ORIGINAL ARTICLE

Protein-Tyrosine Phosphatase-1B Mediates Sleep Fragmentation-Induced Insulin Resistance and Visceral Adipose Tissue Inflammation in Mice

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Study Objectives: Sleep fragmentation (SF) is highly prevalent and has emerged as an important contributing factor to obesity and metabolic syndrome. We hypothesized that SF-induced increases in protein tyrosine phosphatase-1B (PTP-1B) expression and activity underlie increased food intake, inflammation, and leptin and insulin resistance.

Methods: Wild-type (WT) and *ObR-PTP-1b−/−* mice (Tg) were exposed to SF and control sleep (SC), and food intake was monitored. WT mice received a PTP-1B inhibitor (RO-7d; Tx) or vehicle (Veh). Upon completion of exposures, systemic insulin and leptin sensitivity tests were performed as well as assessment of visceral white adipose tissue (vWAT) insulin receptor sensitivity and macrophages (ATM) polarity.

Results: SF increased food intake in either untreated or Veh-treated WT mice. Leptin-induced hypothalamic STAT3 phosphorylation was decreased, PTP-1B activity was increased, and reduced insulin sensitivity emerged both systemic and in vWAT, with the latter displaying proinflammatory ATM polarity changes. All of the SF-induced effects were abrogated following PTP-1B inhibitor treatment and in Tg mice.

Conclusions: SF induces increased food intake, reduced leptin signaling in hypothalamus, systemic insulin resistance, and reduced vWAT insulin sensitivity and inflammation that are mediated by increased PTP-1B activity. Thus, PTP-1B may represent a viable therapeutic target in the context of SF-induced weight gain and metabolic dysfunction.

Keywords: sleep fragmentation, protein tyrosine phosphatase-1B, insulin resistance, macrophage polarity, leptin receptor

Statement of Significance

Chronic sleep fragmentation, a frequent occurrence in modern life and many diseases, induces increased activity of PTP-1B that leads to increased food intake and plasma leptin levels, and visceral white adipose tissue inflammation and insulin resistance via PTP-1B-mediated pathways.

INTRODUCTION

Poor sleep quality and quantity have been implicated in the high prevalence of obesity and associated morbidities around the world.[1,2] In this context, chronic sleep fragmentation (SF), as occurs in many sleep disorders or in suboptimal sleep conditions, has been associated with increased propensity for development of obesity and cardiometabolic disorders.[3,4] We and others have previously shown that SF leads to increased food consumption, accelerated weight gain, along with emergence of visceral white adipose tissue (vWAT) inflammation, and insulin resistance through activation of complex transcriptomic pathways and networks.[5–12] In the context of better understanding, the potential mechanisms underlying such metabolic alterations, evidence implicating protein tyrosine phosphatase 1B (PTP-1B) as altering hypothalamic leptin receptor (ObR) sensitivity, and fostering increased orexigenic behaviors and reduced satiety emerged.[13] However, the potential role of PTP-1B in vWAT inflammation and reduced insulin sensitivity was not explored. Indeed, dephosphorylation of JAK2 is an important mechanism for terminating leptin signal transduction and can be induced by increased PTB-1B activity.[14–16] However, induction of PTP-1B activity can also potentiate inflammatory pathways and promote insulin resistance in vWAT.[17–21] Since selective ablation of PTP-1B from adipocytes does not alter the metabolic perturbations induced by high-fat diet,[22] and to further explore the hypothesis that SF induces increased PTP-1B activity in vWAT and in adipose tissue macrophages (ATM) and that such changes promote the polarity shift in ATM from M2 to M1 and also contribute to adipose tissue insulin resistance following SF in an ObR-dependent manner, we assessed the effect of treatment with a systemic PTP-1B inhibitor, selected among 4-[(5-arylidene-4-oxo-3-thiazolidinyl)methyl]benzoic acids,[23–25] as well as the effect of SF in ObR selective PTP-1B deletion in transgenic mice.

METHODS

Animals

Male C57BL/6J mice weighing 22–25 g were purchased from Jackson Laboratories (Bar Harbor, Maine). In addition, *ObR-Ires-Cre* mice on a C57/B6 background (a generous gift from Dr,. Martin Myers at the University of Michigan) and *PTP1b fl/fl* mice on a C57/B6 background (developed and provided by Dr. Ben Neel at the University of Toronto) were crossed to generate *ObR-PTP-1b−/−* mice that specifically lack PTP-1B in all cells and tissues that express ObR. To ascertain the restricted knockdown of PTP-1B in cells expressing ObR, we performed PTP-1B activity assay experiments in wild-type mice (WT) and in *ObR-PTP-1b−/−* (Tg) mice that confirmed the markedly reduced PTP-1B activity in hypothalamus, liver and visceral white adipose tissues, and adipose tissue macrophages (ATM) but preserved PTP-1B activity in ectorhinal cortex of the transgenic mice, a brain region that has minimal expression of ObR-positive cells (Figure 1; $n = 5$/group; Tg vs. WT: $p < .001$ for all tissues except ectorrhinal cortex;[26,27]) All mice were housed in a 12-hour light–dark cycle (light on 7:00 am to 7:00 pm) at a constant temperature (24 ± 1°C) and were allowed access to food and water *ad libitum*. All the experimental procedures took place after at least a week of habituation to the facility and started at 8–9 weeks of age. The experimental protocols were

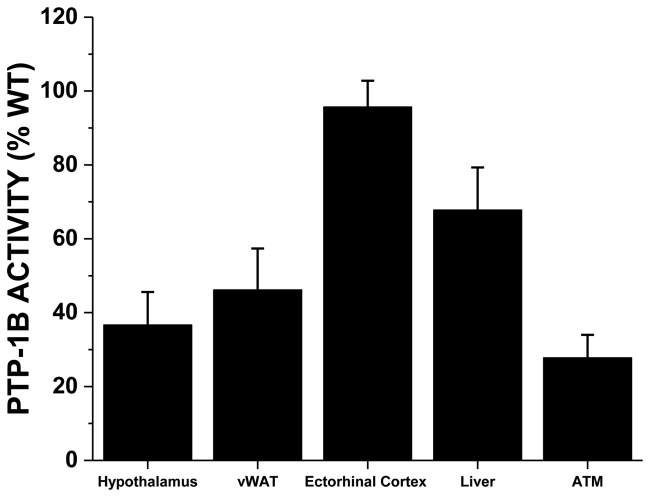

Figure 1—Protein-tyrosine phosphatase-1B (PTP-1B) activity in leptin receptor (ObR)-PTP-1b−/− transgenic (Tg) mice relative to wild-type mice in various tissues. Significant reductions in PTP-1B activity are apparent in ObR-expressing tissues, but not in ectorhinal cortex, in which ObR expression is low or null (n = 6/group). Abbreviations: ATM, adipose tissue macrophages; vWAT, visceral white adipose tissue.

approved by the Institutional Animal Use and Care Committee and are in close agreement with the ARRIVE guidelines and National Institutes of Health *Guide in the Care and Use of Animals*. All efforts were made to minimize animal suffering and to reduce the number of animals used.

PTP-1B Inhibitor

The compound used for all experiments was the 4-{[5-(4-benzyloxybenzylidene)-2-(4-trifluoromethylphenylimino)-4-oxo-3-thiazolidinyl]methyl}benzoic acid (termed RO-7d) which was previously reported.[25] Compound RO-7d showed to be a potent inhibitor of recombinant human PTP1B with IC_{50} value of 1.4 μM.

Moreover, compound RO-**7d** proved to increase the degree of phosphorylation of insulin receptor and also promoted a high level of glucose uptake when it was incubated in mouse C2C12 skeletal muscle cell cultures.[25]

Sleep Fragmentation

The device used to induce SF in rodents has been previously described (catalog #Model 80390, Lafayette Instruments, Lafayette, IN)[28] and employs intermittent tactile stimulation of freely behaving mice in a standard laboratory mouse cage, using a near-silent motorized mechanical sweeper. This method prevents the need for human contact and intervention, introduction of foreign objects or touching of the animals during sleep, and is therefore superior to other existing methods of sleep fragmentation. To induce moderate to severe sleep fragmentation that is present in multiple sleep disorders, such as sleep apnea, we chose a 2-minute interval between each sweep, implemented during the light period (7 a.m. to 7 p.m.). The sweeper required 9 seconds to travel the distance of the cage. Of note, 3–4 mice were housed in each SF cage to prevent isolation stress, and all mice had *ad libitum* access to food and water. Sleep control mice (SC) were housed in the same conditions with the sweeper turned off.

Body Weight and Food Intake

Body weight was measured twice a week and always at the same time of the day (middle of the light cycle period). All animals had free access to regular chow diet. Food intake was monitored on a daily basis, by measuring the amount of food consumed in each cage and dividing it by the number of animals for each cage (usually 3–4 animals per cage). Food intake is presented as grams/mouse/day.

Experimental Protocol

(a) In an initial set of experiments, we administered incrementalintraperitoneal doses of the PTP-1B inhibitor 4-{[5-(4-benzyloxybenzylidene)-2-(4-trifluoromethylphenylimino)-4-oxo-3-thiazolidinyl]methyl}benzoic acid (RO-7d) (25) and assessed PTP-1B activity in both hypothalamus and vWAT (Figure 1). We then assessed PTP-1B activity in these tissues using the selected dose from the initial set of experiments up to 24 hours after administration to ascertain the viability of daily dosage (Figure 2). (b) After determination of optimal dosing, C57/B6 mice were randomly assigned into four treatment groups and were exposed to either SF or sleep control (SC) and treated with either vehicle (Veh) or the PTP-1B inhibitor (Tx) at a daily dose of 250 mg/kg/day. The 4 treatment groups are designated as: Veh-SC, RO-SC, Veh-SF, and RO-SF. After 14 days, animals underwent insulin tolerance tests (ITT; see below) and then hypothalamic and vWAT tissues were harvested and subjected to PTP-1B activity measurements, adipocyte insulin sensitivity (see below), and adipose tissue macrophage (ATM) characterization using FACS procedures (see below). (c) *ObR-PTP-1b−/−* mice and WT mice, all on a C57/B6 background, were exposed to either SF or SC for 4 weeks after which ITT and vWAT tissue-based assays were performed. In addition, PTP-1B activity assays and plasma leptin levels were also assessed.

Tissue Harvesting

At the end of each exposure, mice were euthanized using CO_2 exposures followed by cervical dislocation, the skull was rapidly opened, and the brain was extracted, immediately placed on dry ice, and the hypothalami and cortex were dissected under surgical microscopy and immediately snap frozen in liquid nitrogen (n = 6 for each group). In addition, epididymal adipose tissue was harvested, and blood was collected into EDTA-contianing tubes and immediately centrifuged with plasma samples being stored at −80°C until assay.

ITT and vWAT Insulin Sensitivity Assays

Systemic ITT and vWAT adipocyte insulin sensitivity were performed as previously described.[29,30] In brief, the mice were injected intraperitoneally with humulin (0.25 U/kg of body weight) after 3 hours of fasting. Blood was collected via the tail vein from each mouse, and blood glucose was measured using an OneTouch Ultra2 glucometer (Life Scan; Milpitas, California). Primary adipocytes were isolated using collagenase digestion and flotation centrifugation and were incubated with insulin at 0 or 5 nM at 37°C for 10 minutes with gentle vortexing every 2 minutes. After two washes with cold Krebs–Ringer

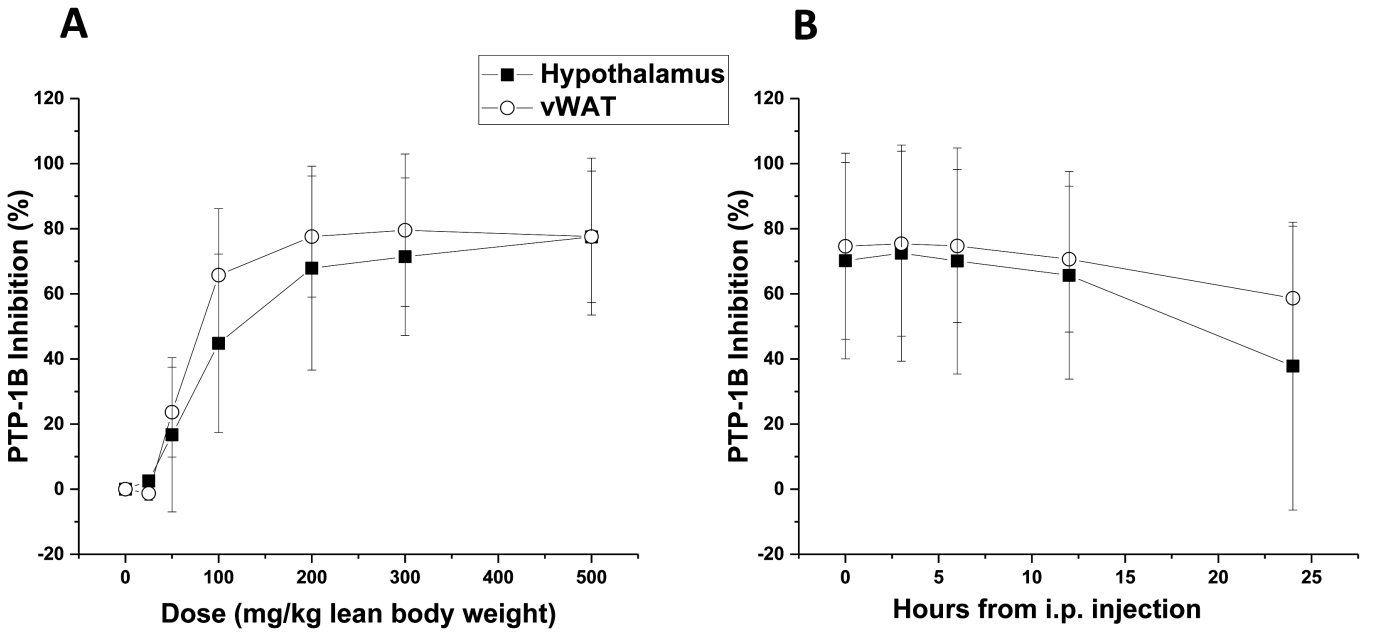

Figure 2—Protein-tyrosine phosphatase-1B (PTP-1B) activity in hypothalamus and visceral white adipose tissue (vWAT) 6 hours following intraperitoneal administration of the PTP-1 inhibitor RO-**7d** at increasing doses (A) and temporal trajectory of the PTP-1B inhibition after administration of a 250 mg/kg dose (B). *n* = 5–6 mice/dose or time point.

buffer (KRB), cells were lysed in radioimmunoprecipitation buffer supplemented with protease and phosphatase inhibitor mixture (Sigma, St Louis, Missouri) and vortexed briefly. The supernatants were collected after centrifugation at 15 000 *g* for 15 minutes at 4°C. Protein concentrations of the cell lysates were determined using BCA kit (Life Technologies). The lysates were separated on 4%–20 nitrocellulose membrane (Millipore, Billerica, Massachusetts) and then transferred to nitrocellulose membranes and incubated in blocking buffer (5% nonfat dry milk in TBST) for 1 hour at room temperature. The membranes were incubated with phospho-Akt (Ser473) antibody (Cell Signaling Technology, Danvers, Massachusetts) or with Akt Antibody (Cell Signaling Technology, Danvers, Massachusetts) overnight at 4°C. Membranes were then washed 3 times for 10 minutes each with 25 mM Tris, pH 7.4, 3.0 mM KCl, 140 mM NaCl, and 0.05% Tween 20 (TBST), incubated with anti-rabbit IgG: HRP-linked Antibody (Cell Signaling Technology, Danvers, Massachusetts) in blocking buffer with gentle agitation for 1 hour at room temperature. Immune-reactive bands were visualized using an enhanced chemiluminescence detection system (Chemidoc XRS+; Bio-Rad, Hercules, California), and quantified by Image Lab software (Bio-Rad, Hercules, California).

Isolation and Analysis of Stromal-Vascular Fraction (SVF) in Adipose Tissues

Epididymal fat pads and mesenteric adipose tissues were gently dissociated in KRB supplemented with 1% BSA after incubation with collagenase (1 mg/mL; Worthington Biochemical Corporation, Lakewood, New Jersey) at 37°C for 45 minutes with shaking. Suspended cells were filtered through a 100-µm mesh and centrifuged at 500 *g* for 5 minutes to separate the floating adipocytes from the stromal-vascular fraction (SVF) pellet. SVF pellets were then resuspended in FACS buffer (phosphate-buffered saline plus 2% fetal bovine serum), and 10^6 cells were used for staining with fluorescence-conjugate primary antibodies or control IgGs at 4°C for 30 minutes. Cells were then washed twice and analyzed with a flow cytometer (Canto II; BD Biosciences, San Jose, California). Data analysis was performed using the FlowJo software (Tree Star, Ashland, Oregon). For flow cytometry analysis, cells were fixed with 1% paraformaldehyde solution for 30 minutes at 25°C and washed twice. Nonspecific binding was blocked with FcR blocker at 1:50 concentration and stained with specific antibodies for analysis. For surface staining, we used the following fluoroconjugated antibodies: CD11b-PB, F4/80-PE/Cy7, CD11c-APC, Ly-6c-APC/APC-Cy7, CD64-PE, CD86-PerCP/Cy5 (BD Biosciences, San Jose, California). Isotype controls were employed to establish background fluorescence. Data were acquired on a FACS CantoII cytometer using the FACS Diva 5.5 software (BD Biosciences) and analyzed by FlowJo software (Tree Star). Adipose tissue macrophages (ATMs) were defined as F4/80+ and CD11b+ cells, from which M1 and M2 macrophages were identified as CD11c+ or CD206+ cells, respectively. In all of the experiments, macrophages were identified as CD11b-F4/80 double positive cells. In addition, proinflammatory activated monocytes were defined as $Ly6c^{high}$ (+) cells[31] and resident ATM as CD64 (+).[32]

Plasma Leptin Levels

Leptin plasma levels were assessed in plasma obtained from blood drawn at the end of each exposure (The assay was carried out using a commercially available enzyme-linked immunosorbent assay [ELISA] kit (Millipore, St. Charles, Missouri) according to the manufacturer's instructions. The linear range was 0.2 ng/mL up to 30 ng/mL with the sensitivity threshold at 0.05ng/mL (~3.13 pM). The intra-assay variation coefficient was up to 1.76%, and the interindividual coefficient of variation was 4.59%.

Leptin Sensitivity and Signaling

For studies involving leptin signaling, SF and SC-exposed WT and Tg mice were fasted for 8 hours (n = 6/experimental group), always starting at 7:00 am and at 3:00 pm received a single intraperitoneal injection (3 µg/g of body weight) of recombinant mouse leptin (Sigma-Aldrich, St Louis, Missouri), with normal saline being used as vehicle. All mice were sacrificed 60 minutes later, and the hypothalamus and vWAT were harvested and processed for protein extraction. To quantify leptin signaling responses, immunoblotting for pSTAT3, STAT3 (Cell Signaling) were performed as described previously.[13]

PTP-1B Activity Assays

Tissue samples were subjected to PTP-1B activity assays using a commercial kit (BPS Bioscience cat #30019, San Diego, California) according to manufacturer's protocol. The protein concentration of supernatants was determined by DC protein assay (Biorad, California). Results were expressed as absorbance per mg tissue per minute.

Statistical Analysis

All data are reported as mean ± SE. Repeated measures analysis of variance (ANOVA) was used to compare food intake

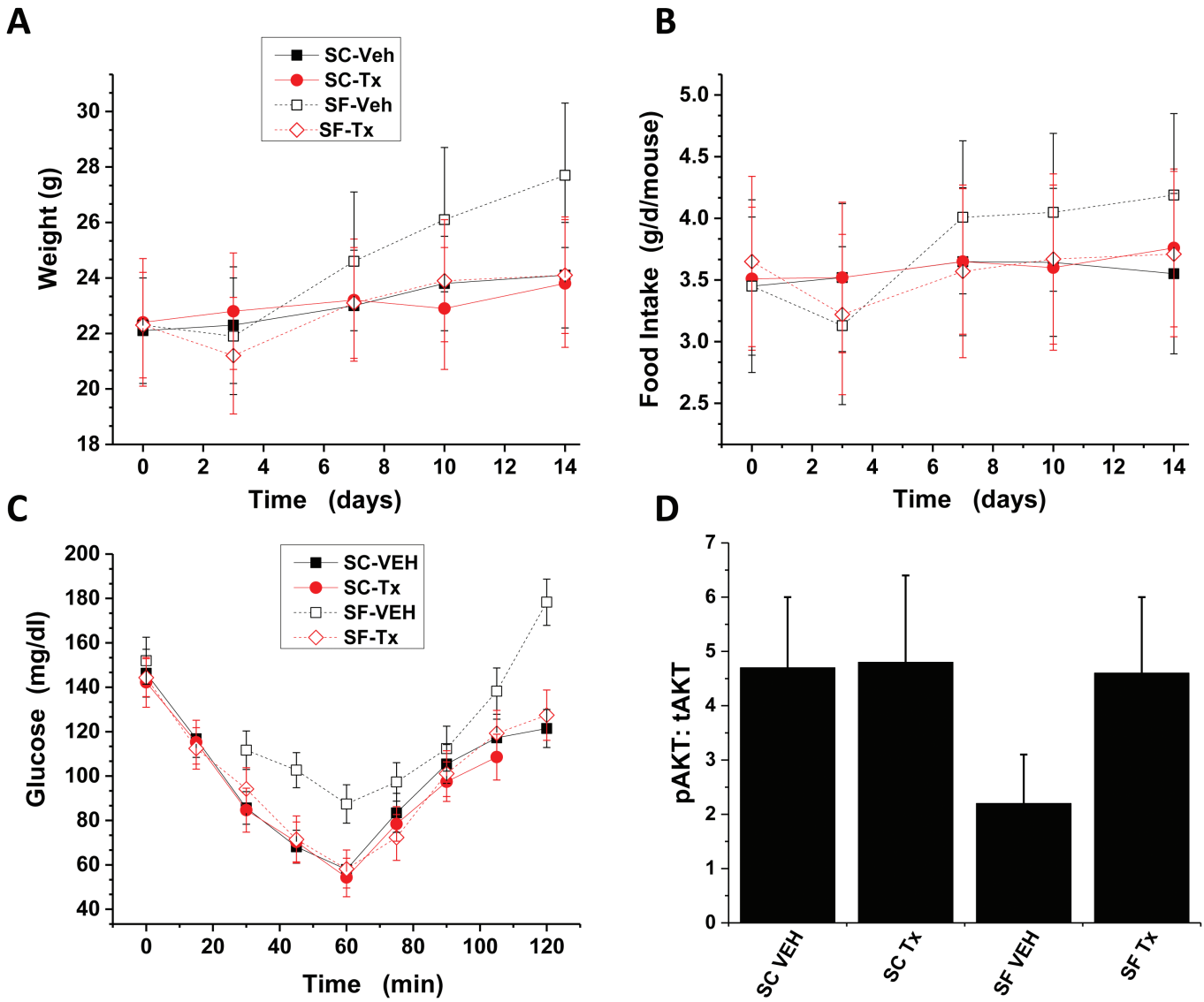

Figure 3—(A) Body weight and (B) food intake in wild-type mice exposed to sleep fragmentation (SF) or sleep control (SC) for 14 days and treated daily with either intraperitoneal (i.p.) vehicle (Veh) or the PTP-1B inhibitor, RO-**7d** (Tx) at a dose of 250 mg/kg (n = 8/experimental group). SF-Veh developed increased food intake and weight gain starting within the first 7 days from initiation of SF (p < .01 vs all other groups). (C) illustrates mean glycemic values over 120 min after i.p. administration of insulin in the four experimental groups, whereby significantly attenuated glycemic responses emerged in SF-Veh treated mice, indicating systemic insulin resistance (SF-Veh vs. all three other groups: p < .001). (D) shows pAKT/AKT changes 30 minutes after adding 5 nM insulin to visceral white adipose tissue (vWAT) dissociated adipocytes mice in each of the experimental treatment groups when compared to 0 nM insulin. Significant attenuation of insulin receptor-induced AKT phosphorylation was apparent in SF-Veh vWAT adipocytes (SF-Veh vs. all three other groups: p < .001).

and weight gain, one-way ANOVA on ranks (Kruskal-Wallis) or Friedman tests followed by unpaired Student's *t*-test with Bonferroni correction were used to compare other time course experiments. Comparison of all other quantitative data between SF and SC conditions was performed using unpaired Student's *t* tests. For all comparisons, a p value < .05 was considered as statistically significant. Statistics were performed using SPSS Statistics 20.0 and Stata 14 for MAC.

RESULTS

Pharmacological Experiments with RO-7d, a PTP-1B Inhibitor

Dose–response experiments in WT mice revealed asymptotic PTP-1B inhibition in both hypothalamus and vWAT around 250 mg/kg dose and beyond (Figure 2). Furthermore, this dosage was associated with sustained PTP-1B inhibition lasting for 24 hours, such that a daily intraperitoneal dosage of 250 mg/kg was employed in all subsequent experiments.

RO-7d Treatment Abrogates SF-Induced Hyperphagic Behaviors, Weight Gain, Insulin Resistance, and vWAT Inflammation

As reported previously, increased food intake became significantly increased after day 7 from the initiation of the SF exposures and was singularly present in mice treated with vehicle and exposed to SF (SF-Veh; p < .05 ANOVA) and was accompanied by accelerated weight gain (Figure 3A and B). In contrast, treatment with RO-7d (Tx) did not alter food intake or weight gain (Figure 3A and B; n = 8/experimental group) throughout the 2 weeks of SF exposures. Upon completion of 2-week SF exposures, ITT showed evidence of systemic insulin resistance in SF-Veh treated mice, but such findings were distinctly absent in SF-Tx mice (p < .001 SF-Veh vs. all three other experimental groups; Figure 3C). Similar results emerged in vWAT, whereby responses to 5 nM insulin resulted in attenuated pAKT–AKT ratios in SF-Veh (p < .001 vs. all three groups) but not in SF-Tx. In addition, Tx prevented SF-induced shift in vWAT ATM polarity toward a proinflammatory M1 phenotype along with reduced counts of the proinflammatory Ly6C^{high} (+) ATM and reduced numbers of resident CD64 (+) ATM (Figure 4). However, Tx did not alter the increases in plasma leptin levels induced by SF (SC-Veh: 0.86 ± 0.20 ng/ml; SC-Tx: 0.89 ± 0.18 ng/ml; SF-Veh:2.06 ± 0.45 ng/ml; SF-Tx: 1.94 ± 0.43 ng/ml; SF vs. SC: p < .001; n = 6/experimental group).

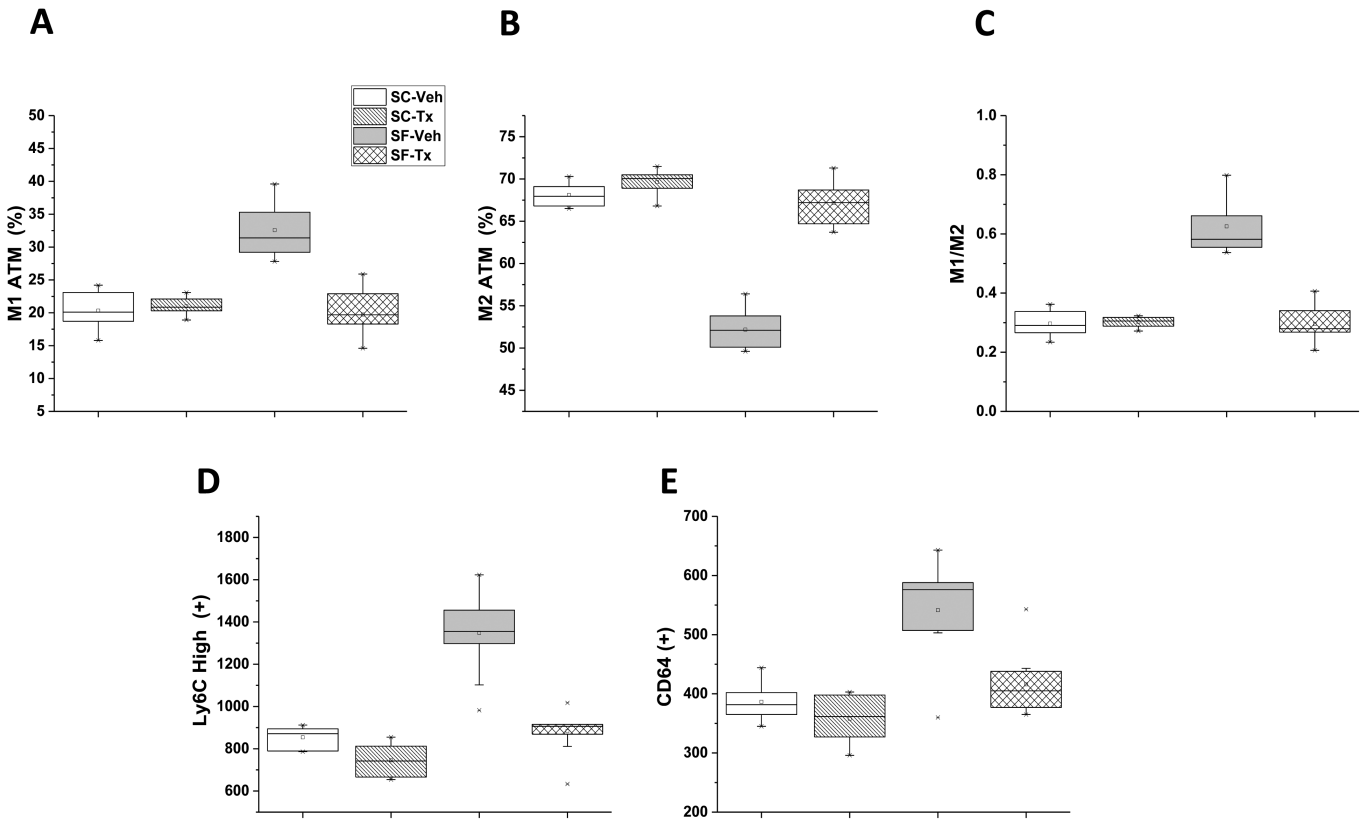

Figure 4—Adipose tissue macrophage (ATM) characteristics in visceral white adipose tissue of wild-type mice exposed to sleep fragmentation (SF) or sleep control (SC) and treated daily with either intraperitoneal (i.p.) vehicle (Veh) or the PTP-1B inhibitor, RO-**7d** (Tx) at a dose of 250 mg/kg. Data are shown as boxplots, with median in the thick line in the box and 90% confidence intervals shown as error bars (n = 8/experimental group). M1 and M2 are displayed of total CD11(+)/F4/80(+) and show significantly increased M1 ATM (A) and reduced M2 ATM (B), increased M1:M2 (C) in SF-Veh compared to all three other experimental groups (p < .001). Significantly increased total visceral white adipose tissue (vWAT) M1 proinflammatory Ly6C^{high} (+) ATM counts (D) and CD64(+) resident macrophages (E) also emerged in SF-Veh mice (p < .01 vs other 3 groups).

PTP-1B Gene Deletion in ObR-Expressing Tissues Restores SF-Induced Leptin Sensitivity and Attenuates Metabolic Dysfunction and vWAT Inflammation

As shown in Figure 1, PTP-1B activity levels in hypothalamus, vWAT, ad ATM were significantly reduced in Tg mice. SF exposures for 4 weeks in Tg mice failed to elicit the increased food intake and body weight accrual that was clearly apparent in WT mice exposed to SF (Figure 5; n = 8–10/experimental group). Similarly, SF exposures for 4 weeks resulted in the anticipated reductions in systemic and vWAT insulin sensitivity which were absent in Tg mice (Figure 5; n = 6/group). In addition, changes in ATM polarity toward a M1 proinflammatory phenotype with reciprocal changes in M2 polarity, and increased LyC6^{high} (+) macrophage counts were not present in Tg mice exposed to SF (Figure 6). However, increased CD64(+) ATM counts occurred in both SF-Tg and SF-WT, albeit of greater magnitude in the latter group (Figure 6).

To further examine the effect of SF on ObR sensitivity in the four experimental groups, we performed leptin injections and found that SF-exposed WT mice exhibited significantly reduced STAT3 phosphorylation in hypothalamus when compared to SC-exposed mice (p < .001; n = 6; Figure 7A). However, Tg mice exposed to SF exhibited similar pSTAT3:total STAT3 responses compared to Tg mice exposed to sleep control

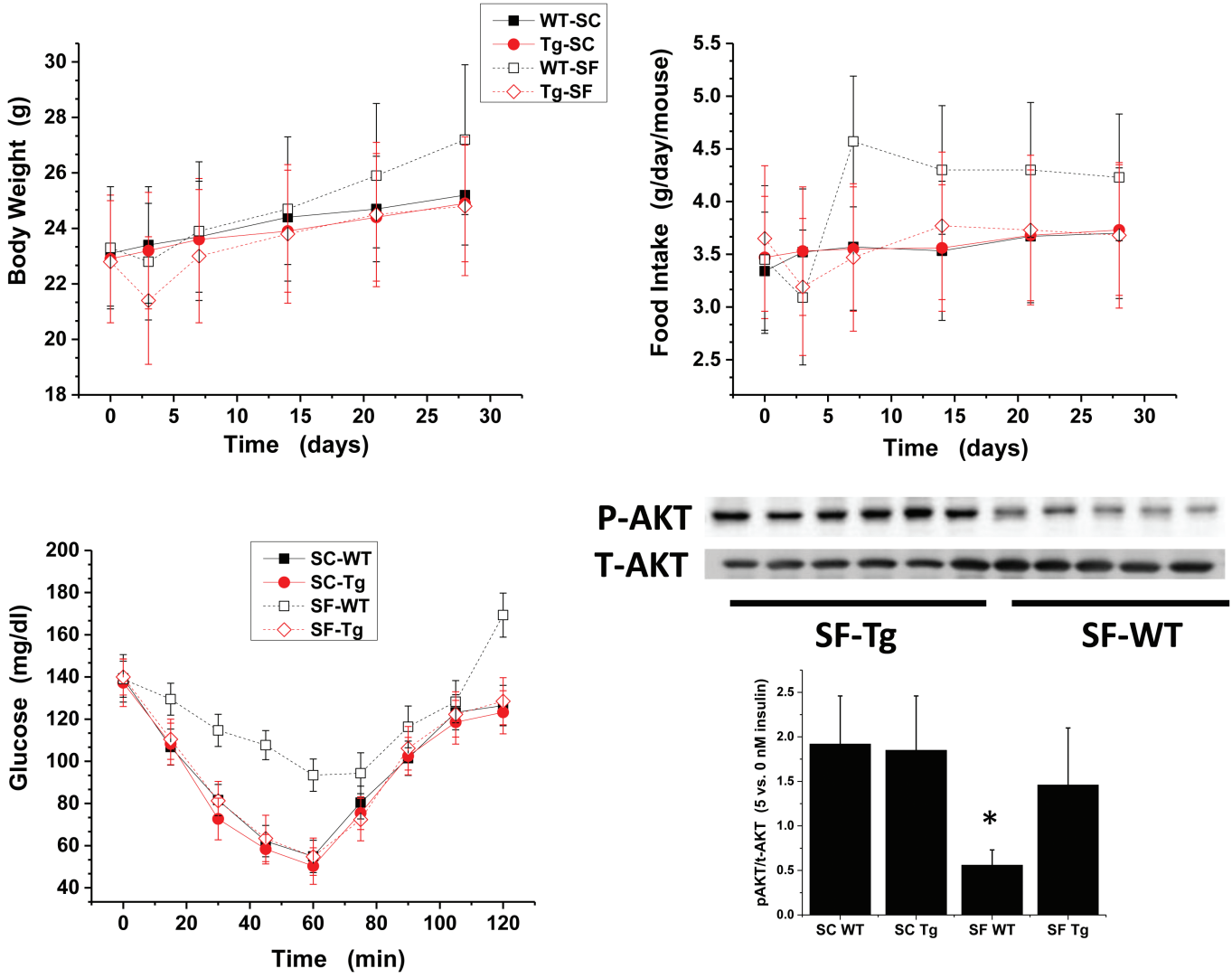

Figure 5—(A) Body weight and (B) food intake in wild-type and transgenic (Tg) mice exposed to sleep fragmentation (SF) or sleep control (SC) for 4 weeks. Only SF-WT mice manifested increased food intake and weight gain (p < .001 vs. 3 other experimental groups; n = 8–10/group). (C) illustrates mean glycemic values over 120 min after intrperitoneal (i.p.) administration of insulin in the four experimental groups, whereby significantly attenuated glycemic responses emerged in WT-SF mice, indicating systemic insulin resistance (SF-WT vs. all three other groups: p < .001; n = 8–10/group). (D) shows pAKT/AKT changes 30 min after adding 5 nM insulin to visceral white adipose tissue (vWAT) dissociated adipocytes mice in each of the experimental treatment groups when compared to 0 nM insulin. Significant attenuation of insulin receptor-induced AKT phosphorylation was apparent in SF-WT vWAT adipocytes (SF-WT vs. all three other groups: p < .001; n = 5–6/group). Representative blots following 5 nM insulin are shown for SF-WT and SF-Tg illustrating the attenuated phosphorylated AKT responses in SF-WT vWAT adipocytes.

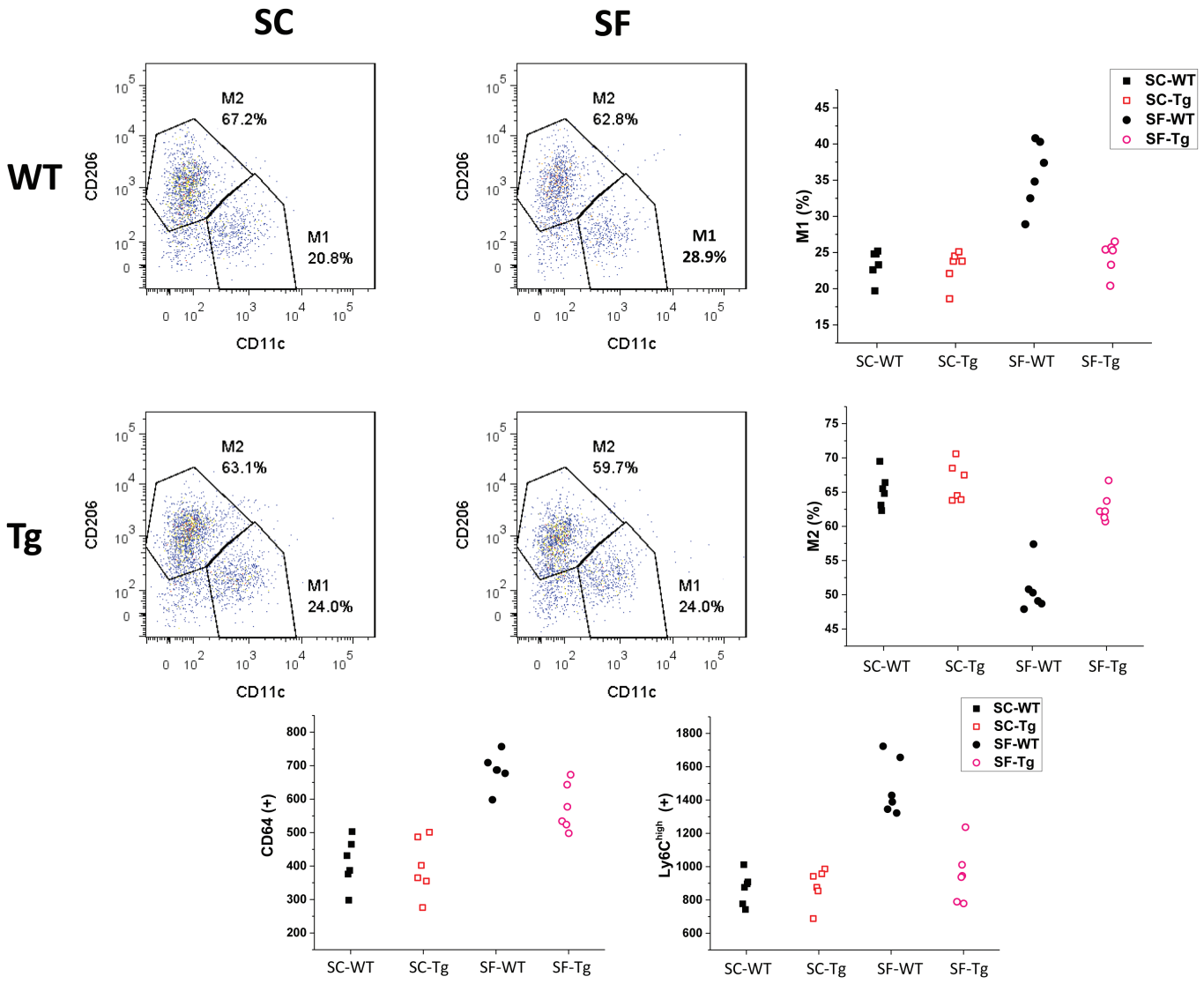

Figure 6—Adipose tissue macrophage (ATM) characteristics in visceral white adipose tissue of transgenic (Tg) or wild-type (WT) mice exposed to sleep fragmentation (SF) or sleep control (SC) for 4 weeks. Number of illustrative example of the flow cytometry strategy for M1 and M2 is shown. Data are shown as individual values for each mouse, (n = 5–6/experimental group). M1 and M2 are displayed of total CD11(+)/F4/80(+) and show significantly increased M1 ATM and reduced M2 ATM in SF-WT mice when compared to all three other experimental groups (p < .01). Significantly increased total vWAT M1 proinflammatory Ly6C^{high} (+) ATM counts and CD64(+) resident macrophages also emerged in SF-WT mice (p < .01 vs. other three groups).

conditions (Figure 7A; n = 6; p > .05). Plasma leptin levels were markedly increased in SF-WT (Figure 7B; p < .001; n = 8) and only mildly increased in SF-Tg mice (p < .04 vs. SC-Tg or SC-WT; p < .001 vs. SF-WT; n = 8; Figure 7B). Further examination of SF-associated changes in PTP-1B activity in hypothalamus revealed significant increases in both WT and Tg mice, but the increases in Tg mice were markedly smaller in Tg mice (Figure 8). SF also induced increases in PTP-1B activity in the vWAT of WT mice, but such changes were not detectable in Tg mice (Figure 8).

DISCUSSION

Obesity and metabolic dysfunction are a highly prevalent condition and constitute a major threat to human health. In this context, disrupted sleep as occurs in sleep apnea has emerged as a potentially important contributor to the generation of a vicious cycle leading to increased food consumption, obesity, and insulin resistance, the latter likely fueled by low-grade systemic and vWAT inflammatory processes.[1,33–35] In a previous study, we uncovered that mice exposed to chronic sleep fragmentation during their natural sleep period exhibited reduced hypothalamic leptin receptor sensitivity, which was mediated by increased PTP-1B activity.[13] Here, we explored whether pharmacological or genetically induced reductions in PTP-1B expression and activity would lead to preservation of hypothalamic ObR sensitivity and would attenuate the magnitude of SF-induced effects on food intake, systemic, and vWAT insulin resistance and inflammation. Taken together, our findings

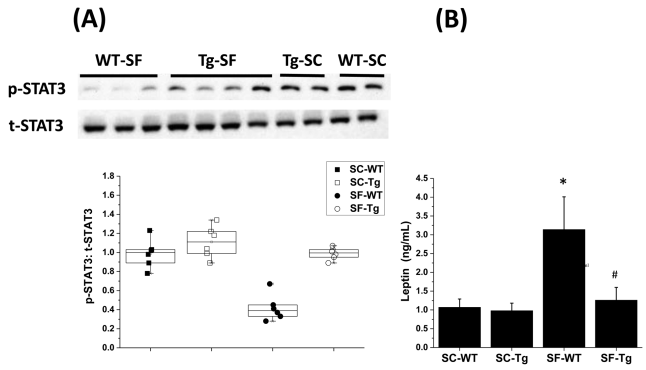

Figure 7—Sleep fragmentation-exposed wild-type (WT) mice but not transgenic (Tg) mice develop hyperleptinemia and attenuation of leptin receptor (ObR) signaling in the hypothalamus. (A) Representative blots of p-STAT3 and t-STAT3 in hypothalamus of sleep fragmentation (SF)-WT, SF-Tg, sleep control (SC)-WT, and SC-Tg. p-STAT3/t-STAT3 was attenuated after intraperitoneal leptin injection in SF-WT mice but not the other groups ($p < .01$; $n = 5$/group). (B) SF-induced ObR resistance as illustrated by elevated leptin plasma levels emerged in both SF-WT mice ($p < .0001$ vs. SC-WT; $n = 8$) but was also apparent, albeit to a significantly lesser degree in SF-Tg mice ($p < .05$ vs. SC-Tg; SF-Tg vs. SF-WT: $p < .001$).

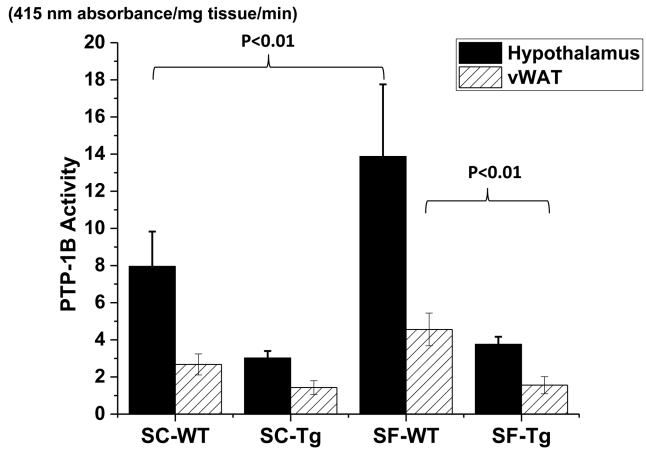

Figure 8—Protein-tyrosine phosphatase-1B (PTP-1B) activity in hypothalamus and visceral white adipose tissue (vWAT)of transgenic (Tg) or wild-type (WT) mice exposed to sleep fragmentation (SF) or sleep control (SC) for 4 weeks. Significant increases in PTP-1B activity emerged in SF conditions, particularly in WT mice (SF-WT: $p < .001$ vs. all three other groups; $n = 6–8$/group) but were also present in the hypothalamus (not in vWAT) of Tg mice albeit to a significantly lesser degree (SF-WT vs. SF-Tg: $p < .001$; SC-Tg vs. SF-Tg: $p < .05$).

implicate PTP-1B as a major driver of not only the altered energy homeostasis that ultimately results in obesity in the context of fragmented sleep but also as a major contributor to metabolic dysfunction and vWAT inflammatory processes.

Before we address the potential implications of our findings, some methodological limitations are worthy of mention. First, we constrained the transgenic PTP-1B mouse model to ObR-expressing tissues such that the role of PTP-1B in other cellular targets was not explored. This is particularly relevant since organ- or tissue-specific PTP-1B ablation has revealed disparate effects on metabolic function.[18,22,36] Furthermore, we did not assess whether the inflammatory processes elicited by SF underlie the increased expression and activity of PTP-1B.[20] However, to circumvent such issues, we also performed parallel experiments using a systemically administered PTP-1B inhibitor, and such experiments revealed substantial concordance between the results in Tg mice and the findings in mice receiving the inhibitor compound.

The impact of our experiments on vWAT was indeed remarkably similar to that reported in a previous study, whereby diet-induced obesity mice exhibited an upregulation of PTP-1B and tumor necrosis factor-α (TNFα) accompanied by a downregulation of PPARγ2 in vWAT, and administration of TNFα recombinant protein prevented PTP-1B reduction and inhibited adipocyte differentiation in vitro.[37] However, only normal chow diet was employed herein to dampen the diet-associated effects on metabolic function. Furthermore, we have previously shown that SF induces increased expression of TNFα in vWAT, and therefore this process could be conducive to the upregulation of PTP-1B.[9] We have also suggested that the increased low-grade inflammatory processes induced by chronic SF may be instigated by alterations in gut microbiome and intestinal permeability that in turn foster systemic inflammatory changes as well as migration, activation, and polarization of vWAT ATM.[11,38,39] Considering that the peripheral actions of PTP-1B in the context of obesity and metabolic dysfunction are still insufficiently resolved,[14] it will be important to further examine the sequence of events leading to up-regulation of PTP-1B in vWAT adipocytes and their role in insulin sensitivity. Furthermore, since PTP-1B plays an important role in macrophage polarization,[40–42] the cascade of events regulating PTP-1B expression and activity in ATM will require future research, particularly as it relates to changes in ObR sensitivity in ATM and the interactions between ObR and PTP-1B in these inflammatory cells.[43–45]

Several studies in both humans and animal models have shown that both shortened sleep duration and disrupted sleep are associated with alterations in food intake and promote increased obesogenic tendencies.[7,8,46–49] In a recent study by Ho et al, 9 days of SF for 18 hours/day was also associated with altered glucose homeostasis provided that high-fat diet was added to the sleep disruption.[50] The present study focused on chronic sleep fragmentation, a more prevalent sleep-related condition that is pervasively encountered in several common disorders (e.g., sleep apnea, depression, and asthma), all of which have been associated with obesity and metabolic dysfunction. We previously showed that SF induces early changes in the unfolded protein response that ultimately affect central ObR-mediated hypothalamic function, eventually promoting weight gain and metabolic dysfunction.[13] However, since both Tg and systemic inhibitor treatment as employed in the current study do not resolve potentially different central and peripheral roles of PTP-1B in the metabolic phenotype induced by SF, more specifically targeted approaches will be required in future studies.

The hypothalamus plays a critical role in appetite regulation and energy metabolism that involve complex feedback signaling loops and coordinated adaptive responses to maintain energy balance, which involve ObR signaling.[51] Current results recapitulate our findings that mice exposed to SF develop increased food intake and leptin resistance (high leptin levels and reduced STAT3 phosphorylation after leptin injection) and that such SF-induced effects were abolished by treatment with a PTP-1B inhibitor or by targeted disruption of PTP-1B in ObR-expressing cells. However, significant increases (albeit of smaller magnitude) in hypothalamic PTP-1B activity emerged in Tg mice exposed to chronic SF in the absence of parallel increases in food intake or reductions in hypothalamic leptin receptor sensitivity. These findings suggest that the physiological effect of PTP-1B on food intake is indeed strictly dependent on ObR signaling in the hypothalamus.

In summary, the documented increases in PTP-1B expression and activity as elicited by SF exposures further suggest that this signaling pathway operates as a major mechanism promoting excessive food intake, weight gain, and both systemic and vWAT metabolic dysfunction. Present findings further highlight the role of preserved and intact sleep architecture in the regulation of energy homeostasis and also provide further evidence that PTP-1B inhibitors are capable of acting on hypothalamic leptin-related pathways and could be proposed as potential anti-obesity agents.

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DISCLOSURE STATEMENT

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