PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) DEFICIENCY REDUCES INSULIN RESISTANCE AND THE DIABETIC PHENOTYPE IN MICE WITH POLYGENIC INSULIN RESISTANCE

Bingzhong Xue*, Young-Bum Kim*, Anna Lee*, Elena Toschi†, Susan Bonner-Weir*, C. Ronald Kahn†, Benjamin G. Neel§# and Barbara B. Kahn*#

*Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, †Joslin Diabetes Center and Harvard Medical School, and §Cancer Biology Program, Division of Hematology-Oncology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215

Running title: Lack of PTP1B rescues insulin resistance in polygenic diabetes

#Address correspondence to: Barbara B. Kahn, Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Research North 325E, Boston, MA 02215, Tel: 617-667-5422, Fax: 617-667-2927, E-mail: bkahn@bidmc.harvard.edu or Benjamin G. Neel, Cancer Biology Program, Division of Hematology/Oncology, Beth Israel Deaconess Medical Center, 77 Avenue Louis Pasteur, Boston, MA 02215, Tel: 617-667-2823, Fax: 617-667-0610, E-mail: bneel@bidmc.harvard.edu.

Mice heterozygous for IR and IRS-1 deficiency provide a model of polygenic type 2 diabetes in which early onset, genetically programmed insulin resistance leads to diabetes. PTP1B dephosphorylates tyrosine residues in IR and possibly IRS proteins, thereby inhibiting insulin signaling. Mice lacking PTP1B are lean and have increased insulin sensitivity. To determine if PTP1B could modify polygenic insulin resistance, we crossed PTP1B/- mice to mice with double heterozygous deficiency of IR and IRS1 (DHet). DHet mice weighed slightly less than wild type mice and exhibited severe insulin resistance and hyperglycemia, with ~35% of DHet males developing diabetes by 9-10 weeks of age. In DHet mice with PTP1B deficiency, body weight was similar to DHet mice. However, absence of PTP1B in DHet markedly improved glucose tolerance and insulin sensitivity at 10-11 weeks of age and reduced the incidence of diabetes and hyperplastic pancreatic islets at 6 months of age. Insulin-stimulated phosphorylation of IR, IRS proteins, Akt/PKB, GSK3β and p70S6K was impaired in muscle and liver of DHet mice and was differentially improved, by PTP1B deficiency. In addition, increased PEPCK expression in liver of DHet was reversed by PTP1B deficiency. In summary, PTP1B deficiency reduces insulin resistance and hyperglycemia without altering body weight in a model of polygenic type 2 diabetes. Thus, even in the setting of high genetic risk for diabetes, reducing PTP1B is partially protective, further demonstrating its attractiveness as a target for prevention and treatment of type 2 diabetes.

Insulin plays a dominant role in regulating glucose homeostasis through a highly orchestrated constellation of effects, which include promoting glucose uptake in peripheral tissues such as muscle and fat, suppressing hepatic glucose output, and regulating lipid metabolism. Failure of peripheral tissues to respond to insulin (i.e., insulin resistance), can initially be overcome by a compensatory increase in insulin secretion from pancreatic β-cells. When this mechanism fails to compensate sufficiently, frank diabetes and its attendant complications ensue (1-4). Insulin action is mediated through a complex network of signaling events, which is initiated by the binding of insulin to its cell surface receptor, the insulin receptor (IR). This triggers the intrinsic protein tyrosine kinase (PTK) activity of the IR, resulting in autophosphorylation of several IR tyrosyl residues and the recruitment and tyrosyl-phosphorylation of insulin receptor substrate (IRS) proteins. Subsequently, molecules such...
as the growth factor receptor binding protein Grb-2, the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and the SH2 domain-containing protein-tyrosine phosphatase Shp2 bind to IRS proteins, leading to the metabolic and growth-promoting effects of insulin (2,3).

The insulin signaling cascade is negatively regulated by other protein-tyrosine phosphatases (PTPs), most notably, protein-tyrosine phosphatase 1B (PTP1B). PTP1B, which is widely expressed and localizes predominantly to the endoplasmic reticulum (5,6), is a major IR phosphatase (7,8), dephosphorylates the IR preferentially at the tandem tyrosine residues 1162 and 1163 \textit{in vitro} (9) and in cultured cells (10), and may also dephosphorylate IRS-1 (11,12). Its expression and activity are increased in obese and insulin resistant human subjects as well as some obese rodent models (13-16), and some studies suggest that PTP1B polymorphisms may be associated with obesity and insulin resistance in humans (17-19). Over-expressing PTP1B in muscle results in insulin resistance (20). Conversely, mice lacking PTP1B have markedly increased insulin sensitivity (21,22), associated with increased or prolonged insulin-stimulated IR and IRS-1 tyrosine-phosphorylation in muscle and liver (21).

The absence of PTP1B in all tissues is also associated with leanness and protection from diet-induced obesity owing in large part to enhanced leptin signaling, because PTP1B also dephosphorylates Jak2 in the leptin signaling cascade (23,24). In general, increased adiposity is a major cause of insulin resistance (25). Therefore, it has been difficult to determine the extent to which enhanced insulin sensitivity in PTP1B-/- mice is due to the direct effect of absence of PTP1B on IR signaling, as opposed to more indirect effects resulting from leanness. We recently found that deletion of PTP1B selectively in neurons increases insulin sensitivity, but, as in total body PTP1B-/- mice, adiposity is reduced in neuron-specific PTP1B-/- mice (26). Reduction of PTP1B expression primarily in liver and adipose tissue of ob/ob mice by antisense oligonucleotides also has been reported to improve insulin sensitivity, but again, largely in association with decreased adiposity (27-29). All of these data strongly support a physiological role for PTP1B in negatively regulating insulin signaling in rodents and humans. However, the conclusions are tempered by the fact that the accompanying changes in adiposity may play a major role in altering insulin signaling and systemic insulin sensitivity.

To determine the role of PTP1B in modulating insulin signaling independent of changes in adiposity in a highly relevant diabetes model, we crossed homozygous PTP1B-/- mice with mice with double heterozygous deficiency in IR and IRS1 alleles (DHet) (30). The DHet model has similarities to human type 2 diabetes in that it is polygenic in nature, has early-onset insulin resistance and is modified by both genetic background and diet (30,31). These mice have a 35-65% reduction in the levels of IR and IRS1 in insulin target tissues. Although there is no apparent phenotype in either IR+/− or IRS1+/− mice, the combination of these allelic deletions results in severe hyperinsulinemia, insulin resistance, β-cell hyperplasia and ultimately, frank diabetes in 50% (30) to 90% (this study) of the mice on a mixed genetic background.

In this study, we show that PTP1B deficiency reduces the severe hyperinsulinemia, insulin resistance, hyperglycemia and β-cell hypertrophy in DHet mice without affecting their body weight. Thus, we demonstrate that absence of PTP1B protects against diabetes:1) even in mice with a high risk for diabetes due to reduction of key signaling molecules and 2) in the absence of alterations in body weight. These data support the notion that even in the setting of genetic defects that markedly increase diabetes risk, PTP1B is an attractive drug target for prevention and treatment of type 2 diabetes.

MATERIALS AND METHODS

\textbf{Animals.} Wild type (WT), PTP1B-/-, double heterozygotes (or doubly heterozygous) for IR and IRS1 alleles (DHet) and homozygous for PTP1B/heterozygous for IR and IRS1
Colonies of DHet and PTP1B-/- mice were first expanded by breeding DHet (30) or PTP1B-/- (22) mice on a C57BL/6Jx129/svJ mixed background with WT FVB mice, to generate DHet and PTP1B+/-. DHet mice (on a background of FVBxC57BL/6Jx129/svJ) were then bred with PTP1B+/+ on the same background to generate mice heterozygous for IR, IRS1 and PTP1B alleles. The resulting triply heterozygous mice (IR+/--IRS1+/--PTP1B+/+) were then bred with mice heterozygous for PTP1B (PTP1B+/+) to generate the four genotypes WT, PTP1B/+, DHet and DHet/PTP1B-/- at a Mendelian frequency of 6.25% for each genotype (Supplemental Figure 1). As a result of this breeding scheme, all mice used in the current study were on a mixed FVBxC57BL/6Jx129/svJ background. Animals were housed with 12-hour light/dark cycle in a temperature-controlled barrier facility and had free access to water and standard laboratory chow (Purina #5053, fat content 4.5% by weight/11.9% by calories). All aspects of animal care were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee and the animal welfare committee at Harvard Medical School. Mice were weighed bi-weekly.

Metabolic measurements. Blood was collected via tail bleed in mice in the fed state between 8-10AM. Blood glucose was measured by a One Touch Ultra glucometer (Lifescan Inc., Milpitas, CA). Serum insulin and leptin levels were measured by enzyme-linked immunosorbent assay (ELISA) (Crystal Chem. Chicago, IL). Serum free fatty acid (FFA) levels were measured by kit from Wako (Wako Pure Chemical Industries, Richmond, VA). For glucose tolerance tests (GTT), mice were fasted overnight, and blood glucose was measured immediately before and 15, 30, 60, 90 and 120 minutes after intraperitoneal (IP) injection of glucose (1.5mg/g body weight). For insulin tolerance tests (ITT), food was removed for 4 hours, and blood glucose was measured immediately before and 15, 30, 60, 90 and 120 minutes after intraperitoneal (IP) injection of human insulin (HumulinR, Eli Lilly Corp., Indianapolis, IN, 1.2U/kg and 1.0U/kg body weight for males and females, respectively). All metabolic measurements were performed when mice were 9-11 weeks and 6 months of age.

Insulin signaling studies. 6-month-old male WT, PTP1B-/-, DHet and DHet/PTP1B-/- mice were fasted overnight. Human insulin (HumulinR, 10U/kg body weight) was injected via tail vein, and 7.5 minutes later, mice were killed by CO2 and tissues were quickly collected and snap frozen in liquid nitrogen. Tissues were stored at -80°C until processing. This time point was chosen to optimize the possibility of seeing increased or prolonged signaling in both muscle and liver in the absence of PTP1B (21). In our studies in normal mice, maximal responses are seen with this insulin dose. Blood glucose levels are between 300-500 mg/dl in the majority of male DHet mice and below 200 mg/dl in the majority of male DHet/PTP1B-/- mice at 6-month-old. Therefore, we chose mice with these blood glucose levels for the insulin signaling studies since we wanted to investigate whether the improved insulin sensitivity in DHet/PTP1B-/- mice could be explained by their improved insulin signaling.

Western blotting and immunoprecipitation. Tissues were homogenized in Lysis buffer (20mM Tris, pH7.4, 5mM EDTA, 10mM Na3P2O7, 100mM NaF, 2mM Na3VO4, 1% NP-40, 10µg/ml aprotinin, 10µg/ml leupeptin and 1mM PMSF), and lysates were solubilized by continuously stirring for 1 hour at 4°C, followed by centrifugation at 14,000xg at 4°C for 15 minutes. Supernatants were stored at -80°C until assays were performed. Lysate protein (50µg) was resolved by SDS-PAGE, and phosphorylation and total levels of specific proteins were measured by immunoblotting. For detecting tyrosine-phosphorylation of IRS-2, liver lysates (500µg) were immunoprecipitated using rabbit polyclonal anti-IRS-2 antibodies (Upstate, Lake Placid, NY) bound to protein A/Sepharose beads (Amersham Biosciences, Piscataway, NJ). Beads were washed 4 times, boiled in Laemmli sample buffer, and
subjected to SDS-PAGE and Western blot analysis. Rabbit polyclonal anti-IR and –IRS-1 antibodies were produced as described previously (32). Antibodies against IRS-2 (rabbit polyclonal), phospho-tyrosine (clone 4G10, mouse monoclonal), and glycogen synthase kinase 3β (GSK3β) (clone 4G-1E, mouse monoclonal) were purchased from Upstate (Lake Placid, NY). Phospho-tyrosine specific antibodies for IRpY972, IRpYpY1162/1163 and IRS-1pY612 (all rabbit polyclonal antibodies) were from BioSource International (Camarillo, CA). Rabbit polyclonal antibodies against phospho-Akt/PKB (Ser473), total Akt/PKB, phospho-GSK3β (S21/9) and phospho-p70 S6 kinase (Thr389) were from Cell Signaling (Beverly, MA). A rabbit polyclonal antibody against total p70 S6 kinase was a gift from Dr. John Blenis (Harvard Medical School, Boston, MA). A rabbit polyclonal antibody against rat PEPCK was a gift from Dr. Daryl K. Granner (Vanderbilt University, Nashville, TN) (33).

**PI3 kinase activity.** Muscle and liver lysates (500 µg of protein) were subjected to immunoprecipitation overnight at 4°C with 3µl of either IRS-1 or IRS-2 antibody coupled to protein A-Sepharose (Sigma, St. Louis, MO). The immune complex was washed, and PI3 kinase activity was determined as described previously (34).

**RNA extraction and real-time PCR.** RNA was extracted from liver using Tri-reagent (Molecular Research Center, Inc., Cincinnati, OH) and quantitated by measuring absorbance at 260nm. mRNA level of SREBP1c was quantified with Quantitative Real-time RT-PCR as previously described (Koza et al. 1996). The RT-PCR conditions are 48°C for 30 min for reverse transcription, followed by 95°C for 10 min and then 40 cycles of 95°C for 15 sec and 60°C for 1 min. The relative copy number of cyclophilin was quantified and used for normalization. mRNA level was calculated using a standard curve with a series of 10-fold dilutions of a mixture of representative RNA samples. Primer and probe sequences are: Srebpc1C (forward, 5’-GACCACGGAGCCATGGA-3’, reverse, 5’-GGGAGAGCTCTTGTGTT-3’ and probe 5’-TGCACTTTGAAGACATGCTCCAGCTCAT-3’), and cyclophilin (forward, 5’-GGTGGAAGACACCAAGACAGA-3’, reverse, 5’-GCCGAGTGCAATGATG-3’ and probe, 5’-TCCTTCAGTGCTGCCTCCGCT-3’).

**Morphological and morphometric analysis of pancreatic islets.** Male mice were sacrificed at six months of age by CO2, and pancreata were removed, cleared of fat and lymph nodes, weighed, spread in anatomical orientation, and fixed in either 10% formalin or Bouin’s solution. Full footprint paraffin sections (5um) were immunostained for β cells using a guinea pig anti-porcine insulin antiserum (Linco, St. Louis, MO) as previously described (35). β cell mass was measured by point-counting morphometry (35). Islet morphology and morphometry were assessed without prior knowledge of the genotype.

**Statistical analysis.** Results are presented as mean ± SEM. Differences between groups were analyzed for statistical significance by ANOVA with Fischer’s probable least-squares difference (PLSD) post hoc testing, ANOVA with repeated measures, or Kruskal-Wallis non-parametric ANOVA by rank, as appropriate.

**RESULTS**

Mice of four genotypes, WT, PTP1B-/-, DHet and DHet/PTP1B-/-, were used in the current study. On a standard chow diet, there was no difference in body weight between WT and PTP1B-/- mice at 5 weeks of age, but by 11 weeks (males) or 15 weeks (females) of age, PTP1B-/- mice weighed less than WT (Figure 1). PTP1B-/- mice also had lower epididymal fat pad mass (Table 1), indicating lower adiposity, as shown previously (22). While liver mass is lower in PTP1B-/- mice (Table 1), the effect is no longer present when
corrected for body weight (Data not shown). Male and female DHet mice weighed less than their WT littermates, as described previously (30,31), and had lower fat pad mass even when corrected for lower body weight, ie fat pad to body weight ratio was lower (Table 1). This most likely reflects insulin resistance in adipose tissue (31). Liver mass in DHet mice tended to be lower than WT but not after correction for lower body weight (Table 1). Superimposition of PTP1B deficiency on the DHet mice (DHet/PTP1B-/-) had no further effect on body weight (Figure 1), fat pad or liver mass, or fat pad to body weight ratio compared to DHet mice with intact PTP1B (Table 1).

At 9-10 weeks of age, male PTP1B-/- mice had lower serum insulin and blood glucose levels than WT (Figures 2A,B, p<0.05 vs. WT), consistent with higher insulin sensitivity in PTP1B-/- mice observed previously (21,22). The glucose x insulin product was also significantly lower in male PTP1B-/- compared to WT mice (Figure 2C, 63.0 ± 9.4 in PTP1B-/- vs. 170.3 ± 33.7 in WT, p<0.05). There was no difference in fed insulin or glucose levels between female PTP1B-/- and WT (Figures 2D,E), similar to our previous results (22), although the glucose x insulin product tended to be lower in female PTP1B-/- than WT (Figure 2F). Due to their genetically determined insulin resistance, most male DHet mice developed severe hyperinsulinemia in the fed state, with fed insulin levels ranging from 2.6- to 20.5-fold elevated above WT levels (Figure 2A). Of 31 male DHet mice examined at 9-10 weeks of age, 11 mice (35%) developed severe fed hyperglycemia of more than 250 mg/dl, while the remaining 65% had normal glycemia (Figure 2B), consistent with the heterogeneity of diabetes incidence in this model (30). A blood glucose value of 250 mg/dl was used as the cut-off because no WT male mice had a higher blood glucose level. The degree of hyperinsulinemia and hyperglycemia was milder in female DHet mice (Figures 2D,E). When insulin sensitivity was expressed as glucose x insulin product, both male and female DHet mice displayed extremely high values (20-fold and 8-fold elevated, respectively, compared to WT, p<0.05, Figures 2C,F.). PTP1B deficiency completely normalized blood glucose levels in the fed state (Figures 2B,E), and partially rescued hyperinsulinemia in male and female DHet/PTP1B-/- mice (Figures 2A,D). PTP1B deficiency in DHet mice also greatly reduced the glucose x insulin product (Figures 2C,F).

Heterogeneity in blood glucose and insulin levels are found in both DHet and DHet/PTP1B-/- mice, most likely reflecting genetic modifiers due to their mixed genetic background (30)(31). Different degrees of severity of insulin resistance have previously been reported in DHet mice on different genetic backgrounds (31).

DHet mice exhibited impaired glucose tolerance and insulin resistance, as determined by intraperitoneal (IP) glucose tolerance test (GTT) and insulin tolerance test (ITT), respectively. In male PTP1B-/- mice, glucose tolerance was enhanced compared to WT, as shown previously (Figure 3A) (21,22). Absence of PTP1B in DHet mice completely normalized glucose tolerance in both males (Figure 3A) and females (Figure 3C). Male PTP1B-/- mice also had enhanced insulin sensitivity compared to WT (Figure 3B) (21,22). Absence of PTP1B also completely prevented the insulin resistance measured by ITT in both male (Figure 3B) and female (Figure 3D) DHet mice.

At 10 weeks of age, serum free fatty acid (FFA) levels in the fed state were lower in male PTP1B-/- and DHet mice than WT (Supplemental Figure 2). This most likely reflects the reduced adipose mass in these genotypes. In DHet mice, the difference in FFAs was more significant on this mixed genetic background (Supplemental Figure 2) than reported previously for inbred DHet mice (31). Serum FFA levels were even lower in DHet/PTP1B-/- mice compared to DHet (Supplemental Figure 2), which could indicate improved insulin sensitivity.

As expected from our previous studies, serum leptin levels were lower in 10 week old male PTP1B-/- compared to WT (Supplemental Figure 3), consistent with improved leptin sensitivity (22). Serum leptin levels tended to be lower in DHet mice due to their genetically determined insulin resistance.
compared to WT (Supplemental Figure 3) although this did not reach statistical significance even when expressed as a ratio of leptin to body weight (data not shown). Previous data showed that serum leptin levels were lower in DHet mice on inbred backgrounds compared to WT (31) most likely reflecting their reduced fat mass. In DHet/PTP1B-/- mice, leptin levels were similar to DHet (Supplemental Figure 3).

At 6 months of age, out of 35 WT males examined, 23% developed hyperinsulinemia of greater than 5 ng/ml (Figure 4A) and 8% developed hyperglycemia of greater than 250 mg/dl (Figure 4B). Previous studies have shown that with aging, WT mice, depending on their genetic background, can develop hyperinsulinemia and hyperglycemia at frequencies similar to those observed herein (31). In contrast, serum insulin and glucose levels remained normal in all PTP1B-/- mice, demonstrating a protective role for PTP1B deficiency against the insulin resistance and diabetes associated with aging. The improved insulin sensitivity also was reflected in the glucose x insulin product, which increased ~7-fold in WT mice between 2 and 6 months of age, but only ~3-fold in PTP1B-/- mice (Figures 2C and 4C).

The hyperinsulinemia and hyperglycemia in DHet mice became even more dramatic at 6 months compared to 9-10 weeks of age (Figures 4A, B). Nearly all (41/42; 98%) male DHet mice developed severe hyperinsulinemia (>5ng/ml), and 77% developed hyperglycemia of greater than 250 mg/dl. DHet mice had even more elevated glucose x insulin product values at 6 months (Compare Figures 2C and 4C :7007 ± 668 at 6 months vs. 3466 ± 577 at 9-10 weeks of age). The mean hyperinsulinemia in male DHet/PTP1B-/- mice was milder (Figure 4A, 17.0 ± 1.0 ng/ml in DHet vs. 11.7 ± 1.3 ng/ml in DHet/PTP1B-/- mice, p<0.05), and 74% of DHet/PTP1B-/- mice had serum insulin levels greater than 5 ng/ml, compared to 98% of DHet mice. PTP1B deficiency also attenuated the incidence of hyperglycemia in DHet mice (Figure 4B, 28% in DHet/PTP1B-/- vs. 77% in DHet mice). The protective effects of PTP1B deficiency on hyperinsulinemia and diabetes became more evident when median glucose and insulin levels were compared. At 6 months of age, the median glucose level for DHet was 437 mg/dl and for DHet/PTP1B-/- mice was 183 mg/dl. The median insulin levels were 19.3 ng/ml for DHet and 10.9 ng/ml for DHet/PTP1B-/- (p<0.001 by Kruskal-Wallis non-parametric ANOVA by rank test).

Of the 38 female DHet mice examined, 61% developed hyperinsulinemia (defined by a serum insulin value of 2.5 mg/ml) because no female WT mice had insulin levels higher than this value). Twenty-eight percent of DHet females developed hyperglycemia (defined by a glucose value of 200 mg/dl since no WT females had blood glucose levels higher than this value). However, only 5% of female DHet/PTP1B-/- mice developed hyperinsulinemia greater than 2.5 mg/ml, and none developed hyperglycemia greater than 200 mg/dl (Figure 4D and 4E). Taken together, these data indicate that PTP1B deficiency can partially protect DHet mice from developing severe insulin resistance and diabetes even with aging. At all ages examined, the insulin resistance phenotype seems milder in female than male DHet mice, and the improvement of insulin sensitivity is greater in female than male DHet/PTP1B-/- mice. Sexual dimorphism in the extent of changes in insulin sensitivity in DHet (30) and PTP1B-/- (22) mice has previously been reported and has been seen in other models of type 2 diabetes (36) or improved insulin sensitivity (37).

Pancreatic sections from 6 month old DHet mice showed enormously enlarged, insulin-staining islets (Figure 5A), as expected for severely insulin resistant mice (30). The β cell mass, determined by point-counting morphometry (35), was increased 3-10-fold in DHet mice (Figure 5B), which is comparable to the values reported previously (30). Serum insulin levels correlated well with glucose levels in DHet mice; i.e., higher insulin levels were found in mice with more severe degrees of hyperglycemia (Figure 5C), similar to a previous description (30). This suggests that insulin secretion is increased in response to insulin resistance in this model, as is seen in humans with Type 2 diabetes. However, even
with hyperplastic islets and markedly increased serum insulin levels, hyperglycemia occurred in the majority of DHet mice due to relative β cell “insufficiency” in the setting of extreme insulin resistance.

PTP1B-/- mice showed smaller islets (Figure 5A), similar to our previous observations (38), probably reflecting their enhanced insulin sensitivity. Importantly, in the DHet mice in which lack of PTP1B (DHet/PTP1B-/-) prevented hyperglycemia and hyperinsulinemia, the increased β cell mass observed in DHet mice was prevented (DHet/PTP1B-/- 2.6 ± 0.5 mg vs DHet 11.2 ± 2.5 mg, p<0.05, Figure 5B). PTP1B deficiency in DHet mice restored β cell mass to the values of WT of the same gender, age and similar genetic background reported previously (Figure 5B) (30), and islet morphology was indistinguishable from that seen in WT (Figure 5A). Thus, improving insulin sensitivity by lowering PTP1B activity appeared to prevent islet enlargement in this model of polygenic insulin resistance. Fed glucose levels in the 6 month old male mice in which pancreatic islet morphology was assessed were: WT 169 ± 8, PTP1B-/- 142 ± 5, DHet 548 ± 10*, and DHet/PTP1B-/- 167 ± 11 mg/dl (n=13-15 per genotype. *p<0.05 vs. all other genotypes).

We evaluated the expression levels of key molecules in the insulin signaling pathway. Deletion of PTP1B did not alter levels of IR or IRS-1 in muscle or liver compared to WT (Supplemental Figure 4). As expected, IR levels were reduced 35-40% in muscle and liver of DHet and DHet/PTP1B-/- mice (p<0.05). IRS-1 levels were reduced by ~65% in muscle of DHet and DHet/PTP1B-/- mice and by ~50% in liver (p<0.05 for both tissues). IRS-2, Akt/PKB, GSK3β and p70 S6 kinase levels were not different among the 4 genotypes (Supplemental Figure 4).

To assess the consequence of PTP1B deficiency on early steps in insulin signaling in DHet mice, we evaluated tyrosyl phosphorylation sites critical for activation of the IR signaling cascade, including: autophosphorylation of IR at (i) the tandem tyrosyl residues Tyr-1162/1163, located in the activation loop of the IR kinase domain and required for IR activation and subsequent phosphorylation of other IR tyrosines (39); and (ii) the juxtamembrane Tyr-972, which contributes to IRS-1 recruitment (40). We also measured phosphorylation on IRS-1 Tyr-612, which is important for full activation of PI3-kinase and subsequent translocation of Glut4 in response to insulin (41).

In muscle, basal (saline-injected) IR, IRS-1 and Akt/PKB phosphorylation were very low in all four genotypes (Figure 6). In WT mice, insulin rapidly stimulated IR phosphorylation at Tyr-1162/1163 and Tyr-972, and phosphorylation of Tyr-612 on IRS-1 (Figures 6A-C). Tyrosyl phosphorylation of IR and IRS1 were further enhanced in PTP1B-/- mice, consistent with their enhanced insulin sensitivity (21,22). Tyrosyl phosphorylation of IRpY1162/1163 and IRpY972 was increased by 30-40% (p<0.05 vs. WT) (Figures 6A,B). Phosphorylation of IRS-1 at Tyr-612 was increased by ~150% vs. WT (Figure 6C). In muscle of DHet mice, IR Tyr-1162/1163 and Tyr-972 phosphorylation were reduced by ~70% vs. WT, and there was a ~60% reduction of IRS-1 Tyr-612 phosphorylation. Lack of PTP1B in DHet mice (DHet/PTP1B-/-) increased IR phosphorylation at both residues compared to Dhet alone (65% and 35% increase at IRpY1162/1163 and IRpY972, respectively, p<0.05 vs. DHet, Figures 6A,B). Even though IR phosphorylation at both sites was restored only partially compared to WT, IRS-1 phosphorylation at Tyr-612 was restored to WT levels (150 ± 12% increase over DHet, p<0.05, Figure 6C).

When IR and IRS-1 tyrosyl phosphorylation levels were corrected for total IR and IRS-1 protein levels, phosphorylation at IR py1162/1163 and pY972 and IRS-1 pY612 was still elevated in muscle of PTP1B-/- mice compared to WT (Figure 6A-C), suggesting that the stoichiometry of phosphorylation at these sites is increased in PTP1B-/- mice. Even after correcting for total IR protein levels, phosphorylation at IR pY1162/1163 (Fig 6A) and pY972 (Fig 6B) was still lower in muscle of DHet than in WT, whereas phosphorylation at IRS-1 pY612 was
similar in muscle of DHet compared to WT mice (Fig 6C). These data suggest that heterozygous deficiency of IR and IRS-1 differentially affects the stoichiometry of phosphorylation of IR and IRS-1 on these important sites. Lack of PTP1B in DHet mice increased phosphorylation at IR pY1162/1163 and pY972 above the levels in DHet mice, but was still lower than that in WT mice (Fig 6A-B). When corrected for total IRS-1 protein levels, PTP1B deficiency resulted in increased stoichiometry of phosphorylation at IRS-1 pY612 in muscle of DHet/PTP1B-/- mice above the levels in WT and similar to the levels in PTP1B-/- mice (Figure 6C).

IRS-1-associated PI3K activity closely paralleled IRS-1 tyrosyl phosphorylation (Figure 6D). Insulin stimulated IRS-1 associated PI3K activity in all 4 genotypes. PI3K activity was 2.8-fold higher in PTP1B-/- mice than in WT mice and was reduced 65% in DHet mice. This was restored to WT levels in DHet/PTP1B-/- mice.

In muscle of WT mice, insulin stimulation resulted in a profound increase of Akt/PKB Ser473 phosphorylation, and PTP1B deficiency led to a further 50% enhancement of Akt/PKB phosphorylation (Figure 6E). Heterozygous deficiency of IR and IRS-1 resulted in a 35% reduction of Akt/PKB phosphorylation in response to insulin stimulation, and lack of PTP1B in DHet mice increased Akt/PKB phosphorylation to the level observed in PTP1B-/- mice (Figure 6E, 1.2-fold increase compared to DHet mice, p<0.05; 45% increase compared to WT, p<0.05). Thus, partial rescue of IR tyrosyl phosphorylation and full restoration of tyrosyl-phosphorylated IRS-1 targets in the downstream target, Akt/PKB, to the levels of phosphorylation seen in PTP1B-/- mice. This indicates that there may be novel PTP1B targets in the insulin signaling pathway distal to IRS-1-associated PI3K activity that also modulate Akt/PKB activity. Insulin also stimulated the rapid phosphorylation of GSK3β and p70S6 kinase in muscle of WT mice (Figures 6F,G), but there was no difference in phosphorylation of these substrates in mice of the different genotypes (Figures 6F,G).

In liver, basal (saline-injected) IR, IRS-1, IRS-2, and Akt/PKB phosphorylation was very low and similar in WT, PTP1B-/-, DHet and DHet/PTP1B-/- mice (Figure 7). Insulin increased the phosphorylation of IR Tyr-1162/1163 and Tyr-972 in WT mice, with an even greater enhancement of phosphorylation observed in PTP1B-/- mice (~65% and 30% increase over WT at Tyr-1162/1163 and Tyr-972, respectively, p<0.05, Figures 7A,B). Heterozygous deficiency of IR and IRS-1 in DHet mice resulted in 50% reduction of IR Tyr-1162/1163 phosphorylation and a 30% reduction of phosphorylation at IR Tyr-972 (p<0.05, Figure 7A and 7B). Lack of PTP1B-/- in DHet mice (DHet/PTP1B-/-) improved IR phosphorylation at both residues (50% and 35% increases at Tyr-1162/1163 and Tyr-972, respectively; p<0.05 vs. DHet). This improved phosphorylation of IR at pY1162/1163 to 70% of WT levels, while phosphorylation at pY972 was completely normalized. DHet mice had a ~35% reduction in insulin-stimulated phosphorylation of IRS-1 at Tyr-612 (p<0.05 vs. WT, Figure 7C), which was restored to WT levels by PTP1B deficiency. Insulin-stimulated IRS-2 phosphorylation also was impaired in liver of DHet mice (40% reduction, p<0.05 vs. WT, Figure 7D), but was completely restored to WT levels in DHet/PTP1B-/- mice.

When corrected for total IR and IRS-1 protein levels, tyrosyl phosphorylation at IR pY1162/1163 and pY972 was still higher in liver of PTP1B-/- mice than in WT (Figure 7A-7C), suggesting increased stoichiometry of phosphorylation at these sites in PTP1B-/- mice. The stoichiometry of phosphorylation at IR pY1162/1163, pY972 and IRS-1 pY612 was similar in WT and DHet mice, whereas PTP1B deficiency increased the stoichiometry of phosphorylation at IR pY1162/1163 to that of WT level (Fig 7A) and IR pY972 (Fig 7B) and IRS-1 pY612 above WT levels (Figure 7C).

In liver, PI3K activity associated with IRS-1 and IRS-2 also closely followed IRS-1 and IRS-2 tyrosyl phosphorylation (Figure.
7E,F). Basal IRS-1-associated PI3K activity in liver tended to be lower in DHet and DHet/PTP1B-/- mice but did not reach statistical significance due to n=3 in the basal state. Insulin stimulated both IRS-1 and IRS-2-associated PI3K activity in liver of WT, PTP1B-/- and DHet/PTP1B-/- mice, but not in DHet mice (Figure 7E,F). Insulin-stimulated IRS-1-associated PI3K activity was greater in liver of PTP1B-/- than in WT mice. In DHet mice PI3K activity associated with IRS-1 and IRS-2 in liver was decreased 60% and 40%, respectively. In DHet/PTP1B-/- mice IRS-1-associated PI3K activity was increased above DHet level and IRS-2-associated PI3K activity was restored to WT levels (Figure 7E, F).

In addition, insulin stimulated Akt/PKB Ser473 phosphorylation in WT mice (Figure 7G), and PTP1B deficiency led to a further enhancement of Akt/PKB Ser473 phosphorylation (Figure 7G, 81% increase vs. WT). Heterozygous deficiency of IR and IRS-1 in DHet mice resulted in a 65% reduction of insulin-stimulated Akt/PKB phosphorylation compared to WT, whereas lack of PTP1B in DHet completely restored Akt/PKB phosphorylation to the levels observed in WT mice (Figure 7G).

As in muscle, insulin stimulated phosphorylation of GSK3β in liver of WT mice, and GSK3β phosphorylation was increased by ~70% in PTP1B-/- mice (Figure 7H). Heterozygous deficiency of IR and IRS-1 in DHet mice resulted in a 40% reduction in GSK3β phosphorylation in liver (p<0.05 vs. WT), which was restored to WT levels in DHet/PTP1B-/- mice (Figure 7H). Insulin stimulated phosphorylation of p70 S6 kinase in WT and PTP1B-/- mice to similar extents (Figure 7I). The level of insulin-stimulated p70 S6 kinase phosphorylation was reduced 38% in DHet and was restored to WT levels in DHet/PTP1B-/- mice (Figure 7I). Thus, our data suggest that there are differential effects of PTP1B deficiency on downstream components of the insulin signaling pathway, and some of these effects are tissue-specific. In addition, expression of the key gluconeogenic enzyme, PEPCK, was elevated in liver of DHet mice, and was restored to WT levels in DHet/PTP1B-/- mice (Figure 7J).

PTP1B induces the expression of sterol regulatory element-binding protein-1 (SREBP-1)(42), which is important in regulating hepatic lipogenesis (43). This appears to be independent of insulin (42). Consistent with this, the expression of SREBP1c was reduced by 60% in liver of PTP1B-/- mice (Figure 7K). SREBP1 expression in liver is higher in hyperinsulinemic and insulin resistant states (43), and its expression is increased by insulin signaling (43). Reduced insulin signaling in DHet mice tended to lower SREBP1c expression, although this does not reach statistical significance. The expression of liver SREBP1c was not different in DHet/PTP1B-/- compared to DHet mice (Figure 7K).

**DISCUSSION**

We investigated the effects of reducing PTP1B expression on insulin sensitivity in a polygenic model of Type 2 diabetes with similarities to the human diabetes. In this model, diabetes results from a combination of relatively modest defects in insulin signaling (haploinsufficiency of IR and IRS-1) that act synergistically to cause severe insulin resistance and diabetes (30). The severity of insulin resistance and the incidence of type 2 diabetes increase with age, which is also similar to type 2 diabetes in humans (30). Notably, the defects in this model parallel insulin signaling defects in humans with type 2 diabetes. For example, reduction of IR and IRS-1 levels to ~50% of that in normal subjects has been reported in muscle of obese, insulin resistant humans. This is associated with impaired insulin-stimulated IR and IRS-1 tyrosyl phosphorylation, IRS-1-associated PI3K activity and glucose uptake (44). Decreased IR levels in adipocytes or circulating monocytes also has been reported in patients with impaired glucose tolerance and type 2 diabetes and these defects are improved with anti-diabetic therapy (45,46). The extent to which reversal of these signaling defects improves systemic insulin sensitivity is an important question. In this paper, we report that deficiency of PTP1B improves insulin
signaling in muscle and liver and reduces hyperinsulinemia, hyperglycemia and the incidence of Type 2 diabetes in a polygenic model of insulin resistance.

Unlike in other animal models in which PTP1B deficiency increases insulin sensitivity concurrent with a reduction in adiposity (21-24), the current study shows that even without changes in body fat content, reduced expression of PTP1B can markedly improve insulin sensitivity and diminish the risk of developing Type 2 diabetes. Absence of PTP1B in all tissues (23,24) or only in neurons (26) results in increased leptin sensitivity. Thus, it is possible that increased leptin sensitivity could contribute to improved insulin sensitivity in DHet/PTP1B-/- mice. Ambient leptin levels were lower in PTP1B-/- mice in the current study, consistent with their enhanced leptin sensitivity shown previously (21,23,24). However, leptin levels in DHet/PTP1B-/- mice are not significantly lower than those in DHet or WT mice (Supplemental Figure 2B). Because there is a range in insulin sensitivity in DHet/PTP1B-/- mice, we investigated whether mice with less hyperinsulinemia have higher leptin levels, but this was not the case (data not shown). Therefore, it is unlikely that enhanced leptin sensitivity in DHet/PTP1B-/- mice contributes to increased insulin sensitivity.

We also find that heterozygous deficiency of IR and IRS1 results in differential effects on key steps in the insulin signaling cascade, as well as differential effects on insulin signaling in muscle and liver (Figures 6 and 7). PTP1B deficiency by itself results in enhanced insulin-stimulated IR, IRS-1, Akt/PKB phosphorylation and PI3K activity compared to WT levels. When PTP1B deficiency is superimposed on heterozygous deficiency of IR and IRS-1, insulin-stimulated IR phosphorylation is partially restored in muscle, consistent with increased insulin sensitivity at the level of IR in the setting of decreased IR protein levels. Even though IRS-1 protein is reduced by 60% in muscle of DHet/PTP1B-/- mice, the total amount of IRS-1 phosphorylated on tyrosyl 612, the binding site for the p85 subunit of PI3 kinase, is restored to WT levels. The stoichiometry of IR and IRS1 phosphorylation after correction for the respective protein levels is also higher in muscle in PTP1B-/- compared to WT and in PTP1B-DHet compared to DHet, also consistent with increased insulin sensitivity. Increased phosphorylation of IRS-1 Tyr612 probably is a critical factor in the improved insulin sensitivity in DHet/PTP1B-/- mice, because activation of PI3K is a critical step for insulin’s metabolic effects in muscle and liver (41). Consistent with improved IRS phosphorylation, the impaired IRS-associated PI3K activity in muscle and liver of DHet mice is improved by PTP1B deficiency in DHet/PTP1B-/- mice.

The total amount of insulin-stimulated IR phosphorylation is restored only partially in DHet/PTP1B-/- muscle compared to WT. Total IRS1 phosphorylation and IRS-1-associated PI3K activity are improved to WT levels. Nevertheless, insulin-stimulated Akt/PKB phosphorylation in muscle of DHet/PTP1B-/- mice is increased above WT levels, to levels comparable to those in muscle of PTP1B-/- mice. Previous studies suggest that ≤45% of maximal tyrosyl phosphorylation on IR and IRS1 and <50% of maximal PI3 kinase activity is sufficient to achieve maximal Akt/PKB phosphorylation and insulin-stimulated glucose uptake (47). Thus, less than complete normalization of IR may be sufficient to increase phosphorylation of downstream targets such as Akt/PKB above WT levels, especially if IR or IRS1 phosphorylation also is sustained as demonstrated by Elchebly et al. for IR in the liver of PTP1B-/- mice (21).

Alternatively, there could be novel PTP1B targets in the insulin signaling pathway distal to IRS-associated PI3K activity that modulate Akt/PKB activity. Insulin stimulation of PI3K activity generates phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3), an important lipid second messenger required for the activation of Akt/PKB (48). Cellular PtdIns(3,4,5)P3 levels are also regulated by lipid phosphatases, including the 3’-lipid phosphatase, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and the 5-lipid phosphatase SH2-containing inositol
phosphatase 2 (SHIP-2), which dephosphorylate PtdIns(3,4,5)P3 at 3'– and 5'– positions, respectively (48). SHIP-2 can be tyrosyl phosphorylated in response to interleukins and growth factors, including insulin (49,50). The regulatory effects of this phosphorylation are not known and may differ for different growth factor receptors and cell types (49,50). Potentially, the absence of PTP1B could decrease the activities of these lipid phosphatases which could increase cellular PtdIns(3,4,5)P3 levels, leading to enhanced Akt/PKB phosphorylation. Other potential modifiers of Akt/PKB activity include TRB3, which has recently been shown to bind to Akt/PKB and inhibit its phosphorylation by insulin (51).

Despite similar reductions of IR and IRS1 levels in muscle and liver of DHet mice, IR and IRS phosphorylation in liver are less severely reduced than in muscle. However, Akt/PKB phosphorylation is reduced more in liver than in muscle. Previous data showed that the degree of impairment of insulin-stimulated Akt/PKB activation in liver of DHet mice depends on the genetic background, ranging from no impairment to a ~50% reduction (31). This reinforces the possibility that there can be important modulators of Akt/PKB activity downstream of IR and IRS1.

As evidence of downstream biologic effects of these alterations in insulin signaling, the expression of key genes involved in gluconeogenesis (PEPCK) and lipogenesis (SREBP1c) in liver are also altered. PEPCK expression is up-regulated in liver of DHet mice, indicating hepatic insulin resistance and increased gluconeogenesis. This defect is corrected by PTP1B deficiency in DHet/PTP1B–/– mice. SREBP1c expression is reduced in liver of PTP1B–/– mice as expected (42). It also tends to be lower in liver of DHet mice probably due to diminished insulin signaling resulting from heterozygous deficiency of IR and IRS-1. The lack of difference in SREBP1c expression in DHet/PTP1B–/– compared to DHet could be due to the opposing effects of PTP1B deficiency (independent of insulin) to decrease SREBP1c expression and improved insulin signaling to increase SREBP1c expression.

We also observed tissue-specific effects of heterozygosity for IR and IRS1 and PTP1B deficiency on other downstream signals. Whereas neither IR/IRS1 heterozygosity nor absence of PTP1B affects insulin-induced phosphorylation of GSK3 and p70 S6K in muscle, insulin-stimulated phosphorylation of both kinases is impaired in liver of DHet mice and is restored by PTP1B deficiency. These differential effects could reflect different upstream signaling pathways in these tissues. For example, IRS-2 plays a more significant role in IR signaling in liver, whereas IRS-1 is more important in muscle (30,52,53). Alternatively, the relative ability of other PTPs to compensate for PTP1B deficiency, or the differential action of other negative regulatory mechanisms (e.g., inhibitory serine/threonine phosphorylation, increased serine/threonine phosphatase activity) also could help to explain tissue-specific differences in the effects of PTP1B deficiency.

We also found that PTP1B deficiency reduced pancreatic islet enlargement. This most likely reflects the reduced peripheral insulin resistance leading to diminished need for insulin secretion. Thus, our studies demonstrate that reduction in PTP1B can markedly reduce the incidence of insulin resistance and frank diabetes in a polygenic model of insulin resistance and diabetes, and also delay the onset of insulin resistance associated with aging. The therapeutic effects of PTP1B deficiency in this diabetic model strongly support the notion that inhibiting PTP1B activity will be effective even in people with a high genetic risk for Type 2 diabetes. Furthermore, even if a pharmacologic inhibitor does not reduce adiposity (as would be the case for agents that do not access the central nervous system (26)), PTP1B inhibition should still be effective in enhancing insulin sensitivity and potentially preventing Type 2 diabetes.
REFERENCES


FOOTNOTES

This work was supported by NIH grants DK60839 (BBK) and the Metabolic Physiology Core of DK57521 (BBK); DK60838 (BGN); DK31036 and DK33201 (CRK); DK66056 (SBW), and ADA grant 7-05-PPG-02 (YBK). The authors thank Fawaz Haj, Kazushi Araki, Kendra Bence and Janice Zabolotny for their important contributions to this work.

FIGURE LEGENDS

Figure 1. Growth curves of WT, PTP1B-/-, DHet and DHet/PTP1B-/- mice. * p<0.05 PTP1B-/- vs. WT; + p<0.05 in (A) for DHet and DHet/PTP1B-/- vs. WT and PTP1B-/- and in (B) for DHet and DHet/PTP1B-/- vs. WT as determined by ANOVA with repeated measures.

Figure 2. Hyperglycemia and hyperinsulinemia in DHet mice are improved by PTP1B deficiency at 9-10 weeks of age. (A) and (D), fed insulin and (B) and (E), fed glucose were measured from tail bleeds in 9-10-week-old mice as described in Materials and Methods. (C) and (F), Glucose x insulin product was calculated by multiplying fed glucose values times fed insulin values as an indication of insulin sensitivity. (A), (B) and (C) are data from male mice and (D), (E) and (F) are data from female mice; note the scales are different. Bars indicate mean values for each genotype. * p<0.05 vs. all other genotypes; # p<0.05 vs. PTP1B-/- and DHet; + p<0.05 vs. DHet and DHet/PTP1B-/-, determined by Kruskal-Wallis non-parametric ANOVA by rank.

Figure 3. PTP1B deficiency reverses glucose intolerance and insulin resistance in DHet mice at 10-11 weeks of age. (A) and (C), GTT was performed in overnight-fasted mice with i.p. injection of 1.5 g/kg glucose. (B) and (D), ITT was performed 4 hrs after food removal. Mice were injected i.p. with 1.2 U/kg (male) or 1 U/kg (female) insulin. (A) and (B) are data from male mice and (C) and (D) are data from female mice. * p<0.05 vs. other genotypes; # p<0.05 for PTP1B-/- vs. WT, determined by ANOVA with repeated measures.

Figure 4. Hyperglycemia and hyperinsulinemia in DHet mice are improved at 6 months of age by PTP1B deficiency. (A) and (D), Fed insulin and (B) and (E), Fed glucose were measured from tail bleeds in 6 month old mice as described in Materials and Methods. (C) and (F), Glucose x insulin product was calculated by multiplying fed glucose values times fed insulin values as an indication of insulin sensitivity. (A), (B) and (C) are data from male mice and (D), (E) and (F) are data from female mice. Note the scales are different. Bars indicate mean values for each genotype. In Figure 4C, SEM for males is too small to be visible. * p<0.05 vs. all other genotypes; # p<0.05 vs. PTP1B-/- and DHet; + p<0.05 vs. WT and DHet; ** p<0.05 vs. DHet, determined by Kruskal-Wallis non-parametric ANOVA by rank.

Figure 5. (A). Pancreatic islet morphology in male WT, PTP1B-/-, DHet and DHet/PTP1B-/- mice at 6 months of age. Pancreata were removed, fixed in 10% Formalin, paraffin-embedded and sectioned as full footprint. Tissue sections were immunostained with anti-insulin antibody and counter-stained with hemotoxylin. (B). β cell mass per pancreas in DHet and DHet/PTP1B-/- mice. The dotted line represents values for WT of the same gender, age and similar genetic
background determined previously (30). (C). Correlation between fed insulin and glucose levels in male DHet mice. * p<0.05 vs. DHet.

Figure 6. Insulin signaling in muscle of WT, PTP1B-/-, DHet and DHet-PTP1B-/- mice. 6-month-old male mice were fasted overnight, injected with 10U/kg insulin via tail vein and killed 7.5 minutes later. Blood glucose in DHet mice was between 300-500 mg/dl in and in DHet-PTP1B-/- was below 200 mg/dl. (A) IRpY1162/1163, (B) IRpY972, (C) IRS-1pY612, (E) Akt/PKB Ser473, (F) GSK3β Ser21/9 and (G) p70 S6K Thr389 phosphorylation in muscle was measured by immunoblot analysis using antibodies specific for each phosphorylation site. The immunoblots shown are representative of 3 blots for each panel. In Figure 6A, 6B and 6C, the upper bar graphs show total tyrosyl phosphorylation of IR and IRS-1 at specific sites, and the lower bar graphs show the specific tyrosyl phosphorylation corrected for IR and IRS-1 protein levels. (D) IRS-1 associated PI3 kinase activity was measured as stated in Materials and Methods. For each genotype, n=3 for saline and n=6-7 for insulin. * p<0.05 vs. insulin-stimulated other groups; # p<0.05 vs. insulin-stimulated PTP1B-/- and DHet; + p<0.05 vs. insulin-stimulated WT and DHet, determined by ANOVA.

Figure 7. Insulin signaling in liver of WT, PTP1B-/-, DHet and DHet-PTP1B-/- mice. 6-month-old male mice were fasted overnight, injected with 10U/kg insulin via tail vein and killed 7.5 minutes later. Blood glucose in DHet mice was between 300-500 mg/dl in and in DHet-PTP1B-/- was below 200 mg/dl. (A) IRpY1162/1163, (B) IRpY972, (C) IRS-1pY612, (G) AKt/PKB S473, (H) GSK3β Ser21/9, (I) p70 S6K Thr389 phosphorylation in liver was measured by immunoblot analysis using antibodies specific for each phosphorylation site. In Figure 7A, 7B and 7C, the upper bar graphs show total tyrosyl phosphorylation of IR and IRS-1 at specific sites, and the lower bar graphs show the specific tyrosyl phosphorylation corrected for IR and IRS-1 protein levels. (D) Tyrosyl-phosphorylation of IRS-2 was assessed by immunoprecipitation of 500µg liver lysates with anti IRS-2 antibody followed by immunoblotting with anti-phospho-tyrosine antibody. The immunoblots shown are representative of 3 blots for each panel. The bar graphs show quantitation of all 3 blots. (E) IRS-1 and (F) IRS-2 associated PI3 kinase activity were measured as stated in Materials and Methods. (J) PEPCK levels were measured using a rabbit polyclonal antibody against PEPCK (33). (K) Liver SREBP1c mRNA levels were measured by Quantitative RT-PCR. For each genotype, n=3 for saline and n=6-7 for insulin. + p<0.05 vs. saline, indicated for panels 7E and 7F only; * p<0.05 vs. insulin-stimulated other groups; # p<0.05 vs. insulin-stimulated PTP1B and DHet, determined by ANOVA.
Table 1. Length and organ weight in male mice at 6 months of age.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight (g)</th>
<th>Length (mm)</th>
<th>Liver (g)</th>
<th>WAT (g)</th>
<th>WAT/BW (%)</th>
<th>Pancreas (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>34.3 ± 1.1</td>
<td>96.6 ± 0.95</td>
<td>1.32 ± 0.05</td>
<td>1.48 ± 0.09</td>
<td>4.30 ± 0.21</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>PTP1B-/-</td>
<td>28.7 ± 0.9*</td>
<td>95.8 ± 0.91</td>
<td>1.14 ± 0.04*</td>
<td>0.76 ± 0.12*</td>
<td>2.58 ± 0.36*</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>DHet</td>
<td>28.3 ± 0.9*</td>
<td>94.3 ± 1.04</td>
<td>1.23 ± 0.04</td>
<td>0.76 ± 0.06*</td>
<td>2.64 ± 0.16*</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>DHet/PTP1B-/-</td>
<td>26.9 ± 0.7*</td>
<td>93.8 ± 0.95</td>
<td>1.17 ± 0.05*</td>
<td>0.66 ± 0.05*</td>
<td>2.48 ± 0.18*</td>
<td>0.20 ± 0.01*†</td>
</tr>
</tbody>
</table>

n=10 for each genotype.  
* p<0.05 vs. WT; † p<0.05 vs. DHet.
Fig 1.

A. Male

B. Female

Body weight (g)

Weeks

WT

DHet

PTP1B-/-

DHet/PTP1B-/-
Fig 2

A. Fed insulin (ng/ml)

B. Fed glucose (mg/dl)

C. Glucose x Insulin

D. Female

E. Female

F. Female
Fig 3

A. Male

GTT

Glucose (mg/dl)

0 50 100 150 200 250

B. Male

ITT

WT

PTP1B/-

DHet

DHet/PTP1B/-

0 50 100 150 200

C. Female

Glucose (mg/dl)

0 50 100 150 200 250 300

D. Female

Glucose (mg/dl)

0 50 100 150 200 250 300

Time (min)

Time (min)
Fig 5

A.  

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>PTP1B-/-</th>
<th>DHet</th>
<th>DHet/PTP1B-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed Glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed insulin (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B.  

**β cell mass (mg/pancreas)**

- WT
- DHet
- DHet/PTP1B-/-

C.  

Fed Glucose (mg/dl) vs. Fed insulin (ng/ml) for DHet

- $R^2 = 0.74$
Fig 6

E. INS

WT 1B/- DHet DHet/1B-/
- + + - + + - + + - + + - + +

Akt/PKB
Ser473

% of insulin-stimulated WT

WT PTP1B/- DHet DHet/PTP1B/-
0 50 100 150 200

F. INS

WT 1B/- DHet DHet/1B-/
+ + + - + + - + + - + + - + +

GSK3β
Ser21/9

% of insulin-stimulated WT

WT PTP1B/- DHet DHet/PTP1B/-
0 40 80 120

G. INS

WT 1B/- DHet DHet/1B-/
- + + + + + + + - + + - + +

p70 S6K
Thr389

% of insulin-stimulated WT

WT PTP1B/- DHet DHet/PTP1B/-
0 40 80 120
Protein tyrosine phosphatase 1B (PTB1B) deficiency reduces insulin resistance and the diabetic phenotype in mice with polygenic insulin resistance
Bingzhong Xue, Young-Bum Kim, Anna Lee, Elena Toschi, Susan Bonner-Weir, C. Ronald Kahn, Benjamin G. Neel and Barbara B. Kahn

*J. Biol. Chem.* published online June 1, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M609680200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2007/07/10/M609680200.DC1