Disruption of Protein Arginine N-Methyltransferase 2 Regulates Leptin Signaling and Produces Leanness In Vivo Through Loss of STAT3 Methylation

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Rationale: Arginine methylation by protein N-arginine methyltransferases (PRMTs) is an important posttranslational modification in the regulation of protein signaling. PRMT2 contains a highly conserved catalytic Ado-Met binding domain, but the enzymatic function of PRMT2 with respect to methylation is unknown. The JAK-STAT pathway is proposed to be regulated through direct arginine methylation of STAT transcription factors, and STAT3 signaling is known to be required for leptin regulation of energy balance.

Objective: To identify the potential role of STAT3 arginine methylation by PRMT2 in the regulation of leptin signaling and energy homeostasis.

Methods and Results: We identified that PRMT2−/− mice are hypophagic, lean, and have significantly reduced serum leptin levels. This lean phenotype is accompanied by resistance to food-dependent obesity and an increased sensitivity to exogenous leptin administration. PRMT2 colocalizes with STAT3 in hypothalamic nuclei, where it binds and methylates STAT3 through its Ado-Met binding domain. In vitro studies further clarified that the Ado-Met binding domain of PRMT2 induces STAT3 methylation at the Arg31 residue. Absence of PRMT2 results in decreased methylation and prolonged tyrosine phosphorylation of hypothalamic STAT3, which was associated with increased expression of hypothalamic proopiomelanocortin following leptin stimulation.

Conclusions: These data elucidate a molecular pathway that directly links arginine methylation of STAT3 by PRMT2 to the regulation of leptin signaling, suggesting a potential role for PRMT2 antagonism in the treatment of obesity and obesity-related syndromes. (Circ Res. 2010;107:992-1001.)

Key Words: PRMT2 ■ leptin ■ methylation ■ STAT3

Protein methylation regulates gene expression by post-translational modification of chromatin, structural proteins, and transcription factors. Methylation of arginine residues is catalyzed by protein N-arginine methyltransferase (PRMT) enzymes using S-adenosylmethionine (Ado-Met) binding domain as the methyl donor. Most mammalian PRMTs (PRMT1 to -8) are classified as type I or type II according to the formation of ω-NG-monomethylarginine and either ω-NG,NG-asymmetrical or ω-NG,NεG-symmetrical dimethylarginine residues, respectively.

PRMT2, a type I enzyme, contains a highly conserved catalytic Ado-Met binding domain and unique Src homology 3 domain that binds proteins with proline-rich motifs.1,2 Although not initially described to have methyltransferase activity,2 subsequent research indicated PRMT2 binds estrogen receptor-α and enhances estrogen-related transcription through its Ado-Met domain, indirectly suggesting methyltransferase activity.3 However, despite further studies indicating PRMT2 exerts biological activity,4–6 in vivo methyltransferase activity has not been convincingly demonstrated, and its designation as a type I enzyme has been challenged.7

Concurrently, mounting evidence suggests JAK-STAT signaling is regulated by arginine methylation.8–11 This includes the finding that PRMT1 associates with and methylates STAT1 on Arg31.9 This is of relevance, because Arg31 is conserved in other STAT members, with both STAT3 and STAT6 also able to undergo arginine methylation.10,11 Thus, methylation of STAT3 by PRMT enzymes may regulate its signaling pathways, possibly including the leptin-induced STAT3-dependent pathway.

Leptin, transcribed from the ob gene, is produced mainly in white adipose tissue and regulates energy status by activating

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Ob-Rb, the leptin long receptor isoform.\textsuperscript{12} Binding of leptin to Ob-Rb induces receptor conformational changes and JAK2 activation, with subsequent phosphorylation of tyrosine residues in Ob-Rb.\textsuperscript{13} Tyrosine phosphorylation of Ob-Rb Tyr1138 recruits STAT3 to an Ob-Rb-JAK2 complex, allowing phosphorylation of a Tyr705 residue in STAT3.\textsuperscript{14,15} Ob-Rb is highly expressed in nuclei of basomedial hypothalamus, including arcuate (ARC), dorsomedial hypothalamic, and ventromedial hypothalamic (VMH) nuclei, collectively known as the “satiety center.”\textsuperscript{16–18} Via a STAT3-dependent pathway, leptin binding to Ob-Rb increases proopiomelanocortin (POMC) production in neurons projecting from the ARC nucleus.\textsuperscript{14,15,17–22} In turn, POMC generates an anorectic signal through a melanocytostimulating hormone binding to melanocortin 3 and 4 receptors.\textsuperscript{23–26} Multiple animal models have now verified the importance of STAT3-dependent signaling in regulating feeding, energy expenditure, and body weight. These models have included rodents with mutant Ob-Rb (Tyr1138→Ser)\textsuperscript{23}; loss of POMC;\textsuperscript{23} and the inactivation of STAT3 in central neural tissues.\textsuperscript{27} POMC-expressing neurons,\textsuperscript{22} or Ob-Rb-expressing neurons.\textsuperscript{28}

Given these reports, we hypothesized that STAT3 methylation may regulate leptin signaling. In particular, based on preliminary observations, we speculated that PRMT2 may fulfill this function.\textsuperscript{4,5} We identified that \textit{PRMT2}\textsuperscript{2−/−} mice exhibit reduced body weight, resistance to obesity, and increased leptin sensitivity. PRMT2 colocalized with STAT3 in hypothalamic nuclei, where it bound and methylated STAT3 through its catalytic domain. Absence of PRMT2 resulted in decreased methylation and prolonged tyrosine phosphorylation of STAT3 and increased expression of hypothalamic POMC following leptin stimulation. These findings delineate a novel mechanism of regulation of the leptin–STAT3–melanocortin pathway and confirm the methylation transferase activity of PRMT2 in vivo.

**Methods**

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

**Generation of \textit{PRMT2}\textsuperscript{2+/-} and \textit{PRMT2}\textsuperscript{2−/−} Mice**

As previously described, \textit{PRMT2}\textsuperscript{2−/−} mice were generated by deleting a 3 helix segment involved in the Ado-Met domain.\textsuperscript{4,5} All Animal care and experimental protocols were reviewed and approved by Animal Care Use Committee of the National Heart, Lung, and Blood Institute in accordance with NIH guidelines.

**Statistical Analyses**

Data are presented as means±SEM. Comparisons between experimental groups were performed using unpaired Student’s \textit{t} test or ANOVA when appropriate. Differences were considered significant if \( P < 0.05 \). At a minimum, all experimental groups were of at least \( n = 3 \).

**Results**

**\textit{PRMT2}\textsuperscript{2−/−} Mice Are Lean and Exhibit Reduced Food Intake**

We observed that \textit{PRMT2}\textsuperscript{2−/−} mice were born at the expected Mendelian frequency, lived for >1.5 years without gross abnormalities, and were fertile. However, we noted that \textit{PRMT2}\textsuperscript{2−/−} male mice gained less weight than age-matched control wild-type mice when weaned onto a chow diet. This weight difference was apparent at 6 weeks and was sustained throughout the period of chow diet administration until 30 weeks of age (Figure 1a and Online Table I). The weight of \textit{PRMT2}\textsuperscript{2+/-} males was intermediate between that of wild-type and \textit{PRMT2}\textsuperscript{2−/−} mice (Online Table I), suggesting that PRMT2 exerts a dose-responsive effect over body weight. Although there were no differences between the weights of female wild-type and \textit{PRMT2}\textsuperscript{2−/−} mice at 12 weeks, by 30 weeks of age, \textit{PRMT2}\textsuperscript{2−/−} females were also lean compared with wild-type females (Online Table I). Measurement of snout–anus length at 12 weeks of age revealed that male and female \textit{PRMT2}\textsuperscript{2−/−} mice were 3% to 4% shorter than wild-type controls (Online Table I), indicating that PRMT2 may also contribute to linear growth regulation.

Leanness in \textit{PRMT2}\textsuperscript{2−/−} mice could result from decreased food intake, increased energy expenditure, and/or malabsorption. To address whether decreased food consumption was responsible for reduced weight gain, the food intake of wild-type and \textit{PRMT2}\textsuperscript{2−/−} mice was monitored for 14 days. Average food intake was significantly decreased in both male and female \textit{PRMT2}\textsuperscript{2−/−} mice compared with age-matched wild-type mice (Online Table I), indicating that reduced food intake by \textit{PRMT2}\textsuperscript{2−/−} mice may play a role in their diminished weight gain. The stools of \textit{PRMT2}\textsuperscript{2−/−} mice did not show any gross changes compared with those from wild-type mice (data not shown), suggesting that malabsorption, in particular lipid malabsorption, was not contributory.

**Reduced Liver Glycogen Content in \textit{PRMT2}\textsuperscript{2−/−} Mice**

Blood pressure recording and complete necropsies were performed to assess for organ abnormalities. We identified that hepatic cytoplasmic vacuoles were less numerous in \textit{PRMT2}\textsuperscript{2−/−} mice and that hepatic cords and sinusoids were relatively more distinct than those of wild-type mice (Figure 1b, top images), suggesting reduced liver glycogen content in \textit{PRMT2}\textsuperscript{2−/−} mice. Indeed, on further analysis, hepatic glycogen content was markedly decreased in \textit{PRMT2}\textsuperscript{2−/−} mice.
No gross or microscopic abnormalities were observed in other PRMT2−/− organs or tissues, including heart, thymus, spleen, pancreas, kidney, skeletal muscle, and brown adipose tissue (data not shown). Normalized heart weight and blood pressure did not differ between wild-type and PRMT2−/− animals (Online Table I).

**Altered Metabolic Homeostasis in PRMT2−/− Mice**

To further explore the possible metabolic derangements caused by PRMT2 deficiency, blood glucose and serum insulin levels were assessed in 8- to 12-week-old mice. Blood glucose levels in fasting and fed PRMT2−/− male mice were significantly lower than wild-type male mice, and fasting glucose levels were also significantly reduced in female PRMT2−/− mice compared with wild-type females (Online Table I). In addition, serum insulin and triglyceride concentrations were reduced in male and female PRMT2−/− mice compared with wild-type mice (insulin: *P*=0.07 and *P*<0.05, respectively; triglycerides: *P*=0.06 and *P*<0.05, respectively) (Online Table I).

**Figure 1.** PRMT2−/− mice are lean, have reduced hepatic glycogen stores, and are resistant to diet-induced obesity. a, Growth curves of age-matched male wild-type and PRMT2−/− mice fed a standard chow diet for 30 weeks after weaning (n=12 in each group). Selected statistical comparisons are provided in Online Table I. b, Hematoxylin/eosin (H & E) (top) and periodic acid–Schiff (PAS) (bottom) staining of liver sections from 8-week-old wild-type and PRMT2−/− male mice. Original magnification, ×40. c, Weight gain of age-matched male wild-type (n=7), PRMT2−/− (n=5), and PRMT2−/− (n=8) mice fed a high-fat diet for 10 weeks. *P<0.001, PRMT2−/− vs wild-type. d, Liver, brown adipose tissue (BAT), and fat pad mass (epididymal, inguinal, axillary, interscapular) of age-matched male wild-type (n=7), PRMT2−/− (n=5), and PRMT2−/− (n=8) mice fed a high-fat diet. *P<0.001, PRMT2−/− and PRMT2−/− vs wild-type.

**Figure 2.** PRMT2−/− mice exhibit enhanced sensitivity to exogenous leptin. Mice at 12 to 16 weeks of age (n=8 males in both groups) were injected IP with PBS for 4 days and then for 6 days with recombinant mouse leptin (0.1 μg/g twice daily). a, Change in body weight in response to leptin in wild-type and PRMT2−/− mice. *P<0.05, **P<0.005, PRMT2−/− vs wild-type. b, Change in total 6 day food intake (%) for wild-type vs PRMT2−/− mice during leptin treatment. *P<0.05.
We also investigated potential changes in proinflammatory cytokine production with high-fat feeding. Wild-type and PRMT2−/− mice were fed a high-fat or normal chow diet for 10 weeks, and the following cytokines were measured: interleukin (IL)-1α, IL-2, IL-4, IL-5, IL-6, IL-17, tumor necrosis factor-α, interferon-γ (Online Table III). We identified that following high-fat feeding, IL-2 was increased in PRMT2−/− versus wild-type mice, whereas IL-17 levels in PRMT2−/− mice were decreased with high-fat feeding as compared with normal chow diet. However, the magnitude of these changes were small, and the majority of the examined cytokines were unchanged (Online Table III). Taken together, these results demonstrate that the absence of PRMT2 results in minimal alterations to inflammatory regulation but significant changes in glucose, insulin, and lipid metabolism.

Resistance to Food-Dependent Obesity in PRMT2−/− Mice

High-fat feeding induces weight gain and obesity, which is associated with increased visceral fat mass, glucose intolerance, and insulin resistance in nonobese rodents.29 To determine whether PRMT2−/− mice are resistant to diet-induced obesity, wild-type and PRMT2−/− mice were fed a high-fat diet for 10 weeks. PRMT2−/− mice gained significantly less weight than heterozygote or wild-type mice (Figure 1c). This relative leanness was associated with reduced fat mass, with epididymal, inguinal, and axillary fat pads weighing significantly less in PRMT2−/− than wild-type mice (Figure 1d). No differences were observed in liver, interscapular fat pad, or brown adipose tissue mass. These data indicate that PRMT2−/− mice are relatively protected against food-dependent obesity and increased adiposity induced by high-fat feeding.

Regulation of Leptin Signaling by PRMT2 In Vivo

The leanness and reduced adiposity of PRMT2−/− mice suggested that PRMT2 may modulate leptin signaling. Therefore, we evaluated leptin regulation in PRMT2−/− mice and identified that serum leptin concentrations in male and female PRMT2−/− mice were significantly decreased compared with wild-type mice (Online Table I). Next, to directly assess leptin sensitivity, we measured the responses of wild-type and PRMT2−/− mice to peripheral leptin injection can vary by rodent body weight,30 we studied wild-type and PRMT2−/− mice are more sensitive to exogenous leptin administration.

PRMT2 and STAT3 Colocalize in the Hypothalamus

It is known that anorexigenic POMC neurons in the ARC nucleus express both Ob-Rb and STAT3 and that the paraventricular hypothalamic (PVH) nucleus is also a prominent site of Ob-Rb and STAT3 expression that detects and integrates anorexigenic signals arising from the ARC nucleus.14,15,17–22,24 Therefore, given our finding that leptin sensitivity is increased in PRMT2−/− mice, we examined whether PRMT2 localizes to the hypothalamus and whether it might interact with STAT3 at these sites. Using in situ hybridization, we observed abundant expression of PRMT2 mRNA in the anterior and medial hypothalamicus of wild-type mice, including the ARC, PVH, VMH, and supraoptic hypothalamic nuclei, whereas PRMT2 mRNA was not detected in PRMT2−/− mice (Figure 3a). In a similar anatomic distribution to PRMT2, STAT3 mRNA was also detected in the ARC, PVH, and VMH nuclei (Figure 3b). Quantification of these results revealed a modest reduction in STAT3 mRNA levels in the PVH nucleus for PRMT2−/− compared with WT mice, with similar STAT3 mRNA levels in the ARC nucleus for WT and PRMT2−/− animals (Figure 3b and Online Figure I).

To confirm this possible association between PRMT2 and STAT3 in vitro, coprecipitation experiments were performed. We transfected 293 cells with Flag-tagged full-length PRMT2 cDNA and/or STAT3 cDNA, immunoprecipitated with anti-Flag antibody, and immunoblotted with anti-STAT3 antibody. As suggested by our in vivo in situ hybridization data, endogenous STAT3 was found to coprecipitate with transfected PRMT2 (Figure 3c, lane 4), with the interaction between PRMT2 and STAT3 increasing when both substrates were cotransfected (Figure 3c, lane 5). These data, particularly when taken in conjunction with our prior finding of enhanced leptin sensitivity in PRMT2−/− mice and the expression pattern of PRMT2 RNA, suggest that PRMT2 may influence appetite and body weight via the hypothalamic leptin–STAT3 signaling pathway.

The Ado-Met Binding Domain of PRMT2 Methylates STAT3 at Arg31

The Arg31 STAT residue is known to undergo methylation in STAT1 and is conserved across other STAT family members.9–11 To investigate the possibility that STAT3 may also undergo methylation at Arg31, we first conducted methylation assays, transfecting wild-type or mutated PRMT2 cDNAs lacking a functional Ado-Met domain into 293 cells or mouse embryonic fibroblasts (MEF) and using a glutathione S-transferase–STAT3 (GST-STAT3) protein as substrate and Flag fusion proteins as an enzyme source. We identified that in 293 cells, wild-type PRMT2 methylated GST-STAT3 (Figure 4a, lane 3), whereas mutant PRMT2 or vector alone did not (Figure 4a, lanes 4 and 5). Cell extracts from wild-type MEFs also methylated GST-STAT3, whereas methylation of STAT3 was abolished in PRMT2−/− MEFs (Figure 4b, lane 2 versus 4). Using wild-type MEFs transfected with an Arg31→Ala GST-STAT3 mutant protein, we observed that methylation was abolished (Figure 4b, lane 3), suggesting that the Arg31 residue of STAT3 is a likely target for PRMT2 methylation.
To further confirm that STAT3 is arginine-methylated by PRMT2 via its Ado-Met domain, we performed transient transfection into 293 cells using wild-type and mutated PRMT2 cDNA lacking a functional Ado-Met domain, followed by immunoprecipitation (IP) with anti-STAT3 antibody and then immunoblotting with an antibody that recognizes free and bound $N^\epsilon$, $N^\epsilon$-dimethyl or -monomethyl arginine. Endogenous methylated STAT3 was observed, at the extreme limits of detection, in cells transfected with either wild-type PRMT2 (Figure 4c, lane 2, top) or STAT3 (Figure 4c, lane 5, top). However, methylated STAT3 was markedly increased when STAT3 was cotransfected with PRMT2 (Figure 4c, lane 3, top). Importantly, methylated STAT3 was not observed following transfection with a catalytic defective PRMT2 mutant (Figure 4c, lane 4, top). As a whole, these 3 separate experiments provide strong evidence to suggest that the Ado-Met binding domain of PRMT2 induces STAT3 methylation at Arg31.

**PRMT2$^{+/+}$ Mice Exhibit Reduced STAT3 Methylation**

To confirm that PRMT2 is implicated in the methylation of STAT3 in vivo, the STAT3 methylation status of wild-type and PRMT2$^{+/+}$ mice was examined in the hypothalamus and other organs with and without leptin stimulation. These experiments identified a significant reduction in baseline STAT3 methylation in the hypothalamus of PRMT2$^{+/+}$ versus wild-type mice (Figure 5a, first row: lane 2 versus lane 6; Figure 5b). After leptin stimulation, hypothalamic STAT3 methylation was decreased in wild-type animals but remained unchanged in PRMT2$^{+/+}$ mice (Figure 5a, first row: lane 4 versus lane 2 and lane 8 versus lane 6; Figure 5b).

We also investigated the methylation status of STAT3 in other tissues that express PRMT2, including skeletal muscle, heart, and liver. Baseline STAT3 methylation was decreased in skeletal muscle in PRMT2$^{+/+}$ versus wild-type mice. Following leptin stimulation, in a similar fashion to the hypothalamus, STAT3 methylation levels were reduced in the skeletal muscle of wild-type animals but were unchanged in PRMT2$^{+/+}$ mice (Figure 5a, second row; Figure 5b). The levels of methylated heart and liver STAT3 were not influenced by PRMT2 deletion or leptin stimulation (Figure 5a, third and forth rows; Figure 5b). Collectively, these data confirm that the absence of PRMT2 induces a decrease in STAT3 methylation in vivo, including in the hypothalamus and skeletal muscle, but not in the heart or liver.

**Prolonged STAT3 Phosphorylation in PRMT2$^{-/-}$ Cells and Tissues**

Arginine methylation of STAT1 plays a critical role in its tyrosine dephosphorylation, with inhibition of STAT1 methylation resulting in prolonged STAT1 tyrosine phosphorylation. Therefore, we examined whether absence of PRMT2 would modulate STAT3 tyrosine phosphorylation. We observed tyrosine phosphorylation of STAT3 in nuclear extracts from wild-type and PRMT2$^{-/-}$ vascular smooth muscle cells (VSMCs) following 10 minutes of stimulation with mouse leptin (Figure 6a), with significantly greater tyrosine phos-
Phosphorylation of STAT3 in peripheral tissues. Phosphorylated STAT3 was not detected in sections of the heart and skeletal muscle in wild-type mice (Figure 6d, right), suggesting that PRMT2 regulates hypothalamic STAT3 phosphorylation in vivo.

To further explore the central effects of leptin stimulation on STAT3 phosphorylation in vivo, cortical sections of wild-type and PRMT2−/− mice were analyzed by immunohistochemistry. In wild-type mice, tyrosine phosphorylation of hypothalamic STAT3 (pY705) peaked at 45 minutes after leptin stimulation, tyrosine phosphorylation of STAT3 (Figure 6c).

It has been reported that the single amino acid substitution of Arg31 for Ala in STAT1 modulates its ability to undergo tyrosine dephosphorylation. To determine the possible involvement of tyrosine phosphatase in the enhanced tyrosine phosphorylation of PRMT2−/− cells, we examined the effect of the phosphatase inhibitor o-vanadate on leptin-stimulated STAT3 tyrosine phosphorylation. These experiments indicated that in nuclear extracts from wild-type VSMCs, pre-treatment with o-vanadate enhanced leptin-stimulated tyrosine phosphorylation of STAT3 (Figure 6e, lanes 1 and 2). In contrast, nuclear extracts from untreated PRMT2−/− VSMCs showed increased STAT3 tyrosine phosphorylation compared with untreated wild-type cells, whereas PRMT2−/− VSMCs did not exhibit any further enhancement of STAT3 tyrosine phosphorylation following o-vanadate treatment (Figure 6e, lanes 3 and 4). Taken together, these data suggest that PRMT2 deletion results in augmented hypothalamic STAT3 tyrosine phosphorylation after leptin stimulation and that augmented tyrosine phosphorylation in PRMT2−/− cells is likely attributable to altered tyrosine phosphatase activity.

Hypothalamic POMC Expression in PRMT2−/− Mice Is Increased in Response to Leptin Stimulation

To determine whether PRMT2 deletion affects hypothalamic levels of anorexigenic POMC and orexigenic neuropeptide Y (NPY) following leptin treatment, mRNA expression was assessed by quantitative real-time PCR. Consistent with our finding that PRMT2−/− mice are more sensitive to exogenous leptin, administration of leptin at a higher dose than given...
Figure 5. STAT3 methylation is reduced in PRMT2−/− tissues in vivo. a, Methylated STAT3 levels were assessed in the hypothalamus, skeletal muscle, heart, and liver of wild-type and PRMT2−/− male mice 90 minutes after peripheral leptin treatment (1.67 μg/g body weight). Total tissue lysates (3 mice per experimental group) were immunoprecipitated with α-metR antibody or with preimmune mouse IgG, followed by immunoblotting with anti-STAT3. Sk muscle indicates skeletal muscle. Experiments were performed in triplicate (36 mice in total). b, Quantification of methylated STAT3 levels in hypothalamic, skeletal muscle, heart, and liver extracts. The relative intensity of methylated STAT3 (as shown in a) was determined by densitometry and normalized to the results from untreated wild-type mice. Data represent the means±SEM of 3 mice per group.

Discussion

Despite abundant evidence that other PRMT enzymes exhibit methyltransferase activity, proof that PRMT2 can function in this fashion in vivo has been elusive. Although in vitro PRMT2 methyltransferase activity has been shown,34 in vivo evidence is limited,7 and we believe our study is the first to fully elucidate and characterize PRMT2 methyltransferase activity in vivo.

This work arose from our observation that PRMT2−/− mice are leaner than wild-type animals. This was associated with decreased food intake, perturbed energy metabolism, and enhanced leptin sensitivity. These data led us to pursue the possibility that PRMT2 modulates leptin signaling. We identified that PRMT2 localizes with STAT3 within the hypothalamus including the ARC, VMH, and PVH nuclei, which are recognized as critical sites for leptin functioning. Absence of PRMT2 was associated with reduced methylation and prolonged tyrosine phosphorylation of hypothalamic STAT3, likely contributing to enhanced leptin-induced POMC function. As part of the leptin signaling machinery, we found that PRMT2 binds STAT3, methylating its Arg31 residue via the PRMT2 catalytic domain. Importantly, despite abundant evidence that other PRMT enzymes exhibit methyltransferase activity in vivo, this work was the first to fully elucidate and characterize PRMT2 methyltransferase activity in vivo.

Previously (0.5 μg/g body weight, twice daily for 3 days) induced weight loss in PRMT2−/− (−0.03±0.14 g versus −1.0±0.2 g; vehicle versus leptin-treatment, P<0.005) but not wild-type mice (−0.12±0.16 g versus −0.59±0.29 g; vehicle versus leptin treatment, P=NS). There was no difference between the baseline expression levels of hypothalamic POMC mRNA in untreated wild-type versus PRMT2−/− mice. Expression of hypothalamic POMC mRNA in wild-type mice treated with leptin was 1.5 times greater than in nontreated wild-type mice (Figure 7a). However, leptin-treated PRMT2−/− mice exhibited significantly greater expression of hypothalamic POMC mRNA compared with leptin-treated wild-type mice (Figure 7a). In contrast and suggesting PRMT2-independence, baseline levels of hypothalamic NPY mRNA expression were slightly higher in PRMT2−/− than wild-type mice. Nevertheless, following leptin treatment, hypothalamic NPY expression was significantly reduced, to a similar extent, in both PRMT2−/− and wild-type mice (Figure 7b). Taken in the context of the existing literature (see above and the introduction), these data suggest that PRMT2 modulates central melanocortin function by regulating hypothalamic Ob-Rb− and specific STAT3-dependent pathways, including POMC signaling.

Finally, we sought to confirm that deletion of PRMT2 was not associated with compensatory changes in other PRMT isoforms that might have confounded these results. We surveyed the mRNA levels of PRMT isoforms 1 to 8 in the hypothalamus, liver, heart, and skeletal muscle (Online Figure III). As expected, PRMT2 mRNA was not detectable in PRMT2−/− mice. Although isolated changes in the levels of certain isoforms were noted in specific tissues, the degree of change was generally modest and no consistent pattern was observed. Therefore, it appears unlikely that secondary alterations in the levels of these alternate PRMT isoforms are implicated in our results.

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energy metabolism. On the other hand, it remains possible that a more direct PRMT2 effect is operative. For example, STAT3 regulates hypothalamic insulin signaling, and it is possible that STAT3 methylation by PRMT2, or PRMT2-mediated methylation of other proteins, may directly regulate insulin activity and the progression to diabetes.

In addition to STAT3, other known PRMT2 targets include histone H4, estrogen receptor-α, the androgen receptor, the retinoblastoma gene product (RB), and hnRNP (heterogeneous nuclear ribonucleoprotein) E1B-AP5. Hypothetically, in addition to attenuated STAT3 methylation, the effects of PRMT2 deletion on any or several of these pathways may be implicated in the PRMT2 phenotype. However, these additional PRMT2 targets are not generally involved in energy regulation, and we are unaware of any evidence to implicate these factors as being contributory to the antiobesity effect of PRMT2 deletion.

Although the mechanism(s) remains to be clarified, our experiments suggest that PRMT2-mediated methylation regulates STAT3 tyrosine phosphorylation. Potentially related to this, an important modulator of STAT3 signaling, protein tyrosine phosphatase (PTP)1B, is known to be a negative regulator of leptin signaling. Interestingly, mice lacking PTP1B display similar characteristics to PRMT2 mice, including reduced adiposity and obesity resistance. However, PTP1B is localized in the cytoplasmic fraction and it is known that leptin-activated JAK2, but not STAT3, is a substrate for PTP1B. Alternatively, it is known that T-cell (TC)-PTP, another member of PTP family, is ubiquitously

Figure 6. PRMT2 modulates leptin-induced STAT3 tyrosine phosphorylation. a, Quiescent wild-type and PRMT2−/− VSMCs were treated with or without mouse leptin (100 nmol/L) for the indicated times. Nuclear extracts were immunoblotted using antibodies against phospho-STAT3 (pSTAT3 [pY705]) (top), STAT3 (middle), and β-actin (bottom). Total cell extracts from HeLa cells prepared with or without interferon-α (IFNα) treatment are shown as positive and negative controls, respectively. b, The pSTAT3 (pY705) and STAT3 signals from blots obtained following 30 minutes leptin treatment were quantified by densitometry, and amounts of pSTAT3 in wild-type and PRMT2−/− cells were normalized to the amount of STAT3 in each sample. Results are expressed as means±SEM of 3 independent experiments. *P<0.05 for PRMT2−/− vs wild-type. c, Tyrosine phosphorylated STAT3 was imaged by immunostaining in quiescent wild-type and PRMT2−/− VSMCs untreated or treated with mouse leptin (100 nmol/L) for 10 or 30 minutes, using anti-pSTAT3 (pY705) antibody. Original magnification, ×40. d, Tyrosine-phosphorylated STAT3 activity in hypothalamic sections from wild-type and PRMT2−/− male mice 90 minutes after peripheral leptin treatment (1.67 μg/g body weight) was imaged by immunohistochemistry using anti-pSTAT3 (pY705) antibody. 3V indicates third ventricle. Original magnification, ×20. e, Quiescent wild-type and PRMT2−/− VSMCs were pretreated with or without α-vanadate (200 μmol/L) for 15 minutes and then stimulated with recombinant mouse leptin (30 minutes, 100 nmol/L). Nuclear extracts were immunoblotted using anti-pSTAT3 (pY705) (top) and anti-STAT3 antibodies (bottom). The results of a representative experiment were quantitated by densitometry and are presented below, with each column representing the corresponding lane. Data are from a representative experiment that was repeated in triplicate with similar results.

Figure 7. Hypothalamic POMC and NPY mRNA levels in wild-type and PRMT2−/− mice. Wild-type and PRMT2−/− male mice were injected with mouse leptin or PBS, and hypothalamic POMC (a) and NPY (b) mRNA were quantitated by quantitative real-time PCR. Data are expressed as percentages of the PBS control and represent the means±SEM of 5 mice per group. *P<0.05, **P<0.01, ***P<0.005.
expressed in various mammalian tissues including mouse brain. Analysis of TC-PTP-null MEFs revealed impaired dephosphorylation of nuclear STAT1 and STAT3, but not STAT5 or STAT6, suggesting TC-PTP may negatively regulate STAT3-mediated signaling. Furthermore, it is also known that inhibition of STAT1 arginine methylation results in decreased association of STAT1 with TC-PTP and delayed STAT1 tyrosine dephosphorylation. Thus, it appears plausible that PRMT2 deletion may result in a failure of STAT3 methylation at Arg31, decreasing the association of nuclear TC-PTP with STAT3, which in turn retards STAT3 dephosphorylation. An unanticipated but provocative finding of this work was that whereas PRMT2 deletion was associated with decreased STAT3 methylation, we also identified that leptin administration reduces the extent of hypothalamic STAT3 methylation in wild-type but not PRMT2−/− animals. Although this finding falls outside the purview of this study, it serves to underscore the importance of the leptin-PRMT2-STAT3 axis in energy regulation, implicating leptin as playing an upstream role in inhibiting the extent of PRMT2-mediated STAT3 methylation. This hypothesis is consistent with the known anorectic effects of both leptin administration and PRMT2 deletion and is the subject of ongoing work in our laboratories.

Importantly, although almost all forms of human obesity involve leptin resistance, the administration of leptin is a generally inadequate treatment for the majority of obese subjects. Therefore, our findings, along with the benign effects of PRMT2 genetic deletion, support the possibility that PRMT2 antagonism may represent an attractive therapeutic target for obesity management. In particular, the reduced body weight and favorable changes in glucose and lipid parameters observed in PRMT2−/− mice argue well for clinical utility.

In conclusion, our results demonstrate that PRMT2 is involved in the regulation of feeding, obesity, and energy metabolism via a leptin–STAT3–dependent pathway. These data also show, we believe for the first time, that PRMT2 exhibits specific methyltransferase activity. Particularly given the looming global obesity epidemic and present lack of efficacious treatment options, we suggest that PRMT2 antagonism may hold promise as a therapeutic tool for the management of obesity.

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Disclosures

None.

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Iwasaki et al. PRMT2 Regulation of Leptin Signaling

Novelty and Significance

What Is Known?

- Protein methylation is an important step in the posttranslational modification and functioning of mammalian proteins.
- Protein methylation is catalyzed by protein methyltransferase enzymes (PRMT1 to -8), although the ability of PRMT2 to perform this function had not been convincingly shown in vivo.
- STAT family members may undergo methylation, and STAT3 is of importance in hypothalamic feeding and energy regulation.

What New Information Does This Article Contribute?

- We show that PRMT2 methylates STAT3 in vivo.
- Genetic deletion of PRMT2 in mice decreases hypothalamic STAT3 methylation and prolongs STAT3 phosphorylation.
- Altered hypothalamic leptin-STAT3 signaling appears responsible for the phenotype of mice with PRMT2 genetic deletion, which have reduced body weight and food intake, resistance to food-dependent obesity, and increased sensitivity to exogenous leptin administration.

Obesity is a major cause of health-related morbidity and mortality, and new insights and treatment modalities to combat this condition are urgently required. Here, we show that genetic deletion of PRMT2, a member of the protein methyltransferase family of enzymes, is associated with a lean, leptin-hypersensitive, and “antidiabetes-like” phenotype in mice. PRMT2 colocalizes with STAT3 in hypothalamic nuclei, where it binds and methylates STAT3. Absence of PRMT2 is associated with reduced methylation and prolonged phosphorylation of hypothalamic STAT3, likely contributing to enhanced downstream anorexigenic signaling. These findings identify a molecular pathway linking arginine methylation of STAT3 by PRMT2 to the regulation of body weight and energy metabolism, suggesting a potential role for PRMT2 antagonism in the treatment of diabetes and obesity.
Disruption of Protein Arginine N-Methyltransferase 2 Regulates Leptin Signaling and Produces Leanness In Vivo Through Loss of STAT3 Methylation

Hiroaki Iwasaki, Jason C. Kovacic, Michelle Olive, Jeanette K. Beers, Takanobu Yoshimoto, Martin F. Crook, Leonardo H. Tonelli and Elizabeth G. Nabel

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