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14. *Arabidopsis thaliana* Landsberg *erecta* suspension culture was maintained by weekly 20-fold dilution (*12*, *13*). Early stationary phases (day 7 after previous subculture) were filtered and washed five times with 200 ml of Murashige and Skoog (MS) medium (containing 3% sucrose, but lacking auxin and cytokinin) and maintained in this medium for 24 hours before addition of inducing agents at time $T = 0$ and sampling at times indicated. Identical results were obtained with exponential (day 3) cells similarly treated. Chx (100 μM) was added 1 hour before other inducers and effectively prevents protein synthesis in *Arabidopsis* [S. Abel, M. D. Nguyen, A. Theologis, *J. Mol. Biol.* **251**, 533 (1995); (*22*)].
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16. Transgenic *Arabidopsis* seedlings carrying the CycD3 promoter linked to a *GUS* reporter gene treated with zeatin also showed twofold to sevenfold induction of GUS but no change in its spatial distribution.
17. Longitudinal sections (8 μm) of 8-week-old vegetative *A. thaliana* Columbia grown under short days, hybridized as described [G. Segers *et al.*, *Plant J.* **10**, 601 (1996)] to 800-nucleotide antisense or full-length sense CycD3 ^{35}S-uridine triphosphate–labeled riboprobes (Promega), were viewed in dark field (autoradiographic signal) or fluorescence (calcofluor staining). For cytokinin induction, 50 μM BAP (Sigma) was applied daily to the apical bud for five successive days.
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19. Calli were induced by *Agrobacterium*-mediated transformation of *Arabidopsis* roots as described (*20*) with a construct expressing CycD3 under the CaMV 35*S* promoter. Calli expressed high levels of CycD3 by Northern (RNA) blot (*22*) but could not be regenerated.
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21. The CycD3 cDNA without 5′ untranslated region (UTR) was cloned in conditional vector pFLP-SWITCH (G. D. Davies, N. J. Kilby, C. Riou-Khamlichi, J. A. H. Murray, in preparation) and crossed to an hsp-FLP line (*20*). F1 embryos were heat shocked 3 days after pollination, and F2 seeds from individual F1 plants were analyzed for reduced *GUS* transmission. F2 plants from such parents were tested for transmission of activated CycD3 expression and absence of hsp-FLP (see supplementary material available at www.sciencemag.org/feature/data/985481.shl).
22. C. Riou-Khamlichi, A. Jacqmard, J. A. H. Murray, data not shown.
23. Explants cut from rosette leaves of 3-week-old F3 CycD3-expressing and wild-type plants were placed on MS medium with 0.45 μM 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4-D and 0.44 μM BAP (control). Calli were propagated and trimmed every 10 days; trimming was unnecessary for wild-type calli on medium lacking cytokinin.
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28. RNA blots on GeneScreen Plus (NEN) were stained with methylene blue to confirm equal loading, hybridized at 42°C, and washed (CycD3 blots: 0.1% standard saline citrate and 0.1% SDS at 42°C for 20 min and at 65°C for 10 min; *Arabidopsis* histone H4 blots: 42°C for 10 min and 55°C for 10 min).
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31. Cells were rendered quiescent by incubation of day 7 cells in sucrose-free medium (with hormones) for 24 hours, followed by readdition of 3% sucrose at $T = 0$ and sampling at times indicated (C. Riou-Khamlichi and J. A. H. Murray, in preparation). Incorporation of [^{3}H]thymidine into acid-insoluble material was measured in triplicate relative to background as described (*10*).
32. We thank colleagues for helpful suggestions; N. Kilby, G. Davies, and A. Sessions for advice on the FLP activation system; L. Dehon for in situ hybridizations; D. Hanke for advice and assistance with cytokinin assays; I. Furner for *Arabidopsis* mutants; and A. Inskip for technical assistance. Support of Biotechnology and Biological Sciences Research Council grant P05114 and Pôles d'attraction interuniversitaires belges (Service du Premier Ministre, Services fédéraux des Affaires scientifiques, techniques et culturelles) P4/15 is acknowledged.

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Increased Insulin Sensitivity and Obesity Resistance in Mice Lacking the Protein Tyrosine Phosphatase-1B Gene

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Protein tyrosine phosphatase–1B (PTP-1B) has been implicated in the negative regulation of insulin signaling. Disruption of the mouse homolog of the gene encoding PTP-1B yielded healthy mice that, in the fed state, had blood glucose concentrations that were slightly lower and concentrations of circulating insulin that were one-half those of their PTP-1B$^{+/+}$ littermates. The enhanced insulin sensitivity of the PTP-1B$^{-/-}$ mice was also evident in glucose and insulin tolerance tests. The PTP-1B$^{-/-}$ mice showed increased phosphorylation of the insulin receptor in liver and muscle tissue after insulin injection in comparison to PTP-1B$^{+/+}$ mice. On a high-fat diet, the PTP-1B$^{-/-}$ and PTP-1B$^{+/-}$ mice were resistant to weight gain and remained insulin sensitive, whereas the PTP-1B$^{+/+}$ mice rapidly gained weight and became insulin resistant. These results demonstrate that PTP-1B has a major role in modulating both insulin sensitivity and fuel metabolism, thereby establishing it as a potential therapeutic target in the treatment of type 2 diabetes and obesity.

PTP-1B is implicated in the attenuation of the insulin signal (*1*). Mice deficient in the heterotrimeric GTP–binding protein subunit $G_{i\alpha 2}$ exhibit a phenotype of insulin resistance characteristic of type 2 diabetes that correlates with the increased expression of PTP-1B (*2*). PTP-1B directly interacts with the activated insulin receptor (*3*), and vanadate, a potent nonselective PTP inhibitor, can function as an insulin mimetic both in vitro and in vivo (*4*). However, PTPs other than PTP-1B can also dephosphorylate the activated insulin receptor (*5*). To clarify the role of PTP-1B in insulin action, we generated mice in which the mouse homolog of PTP-1B was disrupted.

The murine gene encoding PTP-1B was cloned from a 129/Sv mouse genomic library and shown to consist of at least nine exons

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spanning more than 20 kb (*6*). A targeting vector was designed to delete a portion of the gene that included exon 5 and the tyrosine phosphatase active site in exon 6 and to replace it with the neomycin resistance gene. Two separate embryonic stem cell clones that had undergone homologous recombination and possessed a single integration event were used to microinject Balb/c blastocysts. Chimeric males were mated with wild-type Balb/c females, and heterozygotes from this cross were mated to produce animals homozygous for the PTP-1B mutation (Fig. 1A). Immunoblot analysis of liver microsomes revealed that PTP-1B protein was absent in PTP-1B null mice, and heterozygotes expressed about half the amount of PTP-1B as did wild-type mice (Fig. 1B). PTP-1B$^{-/-}$, heterozygous, and wild-type littermates were born with the same appearance and with the expected mendelian ratio of 1:2:1. PTP-1B$^{-/-}$ mice grew normally, did not show any significant difference in weight gain as compared to wild-type mice, have lived longer than 1.5 years without any sign of abnormality, and are fertile. Complete necropsies were done on male and female wild-type, heterozygous, and homozygous PTP-1B mutant mice 7 to 8 weeks old, and no gross or histological (brain, liver, muscle, pancreas, and testes) differences were observed.

If the role of PTP-1B in the insulin signaling pathway is to dephosphorylate the activated insulin receptor, then mice deficient in PTP-1B might have a sustained insulin response because the insulin receptor would remain phosphorylated and hence be activated longer than in PTP-1B$^{+/+}$ mice. We measured glucose and insulin concentrations in fasted and fed animals (*7*) (Fig. 2). In the fed state, the PTP-1B$^{-/-}$ mice had a significant 13% reduction in blood glucose concentrations, whereas the heterozygotes had an 8% reduction when compared to wild-type mice (Fig. 2A). The PTP-1B$^{-/-}$ mice had circulating insulin concentrations that were about half those of control fed animals (Fig. 2B). Thus, PTP-1B–deficient mice appeared to be more insulin sensitive, because they maintained lower glucose concentrations with significantly reduced amounts of insulin. In the fasted state, there were no significant differences in concentrations of glucose or insulin.

We examined insulin sensitivity in PTP-1B$^{-/-}$, PTP-1B$^{+/-}$, and PTP-1B$^{+/+}$ mice with oral glucose and intraperitoneal insulin tolerance tests (*8*). Administration of a bolus of glucose to PTP-1B$^{-/-}$ mice resulted in a more rapid clearance of glucose than was observed in wild-type mice (Fig. 3A). There was a more pronounced hyperglycemia in the wild-type animals at all time points after glucose administration than in PTP-1B–deficient mice. Increased insulin sensitivity was also observed upon injection of insulin (Fig. 3B). Hypoglycemia was evident 30 and 60 min after injection, but by 120 min after injection glucose concentrations were returning to normal values in wild-type mice, whereas the PTP-1B–deficient mice remained hypoglycemic ($P < 0.02$). The PTP-

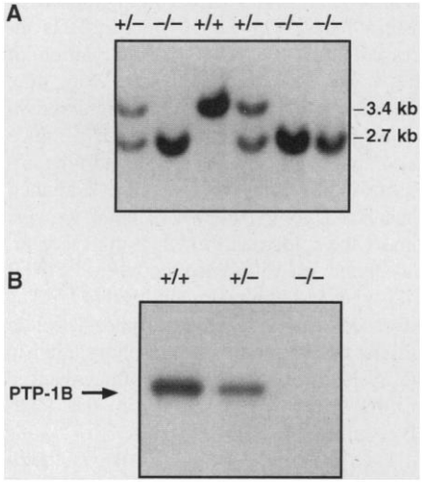

Fig. 1. Gene targeting of the PTP-1B locus. (**A**) Representative genomic Southern blot analysis of tail DNA digested with Bam HI from a heterozygous cross resulting in wild-type (+/+), heterozygous (+/–), and homozygous (–/–) PTP-1B offspring. (**B**) PTP-1B immunoblot analysis of liver membrane samples from PTP-1B$^{+/+}$, PTP-1B$^{+/-}$, and PTP$^{-/-}$ mice.

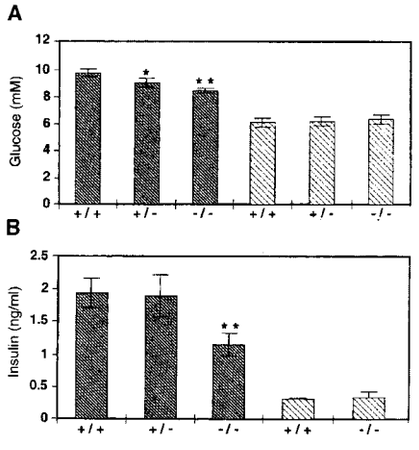

Fig. 2. Serum concentrations of glucose and insulin in animals fed ad libitum or fasted overnight. (**A**) Glucose and (**B**) insulin concentrations were determined as described (*7*). Dark bars indicate fed group [(A) and (B), n = 19 to 21]; light bars indicate fasted group [(A), n = 8 to 10; (B), n = 6]. Values are given as means ± SEM. Statistical analysis was done with a two-tailed, unpaired, Student's t test. Compared to the wild type, $*P = 0.06$ and $**P \leq 0.01$.

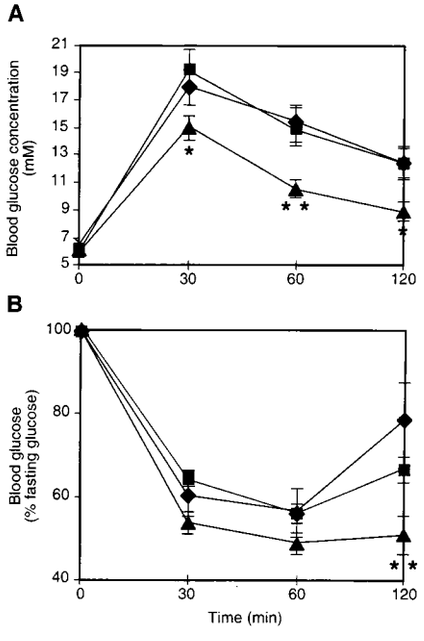

Fig. 3. Glucose and insulin tolerance tests in PTP-1B$^{+/+}$ (diamonds), PTP-1B$^{+/-}$ (squares), and PTP-1B$^{-/-}$(triangles) mice. (**A**) Glucose tolerance of male mice 10 to 14 weeks old (n = 11). (**B**) Insulin tolerance of male mice 10 to 14 weeks old (n = 5 to 6). Data are presented as means ± SEM. Statistical analysis was done with a two-tailed, unpaired, Student's t test. Compared to the wild type, $*P < 0.05$ and $**P < 0.02$.

Table 1. Fasting glucose, triglyceride, and insulin levels of male wild-type, heterozygote, and PT-1B$^{-/-}$ littermates fed a normal or high-fat diet. Values are given as the means ± SEM. Statistical analysis was done with a two-tailed, unpaired, Student's t test. ND, not determined.

Diet	PTP-1B$^{+/+}$		PTP-1B$^{+/-}$		PTP-1B$^{-/-}$	
	Normal	High fat	Normal	High fat	Normal	High fat
Glucose (mM)	6.1 ± 0.3	8.1 ± 0.6*	6.2 ± 0.3	7.3 ± 0.6	6.3 ± 0.3	7.0 ± 0.4
Triglycerides (mM)	1.84 ± 0.76	2.41 ± 0.19	1.43 ± 0.44	2.44 ± 0.32	0.86 ± 0.18*	1.46 ± 0.15*
Insulin (ng/ml)	0.30 ± 0.02	0.98 ± 0.32	ND	0.97 ± 0.30	0.33 ± 0.08	0.45 ± 0.14*

*$P < 0.05$ (n = 6 to 10).

1B$^{+/-}$ mice did not show altered glucose tolerance as compared to that in wild-type mice, although glucose concentration at 120 min after injection of insulin appeared to be intermediate between that of wild-type and PTP-1B$^{-/-}$ mice (Fig. 3).

Binding of insulin to its receptor results in autophosphorylation of the receptor on tyrosines 1146, 1150, and 1151 in the kinase regulatory domain (*9*). This causes activation of the insulin receptor tyrosine kinase, which phosphorylates the various insulin receptor substrate (IRS) proteins that propagate the insulin signaling event (*9*). If PTP-1B dephosphorylates the activated insulin receptor, then the increased insulin sensitivity observed in the PTP-1B$^{-/-}$ mice may be due to increased or prolonged phosphorylation of the receptor (or both). We therefore measured tyrosine phosphorylation of the insulin receptor in liver and muscle tissue (Fig. 4, A and B) after exposure to insulin (*10*). The kinetics of insulin receptor phosphorylation in the liver were significantly different in PTP-1B$^{-/-}$ mice than those observed in the PTP-1B$^{+/+}$ mice. The amount of insulin receptor phosphorylation was the same for both PTP-1B$^{-/-}$ and PTP-1B$^{+/+}$ animals 1 min after injection. However, by 5 min after injection, phosphorylation decreased to about 50% of maximal in the PTP-1B$^{+/+}$ animals, whereas no reduction was observed in the PTP-1B$^{-/-}$ mice. A greater effect on insulin receptor phosphorylation was observed in muscle of the PTP-1B$^{-/-}$ mice. The absolute amount of receptor phosphorylation was increased about twofold in muscle from PTP-1B$^{-/-}$ mice as compared to that in muscle from PTP-1B$^{+/+}$ animals ($P < 0.05$) (Fig. 4B). The amount of insulin receptor phosphorylation in muscle did not significantly change between 2 to 6 min after insulin injection in either PTP-1B$^{-/-}$ or PTP-1B$^{+/+}$ samples. No difference in the amount of insulin receptor expression was detected between PTP-1B$^{+/+}$ and PTP-1B$^{-/-}$ mice, as determined by protein immunoblotting (*11*).

To confirm that the increased phosphorylation of the insulin receptor in the muscle of insulin-treated PTP-1B$^{-/-}$ mice reflects increased kinase activity, phosphorylation of IRS-1 was also measured (*12*) (Fig. 4C). Phosphorylation of IRS-1 was increased in insulin-treated muscle from PTP-1B$^{-/-}$ mice in comparison to muscle from wild-type animals ($P < 0.05$). We have also examined phosphorylation of another receptor tyrosine kinase, the epidermal growth factor receptor, and found no difference between PTP-1B wild-type and deficient animals (*11*). The increased insulin receptor phosphorylation in muscle and its sustained phosphorylation in the liver probably accounts for the enhanced insulin sensitivity observed in the PTP-1B$^{-/-}$ mice.

The disruption of the PTP-1B gene demonstrates that altering the activity of PTP-1B can modulate insulin signaling in vivo. To determine the effect of the loss of PTP-1B activity on insulin resistance, PTP-1B–deficient, wild-type, and heterozygote littermates were subjected to a diet high in fat (50% of calories from fat) and calories (5286 kcal kg^{-1}; Bioserve, NJ) (*13*) that normally results in obesity-induced insulin resistance (*14*). During the 10 weeks the mice were on this diet, male and female wild-type littermates rapidly gained weight, whereas PTP-1B$^{-/-}$ and PTP-1B$^{+/-}$ mice were substantially protected from diet-induced weight gain (Fig. 5). The amount of food consumed by the animals did not differ, which indicates that decreased expression of PTP-1B (heterozygotes have about half the amount of PTP-1B as that in wild-type animals) can influence dietary-induced obesity.

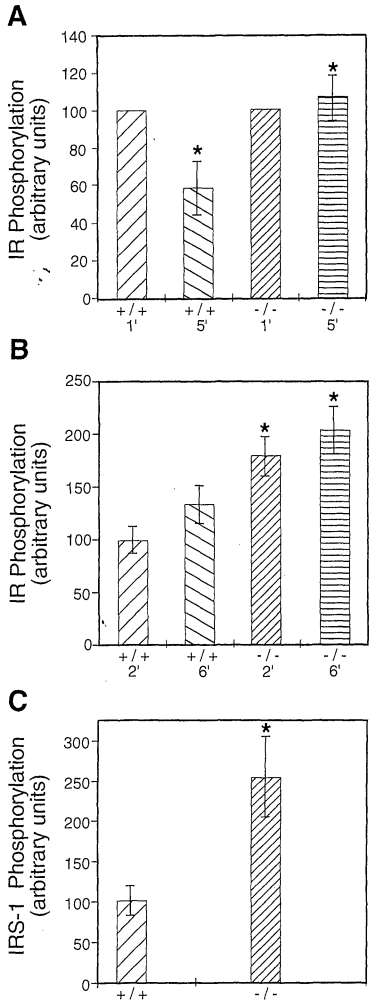

Fig. 4. Increased and prolonged tyrosine phosphorylation of the insulin receptor in PTP-1B$^{-/-}$ mice. (**A**) Time course of tyrosine phosphorylation of the insulin receptor (IR) β subunit in the liver after insulin treatment in PTP-1B$^{+/+}$ and PTP-1B$^{-/-}$ mice. Quantification of the insulin receptor β subunit phosphotyrosine content from immunoblots was performed by densitometry. Data are presented by setting the amount of phosphorylation at 1 min to 100 and the subsequent amount after 5 min for the same animal relative to this value. The results are from five PTP-1B$^{-/-}$ and PTP-1B$^{+/+}$ mice each, from three separate experiments. (**B**) Phosphorylation of the insulin receptor β subunit in muscle of insulin-treated PTP-1B$^{+/+}$ and PTP-1B$^{-/-}$ mice. The quantified data from immunoblots ($n = 6$, from two separate experiments) are presented as arbitrary units. (**C**) Insulin-stimulated tyrosine phosphorylation of IRS-1 from muscle of PTP-1B$^{+/+}$ and PTP-1B$^{-/-}$ mice 2 min after injection ($n = 3$). The quantified data are presented as arbitrary units. Data are means ± SEM. Statistical analysis was done with a two-tailed, unpaired, Student's *t* test comparing in (A) the 5-min to the 1-min time point value and in (B) the PTP-1B$^{-/-}$ mice 2- and 6-min time point values to the respective values of the PTP-1B$^{+/+}$ mice (*$P < 0.05$).

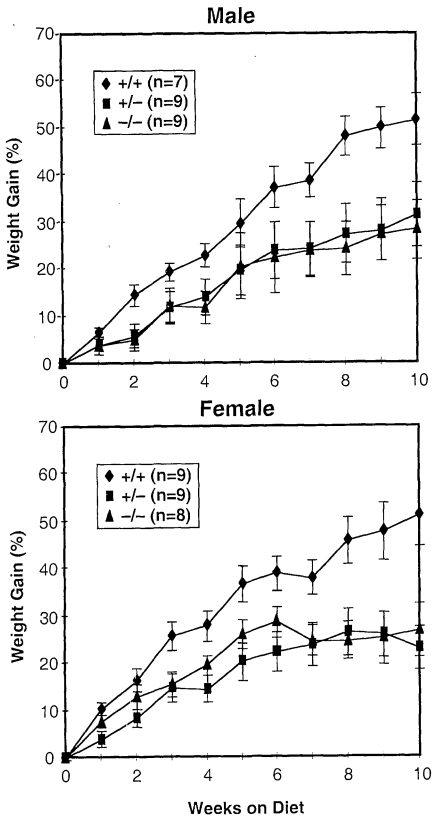

Fig. 5. Resistance of PTP-1B null and heterozygous mice to diet-induced obesity. The percent weight gain of male and female wild-type (diamonds), heterozygous (squares), and homozygous (triangles) littermates fed a high-fat diet for 10 weeks is shown. The starting weight (male: +/+, 27.6 ± 1.4 g; +/–, 28.5 ± 1.2 g; and –/–, 26.3 ± 1.2 g; female: +/+, 22.1 ± 0.8 g; +/–, 22.2 ± 0.8 g; and –/–, 21.5 ± 0.8 g) and final weight (male: +/+, 41.4 ± 1.3 g; +/–, 37.2 ± 2.0 g; and –/–, 33.5 ± 1.6 g; female: +/+, 33.3 ± 1.7 g; +/–, 27.3 ± 1.3 g; and –/–, 27.2 ± 1.4 g) of animals put on the high-fat diet are indicated. The final weight was significantly different ($P < 0.05$) for PTP-1B null and heterozygous mice as compared to wild-type mice, except for male wild-type mice versus heterozygotes ($P = 0.1$).

We examined the effect of the high-fat diet on insulin sensitivity in these animals. Glucose and insulin concentrations in the serum of fasting animals were measured, and glucose and insulin tolerance tests were done on all groups of animals [values for males are presented; females had essentially the same response (11)]. In wild-type mice, the high-fat diet produced a 30% increase ($P < 0.05$) in fasting blood glucose concentrations and a threefold increase in serum insulin concentrations (Table 1) as compared to wild-type mice on a normal diet. In contrast, the PTP-1B$^{-/-}$ animals maintained glucose and insulin concentrations while on the high-fat diet, which were not significantly different from those in animals on a normal diet (Table 1). PTP-1B heterozygotes on a high-fat diet showed increased fasting concentrations of circulating insulin but had fasting glucose concentrations that were not significantly different from those in animals on a normal diet (Table 1). PTP-1B$^{-/-}$ mice also had enhanced insulin sensitivity as compared to their wild-type littermates in both glucose and insulin tolerance tests (Fig. 6, A and B). The difference in insulin sensitivity between the PTP-1B$^{-/-}$ and PTP-1B$^{+/+}$ mice became more augmented on the high-fat diet because of the obesity-induced insulin resistance of the wild-type mice. Obesity-induced insulin resistance results in a reduction in insulin receptor phosphorylation and hence in insulin signaling (*14*). Examination of insulin-stimulated receptor phosphorylation in mice on the high-fat diet revealed that there was a much greater difference in the amount of insulin receptor phosphorylation between the PTP-1B wild-type and deficient animals (Fig. 6C). This increased difference appears to be due to both a reduction in the amount of insulin-stimulated receptor phosphorylation in wild-type mice and a slight increase in the amount of insulin-stimulated receptor phosphorylation in the PTP-1B$^{-/-}$ mice. The PTP-1B heterozygotes on the high-fat diet also appeared to maintain a better response to insulin-stimulated receptor phosphorylation than did wild-type animals (Fig. 6).

The reason for the obesity resistance observed in the PTP-1B$^{-/-}$ mice is unclear at this time, but analysis of triglyceride concentrations indicates that fat metabolism has been affected in these animals. The PTP-1B$^{-/-}$ mice on either diet had significantly lower triglyceride concentrations than did wild-type and heterozygous mice (Table 1). We also examined insulin-stimulated receptor phosphorylation in adipose tissue and found no significant difference between wild-type and PTP-1B-deficient animals on either diet (*11*). Thus, PTP-1B–deficient mice appear to show tissue-specific insulin sensitivity; muscle and liver have an enhanced insulin sensitivity, whereas adipose tissue remains unchanged relative to wild-type mice. The effects that were observed in adipose tissue could be the result of some compensatory mechanism such as the up-regulation of some other PTP [although no difference was observed in the amount of PTP activity between wild-type and PTP-1B$^{-/-}$ tissue extracts (*11*)] or could possibly be due to the overall enhanced insulin sensitivity of the PTP-1B–deficient animal.

The data presented identify PTP-1B as having a major role in the insulin signaling pathway. What this function is remains to be clarified, but the simplest explanation would be that it dephosphorylates the activated insulin receptor. Recently, the disruption of the leukocyte antigen–related (LAR) PTP, which has also been suggested to affect the insulin signaling cascade, has been described. The targeted mutagenesis of LAR produced mice with impaired mammary gland development (*15*) but with blood glucose concentrations within the normal range (*16*). In contrast, the LAR-deficient mice generated by insertional mutagenesis had body weights that were half those of control mice and were insulin resistant (*17*). The reason for the difference in phenotype between these two LAR-deficient mice strains is unknown.

We have shown that the loss of PTP-1B activity causes enhanced insulin sensitivity and resistance to weight gain in mice. These results make PTP-1B a potential therapeutic target for the treatment of type 2 diabetes and obesity.

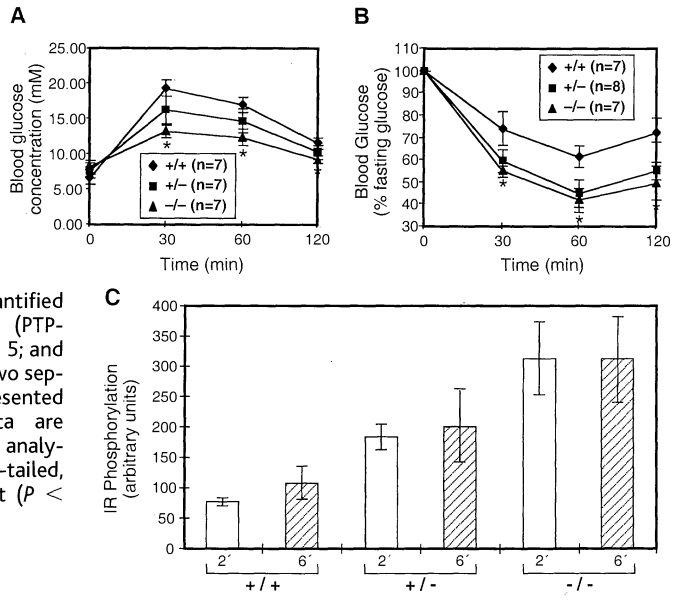

Fig. 6. Insulin tolerance of mice lacking PTP-1B and of wild-type mice on a high-fat diet. (**A**) Glucose and (**B**) insulin tolerance tests of male mice ($n = 7$ to 8) on a high-fat diet. (**C**) Insulin-stimulated insulin receptor tyrosine phosphorylation in muscle of fat-fed mice. The quantified data from immunoblots (PTP-1B$^{-/-}$ and PTP-1B$^{+/+}$, $n = 5$; and PTP-1B$^{+/-}$, $n = 3$; from two separate experiments) are presented as arbitrary units. Data are means ± SEM. Statistical analysis was done with a two-tailed, unpaired, Student's t test ($P < 0.05$).

References and Notes

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6. The mouse PTP-1B gene was isolated by screening a Lambda FIX II 129/SvJ mouse genomic library (Stratagene), and the targeting vector was designed to delete exon 5 and the active site Cys^{215} in exon 6 of the gene. The vector consisted of a 5.5-kb Hind III–Eco RI mouse PTP-1B genomic fragment upstream of exon 5, the neomycin resistance gene driven by the phosphoglycerate kinase (PGK) promoter, a 1.6-kb Xba I–Xho I mouse PTP-1B genomic fragment downstream of exon 6, and the herpes simplex virus–thymidine kinase gene driven by the PGK promoter. Electroporation of the linearized vector into 129/Sv embryonic stem cell line J1 [E. Li, T. H. Bestor, R. Jaenisch, *Cell* **69**, 915 (1992)] and selection of G418 (400 μg/ml)–resistant transformants were done as described [K. E. You-Ten *et al.*, *J. Exp. Med.* **186**, 683 (1997)]. Colonies resistant to G418 were analyzed for homologous recombination by Bam HI digestion of genomic DNA followed by Southern (DNA) blotting and hybridization with a 3′ probe from outside the recombination region. Generation of chimeric and mutant mice was done as described [K. E. You-Ten *et al.*, *J. Exp. Med.* **186**, 683 (1997)]. Genotyping was done by Southern blotting. Immunoblot analysis of PTP-1B expression in liver membranes (25 μg per lane) of PTP-1B$^{+/+}$, PTP-1B$^{+/-}$, and PTP$^{-/-}$ mice was done with a rabbit polyclonal antibody to a PTP-1B NH_2-terminal peptide detected with enhanced chemiluminescence (NEN).
7. Male mice were used. Blood was collected from the orbital sinus of anesthetized mice, and serum was prepared. Serum glucose and triglyceride concentrations were determined with a Vitros analyzer, and a radioimmunoassay (Linco, St. Charles, MO) was used to measure insulin concentrations.
8. Glucose tolerance was performed after an overnight fast by oral administration of glucose [1 g per kilogram of body weight (1 g/kg)]. Insulin tolerance tests were performed after an overnight fast by intraperitoneal injection of 0.75 U/kg of human insulin (1 U ~ 40 μg) (Lilly). Blood was withdrawn from the tail, and glucose concentrations were determined with a One Touch Basic glucometer (Lifescan Canada Ltd., Burnaby, Canada).

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10. After an overnight fast, mice were anesthetized, the abdominal cavity was exposed, and human insulin (5 U) (Lilly) or saline was injected as a bolus into the inferior vena cava [E. Araki *et al.*, *Nature* **372**, 186 (1994); P. L. Rothenberg *et al.*, *J. Biol. Chem.* **266**, 8302 (1991)]. One minute after injection, a small piece of liver was excised and immediately frozen in liquid nitrogen. This was followed at 2 and 3 min for quadriceps muscle and abdominal fat, respectively, and the same procedure was again repeated at 5, 6, and 7 min. Immunoprecipitation of the insulin receptor β subunit was done as follows: the tissue (liver, muscle, or fat) was homogenized on ice in 50 mM tris (pH 7.5); 150 mM NaCl; 1 mM pyrophosphate; 100 μM pervanadate (a potent PTP inhibitor) [G. Huyer *et al.*, *J. Biol. Chem.* **272**, 843 (1997)]; and a protease inhibitor cocktail tablet, Complete (Boehringer Mannheim). A membrane fraction was prepared by centrifugation at 100,000*g* for 1 hour, and the protein concentration was determined. Membrane protein (200 μg from liver or muscle, 100 μg from fat) was solubilized in immunoprecipitation buffer (RIPA) [150 mM NaCl, 10 mM phosphate buffer (pH 7.5), 1% NP-40, 1% Na deoxycholate, and 0.1% SDS], and immunoprecipitation of the insulin receptor β subunit was done overnight at 4°C with the use of antibody to the insulin receptor (1 μg) (C-19, Santa Cruz Biotechnology, Santa Cruz, CA) followed by a 60-min incubation with 50 μl of a 50% slurry of protein G–Sepharose (Pharmacia Biotech). The sample was washed three times in 1 ml of RIPA buffer, and samples were resolved by SDS–polyacrylamide gel electrophoresis (8% gel). The gel was then transferred onto a polyvinyl difluoride membrane, and the phosphotyrosine was detected with antibody to phosphotyrosine (4G10) coupled to horseradish peroxidase (Upstate Biotech, Waltham, MA) and developed with enhanced chemiluminescence (NEN). The same blot was washed in 62.5 mM tris (pH 6.7), 2% w/v SDS, and100 mM β-mercaptoethanol for 30 min at 55°C; rinsed; reprobed with the antibody to the insulin receptor β subunit (C-19, Santa Cruz Biotechnology), followed by a second antibody to rabbit immunoglobulin G coupled to horseradish peroxidase (Amersham); and detected by enhanced chemiluminescence (NEN). The phosphotyrosine and β-subunit signals from the x-ray films of the exposed blots were quantified by densitometry (Molecular Dynamics), and amounts of phosphotyrosine were normalized to the amount of β subunit present in each sample.
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12. Immunoprecipitation of IRS-1 from the cytosolic fraction of muscle from insulin-treated mice was done with two rabbit polyclonal antibodies to IRS-1 (C-20, which binds to the COOH-terminus and A-19, which binds to the NH_2-terminus).
13. Male and female PTP-$1B^{-/-}$ mice and their wild-type and heterozygous littermates at 7 to 8 weeks of age were fed a high-fat high-carbohydrate diet ad libidum (Diet F3282, Bioserve, NJ) and monitored for the following 10 weeks. Body weight was measured weekly, and food consumption was closely monitored. After 10 weeks, concentrations of glucose, insulin, and triglycerides in the serum of fasted animals were measured, and glucose and insulin tolerance tests were done.
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