Sim1 gene dosage modulates the homeostatic feeding response to increased dietary fat in mice

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Sim1 gene dosage modulates the homeostatic feeding response to increased dietary fat in mice. Am J Physiol Endocrinol Metab 287: E105–E113, 2004. First published February 24, 2004; 10.1152/ajpendo.00446.2003.—Haploinsufficiency of the transcription factor gene Sim1 has been previously implicated in hyperphagic obesity in humans and mice. To investigate the relation between Sim1 dosage and hyperphagia, we generated sim1-knockout mice and studied their growth and feeding behavior. Heterozygous mice weaned on standard chow consumed 14% more food per day than controls and developed obesity, hyperinsulinemia, and hyperleptinemia. The sim1 heterozygous mice were also significantly longer than controls. Heterozygous animals had modestly increased feeding efficiency, suggesting reduced energy expenditure, but voluntary wheel-running activity did not differ significantly between the two groups. We studied the effect of dietary fat on the feeding behavior of heterozygous sim1 mutant mice. The tempo and severity of weight gain were much greater in animals weaned on a high-fat diet. When acutely challenged with increased dietary fat, sim1 heterozygous mice weaned on the chow diet markedly increased their food consumption and caloric intake, whereas control mice reduced the mass of food they consumed and maintained approximately isocaloric intake. In wild-type adult mice, we detected Sim1 expression in the paraventricular and supraoptic nuclei, as previously reported in neonates, as well as in the amygdala and lateral hypothalamus, all regions implicated in feeding behavior. Our results indicate that Sim1 gene dosage modulates the homeostatic feeding response to increased dietary fat and likely plays a physiological role in the regulation of energy balance.

hypothalamus; transcription factor; feeding behavior

A MEMBER OF THE basic helix-loop-helix-PAS family of nuclear transcription factors is encoded by the Sim1 gene (6). Mouse Sim1 was originally cloned by virtue of its homology with Drosophila single-minded, a master regulator of central nervous system midline neurogenesis (9, 11). In mammals, Sim1 plays a more specialized role in brain development. The only anatomic defect that has been identified in homozygous sim1-knockout mice, which die shortly after birth, is the complete absence of neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus (22). The PVN produces corticotropin-releasing hormone, thyrotropin-releasing hormone, and somatostatin, which regulate ACTH, thyroid-stimulating hormone, and growth hormone secretion by the anterior pituitary. The PVN and SON also synthesize oxytocin and vasopressin, which are released by the posterior pituitary. In addition to its endocrine functions, the PVN has been known for decades to play a key role in energy balance. Lesions of the PVN in adult dogs (15) or rats (19) result in hyperphagic obesity. The PVN integrates orexigenic (e.g., neuropeptide Y and Agouti-related peptide) and anorexigenic (e.g., α-melanocyte-stimulating hormone and cocaine- and amphetamine-regulated transcript) signals from neurons of other hypothalamic nuclei and translates these signals into changes in feeding behavior and sympathetic nervous system activity (5).

Sim1 was first implicated in body weight regulation by the finding that the gene was disrupted by a balanced translocation between chromosomes 1 and 6 in a girl with early-onset obesity, hyperphagia, and accelerated linear growth (16). Visible deletions of the region of chromosome 6 containing Sim1 have also been associated with early-onset obesity in boys and girls (10, 13, 28, 29), leading us to hypothesize that Sim1 haploinsufficiency causes early-onset obesity in humans. Subsequently, Michaud et al. (21) demonstrated that heterozygous sim1-knockout mice develop hyperphagic obesity with normal metabolism and energy expenditure. They proposed that the obesity was due to a developmental defect resulting in subtle hypocellularity of the PVN, with no effect on any particular cell type.

Here, we report the phenotype of independently generated heterozygous sim1 mutant mice and their feeding behavior in response to change in dietary fat content. Similar to the previously reported mutant, our heterozygotes were hyperphagic and gradually became obese, hyperinsulinemic, and hyperleptinemic when fed a standard rodent chow diet. Mice weaned on a high-fat (HF) diet showed a dramatic increase in their excess energy intake, resulting in earlier-onset obesity and associated insulin and leptin resistance. When mice weaned on the chow diet were challenged with the HF diet, sim1 heterozygotes substantially increased the food mass and calories they consumed, whereas controls reduced the mass of food they ate to maintain approximately isocaloric intake. Our results demonstrate that Sim1 acts in neural circuits that regulate feeding in response to dietary fat. In adult mice, Sim1 was expressed not only in the PVN and SON, as previously noted in newborns (22), but also in the basomedial amygdala and the

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lateral hypothalamic area (LHA), two other regions implicated in feeding. We propose that Sim1 has a physiological as well as a developmental function in the hypothalamic regulation of energy balance.

MATERIALS AND METHODS

Generation of knockout mice and genotyping. A “floxed” pGK-neo cassette and a loxP site in Sim1 exon 2 were introduced into the Sim1 locus by homologous recombination in RW-4 ES cells using positive-negative selection (Fig. 1A). All three loxP sites were in the same orientation. Germ-line deletion of Sim1 exon 2 was induced by crossing mice heterozygous for the three-loxP sim1 allele to FVB transgenic mice expressing Cre recombinase driven by a ubiquitous EIIa promoter (18). Progeny were screened by PCR for loss of the pGK-neo cassette, part of Sim1 intron 2, and part of Sim1 exon 2 containing the translation start site. Animals were genotyped by multiplex PCR using forward primer 1 (5’-CATTCGTCTCTCAGGAGAAACTTC-3’, 500 pM) and reverse primers 2 and 3 (5’-TTTCTGTGCTGCTGGGGTAGGTTT-3’ and 5’-CGAGGAATTCGATCATATTCAAT-3’, 250 pM each). The annealing temperature was 60°C. The wild-type and the three-loxP mutant sim1 alleles yielded a PCR product with primers 1 and 2 only (intron 2 is not drawn to scale), whereas the deleted allele yielded a product with primers 1 and 3. To maintain the line, sim1 heterozygotes were backcrossed to inbred C57BL6 mice. All studies were approved by the UT Southwestern Institutional Animal Care and Use Committee.

Growth and feeding studies. Mice were kept on a 12:12-h light-dark cycle, with lights on at 6 AM. Animals of each gender were housed in groups for measurements of length and body weight and individually caged for 5 days before measurements of food intake. Nose-to-anus length was determined at necropsy.

Mice were fed a low-fat (LF) chow diet (Teklad, Madison, WI; 2.94 kcal/g, with 46.8% available carbohydrate, 4.0% available fat, and 24.0% available protein) or an HF diet (Research Diets, New Brunswick, NJ; 5.24 kcal/g, with 26.3% available carbohydrate, 34.9% available fat, and 26.2% available protein). Feeding efficiency was calculated by dividing the change in mass (mg) by the food intake (kcal), measured over a 7-day period.

Dual-energy X-ray absorptiometry. Six-month-old animals were anesthetized with a mixture of ketamine, xylazine, and acepromazine. Body fat content and bone mineral density (BMD) were measured using a Lunar PIXIImus densitometer calibrated with an aluminum/
Lucite phantom as described by the manufacturer (GE Medical Systems, Madison, WI).

**Wheel-running assay.** Mice were individually housed for ≥7 days before data collection. Wheel turns were measured every 5 min and collected for 5 days in animals fed LF' and HF chow. Each revolution was counted by magnetic switch closures, with magnets placed on the revolving wheel, with use of a data acquisition system (Data Systems International, Overland Park, KS).

**Serum insulin and leptin assays.** Animals were killed by CO₂ asphyxiation at 6 mo of age for mice fed the LF diet or 4 mo of age for mice fed the HF diet. All mice were killed between 1:00 and 2:30 PM. Blood was obtained by exsanguination, and serum was collected. Serum glucose concentration was measured using the One Touch Basic glucometer (Johnson and Johnson, Milpitas, CA). Insulin levels were measured using a rat insulin RIA kit (Linco Research, St. Charles, MO). Leptin levels were measured using a mouse leptin EIA kit (R & D Systems, Minneapolis, MN).

**In situ hybridization.** Adult male C57BL6 mice were anesthetized and killed. Brains were harvested, frozen, and processed with a cryotome to yield 14-μm sections. Slides were fixed for 20 min in 4% paraformaldehyde, acetylated, dehydrated, and air dried. A digoxigenin-labeled Sim1 antisense probe was generated by in vitro transcription of a cDNA containing the Sim1 open reading frame that was digested with XhoI to yield a 650-bp 3’ fragment. Each slide was incubated with 30 ng of digoxigenin-labeled probe in 150 μl of hybridization mix (50% formamide, 5× SSC, pH 7.0, 250 μg/ml yeast RNA, 500 μg/ml salmon testes DNA, and 5× Denhardt solution) at 60°C overnight. Slides were washed for 30 min each in 5× SSC at 60°C and 2× SSC-50% formamide at 65°C and for 5 min each in 2×, 0.2×, and 0.1× SSC. The sections were then blocked with 5% normal rabbit IgG in buffer containing 0.2 M Tris, pH 7.5, 1 M NaCl, and 0.1% Tween (TBST) for 30 min at room temperature, incubated for 60 min in TBST containing a 1:200 dilution of horseradish peroxidase-conjugated rabbit antidigoxigenin antibody (catalog no. P5104, Dako), washed twice for 7 min in TBST, and then incubated for 10 min in TBST containing a 1:50 dilution of horseradish peroxidase-conjugated rabbit antidigoxigenin antibody. The slides were then washed with H2O, dehydrated through 70, 90, and 100% ethanol, washed twice with Citrosolve (Fisher Scientific, Pittsburgh, PA), and counterstained with 4',6-diamidino-2-phenylindole counterstain to visualize cell nuclei. Monochrome images were captured sequentially using an Olympus fluorescence microscope and appropriate filters.

Sim1 and Mc4r mRNA quantitation. Hypothalami were dissected from fresh brains, and total RNA was extracted using RNeasy reagent according to the manufacturer (Qiagen, Valencia, CA). Quantitative

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![Fig. 2. Growth curves of sim1 heterozygotes vs. controls. A, C, and E: low-fat (LF) diet. B, D, and F: high-fat (HF) diet.](http://ajpendo.physiology.org/Downloadedfromhttp://ajpendo.physiology.org/)
real-time PCR was performed using an Opticon instrument and DyNAmo HotStart SYBR green qPCR kit (MJ Research, Waltham, MA). Sim1 and Mc4r measurements for individual samples were normalized to β-actin mRNA levels. Primers sequences were 5’-GAGCAGGCAAGGTACTT-3’ and 5’-CTGACACACTATCTTCAT-3’ (Sim1), 5-GGTCCGGATCATAAGTTGTA-3’ and 5’-CATCAGGAACATGAGCATAAGA-3’ (Mc4r), and 5’-GACGGATGCTCCCCGGGCTGTATTC-3’ and 5’-TCTCTGCTCTGGGCTCTGACC-3’ (β-actin). All reactions were performed at 57°C annealing temperature.

Statistical analysis. Data were analyzed using Microsoft Excel and plotted using Prism software (GraphPad Software, San Diego, CA). Values are means ± SE. Unless otherwise noted, means were compared using two-tailed t-tests, with Welch’s correction if F test results indicated unequal sample variances. Differences were considered statistically significant at P < 0.05.

RESULTS

Targeted inactivation of Sim1. We deleted the translation start site and the first 17 amino acids encoding the basic domain of SIM1 using Cre/loxP technology (Fig. 1). Replacement of the same portion of the Sim1 gene with pGK-neo was previously shown to create a null allele (22). Our homozygous mutants showed uniform perinatal lethality without any gross anatomic defects, as described for the previous sim1−/− knockouts (22). Because our mice had a mixed 129/C57BL6/FVB genetic background, wild-type littermates were used as controls for all experiments.

Growth of sim1 heterozygotes. Three-week-old mice were weaned onto chow containing 4% dietary fat (LF) or 35% dietary fat (HF), allowed to feed ad libitum, and weighed weekly (Fig. 2, A–D). At 8 wk of age, heterozygous females weighed 13% more than controls fed the LF diet (Fig. 2A) and 71% more than controls fed the HF diet (Fig. 2B). Heterozygous males were similarly affected, weighing at the same age 22% more than controls fed the LF diet (Fig. 2C) and 51% more than those fed the HF diet (Fig. 2D). Heterozygotes of either gender continued to gain weight until ≥6 mo of age on the LF diet (Fig. 2, A and C) and 4 mo of age on the HF diet (Fig. 2, B and D).

Heterozygous sim1 mutant mice were also longer than their wild-type littermates (Fig. 2, E and F). The relative differences in length were similar for males and females fed the LF or HF diet.

Body composition of sim1 heterozygotes. To determine whether the increased mass of sim1 heterozygotes was due to increased adiposity, we measured the body composition of 6-mo-old sim1 mutants vs. controls fed the LF diet by dual-energy X-ray absorptiometry (DEXA). Heterozygous males had more than twice as much fat mass as controls, and heterozygous females had more than three times as much (Fig. 3A). Lean mass of male and female mutants was also increased by >20% compared with controls (Fig. 3B), consistent with their increased length. The percent body fat of heterozygotes was also increased compared with controls, with females more severely affected (Fig. 3C). Thus the increased weight of sim1 heterozygotes was primarily due to increased body fat. Whole body BMD of heterozygotes, measured by DEXA, did not significantly differ from that of controls (Fig. 3D).

Feeding behavior of sim1 heterozygotes. We measured daily food intake of mice fed the LF chow diet over a 5-day period (Fig. 4, A and B, 1st data point). Male and female heterozygotes consumed more food per day [+13% for males (P < 0.05) and +14% for females (P < 0.05)] than same-gender

Fig. 3. Dual-energy X-ray absorptiometry measurement of body composition of sim1 heterozygotes vs. controls fed the LF diet. A: fat mass. B: lean mass. C: percent body fat. D: bone mineral density. Values are means ± SE of 5 male sim1+/−, 5 male sim1+/+, 5 female sim1+/+, and 4 female sim1+/− mice.
controls. We then challenged these same mice with the HF diet for 7 days. On day 1 there was an obvious difference between the sim1 mutants and controls. Heterozygous males showed a statistically insignificant 5% increase in the mass of food consumed ($P > 0.5$), whereas controls showed a significant 16% decrease ($P < 0.05$). Females showed similar responses.

Food consumption was summed over the last 5 days of the HF challenge, after daily food consumption had stabilized (Fig. 4, A and B, 1st shaded area). In contrast to the 14% increase in food consumption in animals fed the LF diet, heterozygous males consumed 46% more than controls ($P < 0.0001$) and heterozygous females consumed 68% more than controls ($P < 0.0001$). Thus, on a per-gram basis, there was a three- to fourfold increase in the percent difference in food intake of heterozygotes vs. controls fed the HF vs. the LF diet. When the higher caloric density of the HF diet was taken into account, heterozygotes increased their energy consumption by 57% (males) or 49% (females) when shifted from the LF to the HF diet. By contrast, controls increased their energy consumption by only 20% (males) or 21% (females) after they were switched from the LF to the HF diet.

The mice were then switched back to the LF diet. Food consumption by heterozygotes and controls decreased substantially immediately after the change in diet. This decrease may reflect less palatability of the LF diet. Food consumption gradually returned to normal over the next week. On rechallenge with the HF diet, heterozygotes of either gender were again abnormally hyperphagic (Fig. 4, A and B, 2nd shaded area).

Feeding efficiency and voluntary wheel-running activity of sim1 heterozygotes. Feeding efficiency (weight gained per kilocalorie ingested) of wild-type and heterozygous mice was measured during the first HF challenge week and the subsequent LF diet week. Feeding efficiency was increased in mutant mice fed the LF or HF diet (Fig. 4, C and D). Heterozygous males had 2.5- or 1.9-fold greater feeding efficiency than controls fed the HF or LF diet, respectively. Heterozygous females had two- or threefold greater feeding efficiency than controls fed the LF or HF diet, respectively. Heterozygous mice of either gender fed the HF diet and heterozygous males, but not females, fed the LF diet showed modestly decreased wheel-running activity compared with controls, but the differences were not statistically significant.
Serum glucose, insulin, and leptin levels in sim1 heterozygotes. Serum glucose levels of 6-mo-old heterozygous mice fed the LF diet did not significantly differ from control levels (Fig. 5A). Female, but not male, heterozygotes weaned onto the HF diet had slightly increased serum glucose levels compared with controls (Fig. 5B). Heterozygotes weaned on the LF diet had higher serum insulin levels than controls (Fig. 5C). However, young (6- to 8-wk-old) heterozygotes had normal insulin levels (data not shown), suggesting that the subsequent hyperinsulinemia was secondary to obesity. Weaning onto the HF diet increased the serum insulin levels of mutants and controls to the same extent (Fig. 5D). Serum leptin levels were also elevated in heterozygous mice of either gender fed the LF diet (Fig. 5E). The mean leptin level of the female, but not the male, heterozygotes fed the HF diet tended to be greater than that of the controls, but the difference was not statistically significant (Fig. 5F).

Sim1 expression pattern in adult brain. Sim1 is expressed in the brains of newborn mice at the highest levels in the PVN and SON (22), consistent with the absence of these nuclei in homozygous sim1 mutant mice (21). To better understand the heterozygous phenotype, we used sensitive RNA in situ hybridization with TSA to examine the pattern of expression of Sim1 in the brains of wild-type adult mice (Fig. 6). Sim1 continues to be expressed in the PVN and SON in adults. We also detected Sim1 expression in neurons of the basomedial amygdala and the LHA as well as the anterior hypothalamus.

Sim1 and Mc4r mRNA levels in heterozygous mice. We measured the steady-state levels of Sim1 mRNA in the hypothalamus of mutant vs. wild-type mice by real-time quantitative RT-PCR. Sim1 mRNA levels in heterozygotes were reduced on average by ~27% (P < 0.05) compared with wild-type littermates (Fig. 7A). We also measured hypothalamic Mc4r mRNA levels but saw no difference between wild-type and sim1 heterozygous mice (Fig. 7B).

DISCUSSION
A previous sim1 knockout was made by replacing the first coding exon of Sim1 (exon 2) with a pGK-neo minigene (21), raising the possibility that the targeted mutation interfered with expression of neighboring gene(s). We generated a second
null allele using Cre/loxP technology to delete the same Sim1 exon 2 without leaving a neo cassette. The mutation results in a mean 27% reduction in steady-state hypothalamic Sim1 mRNA levels in heterozygotes. The targeted sim1 allele produces a truncated transcript that is detected by our assay, probably explaining why the reduction in Sim1 mRNA was less than the expected 50%. Michaud et al. (21) detected a similar truncated transcript in their sim1-knockout mice. Alternatively, the heterozygous mutation may be partially compensated during hypothalamic development by upregulation of the normal sim1 allele. Our data confirm that hyperphagic obesity is the result of sim1 haploinsufficiency and corroborate the role of SIM1 in energy balance hypothesized from genetic findings in humans (16). In the present study, we also show that sim1

Fig. 6. In situ hybridization showing Sim1 expression in coronal section of adult wild-type mouse brain [bregma −1.0 mm (26)]. A: composite ×20 inverted 4,6-diamidino-2-phenylindole image of entire section showing cell nuclei. 3V, 3rd ventricle; OT, optic tract; Fx; fornix, PVN, paraventricular nucleus. B: ×20 Cy3 image showing Sim1 hybridization. C: ×40 view of area outlined in B. SON, supraoptic nucleus; LH, lateral hypothalamic area; AH, anterior hypothalamic area; BMA, basomedial amygdala.

Fig. 7. Hypothalamic mRNA levels of Sim1 (A; 1-tailed t-test) and melanocortin receptor type 4 (Mc4r) genes (B) in 6-wk-old Sim1+/+ (n = 9) or sim1+/− (n = 8) female mice.

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heterozygous mice have a defective acute feeding response to increased dietary fat.

It is interesting to compare the behavioral phenotypes of heterozygous sim1 and homozygous mc4r mutant mice. Both mutants significantly increased their acute caloric intake in response to an increase in dietary fat content (4). Homozygous mc4r mutant mice fed a moderate-fat diet showed increased feeding efficiency and decreased wheel-running activity compared with controls (4). The sim1 heterozygotes fed the LF and HF diets showed increased feeding efficiency and a trend toward reduced wheel-running activity on the HF diet, although this difference was not statistically significant because of a large interanimal variation. The previously reported heterozygous sim1 mice had normal feeding efficiency and voluntary locomotor activity (21). This discrepancy could be due to differences in the age of the mice, their diets, their genetic backgrounds, or subtle environmental factors (7). Finally, Sim1- and Mc4r-deficient mice, both have increased linear growth (17), a phenotype not typical of other rodent monogenic obesity models.

The similar growth and feeding behavior phenotypes of sim1 and mc4r mutant mice strengthen the speculation that these two genes act within the same hypothalamic pathway(s) (16). Whether there is a molecular interaction between the two genes is not known. Both are expressed in the PVN and SON (22, 23), although coexpression in individual neurons has not been demonstrated. We found no obvious difference in Mc4r mRNA levels in the hypothalamus of sim1 heterozygotes vs. wild-type littermates (Fig. 7B), suggesting that Mc4r may not be a Sim1 transcriptional target. However, this result must be interpreted with caution. There may be a subset of neurons that coexpress Sim1 and Mc4r in which the Mc4r mRNA level changes, but this difference could be obscured by Mc4r expression in other neurons.

The girl we previously reported with a heterozygous SIM1 mutation also had an elevated BMD (t-score +2.3 SD, age matched, 67 mo). By contrast, heterozygous sim1 mice did not show increased BMD. This may reflect a species difference in the phenotype of sim1 haploinsufficiency. Alternatively, the data may reflect insensitivity of DEXA to detect regional differences in bone mineral content in mice. A similar species difference in BMD effects may be true for mutations affecting melanocortin signaling: humans with MC4R mutations generally have elevated BMD (12), whereas BMD is normal in ApoE mice (8), in which central melanocortin signaling is blocked by ectopic Agouti expression.

The feeding abnormalities of sim1 heterozygous mice were, in general, similar in male and female mice. Hormonal abnormalities were also similar in male and female mice fed the LF diet. Males of both genotypes fed the HF diet were markedly hyperinsulinemic; conversely, females fed the HF diet showed a greater degree of hyperleptinemia. The further increases in serum insulin levels in sim1 heterozygous males and in serum leptin levels in sim1 heterozygous females weaned on the HF diet were not statistically significant, perhaps because the levels in the control animals were already greatly elevated by this diet. Gender × environment × diet effects have been noted in other rodent obesity models and are not well understood (3).

Neuroanatomic, pharmacologic, and genetic studies have identified key pathways within the hypothalamus that modulate food intake and energy expenditure. Most attention has focused on intercellular signaling molecules, such as α-melanocyte-stimulating hormone, neuropeptide Y, melanin-concentration hormone, and their receptors, which are attractive pharmacological targets. However, it is well known that feeding or fasting alters the expression of many neuropeptides. The mechanism of this regulation is unknown but likely involves transcription factors downstream of signaling pathways. Transcription is especially attractive as a mechanism for long-term regulation of food intake and energy expenditure, as has been demonstrated for other complex behaviors such as addiction (24). Mutations in four mouse genes encoding known or putative transcription factors, Tubby, Nlhh2, Sf1, and Sim1, have been shown to cause obesity (2, 14, 20, 21). The molecular mechanisms by which mutations in these genes result in obesity have not been elucidated, largely because their transcriptional targets relevant to feeding behavior have not been identified.

In the central nervous system of newborn mice. Sim1 is expressed at the highest levels in the PVN and SON (22). The PVN has been implicated in regulation of feeding by lesioning studies, and rats with hyperphagic obesity due to electrolytic PVN lesions showed the same diet effect as sim1 mutant mice (19). The hyperphagia of sim1 heterozygotes was previously attributed to a 24% mean decrease in PVN cellularity compared with controls, with no specific neuronal subtype affected (21). However, this subtle defect may not be the sole cause of obesity in these mice. First, even complete bilateral PVN lesioning in weanling female rats did not result in hyperphagia or increased linear growth (1), suggesting that the newborn hypothalamus has functional plasticity that can compensate for congenital lesions. Second, Sim1 is expressed in adult mice not only in the PVN and SON but also in the LHA and the amygdala, brain regions that are implicated in feeding behavior. All these regions also express Mc4r (23). Previous studies that did not report Sim1 expression outside the PVN and SON used less sensitive in situ hybridization techniques or examined only fetal or newborn mice (22, 25). We propose that, in Sim1+/− adults, dysfunction of Sim1-expressing neurons in the PVN, LHA, or amygdala, rather than their absence, causes hyperphagia and obesity.

Conditional inactivation of Sim1 using an inducible Cre/loxP system (27) might tease apart its pre- and postdevelopmental functions in different brain regions. Identification of Sim1 transcriptional targets will also provide insight into its proposed role in modulating feeding behavior. Regardless of the precise mechanism of obesity in sim1+/− mice, these animals provide a model for human hyperphagic obesity and a new tool for dissecting the neural circuits involved in the homeostatic response to increased dietary fat.

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