Protein Tyrosine Phosphatase 1B, a Major Regulator of Leptin-Mediated Control of Cardiovascular Function

Eric J. Belin de Chantemèle, PhD; Kenjiro Muta, BS; James Mintz, BS; Michel L. Tremblay, PhD; Mario B. Marrero, PhD; David J. Fulton, PhD; David W. Stepp, PhD

Background—Obesity causes hypertension and sympathoactivation, a process proposed to be mediated by leptin. Protein tyrosine phosphatase 1B (PTP1B), a major new pharmaceutical target in the treatment of obesity and type II diabetes mellitus, constrains the metabolic actions of leptin, but the extent to which PTP1B regulates its cardiovascular effects is unclear. This study examined the hypothesis that PTP1B is a negative regulator of the cardiovascular effects of leptin.

Methods and Results—PTP1B knockout mice had lower body fat but higher mean arterial pressure (116±5 versus 105±5 mm Hg, P<0.05) than controls. Leptin infusion produced a greater anorexic effect in PTP1B knockout mice and a marked increase in mean arterial pressure (135±5 mm Hg) in PTP1B knockout mice only. The decrease in mean arterial pressure in response to ganglionic blockade was higher in PTP1B knockout mice (−38±3% versus −29±3%, P<0.05), which suggests increased sympathetic tone. PTP1B deletion blunted mean arterial pressure responses to phenylephrine injection (55±10% versus 93±7%, P<0.05). Phenylephrine-induced aortic contraction was reduced in PTP1B knockout mice (57.7±9% versus 96.3±12% of KCl, P<0.05), consistent with desensitization to chronically elevated sympathetic tone. Furthermore, PTP1B deletion significantly reduced gene expression of 3 α1-adrenergic receptor subtypes, consistent with blunted constriction to phenylephrine.

Conclusions—These data indicate that PTP1B is a key regulator of the cardiovascular effects of leptin and that reduced vascular adrenergic reactivity provides a compensatory limit to the effects of leptin on mean arterial pressure. (Circulation. 2009;120:753-763.)

Key Words: hypertension • obesity • receptors, adrenergic, alpha • nervous system, sympathetic • vasoconstriction • arteries • blood pressure

Obesity, a worldwide metabolic disorder that affects 60% of the population, is defined as an increased mass of adipose tissue, and it confers a higher risk for hypertension.1 Although this risk is well documented, the underlying mechanisms are unknown. Obesity is clearly associated with sympathoactivation in humans and animal models,2 and mechanisms underlying the regulation of cardiovascular function by leptin are largely undetermined. Moreover, how the vasculature responds to chronic activation of leptin signaling is unknown. The availability of lean mice that harbor a genetic deletion of PTP1B through activation of the sympathetic nervous system6,7 via Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. Activated receptors induce the transphosphorylation of JAKs, and the phosphorylation of tyrosine residues on the leptin receptor, specifically Tyr1138, facilitates the binding of STAT proteins. Among the regulators of this signaling pathway is the protein tyrosine phosphatase 1B (PTP1B). PTP1B controls this pathway by dephosphorylation of JAK2.8 Cheng et al9 and Zabolotny et al10 reported that genetic deletion of PTP1B increases leptin sensitivity, protecting mice against the development of obesity and type II diabetes mellitus. In addition to its metabolic actions, leptin is involved in the control of cardiovascular function via regulation of sympathetic tone.7,11,12 The molecular mechanisms underlying the regulation of cardiovascular function by leptin are largely undetermined. Moreover, how the vasculature responds to chronic activation of leptin signaling is unknown. The availability of lean mice that harbor a genetic deletion of PTP1B through activation of the sympathetic nervous system6,7 via Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. Activated receptors induce the transphosphorylation of JAKs, and the phosphorylation of tyrosine residues on the leptin receptor, specifically Tyr1138, facilitates the binding of STAT proteins. Among the regulators of this signaling pathway is the protein tyrosine phosphatase 1B (PTP1B). PTP1B controls this pathway by dephosphorylation of JAK2.8 Cheng et al9 and Zabolotny et al10 reported that genetic deletion of PTP1B increases leptin sensitivity, protecting mice against the development of obesity and type II diabetes mellitus. In addition to its metabolic actions, leptin is involved in the control of cardiovascular function via regulation of sympathetic tone.7,11,12 The molecular mechanisms underlying the regulation of cardiovascular function by leptin are largely undetermined. Moreover, how the vasculature responds to chronic activation of leptin signaling is unknown. The availability of lean mice that harbor a genetic deletion of PTP1B through activation of the sympathetic nervous system6,7 via Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. Activated receptors induce the transphosphorylation of JAKs, and the phosphorylation of tyrosine residues on the leptin receptor, specifically Tyr1138, facilitates the binding of STAT proteins. Among the regulators of this signaling pathway is the protein tyrosine phosphatase 1B (PTP1B). PTP1B controls this pathway by dephosphorylation of JAK2.8 Cheng et al9 and Zabolotny et al10 reported that genetic deletion of PTP1B increases leptin sensitivity, protecting mice against the development of obesity and type II diabetes mellitus. In addition to its metabolic actions, leptin is involved in the control of cardiovascular function via regulation of sympathetic tone.7,11,12 The molecular mechanisms underlying the regulation of cardiovascular function by leptin are largely undetermined. Moreover, how the vasculature responds to chronic activation of leptin signaling is unknown. The availability of lean mice that harbor a genetic deletion of PTP1B through activation of the sympathetic nervous system6,7 via Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. Activated receptors induce the transphosphorylation of JAKs, and the phosphorylation of tyrosine residues on the leptin receptor, specifically Tyr1138, facilitates the binding of STAT proteins. Among the regulators of this signaling pathway is the protein tyrosine phosphatase 1B (PTP1B). PTP1B controls this pathway by dephosphorylation of JAK2.8 Cheng et al9 and Zabolotny et al10 reported that genetic deletion of PTP1B increases leptin sensitivity, protecting mice against the development of obesity and type II diabetes mellitus. In addition to its metabolic actions, leptin is involved in the control of cardiovascular function via regulation of sympathetic tone.7,11,12 The molecular mechanisms underlying the regulation of cardiovascular function by leptin are largely undetermined. Moreover, how the vasculature responds to chronic activation of leptin signaling is unknown. The availability of lean mice that harbor a genetic deletion of PTP1B...
allows the examination of the actions of leptin on cardiovascular function without the confounding components of obesity. These mice were used to examine the hypothesis of the present study that PTP1B is a specific negative regulator of the actions of leptin in the cardiovascular system. To test this hypothesis, the regulation of blood pressure and vascular function in mice that harbored a genetic deletion of PTP1B was examined. Taken together, these studies critically test the circumstances under which leptin activation is a potential mechanism for hypertension.

**Methods**

**Animals**

PTP1B null mice were generated on a Balb/c background at Goodman Cancer Center of McGill University.9,13 Balb/c mice, used as a control group, were purchased from the Jackson Laboratory (Bar Harbor, Me). All animals were fed standard mouse chow, and tap water was provided ad libitum. Mice were housed in an American Association of Laboratory Animal Care–approved animal care facility at the Medical College of Georgia, and the Institutional Animal Care and Use Committee approved all protocols.

**Metabolic Measurements**

Fasting blood glucose was assessed with a glucometer (Precision XL, Abbot Laboratories, Alameda, Calif). Plasma total cholesterol and triglycerides were assessed with colorimetric assays (Wako, Richmond, Va). Plasma insulin and leptin, respectively, were determined with the Mercodia insulin ELISA assay and the mouse leptin EIA kit (ALPCO Diagnostics, Salem, NH).

**In Vivo Blood Pressure Measurement**

Mice were instrumented with telemetry transmitters to record arterial pressure and heart rate (PA-C20, Data Sciences, Saint Paul, Minn.). Transmitters were implanted as described previously.14 After 1 week of recovery from surgery, baseline data were recorded for 7 days before implantation of micro-osmotic pumps (ALZET, Cupertino, Calif; model 1007D, 0.5 μL/h). Data are presented as mean±SEM.

### Table. Basic Physiological and Metabolic Parameters of Balb/c and PTP1B Knockout Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Balb/c</th>
<th>PTP1B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>25.7±0.4</td>
<td>27.4±0.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Epididymal fat mass, mg</td>
<td>389±25</td>
<td>163±20</td>
<td>0.000</td>
</tr>
<tr>
<td>Fat/body weight, mg/g</td>
<td>15.2±0.9</td>
<td>5.3±0.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>122±2</td>
<td>149±5</td>
<td>0.003</td>
</tr>
<tr>
<td>Heart/body weight, mg/g</td>
<td>4.7±0.1</td>
<td>4.81±0.1</td>
<td>0.372</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>1.11±0.44</td>
<td>1.16±0.33</td>
<td>0.3</td>
</tr>
<tr>
<td>Insulin, ng/L</td>
<td>57.86±17.01</td>
<td>54.13±15.78</td>
<td>0.437</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>29.2±3.8</td>
<td>35.5±4.2</td>
<td>0.269</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>27.3±5.5</td>
<td>16.8±3.3</td>
<td>0.111</td>
</tr>
<tr>
<td>Glycemia, mg/dL</td>
<td>70.9±6.1</td>
<td>73.8±8.7</td>
<td>0.782</td>
</tr>
<tr>
<td>Hemoglobin %</td>
<td>5.1±0.1</td>
<td>5.3±0.1</td>
<td>0.196</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM.

average food intake during the baseline data collection period. Data were recorded throughout the infusion period. Mice were then euthanized, and tissues and plasma were collected for later analysis. In a different 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 constrictor administration. Changes in MAP were determined after injection of randomized boluses of phenylephrine (0.01 to 1 mg/kg) and expressed as a percent of the baseline blood pressure.19

### Cardiovascular Response to Stress

Whether PTP1B deletion affects the cardiovascular response to stress was determined with the cage-switch stress model that consists of measuring blood pressure variations by telemetry when a male mouse is placed in a cage previously occupied by another male mouse, as described previously.14

### Vascular Reactivity

Thoracic aortas from PTP1B knockout and Balb/c mice were dissected surgically and mounted on a wire myograph (DMT, Aarhus, Denmark) with 1g 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.20 Arterial vasoactivity was determined with a potassium-rich solution (40 mmol/L). Vascular contractility was assessed with cumulative concentration-response curves to norepinephrine (1 pmol/L to 10 μmol/L), phenylephrine (1 pmol/L to 10 μmol/L), and serotonin (5-hydroxytryptamine [5HT]; 1 pmol/L to 10 μmol/L). Concentration–response curves to phenylephrine were repeated in the presence of the nitric oxide (NO) synthase inhibitor N’-nitro-L-arginine methyl ester (L-NAME; 100 μmol/L, 20-minute incubation). Constriction to norepinephrine, phenylephrine, and 5HT was expressed as a percent of KCl-induced constriction. Endothelial function was assessed with concentration–response curves to acetylcholine, and the involvement of NO was determined by inhibition of endothelial NO synthase with L-NAME (100 μmol/L, 20 minutes). Endothelium-independent relaxation was an-
Figure 2. PTP1B deletion increased baseline blood pressure and hemodynamic response to leptin infusion. A, Baseline and MAP response to leptin infusion (10 µg/d, 0.5 µL/h), determined by telemetry, in Balb/c and PTP1B knockout mice (n=5 per group).
B, Baseline and heart rate response to leptin infusion, determined by telemetry and presented as the average of the 7 days of recording, in Balb/c and PTP1B knockout mice (n=5 per group). C, Baseline and MAP response to insulin infusion (2 U/kg/d, 0.5 µL/h), determined by telemetry in PTP1B knockout mice supplemented with glucose. D, Baseline and heart rate response to glucose or glucose plus insulin infusion determined by telemetry and presented as the average of the 7 days of recording, in PTP1B knockout mice. E, Changes in body weight induced by 20% reduction in food intake. F, MAP response to the hypocaloric diet mimicking the anorexigenic effects of leptin (n=5 per group). Data are presented as mean±SEM. *P<0.05 vs Balb/c. Effects of treatment on continuous blood pressure recording and body weight were analyzed by 2-way ANOVA for repeated measurements (A, C, E, and F).
alyzed with a concentration-response curve to sodium nitroprusside (1 pmol/L to 10 μmol/L). Relaxation curves were performed on preconstricted vessels (5HT 1 μmol/L), and relaxation was expressed as a percent of the precontraction.

Relation Between Sympathetic Activity and Vascular Adrenergic Reactivity
A final set of mice were treated with the specific α1-adrenergic receptor inhibitor prazosin (2 mg/kg/d IP)21,22 for 7 days, with or without leptin (10 μg/d).15,16 Cardiovascular consequences of the sympathoinhibition were determined at the vascular level by assessment of vascular reactivity and the gene expression of the 3 subtypes of the α1-adrenergic receptor by quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR), as described below.

Histological Analysis
Mouse aortas were dissected under a microscope, fixed in formalin, and embedded in resin. Sections (10 μm) stained with hematoxylin and eosin were observed under a microscope (Carl Zeiss AX10, Thornwood, NY), and the media cross-sectional area was calculated (AxioVision LE, AxioVs40).

Real-Time RT-PCR
Total aortic mRNA was extracted (Trizol Plus, Invitrogen, Carlsbad, Calif), purified with RNeasy spin columns, and eluted from the column in 30 μL of DEPC-treated water, and the concentration was established with a NanoDrop 1000 (NanoDrop Technologies, Wilmington, Del). Complementary DNA 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, Calif). The sequence of the primers is presented in the Table of the online-only Data Supplement.

Statistical Analysis
All 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 PTP1B deletion on the mouse phenotype were determined by measuring body weight, organ weight, and baseline plasma chemistry. As summarized in the Table, PTP1B deletion induced a slight increase in body weight but a 3-fold decrease in epididymal fat pad mass. Cardiac mass was similar when normalized for body weight. An increase in bone and muscle mass, respectively, triggered by the increased leptin and insulin sensitivity may explain the slight increase in body weight despite a decrease in fat content. As reported previously by Elchebly et al,13 in the same mice, PTP1B deletion had no effect on the fasting plasma level of insulin or leptin and did not affect plasma concentrations of glucose, cholesterol, or triglycerides.

Measurement of Leptin Sensitivity
The effects of PTP1B deletion on metabolic leptin sensitivity were determined by measuring changes in body weight during a 7-day subcutaneous leptin infusion. As shown in Figure 1, PTP1B knockout mice had a larger body weight reduction in response to leptin infusion than Balb/c mice (4.73±0.46% versus 6.93±0.57% for Balb/c versus PTP1B knockout mice, P<0.05).

Effects of PTP1B Deletion on Blood Pressure
The cardiovascular consequences of PTP1B deletion were first assessed by determining blood pressure in vivo via radiotelemetry. As shown in Figure 2A, the deletion of PTP1B resulted in a 20-mm Hg rise in baseline blood pressure (Balb/c 99.4±4.6 mm Hg versus PTP1B 120.4±3.1 mm Hg, P<0.05). Treatment with leptin resulted in a significant increase in blood pressure only in PTP1B knockout mice, with no change in blood pressure in Balb/c mice (Balb/c 100.3±1.5 mm Hg versus PTP1B 130.8±2.1 mm Hg, P<0.05). Treatment with insulin in PTP1B knockout mice under glucose supplementation did not

Figure 3. PTP1B deletion does not affect acute response to stress. A, Representative changes in MAP induced by the cage switch. B, Integrated area under the MAP curve of the cage-switch stress. Data are presented as mean±SEM.
affect MAP (Figure 2C), nor did it affect heart rate (Figure 2D) or nonfasted glycemia (glucose 153±8 mg/dL versus insulin plus glucose 162±10 mg/dL). To confirm that the lack of response to leptin observed in Balb/c mice was not masked by the reduction in blood pressure associated with body weight loss, we determined changes in blood pressure induced by a hypocaloric diet, which mimicked the effects of leptin. The reduction in food intake induced a similar drop in body weight in both Balb/c and PTP1B knockout mice (Balb/c −6.1±0.9% versus PTP1B −5.3±0.7%, P=NS; Figure 2E). As shown in Figure 2F, body weight reduction did not affect blood pressure in either Balb/c or PTP1B knockout mice (Balb/c 100±2 mm Hg versus PTP1B 118±2 mm Hg, P<0.05). As shown in Figure 2B, neither PTP1B deletion nor leptin treatment significantly affected heart rate in any group of mice.

**Effects of PTP1B Deletion on Cardiovascular Response to Stress**

To determine whether the effects of PTP1B deletion on arterial pressure were specific to leptin, male mice were switched to the cage of 1 of their congeners to measure the cardiovascular response to this territorial stress. Cage switch generated a 30-mm Hg increase in blood pressure in both Balb/c and PTP1B knockout mice (Figure 3A). The total pressor response was derived by calculating the integrated area under the curve, a value that was similar in both groups (Figure 3B).

**Effects of PTP1B Deletion on Sympathetic Activity and Adrenergic Tone In Vivo**

To assess the effects of PTP1B deletion on sympathetic activity and adrenergic sensitivity, mice were anesthetized and catheterized. An index of the sympathetic activity was obtained by decreasing changes in blood pressure induced by ganglionic blockade.23 As shown in Figure 4A, the drop in blood pressure induced by the ganglionic blocker mecamylamine was significantly greater in PTP1B knockout mice than in Balb/c (Balb/c −29±3% versus PTP1B −38±3%, P<0.05), which indicates greater sympathetic contribution to arterial pressure in PTP1B knockout mice. Parallel increases in sympathetic tone were observed in Balb/c mice treated with leptin, which suggests consistency of mechanism between leptin-treated and leptin-sensitized mice. No further increase in sympathetic tone was observed in PTP1B knockout mice under leptin treatment, which suggests a saturation of the sympathetic mechanism with deletion of PTP1B.

Cumulative doses of phenylephrine were injected intravenously in mice under mecamylamine blockade to determine vascular adrenergic reactivity in vivo. As shown in Figure 4B, PTP1B deletion significantly blunted the rise in blood pressure induced by α1-adrenergic receptor stimulation (maximum response 63±5 versus 50±4 mm Hg, n>5, P<0.05), which reflects decreased vascular adrenergic reactivity. A similar decrease in vascular adrenergic reactivity was noticed in the Balb/c mice treated with leptin, which links this hormone to the sympathetic overflow and reduced adrenergic reactivity (maximum response 63±5 versus 43±7 mm Hg, n>5, P<0.05). No further reduction in adrenergic sensitivity was observed in PTP1B knockout mice treated with leptin, consistent with saturation of sympathetic drive by the combination of excess leptin stimulation and excess leptin sensitivity.

**Effects of PTP1B Deletion on Vascular Reactivity**

To confirm whether the decreased response to phenylephrine observed in vivo reflects decreased adrenergic reactivity of the vasculature, vasoconstrictor reactivity of aortic rings was assessed in vitro on a wire myograph. As shown in Figure 5, PTP1B deletion resulted in a decreased contraction to both norepinephrine (Figure 5A) and phenylephrine (Figure 5B). Specificity of this response to adrenergic stimulation was assessed by measuring the constriction induced by a depolarizing agent (KCl) and by 5HT, as well as with the relaxation induced by the exogenous NO donor sodium nitroprusside. As shown in Figure 5C, through 5E, PTP1B deletion did not affect the constriction induced by KCl or 5HT, nor did it affect the relaxation induced by sodium nitroprusside, which rules out generalized vascular smooth muscle cell dysfunction as an explanation for the changes in adrenergic sensitivity.

**Effects of PTP1B Deletion on Vascular Structure**

Because PTP1B has been reported to be involved in cell proliferation and migration,24,25 and because changes in vascular...
architecture affect the generation of force,26 we determined whether the deletion of PTP1B affects vascular structure. Analysis of aortic cross sections and measurement of media thickness and wall-to-lumen ratio indicated no significant difference in aortic structure between PTP1B knockout and Balb/c mice (online-only Data Supplement Figure I).

Effects of PTP1B Deletion on Vascular Endothelium

Because endothelial NO modulates adrenergic constriction,27 we examined endothelial function by 2 measures: (1) Relaxation induced by the endothelium-dependent vasodilator acetylcholine and (2) the effect of the endothelial NO synthase inhibitor, L-NAME, on acetylcholine and phenylephrine-induced constriction. Acetylcholine induced a similar relaxation of aortic rings in both PTP1B knockout and Balb/c mice. Similarly, L-NAME completely inhibited relaxation in both groups (online-only Data Supplement Figure II), which indicates an NO dependency of this response. Furthermore, as shown in Figure IIB in the online-only Data Supplement, endothelial NO synthase inhibition had no effect on phenylephrine-induced contraction in either Balb/c or PTP1B knockout mice, which rules out endothelial involvement in the reduced adrenergic reactivity observed in PTP1B knockout mice.

Effects of Sustained Treatment With Leptin on Vascular Function

Vascular effects of sustained leptin treatment were determined by analyzing the reactivity of the aorta taken from the leptin-treated mice. The 7-day leptin treatment had no effect on endothelial function, as reflected by the lack of change in

---

**Figure 5.** PTP1B deletion reduced vascular adrenergic reactivity. Cumulative concentration-response curves to (A) norepinephrine (NE), (B) phenylephrine (PE), and (D) serotonin, performed on aortic rings and expressed as percent of KCl-induced constriction (C). E, Endothelium-independent relaxation (sodium nitroprusside [SNP]) expressed as percent of 5HT-induced constriction. Data are presented as mean±SEM. Comparisons between groups were assessed with 2-way ANOVA for repeated measurements. *P<0.05 vs Balb/c. n=8 to 10 per group.
RT-PCR (Figure 8C), blockade of adrenergic receptors with knockout mice. As evidenced by the results of real-time reduced by leptin and restored adrenergic reactivity in PTP1B 

Vascular Function

Effects of Sustained Treatment With Prazosin on Vascular Function

To determine the extent to which vascular adrenergic changes were caused by sympatoactivation, vascular reactivity and adrenergic receptor expression were assessed by analysis of the vascular reactivity of mice receiving sustained treatment with prazosin in the presence or absence of leptin. As reported in Figure 8A and 8B, α₁-adrenergic receptor inhibition completely blunted the adrenergic desensitization induced by leptin and restored adrenergic reactivity in PTP1B knockout mice. As evidenced by the results of real-time RT-PCR (Figure 8C), blockade of adrenergic receptors with prazosin restored vascular adrenergic reactivity by blunting the reduction in α₁B- and α₁D-adrenergic receptor gene expression induced by both leptin and PTP1B deletion.

Discussion

The goal of the present study was to determine the mechanisms by which leptin, independent of obesity, modulates cardiovascular function. The key findings are that (1) increasing leptin sensitivity by PTP1B deletion enhances MAP; (2) the deletion of PTP1B increases the autonomic and presumably sympathetic contribution to blood pressure; and (3) blood pressure responses to leptin-induced sympatoactivation are associated with reductions in adrenergic reactivity.

PTP1B, Leptin, and MAP

In the present study, we demonstrate for the first time that disruption of the PTP1B gene increases MAP, which supports the clinical association between PTP1B gene variants and systolic blood pressure. More interestingly, the present study establishes a novel regulatory factor that moderates the relationship between blood pressure and leptin. Specifically, subcutaneous leptin infusion at a suppressor dose to raise plasma concentrations into physiological ranges (Balb/c 2.7 ± 0.4 ng/mL versus PTP1B 3.3 ± 0.5 ng/mL, P = NS) failed to produce a pressor response in control Balb/c mice but produced a 20-mm Hg increase in pressure in mice lacking PTP1B. These data provide strong evidence that PTP1B, in addition to limiting the metabolic actions of leptin, also acts as a brake on the hemodynamic actions of leptin.

It is noteworthy in these studies that relatively high levels of leptin did not produce an increase in MAP in control mice. Although several studies reported a mild increase in blood pressure with either an intracerebroventricular leptin infusion (4.1 mm Hg) or a subcutaneous leptin infusion (7 mm Hg), others reported no change in blood pressure despite an up to 50-fold increase in plasma leptin. A potential explanation for this observation is that body weight reduction reduces blood pressure. To test this hypothesis, blood pressure was determined during a 20% calorie restriction, which mimicked the effects of leptin on body weight. Contrary to the data reported by Correia et al and Williams et al., the body weight reduction induced by the hypocaloric diet was not associated with changes in blood pressure, which rules out this hypothesis as an explanation of the lack of response to leptin.

Finally, we observed that baseline blood pressure was also increased in PTP1B knockout mice compared with controls. Although it is tempting to speculate that deletion of PTP1B causes a leptin-mediated hypertension at baseline, caution is warranted, because PTP1B may affect other prohypertensive pathways. Although a role of PTP1B in leptin-mediated hypertension is clear, further studies are needed to determine the mechanisms of baseline hypertension in PTP1B knockout mice.

PTP1B, Leptin, and the Sympathetic Nervous System

The deletion of PTP1B not only increased blood pressure but, as indicated by the greater pressure drop during ganglionic blockade, also resulted in an increased contribution of the
Autonomic nervous system in blood pressure regulation, which was presumably the consequence of a greater sympathetic tone. The ability of leptin to induce sympathetic overdrive was confirmed by the exaggeration of the mecamylamine-induced drop in blood pressure after leptin infusion in control mice. These results are in accordance with several studies that established a clear relationship between leptin concentration and sympathetic activity, and thus, the increased sympathetic tone observed in the PTP1B knockout mice might provide an explanation for the elevated blood pressure. Moreover, the deletion of PTP1B has an element of specificity in its prohypertensive effects: Sympathetically mediated increases in blood pressure and vasoconstriction in response to phenylephrine stimulation, similar results were also observed in leptin-treated Balb/c mice, which indicates that the adrenergic desensitization is attributable to leptin and its associated sympatheoactivation and which suggests that the reduced vascular adrenergic reactivity is the consequence of the leptin-induced sympathetic overflow. This was further supported by data from mice treated with prazosin, which blunted the adrenergic desensitization induced by both leptin and the deletion of PTP1B. Moreover, it suggests that downregulation of adrenergic receptors may be a protective counterregulation, because leptin-treated Balb/c mice did not exhibit elevated blood pressure despite increased sympathetic tone. Indeed, rodent models of obesity with relatively mild blood pressure increases show reduced adrenergic reactivity in the splanchnic circulation, consistent with the concept of adrenergic desensitization in the context of obesity-induced sympatheoactivation.

Vascular Adaptation to Chronic Sympathetic Overdrive

Although many studies of obesity-induced hypertension have documented increased sympatheoactivation and posited this as a mechanism of increased blood pressure, the literature is confounded by examples of sympatheoactivation without hypertension. The missing piece of the puzzle to date has been how the vasculature adapts to chronic sympathetic activity. Through in vivo injection of phenylephrine (Figure 4) and ex vivo analysis of aortic ring reactivity (Figure 5), we determined that PTP1B deletion blunted increases in blood pressure and vasoconstriction in response to phenylephrine stimulation. Similar results were also observed in leptin-treated Balb/c mice, which indicates that the adrenergic desensitization is attributable to leptin and its associated sympatheoactivation and which suggests that the reduced vascular adrenergic reactivity is the consequence of the leptin-induced sympathetic overflow. This was further supported by data from mice treated with prazosin, which blunted the adrenergic desensitization induced by both leptin and the deletion of PTP1B. Moreover, it suggests that downregulation of adrenergic receptors may be a protective counterregulation, because leptin-treated Balb/c mice did not exhibit elevated blood pressure despite increased sympathetic tone. Indeed, rodent models of obesity with relatively mild blood pressure increases show reduced adrenergic reactivity in the splanchnic circulation, consistent with the concept of adrenergic desensitization in the context of obesity-induced sympatheoactivation.
Finally, we have demonstrated that under leptin treatment, PTP1B knockout mice develop an increased blood pressure with no further changes in adrenergic sensitivity. These data suggest that it is possible to saturate the ability to desensitize adrenergic reactivity, and the inability to further decrease adrenergic reactivity in response to sympathetic overdrive might result in a rise in blood pressure. More importantly, these data suggest that the failure to desensitize adrenergic reactivity under conditions of sympathoactivation, such as obesity, may indeed be a major factor in determining whether frank hypertension develops.

Potential Mechanisms for Adrenergic Desensitization

Although increased sympathetic activity leads to \( \alpha \)-adrenergic desensitization, the mechanisms involved in this desensitization remain largely unknown. Because of the stimulatory effects of leptin on endothelial NO synthase activity and the protective effects of PTP1B deletion on flow-mediated dilation, we speculated that the adrenergic desensitization observed in PTP1B knockout mice could be due to improved endothelial function. Both acetylcholine-mediated relaxation curves and phenylephrine-induced constriction curves, performed in the presence of L-NAME, ruled out this hypothesis. Furthermore, both the morphological analysis of the vessel structure and vascular responses to KCl, 5HT, and sodium nitroprusside refuted any smooth muscle cell dysfunction and demonstrated that PTP1B deletion specifically targeted the \( \alpha \)-adrenergic receptor signaling pathway. In further support of this specificity, quantification of \( \alpha \)-adrenergic receptor gene expression by real-time RT-PCR
demonstrated that PTP1B deletion significantly reduced the expression of the $\alpha_1$-adrenergic receptor subtypes in the aorta and that leptin treatment had similar effects. This clearly suggests that the adrenergic desensitization induced by an increase in leptin sensitivity or in leptin concentration is the result of decreased $\alpha_1$-adrenergic receptor expression. The most relevant receptor subtype is the $\alpha_1D$-receptor, because although all receptors were downregulated with leptin/PTP1B-induced sympathoactivation, blockade of leptin-mediated actions by prazosin restored only the $\alpha_1D$-receptor and completely restored vasoconstrictor reactivity.

In summary, by genetic disruption of PTP1B in lean mice, we have been able to identify PTP1B as a new modulator of blood pressure through its ability to regulate sympathetic tone and adrenergic reactivity. Through the experiments performed with subcutaneous leptin infusion in a physiological range, we clearly established a role for leptin in the regulation of blood pressure, a role suggested by several authors but never clearly demonstrated. Finally, the present data suggest that adrenergic desensitization may be a protective mechanism against hypertension, with a failure to properly desensitize the adrenergic signaling pathway leading to the development of hypertension.

Acknowledgments

The authors wish to thank Drs Brenda Lilly and Edward Inscho for technical assistance and the useful exchange of ideas in the generation of this manuscript. The authors acknowledge the helpful editorial assistance of Jessica Osmond in the preparation of the written document.

Sources of Funding

The authors acknowledge research support from National Institutes of Health grant R01 HL067533 to Dr Stepp and an American Heart Association postdoctoral fellowship to Dr Belin de Chantemèle.

Disclosures

None.

References

Clinical Perspective

Protein tyrosine phosphatase 1B (PTP1B) controls insulin and leptin sensitivity through its ability to directly inactivate catalytically phosphorylated tyrosines. Because deletion of PTP1B has been reported to improve insulin and leptin sensitivity and to protect rodents against the development of obesity and type II diabetes mellitus, PTP1B inhibitors are under development to treat metabolic disorders. The present study analyzed the cardiovascular function of PTP1B knockout mice and reports that PTP1B deletion increased sympathetic tone and enhanced mean arterial pressure, which highlights the possible deleterious consequences of global PTP1B inhibitors in obese patients with type II diabetes mellitus, who are already prone to the development of hypertension. This study further reports that the control mice (Balb/c mice) did not develop an increase in blood pressure in response to leptin infusion despite an increased sympathetic tone. The significant decreased vascular adrenergic reactivity reported both in vivo and ex vivo (isolated artery) and supported by the reduction in vascular $\alpha_1$-adrenergic receptor expression suggests that adrenergic desensitization, in response to sympathetic overdrive, may be a protective mechanism against the development of hypertension. Taken together, these data suggest that caution is warranted in the consideration of PTP1B inhibitors as antidiabetic drugs and further suggest that mechanisms that interfere with adrenergic desensitization may contribute to the hypertension observed in obese individuals with high levels of leptin.
Protein Tyrosine Phosphatase 1B, a Major Regulator of Leptin-Mediated Control of Cardiovascular Function

Eric J. Belin de Chantemèle, Kenjiro Muta, James Mintz, Michel L. Tremblay, Mario B. Marrero, David J. Fulton and David W. Stepp

_Circulation_. 2009;120:753-763; originally published online August 17, 2009; doi: 10.1161/CIRCULATIONAHA.109.853077

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/120/9/753

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2009/08/17/CIRCULATIONAHA.109.853077.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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 _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/
## SUPPLEMENTAL MATERIAL

### Supplemental Tables:

Table I: Sequence of the primers used for the quantitative real time RT-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse $\alpha_{\text{ID}}$-adrenergic receptor F</td>
<td>GCTGGTTCCCCTTTTTTCTTC</td>
</tr>
<tr>
<td>Mouse $\alpha_{\text{ID}}$-adrenergic receptor R</td>
<td>ATTGAAGTAGCCCCAGCCAGA</td>
</tr>
<tr>
<td>Mouse $\alpha_{\text{IB}}$-adrenergic receptor F</td>
<td>AACCTTGGGCTATTGAGTCG</td>
</tr>
<tr>
<td>Mouse $\alpha_{\text{IB}}$-adrenergic receptor R</td>
<td>AGGCAGCTGTTGAAAGTAGCC</td>
</tr>
<tr>
<td>Mouse $\alpha_{\text{IA}}$-adrenergic receptor R</td>
<td>CTGCCATTCTCTCCTCGTGAT</td>
</tr>
<tr>
<td>Mouse $\alpha_{\text{IA}}$-adrenergic receptor F</td>
<td>GGCTGGAGCATGGGTATATG</td>
</tr>
<tr>
<td>Mouse GAPDH F</td>
<td>AACTTGGCATTGTGGAAGG</td>
</tr>
<tr>
<td>Mouse GAPDH R</td>
<td>GGATGCAGGGATGATTTCT</td>
</tr>
</tbody>
</table>
Supplemental Figures:

Figure I

A

![Image of Balb/c and PTP1B samples]

B

![Bar graph showing Wall/Lumen ratio for Balb/c and PTP1B]

C

![Bar graph showing Media CSA for Balb/c and PTP1B]
Figure II

A

B

-20
0
20
40
60
80
100
120
140
98765

-\log [ACh] (M)

Relaxation (% preconstriction)

-\log [PE] (M)

Contraction (% KCL)

Balb/c
Balb/c + LNAME
PTP1B
PTP1B + LNAME
Figure IV

A

Balb/c

B

PTP1B

C

D

Contraction (% KCl)

- log [5HT] (M)

Contraction (% KCl)

- log [5HT] (M)

Relaxation (% precontraction)

- log [SNP] (M)

Relaxation (% precontraction)

- log [SNP] (M)

Balb/c

Balb/c + Praz

Balb/c + Praz + Lept

PTP1B

PTP1B + Praz

PTP1B + Praz + Lept

Balb/c PTP1B

AB

C D
Supplemental Legends:

**Figure I: PTP1B deletion did not affect vascular structure.** A) Representative pictures of aortic sections. B) Wall to lumen ration and C) quantification of the media cross sectional area in the Balb/c (n=5) and PTP1B KO mice (n=6). Data are presented as mean ± sem.

**Figure II: PTP1B deletion did not impair endothelial function.** A) Cumulative concentration response curve to Acetylcholine (ACh) performed in the presence or absence of eNOS inhibitor (L-NAME, 10 mM). B) Cumulative concentration response curve to phenylephrine (PE) performed in the presence or absence of eNOS inhibitor (L-NAME, 10 mM). Data are presented as mean ± sem. n= 8-10 per group.

**Figure III: Chronic insulin treatment in PTP1B KO mice did not affect the vascular reactivity.** Cumulative concentration response curve to (A) PE, (B) 5HT and (C) SNP, performed in PTP1B KO mice treated with glucose or with glucose + leptin. Data are presented as mean ± sem.

**Figure IV: Chronic sympatho-inhibition did not affect vascular reactivity to serotonin and sodium Nitroprusside.** Cumulative concentration response curve to 5HT (A, in Balb/c and B, in PTP1B KO mice) and SNP (C, in Balb/c and D, in PTP1B KO mice), in mice treated with prazosin and prazosin + leptin. Data are presented as mean ± sem.