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Improved metabolic phenotype of hypothalamic PTP1B-deficiency is dependent upon the leptin receptor*

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ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is a known regulator of central metabolic signaling, and mice with whole brain-, leptin receptor (LepRb) expressing cell-, or proopiomelanocortin neuron-specific PTP1B-deficiency are lean, leptin hypersensitive, and display improved glucose homeostasis. However, whether the metabolic effects of central PTP1B-deficiency are due to action within the hypothalamus remains unclear. Moreover, whether or not these effects are exclusively due to enhanced leptin signaling is unknown. Here we report that mice with hypothalamic PTP1B-deficiency (Nkx2.1-PTP1B$^{-/-}$) display decreased body weight and adiposity on high-fat diet with no associated improvements in glucose tolerance. Consistent with previous reports, we find that hypothalamic deletion of the LepRb in mice (Nkx2.1-LepRb$^{-/-}$) results in extreme hyperphagia and obesity. Interestingly, deletion of hypothalamic PTP1B and LepRb (Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$) does not rescue the hyperphagia or obesity of Nkx2.1-LepRb$^{-/-}$ mice, suggesting that hypothalamic PTP1B contributes to the central control of energy balance through a leptin receptor-dependent pathway.

1. INTRODUCTION

Obesity continues to be a major public health crisis in the United States and worldwide [1–3]. Given the numerous metabolic comorbidities and an overall increased all-cause mortality associated with obesity [4,5], understanding the underlying biological systems that regulate body weight and adiposity is of great importance. Though obesity is commonly thought of as a disease affecting the periphery (i.e. increased body fat), the central nervous system (CNS) plays a key role in regulating appetite, metabolism, and body weight. Neurons within the hypothalamus integrate neuroendocrine signals from the periphery, gauging short term and long term energy status. The adipocyte-secreted hormone leptin is one such signal whose effects on the central control of energy homeostasis have been studied in depth. Circulating leptin acts on leptin receptors (LepRbs) expressed within the hypothalamus and extrahypothalamic sites (hindbrain nucleus tractus solitarius, parabrachial nucleus) [6,7] to suppress food intake and increase energy expenditure, ultimately promoting negative energy balance. Leptin- (*ob/ob*) and LepRb-deficient (*db/db*) mice are hyperphagic and develop extreme obesity [8–10]. Moreover, deletion of LepRb within the hypothalamus in mice, driven by the ventral forebrain specific Nkx2.1-Cre, recapitulates much of the *db/db* phenotype [11]. LepRb-deficiency within the hindbrain NTS in mice also results in hyperphagia and increased weight gain [12].

At the molecular level, when LepRb is activated, several tyrosine phosphorylation events occur. Initially, leptin binding to LepRb results in a conformational change of the receptor and activation of the associated tyrosine kinase Janus kinase 2 (JAK2). JAK2 autophosphorylates and subsequently phosphorylates tyrosine residues along the intracellular tail of LepRb, which can further recruit downstream signaling molecules necessary for eliciting leptin's physiological effects [13,14]. Protein tyrosine phosphatase 1B (PTP1B) shows enriched expression correlating with areas of LepRb expression [15], and is a known negative regulator of leptin signaling via direct dephosphorylation of JAK2 [15–17]. In mice, PTP1B is encoded by the *Ptpn1* gene, and whole body, whole brain-, LepRb-expressing cell-, or POMC neuron-specific PTP1B-deficiency results in decreased body weight and adiposity on HFD [18–22]. In contrast, deletion of PTP1B in peripheral tissues does not affect body weight [23–26]. Since CNS PTP1B-deficient models to date have used holistic (whole brain) or neuron specific approaches (POMC- or LepRb-targeted), the anatomic specificity of PTP1B's metabolic effects remains unclear. Like the LepRb, POMC is expressed both in the hypothalamus and hindbrain, and there is evidence of enhanced hypothalamic and hindbrain leptin signaling in POMC-PTP1B$^{-/-}$ mice [22,27], suggesting a metabolic role for PTP1B in both regions. Thus, the extent to which the metabolic effects of PTP1B deficiency are due to action within the hypothalamus or in extrahypothalamic sites remains unknown. Here, to determine the metabolic contribution of
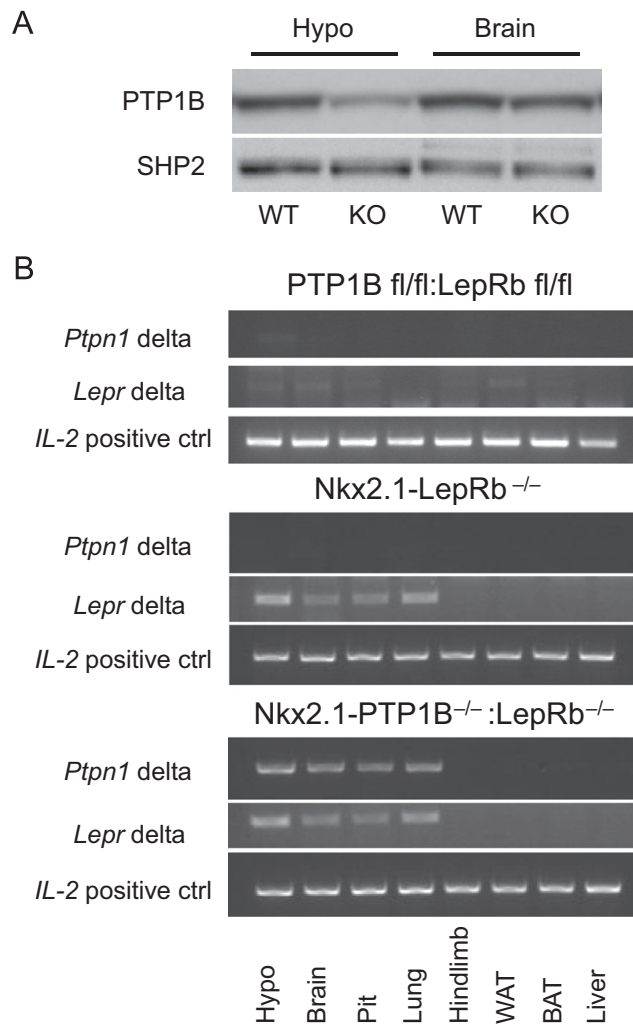
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Figure 1: Detection of PTP1B deletion in Nkx2.1-PTP1B deficient mouse models. (A) PTP1B protein levels in the hypothalamus and brain of Nkx2.1-PTP1B$^{-/-}$ (KO) mice compared with PTP1B fl/fl controls (WT). SHP2 protein levels are shown as a loading control. (B) Detection of deletion of PTP1B or LepRb floxed alleles in PTP1B fl/fl:LepRb fl/fl, Nkx2.1-LepRb$^{-/-}$, and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice. DNA was isolated from different tissues [hypothalamus (Hypo), extrahypothalamic brain, pituitary (Pit), lung, hindlimb, perigonadal white adipose tissue (WAT), brown adipose (BAT), and liver], and deletion of floxed allele was detected by PCR.

hypothalamic PTP1B, we generated a genetic PTP1B deficient mouse model using the Nkx2.1-Cre line, which leads to widespread recombination within the ventral forebrain.

The improved metabolic phenotype of central PTP1B-deficient models is largely attributed to enhanced leptin sensitivity. Interestingly, however, compound *ob/ob*:PTP1B$^{-/-}$ mice show attenuated weight gain in comparison to *ob/ob* mice [17], suggesting that there may be leptin-independent metabolic effects of PTP1B deficiency. Furthermore, *db/db*:PTP1B$^{-/-}$ mice display decreased plasma triglycerides and serum free fatty acids when compared to *db/db* :PTP1B$^{+/-}$ [28], and *ob/ob* mice treated with PTP1B antisense oligonucleotides possess decreased epididymal fat compared to saline-treated controls [29]. Thus, we were interested in examining whether or not the metabolic effects of PTP1B deficiency are exclusively leptin receptor dependent. For these studies, we crossed the Nkx2.1-Cre line with *Ptpn1$^{loxP/loxP}$:Lepr$^{loxP/loxP}$* mice in order to generate compound hypothalamic Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice. Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice were compared to Nkx2.1-LepRb$^{-/-}$ mice as well as wildtype controls to determine whether PTP1B's metabolic effects within the hypothalamus are dependent upon functional leptin receptor signaling.

2. MATERIALS AND METHODS

2.1. Animal care

All animal care protocols and procedures were approved by the University of Pennsylvania Institutional Care and Use Committee. We maintained mice on a 12-h light/12-h dark cycle in a temperature controlled barrier facility, with free access to water and food: standard chow autoclavable Lab Diet 5010 (calories provided by protein [28.7%], fat [12.7%], and carbohydrate [58.5%]) or custom HFD Teklad TD93075 (calories provided by protein [21.2%], fat [54.8%], and carbohydrate [24%]). Age-matched littermates were used for all experiments.

2.2. Generation of Nkx2.1-PTP1B$^{-/-}$, Nkx2.1-LepRb$^{-/-}$, and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice

All mice were on a C57BL/6 background. *Ptpn1$^{loxP/loxP}$* mice were generated previously [20] on a mixed 129Sv/J × C57BL/6 background but were backcrossed at least 10 generations onto C57BL/6 background prior to mating with other lines. *Lepr$^{loxP/loxP}$* mice on a C57BL/6 background were obtained from S. Chua (Albert Einstein College of Medicine) and S. Obici (University of Cincinnati, Ohio). Nkx2.1-Cre transgenic mice were obtained from The Jackson Laboratory (Stock #008661, Bar Harbor, ME). Genotyping primer sequences were as follows: PTP1B fl forward 5′-TGCTCACTCACCCTGCTACAA, reverse 5′-GAAATGGCTCACTCCTACTGG. Lepr fl forward 5′-AACGGTTTTA-CAGTCTCCA, reverse 5′-AAGGCCCATTTAGTCAAC. Nkx2.1-Cre forward 5′-CCACAGGCACCCCACAAAAATG, reverse 5′-GCCTGGCGATCCCTGAACAT.

2.3. Isolating DNA from tissues for detection of recombination of the floxed alleles

Tissues were digested at 55 °C overnight in proteinase K digestion buffer (100 mM Tris–HCl pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 300 μg/ml proteinase K). Saturated NaCl (∼6 M) was added to the digestion, and samples were vortexed vigorously for 1 min. Samples were centrifuged for 20 min at 13,700g, and supernatants were transferred to a fresh tube. DNA was precipitated by adding 1 ml 100% ethanol, and pellets were washed once with 70% ethanol and were resuspended in 100 μl of sterile PCR water for analysis. PCR primers for detection of recombined alleles: *Ptpn1Δ/Δ* forward 5′-GTGGTGCCTGCAAGAGAACTGAC, reverse 5′-GAAATGGCTCACTCCTACTGG. *LeprΔ/Δ* forward 5′-GTCTGATTTGATAGA-TGGTCTT, reverse 5′-ACAGGCTTGAGAACATGAACAC. *IL-2* internal control forward 5′-CTAGGCCACAGAATTGAAAGATCT, reverse 5′-GTAGGTGGAAAT-TCTAGCATCATCC.

2.4. Immunoblotting

Mouse tissues were dissected and immediately frozen in liquid nitrogen. Whole cell lysates were prepared in modified RIPA buffer containing fresh protease inhibitors, and PTP1B and SHP2 immunoblotting was performed as described previously [15,22]. PTP1B immunoblots were normalized to SHP2 (Santa Cruz Biotechnology Inc., sc-280) to control for loading.

2.5. Body composition and food intake

At weaning, mice were placed on diets of either standard laboratory chow or HFD. Body weights were assessed weekly and food intake was measured daily at indicated age. Body length was measured as nose-rump length at indicated age. Epididymal fat pads were dissected and weighed at indicated age. Total fat and lean mass was measured in conscious mice using NMR (Echo Medical Systems) at indicated age in the Penn IDOM Mouse Phenotyping, Physiology and Metabolism Core.

2.6. Energy expenditure measurements

Rectal temperature was measured with a thermistor during the light cycle in animals at 14–17 weeks of age (MicroTherma 2T; ThermoWorks). Feed
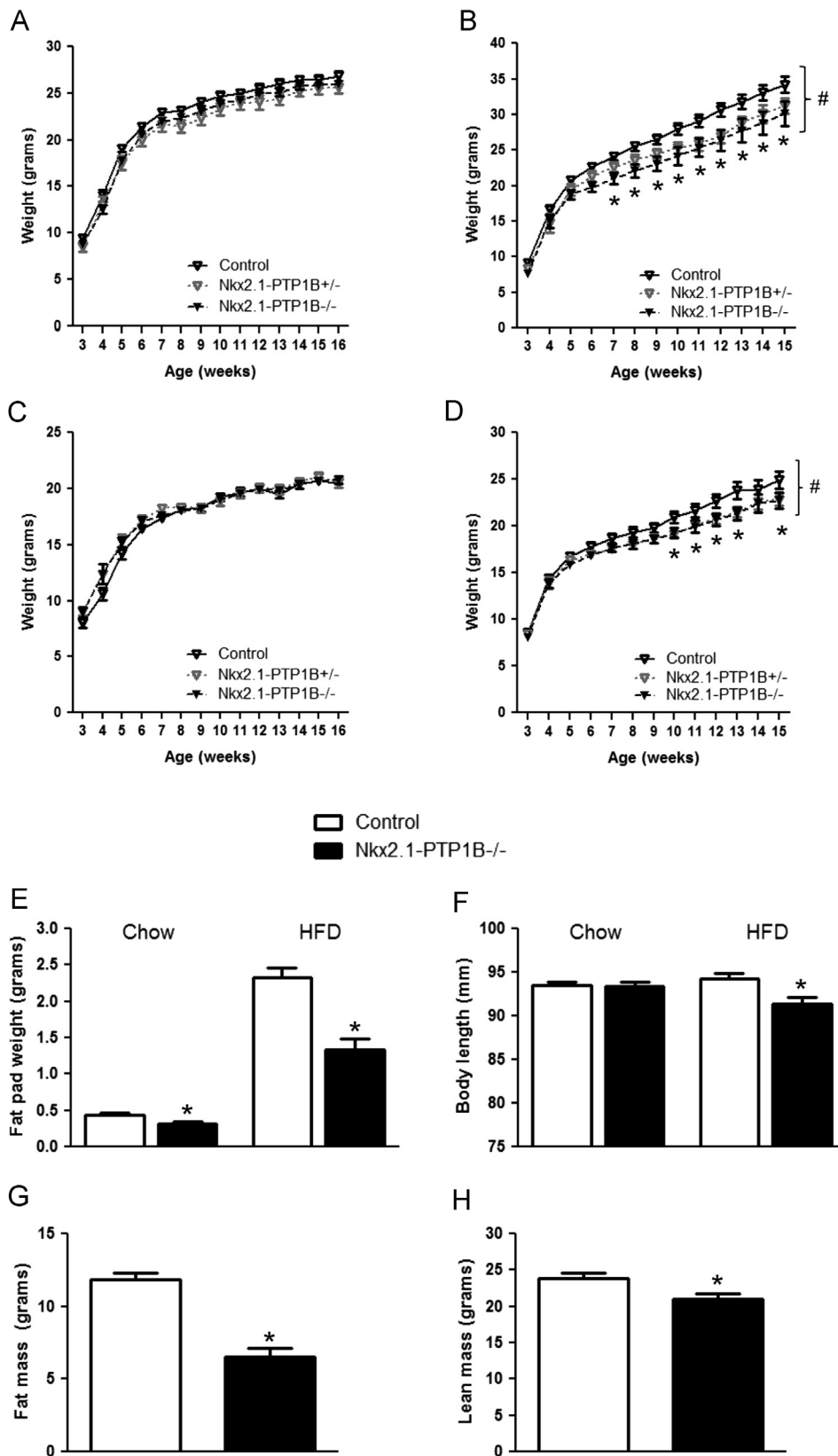
Figure 2: Nkx2.1-PTP1B$^{-/-}$ mice have reduced body weight and adiposity on HFD. (A) Body weights of male Nkx2.1-PTP1B$^{-/-}$ ($n=9$), Nkx2.1-PTP1B$^{+/-}$ ($n=8$), and control PTP1B fl/fl ($n=14$) mice on chow. (B) Body weights of male Nkx2.1-PTP1B$^{-/-}$ ($n=6$), Nkx2.1-PTP1B$^{+/-}$ ($n=6$), and control PTP1B fl/fl ($n=17$) mice on HFD. (C) Body weights of female Nkx2.1-PTP1B$^{-/-}$ ($n=10$), Nkx2.1-PTP1B$^{+/-}$ ($n=8$), and control PTP1B fl/fl ($n=9$) mice on chow. (D) Body weights of female Nkx2.1-PTP1B$^{-/-}$ ($n=15$), Nkx2.1-PTP1B$^{+/-}$ ($n=11$), and control PTP1B fl/fl ($n=17$) mice on HFD. (E) Epididymal fat pad weight for male Nkx2.1-PTP1B$^{-/-}$ ($n=9$ on chow, 6 on HFD) and control PTP1B fl/fl ($n=14$ on chow, 9 on HFD) mice on chow or HFD. (F) Body length for male Nkx2.1-PTP1B$^{-/-}$ ($n=8$ on chow, 6 on HFD) and control PTP1B fl/fl ($n=14$ on chow, 9 on HFD) mice on chow or HFD. (G) Fat mass as determined by NMR of male Nkx2.1-PTP1B$^{-/-}$ ($n=6$) and control PTP1B fl/fl ($n=9$) mice on HFD. (H) Lean mass as determined by NMR of male Nkx2.1-PTP1B$^{-/-}$ ($n=6$) and control PTP1B fl/fl ($n=9$) mice on HFD. All values are mean ± SEM. Weight curves analyzed by two-way ANOVA with repeated measures: #$p \leq 0.05$. Fishers LSD post hoc pairwise comparisons between Nkx2.1-PTP1B$^{-/-}$ and wildtype controls:*$p < 0.05$. Body composition and body length data analyzed by two tailed Student's t-test: *$p < 0.05$.

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Genotype	Chow			HFD		
	1B fl/fl	Nkx2.1-PTP1B+/-	Nkx2.1-PTP1B-/-	1B fl/fl	Nkx2.1-PTP1B+/-	Nkx2.1-PTP1B-/-
Serum leptin (ng/ml)	1.65 ± 0.21	1.14 ± 0.18	1.20 ± 0.16	23.12 ± 4.61	6.69 ± 1.38*	11.50 ± 3.02*
Corticosterone (ng/ml)	89.68 ± 20.25	nd	106.59 ± 28.96	62.35 ± 24.53	nd	82.89 ± 11.36
Fasting blood glucose (mg/dl)	83.4 ± 5.8	75.5 ± 7.5	73.8 ± 6.1	105.3 ± 9.8	92.0 ± 10.9	91.0 ± 10.2
Fasting serum insulin (ng/ml)	0.41 ± 0.03	0.35 ± 0.03	0.34 ± 0.02	0.93 ± 0.17	0.37 ± 0.12*	0.55 ± 0.06^
Genotype	Chow			HFD		
	1B fl/fl:LepRb fl/fl	Nkx2.1-LepRb-/-	Nkx2.1-PTP1B-/-:LepRb-/-	1B fl/fl:LepR fl/fl	Nkx2.1-LepRb-/-	Nkx2.1-PTP1B-/-:LepRb-/-
Serum leptin (ng/ml)	8.18 ± 0.77	90.34 ± 4.79*	89.47 ± 5.88*	16.19 ± 4.91	104.68 ± 3.74*	127.88 ± 2.52*#
Corticosterone (ng/ml)	151.55 ± 29.95	407.29 ± 53.43*	361.75 ± 95.75*	nd	nd	nd
Fasting blood glucose (mg/dl)	62.3 ± 5.6	89.1 ± 18.4	107.6 ± 12.3*	94.3 ± 9.2	219.1 ± 29.3*	245.7 ± 78.2*
Fasting serum insulin (ng/ml)	0.48 ± 0.07	9.40 ± 1.47*	14.43 ± 2.76*#	0.62 ± 0.11	13.25 ± 4.37*	14.78 ± 9.60*

Table 1: Metabolic and neuroendocrine parameters. Measures of fasting blood glucose and fasting serum insulin are from overnight fasted male animals at 12–14 weeks of age for controls, Nkx2.1-PTP1B$^{+/-}$, Nkx2.1-PTP1B$^{-/-}$ on chow or HFD. Fasting blood glucose and fasting serum insulin of Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$, Nkx2.1-LepRb$^{-/-}$, and control PTP1B fl/fl:LepRb fl/fl mice are taken at 12–14 weeks on chow and at 9 weeks on HFD. Measures of serum leptin and corticosterone are from ad lib fed male animals at 7 weeks of age. Fasting blood glucose and serum measures analyzed by one-way ANOVA followed by Fisher's LSD pairwise comparison: *$p < 0.05$, ^$p = 0.08$ indicated group vs. control. #$p < 0.05$ Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ vs. Nkx2.1-LepRb$^{-/-}$. nd, not determined.
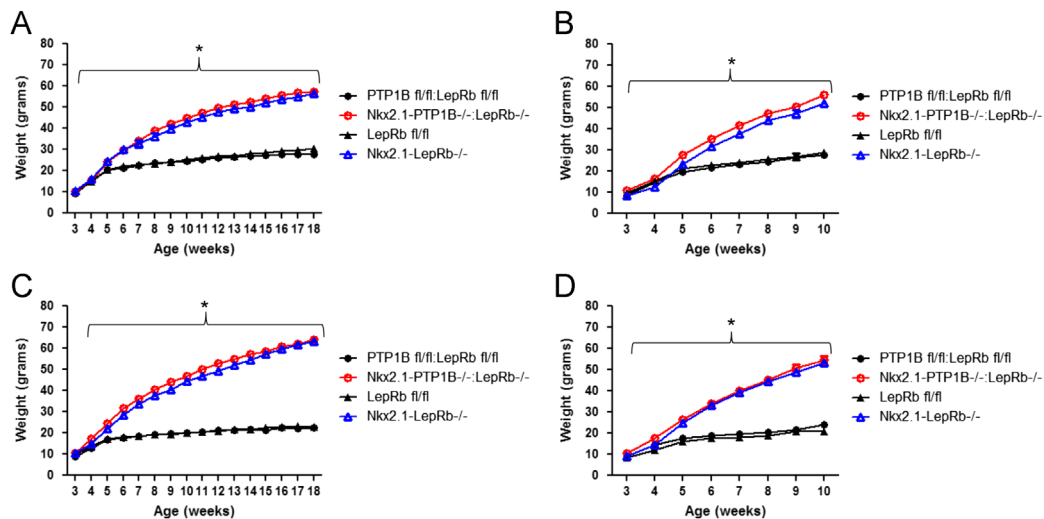

Figure 3: Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice show no difference in body weight compared to Nkx2.1-LepRb$^{-/-}$ mice. (A) Body weights of male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=8$), Nkx2.1-LepRb$^{-/-}$ ($n=9$), control PTP1B fl/fl:LepRb fl/fl ($n=14$) and control LepRb fl/fl ($n=9$) mice on chow. (B) Body weights of male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=5$), Nkx2.1-LepRb$^{-/-}$ ($n=7$), control PTP1B fl/fl:LepRb fl/fl ($n=11$) and control LepRb fl/fl ($n=7$) mice on HFD. (C) Body weights of female Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=13$), Nkx2.1-LepRb$^{-/-}$ ($n=13$), control PTP1B fl/fl:LepRb fl/fl ($n=15$) and control LepRb fl/fl ($n=15$) mice on chow. (D) Body weights of female Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=6$), Nkx2.1-LepRb$^{-/-}$ ($n=13$), control PTP1B fl/fl:LepRb fl/fl ($n=6$) and control LepRb fl/fl ($n=5$) mice on HFD. All values are mean ± SEM. Weight curves analyzed by two-way ANOVA with repeated measures: *$p < 0.05$.

efficiency was calculated as grams weight gained/food consumed over indicated time period. Brown adipose tissue gene expression (*Ucp1* and *Prdm16*) was measured by real-time PCR as described below.

2.7. Glucose homeostasis
Glucose tolerance tests (GTTs) were performed as described previously [19]. Briefly, mice were fasted overnight (14–16 h). Glucose dose used for intraperitoneal injections was 2 mg/g BW (20% solution). Blood glucose was assayed in tail blood using a glucometer (Contour, Bayer). Fasting insulin levels were determined as described below.

2.8. RNA extraction and real-time PCR
Mice were euthanized at the onset of the light cycle (08:00–10:00 a.m.). Tissues were rapidly dissected and flash frozen in liquid nitrogen. Total RNA was extracted from tissues using TRIzol (Invitrogen) and the RNeasy kit (QIAGEN). cDNA was synthesized from 1 μg total RNA using the Advantage RT-for-PCR kit (Clontech). The relative mRNA levels of *Ucp1* and *Prdm16* were assessed and quantified by quantitative real-time PCR (qRT-PCR). The housekeeping gene *hprt1* was used as an internal control. The qRT-PCR

reactions were carried out using RT^2 SYBR Green qPCR Master Mix (SABiosciences), and samples were run using the Eppendorf Mastercycler ep realplex. Primer sequences for *Ucp1* and *Prdm16* were reported previously [30]. Relative mRNA expression was calculated using the comparative Ct method as described previously [20].

2.9. Serum analysis
All blood samples were collected between 08:00–10:30 am at indicated age. Fasting blood samples were collected following overnight fast (14–16 h). Serum was separated by centrifugation at 6000*g*. Serum insulin and leptin (CrystalChem) and serum corticosterone (Immunodiagnostic Systems) were measured by ELISA.

2.10. Statistical analysis
Results are expressed as mean ± SEM. Comparisons between groups were made by unpaired 2-tailed Student's *t*-test, 1-way ANOVA or 2-way ANOVA with repeated measures in one factor followed by Fisher's protected least significant difference (PLSD) or Student–Newman–Keuls pairwise

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comparison, as appropriate. A *p*-value less than 0.05 was considered to be statistically significant.

3. RESULTS

3.1. Generation of mice with hypothalamic-specific PTP1B deficiency or compound PTP1B:LepRb deficiency

In order to generate mice with PTP1B deficiency throughout the hypothalamus, we crossed $Ptpn1^{loxP/loxP}$ mice to a line of transgenic Nkx2.1-Cre mice. The resulting $Ptpn1^{+/loxP}$:Nkx2.1-Cre were subsequently crossed to $Ptpn1^{loxP/loxP}$ mice to generate $Ptpn1^{loxP/loxP}$:Nkx2.1-Cre (hereafter termed Nkx2.1-PTP1B$^{-/-}$) mice, $Ptpn1^{+/loxP}$:Nkx2.1-Cre (hereafter termed Nkx2.1-PTP1B$^{+/-}$) mice, and $Ptpn1^{loxP/loxP}$ and $Ptpn1^{+/loxP}$ wildtype littermate controls. The Nkx2.1-Cre line has been characterized previously [11], and strong Cre recombinase expression is found broadly throughout the majority of the hypothalamus but is not detected in caudal brain sites. In addition to the hypothalamus, Nkx2.1-Cre is known to be expressed within the posterior pituitary, lung, thyroid, and the median ganglionic eminence, which may lead to limited Cre expression within migrating cortical interneurons [31]. Consistent with past reports, Nkx2.1-PTP1B$^{-/-}$ mice indeed show decreased PTP1B protein expression in the hypothalamus relative to wildtype controls, but do not show differences in total PTP1B protein levels in lysates from extrahypothalamic brain (Figure 1A).

To further explore whether the metabolic effects of CNS PTP1B deficiency are dependent upon functional leptin signaling, we generated mice with compound conditional deletion of PTP1B and LepRb in the hypothalamus. $Ptpn1^{loxP/loxP}$ mice were crossed to $Lepr^{loxP/loxP}$ mice to generate $Ptpn1^{+/loxP}$:$Lepr^{+/loxP}$ mice. Subsequently, $Ptpn1^{+/loxP}$:$Lepr^{+/loxP}$ mice were intercrossed to generate $Ptpn1^{loxP/loxP}$:$Lepr^{loxP/loxP}$ mice. $Ptpn1^{loxP/loxP}$:$Lepr^{loxP/loxP}$ mice were then crossed with Nkx2.1-Cre mice to yield $Ptpn1^{+/loxP}$:$Lepr^{+/loxP}$:Nkx2.1-Cre mice. Finally, $Ptpn1^{+/loxP}$:$Lepr^{+/loxP}$:Nkx2.1-Cre mice were crossed to $Ptpn1^{loxP/loxP}$:$Lepr^{loxP/loxP}$ to yield $Ptpn1^{loxP/loxP}$:$Lepr^{loxP/loxP}$:Nkx2.1-Cre (hereafter termed Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$) mice and $Ptpn1^{loxP/loxP}$:$Lepr^{loxP/loxP}$ littermate, wild-type controls. In a parallel cross, Nkx2.1-LepRb$^{-/-}$ mice were generated using the same breeding strategy detailed above for Nkx2.1-PTP1B$^{-/-}$ mice. To verify deletion of the *Ptpn1* and *Lepr* genes, we extracted DNA from a variety of tissues (hypothalamus, extrahypothalamic brain, pituitary, lung, hindlimb muscle, epididymal white adipose, interscapular brown adipose, and liver) and assessed for deletion of the floxed alleles by PCR. Deletion of the *Ptpn1* and *Lepr* floxed alleles was detected in DNA extracted from hypothalamus, extrahypothalamic brain, pituitary, and lung (Figure 1B) of Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice. No deletion was detected in DNA from any tissues isolated from floxed wildtype control animals, and as expected only deletion of the *Lepr* floxed allele was detected in Nkx2.1-LepRb$^{-/-}$ mice.

3.2. Nkx2.1-PTP1B$^{-/-}$ mice have decreased body weight on high-fat diet

We examined weekly body weights of male and female Nkx2.1-PTP1B$^{-/-}$ mice and PTP1B fl/fl controls on chow or high fat diet for at least 12 weeks from weaning. When fed a chow diet, male and female Nkx2.1-PTP1B$^{-/-}$ and Nkx2.1-PTP1B$^{+/-}$ mice have similar body weights compared to wildtype floxed controls (Figure 2A and C). In contrast, HFD-fed male Nkx2.1-PTP1B$^{-/-}$ and Nkx2.1-PTP1B$^{+/-}$ mice show significantly decreased body weight compared to wildtype controls (Figure 2B). Female Nkx2.1-PTP1B$^{-/-}$ and Nkx2.1-PTP1B$^{+/-}$ mice on HFD also display decreased body weight compared to controls (Figure 2D), but to a lesser extent than the males. Overall, these data indicate hypothalamic PTP1B deficiency results in resistance to diet-induced obesity.

3.3. Nkx2.1-PTP1B$^{-/-}$ mice have decreased adiposity on chow or HFD

To determine whether differences in body weight reflect decreased fat mass or total body size, body composition was assessed. Despite no difference in body weight on chow compared to wildtype controls, male Nkx2.1-PTP1B$^{-/-}$ mice display significantly decreased adiposity as determined by epididymal fat pad weight (Figure 2E). Male Nkx2.1-PTP1B$^{-/-}$ mice show no difference in body length relative to controls on chow (Figure 2F). Chow fed female Nkx2.1-PTP1B$^{-/-}$ mice show no difference in either perigonadal fat pad weight or body length compared to controls (Supplemental Figure 1A and B). On HFD, male Nkx2.1-PTP1B$^{-/-}$ mice display decreased adiposity as measured by epididymal fat pad weight as well as total fat mass by NMR (Figure 2E and G). Consistent with their reduced adiposity, male Nkx2.1-PTP1B$^{-/-}$ mice have decreased circulating leptin levels compared to wildtypes (Table 1). On HFD, male Nkx2.1-PTP1B$^{-/-}$ mice also show decreased lean mass and a concomitant decrease in body length (Figure 2H and F). Female Nkx2.1-PTP1B$^{-/-}$ mice display decreased perigonadal fat pad weight relative to wildtypes on HFD, but show no difference in body length (Supplemental Figure 1C and D).

3.4. Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice exhibit obesity on chow or HFD

Since past CNS PTP1B$^{-/-}$ models show a lean metabolic phenotype resulting presumably from enhanced leptin sensitivity [20–22,27], we examined the effects of compound Nkx2.1-PTP1B:LepRb deficiency on body composition compared to Nkx2.1-LepRb$^{-/-}$ mice and wildtype controls to determine if functional leptin receptor signaling is required for any metabolic contribution of PTP1B deficiency in the hypothalamus. Male Nkx2.1-LepRb$^{-/-}$ mice and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ both display significant weight gain compared to wildtype controls (Figure 3A—chow diet and B—HFD). Interestingly, male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ shows no difference in body weight compared to Nkx2.1-LepRb$^{-/-}$ mice on chow or HFD, demonstrating that the metabolic effects of PTP1B deficiency in the hypothalamus are in fact dependent upon functional leptin receptor signaling. Similarly, female Nkx2.1-LepRb$^{-/-}$ mice and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ display significantly increased body weight on chow or HFD relative to controls, and the extent of weight gain is similar in Nkx2.1-LepRb$^{-/-}$ and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice (Figure 3C—chow diet and D—HFD).

3.5. Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice display increased adiposity on chow or HFD

We examined total fat mass, lean mass, and body length in Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice on chow or HFD. On chow diet, male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice display significantly increased total fat and lean mass compared to controls as determined by NMR (Figure 4A and B). Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice also show a nonsignificant trend toward increased body length when compared to wildtype controls (Figure 4C). Like body weight, the extent of increased fat mass, lean mass and length is similar between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice on chow. Total fat mass, lean mass, and body length are also similar between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice on HFD (Figure 4D–F). On chow or HFD, both Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice display significantly elevated serum leptin levels consistent with their increased adiposity (Table 1). Similar body composition and length measures are also seen in female Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice with both groups displaying significantly increased total fat and lean mass compared to wildtype controls on chow or HFD (Supplemental Figure 2 and data not shown), but no differences between the two groups.

Original article

3.6. Food intake, core temperature, and serum corticosterone measurements

To examine the cause of the reduced body weight and adiposity observed in Nkx2.1-PTP1B$^{-/-}$ mice on HFD, we measured daily food intake and core temperature as a measure of thermogenesis. Average daily food intake and cumulative food intake on HFD is reduced in male Nkx2.1-PTP1B$^{-/-}$ compared to wildtype controls (Figure 5A and B). Interestingly, feed efficiency (Δbody weight/Δfood intake) and core temperature are similar in Nkx2.1-PTP1B$^{-/-}$ and control mice suggesting energy expenditure may not be affected in this model of hypothalamic PTP1B deficiency (Figure 5C and D). Consistent with this idea, core temperature is also comparable when comparing Nkx2.1-PTP1B$^{-/-}$ and control mice at 17 weeks of age (Supplemental Figure 3A). Expression of *Ucp1* in interscapular brown adipose tissue (BAT) and *Prdm16*, a gene which plays a major role in brown fat determination, in inguinal white adipose tissue (WAT) were examined to determine if BAT activation or WAT browning was enhanced in Nkx2.1-PTP1B$^{-/-}$ mice. Levels of *Ucp1* expression in BAT and *Prdm16* expression in inguinal WAT were similar in Nkx2.1-PTP1B$^{-/-}$ mice and wildtype controls (Supplemental Figure 3B).

As expected from previous studies, Nkx2.1-LepRb$^{-/-}$ mice display significant hyperphagia as demonstrated by increased average daily and cumulative food intake compared to wildtype controls on chow (Figure 5E and F). There were no differences in food intake across genders, thus food intake data presented is from males and females combined. Notably, Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ show similar levels of hyperphagia compared to Nkx2.1-LepRb$^{-/-}$ mice, suggesting that the food intake reduction seen in Nkx2.1-PTP1B$^{-/-}$ mice is dependent upon functional leptin receptor signaling. Feed efficiency (Figure 5G, males and Supplemental Figure 3C, females) is not different between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$, Nkx2.1-LepRb$^{-/-}$, and wildtype control groups. Core temperature is similar in all three male groups (Figure 5H), but female Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice show significantly decreased core temperature relative to wildtype controls (Supplemental Figure 3D).

To assess whether hypothalamic PTP1B deficiency affects hypothalamus–pituitary–adrenal **(**HPA) function, we measured morning serum corticosterone levels in *ad lib* fed male mice. Nkx2.1-PTP1B$^{-/-}$ mice show similar serum corticosterone levels as wildtype controls on chow or HFD (Table 1). Leptin has been shown to play an important role in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis, and a previous report showed significantly elevated serum corticosterone levels in Nkx2.1-LepRb$^{-/-}$ mice [11,32,33]. Consistent with these findings, we find elevated serum corticosterone in Nkx2.1-LepRb$^{-/-}$ mice and also in Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice compared to wildtype controls on chow (Table 1).

3.7. Nkx2.1-PTP1B$^{-/-}$ show minor improvements in fasting HFD-induced hyperinsulinemia

Given that past CNS-specific PTP1B deficient models show improvements in peripheral glucose tolerance and insulin sensitivity [20–22], we examined glucose homeostasis in Nkx2.1-PTP1B$^{-/-}$ compared to their respective controls. Fasting blood glucose, serum insulin levels, and glucose tolerance are similar in male Nkx2.1-PTP1B$^{-/-}$ and Nkx2.1-PTP1B$^{+/-}$ mice compared to wildtype controls on chow (Table 1, Figure 6A and B). On HFD, fasting blood glucose is comparable in male Nkx2.1-PTP1B$^{-/-}$, Nkx2.1-PTP1B$^{+/-}$ and wildtype mice, whereas fasting serum insulin is significantly decreased in Nkx2.1-PTP1B$^{+/-}$ mice and trending toward a decrease in Nkx2.1-PTP1B$^{-/-}$ mice compared to controls (Table 1). Despite reduced serum insulin levels on HFD, however, Nkx2.1-PTP1B$^{-/-}$ and wildtype controls perform similarly in an intraperitoneal GTT (Figure 6C and D) and an ITT (data not shown). Taken together, these results show that Nkx2.1-PTP1B deficiency results in slight protection against HFD-induced hyperinsulinemia but no overt effects on overall glucose homeostasis.

3.8. Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice show severe impairments in glucose homeostasis

Hypothalamic deficiency of LepRb results in severely impaired glucose homeostasis marked by elevated blood glucose and serum insulin levels and slower glucose clearance during a GTT [11]. Although there are no differences in body weight or composition between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice, we examined whether the additional PTP1B deficiency in Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice could have any beneficial effect on glucose homeostasis independent of energy balance when compared to Nkx2.1-LepRb$^{-/-}$ mice. As expected, obese Nkx2.1-LepRb$^{-/-}$ mice show a trend toward increased fasted blood glucose levels on chow diet with a significant elevation in serum insulin levels (Table 1). Surprisingly, Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice show no protection against hyperglycemia and hyperinsulinemia, and if anything, have further impairments in these parameters compared to Nkx2.1-LepRb$^{-/-}$ mice (Table 1). Glucose tolerance is severely impaired in Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice on chow diet compared to wildtype controls, and the extent of glucose intolerance is similar between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice (Figure 6E and F). On HFD, Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice display similarly elevated fasted blood glucose and increased fasting serum insulin compared to wildtypes (Table 1). Taken together, these data demonstrate that hypothalamus-targeted PTP1B deficiency cannot rescue impaired peripheral glucose homeostasis in the context of hypothalamic leptin receptor-deficiency.

4. DISCUSSION

The anatomical specificity of PTP1B′s metabolic contribution within the CNS is unclear since past studies examined the effects of central PTP1B deficiency using holistic (all neurons) or cell-type specific approaches (POMC-specific, LepRb-specific). Furthermore, while central PTP1B deficiency clearly enhances leptin sensitivity, whether or not the metabolic benefits from CNS PTP1B-deficiency are solely the result of enhanced leptin signaling is unknown. Our data clearly demonstrate an important role for hypothalamic PTP1B in the central control of energy homeostasis in mice. Additionally, our findings clearly show that the metabolic improvements observed with hypothalamic PTP1B deficiency are dependent upon functional leptin receptor signaling.

Whole body, whole brain, LepRb-expressing cell or POMC-neuron specific PTP1B deficiency results in decreased body weight and adiposity on HFD [18–22]. Consistent with past findings, we demonstrate that hypothalamic PTP1B-deficiency reduces body weight and adiposity in mice on HFD. Somewhat surprisingly, the extent of the reduced body weight in hypothalamic PTP1B knockouts is more modest compared to the whole body, whole brain, and LepRb-expressing cell-specific PTP1B-deficient models despite the hypothalamus being long regarded as the major control center for energy balance. While neuronal and LepRb-specific PTP1B$^{-/-}$ mice show suppressed body weight gain even on a chow diet [20,21], Nkx2.1-PTP1B$^{-/-}$ mice do not show any body weight phenotype on chow, suggesting that genetic deletion of PTP1B in the hypothalamus is not enough to confer reductions in body weight when energy balance is not being pushed towards weight gain. Likewise, deleting PTP1B solely within POMC neurons (representing a subpopulation of all LepRb-expressing neurons and a subset of all hypothalamic neurons) also results in no effect on body weight on chow diet [22]. Thus, the anatomic specificity of central PTP1B′s total metabolic contribution clearly includes the hypothalamus (Figure 7), but also extrahypothalamic sites as the additional CNS deletion in pan-neuronal and LepRb-expressing cell specific PTP1B$^{-/-}$ models results in decreased body weights even on chow diet. Evidence of hindbrain PTP1B contribution has been demonstrated using the POMC-specific PTP1B$^{-/-}$ model, whereby hindbrain (4th ventricle) administration of leptin resulted in enhanced food intake and body weight suppression in
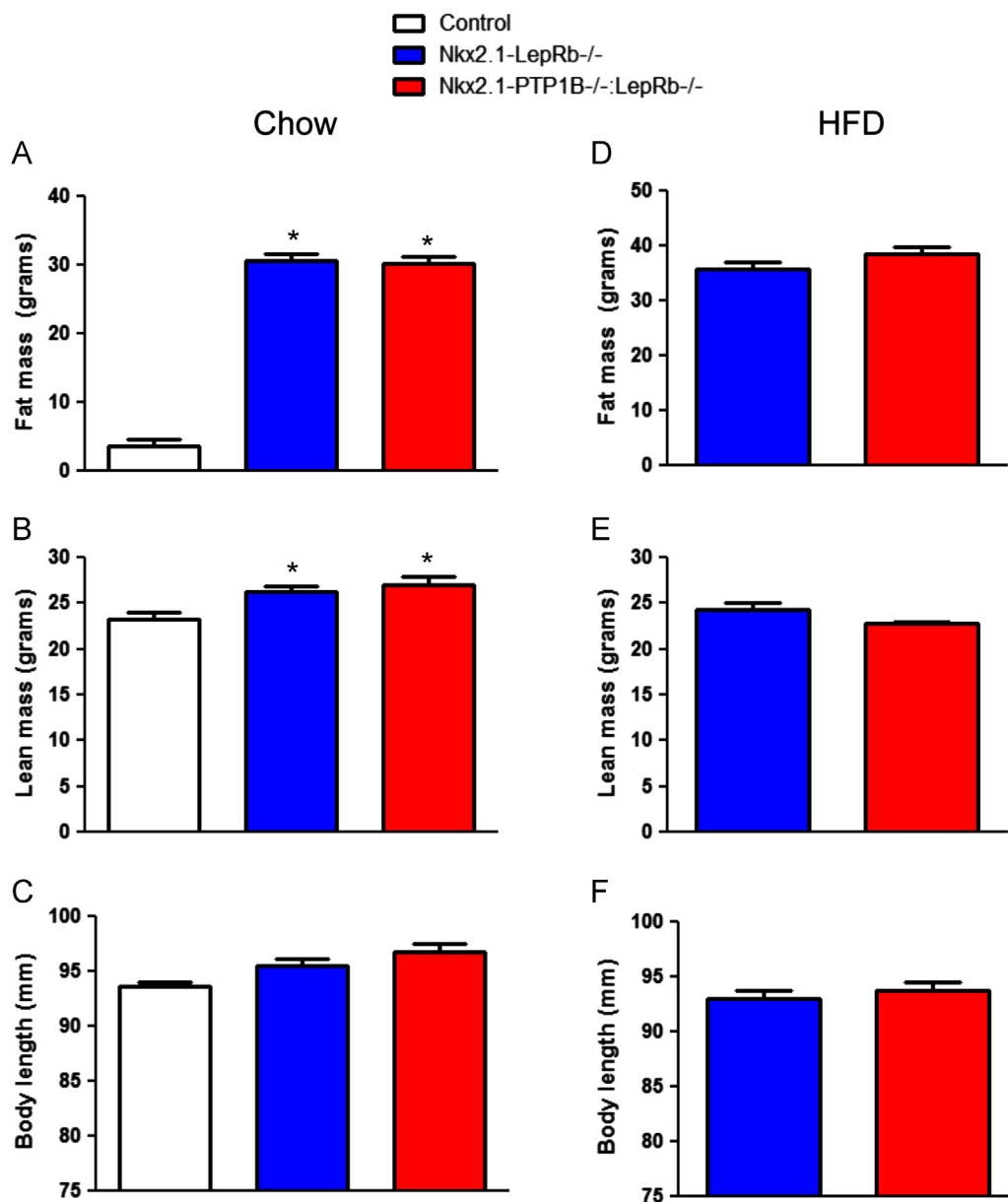
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Figure 4: Male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice show no difference in body composition compared to Nkx2.1-LepRb$^{-/-}$ mice. Fat mass (A) and lean mass (B) as determined by NMR of Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=8$), Nkx2.1-LepRb$^{-/-}$ ($n=8$), and control PTP1B fl/fl:LepRb fl/fl ($n=3$) mice on chow. (C) Body length of Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=8$), Nkx2.1-LepRb$^{-/-}$ ($n=8$), and control PTP1B fl/fl:LepRb fl/fl ($n=3$) mice on chow. Fat mass (D) and lean mass (E) as determined by NMR of Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=5$) and Nkx2.1-LepRb$^{-/-}$ ($n=6$) mice on HFD. (F) Body length of Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=5$) and Nkx2.1-LepRb$^{-/-}$ ($n=6$) mice on HFD. Body composition and body length on chow analyzed by one-way ANOVA followed by Student–Newman–Keuls pairwise comparison: *$p < 0.05$ indicated group vs. control.

POMC-PTP1B$^{-/-}$ mice relative to wildtype controls [27]. Importantly, despite no difference in body weight on chow, male Nkx2.1-PTP1B$^{-/-}$ mice show significantly decreased epididymal fat, suggesting that hypothalamic PTP1B can regulate adiposity independent of body weight. Although the decreased body weight of Nkx2.1-PTP1B$^{-/-}$ mice on HFD is primarily due to decreased fat mass, Nkx2.1-PTP1B$^{-/-}$ mice also display a small decrease in lean mass and body length. Indeed, past CNS PTP1B-deficient models have demonstrated small decreases in linear growth, and there is evidence of PTP1B's regulation of melanocortin action [21,22,27]. In addition to the hypothalamus, the Nkx2.1 promoter drives Cre expression in non-hypothalamic tissues including the pituitary and thyroid. Given that these regions are implicated in neuroendocrine control of metabolism, it raises the possibility that

PTP1B-deficiency in these tissues may contribute to the observed metabolic phenotypes. However, our previous work has shown that pituitary-targeted *Ptpn1* deletion does not result in any metabolic phenotype [22], and thyroid function in whole body and POMC neuron-specific PTP1B$^{-/-}$ mice is normal [19,34], suggesting that the metabolic effects reported here are in fact mediated through the hypothalamus.

The improved metabolic phenotype of central PTP1B-deficient models have been explained by increases in energy expenditure or a combination of suppressed food intake and increased energy expenditure [19–22]. Like whole brain and LepRb-expressing cell specific PTP1B knockouts, Nkx2.1-PTP1B$^{-/-}$ mice demonstrate decreased food intake. Thus, hypothalamic PTP1B deficiency likely contributes to the decreased food intake seen in the neuronal
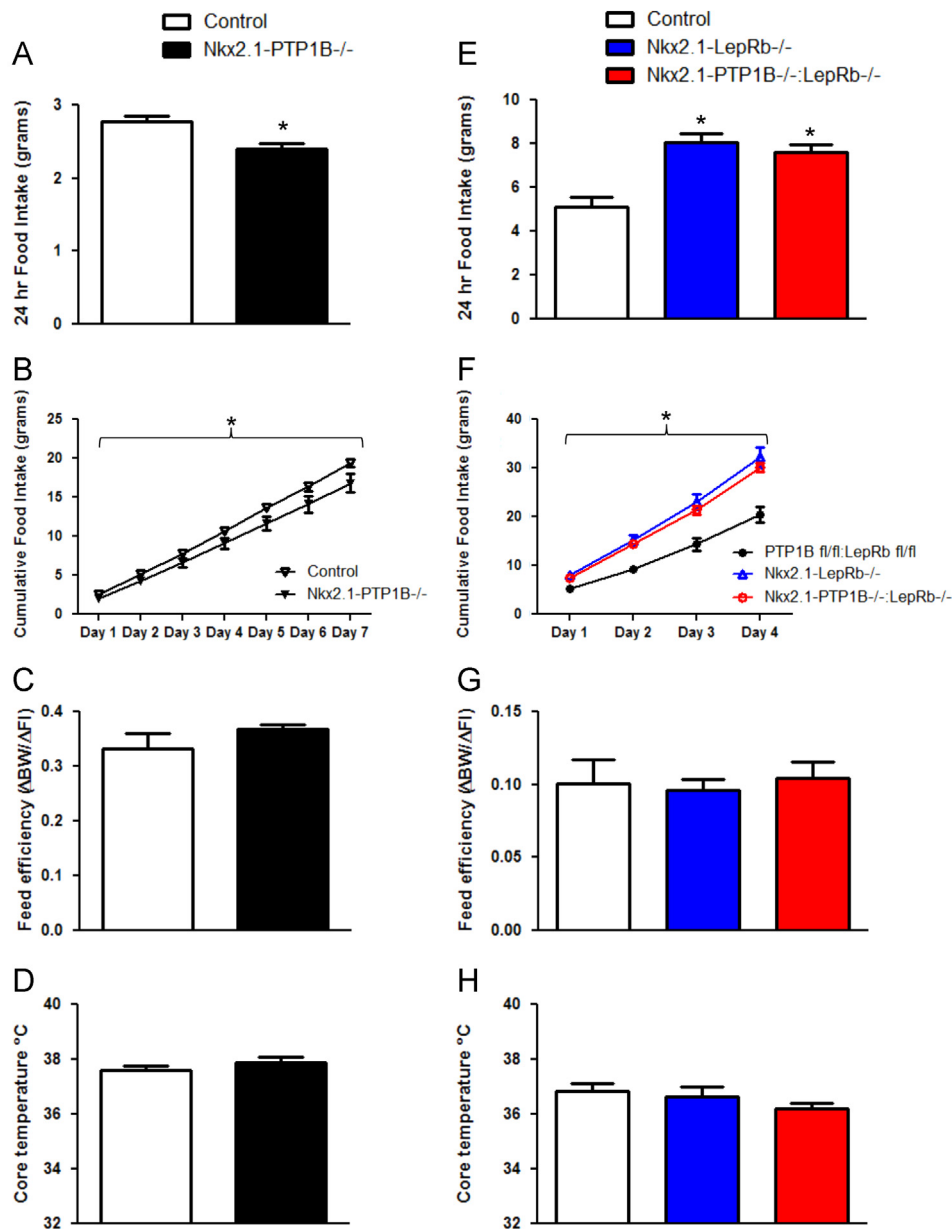
Figure 5: Nkx2.1-PTP1B$^{-/-}$ mice have decreased food intake on HFD whereas Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice show similar hyperphagia as Nkx2.1-LepRb$^{-/-}$ mice. Average daily (A) and cumulative (B) food intake of male Nkx2.1-PTP1B$^{-/-}$ ($n=5$) and control PTP1B fl/fl ($n=6$) on HFD. (C) 7 day feed efficiency of male Nkx2.1-PTP1B$^{-/-}$ ($n=5$) and control PTP1B fl/fl ($n=6$) on HFD. (D) Core temperature of male Nkx2.1-PTP1B$^{-/-}$ ($n=5$) and control PTP1B fl/fl ($n=14$) on HFD at 8 weeks of age. Average daily (E) and cumulative (F) food intake of male and female Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=9$), Nkx2.1-LepRb$^{-/-}$ ($n=6$), and control PTP1B fl/fl:LepRb fl/fl ($n=6$) mice on chow. (G) 4 day feed efficiency of male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=4$), Nkx2.1-LepRb$^{-/-}$ ($n=2$), and control PTP1B fl/fl:LepRb fl/fl ($n=3$) mice on chow. (H) Core temperature of male Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=4$), Nkx2.1-LepRb$^{-/-}$ ($n=2$), control PTP1B fl/fl:LepRb fl/fl ($n=8$) mice on chow at 14 weeks of age. 24 hour food intake analyzed by two tailed Student's t-test or one-way ANOVA followed by Student–Newman–Keuls pairwise comparison: *$p < 0.05$ indicated group vs. control. Cumulative food intake analyzed by two-way ANOVA with repeated measures: *$p < 0.05$.

and LepRb-PTP1B$^{-/-}$ mice; this is consistent with the fact that decreasing hypothalamic PTP1B via 3rd ventricle antisense oligonucleotide treatment results in significantly reduced food intake in rats [35]. Interestingly, no changes in energy expenditure are detected in Nkx2.1-PTP1B$^{-/-}$ mice as determined by feed efficiency, core temperature, or expression of BAT *Ucp1*, suggesting that the energy expenditure effects of CNS PTP1B deficiency may be localized to extrahypothalamic sites. One such site may be hindbrain LepRb/POMC-expressing neurons since both LepRb- and POMC-specific PTP1B knockouts display increased energy expenditure [21,22]. Indeed, hindbrain leptin administration in POMC-PTP1B$^{-/-}$ mice results in greater

increases in spontaneous activity and core temperature compared to wildtype controls [27]. Alternatively, PTP1B might have competing opposing effects on energy expenditure within different subpopulations of neurons in the hypothalamus; this possibility remains to be explored.

In addition to affecting energy balance, central PTP1B has been implicated in the regulation of peripheral glucose homeostasis [20–22]. Whereas whole brain, LepRb-expressing cell-, and POMC neuron-specific PTP1B$^{-/-}$ mice display significant improvements in glucose tolerance, serum insulin levels, and insulin sensitivity, hypothalamic PTP1B deficiency has no effect on glucose tolerance and only displays modest effects on fasting insulin levels.
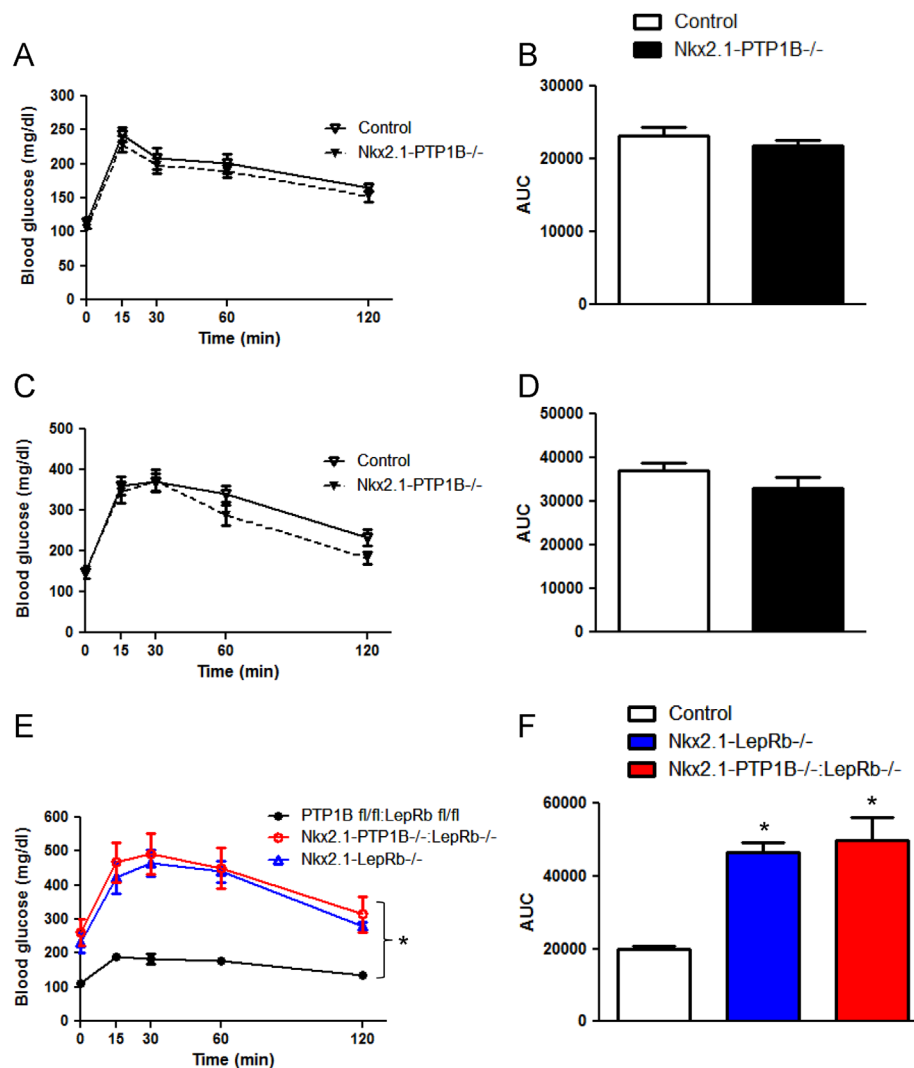
Figure 6: Glucose tolerance is unchanged in Nkx2.1-PTP1B deficient models compared to controls. (A) GTT for male Nkx2.1-PTP1B$^{-/-}$ ($n=9$) and control PTP1B fl/fl ($n=8$) mice on chow at 11 weeks of age. (B) Area under the curve (AUC) for blood glucose during GTT of male chow cohort. (C) GTT for male Nkx2.1-PTP1B$^{-/-}$ ($n=6$) and control PTP1B fl/fl ($n=9$) mice on HFD at 17 weeks of age. (D) AUC for blood glucose during GTT of male HFD cohort. (E) GTT for female PTP1B$^{-/-}$:LepRb$^{-/-}$ ($n=5$), Nkx2.1-LepRb$^{-/-}$ ($n=7$), and control PTP1B fl/fl:LepRb fl/fl ($n=6$) on chow at 13 weeks of age. (F) AUC for blood glucose during GTT of female chow cohort. GTT analyzed by two-way ANOVA with repeated measures: *$p < 0.05$.

Therefore, PTP1B deficiency in extrahypothalamic LepRb and POMC neurons, likely in the hindbrain, may account for the improvements in glucose homeostasis observed in other CNS-PTP1B deficient models.

Hypothalamic LepRb deletion in mice results in significantly increased weight gain and adiposity similar to that seen in *db/db* mice [11]. We demonstrate that compound PTP1B and LepRb deficiency in the hypothalamus results in weight gain comparable to that of Nkx2.1-LepRb$^{-/-}$ mice on both chow and HFD. Consistent with the results of Ring and Zeltser 2010, we also observed that female Nkx2.1-LepRb$^{-/-}$ and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice had increased body weight gain relative to males. In addition to body weight, body composition and length is similar between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice. Both groups similarly demonstrate significant hyperphagia and insulin resistance as demonstrated by elevated fasting serum insulin and glucose intolerance. Interestingly, on chow, Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice display significantly elevated fasting insulin relative to Nkx2.1-LepRb$^{-/-}$ mice, suggesting that knocking out PTP1B on top of LepRb deficiency in the hypothalamus may intensify already impaired glucose homeostasis. However, whether or not a significant statistical difference translates to a relevant physiological difference is unclear as both models demonstrate impairments to glucose homeostasis several fold greater than wildtypes, and the same difference was not observed on HFD. It has been demonstrated that ventromedial hypothalamus (VMH)-specific insulin receptor deletion leads to protection from HFD-induced obesity and hyperglycemia [36]. Therefore, one could speculate that within the context of LepRb-deficiency, additional hypothalamic PTP1B deletion could sensitize VMH insulin signaling, leading to further metabolic impairment. Specifically how hypothalamic PTP1B deletion could exacerbate impaired glucose homeostasis within the context of LepRb deficiency remains unknown.

Taken together, these findings indicate that hypothalamic PTP1B deficiency can elicit metabolic improvements in body weight and adiposity within the context of diet-induced obesity where leptin resistance accumulates over time. In contrast, under the conditions of genetic leptin resistance where intracellular leptin signaling is completely impaired, PTP1B deficiency is ineffective at improving metabolic outcomes, demonstrating that within the hypothalamus, functional leptin receptor signaling is indeed required for PTP1B's metabolic effects. Based upon these findings, we propose two possible models of central
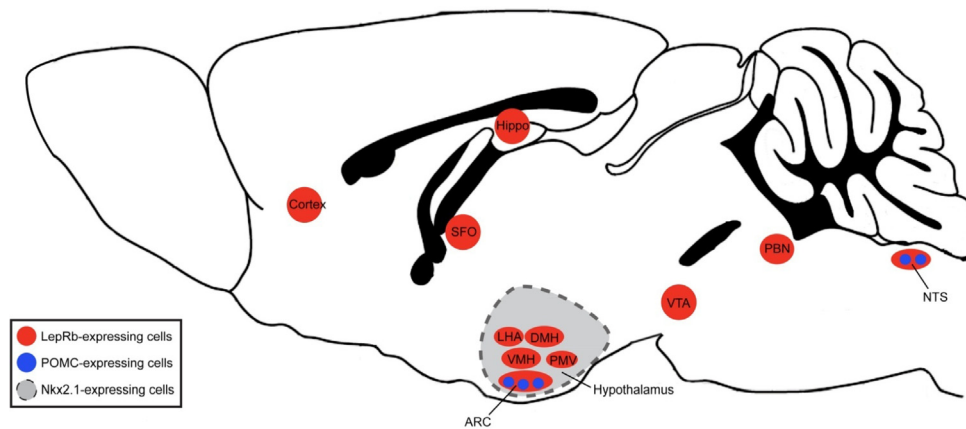
Figure 7: Comparison of the anatomic specificity of various CNS PTP1B deficient mouse models. LepRb and POMC are highly expressed in the arcuate nucleus (ARC) of the hypothalamus and are also found in the nucleus of the solitary tract (NTS) of the medulla. Outside of the NTS and hypothalamic nuclei including the ventromedial hypothalamic nucleus (VMH), dorsomedial hypothalamic nucleus (DMH), lateral hypothalamic area (LHA), and ventral premamillary nucleus (PMV), LepRb-expressing neurons are distributed broadly throughout the brain, with known extrahypothalamic sites including the cortex, hippocampus (Hippo), subfornical organ (SFO), ventral tegmental area (VTA), and the parabrachial nucleus (PBN).

PTP1B action in the control of energy homeostasis: (a) PTP1B's metabolic role in the CNS is exclusively as a negative regulator of leptin signaling, and the metabolic benefit of PTP1B deficiency is solely the result of enhanced leptin sensitivity, or (b) PTP1B acts on multiple signaling pathways (ex. insulin, non-leptin cytokines, growth factors etc.) including leptin, and the metabolic effects of PTP1B deficiency are a combination of numerous sensitized pathways of which leptin is the major contributor. Because PTP1B is a known negative regulator of insulin signaling [37–39] and JAK2 is associated with other non-leptin cytokine pathways implicated in the central control of energy balance, it seems the latter possibility is more likely, but the metabolic effects of these non-leptin pathways are masked by leptin's larger contribution. For example, though insulin and non-leptin cytokines including IL-6 and CNTF have been shown to have weight-reducing effects when administered centrally [40–45], their physiological regulation of energy homeostasis seems much less considerable when compared to leptin's role. Neuron-specific insulin receptor knockout mice only develop mild obesity [46], and global IL-6 knockout mice display an obese phenotype only later in life [47]. In conjunction with our findings here, these studies further suggest that the leptin contribution towards energy homeostasis regulation is far more substantial than non-leptin pathways. Therefore, within the context of central PTP1B deficiency models, sensitized leptin signaling likely underlies the majority of the lean metabolic phenotypes observed.

Alternatively, the lack of any phenotypic difference between Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ and Nkx2.1-LepRb$^{-/-}$ mice could be attributed to loss of leptin signaling during early development resulting in disrupted hypothalamic circuitry. Leptin has been demonstrated to act as a trophic factor and is required during neonatal brain development for formation of projections from the arcuate nucleus [48,49]. Thus, perhaps disrupted hypothalamic connectivity due to early loss of LepRb in Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice prevents any non-leptin pathway sensitization to elicit measurable, beneficial metabolic effects. Future studies utilizing inducible Cre lines may help to distinguish any confounding effects of early hypothalamic development on energy balance function. All in all, these findings highlight the continued importance of central leptin resistance as a main promoter of obesity.

DISCLOSURE STATEMENT

The authors have nothing to disclose.

CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPORTING INFORMATION

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.molmet.2014.01.008.

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