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Knockout Mice Lacking Steroidogenic Factor 1 Are a Novel Genetic Model of Hypothalamic Obesity

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Knockout (KO) mice lacking steroidogenic factor 1 (SF-1) exhibit a phenotype that includes adrenal and gonadal agenesis, impaired gonadotropin expression, and abnormalities of the ventromedial hypothalamic nucleus (VMH). Studies in rodents with lesions of the ventromedial hypothalamus have implicated the VMH in body weight regulation, suggesting that SF-1 KO mice may provide a genetic model of obesity. To prevent death, SF-1 KO mice were rescued with corticosteroid injections, followed by syngeneic adrenal transplants from wild-type (WT) littermates. Corticosterone and ACTH levels in WT and SF-1 KO mice were indistinguishable, documenting restoration of hypothalamic-pituitary-adrenal function. Although weights at earlier ages did not differ significantly from WT littermates, SF-1 KO mice were significantly heavier by 8 wk of age and eventually weighed almost twice as much as WT controls. Obesity in SF-1 KO mice predominantly resulted from decreased activity rather than increased food intake. Leptin was increased markedly, insulin was modestly elevated, and glucose was indistinguishable from WT mice. Although sex steroids in rodents affect weight, ovariectomy did not abolish the weight difference between WT and SF-1 KO mice. These SF-1 KO mice are a genetic model of late-onset obesity that may help elucidate the role of the VMH in weight regulation. (*Endocrinology* 143: 607–614, 2002)

STEROIDOGENIC FACTOR 1 (SF-1; officially designated NR5A1) is an orphan member of the nuclear hormone receptor family that was discovered initially as a regulator of the cytochrome P450 steroidogenic enzymes (1). Subsequent studies have defined broader roles of SF-1 in the development and function of the endocrine system. SF-1 knockout (KO) mice lack gonads and adrenal glands and have impaired function of pituitary gonadotropes (2, 3). Numerous studies have implicated SF-1 in the transcriptional regulation of many different genes in these tissues, including the cytochrome P450 steroid hydroxylases, the steroidogenic acute regulatory protein, the ACTH receptor, anti-Mullerian hormone, the α-subunit of glycoprotein hormones, FSH and its receptor, LH and its receptor, the GnRH receptor, and others (reviewed in Ref. 4). In addition to the aforementioned defects, the structure of the ventromedial hypothalamic nucleus (VMH) of SF-1 KO mice was markedly altered, whereas other hypothalamic nuclei appeared to be intact (5, 6).

Classical stereotactic lesioning studies revealed the importance of the VMH in the regulation of appetite and body weight. Ventromedial lesions in rats caused hyperphagia and obesity, leading to the proposal that this region of the hypothalamus, most likely in concert with other hypothalamic nuclei, acts as a satiety center (7–9). However, neither the molecular events causing obesity in ventromedially lesioned rodents nor the precise role of the VMH in this phenotype are fully understood (10).

Abbreviations: DMH, Dorsomedial hypothalamic nucleus; HPA, hypothalamic-pituitary-adrenal; KO, knockout; MC3-R, melanocortin-3 receptor; MC4-R, melanocortin-4 receptor; MRI, magnetic resonance imaging; OVX, ovariectomized; SF-1, steroidogenic factor 1; VMH, ventromedial hypothalamic nucleus; WT, wild-type.

Body weight in both animals and humans is regulated to balance precisely energy intake and expenditure (reviewed in Refs. 11 and 12). A critical mediator of this regulation is leptin, a hormone produced by white adipose tissue (11–15). The primary hypothalamic site of leptin action is believed to be the arcuate nucleus, although leptin receptors also are expressed in the VMH and the dorsomedial hypothalamic nucleus (DMH) (16, 17). Plasma leptin levels are highly correlated with the amount of white adipose tissue in the body, and increased levels of leptin cause responses in the hypothalamus that ultimately decrease feeding and increase energy expenditure. Several other signaling molecules participate in appetite control by the hypothalamus, at least some of which are thought to act downstream of leptin. Orexigenic hypothalamic signals include neuropeptide Y, agouti-related protein, melanin-concentrating hormone, and the orexins, whereas αMSH, cocaine-amphetamine-regulated transcript, and CRH suppress appetite (11, 12).

In the present study we characterize the phenotype of SF-1 KO mice during postnatal development and in adult life. These SF-1 KO mice become markedly obese, establishing them as a novel, monogenic model of late-onset obesity. Although the precise role of SF-1 remains to be defined, the SF-1 KO mice raise the possibility that impaired SF-1 function in humans causes obesity and provide a novel system for exploring the role of the VMH in weight regulation.

Materials and Methods

SF-1 KO mice, adrenal transplantation, and tissue collection

Mice with the disrupted SF-1 allele (3) were backcrossed for 10 generations to C57BL/6J mice to produce a congenic line (provided by Dr.

Kenn Albrecht, The Jackson Laboratory, Bar Harbor, ME), thus preventing rejection of transplanted adrenals. All mice were housed at constant temperature (20–24 C) in 12-h light/dark cycle and fed standard mouse chow *ad libitum*. Mice were weighed weekly, and food consumption was measured by weighing food every day with careful monitoring of cages for any spills. For activity measurements, mice were housed in individual cages with activity wheels and magnetic counters. Mice (three mice for each group) were placed in the cages for 5 d before data collection to allow them to become familiar with their environment, after which counters were reset each evening before dark, and counts were recorded each morning for 7 consecutive nights. Body length was measured at the time of death (6 months) by extension of anesthetized mice to their full length, always by the same individual. Blood was collected either from the saphenous vein (during experiments) or by cardiac puncture (before sacrifice). All experiments involving mice were approved by the Institutional Review Committee at UT Southwestern Medical Center. To avoid potential confounding effects of sex, all studies described here used genetically female XX mice. Although SF-1 haploinsufficiency in humans is associated with endocrine disease (18) and heterozygous SF-1 KO mice have impaired adrenal proliferation (19), SF-1 heterozygotes had normal VMH structure, body weight, fat composition, and food consumption (Majdic, G., unpublished observation). Despite the apparent lack of a phenotype in heterozygotes, all WT mice described here had the +/+ genotype.

SF-1 heterozygous mice were mated to produce homozygous SF-1 KO offspring. All newborn pups (WT and SF-1 KO) were injected daily with 50 μl of a corticosteroid cocktail in olive oil (400 μg/ml hydrocortisone, 40 ng/ml dexamethasone, and 25 ng/ml fludrocortisone acetate; all from Sigma, St. Louis, MO). Mice were genotyped by PCR assay of tail DNA on d 6 or 7 after birth as previously described (3). Immediately after genotyping, some WT littermates were used as a source of adrenal transplants. After death, the WT adrenal glands were excised, washed in ice-cold PBS, and transferred into ice-cold PBS containing 100 pg/ml fibroblast growth factor (Sigma). A small transdermal puncture was made in the subaxillary region of SF-1 KO pups with a 20-gauge needle, two adrenal glands were placed sc using sharp watchmaker's forceps, and the skin was closed. WT (not operated) and SF-1 KO mice were injected daily with corticosteroid cocktail on the first 2 d after surgery, followed by a 1-d break. Subsequently, steroid injections were given to all mice on d 14, 17, and 21, and they received no further steroid treatment after weaning at 3 wk.

To determine the possible effect of sex steroid deficiency on weight gain, some WT mice were ovariectomized (OVX). Immediately after weaning (and before the onset of ovarian steroidogenesis), mice were anesthetized with a mixture of ketamine (1.25 mg/animal; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA), xylazine (0.125 mg/animal; X-ject SA, Burns Veterinary Supply, Rockville Center, NY), and acepromazine (0.025 mg/animal; Aceproject, Burns Veterinary Supply). Ovaries were excised through a small abdominal incision that was subsequently sutured. As an additional control, the adrenal glands of two WT mice were removed and then autotransplanted at 3 wk of age. These mice received three daily injections of corticosteroid cocktail (as described above) before surgery and steroid injections on d 1, 3, and 6 after the surgery. Mice were anesthetized as described above, and adrenal glands were excised through an incision on the back. The adrenal glands were then transplanted sc into the same mice during the surgery in a manner identical to that used for the SF-1 KO mice.

Mice were killed at 6 months of age using anesthesia as described above, followed by cervical dislocation. Some mice were perfused with 4% paraformaldehyde. Whole brains were dissected from the skull, postfixed for 1 h in 4% paraformaldehyde, and washed overnight in 20% sucrose in PBS. Whole brains were embedded in Tissue-Tek (Sakura Finetek, Torrance, CA), frozen in liquid nitrogen, and stored at −80 C until further use.

For magnetic resonance imaging (MRI) analyses of body fat composition, mice were anesthetized as described above and analyzed in an MRI device (Philips Medical Systems) as previously described (20). MRI data were used to determine lean body mass and to calculate relative food intake.

Histological analyses

Coronal sections of brain (25 μm) were cut with a cryotome, and sections were stained with cresyl violet using standard procedures. Immunohistochemical staining was performed essentially as previously described (21) using rabbit polyclonal antiserum raised against rat ERα (Upstate Biotechnology, Inc., Lake Placid, NY). No signal above background was observed in sections in which the primary antiserum was replaced with nonimmune rabbit serum. An *in situ* hybridization probe for tubby was obtained by RT-PCR of whole brain RNA using primers that amplified a 379-bp sequence (5′ primer, 5′-CTCCAGCAGCATGAGCTTT-3′; 3′ primer, 5′-GGTCCACAGAGATGAGGTAA-3′), followed by cloning into pCRII (Invitrogen, San Diego, CA). These sequences do not include those encoding the highly conserved carboxyl-terminal region of tubby, thus minimizing cross-hybridization with other members of the tubby family. Probes were prepared using T7 (sense) and SP6 (antisense) RNA polymerases in the presence of digoxigenin-labeled UTP (Roche Molecular Biochemicals, Indianapolis, IN). *In situ* hybridizations with cRNA probes were performed essentially as previously described (22), and bound digoxigenin-labeled probe was detected according to the supplier's protocol. No signal above background was detected in sections hybridized with sense probes. Photomicrographs were taken with a Nikon Optiphot 2 microscope (Melville, NY).

Hormone assays

All blood hormone levels were measured using standard RIA procedures. In all cases, mice were bled in the same rooms in which they were housed to minimize stress, and samples were obtained as soon as possible after entering the room. ACTH in EDTA plasma was measured using an RIA kit from Nichols Institute Diagnostics (San Juan Capistrano, CA) following the protocol supplied with the kit. Inter- and intraassay variations were 3.2% and 6.8%, respectively. Corticosterone levels in heparinized plasma were measured by RIA as previously described (23), with inter- and intraassay variations of 7% and 4.8%, respectively. Plasma leptin and insulin were measured using RIA kits (Linco Research, Inc., St. Charles, MO). Inter- and intraassay variations for leptin were 5.7% and 4.6%, respectively. Plasma glucose was measured with a HemoCue glucose analyzer (HemoCue AB, Angelholm, Sweden).

Statistical analyses

Data from each experiment were subjected to ANOVA to identify statistically significant differences between groups. Subgroup comparisons between means for each group were then made using the variance from the experiment as a whole as the measure of error. At least three, but normally more, data points for each group were used in all statistical analyses.

Results

VMH abnormalities seen in newborn SF-1 KO mice persist in adults

Although newborn SF-1 KO mice have marked abnormalities in the VMH (5, 6), the morphology of the VMH in adult SF-1 KO mice has not been described. The ability to keep SF-1 KO mice alive into adulthood afforded an opportunity to examine the fate of VMH neurons at later developmental stages. As shown in Fig. 1, A and B, histological sections of hypothalami of adult SF-1 KO mice closely resembled those reported previously in newborn SF-1 KO mice; the distinct cluster of cells that normally comprise the VMH was not present, whereas the arcuate nucleus and DMH appeared intact. To explore more specifically the effect of the SF-1 KO on the VMH, we examined the expression of two markers that delineate distinct populations of VMH neurons. ERα is expressed specifically in neurons that reside in the ventrolateral region of the VMH, and we previously used ERα

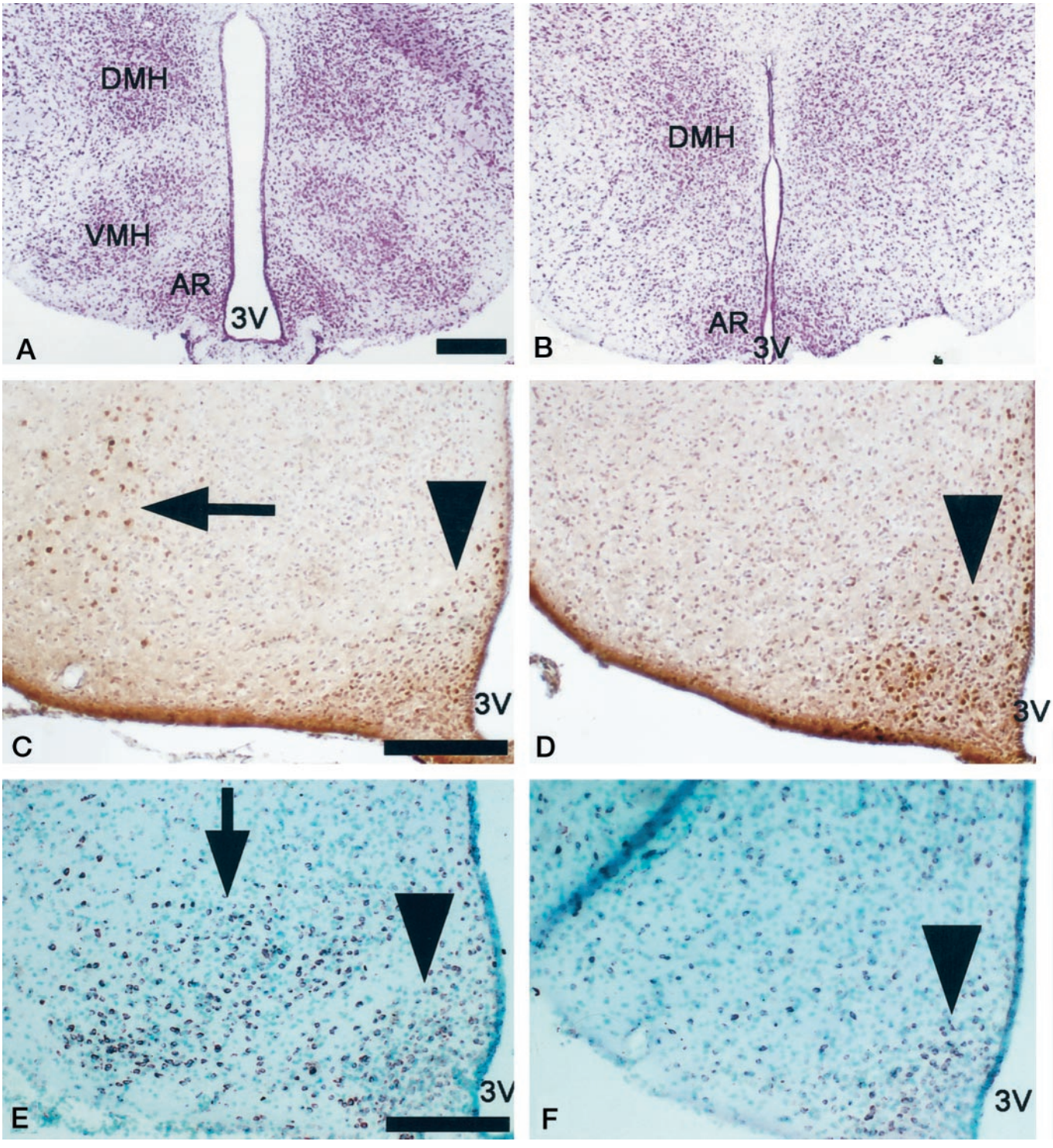


FIG. 1. The VMH is specifically affected in adult SF-1 KO mice. *Top*, Coronal sections of hypothalami from 6-month-old mice were stained with *cresyl violet*. Note the absence of the cluster of cells that normally comprises the VMH in the section from the SF-1 KO mouse (B) *vs.* the well defined structure in the WT section (A). *Middle*, Immunocytochemical detection of ERα demonstrates immunopositive cells in both the VMH (*arrow*) and the arcuate nucleus (*arrowhead*) in the WT hypothalamus (C). In contrast, ERα-positive cells in the SF-1 KO section (D) were detected only in the arcuate nucleus (*arrowhead*), functionally confirming the absence of this subset of neurons in the VMH. *Bottom*, Similarly, tubby mRNA was readily detected in both arcuate nucleus and VMH in the WT section (E), but was diminished markedly in the VMH, but not the arcuate nucleus, in the SF-1 KO section (F). *Scale bar*, 200 μm. Ar, Arcuate nucleus; 3V, third ventricle.

immunoreactivity to define the effect of the SF-1 KO in mouse embryos and newborn pups (22). As in these previous studies, ERα-positive cells in the adult SF-1 KO mice were not seen in their usual position in the ventrolateral quadrant of the VMH, but were readily detected in the WT sections (Fig. 1, C and D). Significantly, comparable numbers of ERα-positive cells were observed in the closely adjacent arcuate nucleus, documenting the specificity of the VMH defect. Moreover, as described in newborn SF-1 KO mice (22), the medial-lateral distribution of ERα-positive cells was apparently altered such that some cells were clustered adjacent to the third ventricle.

A second, more widely expressed marker of VMH neurons is tubby, a gene whose mutation in mice is associated with monogenic obesity (24) (see below for further discussion). Although WT mice expressed tubby in both the VMH and the arcuate nucleus (Fig. 1, E and F), the distribution and number of tubby-positive cells were altered considerably in SF-1 KO mice. This altered expression was most striking in the region where the VMH is normally found, contrasting with the relatively preserved tubby expression in the adjacent arcuate nucleus (Fig. 1) and the DMH (data not shown). These results establish that the absence of SF-1 permanently affects the hypothalamic distribution of two different markers of VMH neurons, suggesting that the VMH defect described previously persists in adult SF-1 KO mice.

Adrenal transplants restore normal corticosteroid levels in SF-1 KO mice

To assess the function of the transplanted adrenal glands, mice were bled at either 1000 or at 1800 h (peak), and plasma corticosterone and ACTH were measured (Table 1). At both 10 wk and 6 months of age, the hormone levels at 1800 h were similar in all three groups (WT, OVX WT, and SF-1 KO). Although the sample size was smaller, comparable corticosterone levels were seen in samples obtained at 1000 h. These values for both WT and SF-1 KO mice were lower than the 1800 h results, suggesting that diurnal rhythm is maintained in adrenal-transplanted SF-1 KO mice; these differences, however, did not achieve statistical significance, precluding any definitive statements about circadian rhythm in the SF-1 KO mice. Collectively, these results suggest that the hypothalamic-pituitary-adrenal (HPA) axis in these mice functions normally. In particular, the ACTH levels in SF-1 KO mice did not differ from those in WT mice, indicating that their corticosterone levels were not elevated sufficiently to suppress their ACTH levels. Thus, the possibility that weight regulation in SF-1 KO mice is affected by primary corticosterone excess is extremely remote.

SF-1 KO mice exhibit late-onset obesity

Based on the long-held association between VMH lesions and obesity, we next examined body weight in the adrenal-transplanted SF-1 KO mice. As shown in Fig. 2, weights were comparable in all groups of mice from weaning until 8 wk of age, when significant differences in body weight first became apparent. This weight difference progressed such that SF-1 KO mice at 6 months of age weighed almost twice as much as WT littermates (50.5 ± 1.2 *vs.* 29.3 ± 0.8 g in WT mice; $P < 0.001$). The differences in weight did not reflect increased linear growth, as body lengths were similar in all groups of mice (~10 cm naso-anal length), suggesting, rather, that SF-1 KO mice might have increased adiposity. Consistent with this model (Fig. 2), MRI analyses at 6 months of age revealed markedly increased body fat in SF-1 KO mice (42 ± 1.3% body fat; n = 4) relative to WT mice (20 ± 1.3% body fat; n = 3).

Consistent with previous analyses of body weight in KO mice with impaired estrogen biosynthesis (25) or action (26), body weight and adiposity also were increased in OVX WT mice (35.7 ± 1.3 g, 28 ± 1.8% body fat). Like the previously described ERα and aromatase KO mice, however, body weight and adiposity in OVX WT females were significantly less than those in SF-1 KO mice, arguing strongly that a lack of sex steroids alone does not account for the near doubling of weight seen in SF-1 KO mice. The weights of two WT mice that were adrenalectomized and then given adrenal autotransplants were comparable to those of WT mice (30.2 and 29.6 g), strongly suggesting that the adrenal transplantation protocol itself does not cause the obesity. In the absence of demonstrable perturbations in the HPA axis or comparable effects of ovariectomy, our results implicate the alterations in VMH structure as the predominant cause of obesity in SF-1 KO mice.

One potential explanation for the increased weight of SF-1 KO mice is that they eat more. From 4–8 wk of age, food consumption did not differ significantly among the groups [4.1 ± 0.2 g/d in WT mice (n = 7), 4.0 ± 0.2 g in OVX WT mice (n = 5), and 4.2 ± 0.2 g in SF-1 KO mice (n = 5); mean ± SE]. Thereafter, food consumption increased in SF-1 KO mice

TABLE 1. Effect of SF-1 KO on biochemical parameters

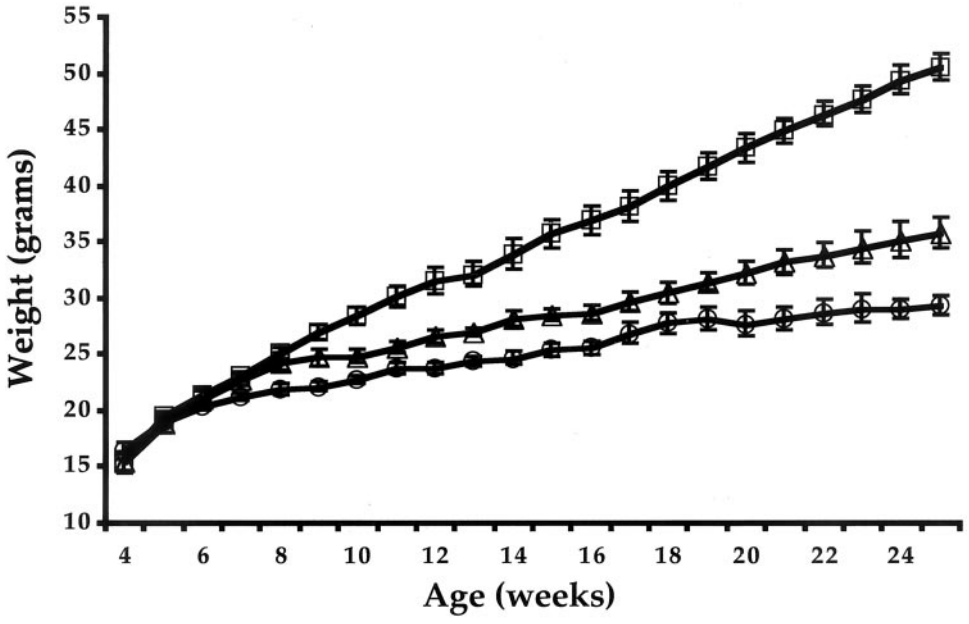
	WT	WT OVX	SF-1 KO
Corticosterone (10 wk, PM; ng/ml)	96.0 ± 23.6 (4)	86.3 ± 24.8 (5)	91.7 ± 27.9 (5)
Corticosterone (AM; ng/ml)	64.3 ± 10.7 (5)	99.8 ± 20.5 (4)	64.6 ± 9.7 (5)
Corticosterone (PM; ng/ml)	133.5 ± 37.0 (6)	137.2 ± 22.0 (5)	128.5 ± 35.8 (9)
ACTH (PM; pg/ml)	173.3 ± 34.2 (4)	500 ± 81.7 (3)	352.5 ± 70.9 (3)
Leptin (ng/ml)	2.3 ± 0.3 (5)	35.8 ± 4.4[a] (5)	57.8 ± 1.84[a] (7)
Insulin (μU/ml)	0.66 ± 0.15 (5)	1.62 ± 0.4[b] (4)	3.38 ± 0.63[b,c] (9)
Glucose (mg/dl)	200 ± 12.7 (5)	205 ± 19.5 (4)	231 ± 15.2 (9)
Glucose/insulin	341.1 ± 78.2	136.2 ± 41.8[b]	97.1 ± 25.3[a,c]

Except where indicated, all data are from 6-month-old mice and are given as the mean ± SEM. The number of animals is in *parentheses.* Statistically significant differences from WT values are indicated.

[a] $P < 0.001$.

[b] $P < 0.05$.

[c] Parameters in which SF-1 KO mice differed significantly from WT, but not WT OVX, mice.



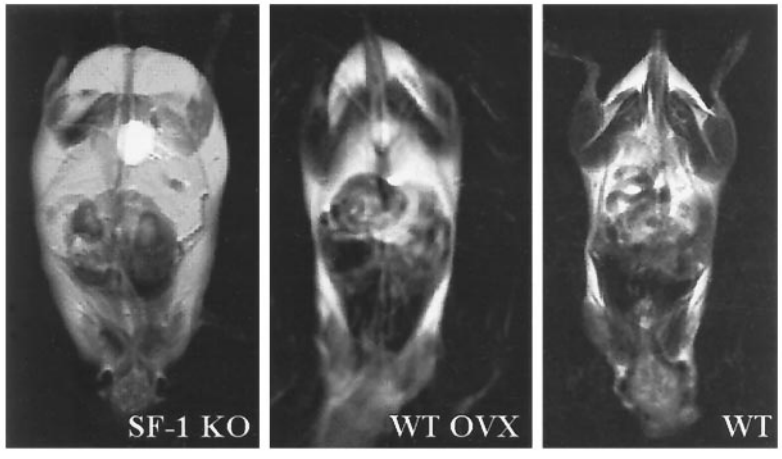


FIG. 2. SF-1 KO mice progressively become obese. *Upper panel*, Body weights were monitored in SF-1 KO (□; n = 10), WT (○; n = 8), and OVX WT (△; n = 6) mice at different ages (mean ± SE). *Lower panel*, Transverse MRI scans of whole mice show striking increased white adipose tissue fat (*light gray color*) in SF-1 KO mice relative to WT mice or WT OVX mice.

(5.0 ± 0.2 g/d at 20 wk of age), whereas it remained relatively constant in WT mice (4.2 ± 0.1 g/d at 20 wk; $P < 0.05$). However, the differences in relative food intake disappeared when the MRI data were used to adjust for lean body mass. Thus, at 20 wk of age, the food intake per d/g (lean body mass)$^{3/4}$ was 0.39 ± 0.02 in SF-1 KO mice (n = 3), 0.40 ± 0.01 in WT mice (n = 3), and 0.37 ± 0.02 in OVX WT mice (n = 3). This finding suggests that increased food intake is only one component of the obesity in SF-1 KO mice.

Given that the hyperphagia in SF-1 KO mice largely disappears after correcting for lean body mass, an alternative explanation for their obesity is decreased energy expenditure. In support of this model, analyses of SF-1 KO mice at 7 wk, before they became overtly obese, revealed a 75% decrease in wheel-turning activity relative to WT mice, a decrease that persisted at 6 months of age (Fig. 3). Although these data ultimately should be refined using metabolic cages to determine total energy consumption and photosensor beam breaks to assess general exploratory activity, these results strongly implicate decreased energy utilization as the primary basis for the late-onset obesity in SF-1 KO mice.

In accord with the increased amount of white adipose tissue seen on MRI, blood levels of leptin were elevated significantly in both SF-1 KO and WT OVX mice relative to those in WT mice (Table 1). Although glucose levels were similar in all three groups of mice (Table 1), insulin levels were elevated significantly in both SF-1 KO and WT OVX mice ($P < 0.05$ compared with WT mice), and their glucose/insulin ratios were reduced significantly relative to those in WT mice ($P < 0.05$). These findings indicate that SF-1 KO mice and WT OVX are markedly insulin resistant despite the absence of overt diabetes.

Discussion

SF-1 is an essential regulator of many genes involved in steroidogenesis and reproduction (4). Its importance is highlighted dramatically by SF-1 KO mice, which are born with-

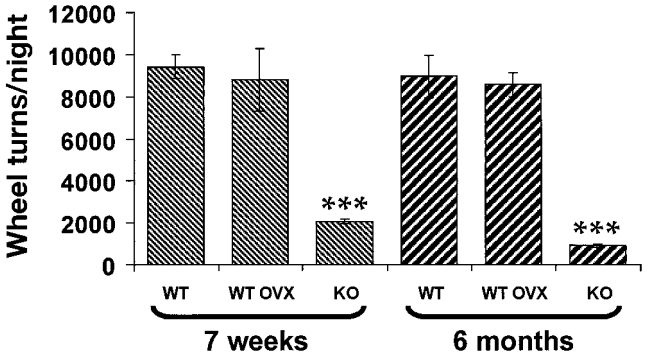


FIG. 3. SF-1 KO mice exhibit decreased activity before they become obese. Activity levels at 7 wk (▨) and 6 months (▨) of age were monitored by spontaneous wheel turning in SF-1 KO, WT, and OVX WT mice as described in *Materials and Methods*. The activity in SF-1 KO mice was significantly lower than that in WT or OVX WT mice (mean ± SE; ***, $P < 0.001$).

out adrenal glands and gonads and die shortly thereafter from adrenal insufficiency. These mice also have impaired expression of gonadotropins in the anterior pituitary and marked alterations in the cellular organization and gene expression patterns of the VMH (2, 3, 5, 22). These effects on the VMH, the only site of SF-1 expression in the adult brain, appear to be specific, as the other hypothalamic nuclei in SF-1 KO mice are morphologically intact. The VMH has been implicated in a broad array of homeostatic and behavioral processes, including the regulation of appetite and body weight (27). Although the VMH first emerges as a distinct cell grouping in the developing mouse hypothalamus on embryonic d 16–17 (28), SF-1-positive cells actually appear in the embryonic diencephalon as early as embryonic d 11.5, subsequently localizing to the VMH (5). Proposed SF-1 target genes in the VMH include glutamic acid decarboxylase (22) and the *N*-methyl-D-aspartate receptor (29), but definitive target genes of SF-1 in the embryonic or adult VMH have not been identified. Thus, it is not known whether the VMH alterations in SF-1 KO mice reflect essential roles of SF-1 in cell migration, cell fate specification, or other events in the function of VMH neurons. However, the striking and selective effects of the SF-1 KO on VMH structure suggest that these mice provide a unique genetic model to study the importance of this nucleus in neurophysiology and to define the roles of SF-1 in VMH development and function.

In the present study SF-1 KO mice were kept alive after birth by transplanting adrenal glands from WT littermates. One concern in these experiments is the function of transplanted adrenals, as glucocorticoids have well known effects on appetite. For example, patients with endogenous or exogenous glucocorticoid excess frequently become obese, and glucocorticoid excess in experimental animals stimulates feeding, reduces thermogenesis in brown adipose tissue, and inhibits leptin action in the brain (30–32). It is highly unlikely that the obesity in SF-1 KO mice results from the corticosteroid replacement regimen, because the WT mice also received steroid injections in the early postnatal period, and steroid administration was limited to the first 3 wk of age. Moreover, we have never observed obesity in KO mice lacking the steroidogenic acute regulatory protein that received a similar corticosteroid replacement regimen (Hasegawa, T., and K. Parker, unpublished observation).

To address the concern that the transplanted adrenals overproduce glucocorticoids, we monitored corticosterone levels at different times of day (presumed peak and trough levels) and found no differences between WT and SF-1 KO mice. In addition, ACTH levels did not differ significantly between WT and SF-1 KO mice, further suggesting that the HPA axis in these mice functions normally. Finally, body weight was normal in two WT mice that were adrenalectomized and given adrenal autotransplants at 3 wk of age. These findings suggest strongly that the obesity in SF-1 KO mice results from alterations in the VMH rather than excessive corticosterone resulting from the steroid replacement regimen or perturbed function of the transplanted adrenal glands. It remains possible, however, that SF-1 KO mice have subtle alterations in their patterns of glucocorticoid secretion, as perturbations in circadian rhythm have been reported in VMH-lesioned rats (33, 34). If similar variations in circadian rhythm ultimately are found in SF-1 KO mice, they may provide a novel model system for exploring the roles of this orphan nuclear receptor in maintaining normal rhythmicity of the HPA axis.

Sex steroids are the other steroid class known to affect body weight regulation, and ovarian agenesis renders the SF-1 KO mice completely deficient in sex steroids. Both ERα- and aromatase KO mice exhibit an obesity phenotype that somewhat resembles that described here. Shared features include the relatively delayed onset of obesity, decreased activity, and marked hepatic steatosis associated with increased lipid deposits in brown adipose tissue (25, 26) (Majdic, G., unpublished observation). However, other features suggest that the marked obesity in SF-1 KO mice differs considerably from that seen in mice with deficient sex steroid action. First, the increased weight and adiposity in SF-1 KO mice exceeded considerably those seen in ERα and aromatase KO mice or in WT mice whose ovaries were removed at 21 d (Fig. 2). This finding argues that the absence of ovarian steroids is but a small part of the obesity phenotype in SF-1 KO mice. In addition, SF-1 KO mice exhibit decreased activity at a much earlier age than aromatase KO mice (Fig. 3). Collectively, these phenotypic disparities highlight important differences in the mechanisms of obesity in SF-1 KO mice *vs.* those in mice with genetic deficiencies in estrogen biosynthesis or action.

A number of genetic models of obesity in mice have been characterized at the molecular level (reviewed in Ref. 35), and their obesity phenotypes establish distinct roles for various components of the hypothalamic circuitry in energy regulation. The most dramatic obesity phenotypes are seen in *ob* and *db* mice, which have mutations in leptin or its receptor, respectively. These mice gain weight excessively from very early life, ultimately weighing up to 80 g, and exhibit a compound endocrine phenotype that includes abnormal reproduction and production of sex steroids. Leptin is secreted by white adipose tissue and correlates closely with total fat deposits in the body (15, 36). In the hypothalamus, the leptin receptor is expressed most highly in the arcuate nucleus, but lower levels of expression also are seen in the DMH, VMH, and paraventricular nucleus (16, 17). Moreover,

leptin accumulates predominantly in the arcuate nucleus, where it modulates the expression of neuropeptide Y and αMSH; thus, the arcuate nucleus has been viewed as the primary site of leptin action (36). Leptin was elevated significantly in SF-1 KO mice, suggesting that SF-1 KO mice, like rodents with lesions of the VMH, are resistant to leptin. We currently do not know whether this effect in SF-1 KO mice reflects impaired leptin action in the VMH or disturbed signaling from other nuclei secondary to the VMH alterations.

Several other mouse genetic models of obesity disrupt various components of a hypothalamic melanocortin signaling network. Yellow agouti mice, which have a mutation that causes ectopic expression of a protein that antagonizes the melanocortin-4 receptor in the hypothalamus, develop obesity and increased body size by about 5 wk of age (37). A similar obese phenotype is observed in KO mice lacking the melanocortin-4 receptor (38). Fat mice have a mutation in carboxypeptidase E, an enzyme that contributes to the conversion POMC to αMSH (35). Presumably, the altered delivery of αMSH in fat mice leads to impaired signaling through melanocortin receptors and thereby alters body weight regulation. Finally, another melanocortin receptor, the melanocortin-3 receptor, is associated with an obesity phenotype that closely resembles that of the SF-1 KO mice. The melanocortin-3 receptor is expressed primarily in the arcuate nucleus and VMH, and melanocortin-3 receptor KO mice have a delayed onset of obesity that results from reduced activity rather than hyperphagia (39) (Butler, A., unpublished observations).

The second genetic obesity model that closely parallels SF-1 KO mice is the tubby mouse mutation. Both tubby and SF-1 KO mice become obese by approximately 8–10 wk of age, ultimately weighing almost twice as much as WT littermates (Ref. 40 and this report). Temperature regulation is not affected, and levels of corticosterone, which are markedly elevated in *ob* and *db* mice, are normal. The gene responsible for the tubby phenotype is a member of a novel gene family that is expressed in the hypothalamus (VMH, DMH, paraventricular nucleus, and arcuate nucleus), as well as in the hippocampus and cerebral cortex (24). Cell culture studies have shown that tubby, upon receptor-mediated activation of G proteins, translocates from the plasma membrane to the nucleus (41), presumably activating the transcription of as yet to be defined target genes to prevent apoptosis (42). The altered expression of tubby in the VMH of SF-1 KO mice appears to be relatively specific, as tubby expression was reportedly normal in *ob*, *db*, and melanocortin-4 receptor KO mice (43). Based on these findings, we speculate that alterations in tubby expression in the VMH may account at least in part for the similar phenotypes in tubby and SF-1 KO mice. Others (41) have likened the tubby phenotype to that of KO mice lacking the 5-HT_{2c} serotonin receptor gene (44), and further studies are needed to compare and contrast the phenotypes in these different obesity models and to define the molecular mechanisms that underlie their delayed onset obesity.

In summary, the present study establishes that adult SF-1 KO mice, with their structural and functional abnormalities of VMH neurons, exhibit relatively severe, late-onset obesity. The obesity in SF-1 KO mice results predominantly from decreased energy expenditure rather than hyperphagia; this result differs from data originally obtained in rodents with lesions of the ventromedial hypothalamic nucleus and is consistent with studies that have targeted more specifically the VMH while preserving the arcuate nucleus (10). The development of strategies for tissue-specific disruption of SF-1 (21) and for using SF-1 regulatory sequences to target transgene expression to the VMH (Stallings, N., and K. Parker, unpublished observation) will provide a powerful approach to explore the roles of SF-1 and the VMH in energy homeostasis.

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References

1. **Lala DS, Rice DA, Parker KL** 1992 Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of *fushi tarazu*-factor I. Mol Endocrinol 6:1249–1258
2. **Ingraham HA, Lala DS, Ikeda Y, Luo X, Shen W, Nachtigal MW, Abbud R, Nilson JH, Parker KL** 1994 The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. Genes Dev 8:2302–2312
3. **Luo X, Ikeda Y, Parker KL** 1994 A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 77:481–490
4. **Parker KL, Schimmer BP** 1997 Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 18:361–377
5. **Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL** 1995 The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 9:478–486
6. **Shinoda K, Lei H, Yoshii H, Nomura M, Nagano M, Shiba H, Sasaki H, Osawa Y, Ninomiya Y, Niwa O, Morohashi K-I, Li E** 1995 Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. Dev Dyn 204:22–29
7. **Brobeck JR** 1946 Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol Rev 26:541–559
8. **Gold RM** 1970 Hypothalamic hyperphagia: males get just as fat as females. J Comp Physiol Psychol 71:347–356
9. **Hetherington AW, Ranson SW** 1940 Hypothalamic lesions and adiposity in rat. Anat Rec 78:149–172
10. **Choi S, Dallman MF** 1999 Hypothalamic obesity: multiple routes mediated by loss of function in medial cell groups. Endocrinology 140:4081–4088
11. **Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS** 1999 Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev 20:68–100
12. **Inui A** 2000 Transgenic approach to the study of body weight regulation. Pharmacol Rev 52:35–61
13. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546
14. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F** 1995 Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269:540–543
15. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–32
16. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG** 1996 Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101–1106

17. **Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, Saper CB** 1998 Distributions of leptin receptor mRNA isoforms in the rat brain. J Comp Neurol 395:535–547
18. **Achermann JC, Ito M, Hindmarsh PC, Jameson JL** 1999 A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet 22:125–126
19. **Bland ML, Jamieson CA, Akana SF, Bornstein SR, Eisenhofer G, Dallman MF, Ingraham HA** 2000 Haploinsufficiency of steroidogenic factor-1 in mice disrupts adrenal development leading to an impaired stress response. Proc Natl Acad Sci USA 97:14488–14493
20. **Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD** 2001 Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. Diabetes 50:123–130
21. **Zhao L, Bakke M, Krimkevich Y, Cushman LJ, Parlow AF, Camper SA, Parker KL** 2001 Steroidogenic factor 1 (SF1) is essential for pituitary gonadotrope function. Development 128:147–154
22. **Dellovade TL, Young M, Ross EP, Henderson R, Caron K, Parker K, Tobet SA** 2000 Disruption of the gene encoding SF-1 alters the distribution of hypothalamic neuronal phenotypes. J Comp Neurol 423:579–589
23. **Gruenewald DA, Hess DL, Wilkinson CW, Matsumoto AM** 1992 Excessive testicular progesterone secretion in aged male Fischer 344 rats: a potential cause of age-related gonadotropin suppression and confounding variable in aging studies. J Gerontol 47:B164–B170
24. **Kleyn PW, Fan W, Kovats SG, Lee JJ, Pulido JC, Wu Y, Berkemeier LR, Misumi DJ, Holmgren L, Charlat O, Woolf EA, Tayber O, Brody T, Shu P, Hawkins F, Kennedy B, Baldini L, Ebeling C, Alperin GD, Deeds J, Lasey ND, Culpepper J, Chen H, Glucksmann-Kuis MA, Carlson GA, Duyk GM, Moore KJ** 1996 Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. Cell 85:281–290
25. **Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER** 2000 Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci USA 97:12735–12740
26. **Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS** 2000 Increased adipose tissue in male and female estrogen receptor-α knockout mice. Proc Natl Acad Sci USA 97:12729–12734
27. **Canteras NS, Simerly RB, Swanson LW** 1994 Organization of projections from the ventromedial nucleus of the hypothalamus: a *Phaseolus vulgaris*-leucoagglutinin study in the rat. J Comp Neurol 348:41–79
28. **Tobet SA, Henderson RG, Whiting PJ, Sieghart W** 1999 Special relationship of γ-aminobutyric acid to the ventromedial nucleus of the hypothalamus during embryonic development. J Comp Neurol 405:88–98
29. **Pieri I, Klein M, Bayertz C, Gerspach J, van der Ploeg A, Pfizenmaier K, Eisel U** 1999 Regulation of the murine NMDA-receptor-subunit NR2C promoter by Sp1 and fushi tarazu factor1 (FTZ-F1) homologues. Eur J Neurosci 11:2083–2092
30. **Solano JM, Jacobson L** 1999 Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am J Physiol 277:E708–E716
31. **Tempel DL, Leibowitz SF** 1994 Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism. J Neuroendocrinol 6:479–501
32. **Arvaniti K, Ricquier D, Champigny O, Richard D** 1998 Leptin and corticosterone have opposite effects on food intake and the expression of UCP1 mRNA in brown adipose tissue of lep(ob)/lep(ob) mice. Endocrinology 139: 4000–4003
33. **Choi S, Wong LS, Yamat C, Dallman MF** 1998 Hypothalamic ventromedial nuclei amplify circadian rhythms: do they contain a food-entrained endogenous oscillator? J Neurosci 18:3843–3852
34. **Parkinson WL, Weingarten HP** 1990 Dissociative analysis of ventromedial hypothalamic obesity syndrome. Am J Physiol 259:R829–R835
35. **Robinson SW, Dinulescu DM, Cone RD** 2000 Genetic models of obesity and energy balance in the mouse. Annu Rev Genet 34:687–745
36. **Elmquist JK, Elias CF, Saper CB** 1999 From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 22:221–232
37. **Yen TT, Gill AM, Frigeri LG, Barsh GS, Wolff GL** 1994 Obesity, diabetes, and neoplasia in yellow A(vy)/− mice: ectopic expression of the agouti gene. FASEB J 8:479–488
38. **Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F** 1997 Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131–141
39. **Butler AA, Kesterson RA, Khong K, Cullen MJ, Pelleymounter MA, Dekoning J, Baetscher M, Cone RD** 2000 A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141: 3518–3521
40. **Coleman DL, Eicher EM** 1990 Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. J Hered 81: 424–427
41. **Santagata S, Boggon TJ, Baird CL, Gomez CA, Zhao J, Shan WS, Myszka DG, Shapiro L** 2001 G-protein signaling through tubby proteins. Science 292:2041–2050
42. **Noben-Trauth K, Naggert JK, North MA, Nishina PM** 1996 A candidate gene for the mouse mutation tubby. Nature 380:534–538
43. **Stubdal H, Lynch CA, Moriarty A, Fang Q, Chickering T, Deeds JD, Fairchild-Huntress V, Charlat O, Dunmore JH, Kleyn P, Huszar D, Kapeller R** 2000 Targeted deletion of the tub mouse obesity gene reveals that tubby is a loss-of-function mutation. Mol Cell Biol 20:878–882
44. **Nonogaki K, Strack AM, Dallman MF, Tecott LH** 1998 Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. Nat Med 4:1152–1156