Knockdown of neuropeptide Y in the dorsomedial hypothalamus reverses high-fat diet-induced obesity and impaired glucose tolerance in rats

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Knockdown of neuropeptide Y in the dorsomedial hypothalamus reverses high-fat diet-induced obesity and impaired glucose tolerance in rats. Am J Physiol Regul Integr Comp Physiol 310: R134–R142, 2016. First published November 11, 2015; doi:10.1152/ajpregu.00174.2015.—Neuropeptide Y (NPY) in the dorsomedial hypothalamus (DMH) plays an important role in the regulation of energy balance. While DMH NPY overexpression causes hyperphagia and obesity in rats, knockdown of NPY in the DMH via adeno-associated virus (AAV)-mediated RNAi (AAVshNPY)ameliorates these alterations. Whether this knockdown has a therapeutic effect on obesity and glycemic disorder has yet to be determined. The present study sought to test this potential using a rat model of high-fat diet (HFD)-induced obesity and insulin resistance, mimicking human obesity with impaired glucose homeostasis. Rats had ad libitum access to rodent regular chow (RC) or HFD. Six weeks later, an oral glucose tolerance test (OGTT) was performed for verifying HFD-induced glucose intolerance. After verification, obese rats received bilateral DMH injections of AAVshNPY or the control vector AAVshCTRL, and OGTT and insulin tolerance test (ITT) were performed at 16 and 18 wk after viral injection (23 and 25 wk on HFD), respectively. Rats were killed at 26 wk on HFD. We found that AAVshCTRL rats on HFD remained hyperphagic, obese, glucose intolerant, and insulin resistant relative to lean control RC-fed rats receiving DMH injection of AAVshCTRL, whereas these alterations were reversed in NPY knockdown rats fed a HFD. NPY knockdown rats exhibited normal food intake, body weight, glucose tolerance, and insulin sensitivity, as seen in lean control rats. Together, these results demonstrate a therapeutic action of DMH NPY knockdown against obesity and impaired glucose homeostasis in rats, providing a potential target for the treatment of obesity and diabetes.

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energy demands, such as food restriction (7) and increased physical activity (20). Using the recombinant vector of adeno-associated virus (AAV)-mediated NPY-specific shRNA (AAVshNPY), we have found that knockdown of NPY in the DMH via bilateral injections of AAVshNPY into the DMH ameliorates hyperphagia, obesity, and glucose intolerance in OLETF rats (49). Overexpression of NPY in the DMH of rats via delivering the recombinant vector of AAV-mediated NPY expression into the DMH mimics obese phenotypes of OLETF rats and exacerbates high-fat diet (HFD)-induced hyperphagia and obesity in rats (49, 52). Furthermore, we have found that DMH NPY affects food intake, body adiposity, thermogenesis, energy expenditure, and physical activity in rats (9). DMH NPY knockdown also prevents HFD-induced hyperphagia, obesity, and glucose intolerance in rats (9). Importantly, NPY-expressing neurons have been identified in the DMH of non-human primates (5). Together, these results demonstrate a critical role for DMH NPY in body weight regulation and also underscore DMH NPY as a potential target for therapies aimed at fighting against obesity and its comorbidities such as Type 2 diabetes.

The present study sought to test whether DMH NPY knockdown can produce therapeutic actions against obesity and/or diabetes using a rat model of HFD-induced obesity and impaired glucose homeostasis, mimicking the most common condition of obesity and its associated diabetes in humans. After rats had ad libitum access to HFD for 6 wk to develop HFD-induced obesity and impaired glucose tolerance, we delivered the vector AAVshNPY into the DMH of HFD-induced obese rats for specific knockdown of NPY in the DMH, and determined the effects of this knockdown on food intake, body weight gain, and fat mass. We also evaluated the effects of DMH NPY knockdown on glucose tolerance and insulin sensitivity in these animals. The results from this study demonstrate that DMH NPY knockdown reverses HFD-induced obesity and impaired glucose tolerance in rats.

METHODS

Animals. Male Sprague-Dawley rats were purchased from Charles River Laboratories and were individually housed on a 12:12-h light-dark cycle (lights on at 0600) in a temperature-controlled colony room (22–24°C) with ad libitum access to tap water and standard laboratory rodent chow, except where noted. All procedures were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University.

High-fat diet-induced obesity and impaired glucose homeostasis. After 1-wk acclimation, rats at 8 wk of age were randomly assigned to one of two groups. One group of rats (n = 6) was maintained on a regular chow diet (RC; 15.8% fat, 65.6% carbohydrate, and 18.6% protein in kcal%; 3.37 kcal/g; PMI Nutrition International, LLC), and the other group of rats (n = 12) were switched to ad libitum access to a HFD (60% fat, 20% carbohydrate, and 20% protein in kcal%; 5.2 kcal/g; Research Diets). Body weight was determined daily, and food intake was measured weekly. Following 6 wk on HFD, an oral glucose tolerance test (OGTT) was conducted for determination of HFD-induced alterations in glucose homeostasis.

Effects of DMH NPY knockdown on HFD-induced obesity and impaired glucose tolerance. AAVshNPY vectors for NPY knockdown and control vectors AAVshCTL containing scrambled shRNA were generated, as described previously (49). After verification of HFD-induced obesity and impaired glucose tolerance, at week 7, rats fed HFD were randomly divided into two groups (n = 6): one group received bilateral injections of AAVshNPY in the DMH and the other group received bilateral DMH injections of AAVshCTL. Both groups of rats remained on HFD. Six rats on RC received bilateral DMH injections of AAVshCTL and remained on RC, DMH viral injection was made as previously described (49). Briefly, 0.5 µl/site (~1 x 10^9 particles/site) of recombinant AAV vectors were injected into the DMH with coordinates: 3.1 mm caudal to bregma, 0.4 mm lateral to midline, and 8.6 mm ventral to skull surface (39) at a rate of 0.1 µl/min for 5 min and the injector remained in place for additional 5 min before removal. Body weight was determined daily, and food intake was measured weekly. Sixteen weeks after viral injection (23 wk on HFD), OGTT was conducted again. Two weeks later (25 wk on HFD), an insulin tolerance test (ITT) was conducted. Nineteen weeks after viral injection (26 wk on HFD), rats were euthanized, brains were saved for subsequent verification of DMH NPY knockdown, and the left side of epidydimal white adipose tissue (EWAT) and inguinal WAT (IWAT), as well as interscapular brown adipose tissue (IBAT), were harvested and weighed.

Glucose tolerance test. Following an overnight fast, rats were given an intraperitoneal injection of insulin (0.5 U/kg). Tail blood was sampled before and 15, 30, 45, 60, and 120 min after giving glucose for measurements of blood glucose and plasma insulin concentrations. Blood glucose levels were determined using a FreeStyle glucometer (TheraSense). Plasma insulin concentrations were determined using a rat insulin radioimmunoassay kit (Linco Research).

Insulin tolerance test. Following a 6-h fast during the light, rats were given an intraperitoneal injection of insulin (0.5 U/kg). Tail blood was sampled before and 15, 30, 45, 60, and 90 min after intraperitoneal insulin, and blood glucose levels were determined using a FreeStyle glucometer (TheraSense).

Verification of DMH NPY knockdown. We have previously documented that injection of AAVshNPY vectors into the DMH produced significant knockdown of Npy mRNA expression in the DMH by a range from 35% to 67% over 20 wk relative to control rats receiving DMH AAVshCTL injection (9, 49). On the basis of these results, we defined a minimal 35% reduction of Npy mRNA expression in the DMH as a criterion for successful knockdown of NPY in the DMH. Levels of Npy mRNA expression in the DMH were determined using real-time RT-PCR, as previously described (49, 52). Briefly, brains at the DMH level were sliced via a cryostat, and individual DMH was punched out. Total RNA was extracted from each punched sample using TRIzol reagent (Life Technologies, Grand Island, NY). Two-step quantitative real-time RT-PCR was performed. β-actin was used as an internal control for quantification of individual mRNA. A list of primer sets included: β-actin, forward 5'-tgctacaagggacagct-3', and reverse primer, 5'-agtgtgctgtcagctg-3'; and Npy, forward primer, 5'-agagatccagccctgga-3', and reverse primer, 5'-aagagacaaaggaatc-3'. The relative quantification of Npy mRNA expression in the DMH was calculated using the equation of 2^-ΔΔCt. One out of six rats receiving DMH AAVshNPY injection was excluded from the study due to insufficient knockdown of NPY in the DMH.

Glycemic effect of DMH NPY knockdown. Two additional groups of rats received bilateral DMH injections of AAVshCTL or AAVshNPY, as described above. All rats had ad libitum access to RC. Body weight was determined daily, and food intake was measured weekly. Four weeks after viral injection, an OGTT was performed in the two groups of AAVshCTL and AAVshNPY rats with body weight matched. Following OGTT, rats were euthanized, and brains were saved for subsequent verification of DMH NPY knockdown, as described above.

Statistical analysis. All values are presented as means ± SE. Data were analyzed by StatSoft Statistica 7 software. Data for body weight and food intake were analyzed using two-way repeated-measures ANOVA. Data for blood glucose, plasma insulin, and fat mass were analyzed using either two-way repeated-measures ANOVA or one-way ANOVA. All ANOVAs were followed by pairwise multiple
Fisher’s LSD comparisons. \( P < 0.05 \) was considered as a statistically significant difference.

RESULTS

High-fat diet induces obesity and impaired glucose tolerance. Prior to AAV-mediated knockdown of NPY in the DMH, rats were given ad libitum access to a HFD for inducing obesity and impaired glucose homeostasis. Initial body weight did not differ significantly between the rats on RC and HFD (205 ± 8 g in rats on RC vs. 203 ± 3 g in rats on HFD). As expected, HFD access resulted in a significant increase in body weight gain (Fig. 1A). Over a 6-wk period, rats fed HFD gained significantly more weight by 2 wk (\( P = 0.03 \)) and had gained 26% more weight by 6 wk (\( P < 0.001 \)) compared with control rats on RC (Fig. 1A). Consistent with HFD-induced hyperphagia, rats fed HFD consumed significantly more calories (Fig. 1B). At week 6 (prior to AAVshNPY injection), the daily caloric intake of rats fed a HFD was 24% higher than that of control rats on RC (\( P = 0.017 \), Fig. 1B).

After 6 wk of HFD access, we assessed whether HFD causes impaired glucose homeostasis in these animals using an OGTT. We found that a gastric glucose load resulted in a greater increase in blood glucose levels in rats fed HFD, and their 2-h glucose levels remained 54% higher compared with control rats on RC (\( P < 0.01 \), Fig. 2A), indicating that rats fed HFD had impaired glucose tolerance. Consistent with this impairment, fasting blood glucose levels and the area under the curve (AUC) of blood glucose response were significantly elevated in rats fed HFD relative to control rats on RC (\( P < 0.01 \) and \( P = 0.018 \), respectively, Fig. 2, A and B). Determination of plasma insulin further revealed that plasma insulin levels were significantly increased in rats fed a HFD both at fasting (by a more than three-fold increase) and after a gastric glucose load (by a more than four-fold increase at 2 h) compared with control rats on RC (\( P = 0.027 \) and \( P < 0.01 \), respectively, Fig. 2C). Overall, the AUC of plasma insulin response in rats fed a HFD was almost double that of rats on RC (\( P < 0.001 \), Fig. 2D). Despite such high levels of insulin, blood glucose was still not normalized in rats fed a HFD (Fig. 2A), indicating that these animals became insulin resistant.

Reversal effects of DMH NPY knockdown on HFD-induced obesity. After verification of HFD-induced obesity and impaired glucose tolerance, rats on HFD received bilateral injections of AAVshCTL into the DMH as the obese control group (indicated as AAVshCTL-HFD) or bilateral DMH injections of AAVshNPY for knockdown of NPY in the DMH (indicated as AAVshNPY-HFD) to examine whether DMH NPY knockdown has a therapeutic effect on HFD-induced obesity and impaired glucose tolerance. Examination of body weight gain revealed that obese control rats on a HFD continued to gain significantly more weight and had gained 32% more weight by 26 wk compared with lean control rats on RC receiving bilateral DMH injections of AAVshCTL (indicated as AAVshCTL-RC, \( P < 0.001 \), Fig. 1A). Although NPY knockdown rats maintained ad libitum access to HFD, they had a significant reduction of body weight gain by week 19 (12 wk after viral injection, \( P = 0.036 \)) and gained 21% less weight compared with obese control rats by 26 wk (19 wk after viral injection, \( P < 0.01 \), Fig. 1A). As a result, over the 19 wk following AAVshNPY injection, the body weight gain between NPY knockdown rats on HFD and lean control rats on RC did not differ significantly (\( P = 0.999 \), Fig. 1A).

Following viral injection, obese control rats on HFD remained hyperphagic, and on average, they consumed 21% more daily calories than lean control rats on RC (the overall mean daily intake: 105.6 ± 5.3 kcal in AAVshCTL-HFD vs. 87.2 ± 4.5 kcal in AAVshCTL-RC, \( P < 0.05 \), Fig. 1B). In contrast, the daily intake of NPY knockdown rats on HFD was...
similar to that of lean control rats on RC (the overall mean daily intake: 90.5 ± 5.4 kcal in AAVshNPY-HFD, \( P = 0.295 \), Fig. 1B), indicating that DMH NPY knockdown reversed HFD-induced hyperphagia.

**Reversal effects of DMH NPY knockdown on HFD-induced glucose intolerance.** We reassessed glucose tolerance in rats after 23 wk on HFD (16 wk after viral injection). Again, we found that obese control rats had a sustained elevation of blood glucose in response to a gastric glucose load (Fig. 3, A and B), even though their plasma insulin levels were increased at an even greater degree compared with lean control rats (Fig. 3, C and D), indicating that these obese rats remained glucose intolerant and insulin resistant. Consistent with these alterations, both fasting blood glucose and plasma insulin levels were significantly elevated in obese control rats by 42% and 66%, respectively, relative to lean control rats (Fig. 3, A and C). Notably, these alterations were ameliorated in NPY knockdown rats, as their blood glucose and plasma insulin levels

![Figure 2](http://ajpregu.physiology.org/)

**Fig. 2. OGTT determination of impaired glucose tolerance in rats fed a HFD for 6 wk.** A: blood glucose levels in response to a gastric glucose load. B: area under the curve (AUC) of blood glucose levels. C: plasma insulin levels in response to a gastric glucose load. D: AUC of plasma insulin levels. RC indicates a group of rats on RC and HFD indicates a group of rats on HFD. Values are expressed as means ± SE; \( n = 6 \) or 12/group. *\( P < 0.05 \) vs. RC group.

![Figure 3](http://ajpregu.physiology.org/)

**Fig. 3. Effect of DMH NPY knockdown on HFD-induced glucose intolerance.** A: blood glucose levels in response to a gastric glucose load. B: AUC of blood glucose levels. C: plasma insulin levels in response to a gastric glucose load. D: AUC of plasma insulin levels. OGTT was conducted at 16 wk after viral injection (23 wk on HFD). Values are expressed as means ± SE; \( n = 5 \) or 6 rats per group. *\( P < 0.05 \) vs. AAVshCTL-RC. #\( P < 0.05 \) vs. AAVshCTL-HFD.
both at fasting and after a gastric glucose load were no longer significantly different from those of lean control rats (Fig. 3, A and D).

We further tested insulin sensitivity in rats after 25 wk on a HFD (18 wk after viral injection) using an ITT. We found that intraperitoneal administration of insulin to lean control rats resulted in a significant decrease in blood glucose levels and caused a 28% reduction at 60 min over a 90-min test (Fig. 4A), but this insulin-induced hypoglycemic effect was impaired in obese control rats, as their blood glucose levels remained high following intraperitoneal insulin (Fig. 4A). Overall, the AUC of blood glucose response in obese control rats was 25% greater than that of lean control rats (P < 0.01, Fig. 4B). These data provide additional support for the view that obese control rats fed HFD were insulin resistant. Moreover, we found that this diet-induced insulin resistance was reversed by DMH NPY knockdown. As shown in Fig. 4A, NPY knockdown rats exhibited a strong response to intraperitoneal insulin, resulting in a 36% decrease in blood glucose levels at 60 min. Determination of the AUC of blood glucose response revealed an overall 37% reduction in NPY knockdown rats compared with obese control rats (P < 0.001, Fig. 4B). Strikingly, NPY knockdown rats were likely even more insulin sensitive than lean control rats, as blood glucose levels and the AUC of blood glucose in response to intraperitoneal insulin were lowered in NPY knockdown rats relative to lean control rats, but the differences did not reach statistical significance (P > 0.05, Fig. 4).

Effects of DMH NPY knockdown on fat mass. At death, we determined the weights of selected fat pads: intraabdominal EWAT, subcutaneous IWAT, and energy-burning IBAT. We found that obese control rats had significant increases in fat weights in EWAT and IWAT depots compared with lean control rats (P < 0.05, Fig. 5A), indicating HFD-induced excessive fat accumulation, whereas such fat accumulation was reduced significantly by DMH NPY knockdown, as the weights of EWAT and IWAT in NPY knockdown rats were significantly lower than those of obese control rats (P < 0.05, Fig. 5A). In fact, NPY knockdown rats had the same amount of EWAT as lean control rats (P = 0.966, Fig. 5A). Although the weights of IWAT in NPY knockdown rats appear more than those of lean control rats, this increase did not reach statistical significance (P = 0.360, Fig. 5A). Analysis of IBAT data did not reveal any significant difference among the three groups (P = 0.142, Fig. 5A).

DMH AAVshNPY injection produces NPY-specific knockdown effect in the DMH. Consistent with our previous reports (9, 49), we verified that DMH injection of AAVshNPY caused a 46% reduction of Npy expression in the DMH of AAVsh-NPY-HFD rats by a range from 38% to 62% reductions compared with AAVshCTL-RC rats (Fig. 5B). Since DMH NPY neurons contain CCK-1 receptors (CCK1R) (8), we further examined expression of Cck1r in the DMH of AAVsh-
NPY rats. Similar to our previous report (49), DMH AAVshNPY injection did not affect levels of Cck1r expression in the DMH (data not shown). Together, these results indicate that DMH AAVshNPY injection produced NPY-specific knockdown effect in the DMH.

In addition, we found that HFD access resulted in a 22% reduction of Npy expression in the DMH of AAVshCTL-HFD rats relative to AAVshCTL-RC rats, but the degree of this reduction was significantly smaller than that produced by AAVshNPY injection in AAVshNPY-HFD rats (Fig. 5B). ARC expression of Npy was decreased equally in AAVshCTRL-HFD and AAVshNPY-HFD rats relative to AAVshCTRL-RC rats (Fig. 5B). Thus, these data suggest that physiologically, the expression of ARC NPY and DMH NPY is downregulated in response to HFD-induced increases in energy intake and/or body weight to maintain energy balance, but such responsiveness is not sufficient to overcome HFD-induced hyperphagia and obesity.

Glycemic effect of DMH NPY knockdown. Since changes in body weight may also contribute to the glycemic effect of DMH NPY knockdown, we conducted an additional experiment specifically to evaluate the glucose and insulin responses to oral glucose administration in NPY knockdown rats with body weight matched to control rats. Four weeks after viral injection, at a time when the body weight of AAVshNPY and AAVshCTRL rats did not differ significantly (351 ± 8 g in AAVshNPY vs. 366 ± 10 g in AAVshCTRL, P = 0.275), we performed OGTT. We found that although blood glucose levels did not differ between AAVshNPY and AAVshCTRL rats in fasting and glucose-challenging conditions, (Fig. 6A), insulin levels were significantly decreased in AAVshNPY rats in both conditions (Fig. 6B), indicating that AAVshNPY rats require less insulin secretion to maintain euglycemia and suggesting that this knockdown increases insulin sensitivity. Thus, these data provide support for the view that DMH NPY knockdown enhances insulin sensitivity and glucose homeostasis independently of changes in body weight.

DISCUSSION

Previous reports have demonstrated an important role for DMH NPY in the control of energy balance (5). Overexpression of NPY in the DMH causes hyperphagia and obesity, whereas knockdown of NPY in the DMH ameliorates the hyperphagia and obesity of OLETF rats and prevents the development of obesity in rats with HFD access (9, 49, 52). In this study, we have extended the previous findings to ascertain the potential action for DMH NPY knockdown in the treatment of obesity and impaired glucose homeostasis using a common rat model of HFD-induced obesity. We reported that rats fed a HFD developed hyperphagia, obesity, glucose intolerance, and insulin resistance. DMH NPY knockdown via injection of AAVshNPY into the DMH reverted these diet-induced alterations; following viral injection, NPY knockdown rats exhibited normal caloric intake, body weight, glucose tolerance, and insulin sensitivity as seen in lean control rats. These results provide compelling support for the approach of DMH NPY knockdown as a therapeutic strategy to combat obesity and impaired glucose homeostasis.

We have previously reported a detailed identification of AAV-mediated NPY-specific shRNAs for the study of the function of hypothalamic NPY in the control of food intake and energy balance using both in vitro and in vivo settings (49). Particularly, we have documented that bilateral injections of AAVshNPY into the ARC of adult Sprague-Dawley rats decrease expression levels of Npy, but not Agrp in the same ARC neurons (49). In support of a role for ARC NPY in the mediation of food deprivation-induced feeding, this NPY knockdown results in decreased feeding response to 24 h of food deprivation (49). We have also used this vector success-

![Fig. 6](http://ajpregu.physiology.org/)

**Fig. 6.** Glycemic effect of DMH NPY knockdown in rats with body weight matched to AAVshCTRL rats. A: blood glucose response to a gastric glucose load. B: plasma insulin response to a gastric glucose load. Values are expressed as means ± SE; n = 5 rats per group. *P < 0.05 vs. AAVshCTRL.
fully to identify a critical role for DMH NPY in maintaining energy homeostasis (9, 49). In the present study, we have further verified the previous result of NPY-specific knockdown effect of AAVshNPY (49); injection of AAVshNPY into the DMH of rats resulted in decreased expression of Npy, but not Cck1r in the same DMH neurons. Together, these results provide clear evidence demonstrating the feasibility of using the vector AAVshNPY as a valuable tool specifically to target NPY.

Elevation of Npy expression in the DMH has been found in several animal models of hyperphagia and obesity (6, 16, 17, 22, 45), implying a potential role for dysregulation of DMH NPY in disordered energy balance. Consistent with this point, we have previously found that DMH NPY overexpression contributes to the hyperphagia and obesity of OLETF rats (6, 41, 49). Specifically, DMH NPY overexpression causes increased meal size, leading to overall increases in food intake and body weight in OLETF rats, whereas DMH NPY knockdown reduces this increased meal size and results in decreased food intake and body weight (49). We now appreciate that DMH NPY is not under the control of leptin, but is affected by CCK and other yet identified molecules to modulate food intake and body weight (5). This is supported by the observations that 1) DMH NPY neurons do not coexpress leptin receptors and DMH Npy expression is not altered by a change of circulating leptin levels in rats (7), 2) DMH NPY neurons contain CCK-1 receptors in rats (8), 3) congenital lack of CCK-1 receptors in OLETF rats results in elevated expression of Npy in the DMH (6) and the resulting elevation contributes to their hyperphagia and obesity (49), and 4) parenchymal microinjection of CCK into the DMH of intact rats inhibits DMH Npy expression and limits food intake (10). Intriguingly, both CCK-1 receptors and NPY are found in the DMH of rats and nonhuman primates (3, 18), but undetectable in the DMH of normal-weight mice (3, 8), suggesting that the action of DMH NPY in the control of energy balance in rats is better to reflect the function of primate DMH NPY than mice. Thus, we have used a rat model in the present study aimed at assessing the potential for DMH NPY knockdown in the treatment of obesity and impaired glucose homeostasis.

The findings here that DMH NPY knockdown reduced HFD-induced increases in food intake and body weight are consistent with the concept that DMH NPY acts as an orexigenic neuromodulator in the regulation of energy homeostasis (5). Notably, we have found that a high-fat diet resulted in a 22% reduction of Npy expression in the DMH (Fig. 5B), which is similar to the previous reports showing that Npy expression is decreased (~25% reduction) in the DMH of HFD-induced obese rats (4), suggesting that this decrease is a response to diet-induced increases in energy intake and body weight rather than a causal action, but this decrease is not sufficient for overcoming diet-induced hyperphagia and obesity. In support of this view, our previous report has demonstrated that DMH NPY knockdown can prevent these diet-induced alterations (9), and the present data further show that this knockdown can also reverse HFD-induced hyperphagia and obesity. Together, these results indicate that limiting DMH NPY signaling can be an effective means for treating hyperphagia and obesity.

We have recently reported that DMH NPY knockdown results in the browning of IWAT and promotes both IBAT and IWAT thermogenic activity, leading to increased energy expenditure (3, 9). Although we did not examine this effect in the present study, NPY knockdown rats on a HFD consumed a similar amount of calories (90.5 kcal/day in average in AAVshNPY-HFD vs. 87.2 kcal/day in AAVshCTL-RC, Fig. 1B), but gained 5% less body weight over 19 wk after viral injection compared with lean control rats (222 g in AAVshNPY-HFD vs. 234 g in AAVshCTL-RC, Fig. 1A). This implies that a thermogenic effect of DMH NPY knockdown may contribute to its action against diet-induced obesity in addition to the dietary effect. Recently, active BAT has been found in adult humans, particularly exposed to a cold environment (13, 35, 40, 46, 47), and adult humans have lowered BAT activity when they are overweight or obese (13, 40, 46, 47), implicating BAT in thermogenesis and energy balance regulation in adult humans. Thus, follow-up studies will be of extreme interest to assess a specific role for fat browning and BAT thermogenesis produced by DMH NPY knockdown in curbing obesity in this rat model.

Type 2 diabetes and/or insulin resistance syndrome are serious comorbidities associated with obesity. Previous studies have demonstrated that DMH NPY knockdown ameliorates hyperglycemia, hyperinsulinemia, and impaired glucose tolerance in diabetic OLETF rats (49), and this knockdown also increases insulin sensitivity and improves glucose homeostasis in both normal weight and diet-induced obese rats (9), but in these studies, AAVshNPY injection was made in preobese OLETF rats (49) or intact rats prior to access to HFD (9) and whether DMH NPY knockdown could produce a therapeutic effect on impaired glucose tolerance and insulin resistance had not been determined. To this end, we injected AAVshNPY into the DMH of rats with HFD-induced obesity and impaired glucose tolerance in the present study. We found that DMH NPY knockdown reverses HFD-induced glucose intolerance (Fig. 3) and insulin insensitivity (Fig. 4). Intriguingly, we also noticed a trend for increased insulin sensitivity in NPY knockdown rats on HFD relative to lean control rats on RC (Fig. 4), even though the body weight of NPY knockdown rats was slightly (but not significantly) higher than lean control rats, suggesting that this glycemic effect appears independent of changes in body weight. To further determine this effect, we examined glucose and insulin responses to oral glucose administration in an additional cohort of NPY knockdown rats that were maintained on RC, and their body weight was matched to AAVshCTL rats on RC. We found that AAVshNPY rats require less insulin secretion to maintain euglycemia, as their insulin levels were significantly decreased at both fasting and glucose-challenging conditions, while their blood glucose levels were comparable to control rats (Fig. 6). Together, these results demonstrate that DMH NPY knockdown can enhance insulin sensitivity and glucose homeostasis independently of changes in body weight. Since BAT acts as a major sink for glucose disposal (1, 34), it is possible that DMH NPY knockdown-induced elevation of BAT activity contributes to this glycemic effect. In addition, we have found that DMH NPY neurons modulate brain stem vagal activity (49), which has been shown to affect hepatic metabolic function, implying that the glycemic effect of DMH NPY knockdown may be derived from its modulation of hepatic glucose production. Nevertheless, the detailed mechanism underlying the glycemic effect of DMH NPY knockdown merits further investigation.
Perspectives and Significance

Previous reports have indicated an important role for the DMH in maintaining energy homeostasis. DMH lesions result in hypophagia and decreased body weight (2), and activation of neurons in the DMH provokes nonshivering thermogenesis and elevates core body temperature (50). Despite these observations, the neural mechanisms through which the DMH act to affect food intake and energy expenditure remain less clear. Recently, we have established critical roles for NPY in the DMH in the controls of energy balance. DMH NPY affects both food intake and thermogenesis/energy expenditure, and DMH NPY overexpression can cause hyperphagia and obesity (9, 49). Having demonstrated that DMH NPY knockdown can prevent the development of obesity and glucose intolerance in rats on a HFD (9), here, we have further investigated the approach of AAVshNPY-mediated knockdown of NPY in the DMH as a therapy for obesity and impaired glucose homeostasis using a rat model. We demonstrate that DMH NPY knockdown reverses HFD-induced hyperphagia, obesity, glucose intolerance, and insulin resistance in rats. Given that the pattern of Npy expression in the DMH is similar between rats and primates, our results not only provide further support for the importance of DMH NPY in the regulation of body weight and glucose homeostasis, but also provide a potential target for the treatment of obesity and diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: Y.J.K. performed experiments; Y.J.K. and S.B. analyzed data; Y.J.K. and S.B. prepared figures; Y.J.K. and S.B. drafted manuscript; Y.J.K. and S.B. edited and revised manuscript.

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