Steroidogenic factor 1 directs programs regulating diet-induced thermogenesis and leptin action in the ventral medial hypothalamic nucleus

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The transcription factor steroidogenic factor 1 (SF-1) is exclusively expressed in the brain in the ventral medial hypothalamic nucleus (VMH) and is required for the development of this nucleus. However, the physiological importance of transcriptional programs regulated by SF-1 in the VMH is not well defined. To delineate the functional significance of SF-1 itself in the brain, we generated pre- and postnatal VMH-specific SF-1 KO mice. Both models of VMH-specific SF-1 KO were susceptible to high fat diet-induced obesity and displayed impaired thermogenesis after acute exposure to high fat diet. Furthermore, VMH-specific SF-1 KO mice showed significantly decreased LepR expression specifically in the VMH, leading to leptin resistance. Collectively, these results indicate that SF-1 directs transcriptional programs in the hypothalamus relevant to coordinated control of energy homeostasis, especially after excess caloric intake.

The nuclear receptor steroidogenic factor 1 (SF-1; officially named NR5A1) is a transcription factor whose expression is required for the development of the ventral medial hypothalamic nucleus (VMH) (1). The VMH is a nucleus long associated with the regulation of food intake and body weight (2). Global SF-1 knockout mice (SF-$1^{-/-}$) are not viable but can be rescued by corticosteroid injections and syngeneic adrenal transplants from WT littermates. These mice displayed robust weight gain, suggesting that SF-1 deficiency results in obesity (3). However, inherent secondary effects from the transplantation and steroid injections could contribute to the obese phenotype of the global SF-1 KO mice. Further, the developmental impairment of VMH structure evoked by germ-line deletion of SF-1 could not differentiate whether the obesity seen in SF-1 $KO^{-/-}$ mice is due to direct effect of SF-1 deletion or secondary to the malformation of the VMH (3, 4). Thus, the role of SF-1 regulated gene programs in regulating energy balance has remained obscure.

In humans, mutations of the *SF-1* gene often result in mild to severe obesity. However, the studies describing these patients mainly focused on adrenal or gonadal deficits (5–7). Notably, patients harboring a mutation in Gly-146 of the *SF-1* gene exhibited obesity affecting insulin sensitivity and type II diabetes, implying that SF-1 function is necessary for normal body weight regulation and insulin sensitivity in humans (8). However, there is no direct evidence of the effects of SF-1 on body weight regulation in humans.

Recently, several studies have suggested that neurons expressing SF-1 in the VMH play critical roles in regulating energy balance and glucose homeostasis. For example, leptin receptor and PI3K (p110α) expression in SF-1 neurons of the VMH is required for normal body weight homeostasis (9–11). Additionally, blocking glutamate release from SF-1 neurons was shown to play important roles in regulating glucose homeostasis (12). Furthermore, mice lacking suppressor of cytokine signaling 3 (Socs3) in SF-1 neurons showed enhanced sensitivity to exogenous leptin without body weight change in both normal and high fat chow-fed mice (13). Thus, these findings suggest that SF-1 neurons in the VMH play important roles in energy homeostasis. However, the importance of the transcription factor SF-1 itself and gene programs regulated by SF-1 in the regulation of metabolic homeostasis remain to be established. In this study, we inactivated SF-1 specifically in the VMH to determine the roles of SF-1 in the hypothalamus.

Results

Prenatal SF-1 Deletion in the VMH Leads to Late Onset Obesity. Given that the dysfunction of the VMH is associated with obesity and neurons expressing SF-1 play important roles in regulation of energy homeostasis in the VMH (2, 10), we examined whether SF-1 is a crucial transcription factor for metabolic regulation in the VMH. To directly address this question, we generated prenatal VMH-specific SF-1 KO (SF-1 $KO^{nCre;F/-}$) and control (SF-$1^{nCre;F/+}$) mice by crossing female SF-$1^{F/F}$ mice with male SF-$1^{+/-}$ mice carrying nestin-cre. To account for the potential metabolic effects of the nestin-cre transgene (14), we directly assessed littermate SF-$1^{nCre;F/+}$ mice. We first examined cohorts of mice fed with regular fat (4%) chow up to 56 wk. Body weights were comparable between SF-$1^{nCre;F/+}$ and SF-1 $KO^{nCre;F/-}$ mice from weaning to 42 wk of age. At older ages, significant differences in body weight became apparent in females (Fig. S1*C*). This late-onset obesity also was obvious in male SF-1 $KO^{nCre;F/-}$ mice compared with control counterparts at 56 wk old (Fig. S1*A*). The difference in weight did not reflect increased linear growth of mice, because body lengths (nasal-anal) were not significantly different in all groups of mice (SF-$1^{nCre;F/+}$, 9.5 ± 0.18 versus SF-1 $KO^{nCre;F/-}$, 9.58 ± 0.17 cm; $n = 11$ and 12, respectively). Minispec NMR analysis demonstrated that the increased body weight in SF-1 $KO^{nCre;F/-}$ mice reflects increased adiposity in all groups of mice fed normal fat chow (Fig. S1 *B* and *D*).

We have reported that SF-1 neurons in the VMH are direct targets of leptin and are potentially critical in resisting diet-induced obesity (9, 10). Together with the previous results, we found that expression of the leptin receptor (LepR) is specifically decreased in the region of the VMH of SF-1 $KO^{nCre;F/-}$ (Fig. S1*E*). In parallel with in situ hybridization, quantitative RT-PCR (Q-PCR) studies using RNA samples prepared from FACS-sorted SF-1 neurons from WT and SF-1 KO (SF-$1^{-/-}$) embryos [embryonic day (E)18.5] carrying a SF-1/enhanced green fluorescent protein (eGFP) (15) showed significantly decreased ex-

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pression of LepR in SF-1 KO$^{-/-}$ compared with WT littermates (Fig. S1 *F* and *G*). Because normal leptin signaling plays a role in resisting calorie surplus in SF-1 neurons of the VMH, we hypothesized that SF-1 may play a critical role in diet-induced obesity (9, 10). Thus, we challenged SF-1$^{nCre;F/+}$ and SF-1 KO$^{nCre;F/-}$ mice with a high fat diet (HFD) from 8 wk of age. The mean weight of SF-1 KO$^{nCre;F/-}$ mice on regular chow in both sexes did not differ significantly from that of control mice by 8 wk of age. However, at 1 wk (male) and 3 wk (female) after HFD challenge, the SF-1 KO$^{nCre;F/-}$ mice showed marked increase of body weight compared with their control littermates (Fig. 1 *A* and *D*). This response occurred without significant differences in food intake (Fig. 1 *B* and *E*). The SF-1 KO$^{nCre;F/-}$ mice in both sexes showed elevated fat mass and decreased lean body mass compared with control littermates (Fig. 1 *C* and *F*).

Postnatal SF-1 Deletion Preserves the VMH and Leads to Diet-Induced Obesity. An important point to consider regarding prenatal deletion of SF-1 in the VMH is that this results in developmental disorganization of the nucleus (16, 17). Hence, the phenotype exhibited in prenatal VMH-specific SF-1 KO mice could reflect results of either disorganization of the VMH architecture or loss of SF-1 action within the nucleus. Moreover, as noted above, a recent study has suggested that there are limitations regarding the use of nestin-cre mice because of effects of the transgene itself on body weight (14). Therefore, we assessed whether absence of SF-1 in the context of an intact VMH results in similar phenotypes as seen in prenatal VMH-specific SF-1 KO mice. To address this question, we produced postnatal VMH-specific SF-1 KO mice by using CamKII-Cre (SF-1 KO$^{ckIICre;F/F}$) (18, 19). This model expresses Cre recombinase postnatally (approximately day 15) and circumvents the developmental confounds caused by prenatal deletion of SF-1 using nestin-Cre mice (17–19).

We first confirmed whether Cre recombinase expression was restricted to the brain and whether SF-1 was deleted in the VMH without effects on VMH development. Because SF-1 is also expressed in peripheral tissues including the pituitary gland, adrenal gland, and in the gonads, we examined SF-1 expression in these tissues. Hematoxylin and eosin (H&E) staining showed that all organs expressing SF-1 were intact both in control and SF-1 KO$^{ckIICre;F/F}$ mice (Fig. S2 *A–F*). In addition, the postnatal VMH-specific SF-1 KO mice showed normal SF-1 immunoreactivity in the anterior pituitary, adrenal cortex, and ovaries (Fig. S2 *a–f*). In contrast, SF-1 immunoreactivity was markedly decreased in the VMH of SF-1 KO$^{ckIICre;F/F}$ mice, regardless of the diet administered (Fig. 2 *A–E*). Importantly, the VMH of SF-1 KO$^{ckIICre;F/F}$ mice was structurally intact as assessed by Nissl staining (Fig. S3 *A* and *B*). In addition to the structural integrity, we confirmed that SF-1 KO$^{ckIICre;F/F}$ mice have a normal population of SF-1 neurons in the nucleus by using a SF-1/eGFP transgene, which allows us to label SF-1 neurons in the VMH of the SF-1$^{F/F}$ and SF-1 KO$^{ckIICre;F/F}$ mice (15) (Fig. S3 *C* and *D*). Therefore, these results highlight that the CamKII-Cre model specifically ablates SF-1 in the postnatal VMH without disrupting the normal formation of the nucleus.

We next monitored the body weight of controls (SF-1$^{F/F}$ or SF-1$^{ckIICre;F/+}$) and SF-1 KO$^{ckIICre;F/F}$ mice on chow and HFDs from 8 wk of age. As shown in Fig. 2 *F* and *G*, no difference in body weight and fat and lean mass was detected between genotypes when mice were fed chow. However, SF-1 KO$^{ckIICre;F/F}$ mice fed HFD starting at 8 wk of age showed significantly increased body weight, exhibiting markedly increased adiposity and decreased lean mass (Fig. 2 *H* and *I*). In addition, the SF-1 KO$^{ckIICre;F/F}$ mice showed increased glucose, insulin, and leptin levels in both the fed and fasted states (Fig. 2 *J–L*). These results demonstrate that deletion of SF-1 specifically in the VMH increases sensitivity to diet-induced obesity and suggest that SF-1 and its target genes may play essential roles in regulating energy balance, especially after HFD feeding.

SF-1 in the VMH Is Required for Thermogenic Responses After HFD Exposure. Several reports have suggested that the VMH plays important roles in the regulation of diet-induced thermogenesis (9–11). In addition, lesions of the VMH cause susceptibility to HFD (11, 20). To assess the mechanisms underlying the increased sensitivity to HFD in VMH-specific SF-1 KO mice, we performed indirect calorimetry studies. Body weight- and body composition-matched SF-1 KO$^{ckIICre;F/F}$ and control littermates (8–10 wk) were maintained on regular chow and then acutely switched to HFD. Control mice exhibited expected increases in energy expenditure when exposed to the HFD (Fig. 3 *A* and *B*, black line). However, the increased energy expenditure and O_2 consumption induced by acute HFD exposure was completely abolished in VMH-specific SF-1 KO mice (Fig. 3 *D* and *E*). This result was most pronounced in the dark phase. Furthermore, CO_2 production in the dark phase was also significantly impaired in VMH-specific SF-1 KOs compared with controls (Fig. 3 *C* and *F*). The average RER was not significantly different between SF-1 KO$^{ckIICre;F/F}$ and control littermates (Fig. S4 *B* and *E*). In addition, SF-1 KO$^{ckIICre;F/F}$ and control mice displayed comparable total movement (X+Y+Z

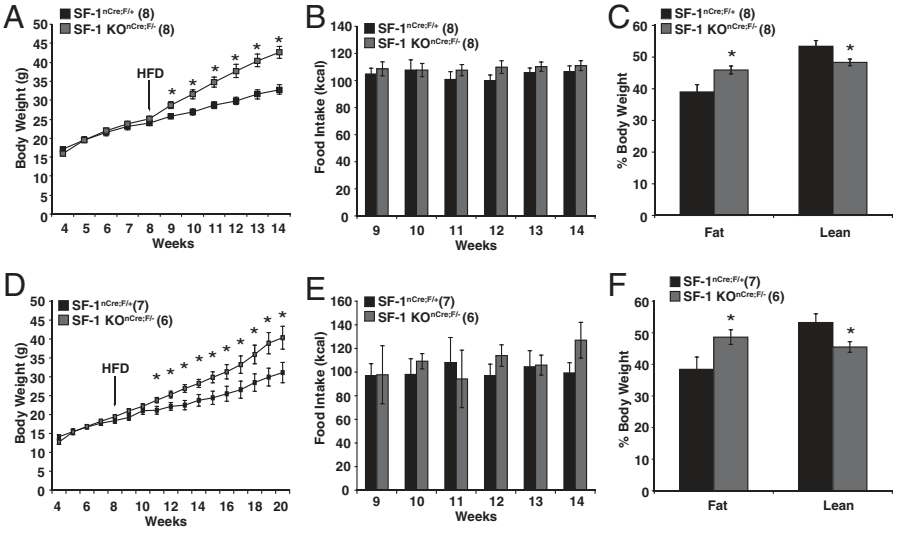

Fig. 1. Effect of HFD on body weight in prenatal VMH-specific SF-1 KO mice. (*A*) Effects of HFD on body weight in male mice lacking SF-1 in the VMH. (*B* and *C*) Weekly food intake and body composition (15 wk old) in male mice fed HFD. (*D*) Effects of HFD on body weight in female mice lacking SF-1 in the VMH. (*E* and *F*) Weekly food intake and body composition (21 wk old) in female mice fed HFD. Number of animals examined was expressed in parentheses in each graph. Values represent mean ± SEM (*$P < 0.05$).

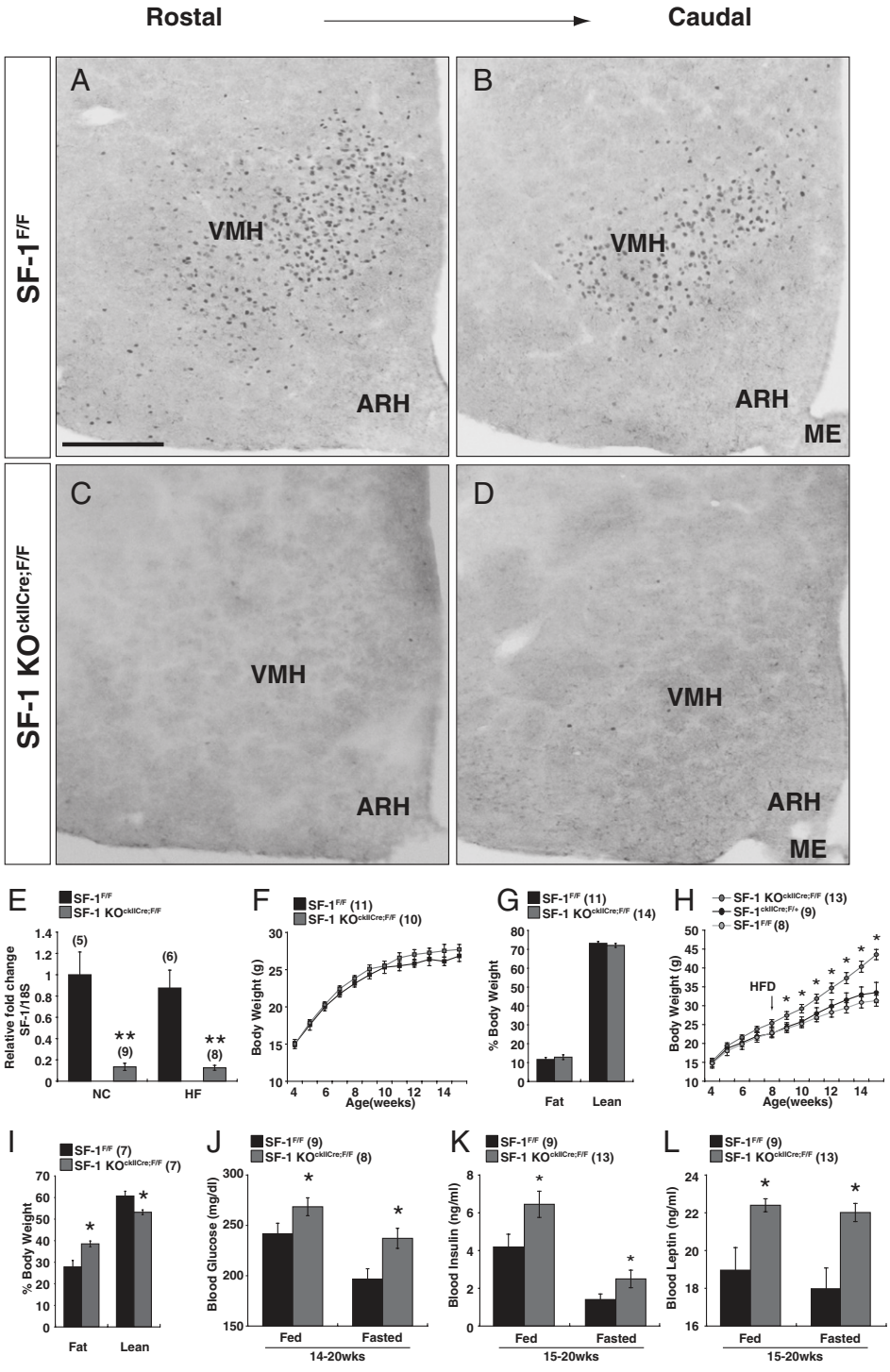

Fig. 2. Phenotype of postnatal VMH-specific SF-1 KO mice. (*A–D*) Immunohistochemistry for SF-1 to confirm CamKII-Cre–mediated SF-1 ablation in the VMH of SF-1 KO$^{ckIICre;F/F}$ mice. Two different hypothalamic levels were selected from SF-1$^{F/F}$ and SF-1 KO$^{ckIICre;F/F}$ mice (n = 8 per each genotype). (*E*) Expression level of SF-1 in SF-1$^{F/F}$ and SF-1 KO$^{ckiiCre;F/F}$ mice in NC and HFD condition. (*F*) Body weight of male mice fed with NC. (*G*) Body composition of male mice fed with NC aged 8–13 wk. (*H*) Body weight of male mice before and after HFD [*genotype* × *time*$_{rm}$ interaction ($F_{22,\ 276}$ = 43.94, $P < 0.005$)]. (*I*) Body composition of male mice fed HFD aged 13–15 wk. (*J–L*) Blood glucose, insulin, and leptin levels in fed and fasted state in mice fed HFD from 8 wk old. Ages of tested mice were indicated in the bottom of each graph. Values are represented as mean ± SEM (*$P < 0.05$ and **$P < 0.01$, Student's t test). Number of experimental animals was expressed in parentheses. rm, repeated measure. (Scale bar: 200 μm.)

beam breaks) and food intake (total and daily) (Fig. S4 *A*, *C*, *D*, and *F*). Collectively, these results indicate that the susceptibility to HFD shown in VMH-specific SF-1 KO mice is due to impaired thermogenic responses after exposure to HFD.

It has been reported that defective brown adipose tissue (BAT) thermogenesis is closely associated with diet-induced obesity in rodents and VMH-lesioned rodents display impaired thermogenic responses, mainly due to impairment of the BAT activity (21–23). Because ablation of SF-1 in the VMH resulted in impaired thermogenesis after HFD exposure, we isolated BAT from SF-1$^{F/F}$ and SF-1 KO$^{ckIICre;F/F}$ mice and examined mRNA level for several genes regulating thermogenic responses (24). Postnatal VMH-specific SF-1 KO mice exhibited significantly decreased expression of UCP1, UCP3, and PGC1α (Fig. 3 *G–I*). Collectively, our results suggest that SF-1 directs transcriptional programs within VMH neurons that result in altered BAT gene expression and BAT activity. Moreover, these programs are required for the appropriate regulation of thermogenesis following exposure to HFD.

Blunted Actions of Leptin in VMH-Specific SF-1 KO Mice. We next examined the effect of SF-1 deletion in the VMH on leptin sensitivity. As noted above, prenatal VMH-specific SF-1 KO mice

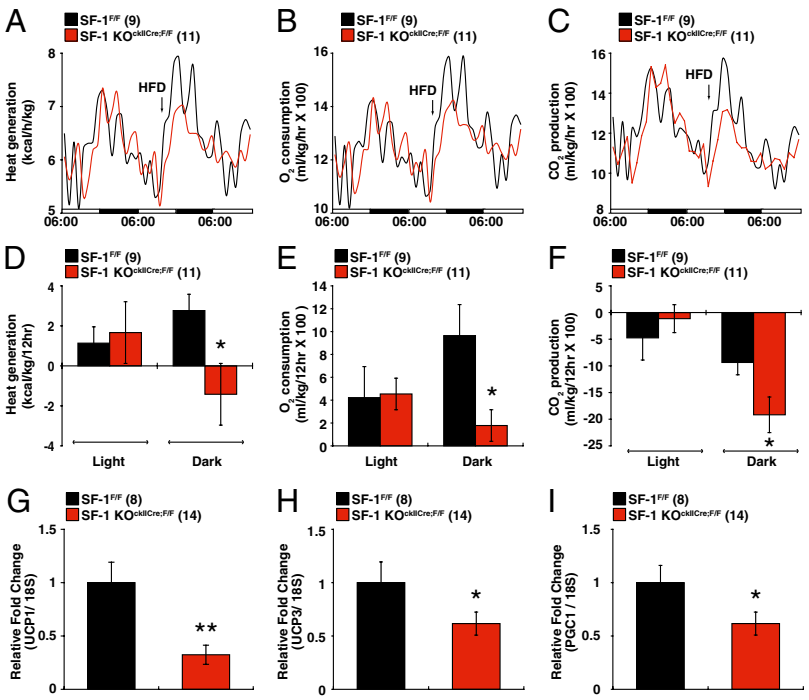

Fig. 3. Response of postnatal VMH-specific SF-1 KO mice to acute HFD challenge. Heat generation (*A*), O_2 consumption (*B*), and CO_2 production (*C*) were measured before and after HFD in SF-1$^{F/F}$ and SF-1 KO$^{cklICre;F/F}$ mice. Changes in heat production (*D*), O_2 consumption (*E*), and CO_2 production (*F*) were measured between the 12 h [light (0600–1800) or dark (1800–0600)] before and after HFD. The mRNA levels of UCP1 (*G*), UCP3 (*H*), and PGC1α (*I*) in BAT from SF-1$^{F/F}$ and SF-1 KO$^{cklICre;F/F}$ mice aged 13–15 wk were measured by Q-PCR analysis. All mice used for Q-PCR were fed with HFD from 8 wk old. Number of animals examined is expressed in parentheses in each graph. The data were expressed as either average (*A*–*C*) or mean ± SEM (*$P < 0.05$ and **$P < 0.001$, Student's *t* test). UCP, uncoupling protein. PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α.

showed blunted expression of LepR specifically in the VMH (Fig. S1). In addition, we also found that the expression of LepR was also significantly blunted in the VMH of the postnatal SF-1 KO mice (Fig. 5*A*). Based on these results, we hypothesized that the transcriptional programs regulated by SF-1 in the VMH may play important roles in regulation of LepR expression and downstream signaling pathways.

To clarify the effects of SF-1 deficiency in the VMH on leptin action, murine recombinant leptin was administrated to control and postnatal SF-1 KO mice and immunohistochemistry was performed to examine the phosphorylation of Stat3. Stat3 phosphorylation has been shown to be indicative of leptin receptor activation and has been used to monitor leptin sensitivity (13, 25, 26). Notably, phosphorylation of Stat3 (pStat3) was significantly impaired specifically in the VMH of the knockout mice compared with control brains. However, pStat3 immunoreactivity was not altered in other brain sites of the SF-1 KO$^{cklICre;F/F}$ mice (Fig. 4 *A*–*F*). As shown in several previous studies (14, 25–27), phosphorylation of Stat3 was evident in many hypothalamic nuclei including the dorsomedial hypothalamus (DMH), VMH, ARH, and ventral premammillary nucleus (PMV). Immunoreactivity was also observed in the ventral tegmental area, dorsal raphe (DR), parabrachial nucleus (PBN), periaqueductal gray (PAG), and nucleus of the solitary tract.

Deletion of Socs3, a potential mediator of central leptin resistance, in SF-1 neurons has been shown to enhance the effects of exogenous leptin administration (13). Further, ablation of PI3K, a downstream component of the leptin signaling pathway, in SF-1 neurons showed blunted responses against acute ICV leptin injection (11). Thus, we next examined the effect of exogenous leptin on several parameters including body weight and food intake in SF-1$^{F/F}$ and SF-1 KO$^{cklICre;F/F}$ mice. Administration of leptin to control mice inhibited food intake by reducing meal frequency and size (Fig. 5*B*). Body weights of WT mice also were significantly reduced in response to leptin injection (Fig. 5*C*). However, the effect of leptin to reduce food intake and body weight was significantly blunted in SF-1 KO$^{cklICre;F/F}$ mice (Fig. 5 *B* and *C*). In addition, the ability of leptin to reduce meal size was also significantly blunted. However, the meal frequency was not changed in SF-1 KO$^{cklICre;F/F}$ mice. These data suggest that SF-1 in the VMH is required not only for the proper expression of the LepR, but also for mediating the acute effects of exogenous leptin administration.

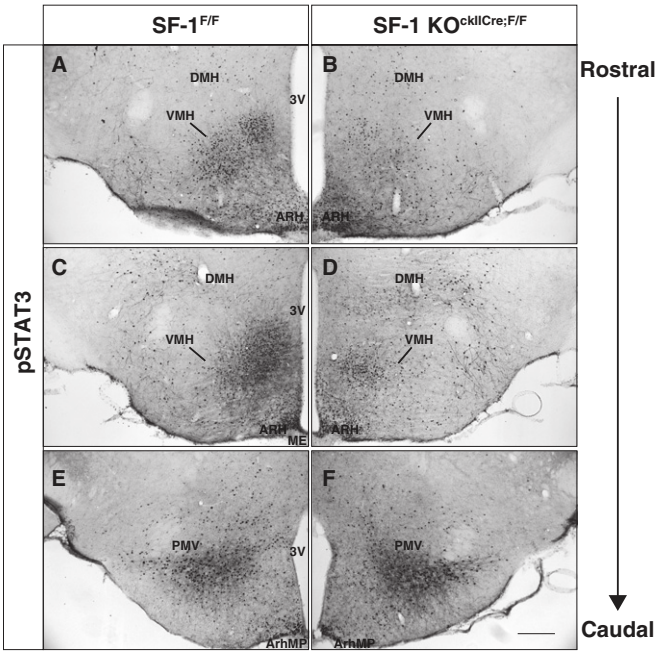

Fig. 4. Blunted pSTAT3 expression in response to acute leptin administration in postnatal VMH-specific SF-1 KO mice. Immunohistochemistry for pStat3 was performed in 8-wk-old chow-fed male SF-1$^{F/F}$ and SF-1 KO$^{cklICre;F/F}$ mice. Three different hypothalamic levels were selected from SF-1$^{F/F}$ and SF-1 KO$^{cklICre;F/F}$ mice (n = 6 per each genotype). DMH, dorsomedial hypothalamus; 3V, third ventricle; ARH, arcuate nucleus of hypothalamus; ME, median eminence; PMV, ventral premammillary nucleus; ArhMP, medial posterior part of arcuate nucleus of hypothalamus. (Scale bar: 200 μm.)

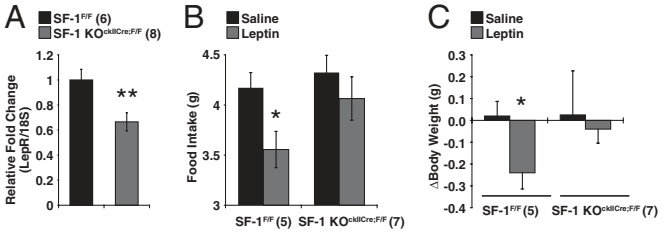

Fig. 5. Blunted response to acute leptin administration in postnatal VMH-specific SF-1 KO mice. (*A*) Expression level of LepR in SF-1$^{F/F}$ and SF-1 KO$^{cklICre;F/F}$ mice aged 8 wk. (*B*) Changes in food intake after exogenous leptin injection [significant leptin effect ($F_{1,20} = 5.19$, $P = 0.03$)]. (*C*) Changes in body weight after exogenous leptin administration. Number of animals used are expressed in parentheses in each graph. The data are expressed as mean ± SEM (*$P < 0.05$ and **$P < 0.01$, Student's *t* test).

Discussion

The VMH has long been considered a critical hypothalamic nucleus responsible for regulating food intake and body weight since the classic lesion studies of Hetherington and Ranson (28). Over the years, the role of the VMH in regulating food intake has been questioned (29). However, a number of studies based on physical lesions or electric stimulation approaches suggested that neurons in the VMH regulate food intake and body weight by modulating the sympathetic nervous system (21, 22, 30, 31). Initial genetic evidence using germ-line SF-1 KO mice supported the model that neurons in the VMH were key regulators of energy balance (3).

Although the germ-line SF-1 KO mice provided important insights into the roles of the VMH in regulation of body weight, conclusions from the studies were limited by the confounding effects of the requirement of concomitant steroid injections and the malformation of the VMH seen in the knockout mice. In this study, therefore, we circumvented these inherent limitations of the germ-line SF-1 KO model by producing and analyzing pre- and postnatal VMH-specific SF-1 KO mice. In these studies we found that early deletion of SF-1 by using the nestin-cre model resulted in malformation of the VMH. Thus, components of the phenotype in this model may be due to developmental effects of SF-1 deletion. However, we found that postnatal deletion produced an energy expenditure defect. The phenotype of postnatal VMH-specific KO mice suggests that ablation of SF-1 and, presumably, altered expression of target genes underlies the phenotypes we have observed. Specifically, we provide evidence that the expression of SF-1 in the VMH is required for the regulation of body weight homeostasis, especially after exposure to HFD. This was not due to increased food intake, but rather a defect in thermogenesis in high calorie condition. In addition, our results suggest that SF-1 is necessary for normal leptin action specifically in the VMH and blunted leptin action induced by ablation of SF-1 action may contribute, at least in part, to the impaired diet-induced thermogenesis seen in VMH-specific SF-1 KO mice. Thus, identification of direct SF-1 targets in VMH neurons could add to the understanding of the mechanisms underlying diet-induced obesity.

One point to clarify is that in the current context we define diet-induced thermogenesis as the heat production in response to eating a HFD. It functions as a mechanism to maintain body weight homeostasis against an overload of energy (32). In rodents, BAT has been considered a major site for the regulation of diet-induced thermogenesis through the sympathetic nervous system and β-adrenergic receptors (33). Several lines of evidence suggest that the VMH plays an important role in regulating the activity of the sympathetic nervous system, including a multisynaptic functional connection between the VMH and BAT. For example, electrical stimulation of the VMH activates the sympathetic nervous system (34). Further, the energy dissipation effect of BAT from VMH-lesioned rats was significantly impaired compared with controls, demonstrating that the VMH mediates a stimulatory effect on BAT, presumably by activating its sympathetic nervous system (35, 36). In addition, studies using obese-resistant rats showed that the resistant rats became susceptible to HFD-induced obesity after VMH lesions. This result suggested that the VMH might contribute to diet-induced thermogenesis in rodent models (20).

Several reports have established the functional linkage between the VMH and BAT (22, 36–38). Thus, we measured expression levels of genes involved in thermogenic regulation in BAT, whether the blunted response to HFD in the VMH-specific SF-1 KO mice attributed to impaired links between the VMH and BAT. Indeed, expression of UCP1 and PGC1α, which are closely related to the regulation of energy dissipation in the BAT, was significantly blunted in both pre- and postnatal VMH-specific SF-1 KO mice compared with control littermates. These findings support our hypothesis that SF-1 regulates transcriptional programs that play a critical role in mediating diet-induced thermogenesis in the BAT.

In addition, anatomic observations support the model that the VMH plays a role in regulating diet-induced thermogenesis. For example, genes known to regulate metabolic state including leptin receptors, NPY Y2 receptors, and estrogen receptors (ERα) are all expressed in the VMH (9, 10, 39–43). More specifically, several studies have suggested that metabolic signals in SF-1 neurons of the VMH play important roles in resisting a high calorie load by regulating thermogenesis (9–12). In the current study, we examined whether the function of SF-1 is linked to diet-induced thermogenesis in the VMH. Indeed, VMH-specific SF-1 knockout mice gained significantly more weight compared with WT littermates in a relatively short time period in HFD condition. In addition, weight-matched WT mice aged 8–10 wk showed increased O_2 consumption and heat generation when they were exposed to HFD. However, the increased energy expenditure induced by the HFD was completely abolished in our VMH-specific SF-1 KO mice. Collectively, these results highlight that the transcriptional regulation by SF-1, especially in the HF condition, is necessary for normal body weight homeostasis.

Questions remain regarding the regulatory roles of SF-1 in the VMH and whether SF-1 is participating in metabolic regulation by modulating its target genes. Previous studies demonstrated that several metabolically relevant genes including the cannabinoid receptor 1 (CB1), brain-derived neurotrophic factor (BDNF), and the type 2 receptor for CRH (Crhr2) are potential SF-1 targets in the VMH (17, 44, 45). Mice with selective ablation of BDNF in the VMH/DMH exhibited hyperphagia and obesity (46). In addition, pharmacological approaches showed that the expression of CB1 in the VMH might regulate body weight and food intake (44, 47). Consistently, we found that the expression of potential SF-1 targets in the VMH such as CB1 and BDNF were down-regulated in the postnatal SF-1 knockout mice (Fig. S5).

In this study, we focused on the leptin-signaling pathway as potential candidates whose action may be regulated by transcriptional activity of SF-1 in the VMH. This hypothesis is due in part to our previous studies that found leptin action in SF-1 neurons is required for regulating body weight, especially after exposure to HFD (9–11). We found that leptin receptor mRNA expression is reduced VMH-specific SF-1 knockout mice. This effect was not due to the VMH developmental defects induced by SF-1 ablation, but to the absence of SF-1 itself in the VMH. The presence of a cognate SF-1 binding site in 5′ promoter region of the LepR raises the possibility that the LepR could be directly regulated by SF-1. In parallel with the decreased LepR, we also found blunted responses to exogenous leptin administration in knockout mice. Based on our previous findings and our current results, we suggest that blunted leptin actions in SF-1 neurons in the VMH contribute

to the impaired thermogenic responses in the VMH-specific SF-1 KO mice.

In summary, we explored functional roles of SF-1 expressed in VMH neurons by creating postnatal VMH-specific SF-1 KO mice. Using this approach, we avoided the complication of malformation of the VMH caused by absence of SF-1 during development. Our results support the model that the transcriptional programs regulated by SF-1 are required for the regulation of body weight homeostasis and leptin action in the VMH.

Methods

Animal Care. All mouse care and experimental procedures were approved by the Institutional Animal Care and Use Committee at UT Southwestern and the National Institute of Biological Science. Mice were housed at room temperature (22–24 °C) with a 12-h light/dark cycle (lights on at 6 AM) with regular mouse chow (Teklad mouse/rat diet #7001; 4.25% kcal from fat, 3.82 kcal/g) or HFD (Research Diet D12331; 58% kcal from fat, 26% from sucrose, 5.56 kcal/g) and water provided ad libitum. The generation of prenatal VMH-specific SF-1 KO (SF-1 KO$^{nCre;F/-}$) mice was described previously (17). To generate postnatal VMH-specific SF-1 KO (SF-1 KO$^{ckIICre;F/F}$) mice, female mice homozygous for the floxed (F) SF-1 allele (17) and heterozygous for the α-calcium/calmodulin-dependent protein kinase II (CamKII) cre transgene (18, 19) were crossed with male mice homozygous for the floxed SF-1 allele. All mice have been backcrossed on the c57BL/6 background for over ten generations to minimize the potential confounding effects of genetic background.

Additional details regarding methods can be found in *SI Methods*.

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