Impact of Leptin-Mediated Sympatho-Activation on Cardiovascular Function in Obese Mice

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Abstract—Although the anorexic effects of leptin are lost in obesity, leptin-mediated sympatho-activation is preserved. The cardiovascular consequences of leptin-mediated sympatho-activation in obesity are poorly understood. We tested the hypothesis that 32 weeks of high-fat diet (HFD) induces metabolic leptin resistance but preserves leptin-mediated sympatho-activation of the cardiovascular system. HFD in mice significantly increased body weight and plasma leptin concentrations but significantly reduced the anorexic effects of leptin.  HFD increased heart rate, stroke volume, cardiac output, and plasma aldosterone levels but not blood pressure. As reflected by the contractile response to phenylephrine measured both in vivo and ex vivo, vascular adrenergic reactivity was reduced by HFD, suggesting that reductions in sympathetic tone to the periphery vasculature may mitigate sympatho-activation of the heart and the renin-angiotensin-aldosterone system. Tachyphylaxis was partially restored by symptho-inhibition and not present in ob/ob and db/db mice, despite obesity, arguing for a sympatho-mediated and leptin-specific mechanism. Although infusion of leptin in HFD mice had no effect on heart rate or blood pressure, it further increased aldosterone levels and further reduced vascular adrenergic tone in the absence of weight loss, indicating persistent leptin-mediated stimulation of the cardiovascular system in obesity. In conclusion, these data indicate that, despite metabolic leptin resistance, leptin-mediated stimulation of the heart, the vasculature, and aldosterone production persists in obesity. Blood pressure effects in response to leptin may be limited by a tachyphylactic response in the circulation, suggesting that failure of adrenergic desensitization may be a requisite step for hypertension in the context of obesity. (Hypertension. 2011;58:271-279.) • Online Data Supplement

Key Words: blood pressure ■ adrenergic reactivity ■ cardiac output ■ aldosterone ■ high fat diet

Obesity is a metabolic disorder associated with excess body weight affecting 30% to 60% of the American population.1-3 Although substantial evidence shows that obesity is a major risk factor for the development of hypertension,1,2 the relationship between body weight and blood pressure (BP) is complex and incompletely understood. Indeed, although many obese patients develop hypertension,4 40% to 60% of them do not.5-8 Sympatho-activation is thought to be a major component of obesity-induced hypertension.9-11 It remains to be fully determined.

Leptin is an adipocyte-derived cytokine, the concentration of which increases proportionally to the level of adiposity.12,13 Leptin is involved in the control of the metabolism, communicating repletion of body energy stores to the central nervous system to suppress food intake and permit energy expenditure.12 Despite high circulating levels, leptin fails to promote weight loss in obesity, a state defined as leptin resistance.14 Other than its metabolic actions, leptin also stimulates the cardiovascular system,15-19 increasing sympathetic outflow via renal, lumbar, and adrenal sympathetic nerves.20,21 Recent studies of leptin action16,22 describe a phenomenon of “selective leptin resistance” in which leptin-mediated stimulation of brown adipose tissue innervation is lost with obesity, but leptin-mediated activation of renal nerve activity is preserved. Although nerve and BP responses to pharmacological infusion of leptin are well documented,22-26 the response of specific target organs in the cardiovascular system to physiological and pathophysiological levels of leptin remains to be clearly defined. Recent studies from our laboratory have indicated that leptin stimulation results in a sympathetically mediated stimulation of the vasculature, as indicated by an adaptive downregulation of adrenergic vasoconstriction and \( \alpha_1 \)-adrenergic receptor mRNA levels.15 The extent to which selective leptin resistance impacts sympathetic targets in the cardiovascular system is unknown.

A major gap in our knowledge is how leptin-mediated stimulation of the cardiovascular system is affected by obesity and whether there are adaptive responses that parallel selective leptin resistance. The goal of the current study was to test the hypothesis that leptin-stimulated activation of the cardiovascular system was preserved in obesity. To test this hypothesis, obesity was generated in C57Bl/6 mice using 32 weeks of a high-fat diet.
diet. Metabolic function was assessed in terms of metabolic profiling and body weight changes in response to leptin. Cardiovascular responses to obesity were examined in parallel, including cardiac function, vascular reactivity, adrenergic receptor expression, aldosterone production, and BP. The goal of these studies was to determine the extent to which leptin-mediated stimulation of cardiovascular target organs contribute to the regulation of BP in obesity.

Methods

Animals
Male C57Bl/6 mice (8 weeks of age, Jackson Laboratory, Bar Harbor, ME) were divided in 2 groups and fed either a control diet (CD; 4% of fat calories) or a high-fat diet (HFD; 60% of fat calories from lard, Diet F3282, Bioserve, Frenchtown, NJ) ad libitum, as described previously.27 Mice were monitored for the following 32 weeks, and body weight was measured weekly. After 32 weeks, plasma concentrations of glucose, insulin, triglycerides, and leptin of fasted animals were measured following the procedures described previously.15 In a separate set of experiments, leptin-deficient (ob/ob), and leptin receptor–deficient mice (db/db) were purchased from the Jackson Laboratory. Mice were housed in an American Association of Laboratory Animal Care–approved animal care facility at Georgia Health Sciences University, and the Institutional Animal Care and Use Committee approved all of the protocols.

Leptin Sensitivity

To determine the effects of a 32-week HFD on metabolic and cardiovascular sensitivity to leptin, CD and HFD mice were submitted to a 7-day treatment of subcutaneous infusion of leptin at the doses of 10 and 40 µg/d (ALZET, Cupertino, CA; model 1007D, 0.5 µL/h),13 based on the fact that obesity does not increase blood volume in rodents.28

In Vivo Vascular Adrenergic Reactivity

In a separate set of mice, the carotid artery and jugular vein were catheterized under isoflurane anesthesia (1.5%) for the measurement of mean arterial pressure (MAP) and drug delivery, respectively. To eliminate endogenous sympathetic vasomotor tone and baroreceptor-reflex–mediated responses, animals were given the ganglionic blocker mecamylamine (2 mg/kg IV). Effective blockade was confirmed by the absence of reflex bradycardia after vasoconstrictor administration. Changes in MAP were determined after injection of randomized boluses of phenylephrine (PE; 0.01 to 1.00 mg/kg).15

Ex Vivo Aortic Reactivity

Thoracic aortas were dissected surgically, cleaned of fat, and mounted on a wire myograph (DMT, Aarhus, Denmark) with 1 g of basal tension. Briefly, 2 tungsten wires were inserted into the lumen of the arteries and fixed to a force transducer and a micrometer. Arteries were bathed in a physiological salt solution, as described previously.29 Arterial viability was determined with a potassium-rich solution (40 mmol/L). Vascular contractility was assessed with cumulative concentration-response curves to PE (1 nmol/L to 1000 µmol/L) and serotonin (5-hydroxytryptamine [5HT]; 1 µmol/L to 10 µmol/L). Constriction to PE and 5HT were expressed as a percentage of KCl-induced constriction. Endothelial function was analyzed with concentration-response curves to acetylcholine (1 nmol/L to 10 µmol/L). Endothelium-independent relaxation was analyzed with a concentration-response curve to sodium nitroprusside (1 nmol/L to 10 µmol/L). Relaxation curves were performed on preconstricted vessels (5HT; 0.1 µmol/L), and relaxation was expressed as a percentage of the precontraction.

Relationship Between Sympathetic Activity and Vascular Adrenergic Reactivity

A set of CD and HFD mice was treated with the specific α1-adrenergic receptor inhibitor prazosin (2 mg/kg per day, IP, daily injection)15 or with the combination of leptin (10 µg/d) plus prazosin for 7 days. Cardiovascular consequences of the α1-adrenergic receptor inhibition were determined at the vascular level by assessment of aortic reactivity and the gene expression of the α1-D-adrenergic receptor by quantitative real-time RT-PCR, as described below.

Real-Time RT-PCR

Total aortic mRNA was extracted (TRIzol Plus, Invitrogen, Carlsbad, CA), purified with RNeasy spin columns, and eluted from the column in 30 µL of diethylpyrocarbonate-treated water, and the concentration was established with a NanoDrop 1000 (NanoDrop Technologies, Wilmington, DE). CDNA was generated by RT-PCR with SuperScript III (Invitrogen) from 400 ng of total RNA using random hexamers. Reverse transcription was performed at 50°C for 50 minutes; the enzyme was heat inactivated at 85°C for 5 minutes; and real-time quantitative RT-PCR was performed with the SYBR-Green Supermix (Bio-Rad Laboratories, Hercules, CA) and the primers described previously.15

Echocardiographic Studies

At the end of the 32 weeks of CD or HFD, mouse heart was imaged in anesthetized animals (isoflurane 2%) in a supine position on a THM100 MousePad with integrated temperature sensor, heater, and ECG electrodes (Indus Instruments, Houston, TX). Ultrasound imaging was performed using the Vevo 770 system (VisualSonics, Toronto, Ontario, Canada) and the RMV 707B scanhead designed for high frame rate and real-time small animal imaging applications with a center frequency of 30 MHz and a frequency band of 15 to 45 MHz. B-mode images of the parasternal long axis view of the heart were used to measure the left ventricular outflow tract (LVOT) and left ventricle (LV) dimensions, and pulse wave mode was used to determine aortic velocity time integral (Ao VTI). Stroke volume (SV), cardiac output (CO), and fractional shortening were calculated according to the following formulas:

\[
(1) \quad SV = 7.85 \times LVOT^2 \times Ao \ VTI
\]

\[
(2) \quad CO = (SV \times heart \ rate/1000)
\]

\[
(3) \quad FS = \frac{LV \ end \ diastolic \ diameter - LV \ end \ systolic \ diameter}{LV \ end \ diastolic \ diameter}
\]

Measurement of Plasma Aldosterone

To assess the effects of obesity and leptin on the activation of the renin-angiotensin-aldosterone system (RAAS), plasma aldosterone levels were measured by radioimmunoassay (Siemens Medical Diagnostic Deerfield, IL) in plasma samples collected from CD and HFD mice submitted to a 7-day treatment with either saline, leptin (10 µg/d), prazosin or leptin (10 µg/d) plus prazosin.

In Vivo BP Measurement

At the end of the 32 weeks of treatment, C57Bl/6 and ob/ob mice were instrumented with telemetry transmitters to record BP and heart rate (PA-C10, Data Sciences, St Paul, MN) and kept on their specific diet. Transmitters were implanted as described previously.15 After 7 to 12 days of recovery from surgery, necessary for the mice to gain their initial body weight, baseline data were recorded for 7 days before implantation of micro-osmotic pumps (ALZET; model 1007D, 0.5 µL/h) for subcutaneous infusion of leptin (10 or 40 µg/d) or leptin plus prazosin (2 mg/kg per day, IP).15 After 1 week of leptin treatment, mice were euthanized and tissues and plasma were collected for later analysis.

Statistical Analysis

All of the data are presented as mean±SEM. Differences in means among groups for nonrepeated variables were compared by 1-way ANOVA. Differences in means among groups and treatments, with repeated variables, were compared by 2- or 3-way ANOVA with
repeated measures, when appropriate. Bonferroni and Fisher least significant difference tests were used as the post hoc test (SigmaStat).

Results

Baseline Phenotypes
The effects of 32 weeks of HFD were determined by measuring body weight and baseline plasma chemistry. As shown in Table 1 and Figure S1 (available in the online Data Supplement at http://hyper.ahajournals.org), the 32-week HFD induced a much greater increase in body weight compared with CD (HFD: 48±6% versus CD: 12±3% increase; P<0.0001) and significantly raised circulating leptin levels (HFD: 18.6±3.0 versus CD: 1.2±0.8 ng/mL; P<0.05; Table 1). As summarized in Table 1, HFD was associated with increased plasma cholesterol, triglycerides, glycemia, hemoglobin 1Ac, and plasma insulin concentration, confirming that our model of diet-induced obesity leads to metabolic dysfunction. As shown in Table 1, diet-induced obesity completely abolished the decrease in body weight induced by low (10 µg/d) and high (40 µg/d) doses of leptin infusion, demonstrating that sustained HFD leads to a resistance to the metabolic effects of leptin, as described previously.22

Effects of HFD on Cardiac Function and Aldosterone Secretion
The consequences of obesity and hyperleptinemia on cardiac functions were determined by ultrasonography. As reported in Table 2, HFD promoted an increased CO, notably elevating Ao VTI and LVOT observed in these mice.

Obesity has often been associated with an increased activation of the RAAS, both in human30 and animals models.31 To assess the effects of the HFD on the RAAS, we measured plasma aldosterone levels. As shown in Figure 1, plasma aldosterone levels were increased with HFD and further increased with sustained leptin infusion. Chronic α1-adrenergic receptor inhibition with prazosin completely abolished HFD-induced aldosterone secretions and also blunted leptin-stimulated aldosterone secretion in obese mice. This suggests a key role for leptin in the control of aldosterone secretion, most likely through the activation of the sympathetic nervous system in HFD mice.

Effects of Obesity and Leptin on BP and Heart Rate
The effects of obesity and hyperleptinemia on BP and heart rate are shown in Figure 2. Thirty-two weeks of HFD in mice increased neither systolic nor diastolic BP compared with controls. Sustained leptin infusion at low (Figure 2) or high doses (Figure S2) did not affect the BP of the CD and HFD mice. Prazosin did not alter responses in either group (Figure

Table 1. Basic Physiological and Metabolic Parameters of Control and High-Fat Diet Mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Diet</th>
<th>Control Diet + Leptin 10 µg/d</th>
<th>Control Diet + Leptin 40 µg/d</th>
<th>High-Fat Diet</th>
<th>High-Fat Diet + Leptin 10 µg/d</th>
<th>High-Fat Diet + Leptin 40 µg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>34.5±0.5</td>
<td>31.2±0.8*</td>
<td>29.4±0.6*</td>
<td>47.7±1.5†</td>
<td>46.4±0.8†</td>
<td>49.0±2.1†</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>141±13</td>
<td>121±7</td>
<td>123±8</td>
<td>203±19*</td>
<td>166±8*</td>
<td>197±14*</td>
</tr>
<tr>
<td>Hemoglobin 1Ac</td>
<td>4.8±0.1</td>
<td>4.6±0.1</td>
<td>4.9±0.1</td>
<td>5.7±0.2*</td>
<td>5.6±0.1*</td>
<td>5.4±0.1*</td>
</tr>
<tr>
<td>Insulin, µg/mL</td>
<td>61.4±4.0</td>
<td>48.6±8.4</td>
<td>33.3±7.0*</td>
<td>603.8±278.5*</td>
<td>398.6±145.3*</td>
<td>419.1±52.3*</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>56.5±7.5</td>
<td>50.4±7.0*</td>
<td>87.4±5.9</td>
<td>93.3±12†</td>
<td>84.8±7.35*</td>
<td>180.9±13.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>37.6±8.2</td>
<td>26.1±0.9</td>
<td>36.6±3.0</td>
<td>38.7±8.9</td>
<td>35.9±5.7</td>
<td>58.2±8.02</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM.

*P<0.05 vs control diet.
†P<0.0001 vs control diet.
n=10 to 12 per group.

Table 2. Echocardiographic Parameters of Control and High-Fat Diet Mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Diet</th>
<th>High-Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao VTI, cm</td>
<td>1.39±0.13</td>
<td>1.91±0.16*</td>
</tr>
<tr>
<td>LVOT, mm</td>
<td>1.40±0.05</td>
<td>1.54±0.03*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>436±63</td>
<td>467±14</td>
</tr>
<tr>
<td>SV, µL</td>
<td>21.1±1.0</td>
<td>35.9±3.7*</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td>9.2±1.5</td>
<td>16.8±1.9*</td>
</tr>
<tr>
<td>FS, %</td>
<td>17.9±1.1</td>
<td>18.6±0.9</td>
</tr>
</tbody>
</table>

Ao VTI indicates aortic velocity time integral; LVOT, left ventricular outflow tract; HR, heart rate; SV, stroke volume; CO, cardiac output; and FS, fractional shortening obtained from the ultrasound analysis of mouse’s heart. Data are presented as mean±SEM.

*P<0.05 vs control, n=8 per group.

Figure 1. Diet-induced obesity and sustained leptin infusion increase plasma aldosterone levels. Plasma aldosterone levels measured in control and high-fat diet mice submitted to 7 days of saline, leptin (10 µg/d), or prazosin or leptin (10 µg/d) plus prazosin (2 mg/kg per day, IP) infusion. Data are presented as mean±SEM. #P<0.05 vs saline, #P<0.05 vs leptin, *P<0.05 vs control diet. n=6 to 12 per group.
S3). As further shown in Figure 2, leptin-deficient mice (ob/ob) presented systolic and diastolic BP values similar to those of the CD mice, despite obesity. BP data from the HFD and ob/ob mice suggest that leptin does not directly affect BP.

In contrast to the lack of effect on BP, HFD induced a 23% increase in heart rate. Obesity without leptin in ob/ob mice had no significant effect on HR, suggesting the leptin is required for obesity-related tachycardia. Further increases in plasma leptin via leptin infusion did not affect heart rate in CD and HFD mice. Despite obesity, sympatho-activation of the heart, and an increased RAAS activation, HFD mice did not present an elevated BP. These data indicate that HFD mice may evidence parallel counterregulatory mechanisms to offset the sympatho-excitatory effects of leptin and maintain their BP in a physiological range.

Effects of HFD on Adrenergic Tone

Total peripheral resistance is determined in large part by sympathetic innervation of the vasculature. Because sympathetic innervation is the most likely aspect of peripheral control to be influence by obesity-related hyperleptinemia, we assessed changes in MAP induced by 1-adrenergic stimulation (phenylephrine) in anesthetized mice under ganglionic blockade. Under these conditions, the rise in MAP reflects the constriction of the resistance vasculature. As shown in Figure 3, HFD significantly blunted PE-mediated rises in MAP (CD: baseline 72.2±5.8, peak 129.6±4.1 mm Hg; HFD: baseline 96.6±3.4 peak 140.8±1.07 mm Hg), suggesting that obesity decreases vascular adrenergic tone and likely total peripheral resistance. To localize this effect directly to the vasculature, we assessed aortic ring reactivity on a wire myograph. As observed in Figure 4A, diet-induced obesity blunted vascular adrenergic constriction in vitro. To determine the effect of leptin on adrenergic reactivity, PE-induced constriction was assessed in CD and HFD mice treated with low and high doses of leptin for 7 days. Sustained leptin treatment blunted aortic reactivity to PE in CD mice (Figure 4B) and further reduced it in HFD mice (Figure 4C). In CD mice, sustained leptin infusion reduced vascular adrenergic reactivity to the level of the HFD mice. To determine the role of the sympathetic activity in the development of these defects, we measured the vascular consequences of a chronic peripheral 1-adrenergic receptor inhibition with prazosin. As reported in Figure 4B and 4C, 1-adrenergic receptor inhibition alone or in combination with leptin did not affect adrenergic reactivity in CD mice, whereas it restored it in HFD mice treated with saline and leptin. As reported previously by our group, leptin-induced vascular adrenergic desensitization is linked to decreased expression of the 1-adrenergic receptor. As reported in Figure 4D, sustained leptin infusion, as well as diet-induced obesity, significantly reduced 1-adrenergic receptor gene expression as measured by quantitative real-time RT-PCR. Chronic 1-adrenergic receptor inhibition restored 1-adrenergic receptor gene expression in obese mice and blunted the leptin effects.

To confirm that leptin specifically affect vascular adrenergic reactivity, we analyzed endothelium-dependent relaxation and assessed the properties of the aorta to constrict in response to 5HT infusion and to relax to sodium nitroprusside. As shown in Figures S4 to S6, obesity-induced high levels of leptin, as well as sustained leptin infusion, did not affect the endothelium-dependent relaxation or the vascular smooth muscle cell function, as reflected by the reactivity to KCl, 5HT, and sodium nitroprusside. These results argue for

![Figure 2](image-url)  
**Figure 2.** High-fat diet or leptin infusion does not increase blood pressure. Systolic blood pressure, diastolic blood pressure, and heart rate measured via radiotelemetry in baseline condition and during 7-day leptin infusion at the dose of 10 μg/d in mice submitted to 32 weeks of control or high-fat diet, as well as in leptin-deficient mice (ob/ob). Data are presented as mean±SEM. P<0.05 vs control diet, ##P<0.001 vs control diet, *P<0.05 vs ob/ob. **P<0.0001 vs ob/ob. n=6 to 8 per group.

![Figure 3](image-url)  
**Figure 3.** Thirty-two weeks of high-fat diet reduces vascular adrenergic tone in resistance arteries. Changes in mean arterial pressure (MAP) induced by phenylephrine injection in mice under mecamylamine blockade. Data are presented as mean±SEM. Effects of the diet were assessed with ANOVA. P<0.05 vs control diet; n=5 per group.
a specific effect of leptin on vascular adrenergic reactivity. Moreover, 7 days of leptin treatment also did not alter nonadrenergic vascular reactivity (Figure S6).

Role of Obesity and Leptin Receptor in Vascular Adrenergic Desensitization

To determine the role of leptin in obesity-related vascular adrenergic tachyphylaxis, we measured adrenergic reactivity in leptin receptor- (db/db) and leptin-deficient (ob/ob) mice. As reported in Figure 5A and 5B, neither db/db nor ob/ob mice, both obese, presented a reduced vascular adrenergic reactivity or a decrease in α1-adrenergic receptor gene expression (Figure 5E and 5F) compared with their respective control, suggesting that intact leptin signaling is required for adrenergic desensitization with obesity-related sympathoactivation. This was further supported by the lack of effect of leptin on adrenergic tone in db/db mice (Figure 5C) and the reduced adrenergic reactivity in ob/ob treated with leptin (Figure 5D and 5G). Altogether, these data suggest that obesity-induced high leptin secretion triggers a sympathomediated vascular adrenergic tachyphylaxis, likely decreasing total peripheral resistance, which may offset the prohypertensive effects of the high CO and RAAS activation.

Discussion

The goal of the present study was to determine the effects of leptin on sympato-activation of the cardiovascular system in the context of obesity. The key observations of the current study are as follows: (1) obesity induces resistance to the metabolic action of leptin; (2) sympato-activation of the cardiovascular system as indicated by increases in heart rate and SV persist in obesity, despite metabolic leptin resistance; (3) HFD- and leptin-mediated sympato-activations stimulate the RAAS, as reflected by the plasma aldosterone levels; (4) vascular adrenergic tachyphylaxis caused by leptin-mediated sympato-activation persists in obesity, indicating a lack of resistance to the effects of leptin in the vasculature; (5) obesity in the absence of leptin signaling does not result in vascular adrenergic tachyphylaxis; and (6) the decreased adrenergic tone in the vasculature correlates with an increase in heart rate, CO, and RAAS activation but not with changes in pressure. Relevant to these observations are the concepts of selective leptin resistance, the mechanisms of sympato-activation of the vasculature in obesity, and the balance of hemodynamic forces that regulate BP in obesity.

Selective Leptin Resistance and the Cardiovascular System

Although many studies have reported that obesity leads to a resistance to the metabolic effects of leptin,14 whether the vasculature becomes resistant to the high circulating leptin levels remained to be determined. As demonstrated in our previous study, a “footprint” of the leptin-mediated innerva-
tion of the vasculature can be detected by a compensatory vascular adrenergic tachyphylaxis and a reduction in the gene expression of the vascular adrenergic receptor.15 Whether this chronic effect of leptin in obese animals is preserved was unknown. In the current study, we observed that sustained exogenous leptin infusion caused a sympatho-mediated vascular adrenergic tachyphylaxis in lean leptin sensitive and in obese mice with metabolic leptin resistance, sug-
gest that the ability of leptin to increase sympathetic nerve activity to the vasculature is preserved in obese animals and is in support of the theory of selective resistance to the metabolic effects of leptin.16,22,32

Another major observation in the current study is that obesity increases aldosterone secretion through a leptin-
dependent mechanism requiring sympatho-activation. Indeed, sustained leptin infusion increased aldosterone secretion in CD mice and further enhanced it in HFD mice. Furthermore, sympa-tho-inhibition restored aldosterone levels in HFD and blunted the increase induced by leptin infusion. One potential mechanism of this is an increase in renal nerve traffic in response to leptin,21,22 leading to an increase in renin release,33,34 although this has historically been thought to be β-adrenergic in character. Although detailed mechanisms will require further study, the prazo-sin data further support the concept that neural components related to the metabolic function develop a resistance to high leptin levels, whereas components relevant to cardio-
vascular control do not.16,22,32

Figure 5. Leptin-induced vascular adrenergic desensitization requires functional leptin receptor. Cumulative concentration-response curves to phenylephrine (PE) performed on aortic rings taken from leptin receptor (db/db, A) or leptin-deficient (ob/ob, B) mice and in mice submitted or not to a 7-day subcutaneous leptin infusion (C and D). Effects of leptin receptor deletion and of leptin treatment were determined with ANOVA for repeated measurements. Quantification of α1D-adrenergic receptor subtype gene expression by quantita-tive real-time RT-PCR performed on aortas taken from db/db (E), ob/ob (F), and ob/ob mice treated with leptin (G). Data are pre-
sented as mean±SEM, **P<0.0001 vs db/+, # #P<0.0001 vs ob/ob, n=5 to 8 per group.
Hemodynamic Impact of Leptin in Obesity

Although leptin’s role in sympatho-activation is well documented, the impact on the regulation of BP is incompletely understood. Indeed, the role of leptin as a pressor agent in normal and obese states remains to be fully determined. Although several studies reported an increased BP with acute or sustained leptin infusion,39,22–24 several others did not report any changes.15,21,24,35,36 Some of these differences between these studies come from the techniques used to administrate leptin (intravenous versus intracerebrovascular or intraperitoneal), the duration of the treatment (acute versus chronic), the dose (physiological versus pharmacological), and the technique used to measured BP (tail cuff plethysmography and intra-arterial catheter versus telemetry). Because leptin is mainly secreted from the subcutaneous adipose tissue into the bloodstream, we infused leptin subcutaneously with osmotic minipumps. BP was recorded in conscious animals via radiotelemetry to avoid the manipulation of animals with exaggerated cardiovascular response to stress.38

Thus, we believe that the experimental approach used in this study provides the best experimental reflection of physiological control of leptin.

Another deficit in our understanding of leptin’s effects stems from a lack of information of how the complex mechanisms that control BP are impacted by obesity and increases in leptin. In the current study, we use gold-standard measurements of arterial pressure combined with examination of indices of each major compartment of BP control. In obese mice characterized either by a high (HFD) or low (ob/ob) circulating levels of leptin, we did not observe any difference in BP between lean and obese animals. However, high leptin levels were associated with an increased heart rate, whereas increased heart rate was absent in mice that lack leptin (ob/ob), consistent with leptin mediated sympatho-activation of the heart, a relationship between leptin and heart rate documented previously both in humans and animal models.19 This increase in heart rate was paralleled by an increase in CO, further supporting the concept of sympatho-activation of the heart in obesity. When the effects of obesity and leptin on aldosterone levels are added to these cardiac indices, it is clear that obesity and leptin have direct sympathetically mediated actions on the cardiovascular system. The observation that leptin does not increase BP despite increases in heart rate and sympathetic activity suggest that counter-regulatory mechanisms oppose the prohypertensive influence of the sympathetic activation.

The absence of an increased in BP despite these increases in cardiac and RAAS mechanisms suggests that parallel changes in the vasculature must occur that limit the expression of hypertension in these animals. The results of the present study and several others36,40 do not support the vasodilator effects of leptin as the primary mechanism moderating the BP response to leptin and instead propose that this is mediated by, rather, a vascular adrenergic tachyphylaxis. Interestingly, human studies also support the role of altered adrenergic tone in the modulation of the BP response to obesity. While studying obese normotensive patients, Agapitov et al41 observed a dissociation between the increased forearm muscle sympathetic nerve activity and reduced brachial adrenergic tone, suggesting that the reduced vascular adrenergic tone could compensate or counterbalance increased sympathetic activity. Moreover, Egan et al42 noted that obese patients with increased forearm vascular adrenergic tone were hypertensive, suggesting that loss of compensatory reductions in adrenergic tone favors the actions of prohypertensive factors. Altogether these data suggest that the failure to induce a vascular adrenergic tachyphylaxis under conditions of sympatho-activation, such as obesity, may indeed be a major factor in determining whether frank hypertension develops.

Mechanisms of Adrenergic Desensitization in Obesity

Although a vascular adrenergic tachyphylaxis, reflected by a reduced vascular adrenergic tone, has often been reported in human obese patients,41 as well as in animals models of obesity,28,43 the underlying mechanisms of theses vascular changes remain undetermined. After 32 weeks of HFD, mice of the present study presented a significant reduction in their vascular adrenergic tone, as reflected, in vivo, by the reduced PE-mediated increase in BP, as well as in vitro with the blunted aortic constriction to PE. Without effects in control mice, sustained peripheral inhibition of the α1-adrenergic receptor with daily prazosin injection partially restored vascular constriction to PE in HFD mice and completely restored α1D-adrenergic receptor gene expression. These results confirmed our previous study15 and present vascular adrenergic tachyphylaxis as a compensatory mechanism to obesity-induced sympatho-activation. Moreover, sustained leptin infusion further decreased aortic adrenergic sensitivity in HFD mice and reduced the gene expression of the α1D-adrenergic receptor both at the levels of the conduit arteries (aorta) and resistance arteries (mesenteric and tail caudal arteries; Figure S7), an effect that was absent in mice lacking functional leptin receptors (db/db). Leptin-deficient ob/ob mice likewise demonstrated no tachyphylaxis to PE despite obesity, but when leptin was restored by subcutaneous infusion, desensitization was evident. These data indicate that sympathetic link between obesity and vascular adrenergic tone is mediated by leptin. The mechanism of this tachyphylaxis and whether it is defective in obese hypertensives is worthy of further study.

In summary, by studying the cardiovascular response to leptin in obese mice resistant to the metabolic effects of leptin, we have been able to demonstrate that leptin, despite increasing heart rate, CO, and RAAS, is also responsible for a vascular adrenergic tachyphylaxis most likely protecting against the development of hypertension (Figure 6).

Perspectives

Known for its ability to increase the sympathetic tone, leptin has been suggested to be the link between the metabolic and cardiovascular dysfunctions associated with obesity and to be a potential player in the development of hypertension related to obesity. In the present study, we observed that diet-induced obesity leads to a resistance to the metabolic effects of leptin. The cardiovascular consequences of leptin stimulation in obesity are complex. Prohypertensive effects, such an increased CO and aldosterone secretion, are parallel by sym-
pathetically driven tachyphylaxis of adrenergic tone. These results suggest that the reduction in the vascular adrenergic tone could be a compensatory mechanism reducing the total peripheral resistances and limiting the development of frank hypertension. These data obtained in a mouse model of obesity are supported by 2 clinical studies reporting that obese normotensive patients presented a decrease adrenergic tone, whereas obese hypertensive patients developed an increased response to adrenergic stimulation. Altogether these data suggest that the failure to reduce adrenergic tone in the face of chronic sympatho-activation may be important to the development of hypertension in obesity.

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None.

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Impact of leptin-mediated sympatho-activation on cardiovascular function in obese mice.

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**Figure S1:** 28 weeks of high fat diet significantly increased body weight gain in mice. Changes in body weight induced in C57Bl/6 male by feeding mice with either a control or a high fat diet during 28 weeks. Starting weights are 23.9± 0.3g for the control diet group and 23.8 ± 0.4g for the high fat diet group. Data are presented as mean ± sem, n=10 per group, **p<0.001 vs. control diet.
Figure S2: High fat diet or leptin infusion does not increase blood pressure. Systolic, diastolic blood pressure and heart rate measured via radio telemetry, in baseline condition and during 7 days leptin infusion at the dose of 40μg/day in mice submitted to 32 weeks of control or high fat diet. Data are presented as mean ± sem, # p<0.05 vs. control diet. n=6-8 per group.
Figure S3: Chronic inhibition of $\alpha_1$-adrenergic receptors, in the presence of leptin, did affect systolic and diastolic blood pressure. Systolic and diastolic blood pressure measured in control and high fat diet mice under baseline conditions and during a 7 day treatment with the combination of leptin (10$\mu$g/day) and prazosin (2mg/kg). Data are presented as mean ± sem, n=6-7 per group.
Figure S4: 32 weeks of high fat diet did not affect vascular constriction to KCl and serotonin (5HT) nor did affect endothelium-dependent (ACh) and -independent (SNP) vascular relaxation. Data are presented as mean ± sem, n=5 per group.
Figure S5: Sustained leptin infusion did not affect vascular contraction to KCl and serotonin (5HT). Data are presented as mean ± sem, n=5 per group.
Figure S6: 7 days of leptin infusion do not affect the endothelium-dependent (ACh) and independent (SNP) relaxation in both control and high fat diet mice. Data are mean ± sem, n=5-8 per group.
Figure S7: 7 days of leptin treatment reduced the expression of the α1D-adrenergic receptor in resistance arteries. Quantification of α1D-adrenergic receptor subtype gene expression by quantitative real time RT-PCR performed on mesenteric (Mes Art.) and tail caudal arteries (Tail caudal Art.) taken from control mice treated with either saline or leptin (10 μg/day, osmotic minipump) for 7 days. N=8 per group, *p<0.05 vs saline.