Loss of PDGF-B activity increases hepatic vascular permeability and enhances insulin sensitivity

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Raines SM, Richards OC, Schneider LR, Schueler KL, Rabaglia ME, Oler AT, Stapleton DS, Genovie G, Dawson JA, Betsholtz C, Attie AD. Loss of PDGF-B activity increases hepatic vascular permeability and enhances insulin sensitivity. Am J Physiol Endocrinol Metab 301: E517–E526, 2011. First published June 14, 2011; doi:10.1152/ajpendo.00241.2011.—Hepatic vasculature is not thought to pose a permeability barrier for diffusion of macromolecules from the bloodstream to hepatocytes. In contrast, in extrahepatic tissues, the microvasculature is critically important for insulin action, because transport of insulin across the endothelial cell layer is rate limiting for insulin-stimulated glucose disposal. However, very little is known concerning the role in this process of pericytes, the mural cells lining the basolateral membrane of endothelial cells. PDGF-B is a growth factor involved in the recruitment and function of pericytes. We studied insulin action in mice expressing PDGF-B lacking the proteoglycan binding domain, producing a protein with a partial loss of function (PDGF-B<sup><sub>revert</sub></sup>). Insulin action was assessed through measurements of insulin signaling and insulin and glucose tolerance tests. PDGF-B deficiency enhanced hepatic vascular transendothelial transport. One outcome of this change was an increase in hepatic insulin signaling. This correlated with enhanced whole body glucose homeostasis and increased insulin clearance from the circulation during an insulin tolerance test. In obese mice, PDGF-B deficiency was associated with an 80% reduction in fasting insulin and drastically reduced insulin secretion. These mice did not have significantly higher glucose levels, reflecting a dramatic increase in insulin action. Our findings show that, despite already having a high permeability, hepatic transendothelial transport can be further enhanced.

The liver displays a unique sinusoidal structure, characterized by gaps between endothelial cells, large fenestrae, and lack of a well-formed basement membrane. Fenestrae occupy 10% of the sinusoidal surface and are organized into clusters, termed “sieve plates.” They connect the sinusoidal lumen directly to the space of Disse, which lines the basolateral surface of the endothelium and interacts with hepatocyte cell membranes and microvilli (4). These fenestrated discontinuous hepatic sinusoids are highly permeable and allow particles smaller than 100 nm to freely diffuse into the space of Disse (34). Therefore, unlike muscle, the liver vasculature is considered not to be rate limiting for nutrient transport under normal circumstances.

However, hepatic nutrient transport out of the bloodstream and diffusion through the space of Disse is hindered during certain pathological conditions. “Capillarization” is the process by which hepatic sinusoids undergo morphological changes that include sinusoidal fenestration, the development of basement membrane, and excessive distortion of the space of Disse due to extracellular matrix (ECM) deposition (34). These ultrastructural changes result from the activation of liver pericytes, also termed hepatic stellate cells (HSCs) (45), and PDGF-B signaling can induce HSC activation (20). Capillarization-like phenotypes have been noted in fibrosis (34), aging (34), and type 1 diabetes (8, 9, 30, 33). Defenestration and perisinusoidal fibrosis are also present in nonalcoholic fatty liver disease.
liver disease (NAFLD) and nonalcoholic steatohepatitis, the two most common manifestations of hepatic insulin resistance (22). Capillarization can impede the delivery of nutrients and small molecules to hepatocytes (15, 34), although hepatic insulin delivery has not been examined under these conditions.

Few studies have examined the effects of PDGF-B signaling and pericytes in insulin action. PDGF-B signaling can induce glucose transporter 4 translocation in both cultured adipocytes (52) and mouse skeletal muscle (55), but only under conditions of PDGFRβ overexpression. The effect of PDGF-B on insulin action in hepatocytes has not been examined, although the low endogenous expression of PDGFRβ in these cells suggests that PDGF-B would have little direct effect on hepatocyte function (46) but rather through modulation of pericytes and the vasculature.

One pathway potentially modulated by PDGF-B is the maintenance of vascular barrier function. Mice with reduced pericyte coverage of the vasculature display widespread and often lethal vascular hemorrhage and edema (27, 28, 35, 36, 47). Loss of pericyte retention on blood vessels in tumors leads to vascular leakage, with an increased potential for tumor cell metastasis (1, 53). Pericytes also play a critical role in the development and maintenance of the blood-brain barrier, and pericyte deficiency both increases the permeability of the blood-brain barrier (5, 7, 21) and reduces brain capillary perfusion (7). Diabetic retinopathy is characterized by excessive vascular permeability in the retina and is caused by continual pericyte loss over time (25). Finally, high doses of Gleevec, a pharmacological inhibitor of PDGF-B signaling, lead to increased risk of edema and interstitial leakage of fluid (31). These data led us to hypothesize that reduced PDGF-B signaling may increase vascular permeability and thereby improve insulin delivery to its target cells.

To address this hypothesis, we studied PDGF-B retention-deficient (PDGF-B<sup>ret/ret</sup>) mice. PDGF-B is secreted as a homodimer from endothelial cells, where it is retained on the cell surface by a COOH-terminal heparan-sulfate proteoglycan-binding retention motif (40). Deletion of this motif in the PDGF-B<sup>ret/ret</sup> mice leads to decreased retention of PDGF-B and a reduction in PDGF-B signaling (40, 43). PDGF-B<sup>ret/ret</sup> mice display a loss of pericyte density, pericyte detachment, and abnormal capillary morphology in the developing brain, kidney, and retina as well as the postnatal kidney and retina (37).

In the present study, we demonstrate that impaired PDGF-B signaling enhances vascular transendothelial transport in the liver. The resulting elevations in hepatic insulin signaling lead to improved whole body glucose clearance. To metabolically challenge these animals, we bred the Leptin<sup>ob</sup> (Lept<sup>ob</sup>) mutation (56) into the PDGF-B<sup>ret/ret</sup> background to generate obese PDGF-B retention-deficient mice. Despite an 88% reduction in plasma insulin, the mice had normal glucose levels, reflecting a drastic improvement in insulin action. These phenotypes appear to result from elevated insulin transport and signaling rather than a loss of β-cell secretory capacity. To the best of our knowledge, this represents the first study that links reduced PDGF-B signaling to increased insulin action and establishes that hepatic vasculature can be made even more permeable.

**RESEARCH DESIGN AND METHODS**

**Animals.** CS7BL/6J (B6) Lept<sup>ob/+</sup> animals were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at the University of Wisconsin-Madison. PDGF-B<sup>ret/ret</sup> Lept<sup>ob/ob</sup> mice were created by crossing B6 Lept<sup>ob/+</sup> PDGF-B<sup>ret/ret</sup> (37) and B6 Lept<sup>ob/+</sup> PDGF-B<sup>+/+</sup> mice. For all studies, either wild-type or PDGF-B<sup>ret/ret</sup> littermates (indistinguishable from wild type) were used as controls. Either Lept<sup>ob/+</sup> or Lept<sup>ob/ob</sup> mice were used in studies involving lean mice. Mice were housed in an environmentally controlled facility (12:12-h light-dark cycles) with free access to food (Purina) and water. All animal procedures were approved by the University of Wisconsin Animal Care and Use Committee.

**FITC-dextran leakage.** Mice were anesthetized with a 250 mg/kg dose of Avertin. The right jugular was cannulated and injected with 200 ml of 2 mg/ml lysine-fixable FITC-dextran [10 kDa molecular weight (MW); Invitrogen]. The FITC-dextran circulated for 30 min. Frozen 12-μm sections were blocked with 5% donkey serum and stained overnight at +4°C with 1:50 rat anti-platelet endothelial cell adhesion molecule-1 (PECAM-1) antibody (BD Pharmingen) to mark the vasculature. Sections were stained with 1:100 Cy3-conjugated donkey anti-rat secondary antibody (Jackson ImmunoResearch). Confocal images were analyzed using Metamorph software in a blinded manner, and FITC-dextran associated with the vasculature was quantified as a signal that colocalized with PECAM-1. Extravascular FITC-dextran intensity was quantified as a signal that did not colocalize with PECAM-1.

**Pericyte coverage immunohistochemistry.** Two frozen 10-μm sections/animal were blocked with 5% donkey serum containing 1% Triton X-100 and stained overnight at +4°C with 1:50 rat anti-PECAM-1 antibody (as described in FITC-dextran leakage) and 1:100 rabbit anti-desmin antibody (Abcam) to mark HSCs. Sections were stained with anti-rat (above) or 1:100 FITC-conjugated goat anti-rabbit (Jackson ImmunoResearch) secondary antibodies. We imaged three random images per section, totaling six images per animal. Pericyte coverage was calculated as the desmin area divided by the PECAM-1 area per animal as determined by Metamorph.

**Transmission electron microscopy of liver sinusoids.** Sections were cut and contrasted with 8% uranyl acetate in 50% ethanol for 7 min at room temperature, followed by Reynolds lead citrate for 5 min at room temperature. Images of hepatic sinusoids were taken at 8,800 magnification on a Philips CM120 transmission electron microscope. Sinusoids were scored in a blinded manner as “intact” (fenestrated with a well-formed space of Disse) or “disrupted” (defenestrated with a disorganized, electron-dense space of Disse). Results are presented as intact or disrupted sinusoids as a percentage of total images analyzed per animal.

**Insulin signaling Western blots.** Mice were given a 5-min portal vein injection of 5 U of insulin to measure insulin receptor-β (IRβ) signaling or were injected with 0.1 U/kg insulin via jugular vein for 15 min to measure Akt signaling. Livers were lysed on ice for 30 min in 50 mM HEPES buffer (pH 7.4) containing 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, and 10% glycerol and supplemented with a protease and phosphatase inhibitor cocktail (Roche). Supernatants were collected via centrifugation at 15,000 x g for 20 min, and protein concentration was measured by BCA assay.

Total liver homogenates (200 mg for IRβ, 50 mg for Akt) were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and blotted with a mouse anti-phosphotyrosine antibody (1:1,000; Millipore) for IRβ or a mouse anti-phospho-Akt (Ser<sup>473</sup>) antibody (1:1,000; Cell Signaling Technology) overnight. To detect total protein, blots were stripped and probed overnight with a rabbit anti-IRβ antibody (1:1,000; Santa Cruz Biotechnology), or a rabbit anti-Akt antibody (1:1,000; Cell Signaling Technology).

**2-[<sup>14</sup>C]deoxyglucose uptake.** Nonfasted animals were injected via right jugular cannulation with 0.1 U/kg insulin, 2 g/kg glucose, and 300 μCi/kg 2-[<sup>14</sup>C]deoxy-D-glucose (PerkinElmer). Mice were bled 15 min after tracer injection, and organs were snap-frozen.

Plasma was precipitated with 0.3 N NaOH and 0.3 N Zn(SO)<sub>4</sub> and centrifuged for 5 min at high speed. Glucose-specific activity (disintegrations·min<sup>-1</sup>·mg<sup>-1</sup>) was calculated by dividing the supernatant radioactivity (disintegrations·min<sup>-1</sup>·ml<sup>-1</sup>) by the plasma glucose concentration (mg/ml).
Tissues (50 mg) were homogenized in 0.5% perchloric acid and centrifuged for 20 min at 2,000 g. Radioactivity was counted from the initial supernatant (supernatant 1) and the supernatant following Ba(OH)₂ and Zn(SO)₄ precipitation (supernatant 2). 2-[¹⁴C]deoxyglucose radioactivity (disintegrations·min⁻¹·mg⁻¹) was calculated as the difference between supernatants 1 and 2 divided by the sample weight. This value, divided by the glucose-specific activity, generated the amount of 2-[¹⁴C]deoxyglucose phosphate per tissue (mg ¹⁴C/mg tissue).

Fig. 1. Enhanced vascular leakage resulting from reduced sinusoidal disruption in platelet-derived growth factor B (PDGF-B) ret/ret liver. Eight-week-old male Leptinob (Lepob/+) control (A) and PDGF-B ret/ret mice (B) were infused with FITC-dextran (green; molecular weight = 10 kDa) for 30 min. After circulation, livers were stained for platelet endothelial cell adhesion molecule-1 (PECAM-1; red) and imaged via confocal microscopy. Scale bars, 50 μm. C: the intensity of extravascular FITC-dextran was quantified. Liver sections from 8-wk-old male Lepob/+ wild-type (D) and PDGF-B ret/ret mice (E) were stained for PECAM-1 (red) and desmin (green) and imaged via confocal microscopy. Scale bars, 75 μm. F: hepatic stellate cell (HSC) coverage of liver vessels was quantitated. G and H: transmission electron microscopy (TEM) of 8-wk-old male Lepob/+ wild-type (G) and PDGF-B ret/ret liver sinusoids (H). Scale bars, 2 μm; RBC, red blood cell; KC, Kupffer cell; SD, space of Disse. Arrows represent fenestrae and endothelial cell gaps. I: sinusoidal disruption was quantified as loss of fenestrae and a disordered, electron-dense SD. All results are presented as means ± SE. *P < 0.05 by unpaired Student t-test.
Insulin and glucose tolerance tests and glucose-stimulated insulin secretion from isolated islets. Insulin tolerance tests were performed on nonfasted animals. Blood samples were drawn before insulin injection (Novolin R; Novo Nordisk) and at 5, 15, 30, 60, and 120 min after intraperitoneal injection. Lep<sup>+/−</sup> mice were given 0.1 U/kg body wt insulin in sterile saline, whereas Lep<sup>−/−</sup> mice were given 20 U/kg body wt. Human plasma insulin was measured using a radioimmunoassay (Millipore).

Glucose tolerance tests were performed on mice fasted overnight. Glucose (2 mg/g body wt in sterile saline) was administered intraperitoneally. Blood samples were drawn before glucose injection and at 15, 30, 60, 120, 240, and 360 min after injection. Plasma C-peptide levels were measured using a radioimmunoassay (Linco-Millipore).

In vitro insulin secretion assays were performed as described previously (42).

Statistical analysis. Fasting plasma insulin values did not have a normal distribution and were thus analyzed using a Mann-Whitney test. Area under the curve was determined for insulin and glucose tolerance tests and analyzed using unpaired Student t-tests. Curves were not corrected for baseline in these analyses. Individual time points during the insulin and glucose tolerance tests were analyzed by unpaired Student t-tests without adjusting for multiple comparisons;

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Fig. 2. Increased insulin signaling and glucose clearance in PDGF-B<sup>−/−</sup> liver. A: representative Western blots of insulin receptor-β (IRβ) and Akt phosphorylation. Phosphotyrosine and total IRβ were measured in 8-wk-old Lep<sup>−/−</sup> male wild-type and PDGF-B<sup>−/−</sup> liver 5 min after insulin treatment. Phosphoserine 473 (Ser473) on Akt and total Akt were measured in 9- to 10-wk-old lean female wild-type and PDGF-B<sup>−/−</sup> liver mice 15 min after insulin treatment. B: quantification of phosphorylation signal relative to total protein. C: quantification of glucokinase and glucose-6-phosphatase mRNA expression from 8-wk-old Lep<sup>−/−</sup> wild-type and PDGF-B<sup>−/−</sup> livers. D: hepatic glucose uptake in 8-wk-old lean female wild-type and PDGF-B<sup>−/−</sup> liver. Insulin, glucose, and 2-[14C]deoxyglucose tracer were injected into the jugular vein, and accumulation of hepatic 2-[14C]deoxyglucose phosphate was measured after 15 min of circulation. E: plasma glucose during insulin tolerance tests on 8-wk-old female wild-type (n = 6) and PDGF-B<sup>−/−</sup> (n = 6) mice. All results are presented as means ± SE. *P ≤ 0.05, ***P < 0.001 by unpaired Student t-test; #P ≤ 0.05 by area under the curve analysis through 30 min.
during the insulin tolerance tests, raw (not normalized to time 0) glucose values were used for this analysis. All other studies were analyzed using unpaired Student t-tests.

RESULTS

**PDGF-B<sup>−/−</sup>** livers display increased vascular permeability. To study vascular permeability in liver, gastrocnemius muscle, and heart, we carried out intrajugular injection of 10 kDa FITC-dextran. The most striking difference was found in liver, where after only 30 min of circulation, we observed an approximately threefold increase in FITC-dextran accumulation in PDGF-B<sup>−/−</sup> relative to PDGF-B<sup>+/+</sup> control mice (Fig. 1, A–C). Of the accumulated FITC-dextran, 38% remained associated with the vasculature in control liver compared with only 14% in PDGF-B<sup>−/−</sup> liver, suggesting more rapid diffusion of dextran out of the vascular space in PDGF-deficient mice. We did not observe leakage in skeletal muscle but found increased leakage in PDGF-B<sup>−/−</sup> heart after only 24 h of circulation.

PDGF-B is a known mitogen and chemotactic growth factor for liver pericytes, also known as hepatic stellate cells (HSCs) or Ito cells (45). In addition, PDGF-B induces pericyte recruitment and attachment to endothelial cells in other vascular beds (23). Pericyte coverage is believed to play an important role in the maintenance of vascular barrier function (44). Therefore, loss of pericyte coverage induced by the PDGF-B<sup>−/−</sup> mutation may explain the difference in vascular permeability noted in the PDGF-B<sup>−/−</sup> liver.

To quantify hepatic pericyte coverage, we performed immunohistological analysis of wild-type and PDGF-B<sup>−/−</sup> liver vasculature and staining for sinusoidal endothelial cells (PECAM-1) and hepatic stellate cells (desmin). Despite the loss of pericytes in numerous other tissues, we did not observe a loss of HSC coverage in PDGF-B<sup>−/−</sup> liver (Fig. 1, D–F). This implies that PDGF-B deficiency increases hepatic vascular permeability independently of its effects on liver pericyte density.

PDGF-B signaling also contributes to hepatic stellate cell activation (20). This process is characterized by sinusoidal capillarization, a process by which liver sinusoids become fenestrated and display fibrotic alterations to the space of Disse (22). Capillarization can prevent the delivery of macromolecules to hepatocytes in models of liver fibrosis, injury, obesity, and aging (34, 38). Therefore, we analyzed PDGF-B<sup>−/−</sup> and PDGF-B<sup>+/+</sup> sinusoidal ultrastructure by transmission electron microscopy. The majority of PDGF-B<sup>−/−</sup> sinusoids (80%) were intact, as marked by a highly fenestrated endothelium and well-formed space of Disse with numerous hepatocyte microvilli (Fig. 1, H and I). In contrast, ~60% of PDGF-B<sup>+/+</sup> sinusoids displayed some form of disruption, including fenestration and a disordered electron-dense space of Disse (Fig. 1, G and I). Together, these data suggest that PDGF-B deficiency prevents capillarization-like alterations to the hepatic sinusoidal ultrastructure, thereby enhancing trans-endothelial transport in the liver.

**PDGF-B<sup>−/−</sup>** mice display enhanced hepatic insulin signaling and whole body glucose uptake. PDGF-B<sup>−/−</sup> mice have elevated hepatic sinusoidal transport that appears to result from a reduction in capillarization-like changes to the sinusoidal architecture. Given that the molecular weight of the FITC-dextran we used was 10 kDa and that of insulin was ~6 kDa, we hypothesized that PDGF-B-deficient sinusoids might be more permissive for insulin delivery to hepatocytes. This would lead to greater downstream insulin signaling. Therefore, we examined phosphorylation of the IRβ and Akt at 5 and 15 min, respectively, after intraportal injection of insulin. Phosphorylation of both IRβ and Akt was increased in the livers of PDGF-B<sup>−/−</sup> mice (3-fold increase over wild type for IRβ, 5-fold for Akt; Fig. 2, A and B).

To evaluate the downstream effects of insulin stimulation, we quantified the mRNA abundance of insulin-regulated genes. Insulin signaling induces the expression of glucokinase, and we observed a twofold elevation in glucokinase mRNA in PDGF-B<sup>−/−</sup> liver (Fig. 2C). Insulin signaling also suppresses the expression of glucose-6-phosphatase, and accordingly, we observed an 80% reduction in glucose-6-phosphatase mRNA in PDGF-B<sup>−/−</sup> liver (Fig. 2C). These results support our hypothesis that elevated hepatic vascular permeability increases insulin signaling as a consequence of PDGF-B deficiency.

We next asked whether this increased hepatic insulin signaling could affect hepatic glucose uptake. In hepatocytes, glucose transport is not determined by the abundance of glucose transporters but is largely determined by the capacity of hepatocytes to phosphorylate glucose, i.e., the abundance of glucokinase. Mice were injected intravenously with insulin (0.1 U/kg), glucose, and a 2-[14C]deoxyglucose tracer. After 15 min of circulation, 14C radioactivity was measured in the liver. Hepatic glucose uptake was increased twofold in PDGF-B<sup>−/−</sup> mice compared with PDGF-B<sup>+/+</sup> mice (Fig. 2D). This measurement correlates with increased whole body insulin-stimulated glucose clearance in PDGF-B<sup>−/−</sup> mice following an intraperitoneal insulin injection (Fig. 2E). Taken together, these results indicate that increased hepatic insulin signaling present during PDGF-B deficiency contributes to whole body insulin sensitivity and increased glucose clearance.

**PDGF-B<sup>−/−</sup>** partially restores insulin sensitivity to Lep<sup>ob</sup> mice. To study the role of PDGF-B signaling in insulin and glucose homeostasis under conditions of metabolic stress evoked by obesity, we created obese PDGF retention-deficient mice by breeding the Lep<sup>ob</sup>/Lep<sup>ob</sup> with PDGF-B<sup>−/−</sup>/PDGF-B<sup>−/−</sup> mice (Table 1). Lep<sup>ob</sup> mice normally have severe insulin resistance and hyperinsulinemia (typical fasting insulin levels are elevated ~8-fold). PDGF-B<sup>−/−</sup> Lep<sup>ob</sup> mice displayed improved peripheral insulin sensitivity. When challenged by

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**Table 1. PDGF-B<sup>−/−</sup> Lep<sup>ob</sup> fasting plasma phenotypes**

<table>
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<th>Glucose, mg/dl</th>
<th>Insulin, ng/dl</th>
<th>HOMA-IR</th>
<th>Triglyceride, mg/dl</th>
<th>Weight, g</th>
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<tr>
<td>Lep&lt;sup&gt;ob&lt;/sup&gt; (n = 13)</td>
<td>279 ± 29</td>
<td>47.7 ± 16.0</td>
<td>930 ± 384</td>
<td>108 ± 13</td>
<td>58.4 ± 2.0</td>
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<tr>
<td>PDGF-B&lt;sup&gt;−/−&lt;/sup&gt; Lep&lt;sup&gt;ob&lt;/sup&gt; (n = 5)</td>
<td>342 ± 40</td>
<td>5.7 ± 2.1**</td>
<td>122 ± 56*</td>
<td>136 ± 26</td>
<td>47.9 ± 4.9*</td>
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Data are expressed as means ± SE. PDGF-B, platelet-derived growth factor B; Lep<sup>ob</sup>, Lep<sup>tert</sup>; HOMA-IR, homeostasis model assessment of insulin resistance. Male mice were fasted for 4 h before blood collection at 14 wk of age. Glucose, insulin, and triglyceride were measured using commercially available kits. HOMA-IR was calculated as [insulin (mU/ml) × glucose (mmol/l)]/22.5. Insulin and HOMA-IR were analyzed using a Mann-Whitney test. Body weight was analyzed using an unpaired Student t-test; *P < 0.05 and **P < 0.01 compared with Lep<sup>ob</sup> control.
Fig. 3. Enhanced whole body glucose and insulin clearance in PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice. Plasma glucose (%time 0; A) and human insulin concentration in plasma (B) during insulin tolerance tests on 8-wk-old male control Lep<sup>ob</sup> (n = 8) and PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice (n = 4). Results are presented as means ± SE. *P ≤ 0.05 and **P ≤ 0.01 by unpaired Student t-test; vertical lines indicate area under the curve analysis.

PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice display severely reduced plasma insulin without elevated hyperglycemia. The improved insulin sensitivity of the PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice would predict an amelioration of the severe obesity-induced hyperinsulinemia normally seen in Lep<sup>ob</sup> mice. Indeed, the fasting plasma insulin levels of the PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice were reduced by 88% at 14 wk of age (Fig. 4A). Despite a severe reduction in fasting plasma insulin and a severe reduction in the insulin secretory response to a glucose challenge (Fig. 4C), fasting glucose (Fig. 4B) and postchallenge glucose (Fig. 4D) were at the normal range of Lep<sup>ob</sup>. Together, these data show that the loss of PDGF-B function greatly enhances insulin action in vivo, enabling severely obese mice to avoid hyperglycemia despite a very low insulin level.

PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice display intact β-cell secretory capacity. A surprising result was the essentially flat insulin response of the PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice after a glucose challenge (Fig. 4C). This is a consequence of very rapid insulin...
clearance (Fig. 3B), but it may also reflect reduced insulin secretion. To assess β-cell secretion in vivo, we quantified C-peptide levels. After a glucose challenge, the PDGF-B^{ret/ret} Lep^{ob} C-peptide area under the curve was reduced by 36% relative to Lep^{ob} controls (Fig. 5A).

The C-peptide/insulin ratio can be used as a surrogate measurement of insulin clearance because C-peptide is released in equimolar amounts to insulin but is cleared from circulation at approximately one-tenth the rate of insulin (48). The C-peptide/insulin ratio was significantly higher over time in PDGF-B^{ret/ret} Lep^{ob} mice (Fig. 5B), consistent with the reduced area under the curve of plasma insulin during the insulin tolerance curve (Fig. 3B).

To directly assess β-cell insulin secretory function, we measured glucose-stimulated insulin secretion from isolated islets in vitro. Both insulin secretion in response to high glucose and total cellular insulin content were reduced significantly in islets from PDGF-B^{ret/ret} Lep^{ob} mice (Fig. 5C). However, when normalized for insulin content, PDGF-B^{ret/ret} Lep^{ob} fractional insulin release was not significantly different from control Lep^{ob} islets (Fig. 5D), indicating that the secretory capacity of PDGF-B^{ret/ret} islets is intact and that the reduced insulin secretion is due to a large reduction in the pool of available insulin in the β-cells. The β-cell area (Fig. 5E), islet size (data not shown), and β-cell architecture (data not shown) were not significantly different between PDGF-B^{ret/ret} Lep^{ob} and PDGF-B^{ret/ret} Lep^{ob} mice, suggesting that the reduced insulin content results from diminished insulin synthesis in the β-cells rather than a loss of β-cells.

**DISCUSSION**

In the present study, we demonstrate that PDGF-B deficiency prevents hepatic capillarization and thereby increases hepatic vascular permeability. This results in increased hepatic insulin signaling, suggesting improved insulin delivery to hepatocytes. In obese mice, this enhanced hepatic insulin signaling partially ameliorates the insulin resistance and hyperinsulinemia normally associated with obesity (Fig. 6) and is associated with a far lower glucose level than one would normally expect in obese mice with low insulin levels.

Unlike muscle, where transendothelial transport is known to be rate limiting for insulin delivery (6, 18), the liver is considered to be freely permeable to most solutes, and vascular delivery to hepatocytes has not been believed to limit hormone action (34). Although denefestration and alterations to the perisinusoidal space can impede the delivery of macromolecules to hepatocytes (34, 38), most studies have focused on
In our study, H11011 trigger HSC activation and fibrosis independently of TGF-PDGF-B overexpression has more recently been shown to growth factor-ECM production are classically attributed to transforming synthesis of ECM (45). HSC activation and the resulting activation, the process by which normally quiescent HSCs begin synthesizing ECM (45). HSC activation and the resulting upregulation of glucokinase and suppression of glucose-6-phosphatase should trigger elevated hepatic glucose flux while reducing glucose output into circulation, resulting in better maintenance of glucose homeostasis. In the obese state, this enhanced glycemic control likely triggers feedback mechanisms that signal to the ß-cells to reduce insulin secretion, thereby alleviating the hyperinsulinemia typically associated with obesity. ECM, extracellular matrix.

cases of advanced cirrhosis and fibrosis. We show that hepatic sinusoidal transendothelial transport can be improved even in animals lacking overt liver fibrosis and that such changes affect whole body insulin and glucose homeostasis.

We were surprised to observe equal HSC coverage of sinusoidal vessels in PDGF-B<sup>wt/wt</sup> and PDGF-B<sup>ret/ret</sup> liver. However, in PDGF-B<sup>−/−</sup> embryos, which do not survive to birth, HSCs develop normally in the liver (28), suggesting that another factor can induce HSC recruitment in the face of reduced hepatic PDGF-B signaling. Multiple growth factors that function in signaling and recruitment of pericytes have been identified (23), and the identity of the pericyte recruitment factor in the liver remains to be elucidated. Our data imply that PDGF-B controls hepatic sinusoidal function independently of its role in pericyte recruitment in the liver.

One such potential function for PDGF-B involves HSC activation, the process by which normally quiescent HSCs begin synthesizing ECM (45). HSC activation and the resulting ECM production are classically attributed to transforming growth factor-ß (TGFß) signaling (11). However, hepatic PDGF-B overexpression has more recently been shown to trigger HSC activation and fibrosis independently of TGFß (20). In our study, ~60% of PDGF-B<sup>−/−</sup> sinusoids were noticeably altered. These disruptions resembled the sinusoidal changes reported for HSC activation, including loss of endothelial fenestration and accumulation of electron-dense ECM-like material in the space of Disse (45). In contrast, hepatic sinusoidal architecture was noticeably improved in PDGF-B<sup>−/−</sup> mice. The results of our study provide further evidence that PDGF-B plays an active role in sinusoidal remodeling.

Hepatic steatosis/NAFLD is the most common manifestation of liver insulin resistance, and B6 mice are predisposed to the development of fatty liver, even in the lean state (10). Ultrastructural changes to the hepatic sinusoids are common in NAFLD (3), and this may explain why reduced PDGF-B signaling actually improves hepatic sinusoidal structure in the B6 background. Indeed, black and tan, tufted mice are resistant to the development of fatty liver, and they display reduced sinusoidal alterations compared with B6 mice (data not shown). These studies suggest that sinusoidal dysfunction plays a role in the development of hepatic insulin resistance.

Our data illustrate that changes in liver transendothelial transport due to loss of PDGF-B retention result in elevated whole body insulin and glucose clearance. This is a unique mechanism for enhancement of insulin sensitivity, because insulin resistance is classically thought to occur through defects in the intracellular insulin-signaling cascade downstream of the insulin receptor. Our findings demonstrate a novel role for PDGF-B in hepatic insulin signaling and maintenance of euglycemia and emphasize that hepatic delivery of insulin is an important step in insulin action.

One of the major observations in the present study is that the loss of PDGF-B retention causes a profound increase in insulin sensitivity in PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice. This improvement in insulin sensitivity prevents the expected obesity-induced insulin resistance and hyperinsulinemia of PDGF-B<sup>ret/ret</sup> Lep<sup>ob</sup> mice. The mice maintain the same abnormal fasting glucose values as the Lep<sup>ob</sup> controls and similar glucose tolerance despite drastically reduced fasting insulin levels and reduced glucose-stimulated insulin secretion. The ß-cell secretory machinery remains functional upon PDGF-B deficiency, as demonstrated by normal in vitro fractional insulin secretion from isolated islets. The observed decrease in insulin secretion is most likely due to reduced insulin demand. Improvements in
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insulin sensitivity are often accompanied by decreasing decreases in insulin secretion and β-cell insulin content (16).

The present study provides new insights into the effect of imatinib mesylate (Gleevec) on diabetes. Imatinib mesylate inhibits several receptor tyrosine kinases, including PDGFRβ (13). Imatinib mesylate lowers fasting blood glucose levels in diabetic patients treated for chronic myeloid leukemia (2, 12, 13, 49). Treatment of db/db mice with imatinib mesylate improves insulin sensitivity and decreases in vivo insulin secretion in response to a glucose challenge (26). It is possible that the observed increase in insulin sensitivity may involve PDGFRβ inhibition and subsequent effects on hepatic sinusoidal structure. Sinusoidal fibrosis is reduced upon imatinib mesylate treatment (54). This suggests that further investigation into the effects of imatinib mesylate and other PDGFRβ inhibitors on HSC function might uncover new avenues, leading to the treatment of insulin resistance and type 2 diabetes.

Our results also provide a basis for further study into the mechanisms underlying the action of Sorcs1, a PDGFB-binding protein (29). Several studies have suggested that Sorcs1 expression modulates diabetes in both rodents and humans (19, 24, 41), although its mechanism of action is not clear. The present study demonstrates that modulation of PDGFB-B levels can affect insulin action, and Sorcs1 binding to PDGF-B could potentially affect PDGFB availability, clearance, or function.

In conclusion, this study shows that loss of PDGF-B enhances hepatic insulin action, resulting in improved glucose tolerance. This enhanced glycolysis control reduces the need for excessive insulin secretion, alleviating the hyperinsulinemic state normally seen in obese animals. Most importantly, our work reveals a novel role for PDGFB in hepatic insulin delivery and supports the concept that insulin access to its target tissues is a vital part of insulin sensitivity.

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DISCLOSURES

D. Attie is a member of Pfizer’s Cardiovascular Medicine TASAP Committee.

REFERENCES


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