

Neuronal PTP1B regulates body weight, adiposity and leptin action

Kendra K Bence^{1,4}, Mirela Delibegovic¹, Bingzhong Xue², Cem Z Gorgun³, Gokhan S Hotamisligil³, Benjamin G Neel¹ & Barbara B Kahn²

Obesity is a major health problem and a risk factor for type 2 diabetes. Leptin, an adipocyte-secreted hormone, acts on the hypothalamus to inhibit food intake and increase energy expenditure. Most obese individuals develop hyperleptinemia and leptin resistance, limiting the therapeutic efficacy of exogenously administered leptin. Mice lacking the tyrosine phosphatase PTP1B are protected from diet-induced obesity and are hypersensitive to leptin, but the site and mechanism for these effects remain controversial. We generated tissue-specific PTP1B knockout (*Ptpn1^{-/-}*) mice. Neuronal *Ptpn1^{-/-}* mice have reduced weight and adiposity, and increased activity and energy expenditure. In contrast, adipose PTP1B deficiency increases body weight, whereas PTP1B deletion in muscle or liver does not affect weight. Neuronal *Ptpn1^{-/-}* mice are hypersensitive to leptin, despite paradoxically elevated leptin levels, and show improved glucose homeostasis. Thus, PTP1B regulates body mass and adiposity primarily through actions in the brain. Furthermore, neuronal PTP1B regulates adipocyte leptin production and probably is essential for the development of leptin resistance.

Obesity is increasing at an alarming rate worldwide and is an important risk factor for type 2 diabetes, cardiovascular disease and the metabolic syndrome¹. Effective antiobesity therapies are urgently needed. Leptin, a hormone secreted by adipocytes², is a key regulator of adiposity that acts primarily in hypothalamic nuclei to suppress food intake and increase energy expenditure³. In principle, leptin should be an effective antiobesity agent. Leptin levels increase in proportion to adiposity; indeed, in most obese states, leptin levels are high. Unfortunately, obese individuals typically develop resistance to the physiological effects of leptin ('leptin resistance'), limiting its therapeutic utility⁴. Overcoming leptin resistance, therefore, is a potentially attractive strategy to combat obesity and its complications.

Leptin signals in the hypothalamus by binding to a Type I cytokine receptor (the leptin receptor, LRb), and activating the associated tyrosine kinase Jak2 (Janus kinase 2)⁵. Activated Jak2, in turn, phosphorylates itself and residues Tyr985 and Tyr1138 within the LRb cytoplasmic tail. Jak2 also phosphorylates insulin receptor substrate protein(s), resulting in activation of the phosphatidylinositol 3-kinase pathway^{5,6}. Phosphorylated Tyr985 recruits the tyrosine phosphatase Shp2 (SH2-containing protein-tyrosine phosphatase-2), resulting in leptin-evoked activation of Erk (extracellular signal-regulated kinase)^{7,8}. Tyr1138 recruits and activates the transcription factor Stat3 (signal transducer and activator of transcription 3)³.

Leptin also suppresses the activity of AMP kinase (AMPK) in the hypothalamus, through an unknown pathway⁹.

Leptin receptor signaling alters the transcription of key hypothalamic neuropeptides. In the arcuate nucleus, expression of neuropeptide Y (Npy) and agouti-related protein (Agrp) is elevated upon fasting and suppressed by leptin. Npy and Agrp stimulate food intake and decrease energy expenditure, and are coexpressed in a subset of arcuate neurons, distinct from those that express the anorexigenic neuropeptide precursor pro-opiomelanocortin (Pomc)^{10,11}. Cleavage of Pomc yields alpha melanocyte-stimulating hormone, which inhibits food intake and promotes energy expenditure. Leptin-evoked activation of Stat3 suppresses expression of Agrp and stimulates expression of Pomc in the arcuate nucleus; other LRb pathways regulate Npy¹². The integrated output of these neural pathways regulates food intake, energy expenditure and, ultimately, adiposity. Recent studies, however, show that LRb-expressing arcuate neurons mediate only part of the effects of leptin on adiposity¹³, with SF-1-expressing neurons in the ventral medial hypothalamus and other, as yet unidentified, LRb-expressing neurons also required¹⁴.

Targeting molecules required for the development of leptin resistance is a potentially powerful therapeutic approach for combating the obesity epidemic. One such target may be the ubiquitously expressed nonreceptor protein-tyrosine phosphatase PTP1B encoded by *Ptpn1*. Consistent with early studies implicating PTP1B as a negative

¹Cancer Biology Program, Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, NRB 1030, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA. ²Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Research North 380C, 99 Brookline Avenue, Boston, Massachusetts 02215, USA. ³Department of Genetics and Complex Diseases, Harvard School of Public Health, Building I Room 207, 655 Huntington Avenue, Boston, Massachusetts 02115, USA. ⁴Current address: Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, Pennsylvania 19104, USA. Correspondence should be addressed to B.B.K. (bkahn@bidmc.harvard.edu), K.K.B. (kbence@vet.upenn.edu) or B.G.N. (bneel@bidmc.harvard.edu).

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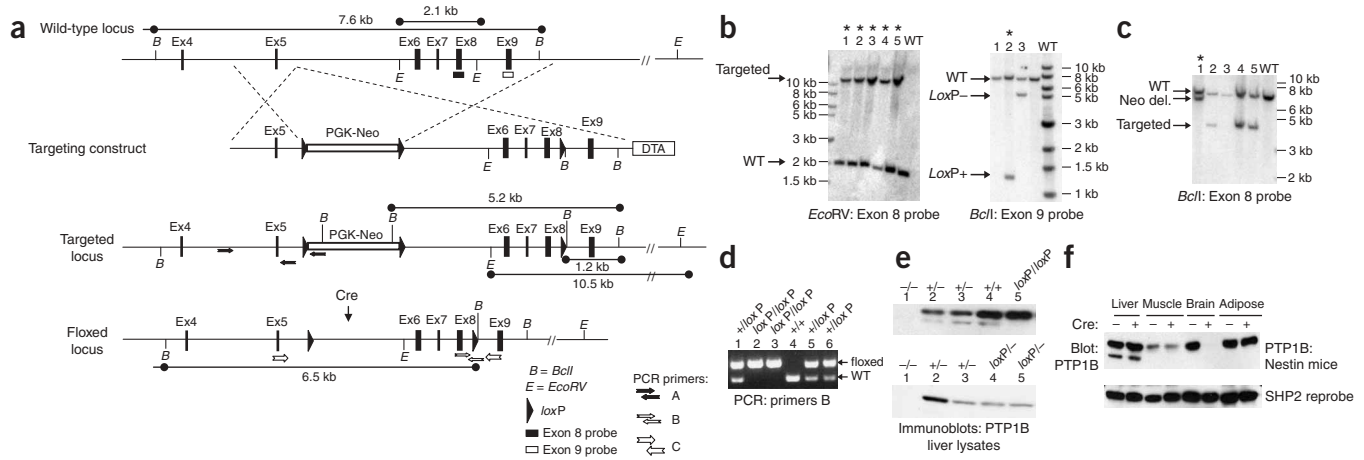


Figure 1 Generation and characterization of mice with tissue-specific deletion of PTP1B. **(a)** *Ptpn1* genomic locus and targeting design. **(b,c)** Southern blots of ES cell clone DNA from initial targeting **(b)**, and after Cre-mediated deletion of the Neo cassette **(c)**. Asterisk indicates properly targeted clones. **(d)** PCR analysis of progeny of *Ptpn1*^{loxP/+} F1 cross. **(e)** PTP1B protein levels in *Ptpn1*^{loxP/loxP} (top panel) and *Ptpn1*^{loxP/-} (bottom panel) mice compared to *Ptpn1*^{+/+} (WT), *Ptpn1*^{+/-} and *Ptpn1*^{-/-} mice, as shown by immunoblotting of mouse liver extracts. **(f)** PTP1B levels in neuronal-specific knockout mice as determined by immunoblotting (top panel); Shp2 reprobe (bottom panel) is shown as a loading control. The specific tissue analyzed is shown at the top. Plus sign (+) indicates the presence of the nestin-Cre gene.

regulator of insulin signaling¹⁵, mice lacking PTP1B in all tissues are hypersensitive to insulin. Unexpectedly, though, whole-body *Ptpn1*^{-/-} mice also are lean and resistant to high-fat diet-induced obesity (DIO)^{16,17}.

The relationship between insulin hypersensitivity and resistance to obesity in *Ptpn1*^{-/-} mice, and the tissue(s) responsible for each phenotype, are controversial. Previous studies suggested that PTP1B negatively regulates hypothalamic leptin signaling and, consequently, controls adiposity, by dephosphorylating Jak2 (refs. 18,19). Indeed, *Ptpn1*^{-/-} mice are hypersensitive to leptin compared to wild-type littermates and most (although not all) of the weight gain in leptin-deficient *ob/ob* mice persists in compound *ob/ob Ptpn1*^{-/-} mice¹⁹. But PTP1B deficiency only partially reverses obesity caused by ablation of leptin-responsive hypothalamic neurons with gold thioglucose, leaving open the possibility of PTP1B effects on adiposity distal to these neurons¹⁸. Furthermore, studies using *Ptpn1* antisense oligonucleotides, which lower PTP1B levels only in liver and fat, argued that PTP1B regulates glucose homeostasis and adiposity primarily, if not exclusively, through actions in these peripheral tissues^{20,21}. Thus, the crucial site(s) of PTP1B's metabolic actions *in vivo* remain unclear.

Knowing the tissue(s) in which PTP1B must be inhibited for salutary effects should aid ongoing development of PTP1B inhibitors²². To resolve the site(s) and mechanism of PTP1B action on adiposity, we generated and analyzed mice with tissue-specific deletions of PTP1B in brain, muscle, liver or fat. Our results show that only mice lacking neuronal PTP1B are resistant to high-fat DIO and are protected from developing leptin resistance. Neuronal PTP1B also regulates adipocyte leptin production and insulin sensitivity independent of changes in body weight. Accordingly, for effective obesity treatment and optimal therapy for type 2 diabetes, PTP1B inhibitors must be directed to the brain.

RESULTS

Generation of mice with a conditional allele of PTP1B

To generate mice expressing a conditional *Ptpn1* allele, we used gene targeting to introduce a modified *Ptpn1* locus into J1 embryonic stem (ES) cells (Fig. 1a). We inserted Cre recombinase-specific *loxP* sites

into the intronic sequence surrounding exons 6–8, which encode the PTP1B active site and surrounding parts of the catalytic domain. In a first round of ES cell targeting, we screened drug-resistant clones by PCR and Southern blotting using exon- (Fig. 1b) and Neo- (data not shown) specific probes. Two primary clones were then transfected with a plasmid encoding Cre recombinase (pCMV-Cre), and secondary clones were screened for deletion of the Neo cassette (Fig. 1c). We injected two properly excised secondary clones into C57BL/6J blastocysts, and bred high-percentage chimeras to wild-type C57BL/6J mice. *Ptpn1*^{loxP/+} progeny were intercrossed to generate all possible allelic combinations (Fig. 1d). *Ptpn1*^{loxP/loxP} mice displayed appropriate 'wild-type' levels of PTP1B, as determined by immunoblotting (Fig. 1e); likewise, *Ptpn1*^{loxP/-} mice (generated by crossing *Ptpn1*^{loxP/+} to whole-body *Ptpn1*^{-/-} mice) displayed 'heterozygous' levels of PTP1B (Fig. 1e). Thus, the inserted *loxP* sites do not interfere with normal expression of *Ptpn1*.

Effects of tissue-specific deletions of *Ptpn1* on body mass

We crossed *Ptpn1*^{loxP/loxP} mice to nestin-, albumin-, MCK- or aP2-Cre recombinase-expressing strains to generate *Ptpn1*^{loxP/+}-Cre mice for each strain. Subsequently, we crossed these mice to *Ptpn1*^{loxP/loxP} mice to yield *Ptpn1*^{loxP/loxP}-Cre mice lacking PTP1B in brain, liver, skeletal muscle or adipose tissue, respectively (Fig. 1f and Supplementary Fig. 1 online). The resultant tissue-specific knockout mice were placed on a high-fat diet (HFD; 55% fat by weight) at weaning. As expected (given their normal PTP1B expression; Fig. 1e), control *Ptpn1*^{loxP/loxP} (no Cre) and wild-type mice on a similar strain background gained comparable amounts of weight on HFD (Fig. 2a). Muscle- or liver-specific PTP1B-knockout mice also showed comparable weight gain on HFD as *Ptpn1*^{loxP/loxP} littermate controls (Supplementary Fig. 1).

In contrast, mice with neuron-specific deletion (*Ptpn1*^{loxP/loxP}-nes Cre) had markedly reduced weight on HFD (Fig. 2b), comparable to that seen in whole-body *Ptpn1*^{-/-} mice¹⁷. Fifty percent reduction of neuronal PTP1B protein levels (that is, in *Ptpn1*^{loxP/+}-nes Cre mice) also resulted in reduced weight on HFD (Fig. 2b). Similar, although less severe, effects of neuronal PTP1B deficiency on adiposity were observed in female mice (Fig. 2c).

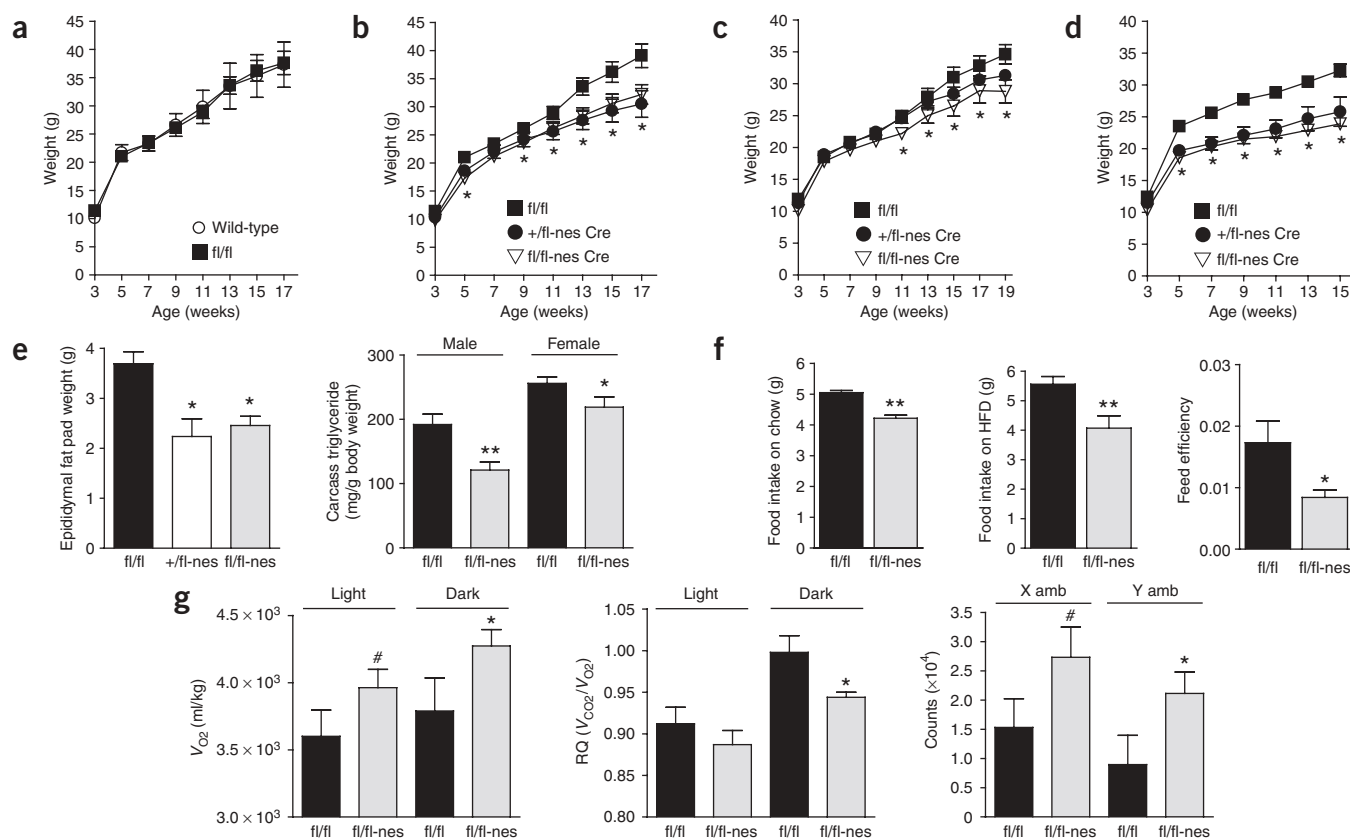


Figure 2 Neuronal *Ptpn1*^{-/-} mice are lean, and have increased energy expenditure and activity. Weight curves of neuronal *Ptpn1*^{-/-} mice (fl/fl-nes) and *Ptpn1*^{loxP/loxP} controls (fl/fl) on HFD or chow diet, as indicated. **(a)** Wild-type 129SvJ × C57BL/6J (*n* = 8) versus *Ptpn1*^{loxP/loxP} (fl/fl) controls (*n* = 14) on HFD. **(b)** Male neuronal *Ptpn1*^{-/-} (*n* = 8) and *Ptpn1*^{+/-} (*n* = 7) mice versus *Ptpn1*^{loxP/loxP} controls (*n* = 14) on HFD. **(c)** Female neuronal *Ptpn1*^{-/-} (*n* = 10) and *Ptpn1*^{+/-} (*n* = 12) mice versus *Ptpn1*^{loxP/loxP} controls (*n* = 12) on HFD. **(d)** Male neuronal *Ptpn1*^{-/-} (*n* = 9) and neuronal *Ptpn1*^{+/-} (*n* = 5) mice versus *Ptpn1*^{loxP/loxP} controls (*n* = 11) on chow diet. **(e)** Neuronal *Ptpn1*^{-/-} and neuronal *Ptpn1*^{+/-} mice have decreased epididymal fat pad weight on HFD (left panel, *n* = 8 per genotype), and neuronal *Ptpn1*^{-/-} mice have decreased carcass triglyceride normalized to body weight compared to *Ptpn1*^{loxP/loxP} controls (right panel, *n* = 6–8 mice per group) on HFD. **(f)** Neuronal *Ptpn1*^{-/-} mice have decreased food intake on chow diet (left panel) and HFD (middle panel), and decreased feed efficiency (right panel) compared to *Ptpn1*^{loxP/loxP} controls (*n* = 6–8 mice/group). **(g)** Neuronal *Ptpn1*^{-/-} mice have increased energy expenditure as indicated by increased *V*_{O₂} (left panel), decreased respiratory quotient (RQ) during the dark cycle (middle panel), and increased ambulatory movement (activity, right panel). * *P* < 0.05, ** *P* < 0.01 by two-tailed Student *t*-test; # *P* = 0.07 (left panel), # *P* = 0.06 (right panel) by 2-tailed Student *t*-test versus *Ptpn1*^{loxP/loxP} control.

Notably, neuronal *Ptpn1*^{-/-} mice also weighed less on a chow diet (4.5% fat by weight; **Fig. 2d**). On the same diet, mice lacking PTP1B in all tissues have the same weight as wild-type littermates^{16,18}, suggesting that absence of PTP1B in another tissue might increase susceptibility to obesity. PTP1B deletion was variable and less efficient in adipocytes from *Ptpn1*^{loxP/loxP}-aP2 Cre or *Ptpn1*^{loxP/+}-aP2 Cre mice, compared to the other Cre strains used (**Supplementary Fig. 1**). Nevertheless, mice with only 50–60% reduction of PTP1B levels in adipocytes gained more weight on HFD than littermate *Ptpn1*^{loxP/loxP} controls (**Supplementary Fig. 1**). Therefore, the body mass phenotype in whole-body *Ptpn1*^{-/-} mice may reflect the combined effects of neuronal and adipose PTP1B deficiency.

Neuronal *Ptpn1*^{-/-} mice had reduced adiposity after high-fat feeding, as shown by lower epididymal fat pad weight (**Fig. 2e**) and carcass triglyceride content (**Fig. 2e**). Food intake was slightly less in these mice compared to controls (**Fig. 2f**). More notably, feed efficiency (grams weight gained/food consumed)²³ was decreased markedly in the absence of neuronal PTP1B (**Fig. 2f**), suggesting increased energy expenditure.

To assess energy balance directly, we monitored 8–10-week-old neuronal *Ptpn1*^{-/-} and *Ptpn1*^{loxP/loxP} control mice in open-circuit indirect calorimetry cages. Neuronal *Ptpn1*^{-/-} mice showed increased O₂ consumption (VO₂; **Fig. 2g**), as well as a decreased respiratory quotient (RQ; **Fig. 2g**), indicating preferential metabolism of fat as an energy source. Mice lacking neuronal PTP1B also had increased ambulatory movement (**Fig. 2g**). Thus, both decreased food intake and increased energy expenditure cause the decreased adiposity in neuronal *Ptpn1*^{-/-} mice.

Increased leptin sensitivity in neuronal *Ptpn1*^{-/-} mice

Whole-body *Ptpn1*^{-/-} mice are hypersensitive to leptin^{18,19}. To determine whether this is mediated by neuronal pathways, we injected neuronal *Ptpn1*^{-/-} and littermate *Ptpn1*^{loxP/loxP} control mice twice daily over a 60-h period with saline or a low dose of leptin (**Fig. 3a**). Compared to littermate controls, neuronal *Ptpn1*^{-/-} mice showed enhanced suppression of body weight (**Fig. 3a**) and food intake (**Fig. 3a**) in response to leptin. Leptin acts through hypothalamic neurons to reduce bone formation and bone mass²⁴. Consistent with increased leptin action, neuronal *Ptpn1*^{-/-} mice had decreased bone

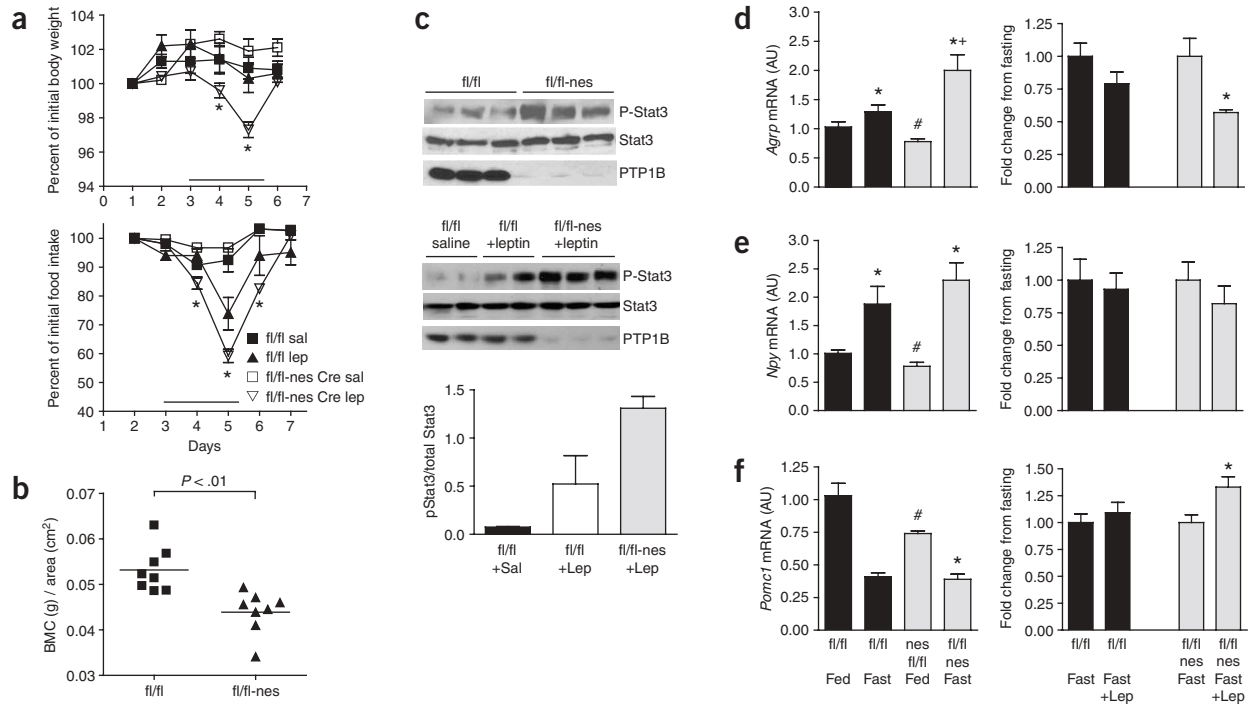


Figure 3 Leptin sensitivity and neuropeptide expression in neuronal *Ptpn1*^{-/-} mice. **(a)** Leptin sensitivity is increased in neuronal *Ptpn1*^{-/-} mice (fl/fl-nes). Male mice (8–10 weeks of age on chow diet) were injected with leptin (lep; 1 μg/g body weight/d) or saline (sal) during the indicated period (bar). Neuronal *Ptpn1*^{-/-} mice show a larger decrease in body weight (top panel) and food intake (bottom panel) than *Ptpn1*^{loxP/loxP} (fl/fl) controls. **P* < 0.05 by ANOVA between *Ptpn1*^{loxP/loxP} + leptin and *Ptpn1*^{loxP/loxP}-nes Cre +leptin. **(b)** Decreased bone mineral density (bone mineral content (BMC) per unit area) in male neuronal *Ptpn1*^{-/-} mice (8–10 weeks of age on chow diet) determined by DEXA analysis. **(c)** Increased hypothalamic phosphorylated Stat3 in neuronal *Ptpn1*^{-/-} mice in both the saline- (top panel) and leptin-stimulated (middle panel) states. Immunoblots for phosphorylated Stat3, total Stat3 and PTP1B, as indicated. Bottom panel in c is a quantification of the blot in the middle panel by densitometry. **(d–f)** Hypothalamic expression of genes encoding neuropeptides in neuronal *Ptpn1*^{-/-} mice versus *Ptpn1*^{loxP/loxP} controls in the fed, fasted and fasted + leptin states. **(d)** *AgRP*, **(e)** *Npy* and **(f)** *Pomc1*, all measured by quantitative real-time PCR. In the left panels, **P* < 0.05 comparing fed (Fed) and fasted (Fast) gene expression within the same genotype; #*P* < 0.05 comparing *Ptpn1*^{loxP/loxP} Fed versus *Ptpn1*^{loxP/loxP}-nes Fed; +*P* < 0.05 comparing fl/fl Fast vs. fl/fl-nes Fast. In the right panels, fasted levels for each genotype were normalized to 1.0, and fasted + leptin (Fast+Lep) response is shown as fold difference from the respective fasted levels; **P* < 0.05 comparing fl/fl-nes Fast to fl/fl-nes Fast+Lep.



mineral density (BMD) compared to controls (**Fig. 3b**). Furthermore, hypothalamic leptin signaling was increased in neuronal *Ptpn1*^{-/-} mice, as indicated by their enhanced fasting (**Fig. 3c**) and leptin-evoked (**Fig. 3c**) phosphorylation of Stat3 in the hypothalamus.

We also analyzed hypothalamic expression of genes encoding neuropeptides in fed, fasted or fasted plus leptin-treated neuronal *Ptpn1*^{-/-} mice. Compared to littermate controls, neuronal *Ptpn1*^{-/-} mice had reduced *AgRP* and *Npy* levels in the fed state (**Fig. 3d,e**), consistent with their decreased food intake and increased metabolic rate¹¹. *AgRP* levels rose upon fasting in both control and neuronal *Ptpn1*^{-/-} mice, although the increase was more robust in neuronal *Ptpn1*^{-/-} mice (**Fig. 3d**). Consistent with enhanced leptin sensitivity, in neuronal *Ptpn1*^{-/-} mice, *AgRP* levels were suppressed more by leptin than in controls (**Fig. 3d**). We did not observe a leptin effect on *Npy* levels under these conditions in either genotype (**Fig. 3e**). Although *Pomc1* levels were reduced in the fed state in neuronal *Ptpn1*^{-/-} hypothalami (**Fig. 3f**), induction of *Pomc1* in response to leptin was clearly enhanced (**Fig. 3f**).

Abnormal serum leptin levels in neuronal *Ptpn1*^{-/-} mice

Lean animals normally have low serum leptin levels, and leptin levels typically increase in obesity. Whole-body *Ptpn1*^{-/-} mice have low leptin levels concomitant with their increased leptin sensitivity and

leanness^{18,19}. Notably, despite their lower adiposity, neuronal *Ptpn1*^{-/-} mice showed increased serum leptin levels (2.3-fold on chow and 4.4-fold on HFD) compared to *Ptpn1*^{loxP/loxP} littermates (**Fig. 4a**). This elevation was detected by 4 weeks after birth, and depended on *Ptpn1* dosage (**Fig. 4b**). Leptin protein (**Fig. 4c**) and mRNA (**Fig. 4d**) were elevated in white adipose tissue of neuronal *Ptpn1*^{-/-} mice, indicating that their increased serum leptin levels reflected, at least in part, enhanced leptin production by adipocytes.

Other adipose tissue hormones and cytokines (adipokines) contribute to the regulation of fat mass and fuel metabolism. Adiponectin is an insulin-sensitizing adipokine, and its levels are normally reduced in insulin-resistant states²⁵. Serum adiponectin levels in neuronal *Ptpn1*^{-/-} mice were normal at 4 weeks of age on chow diet (**Fig. 4e**). Control but not neuronal *Ptpn1*^{-/-} mice showed the expected decrease in adiponectin upon high-fat feeding (**Fig. 4e**). Resistin is elevated in obesity and antagonizes insulin action²⁶. Serum resistin levels in neuronal *Ptpn1*^{-/-} mice were similar to controls on chow and HFD (**Fig. 4f**). In summary, adiponectin levels were elevated in neuronal *Ptpn1*^{-/-} mice, consistent with their lean phenotype, whereas leptin levels were inappropriately high and resistin levels were unexpectedly normal. These data point to a novel pathway by which neuronal PTP1B differentially regulates adipokine production in white adipose tissue.

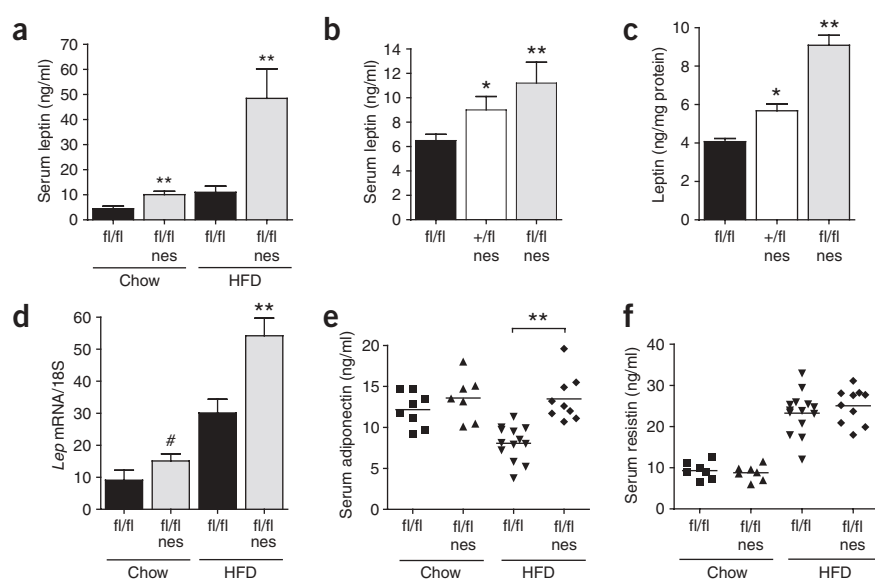


Figure 4 Altered adipokine levels in neuronal *Ptpn1*^{-/-} mice. (a) Fed serum leptin levels are elevated in neuronal *Ptpn1*^{-/-} mice (fl/fl nes) at 8 weeks of age on chow diet or HFD compared to *Ptpn1*^{loxP/loxP} (fl/fl) controls. (b) Serum leptin is elevated in 4-week-old male neuronal *Ptpn1*^{-/-} mice on chow diet. (c) Elevated leptin content in white adipose tissue (WAT) of neuronal *Ptpn1*^{-/-} mice on HFD (*n* = 6–9 mice per genotype). (d) Elevated *Lep* mRNA in WAT of neuronal *Ptpn1*^{-/-} mice on chow or HFD (*n* = 6–9 mice per genotype on each diet); #*P* < 0.07 *Ptpn1*^{loxP/loxP}-nes Cre versus control fl/fl mice on chow; **P* < 0.05, ***P* < 0.01 *Ptpn1*^{loxP/loxP}-nes Cre versus control *Ptpn1*^{loxP/loxP} mice on HFD. (e) Serum adiponectin levels are comparable at 4 weeks of age on chow diet, but increased in neuronal *Ptpn1*^{-/-} mice compared to *Ptpn1*^{loxP/loxP} controls on HFD, ***P* < 0.01 by Student *t*-test. (f) Serum resistin levels are similar in the two genotypes on both diets.

Improved glucose homeostasis in neuronal *Ptpn1*^{-/-} mice

Neuronal *Ptpn1*^{-/-} mice had lower fed blood glucose and serum insulin on chow and HFD (Table 1) and lower fasting serum insulin levels on HFD. Even heterozygous mice (*Ptpn1*^{loxP/+}-nes Cre) had lower blood glucose and serum insulin levels on both diets. No differences were detected in serum free fatty acids (FFA) or serum triglycerides in males (Table 1) or females (data not shown).

These results suggest that mice lacking PTP1B in the brain have increased peripheral insulin sensitivity. Indeed, male neuronal *Ptpn1*^{-/-} mice showed improved insulin sensitivity (by insulin-tolerance test; Fig. 5a), and enhanced glucose tolerance (by glucose-tolerance test; Fig. 5b), compared to littermate *Ptpn1*^{loxP/loxP} controls. Improved insulin sensitivity was accompanied by increased insulin receptor phosphorylation in muscle and liver (Supplementary Fig. 2 online).

Obesity is often associated with insulin resistance, complicating the interpretation of differences in insulin sensitivity between male neuronal *Ptpn1*^{-/-} and controls (Fig. 2b,d). However, female neuro-

nal *Ptpn1*^{-/-} mice that were the same weight as *Ptpn1*^{loxP/loxP} littermate controls (at 8 weeks on HFD; Fig. 2c) and had similar FFA levels (0.431 ± 0.030 neuronal *Ptpn1*^{-/-} versus 0.434 ± 0.280 *Ptpn1*^{loxP/loxP} controls) also showed substantially improved insulin sensitivity. In fact, their insulin sensitivity and glucose tolerance were similar to that of whole-body *Ptpn1*^{-/-} mice (Fig. 5c,d). Hence, neuronal PTP1B may also have an important role in central signaling pathways, presumably in the hypothalamus, that regulate peripheral insulin sensitivity independent of changes in body weight²⁷.

DISCUSSION

To ascertain the cellular basis for the reduced adiposity, decreased weight gain on HFD, and improved glucose and insulin homeostasis in whole-body *Ptpn1*^{-/-} mice, we generated mice lacking PTP1B in specific tissues. Loss of PTP1B only in neurons (*Ptpn1*^{loxP/loxP}-nes Cre) causes a marked decrease in adiposity. Deletion of PTP1B in liver or muscle has no effect on adiposity, whereas unexpectedly,

Table 1 Metabolic parameters in neuronal *Ptpn1*^{-/-} mice on chow and high-fat diet

	Chow diet			High-fat diet		
	<i>Ptpn1</i> ^{loxP/loxP}	<i>Ptpn1</i> ^{+/loxP} -nes Cre	<i>Ptpn1</i> ^{loxP/loxP} -nes Cre	<i>Ptpn1</i> ^{loxP/loxP}	<i>Ptpn1</i> ^{+/loxP} -nes Cre	<i>Ptpn1</i> ^{loxP/loxP} -nes Cre
Blood glucose (mg/dl)						
Fed	107 ± 4.6	101 ± 3.3	86 ± 4.3 **	121 ± 5.4	109 ± 4.2 *	103 ± 5.3 **
Fasted	51 ± 2.0	53 ± 5.6	55 ± 4.2	69 ± 3.8	56 ± 0.9	58 ± 5.1
Serum insulin (ng/ml)						
Fed	0.93 ± 0.07	0.62 ± 0.04 **	0.62 ± 0.03 **	5.8 ± 1.1	1.7 ± 0.4 **	1.4 ± 0.3 **
Fasted	nd	nd	nd	0.61 ± .07	nd	0.46 ± 0.05 *
Serum FFA (mM)						
Fed	0.48 ± 0.04	0.52 ± 0.06	0.55 ± 0.04	0.52 ± 0.04	0.53 ± 0.03	0.54 ± 0.05
Fasted	nd	nd	nd	0.96 ± 0.12	0.94 ± 0.19	0.82 ± 0.10
Serum TG, fed (μg/ml)	nd	nd	nd	449 ± 48	552 ± 29	581 ± 25

Blood glucose, serum insulin, serum free fatty acids (FFA) and serum triglycerides (TG) were analyzed from *Ptpn1*^{loxP/loxP} controls, *Ptpn1*^{+/loxP}-nes Cre and *Ptpn1*^{loxP/loxP}-nes Cre mice. Left side shows values from male mice on chow diet at 8–10 weeks of age. Right side shows male mice on HFD for 8 weeks (11 weeks of age). **P* < 0.05, ***P* < 0.01 for indicated genotype compared to *Ptpn1*^{loxP/loxP} littermate controls. nd, not determined.

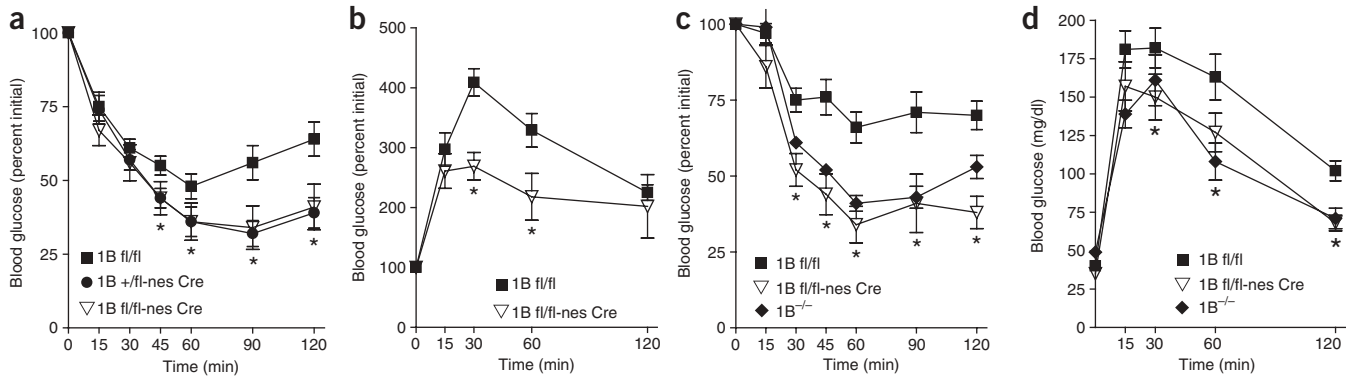


Figure 5 Improved insulin sensitivity and glucose homeostasis in neuronal *Ptpn1*^{-/-} mice. (a) Insulin-tolerance test (ITT) for male neuronal *Ptpn1*^{-/-} (fl/fl-nes Cre), neuronal *Ptpn1*^{+/-} (+/fl-nes Cre) and *Ptpn1*^{loxP/loxP} (fl/fl) control mice. (b) Glucose-tolerance test (GTT) for male neuronal *Ptpn1*^{-/-} and *Ptpn1*^{loxP/loxP} control mice. (c) ITT for female neuronal *Ptpn1*^{-/-}, *Ptpn1*^{loxP/loxP} and total *Ptpn1*^{-/-} (-/-) mice. (d) GTT female neuronal *Ptpn1*^{-/-}, *Ptpn1*^{loxP/loxP} and total *Ptpn1*^{-/-} mice. * *P* < 0.05 for neuronal *Ptpn1*^{-/-} versus *Ptpn1*^{loxP/loxP} controls by ANOVA. 1B, PTP1B.

adipose-specific deficiency of PTP1B promotes weight gain. Therefore, the decrease in adiposity in whole-body *Ptpn1*^{-/-} mice results from a dominant effect on leanness caused by neuronal deficiency of PTP1B. Our data show that neuronal PTP1B controls whole-body leptin sensitivity, and may be required for the development of leptin resistance. Deficiency of PTP1B in the brain also affects production of leptin by adipocytes, glucose homeostasis and insulin sensitivity.

Previous work suggested that leanness in *Ptpn1*^{-/-} mice was due, at least in part, to increased hypothalamic leptin sensitivity^{18,19}. These conclusions were challenged by studies of the effects of *Ptpn1* antisense oligonucleotides in *ob/ob* mice, which concluded that decreased PTP1B expression in liver and/or fat causes weight loss^{20,21}. Deletion of *Ptpn1* in the liver does not affect body mass, whereas deficiency in fat cells causes increased weight gain. Hence, lowering expression of PTP1B in these two tissues alone should, if anything, result in increased adiposity. We suspect that the weight loss evoked by *Ptpn1* antisense oligonucleotides may reflect off-target effects, a recognized problem of these agents²⁸. Our data strongly suggest that PTP1B inhibitors must act in the brain to provide effective therapy for obesity.

Whole-body *Ptpn1*^{-/-} mice are hypersensitive to leptin, and have low leptin levels. Leptin hypersensitivity is usually associated with low leptin levels⁴, but neuronal *Ptpn1*^{-/-} mice have elevated serum leptin. Both whole-body and neuronal *Ptpn1*^{-/-} mice show increased leptin signaling in the hypothalamus and enhanced biological responses to leptin (food intake, body mass, bone density) in spite of opposite effects on circulating leptin levels. Thus, PTP1B acts directly on the leptin signaling pathway in the central nervous system, and neuronal PTP1B is a key regulator of the systemic effects of leptin. Furthermore, neuronal PTP1B is probably required for the development of leptin resistance.

These effects of PTP1B deficiency probably are mediated, at least in part, by increased hypothalamic activation of Stat3, although the precise downstream pathway(s) remain unknown. Both Stat3 and AMPK regulate some arcuate neuropeptide genes^{9,12}, but our analysis reveals complex effects of neuronal PTP1B deficiency on hypothalamic expression of genes encoding neuropeptides. Deficiency of PTP1B clearly augments the effects of leptin on expression of *Agrp* and *Pomc1*, consistent with the increased leptin sensitivity of neuronal *Ptpn1*^{-/-} mice. Moreover, the decreased *Npy* and *Agrp* levels in the fed state suggest that some of the effects of neuronal PTP1B deficiency on adiposity may be caused by altered expression of hypothalamic

neuropeptides. Nevertheless, the lower levels of *Pomc1* after feeding and the exaggerated response of *Npy* and *Agrp* to fasting suggest complicated compensatory responses to neuronal PTP1B deficiency and/or effects of PTP1B on LRb signaling elsewhere in the brain. Indeed, recent work suggests that substantially less than 50% of LRb actions are mediated in arcuate neurons, and point to key leptin actions in SF-1-expressing and other, as yet unidentified, neuronal groups^{13,14}. Although our results clearly implicate increased activation of Stat3, other pathways downstream of LRb, such as AMPK⁹, may be affected by neuronal deficiency of PTP1B and might help promote leanness in neuronal (and whole-body) *Ptpn1*^{-/-} mice.

Unexpectedly, adipose *Ptpn1*^{-/-} mice have increased propensity to obesity. Fat-specific disruption of the insulin receptor results in the converse phenotype: protection against obesity and reduced adipose tissue mass²⁹. Conceivably, the increased body mass in adipose *Ptpn1*^{-/-} mice reflects enhanced insulin action in adipocytes, although phosphorylation of insulin receptor in adipose tissue¹⁶ and insulin-stimulated glucose transport in isolated adipocytes of whole-body *Ptpn1*^{-/-} mice seemed to be normal¹⁷.

Our results uncover unanticipated new connections between the nervous system and adipose tissue that control production of some adipokines. The paradoxically elevated serum leptin levels in neuronal *Ptpn1*^{-/-} mice are due to enhanced leptin gene expression in white adipose tissue. Leptin levels surge between neonatal days 7 and 10, and then fall off rapidly⁴. Leptin levels also are increased by overeating, increased fat mass, glucose and insulin, and decreased by food restriction, chronic exercise, cold exposure and sympathetic nervous system activation³⁰. Neuronal *Ptpn1*^{-/-} mice have decreased glucose and insulin levels and reduced food intake. Therefore, other mechanisms must account for their increased leptin levels. White adipose tissue receives inputs from sympathetic nerves³¹⁻³³. However, the increased energy expenditure in whole-body and neuronal *Ptpn1*^{-/-} mice is more consistent with increased, rather than decreased, overall sympathetic activity. Recent evidence suggests parasympathetic innervation of some fat depots³⁴; conceivably, parasympathetic inputs regulate adipocyte leptin production. Alternatively (or in addition), because leptin levels are increased even in young (4-week-old) neuronal *Ptpn1*^{-/-} mice, pathways controlled by neuronal PTP1B may be required for the postnatal decline in leptin levels³⁵. Neuronal PTP1B could regulate secretion of an unknown humoral factor that controls adipokine secretion. Notably, elevated leptin levels alone are unlikely to account for the leanness caused by neuronal PTP1B

deficiency, as transgenic mice overexpressing leptin become obese when placed on a HFD³⁶.

Whole-body *Ptpn1*^{-/-} mice have enhanced systemic insulin sensitivity and increased insulin receptor phosphorylation in skeletal muscle and liver^{16,17}. Accordingly, skeletal muscle and/or liver have been presumed to mediate the insulin-sensitizing effects in *Ptpn1*^{-/-} mice. Indeed, our preliminary studies of liver- and muscle-specific *Ptpn1*^{-/-} mice demonstrate a role for PTP1B in these tissues. However, neuronal *Ptpn1*^{-/-} mice also show improved insulin sensitivity and glucose tolerance, even without differences in weight or FFA (for example, in 8-week-old females). Although female neuronal *Ptpn1*^{-/-} mice might have subtle differences in adiposity at this age, these differences are unlikely to explain such substantial improvements in whole-body insulin sensitivity. Thus, our results suggest a previously unknown role for neuronal PTP1B in the regulation of peripheral insulin sensitivity. Possibly, the increased physical activity leads to enhanced insulin sensitivity. Neuronal pathways controlling hepatic glucose production^{27,31,37–40} or affecting adipokine secretion also could contribute. Therefore, inhibiting neuronal PTP1B should be sufficient to promote appreciable weight reduction, and access to the brain may also be necessary to optimally improve insulin sensitivity and glucose homeostasis.

METHODS

Mice with tissue-specific deletion of PTP1B. We subcloned a 9.2-kb fragment containing exons 5–9 of the mouse *Ptpn1* gene¹⁷ into pBluescript (Stratagene). We designed a targeting construct to insert a neomycin-resistance (Neo) cassette flanked by *loxP* sites between exons 5 and 6, and a third *loxP* site between exons 8 and 9, using the pSABgal-pgkneo⁺2PGKDTA vector (details available from K.K.B. upon request). Properly targeted clones and, subsequently, *Ptpn1*^{loxP/loxP} mice were obtained as described in **Supplementary Methods** online. We genotyped mice by PCR (**Supplementary Methods**) and analyzed tissues from experimental mice for deletion of *Ptpn1* by immunoblotting. All experiments used *Ptpn1*^{loxP/loxP} mice on a mixed 129SvJ × C57BL/6J background. We obtained Nestin-Cre and Albumin-Cre mice from Jackson Labs, MCK-Cre mice⁴¹ from C. Ronald Kahn (Joslin Diabetes Center), and we generated aP2-Cre mice previously⁴².

Mouse studies. We maintained mice on a 12-h light-dark cycle in a temperature-controlled barrier facility, with free access to water and food (standard lab chow Purina #5058 or custom high-fat diet Teklad #TD93075)¹⁷. We used age-matched littermates for all experiments. We conducted mouse studies according to federal guidelines; studies were approved by the Harvard Medical Area Standing Committee on Animals.

Immunoblotting. We dissected mouse tissues and immediately froze them in liquid nitrogen. We prepared whole-cell lysates and performed PTP1B immunoblotting as described¹⁸. For adipose *Ptpn1*^{-/-} mice, deletion efficiency was assessed in isolated adipocytes⁴³, and blots were normalized by immunoblotting for Erk to control for loading. We quantified blots using the Odyssey Imaging System (LI-COR Biotechnology) or by densitometry using NIH Image.

Body composition and food intake. At weaning, we placed mice on standard lab chow or HFD and followed them for at least 20 weeks. We weighed mice weekly and measured food intake daily. Feed efficiency was calculated as grams of weight gained per grams of food consumed²³. We measured epididymal fat pad weight and carried out carcass analysis on mice on HFD for 18 weeks¹⁷.

Metabolic measurements. We acclimated mice (8–10-week-old males on chow diet) for 12 h and then monitored them for 24 h in an indirect open-circuit calorimeter (Oxymax System, Columbus Instruments). We measured oxygen and carbon dioxide concentrations by volume⁴⁴. Respiratory quotient was calculated as the ratio of V_{CO_2}/V_{O_2} . We monitored

physical activity of the mice with the OPTO-M3 Activity Application Device (Columbus Instruments). Ambulatory movements were determined by infrared beams in *x* and *y* axes. We determined bone density by DEXA scanning.

Leptin sensitivity and signaling. For *in vivo* studies, we administered mouse leptin (A.F. Parlow, National Hormone and Peptide Program) intraperitoneally to male mice on chow diet (12 weeks of age). We monitored body weight and food intake daily for 7 d. Measurements for the 3 d before the start of the experiment were averaged and used to calculate percent basal. We administered leptin intraperitoneally twice daily (morning and evening; dose, 0.5 μg/g; total dose for the 24-h period, 1.0 μg/g). We performed hypothalamic signaling experiments using mice fasted for 15–18 h¹⁸. We performed immunoblotting for pStat3, Stat3 (Cell Signaling) or PTP1B.

RNA extraction and real-time PCR. We extracted RNA using Trizol (Invitrogen) and quantified neuropeptide and leptin mRNA levels by quantitative real-time PCR⁴⁵. The relative copy number of 18S or *Gapdh* RNA was quantified and used for normalization, and data were calculated as described⁴⁵. Primer and probe sequences for neuropeptide transcripts and RT-PCR conditions are in **Supplementary Methods**. We quantified leptin transcripts using the Leptin Assay-on-Demand (Mm00434759_m1, Applied Biosystems).

Leptin content. We dissected and immediately froze epididymal fat pads. We then homogenized fragments in lysis buffer (0.3 M NaCl, 1 mM EDTA, 5 mM Tris-HCl, pH 7.4, 1% Triton X-100, 1 mM PMSF), sonicated them briefly and cleared them by centrifugation at 14,000g at 4 °C. We determined protein concentrations by BCA assay (Pierce) and leptin content by immunoassay (Quantikine Mouse Leptin Immunoassay, R&D Systems).

Adipokine measurements. We collected blood by tail bleeding fed mice between 08:00 and 10:00 and separated serum by centrifugation at 6,000g. We measured serum insulin and leptin (CrystalChem), and serum adiponectin and resistin (Linco Research), by enzyme-linked immunosorbent assays.

Glucose homeostasis. We assayed blood glucose in tail blood with a glucometer (Lifescan One-Touch Basic). Fed measurements were taken between 08:00 and 10:00. Where indicated, we obtained blood from mice fasted for 12–16 h. We quantified FFA (Wako) and serum triglycerides (Sigma) by enzymatic assay. We performed insulin-tolerance tests and glucose-tolerance tests as previously described¹⁷. Male mice were 12 weeks of age and on HFD for 9 weeks at the time of analysis. Female mice were 18 weeks of age and on HFD for 15 weeks.

Statistical analysis. Results are expressed as mean ± s.e.m. Comparisons between groups were made by unpaired two-tailed Student *t*-test or analysis of variance (ANOVA) with repeated measures and Bonferroni post-test analysis, as appropriate. *P* < 0.05 was considered to be statistically significant.

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Medicine* website for details).

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