Obesity

Increasing Peripheral Insulin Sensitivity by Protein Tyrosine Phosphatase 1B Deletion Improves Control of Blood Pressure in Obesity


Abstract—Obesity is a major risk factor for hypertension. The copresentation of hypertension and insulin resistance (IR) suggests a role for IR in blood pressure (BP) dysregulation. To test this hypothesis, peripheral IR has been genetically subtracted in a model of obesity by crossing leptin receptor mutant mice (K_{db}H_{PTP}) with mice lacking protein tyrosine phosphatase 1B (insulin desensitizer, H_{db}K_{PTP}) to generate obese insulin-sensitive mice (K_{db}K_{PTP}). BP was recorded in lean (H_{db}H_{PTP}, H_{db}K_{PTP}) and obese (K_{db}H_{PTP}, K_{db}K_{PTP}) mice via telemetry, and a frequency analysis of the recording was performed to determine BP variability. Correction of IR in obese mice normalized BP values to baseline levels (H_{db}H_{PTP}: 116±2 mmHg; K_{db}H_{PTP}: 129±4 mmHg; K_{db}K_{PTP}: 114±5 mmHg) and restored BP variability by decreasing its standard deviation and the frequency of BP values over the upper autoregulatory limit of the kidneys. However, although IR-induced increases in proteinuria (versus 53±13 μg/d, H_{db}H_{PTP}) were corrected in K_{db}K_{PTP} (112±39 versus 422±159 μg/d, K_{db}K_{PTP}), glomerular hypertrophy was not. IR reduced plasma aldosterone levels ruling out a role for mineralocorticoids in the development of hypertension. Taken together, these data indicate that correction of IR prevents hypertension, BP variability, and microalbuminuria in obese mice. Although the mechanism remains to be fully determined, increases in aldosterone or sympathoactivation of the cardiovascular system seem to be less likely contributors. (Hypertension. 2012;60:1273-1279.)

Key Words: aldosterone ■ obese mice ■ db/db mice ■ pressure variability

Hypertension is a major risk factor for cardiovascular disease, affecting one third of the American population. Although obesity has been clearly identified as a major risk factor for the development of hypertension, mechanisms by which obesity increases blood pressure (BP) are incompletely understood. Because hypertension and insulin resistance (IR) commonly present together in patients with obesity, IR has been presented as a potential link between metabolic dysfunctions induced by obesity and hypertension.

Whether and how IR might produce an increase in BP have been the subject of intense investigation. Supporting evidence stems from observations in rats on a high-sugar diet in which moderate increases in IR are associated with increases in tail cuff pressure. Moreover, insulin stimulates pathways that would be considered prohypertensive, such as sympathetic outflow or vasoconstriction in compromised beds. In contrast with these observations is the emerging evidence that high-sugar diets do not cause hypertension when pressure is assessed by catheter-based methods and BP effects of pharmacological modulators of insulin signaling are neutral, mixed, or lacking sufficient data to draw conclusions. The question of whether IR in the context of obesity elevates BP remains unanswered.

To address the role of IR in the development of hypertension associated with obesity, we generated an obese insulin-resistant mouse model by deleting the molecular restraint of the insulin signaling pathway, protein tyrosine phosphatase 1B (PTP1B), in leptin receptor–deficient mice (db/db). We hypothesized that improving insulin sensitivity without affecting body weight, in obese animals, would improve BP. Metabolic profiling was used to assess the degree of insulin sensitivity of the animals while radiotelemeter probes were implanted to record their BP. Potential mechanisms of hypertension were assessed by examining pressure variability, aldosterone production, and kidney injury.

Material and Methods

Animal Model

To study the cardiovascular consequences of correcting IR in obese mice, 4 groups of mice were generated by crossing obese leptin receptor–deficient (db/db) mice with mice presenting an increase

Received April 5, 2012; first decision May 3, 2012; revision accepted August 26, 2012.

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The online-only Data Supplement is available with this article at http://hyper.ahajournals.orglookup/suppl/doi:10.1161/HYPERTENSIONAHA.112.196295/-/DC1.

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Hypertension is available at http://hyper.ahajournals.org

DOI:10.1161/HYPERTENSIONAHA.112.196295

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in insulin sensitivity, thanks to the deletion of PTP1B.²⁶,²⁷ As previously described,³ this breeding strategy yields the following: (1) dual heterozygous littermates (HdbKPTP1B); (2) lean insulin-sensitive mice heterozygous for db but deficient in PTP1B (HdbKPTP1B); (3) obese insulin-resistant animals deficient in db and heterozygous for PTP1B (KdbKPTP1B); and (4) obese insulin-sensitive mice deficient in db and PTP1B genes (KdbHPTP1B). 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 protocols.

**Metabolic Measurements**

Details regarding plasma measurements of glucose, cholesterol, triglycerides, non-esterified fatty acids, insulin, leptin, and thyroid hormones can be found in the online-only Data Supplement.

**In Vivo Blood Pressure Measurement**

Details regarding BP measurements in conscious and unconscious mice can be found in the online-only Data Supplement.

**Vascular Adrenergic Tone**

Mesenteric arteries from another set of mice were isolated and mounted on a pressurized myograph to determine their contractile response to adrenergic stimulation (phenylephrine, 10 nmol/L to 100 μmol/L), as previously described.²⁸

**Renal Morphology**

Details regarding the analysis of the renal morphology can be found in the online-only Data Supplement.

**Statistical Analysis**

All data are presented as means±SEM. Differences in means among the groups for nonrepeated variables were compared by 1-way ANOVA. Differences in means among the 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**

**Indices of the Metabolic and Renal Function**

The effects of the deletion of the db and PTP1B genes on the mice phenotype were determined by measuring body weight and baseline plasma chemistry. As summarized in Table 1, obesity, induced by the deletion of the leptin receptor, was associated with a significant increase in fasting blood glucose, in the percentage of glycosylated hemoglobin, and in plasma insulin levels but also with increased circulating lipids levels (cholesterol, triglycerides, and non-esterified fatty acids).

Data from measurements in metabolic cages are reported in Table 2. Obesity was associated with a significant increase in food and water intake, independent of the presence or absence of the PTP1B gene, and likely leading to the increase in urinary output. Although improving insulin sensitivity by PTP1B deletion did not affect Na⁺ and K⁺ excretion in lean and obese mice, it significantly reduced albumin excretion, likely suggesting that improving insulin sensitivity in obese animals prevented kidney damage (Figure 1).

**Effects of Obesity and PTP1B Deletion on BP and Heart Rate**

The effects of obesity on BP were assessed with a 7-day continuous BP recording via radiotelemetry. As reported in Figure 2, obesity (KdbHPTP1B) induced a significant increased in continuous BP recording via radiotelemetry. As reported in Figure 2, obesity (KdbHPTP1B) induced a significant increase in MAP, as well as systolic and diastolic BP (Table 3), with no effect on circadian variation. Although the deletion of PTP1B in lean mice (HdbKPTP1B) did not affect BP, improving peripheral insulin sensitivity by the deletion of PTP1B in obese mice significantly improved BP in KdbKPTP1B mice. Indeed, despite obesity, KdbKPTP1B mice present an MAP.

**Table 1. General Characteristics of the 4 Groups of Mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>HdbKPTP1B</td>
<td>KdbKPTP1B</td>
</tr>
<tr>
<td></td>
<td>HdbHPTP1B</td>
<td>KdbHPTP1B</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>30±0.5</td>
<td>53±1*</td>
</tr>
<tr>
<td></td>
<td>31±0.5</td>
<td>50±1*</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>63±5</td>
<td>144±13*</td>
</tr>
<tr>
<td></td>
<td>67±4</td>
<td>131±10*</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>0.45±0.1</td>
<td>5.44±0.7*</td>
</tr>
<tr>
<td></td>
<td>0.54±0.1</td>
<td>4.68±0.6*</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>2.57±0.3</td>
<td>9.8±0.9*</td>
</tr>
<tr>
<td></td>
<td>1.38±0.3*</td>
<td>8.5±1.1</td>
</tr>
<tr>
<td>T3, ng/dL</td>
<td>18±3</td>
<td>23±1.4</td>
</tr>
<tr>
<td></td>
<td>20±1.2</td>
<td>25±1.3</td>
</tr>
<tr>
<td>T4, μg/dL</td>
<td>11.3±0.6</td>
<td>11.1±0.7</td>
</tr>
<tr>
<td></td>
<td>12.1±1.2</td>
<td>9.5±0.6</td>
</tr>
<tr>
<td>Intact PTH, pg/mL</td>
<td>16.8±2.1</td>
<td>15.1±4.1</td>
</tr>
<tr>
<td></td>
<td>14.4±2.2</td>
<td>19.4±4.8</td>
</tr>
<tr>
<td>C-reactive protein, ng/mL</td>
<td>76±5</td>
<td>69±4</td>
</tr>
<tr>
<td></td>
<td>80±3</td>
<td>72±4.4</td>
</tr>
<tr>
<td>Resistin, ng/mL</td>
<td>3.9±0.2</td>
<td>3.9±0.2</td>
</tr>
</tbody>
</table>

Data are means±SEM, n≥8 per group.

**Table 2. Metabolic Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HdbKPTP1B</td>
<td>KdbKPTP1B</td>
</tr>
<tr>
<td></td>
<td>HdbHPTP1B</td>
<td>KdbHPTP1B</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>2.5±0.5</td>
<td>5.0±1.9</td>
</tr>
<tr>
<td></td>
<td>2.0±0.6</td>
<td>4.6±0.7</td>
</tr>
<tr>
<td>Water intake, mL/d</td>
<td>3.9±0.3</td>
<td>9.0±2.0*</td>
</tr>
<tr>
<td></td>
<td>3.5±0.8</td>
<td>8.0±1.5*</td>
</tr>
<tr>
<td>Urine volume, mL</td>
<td>1.0±0.2</td>
<td>4.7±1.7*</td>
</tr>
<tr>
<td></td>
<td>0.8±0.2</td>
<td>5.0±1.2*</td>
</tr>
<tr>
<td>Na⁺ excretion, mg/d</td>
<td>2.1±0.6</td>
<td>4.3±1.0*</td>
</tr>
<tr>
<td></td>
<td>1.6±0.4</td>
<td>4.8±1.2*</td>
</tr>
<tr>
<td>K⁺ excretion, mg/d</td>
<td>13.4±3.9</td>
<td>36.3±8.8*</td>
</tr>
<tr>
<td></td>
<td>10.4±2.9</td>
<td>38.2±10.2*</td>
</tr>
</tbody>
</table>

Data are means±SEM, n≥8 per group.

*P<0.05 vs HdbKPTP1B.
as well as a systolic and diastolic BP, that are similar to the BP of the lean mice (HdbHPTP1B and HdbKPTP1B). Heart rate was neither affected by obesity nor by the deletion of PTP1B (Table 3). The distribution of the MAP values was analyzed over the 7-day BP recording and plotted on Figure 3. As represented in Figure 3A, and summarized in Table 3, lean control mice (HdbHPTP1B) present a Gaussian distribution of their MAP distribution centered around a value of 114±2 mmHg. Obesity (KdbHPTP1B) not only induced a shift of the MAP distribution toward higher MAP values but also increased the SD of the MAP and the frequency of the MAP values >140 mmHg (Table 3). This suggests that the vasculature and the kidney of the KdbHPTP1B mice are more often submitted to higher pressure values, likely above the limits of autoregulation. Restoration of insulin sensitivity in obese mice with the deletion of PTP1B completely restored the distribution of the MAP. The deletion of PTP1B in lean mice did not affect the distribution of the MAP (Figure 3B), confirming that the effects observed are not the direct consequence of the deletion of PTP1B.

### Sympathetic and Vascular Adrenergic Tone

Sympathetic contribution to BP control was determined by measuring BP response to ganglionic blockade. As reported in Table 3, obesity increased BP response to mecamylamine, supporting a sympathoactivation. Increasing insulin sensitivity with PTP1B deletion did not affect sympathetic control of BP in obese or in lean animals. Indices of sympathetic tone were also obtained by measuring vascular adrenergic tone. As represented in Figure 4, obesity reduced mesenteric adrenergic constriction to phenylephrine consistent with the aforementioned sympathoactivation. Correction of IR in obese mice did not restore vascular adrenergic tone, suggesting that obesity-induced sympathoactivation is not driven by IR. PTP1B deletion in lean mice did not affect vascular adrenergic reactivity, ruling out a direct effect of PTP1B on mesenteric adrenergic tone.

### Plasma Aldosterone Levels

Because the renin-angiotensin-aldosterone system (RAAS) has been reported to be activated in obesity,10,20 we measured plasma aldosterone levels in our 4 groups of mice. As reported in Figure 5, obese insulin-resistant mice (KdbHPTP1B) showed a reduction in plasma aldosterone levels compared with HdbHPTP1B mice, consistent with increased pressure and salt intake. Deletion of PTP1B did not affect plasma aldosterone levels in lean HdbHPTP1B mice but restored its levels in obese KdbKPTP1B mice, suggesting that the decrease observed in obese mice likely reflects increased BP rather than salt intake in hyperphagic mice.

### Renal Morphology

Histomorphological analysis of the kidneys revealed that obesity (KdbHPTP1B) significantly affects renal structure by increasing glomerular and matrix areas. PTP1B deletion neither affected renal structure in lean mice (HdbHPTP1B) nor restored it in obese insulin-sensitive mice (KdbKPTP1B) (Figure 6).

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**Table 3.** Baseline Cardiovascular Parameters, Blood Pressure Response to Ganglionic Blockade, and Statistical Descriptors of Individual Frequency Distribution Curves From HdbHPTP1B, HdbKPTP1B, KdbHPTP1B, and KdbKPTP1B Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean HdbHPTP1B</th>
<th>Lean HdbKPTP1B</th>
<th>Obese KdbHPTP1B</th>
<th>Obese KdbKPTP1B</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>114±2</td>
<td>115±2</td>
<td>127±2*</td>
<td>127±2*</td>
</tr>
<tr>
<td>DBP</td>
<td>105±3</td>
<td>104±3</td>
<td>114±2*</td>
<td>114±2*</td>
</tr>
<tr>
<td>SBP</td>
<td>124±3</td>
<td>119±4</td>
<td>133±3*</td>
<td>125±3</td>
</tr>
<tr>
<td>HR</td>
<td>522±15</td>
<td>551±16</td>
<td>488±9</td>
<td>492±18</td>
</tr>
</tbody>
</table>

Response to ganglionic blockade

| Δ MAP, %        | −26±3          | −31±2          | −39±4*           | −35±4*          |

Statistical descriptors of frequency distribution curves (Figure 2)

| Skew            | −0.06±0.15     | −0.79±0.44     | −0.22±0.35       | 0.17±0.05       |
| Frequency >140  | 14.6±0.8       | 13.1±3.5       | 21.4±2.2*        | 14.1±1.7†       |

MAP indicates mean arterial pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; HR, heart rate.

*P<0.05 vs HdbHPTP1B.
†P<0.05 vs KdbHPTP1B.
The goal of the present study was to determine whether restoring insulin sensitivity in obese mice would prevent the development of hypertension associated with obesity. We observed the following: (1) obesity is associated with reduced insulin sensitivity and impaired control of BP, (2) deletion of the molecular restraint of insulin signaling pathway (PTP1B) improves peripheral insulin sensitivity in obese mice and prevents increases in BP and BP variability, and (3) mechanisms of this improvement are likely independent of thyroid status, the RAAS, and renal injury. Relevant to these observations are the concepts of IR and its role in the development of hypertension.

**Model**

The metabolic dysfunction associated with obesity is characterized by a loss of sensitivity to insulin in metabolically active tissues. The pervasiveness of this observation along with significant levels of hypertension in these obese populations has led to the intriguing hypothesis that IR is indeed the root cause of hypertension in obesity. Hyperinsulinemia is highly correlated with hypertension in human patients and has been shown to raise BP in insulin-resistant rats. However, controversies have emerged, including the lack of effects in obese insulin-resistant dogs and rats. Previously, we generated an insulin-sensitive mouse model despite obesity by deleting the molecular restraint of the insulin signaling pathway (PTP1B). Deletion of PTP1B did not affect body weight but significantly improved peripheral insulin sensitivity, as reflected by the reduced glycosylated hemoglobin, improved glucose clearance, and reduced levels of circulating lipids. However, PTP1B deletion in db/db mice does not correct fasting blood glucose, suggesting that these mice retain a defect in their hepatic gluconeogenesis that likely explains the persistent hyperglycemia observed in the KdbKPTP1B mice. The deletion of PTP1B did not affect the metabolic profile or the BP of the lean mice. A previous study from our group demonstrated that PTP1B deletion only modifies leptin-mediated control of BP in mice on a Balb/C background. Therefore, the mouse model generated, on a C57Bl/6 background, provides us an important tool to assess the role of insulin and IR in hypertension related to obesity without the confounding effects of changes in body weight or effects of PTP1B on leptin signaling.

**Insulin Sensitivity and BP Regulation**

In the present study, we observed that our obese insulin-resistant mice (KdbHPTP1B) not only develop hypertension but also present an increase in BP variability. As a consequence, the distribution of their BP values is shifted toward...
Obesity has long been associated with activation of the RAAS.19,20 In the current study, we found that aldosterone, a major end-product of the RAAS, is not increased but rather decreased. This finding is consistent with previous studies of obesity showing reduced renin and aldosterone in non-diabetic rodent models of obesity28 and also consistent with humans in obesity showing reduced renin and aldosterone in non-diabetic decreased. This finding is consistent with previous studies of major end-product of the RAAS, is not increased but rather the increase in BP observed in the KdbHPTP1B mice. We further functional consequence (the development of kidney damage) to the increase in BP observed in the K-db-HPTP1B mice. We further demonstrated that restoring insulin sensitivity in the obese K-db-KPTP1B mice prevented the development of hypertension associated with obesity and completely restored BP variability. This improvement occurred despite no improvement in the structural effects of obesity and metabolic dysfunction on the kidney, indicating that the improvement in albumin excretion likely reflects the improvement of BP. Finally, the analysis of the cardiovascular phenotype of the K-db-KPTP1B mice did not reveal any effects of PTP1B deletion on the BP and BP variability of the lean mice, ruling out a direct effect of PTP1B deletion on BP regulation. Taken together, these data support the hypothesis that, in the context of obesity, the IR state is a major determinant of altered regulation of BP.

Potential Mechanisms
The central hypothesis of the current study is that IR is a contributing mechanism to impaired regulation of BP in obesity. This hypothesis is supported by the primary data of the study but the mechanisms are only indirectly revealed.

Aldosterone
Obesity has long been associated with activation of the RAAS.19,20 In the current study, we found that aldosterone, a major end-product of the RAAS, is not increased but rather decreased. This finding is consistent with previous studies of obesity showing reduced renin and aldosterone in non-diabetic rodent models of obesity28 and also consistent with humans in early stages of obesity in which salt-insensitive increases in BP are evident.29 Moreover, aldosterone is restored to normal levels when IR is corrected by deletion of PTP1B, suggesting that the decrease in aldosterone was simply a reflection of the increase in BP, because sodium intake was similar between groups. Although aldosterone does not account for all of the pressor actions of angiotensin,30 there were also no differences in sodium excretion between obese groups of mice, whether IR was corrected or not. Thus, although an effect of angiotensin independent of aldosterone cannot be entirely ruled out as a mechanism, it would require a pathway with no impact on sodium balance. The simplest conclusion, therefore, is that the increase in BP and its normalization with deletion of PTP1B does not involve the RAAS to a major extent.

Renal Injury
Another common hemodynamic insult in obesity and metabolic disease is damage to the kidney, reflected by glomerular injury, matrix expansion, and the presence of protein in the urine.31 In the current study, we observed that the combination of high BP and hyperglycemia resulted in all 3 of these insults but that only microalbuminuria was resolved by correction of IR. From these observations, we conclude that IR as manifested in elevated lipids and glycosylated hemoglobin is not the root cause of an atomic injury to the kidney. Because fasting hyperglycemia persists in these obese mice with deletion of PTP1B, this finding is consistent with clinical observations that aggressive management of blood glucose limits diabetic nephropathy.32 The correction of albuminuria may reflect the correction of BP rather than a direct effect on glomerular function. Thus, although it is clear that the kidney is damaged in the obese, metabolically compromised state, this injury may be the resultant rather than the cause of derangements in BP control.

Sympathetic Tone
A third major component of the cardiovascular dysfunction associated with obesity is the overactivation of the sympathetic nervous system. Consistent with previous studies performed with obese patients or animal models of obesity, we find that obesity enhances sympathetic contribution to BP. Indeed, an increased BP response to ganglionic blockade was observed in K-db-HPTP1B and K-db-KPTP1B mice (Table 3). Obesity-induced sympathoactivation was further supported by a vascular adrenergic escape reported in mesenteric artery (Figure 4). As we and others documented previously,18,26,33,34 sympathoactivation is associated with reduced vascular adrenergic tone. In the present study, we made the singular observation that correction of peripheral IR does not prevent sympathoactivation. These
data minimalize the role of sympathoactivation in the development of hypertension associated with obesity and suggest that IR is not the factor triggering sympathoactivation in mice. Nevertheless, these data do not rule out a role for insulin, per se, in sympathoactivation. Indeed, whereas PTP1B deletion corrected peripheral IR, it did not restore insulin levels in obese mice. This is consistent with studies reporting that hyperinsulinemia increases sympathetic tone but not BP in human9,10,39 and dogs32 but in contradiction with studies demonstrating that hyperinsulinemia raises BP although as a sympathomediated mechanism in rats.21,36 These data present mice as a better model than rats to study the role of insulin and IR in the development of obesity-induced hypertension.

Nitric Oxide

Another pathway implicated in the cardiovascular effects of obesity is a reduction in the actions or production of nitric oxide (NO). In consideration of cardiovascular control, the 2 primary sites of NO action are in the vasculature, where it regulates peripheral resistance,37,38 and in the kidney, where it regulates salt balance.39 An extensive characterization of our mouse model19 discovered that NO-mediated dilation is sharply curtailed in K_\text{dbHPTP1B} mice, an effect almost completely corrected by deletion of PTP1B. Vascular NO has been documented to be an important contributor of BP variability in animals,40 and humans,41,42 and BP variability, most likely because of progressive renal injury, contributes to fixed hypertension.43

The concept that loss of vascular NO is the cause of obesity-related increases in BP is further supported by studies in other disease states and conditions. Brands et al44 have documented that type 1 diabetes mellitus fails to produce hypertension unless NO production is blocked, despite the significant renal injury present in this model. Moreover, recent work from do Carmo et al45 demonstrated that peripheral NO was essential to prevent hypertension with activation of the melanocortin system.

In the present study, we demonstrate that IR plays a key role in the loss of BP control, both mean and variability, related to obesity. In this experimental model, obesity in young animals produces these defects without involvement of aldosterone or other endocrine deficits. A survey of potential mechanisms suggests that BP variability associated with the loss of vascular NO is a likely culprit, especially when superimposed on a compromised kidney. Correction of NO-mediated dilation restores BP control. We conclude that, at least during early stages of obesity and metabolic dysfunction, correction of IR would be an important step in the prevention of long-term hypertension. The extent to which IR is involved in more established hypertension associated with more pervasive renal damage remains to be determined.

Perspectives

The growing epidemic of obesity and type II diabetes mellitus increases the need for pharmacological therapies to prevent body weight gain and its adverse effects on the metabolic and cardiovascular functions. Because of its key role in the control of insulin and leptin signaling, the PTP1B is suggested to be a key therapeutic target for the treatment of obesity and type II diabetes mellitus. The potential of this therapeutic target has been greatly enhanced by the demonstration that PTP1B KO mice are protected against obesity and type II diabetes mellitus. However, whether PTP1B inhibition could be beneficial for the cardiovascular function remained to be fully elucidated. In the present study, we tested the hypothesis that improving insulin sensitivity, by the deletion of PTP1B, will prevent the development of hypertension associated with obesity. By deleting PTP1B in leptin receptor–deficient mice, we demonstrated that improving peripheral insulin sensitivity is sufficient to prevent obesity-induced hypertension in mice. Although further studies are still needed to determine the precise mechanisms involved, this study clearly supports the role of PTP1B as a potential therapeutic target against obesity-related cardiovascular dysfunction and presents IR as a key component of the development of hypertension associated with obesity.

Acknowledgments

We acknowledge the helpful editorial assistance of J. Blake Norman in the preparation of this article.

Sources of Funding

This work was supported by the National Institutes of Health (R01, Dr Stepp) and the American Heart Association (11SDG5060006, Dr Belin de Chantemèle).

Disclosures

None.

References


**Novelty and Significance**

**What Is New?**

The new information in the current study is as follows:

- Correction of insulin signaling in muscle and fat of obese mice via deletion of protein tyrosine phosphatase 1B normalizes elevated blood pressure.
- Obese mice have reduced aldosterone levels that are driven by the increased pressure, not their elevated salt intake.
- Obese mice have renal injury and sympathoactivation but this cannot be explained by improved in peripheral insulin sensitivity.
- The risk factors that determine increased blood pressure in obese individuals remain poorly understood. These data indicate that some component of the insulin-resistant state is driving increases in blood pressure in a manner that does not related to increased aldosterone, sympathetic nervous system activity, or physical injury to the kidney. Exact mechanisms are likely complex and remain to be fully elucidated.

**Summary**

Obesity increases arterial pressure in a manner that is driven in part by the metabolic defects caused by obesity. Correcting these defects, perhaps with drugs that target protein tyrosine phosphatase 1B, may improve hypertension in patients with obesity.
Increasing Peripheral Insulin Sensitivity by Protein Tyrosine Phosphatase 1B Deletion Improves Control of Blood Pressure in Obesity

Hypertension. 2012;60:1273-1279; originally published online October 8, 2012; doi: 10.1161/HYPERTENSIONAHA.112.196295

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Increasing peripheral insulin sensitivity by PTP1B deletion improves control of blood pressure in obesity

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Short title: Correction of hypertension in db/db mice

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Material and Methods

Animal model
To study the cardiovascular consequences of correcting insulin resistance in obese mice, four groups of mice were generated by crossing obese leptin receptor deficient (db/db) mice with mice presenting an increased in insulin sensitivity thanks to the deletion of the molecular restraint of the insulin signaling pathway: the protein tyrosine phosphatase 1B (PTP1B, Goodman Cancer Center of McGill University). Because db/db mice are sterile, progeny were generated from dual heterozygotes (H_{db}, heterozygous for mutant leptin receptor, H_{PTP1B}, heterozygous for PTP1B gene). As previously described, this breeding strategy yielded: i) dual heterozygous littermates (H_{db}H_{PTP1B}), used as lean control, ii) lean insulin sensitive mice heterozygous for db but deficient in PTP1B (H_{db}K_{PTP1B}), iii) obese insulin resistant animals deficient in db and heterozygous for PTP1B (K_{db}H_{PTP1B}) and iii) obese insulin sensitive mice deficient in db and PTP1B genes (K_{db}K_{PTP1B}). Males only were used for the study. 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 protocols.

Metabolic Measurements:
Fasting blood glucose was assessed using a glucometer (Medisense, Bedford, MA, USA). Plasma total cholesterol, triglycerides and NEFA were assessed with colorimetric assays (Wako, Richmond, VA, USA). Plasma insulin, leptin, T3, T4, PTH, C-Reactive Protein and Resistin levels were determined using colorimetric assays from ALPCO Diagnostics (Salem, NH, USA). Plasma aldosterone levels were measured by radioimmunoassay (Siemens Medical Diagnostic Deerfield, IL). A different set of mice was used to measure food and water intake as well as urine electrolytes concentration and albuminuria (Elisa kit, ALPCO Diagnostics, Salem, NH, USA). Mice form the 4 groups were placed in metabolic cages. After 3 days of acclimation to the cages, food and water consumption were determined and urine collected for 24 hours.

In vivo blood pressure measurement
At 10-12 weeks of age, mice were instrumented with telemetry transmitters to record blood pressure (BP) and heart rate (PA-C10, Data Sciences, SaintPaul, Minn). Transmitters were implanted as described previously. 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. After one week of treatment, mice were euthanized. Tissues and plasma were collected for later analysis. Blood pressure values were obtained at 10 minute intervals for the duration of the study. Mean values were collected from 24-hour averages. To assess variability in blood pressure, frequency analysis was performed on 7 complete days of telemetry recording (~1400 pts) and the standard deviation of the data as well as the frequency of blood pressure over 140 mmHg were compared. 140 mmHg was selected as a reference for the limit of autoregulation in many vascular beds and a likely threshold of tissue injury. In a separate set of mice, the carotid artery and jugular vein were catheterized under isoflurane anesthesia to measure BP response to ganglionic blockade, as previously described.
Renal Morphology
Renal structural alterations were analyzed by the UW Pathology Research Services Laboratory. Briefly, kidneys obtained from HdbHPTP1B, HdbKPTP1B, KdbHPTP1B and KdbKPTP1B mice were immersion-fixed in 10% neutral-buffered formalin. Tissues were embedded in paraffin using standard methods; sectioned (2 μm); and stained with silver methenamine. For each animal, section areas were randomly photographed under ×100 magnification. The glomerular cross-sectional area and the amount of silver stained matrix were measured in 15 glomeruli from each animal. The percentage of matrix was calculated using ImagePro Plus image analysis software as described previously6, 7.

Reference: