However, the finding that leptin decreased plasma glucose suggests additional effects besides a simple inhibition of insulin secretion. Another possibility is that leptin increased glucose utilization or improved insulin sensitivity in peripheral tissues, which enhanced glucose disposal in skeletal muscle and fat cells and suppressed glucose output by the liver. Thus, the decrease in plasma insulin could be a compensatory response to a fall in plasma glucose. Additional studies are needed, however, to determine how leptin affects glucose control and insulin secretion.

Leptin, at the higher dose, did not alter plasma renin activity, but corticosterone and aldosterone tended to decrease, although not significantly. The reduced plasma aldosterone levels may be related to reduced potassium intake associated with reduced food consumption.

**Arterial Pressure and Heart Rate Responses to Leptin**

Previous studies have reported that arterial pressure and heart rate were not changed by acute leptin infusion. One possible explanation is that arterial pressure and heart rate were measured under anesthesia in those studies, whereas our studies were conducted in conscious rats. In addition, leptin was administered acutely in previous studies, either by a single bolus injection or by short-term infusion. Our results are consistent with those findings, because the increases in arterial pressure and heart rate were not apparent until 3 to 4 days after the leptin infusion was started. This was true even for the low infusion rate, which appeared to have a small centrally mediated effect on arterial pressure, since there was no significant change in systemic plasma leptin concentration and IV leptin infusion at this rate caused no changes in heart rate and arterial pressure. Thus, both the arterial pressure and heart rate responses to physiological increases in circulating leptin levels appear to be slow in onset.

The mechanisms by which increased circulating levels of leptin elevate arterial pressure and heart rate chronically are not entirely clear. One possible explanation is activation of the sympathetic nervous system. The finding that animals with deficient leptin production (ob/ob mice) or defective leptin receptors (db/db mice) also have decreased metabolic activity and hypothermia is consistent with the concept that leptin interacts with hypothalamic receptors to reduce food intake and activate the SNS. Furthermore, leptin has been shown to increase norepinephrine turnover in interscapular brown adipose tissue, and a recent study by Haynes and colleagues reported that acute IV infusion of leptin in rats increased sympathetic nerve activity in the adrenals, brown adipose tissue, and the kidneys.

Our finding that there was a tendency for arterial pressure to increase with low-dose carotid artery infusion suggests a possible CNS mechanism, but our studies cannot determine the precise role of CNS compared to systemic actions of leptin in chronic blood pressure regulation. Further studies are needed to determine the role of the CNS and peripheral effects of leptin in long-term blood pressure regulation.

Heart rate also increased significantly with chronic carotid artery and IV leptin infusion at 1 $\mu$g/kg/min. Although leptin receptor mRNA has been shown to be expressed in the heart, the physiological role of this receptor remains to be determined. Leptin could increase heart rate by increasing cardiac sympathetic activity or by withdrawal of parasympathetic tone. A recent study in our laboratory, for example, demonstrated that the increased heart rate associated with 5 weeks of a high-fat diet in dogs was due mainly to decreased cholinergic activity. However, additional studies are required to determine the contribution of sympathetic and parasympathetic mechanisms in mediating leptin’s effect on heart rate.

**Renal Effects of Leptin**

Previous acute studies have shown that infusion of leptin at high doses caused natremia and diuresis. For example, Jackson and Le reported that intravenous leptin injection at 30 $\mu$g/min caused a twofold increase in urine volume and sodium excretion in normal rats. Reams et al also reported that bolus IV injection of leptin at 400 $\mu$g/kg significantly increased urine volume and sodium excretion in normotensive Sprague-Dawley rats, although natremia was not observed in spontaneously hypertensive rats.

In the present study, we found no significant changes in sodium excretion or urine volume during chronic infusion of leptin at a rate that produced physiological increases in plasma leptin concentration. However, the absence of significant changes in sodium excretion in our study does not necessarily imply that leptin had no effect on renal function. In fact, IV leptin infusion significantly decreased RPF and increased RVR. Moreover, the observation that leptin did not increase sodium excretion, despite raising arterial pressure, provides evidence that leptin, infused IV or via the carotid arteries, shifted the renal-pressure natriuresis relationship to higher

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leptin (ng/mL)</th>
<th>Ins (mU/mL)</th>
<th>Glu (mg/dL)</th>
<th>PRA (ng Al/mL/h)</th>
<th>Aldo (ng/dL)</th>
<th>Cort (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9 ± 0.1</td>
<td>52.6 ± 10.2</td>
<td>113.8 ± 35</td>
<td>3.8 ± 0.5</td>
<td>45.8 ± 6.5</td>
<td>137.6 ± 20.2</td>
</tr>
<tr>
<td>Leptin (0.1 $\mu$g/kg/min)</td>
<td>2.3 ± 0.7</td>
<td>37.4 ± 10.9</td>
<td>114.2 ± 2.4</td>
<td>3.6 ± 0.6</td>
<td>36.9 ± 5.9</td>
<td>118.0 ± 35.8</td>
</tr>
<tr>
<td>Leptin (1.0 $\mu$g/kg/min)</td>
<td>94.0 ± 0.9*</td>
<td>10 ± 2.3*</td>
<td>103.4 ± 6*</td>
<td>3.4 ± 0.4</td>
<td>23.8 ± 7.7</td>
<td>106.6 ± 38.7</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.7 ± 0.1</td>
<td>53.8 ± 15.8</td>
<td>114.0 ± 3.3</td>
<td>4.0 ± 0.3</td>
<td>35.4 ± 10.4</td>
<td>116.2 ± 26.8</td>
</tr>
</tbody>
</table>

*Values are mean ± SE, n=5 rats
*P<0.05 compared with control, leptin (0.1 $\mu$g/kg/min), and recovery.
blood pressures. In the absence of an impaired pressure natriuresis, increased arterial pressure would tend to increase renal sodium and water excretion. Whether this effect of leptin to shift pressure natriuresis is due to direct renal action or to other effects, such as sympathetic stimulation, is still unclear.

In summary, our studies demonstrated that leptin, at physiological concentration, markedly decreased food intake and plasma insulin, supporting the concept that leptin plays an important role in regulating energy balance. Our studies also indicate that increases in circulating leptin concentration, to levels similar to those found in obesity, significantly elevate arterial pressure and heart rate, findings suggesting a possible role for leptin in obesity hypertension. Further studies are needed, however, to determine the precise role of the CNS and peripheral effects of leptin in long-term blood pressure regulation in obesity.

Acknowledgments
We thank Dr. Mans J. Smith, Jr. for the radioimmunoassays and Dr. Margery Nicolson (Amgen Inc.) for the enzyme immunoassay used in these experiments. We also thank Amgen Inc. for supplying the murine leptin.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-51971.

References
Chronic Leptin Infusion Increases Arterial Pressure
Eugene W. Shek, Michael W. Brands and John E. Hall

*Hypertension*. 1998;31:409-414
doi: 10.1161/01.HYP.31.1.409

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/1/409

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org/subscriptions/