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Protein tyrosine phosphatase alpha inhibits hypothalamic leptin receptor signaling and regulates body weight *in vivo*

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ABSTRACT: Understanding how body weight is regulated at the molecular level is essential for treating obesity. We show that female mice genetically lacking protein tyrosine phosphatase (PTP) receptor type α (PTPRA) exhibit reduced weight and adiposity and increased energy expenditure, and are more resistant to diet-induced obesity than matched wild-type control mice. These mice also exhibit reduced levels of circulating leptin and are leptin hypersensitive, suggesting that PTPRA inhibits leptin signaling in the hypothalamus. Male and female PTPRA-deficient mice fed a high-fat diet were leaner and displayed increased metabolic rates and lower circulating leptin levels, indicating that the effects of loss of PTPRA persist in the obese state. Molecularly, PTPRA down-regulates leptin receptor signaling by dephosphorylating the receptor-associated kinase JAK2, with which the phosphatase associates constitutively. In contrast to the closely related tyrosine phosphatase ε, leptin induces only weak phosphorylation of PTPRA at its C-terminal regulatory site Y789, and this does not affect the activity of PTPRA toward JAK2. PTPRA is therefore an inhibitor of hypothalamic leptin signaling *in vivo* and may prevent premature activation of leptin signaling, as well as return signaling to baseline after exposure to leptin.—Cohen-Sharir, Y., Kuperman, Y., Apelblat, D., den Hertog, J., Spiegel, I., Knobler, H., Elson, A. Protein tyrosine phosphatase alpha inhibits hypothalamic leptin receptor signaling and regulates body weight *in vivo*. FASEB J. 33, 5101–5111 (2019). www.fasebj.org

KEY WORDS: obesity · leptin resistance · JAK2 · PTPRA

The hormone leptin is a key regulator of body weight and energy balance. Leptin is produced by white adipose tissue and serves as a lipostatic signal, conveying critical information regarding metabolic state to the brain to reduce food intake and increase energy expenditure (1–3). Leptin acts on specific neuronal cells located in the hypothalamus. Among these, the hormone inhibits activity in specific neurons of neuropeptide Y and agouti-related protein, which stimulate food intake and inhibit energy expenditure. Leptin also stimulates a different set of neurons to produce pro-opiomelanocortin, thereby reducing food intake and increasing energy expenditure. Molecularly, binding of leptin to the leptin receptor (LEPR) activates the JAK2 tyrosine kinase, which is associated with the receptor. JAK2 then phosphorylates specific sites on the LEPR that enable activation of the RAS-MAPK, PI3-kinase, STAT3, and STAT5 signaling cascades (1, 3, 4). High levels of body fat in obesity are associated with elevated levels of circulating leptin but, paradoxically, also with leptin resistance that reduces the intensity of LEPR signaling and blunts its physiologic effects (5). Clarifying the molecular basis for leptin resistance is required for more complete understanding of obesity and for designing strategies to treat obese individuals.

Leptin signaling is tightly controlled. For example, SOCS3, a molecule whose expression is induced by STAT3 after LEPR activation, inhibits leptin signaling by targeting the LEPR and JAK2 (1). Another example is the PH and SH2 domain-containing protein SH2B1, which supports signaling by the receptor. Mice lacking SH2B1 are obese and hyperglycemic, and experience insulin and leptin

ABBREVIATIONS: AKO, receptor-type protein tyrosine phosphatase α knockout; EKO, receptor-type protein tyrosine phosphatase ε knockout; HFD, high-fat diet; JAK2, Janus kinase 2; LEPR, leptin receptor; PTP, protein tyrosine phosphatase; PTPRA/E, PTP receptor type α/ε; RPTP, receptor-type PTP; WT, wild type

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resistance (6). Several protein tyrosine phosphatases (PTPs) also regulate LEPR signaling, in a manner consistent with the prominence of tyrosine phosphorylation in this process. The first PTP to be described in this respect was PTP1B; mice lacking this PTP exhibit increased energy expenditure, resistance to diet-induced obesity, increased sensitivity to leptin and insulin, and reduced adiposity relative to wild-type (WT) controls (7–10). These effects are caused at least in part by PTP1B dephosphorylating JAK2 downstream of the LEPR in brain neurons (9–13). The related but distinct phosphatase TCPTP down-regulates LEPR signaling by dephosphorylating STAT3, leading to leptin hypersensitivity and resistance to diet-induced obesity in mice that lack this phosphatase in nestin-positive neurons (14). Loss of both PTP1B and TCPTP in nestin-positive or pro-opiomelanocortin–positive neurons exerts additive effects in preventing diet-induced obesity by increasing the browning of white adipose tissue and increasing energy expenditure (14, 15). Another nonreceptor PTP, the SH2 domain-containing phosphatase SHP2, up-regulates leptin signaling by binding to the LEPR at pY985 and activating ERK. Of note, SHP2 can also down-regulate LEPR signaling by inhibiting JAK2/STAT3 activity (3, 16). Mice lacking SHP2 in neurons exhibit increased adiposity, decreased leptin sensitivity, and reduced energy expenditure, which indicate decreased leptin signaling; the overall role of SHP2 is therefore to up-regulate LEPR signaling (11, 16, 17). The PTP receptor type ε (PTPRE) down-regulates LEPR signaling by dephosphorylating JAK2 at Y1007/1008. PTPRE targets JAK2 following its own phosphorylation at its C-terminal Y695, by JAK2. PTPRE then participates in a negative feedback loop that down-regulates LEPR signaling after its activation and returns it to baseline (18). Accordingly, mice genetically lacking PTPRE are leptin hypersensitive and are resistant to diet-induced obesity, a phenotype that is detected predominantly in females. More recently, PTPRJ was shown to inhibit leptin signaling by dephosphorylating JAK2 at Y813 and Y868; loss of PTPRJ induced leptin hypersensitivity and protected mice from diet-induced obesity (19). Interestingly, a high-fat diet (HFD) up-regulated expression of PTPRJ in the hypothalamus, suggesting that this phosphatase helps induce leptin resistance in obese mice.

The PTP receptor type α (PTPRA) is closely related to PTPRE. Both PTPs are orphan receptors that possess short extracellular domains with distinct sequences; however, their cytosolic regions, which include 2 PTP catalytic domains in each, are 72% identical. PTPRA is expressed ubiquitously and has been implicated in, among other functions, cytoskeletal reorganization and cell adhesion and migration (20–24), promotion of neural cell adhesion molecule–dependent neurite outgrowth (25), regulation of oligodendrocyte differentiation (24, 26), and cell transformation (27–30). PTPRA often functions by dephosphorylating and activating Src or one of its related kinases (20, 21, 27, 31), an ability that PTPRE also shares (32–34). Nonetheless, studies of the roles of both PTPs in bone-resorbing osteoclasts and in Schwann cells indicate that despite their similarities, PTPRA and PTPRE can fulfill distinct physiologic roles (35, 36).

The present study shows that PTPRA down-regulates hypothalamic LEPR signaling by targeting JAK2 at Y1007/1008. We also show that mice lacking PTPRA are leaner than WT control mice and are resistant to diet-induced obesity. Unlike PTPRE, leptin-induced C-terminal phosphorylation of PTPRA does not play a significant role in its regulation of LEPR signaling, suggesting that PTPRA can constitutively down-regulate LEPR signaling to prevent its activation in the absence of ligand. The functional variability among PTPs that regulate LEPR signaling underscores the importance of this pathway and enables it to be regulated by distinct physiologic inputs.

MATERIALS AND METHODS

Mice

PTPRA knockout (AKO) (25) or PTPRE knockout (EKO) (37) mice have been described. Mice were kept in a pathogen-free facility and fed regular chow (18% of calories from fat, 2018SC; Harlan Teklad, Madison, WI, USA) and water *ad libitum*. On occasion, mice were fed high-fat food [diet D12492 (60% of calories from fat); Research Diets, New Brunswick, NJ, USA]. AKO and control WT mice were derived from littermates. Mice were in a pure C57Black/6 background or in a mixed C57Black/6: 129SvEv (50:50) background. All mice in an experiment were of the same background, and AKO mice were always compared with WT mice in the same background. All experiments were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute and were performed in accordance with Israeli law.

Reagents

cDNAs for mouse WT, Y789F or D401A PTPRA, as well as cyt-PTPa (35) were cloned into pcDNA3 (Thermo Fisher Scientific, Waltham, MA, USA); all were FLAG-tagged at their C terminus. Plasmids for expression of HA-tagged human JAK2 and leptin receptor were generous gifts from Dr. Michel Tremblay (McGill University, Montreal, Quebec, Canada). The following mAbs were used: anti-FLAG (clone M2; MilliporeSigma, Burlington, MA, USA), PTP1B (clone 15; BD Biosciences, San Jose, CA, USA), Actin (clone C4; MP Biomedicals, Illkirch-Graffenstaden, France), anti-oxidized PTP (38) (clone 335636; R&D Systems, Minneapolis, MN, USA), STAT3 and pY705 STAT3 (clones 124H6 and 3E3, respectively; Cell Signaling Technology, Danvers, MA, USA), and SHP2 (clone 79; BD Biosciences). The pAbs used included anti-PTPRE/PTPRA (39), pY695 PTPRE/pY789 PTPRA (32), HA (probe Y11; Santa Cruz Biotechnology, Dallas, TX, USA), JAK2 and pY1007/1008 JAK2 (MilliporeSigma), and PTPRJ (R&D Systems). Leptin was purchased from Protein Laboratories Rehovot, (Rehovot, Israel).

Metabolic rate, physical activity, and adiposity

Indirect calorimetry, locomotor activity and food and water intake were measured by using the Phenomaster/Labmaster system (TSE Systems, Bad Homburg, Germany). Mice were placed in individual cages with a light/dark cycle of 12 h. After 24 h adaptation, the system determined oxygen consumption, carbon dioxide production, heat production, respiratory exchange ratio, and food and water consumption for each mouse individually for an additional 72 h. A photobeam-based monitoring system was

used to track animal movement (including rearing). Lean and fat body composition were determined by using a Minispec LF50 Body Composition Analyzer (Bruker, Germany).

Leptin stimulation and serum leptin determination

For acute stimulation, mice were unfed overnight and injected intraperitoneally with leptin (5 μg/g body weight). Forty-five minutes later, mice were euthanized by cervical dislocation, and their hypothalami were rapidly dissected and snap-frozen in liquid nitrogen. For chronic stimulation, mice were housed individually and injected intraperitoneally with PBS twice daily. After return of body weight and food consumption to baseline values (7–9 d), PBS was replaced with leptin (in PBS) (1 μg/g body weight per injection, 2 μg/g body weight/day). After 6 d, all mice received PBS for 3 additional days. Body weights and food consumption were measured daily. Leptin concentrations in serum were measured in fed control mice by using an antibody-based kit from Crystal Chem (Elk Grove Village, IL, USA).

Tissue culture

HEK 293 cells were grown in DMEM (MilliporeSigma) supplemented with 10% fetal calf serum (Thermo Fisher Scientific), 4 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin, at 37°C, 5% carbon dioxide. Cells were transfected by using the calcium phosphate method (40).

Protein analysis

Samples were lysed in Buffer A [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40], or in RIPA buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP40, 1% sodium deoxycholate]. Lysis buffers were supplemented with protease inhibitors [1 mM *N*-(α-aminoethyl) benzene-sulfonyl fluoride, 40 μM bestatin, 15 μM E64, 20 μM leupeptin, 15 μM pepstatin; MilliporeSigma]. In some cases, 0.5 mM sodium pervanadate (PTP inhibitor) and 0.1 μm okadaic acid/0.4 nm calyculin A/1 mM NaF/25 mM β-glycerophosphate (Ser/Thr phosphatase inhibitors) were added. Immunoprecipitation (32), SDS-PAGE, and protein blotting (41) were performed as previously described. Lysates to be analyzed with the anti-oxidized PTP antibody (38) were treated with 0.5 mM sodium pervanadate for 10 min before SDS-PAGE. Purification of transfected, FLAG-tagged WT or D401A cyt-PTPa (numbered as in PTPRA) from HEK 293 cells was performed as previously described (42). HA-JAK2 was immunoprecipitated from HEK 293 cells with HA antibodies; phosphorylated JAK2 was isolated from cells that had been serum starved for 12 h and then stimulated with 100 mM leptin for 5 min. For *in vitro* dephosphorylation of pJAK2, 200 ng cyt-PTPa and beads carrying 200 ng phosphorylated JAK2 were incubated at 37° with occasional shaking for 30–60 min in 25 μl of a buffer consisting of 50 mM MES (pH 7.3), 0.5 mM DTT, and 0.5 mg/ml bovine serum albumin; 0.5 mM sodium pervanadate was added to some reactions. ECL signals were visualized by using an ImageQuant LAS 4000 Mini instrument (GE Healthcare Biosciences, Uppsala, Sweden) and quantified (GelPro Analyzer; Media Cybernetics, Rockville, MD, USA).

Statistics

Data were analyzed by using a 2-tailed Student's *t* test, or by 2-way ANOVA with repeated measurements and *post hoc* tests as indicated, with significance set at $P < 0.05$. Data are presented as means ± SE.

RESULTS

PTPRA-deficient mice are lean and resistant to obesity induced by a high-fat diet

To explore the possible role of PTPRA in regulating body weight and growth of mice, we examined the effects of diet on weight gain and body adiposity of mice lacking this phosphatase [AKO mice (25)]. When fed a regular laboratory chow diet for 10 wk, female AKO mice gained weight more slowly (**Fig. 1*A***) and their body fat content was decreased (Fig. 1*E*) relative to WT control mice. Male chow-fed AKO mice exhibited a weaker phenotype. Despite being somewhat leaner and weighing less (Fig. 1*B*) than matched WT mice, their body weight increased at the same rate as WT control mice (Supplemental Fig. S1). When fed an HFD, female AKO mice tended to gain weight more slowly than WT control mice; these AKO mice weighed significantly less during the 5 final weeks of this study (wk 9–13) (Fig. 1*C*) and exhibited reduced adiposity relative to WT control mice, indicating that female HFD-fed AKO mice are resistant to diet-induced obesity. This finding was subsequently confirmed by additional studies presented later. Male AKO mice fed an HFD also weighed less and exhibited reduced adiposity relative to control mice (Fig. 1*D, E*), indicating that AKO mice of both sexes are protected from diet-induced obesity.

AKO mice exhibit increased metabolic rates

To examine the basis for the reduced weight gain of AKO mice, we placed mice individually in metabolic cages and examined their food intake, heat production, oxygen consumption, carbon dioxide production, and physical activity continuously for 72 h. Mice were fed either regular chow or an HFD; the latter mice had been fed fatty food for 14 wk before initiation of this study. In all cases studied, food consumption of AKO mice and their matched WT controls was similar (**Fig. 2*A***). Heat production, oxygen consumption, and carbon dioxide production were increased in female AKO mice fed regular chow and in AKO mice of both sexes fed an HFD (Fig. 2*B, D, E*). Locomotion of AKO mice of both sexes fed an HFD was also significantly increased (Fig. 2*C*). In agreement with their normal patterns of weight gain (Fig. 1*B* and Supplemental Fig. S1), male chow-fed mice displayed no metabolic abnormalities (Fig. 2*B–E*). The relative use of carbohydrates or fat as an energy source, indicated by the respiratory exchange ratio, did not differ in AKO *vs.* WT mice fed similar food; however, as expected, mice fed an HFD exhibited reduced respiratory exchange ratio values compared with chow-fed mice (Fig. 2*F*).

The differences in increased energy expenditure parameters found in female AKO mice fed chow and in AKO mice of both sexes fed an HFD correlate with the slower rate of weight gain of these mice (Fig. 1*A, C, D*), suggesting

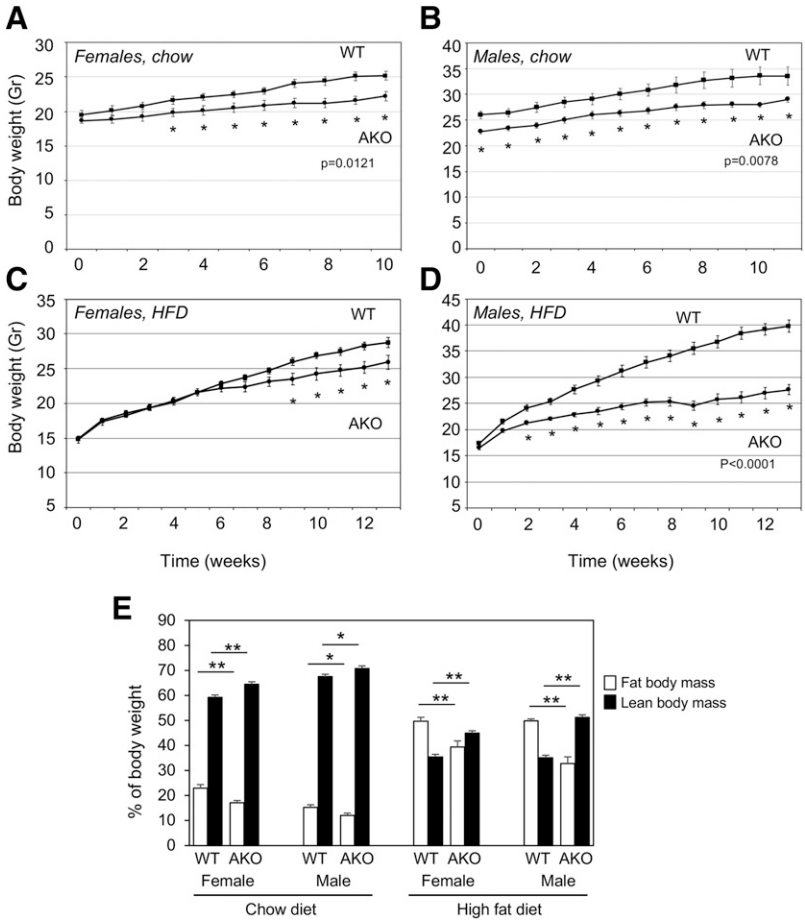

Figure 1. Reduced body weight and fat content in AKO mice. *A–D*) Weekly weights of WT and AKO mice fed standard lab chow (*A*, *B*) and an HFD (*C*, *D*). Data shown are means ± SE; n = 8–16 mice per sex and genotype. At the start of the study, mice were 8–9 (chow) or 6–7 (HFD) wk old. Data were analyzed by using 2-way ANOVA with repeated measurements followed by Student's t test *post hoc* analysis. ANOVA significance values between the complete WT and AKO datasets are indicated in panels *A*, *C*, and *D*. Asterisks note individual weeks in which genotypes are distinct at $P < 0.05$. Similar data normalized to the starting weight of each mouse is shown in Supplemental Fig. S1. *E*) Percentage of lean and fat body content in WT and AKO mice at age 4 mo (chow) or 5 mo (after 14 wk HFD). $*P \leq 0.05$, $**P \leq 0.01$ (Student's t test).

that higher basal energy expenditure plays a significant role in the reduced weight gain of AKO mice. Increased locomotion, which was observed in HFD-fed mice of both sexes, could also contribute to this effect. Examination of younger mice revealed that the metabolic parameters of chow-fed AKO mice of both sexes did not differ from WT control mice, whereas young AKO mice fed an HFD for 8–10 wk exhibited trends or actual increases in oxygen consumption, carbon dioxide production, and heat production (Supplemental Fig. S2). This weaker effect could be due to the need for additional time for phenotype development and to the fact that these mice were exposed to HFD for 4–6 wk less than the older mice in Fig. 2.

Increased leptin sensitivity in AKO mice

Leptin is a prominent regulator of body weight and fat content; hence we examined if, in light of their reduced weight and adiposity, AKO mice also exhibit abnormalities in leptin signaling. Plasma levels of leptin in male and female AKO mice fed a regular chow diet were decreased relative to matched WT mice; similar results were obtained when HFD-fed mice were examined (**Fig. 3*A*, *C***). Levels of circulating leptin were lower in AKO mice also when the data were normalized to the weight of the mice (Fig. 3*B*, *D*). To examine the sensitivity of AKO mice to leptin, we subjected chow-fed AKO mice to acute leptin stimulation by injecting them with the hormone and examining phosphorylation of STAT3 in the hypothalamus as a readout of leptin signaling. Acute stimulation of female AKO mice resulted in a significantly stronger pSTAT3 signal than in matched WT female mice (Fig. 3*E*), indicating that these mice are leptin hypersensitive. This conclusion was further confirmed in a chronic leptin stimulation study, in which mice were exposed to daily leptin injections over the course of several days. Injection of leptin reduced body weight and food intake; both parameters returned to baseline values when leptin was replaced with PBS (Fig. 3*F*, *G*). Administration of leptin resulted in an increased rate of weight loss in chow-fed female AKO mice relative to matched WT control mice. Food intake was reduced by similar extents in both genotypes. In contrast, male chow-fed AKO mice did not differ from control WT mice in both acute and chronic leptin stimulation challenges (Fig. 3*E*, *F*, *G*). We conclude that under the dietary conditions of this study, the chow-fed male AKO mice are not leptin hypersensitive, in agreement with their normal rate of weight gain and metabolic parameters.

The finding that acute exposure of AKO mice to leptin induces increased phosphorylation of STAT3 in the hypothalamus suggests that loss of PTPRA dysregulates leptin signaling in hypothalamic neurons. Protein-blotting studies indicated that PTPRA is expressed in hypothalami

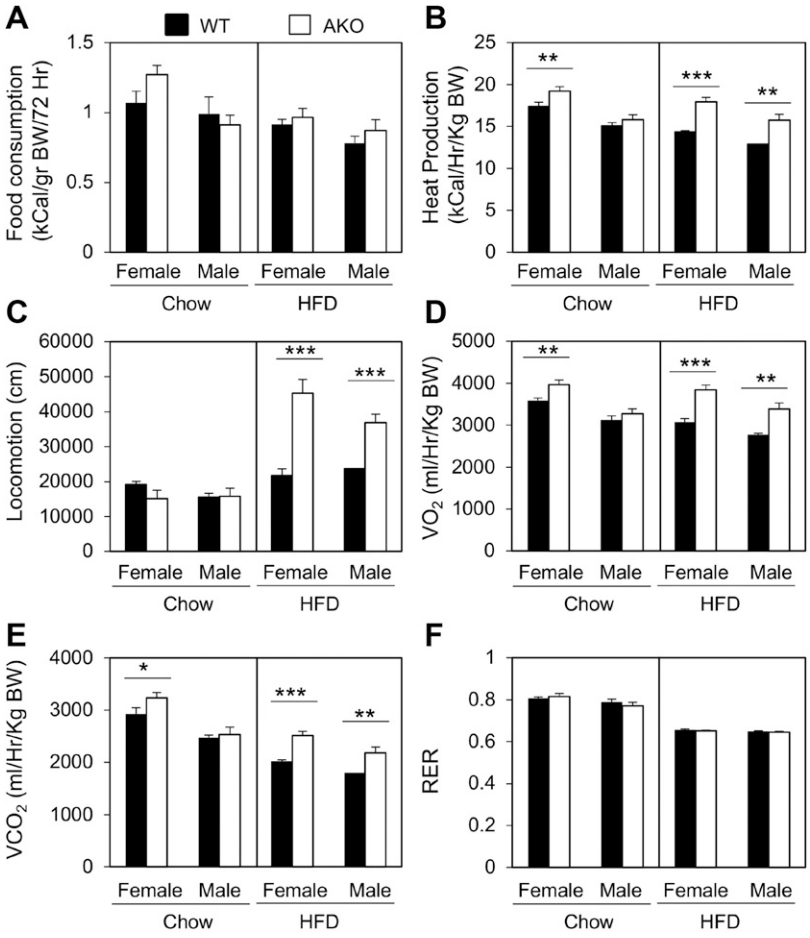

Figure 2. Increased energy expenditure in AKO mice. Mice of both sexes were housed individually in metabolic cages and fed either regular chow or an HFD as indicated. The following parameters were measured: food intake (total intake per mouse over a period of 72 h/g body weight) (*A*), heat production (*B*), locomotion (average distance traveled per mouse) (*C*), oxygen consumption (*D*), carbon dioxide production (*E*), and calculated respiratory exchange ratio (*F*). Except where noted, data are the average values of 3 consecutive dark, active, phases. Mice analyzed here were 5–6 mo old, and were the same mice used in Fig. 1*A–D*. Data are means ± SE; $n = 7$–14 mice per bar. $^*P = 0.025$, $^{**}P \leq 0.0087$, $^{***}P \leq 0.0005$ (Student's *t* test). Similar analyses of younger mice are shown in Supplemental Fig. S2.

isolated from WT mice but is absent from similar samples obtained from AKO mice (**Fig. 4*A***). Hypothalamic expression levels of the related PTPRE and of SHP2, PTP1B, and PTPRJ, all PTPs that regulate hypothalamic leptin signaling, were not affected by loss of PTPRA (Fig. 4*A, B*). In addition, the Ox-PTP antibody, which can be used in conjunction with sodium pervanadate to identify several PTPs by binding to their oxidized catalytic cysteine (38), marked a similar pattern of bands in protein blots prepared from WT and AKO hypothalami. The only differences observed were absence of PTPRA or PTPRE from hypothalamic lysates of mice lacking either PTP (Fig. 4*C*). We conclude that loss of PTPRA does not induce obvious compensatory changes in expression of other PTPs in the hypothalamus.

PTPRA constitutively associates with and dephosphorylates JAK2

The increased leptin sensitivity observed in AKO mice and expression of PTPRA in the hypothalamus suggests that PTPRA down-regulates LEPR signaling in hypothalamic neurons, prompting us to examine this process in greater molecular detail. Increased phosphorylation of hypothalamic STAT3 after acute leptin stimulation of AKO mice (Fig. 3*E*) suggests that this PTP dephosphorylates STAT3 itself or molecules further upstream, such as JAK2. Expression of PTPRA in HEK 293 cells significantly attenuated leptin-induced phosphorylation of JAK2 at Y1007/Y1008, a site required for JAK2 activity (43) (**Fig. 5*A***); smaller effects were noted with STAT3 (data not shown). To examine if PTPRA can directly dephosphorylate JAK2, we purified both molecules and examined if PTPRA can dephosphorylate JAK2 *in vitro*. Due to difficulties in purifying full-length PTPRA, these studies used cyt-PTPa, which contains the entire cytosolic sequence of PTPRA, including its PTP catalytic domain, fused at its N-terminus to the 12 N-terminal amino acids from the nonreceptor isoform of PTP epsilon (35). Purified cyt-PTPa was able to dephosphorylate purified phospho-JAK2 in a time-dependent manner in *in vitro* assays (Fig. 5*B*). Importantly, adding the PTP inhibitor sodium pervanadate to the reaction or replacing active WT PTPRA with its inactive D401A mutant abolished JAK2 dephosphorylation, findings that are all consistent with direct dephosphorylation of JAK2 by the catalytic domain of PTPRA. JAK2 coprecipitated with PTPRA both in the presence or absence of LEPR stimulation (Fig. 5*C*), indicating that both molecules are part of a complex that exists irrespective of LEPR stimulation and in which PTPRA dephosphorylates JAK2 and down-regulates its activity.

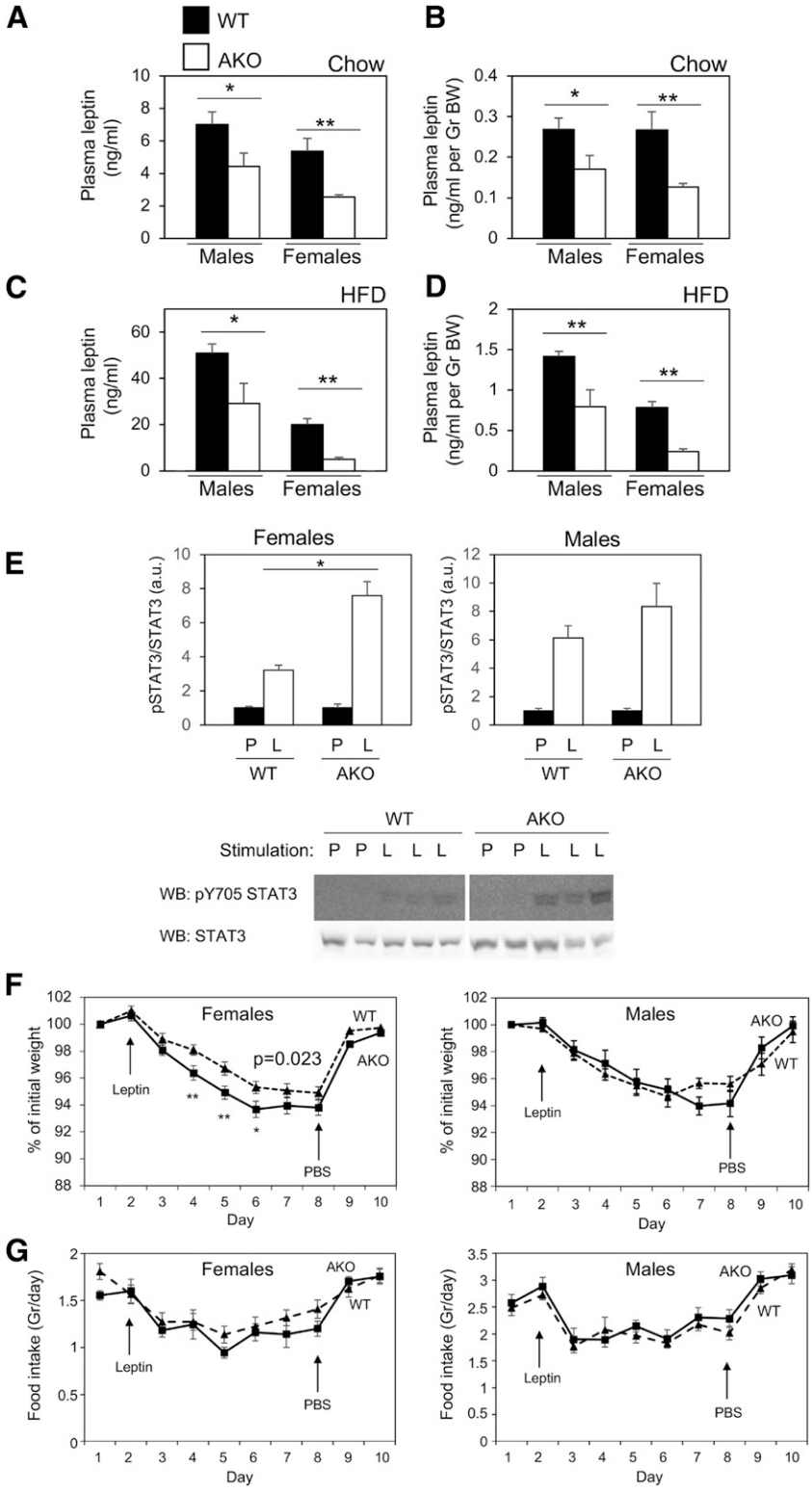

Figure 3. AKO mice are leptin hypersensitive. *A*) Plasma leptin levels of male and female AKO mice fed chow. *B*) Data shown in *A*, normalized to body weight of the mice. *C*) Plasma leptin levels of male and female AKO mice fed an HFD. *D*) Data of *C* normalized to body weight of the mice. $*P < 0.05$, $**P \leq 0.01$ (Student's *t* test; n = 7–9 mice/bar, aged 3.5–4 mo). *E*) Top: STAT3 phosphorylation in hypothalami of chow-fed, female and male AKO mice after acute leptin stimulation. Data, obtained from protein blotting studies, are from 8-wk-old mice after injection of leptin (L) relative to injection of PBS (P). $**P = 3.8 \times 10^{-4}$ (Student's *t* test; n = 10–14 mice/bar). Bottom: representative protein blot showing STAT3 phosphorylation in hypothalami of mice after injection of PBS (P) or leptin (L). *F*) Chronic leptin stimulation. Female or male chow-fed mice were injected with leptin and weighed daily. Shown is change from initial mouse body weight; n = 21–22 mice/genotype, $P = 0.023$ (2-way ANOVA with repeated measurements, using the original false discovery rate method of Benjamini and Hochberg *post hoc* analysis). $*P = 0.01$, $**P \leq 0.008$. Asterisks denote days in which the data points are distinct. Arrows denote start (d 2) and end (d 8) of leptin treatment. Initial body weights of WT *vs.* AKO mice were similar in each sex. All data are presented as means ± SE. *G*) Similar to *F*, showing the effect of leptin on food intake.

C-terminal phosphorylation of PTPRA is not required for its activity toward JAK2

The extreme C-terminal sequences of PTPRA (AFSDYANFK) and of the related PTPRE (IFSDYANFK) are almost identical; this includes their C-terminal tyrosine residues (Y789 in PTPRA, Y695 in PTPRE, underlined), whose phosphorylation helps regulate the function of either PTP (18, 32, 33, 42, 44, 45). To determine if phosphorylation of PTPRA at Y789 is required for regulation of leptin signaling, we examined phosphorylation of PTPRA in response to activation of the LEPR. Initial studies in HEK 293 cells indicated that PTPRA is basally phosphorylated at Y789, and that phosphorylation increases somewhat after leptin stimulation of cells which also express the LEPR and JAK2 (**Fig. 6*A***). No

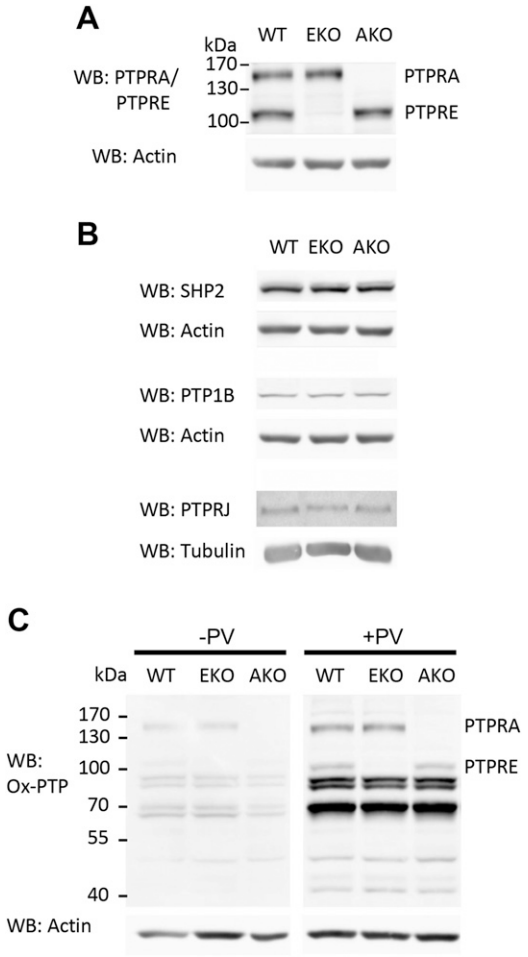

Figure 4. Expression of PTPs that regulate LEPR signaling in mouse hypothalami. *A*) Protein blot documenting expression of PTPRA and PTPRE in hypothalami of WT, PTPRE-deficient (EKO), and PTPRA-deficient (AKO) mice. *B*) Similar to *A*, showing expression of SHP2, PTP1B, and PTPRJ. *C*) Lack of obvious changes in expression levels of PTPs in hypothalami of WT, EKO, and AKO mice. Protein blots prepared from hypothalamic lysates that were either treated (+PV) or not treated (−PV) with 0.5 mM sodium pervanadate were probed with the anti–Ox PTP antibody that recognizes the oxidized, sulfonic acid form of the catalytic cysteine of PTP domains. Molecular mass standards are in kilodaltons.

phosphorylation was detected in cells expressing Y789F PTPRA, confirming the specificity of the phosphorylation signal.

To examine leptin-induced phosphorylation of PTPRA *in vivo*, we injected chow-fed WT female mice with leptin, isolated hypothalamic tissue at various time points thereafter, and examined phosphorylation of endogenous PTPRA at Y789. Phosphorylation of PTPRE at Y695, which is induced under these conditions (18), was also determined in the same samples as a control. Similar to HEK 293 cells, significant basal phosphorylation of PTPRA at Y789 was observed in hypothalamic tissue before leptin stimulation. Stimulation with leptin did not significantly affect PTPRA phosphorylation; it remained at prestimulation levels for the first 60 min after stimulation, and increased slightly thereafter (Fig. 6*B, C*, left). In contrast, PTPRE phosphorylation followed the pattern observed previously (18): basal phosphorylation was low, increased significantly 60–75 min post-stimulation, and receded thereafter (Fig. 6*B, C*, right). We conclude that although LEPR stimulation induces some phosphorylation of PTPRA at Y789, this increase is relatively small and occurs in addition to significant preexisting phosphorylation at this site. Moreover, Y789F PTPRA, in which position 789 cannot be phosphorylated, bound JAK2 and attenuated LEPR-induced JAK2 phosphorylation (Fig. 5*A, C*), suggesting that

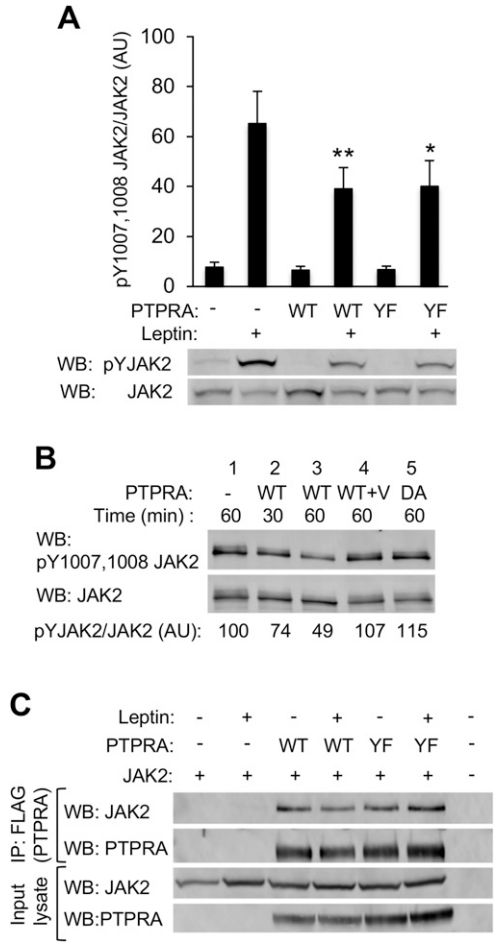

Figure 5. Regulation of JAK2 by PTPRA. *A*) Leptin-induced phosphorylation of JAK2 at Y1007/1008 is reduced in HEK 293 cells after expression of WT PTPRA or Y789F PTPRA. Shown are means ± SE of n = 12–14 repeats/bar. *P = 0.03, **P = 0.0008 (Student's t test, relative to leptin-induced phosphorylation in the absence of PTPRA). *B*) PTPa dephosphorylates JAK2 at pY1007/1008 *in vitro*. Purified cyt-PTPa and purified, phosphorylated JAK2 were incubated together for 30–60 min at 37°C as indicated. Values below the figure indicate JAK2 phosphorylation (normalized to JAK2 amounts), relative to lane 1. WT, WT cyt-PTPa; WT + V, WT cyt-PTPa with 0.5 mM sodium pervanadate; DA, inactive D401A cyt-PTPa (numbered as in PTPRA). *C*) PTPRA physically associates with JAK2 irrespective of leptin signaling or PTPRA phosphorylation at Y789. HEK 293 cells were transfected with the LEPR, with JAK2, and with WT or Y789F (YF) PTPRA as indicated; some cells were stimulated with 100 nM leptin for 5 min. PTPRA was precipitated and coprecipitation of JAK2 was determined by protein blotting.

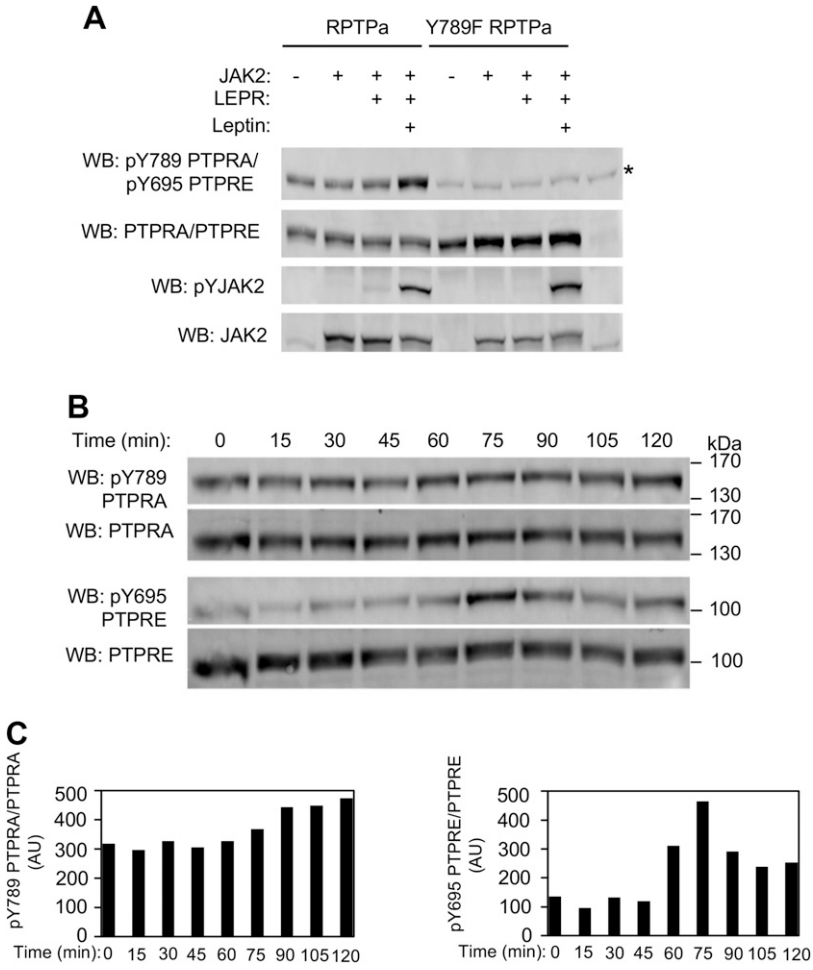

Figure 6. Phosphorylation of PTPRA at Y789. *A*) HEK 293 cells were transfected with WT or Y789F PTPRA, JAK2, and the LEPR, serum-starved for 12 h and stimulated with 100 nM leptin for 5 min as indicated. Asterisk notes nonspecific band present also in nontransfected cells (far right lane). *B*) Different *in vivo* phosphorylation kinetics of PTPRA and PTPRE in response to leptin stimulation. Eight-wk-old female WT C57Black/6 mice were injected intraperitoneally with leptin; their hypothalami were isolated at the indicated time points postinjection and analyzed for phosphorylation of PTPRA at Y789 and of PTPRE at Y695 by protein blotting. *C*) Quantification of PTPRA and PTPRE phosphorylation (relative to expression of PTPRA and PTPRE, respectively) in the blot shown in *B*. Antibodies to PTPRA and to pY789 PTPRA also detect PTPRE and pY695 PTPRE, respectively. *B*, *C*) Values are from 1 experiment representative of 3.

phosphorylation at Y789 is not required for these functions of PTPRA. We conclude that PTPRA down-regulates LEPR signaling in hypothalamic neurons by targeting JAK2 but that, contrary to PTPRE, C-terminal phosphorylation is not required for this activity.

DISCUSSION

Chow-fed female AKO mice and diet-induced obese AKO mice of both sexes weigh less, exhibit reduced fat content and reduced levels of circulating leptin, and are more metabolically active than matched WT control mice. Chow-fed male AKO mice display a much weaker metabolic phenotype, indicating a strong sex-specific effect of loss of PTPRA in the nonobese state. The responses of chow-fed female AKO mice to leptin exposure in both acute and chronic paradigms of leptin stimulation are increased compared with WT control mice, indicating that they are leptin hypersensitive. The finding that acute exposure of AKO mice to leptin generates a stronger hypothalamic signaling response than in WT mice suggests that hypothalamic LEPR signaling is a major target through which PTPRA affects body mass. Collectively, these data indicate that loss of PTPRA induces a leptin-hypersensitive state and that PTPRA down-regulates metabolic signaling by leptin. Importantly, these effects are retained in obese AKO mice, suggesting that this phosphatase is required for establishing or maintaining obesity-associated leptin resistance.

PTPRA down-regulates LEPR signaling in the hypothalamus by dephosphorylating JAK2 at Y1007/1008, a site whose phosphorylation is required for activation of the kinase (43). Although several studies have shown that C-terminal phosphorylation of PTPRA at Y789 helps regulate the biologic function of this phosphatase in other contexts, this phosphorylation event does not play a major role in down-regulating LEPR signaling. This conclusion is based on significant pre-existing phosphorylation of PTPRA at Y789, the relatively small increase in phosphorylation at this site after LEPR activation, and the finding that WT PTPRA and its non-phosphorylatable Y789F mutant bind JAK2 and reduce its phosphorylation at Y1007/1008 to similar extents. Together with the ability of purified PTPRA to dephosphorylate purified JAK2 *in vitro*, these findings suggest that PTPRA does not require previous priming to target JAK2. PTPRA might therefore participate in preventing inappropriate activation of leptin signaling before leptin exposure, as well as in down-regulating LEPR signaling after its activation and returning leptin signaling to baseline levels (**Fig. 7**).

Despite their similarities, the leptin-related phenotypes of AKO mice and of mice lacking PTPRE (EKO mice) are not identical. Chow-fed, female AKO mice are lean, whereas EKO mice of both sexes fed the same diet are of normal weight; AKO mice of both sexes are resistant to diet-induced obesity, while in EKO mice this phenotype is limited to female mice (18). The leptin-related phenotype of AKO mice then seems to be stronger than that of EKO mice. At the molecular level, this distinction may arise from the requirement of RPTPE to be phosphorylated after LEPR activation to target JAK2. This mechanism restricts PTPRE activity toward LEPR signaling, mainly to poststimulation events, whereas PTPRA can target this signaling system also before its stimulation by leptin. PTPRE has potent autodephosphorylating activity (32), which provides an attractive mechanism to limit the participation of PTPRE in LEPR signaling. Although PTPRA can autodephosphorylate at Y789 (46), the minor role, if any, that phosphorylation at this site plays in leptin signaling indicates that autodephosphorylation does not down-regulate RPTPa function in this context.

In chow-fed mice, PTPRA affected body weight and hypothalamic leptin signaling in female mice but not in male mice. In contrast, metabolic phenotypes were observed in HFD-fed AKO mice of both sexes, indicating that sex specificity is lost when these mice are stressed metabolically. A female-specific effect of PTPRE on LEPR signaling was observed in EKO mice (18), whereas an opposite sex preference that was attributed to diet or genetic background was described in a study of PTP1B-deficient mice (8). Although these factors may be relevant for AKO mice as well, estrogen is known to help regulate energy status by affecting leptin sensitivity in the brain (47), more prominently in females (48, 49). The differential roles of estrogen in metabolic regulation in male mice *vs.* female mice may help reduce the magnitude of the metabolic phenotype in chow-fed male AKO mice. However, challenging these mice with HFD evidently stressed these mice sufficiently to overcome sex specificity and to induce effects in both sexes.

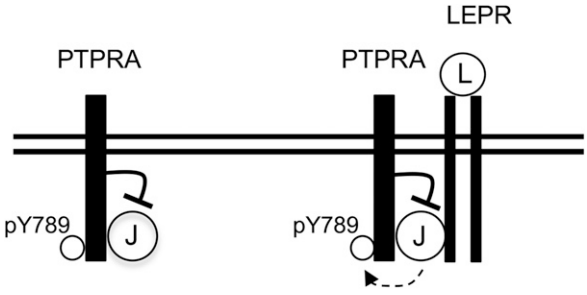

Figure 7. Model of PTPRA activity toward JAK2 in leptin receptor signaling. Left: PTPRA and JAK2 (J) constitutively associate in the absence of the leptin receptor; PTPRA can dephosphorylate JAK2 at Y1007/1008. PTPRA may be phosphorylated at Y789 (as drawn), but this action is not required for dephosphorylation of JAK2. Right: PTPRA and JAK2 associate with the LEPR after its activation by leptin (L), and PTPRA dephosphorylates JAK2 at Y1007/1008. JAK2 weakly phosphorylates PTPRA at Y789 (dashed arrow), but this action is not required for PTPRA to dephosphorylate JAK2.

PTPRA therefore joins an expanding group of PTPs that down-regulate hypothalamic leptin signaling by targeting JAK2, a group that currently includes PTP1B, PTPRE, and PTPRJ. Other PTPs, including TC-PTP, SHP2, and PTEN, regulate hypothalamic LEPR signaling also by targeting other molecules (3, 14–17, 50). It is not surprising that this pathway, which affects the core physiologic properties of an organism, is regulated at several levels by multiple distinct regulators. In particular, the key role of JAK2 as an activator of signaling downstream of the LEPR makes it a major target of various regulatory mechanisms. Each of the known PTPs that targets JAK2 at Y1007/1008 does so on different terms: HFD or old age increase expression of hypothalamic PTP1B (51), but not of PTPRE and PTPRA (data not shown), suggesting that PTP1B is regulated also through its protein levels. Differences between the leptin-related phenotypes of mice lacking PTPRE or PTPRA exist and have been discussed earlier. PTPRJ targets other tyrosine residues in JAK2 and, similar to PTP1B but distinct from PTPRE and PTPRA, its expression is induced by an HFD (19). Each PTP may therefore enable regulation of hypothalamic leptin signaling by distinct upstream stimuli, providing added flexibility but also redundancy in the regulation of this central pathway. Finally, obesity-induced leptin resistance precludes use of leptin to treat obesity in humans. The metabolic effects of loss of PTPRA are evident also in the obese state, suggesting that inhibition of PTPRA, possibly in combination with inhibition of other PTPs that target LEPR signaling, may help avoid leptin resistance in obese individuals and enable their treatment with leptin. FJ

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AUTHOR CONTRIBUTIONS

Y. Cohen-Sharir, H. Knobler, and A. Elson designed the research; Y. Cohen-Sharir, Y. Kuperman, and D. Apelblat performed the research; J. den Hertog contributed new reagents; Y. Cohen-Sharir, Y. Kuperman, D. Apelblat, I. Spiegel, H. Knobler, and A. Elson analyzed the data; and A. Elson wrote the manuscript.

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