The stimulatory G protein Gsα is required in melanocortin 4 receptor–expressing cells for normal energy balance, thermogenesis, and glucose metabolism

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Edited by Jeffrey E. Pessin

Central melanocortin 4 receptors (MC4Rs) stimulate energy expenditure and inhibit food intake. MC4Rs activate the G protein Gsα, but whether Gsα mediates all MC4R actions has not been established. Individuals with Albright hereditary osteodystrophy (AHO), who have heterozygous Gsα-inactivating mutations, only develop obesity when the Gsα mutation is present on the maternal allele because of tissue-specific genomic imprinting. Furthermore, evidence in mice implicates Gsα imprinting within the central nervous system (CNS) in this disorder. In this study, we examined the effects of Gsα in MC4R-expressing cells on metabolic regulation. Mice with homozygous Gsα deficiency in MC4R-expressing cells (MC4RGsKO) developed significant obesity with increased food intake and decreased energy expenditure, along with impaired insulin sensitivity and cold-induced thermogenesis. Moreover, the ability of the MC4R agonist melanotan-II (MTII) to stimulate energy expenditure and to inhibit food intake was impaired in MC4RGsKO mice. MTII failed to stimulate the secretion of the anorexigenic hormone peptide YY (PYY) from enterocortine L cells, a physiological response mediated by MC4R–Gsα signaling, even though baseline PYY levels were elevated in these mice. In Gsα heterozygotes, mild obesity and reduced energy expenditure were present only in mice with a Gsα deletion on the maternal allele in MC4R-expressing cells, whereas food intake was unaffected. These results demonstrate that Gsα signaling in MC4R-expressing cells is required for controlling energy balance, thermogenesis, and peripheral glucose metabolism. They further indicate that Gsα imprinting in MC4R-expressing cells contributes to obesity in Gsα knockout mice and probably in individuals with Albright hereditary osteodystrophy as well.

Gsα is a ubiquitously expressed G protein α-subunit that couples various hormone and neurotransmitter receptors, including melanocortin receptors, to adenylly cyclase, leading to increased generation of intracellular cAMP. Central melanocortins act via melanocortin receptors, such as melanocortin 4 (MC4R) receptors, which are expressed in distinct regions of the central nervous system (CNS) (1) and have divergent effects on energy and glucose homeostasis, thermogenesis, and cardiovascular function (2–5). Both patients with MC4R mutations (6, 7) and MC4RKO mice (8, 9) develop obesity associated with hyperphagia and reduced energy expenditure, as well as increased linear growth.

Gsα is encoded by the gene GNAS (Gnas in mice) that undergoes genomic imprinting, an epigenetic phenomenon leading to differential expression from the two parental alleles. Heterozygous loss-of-function mutations of Gsα lead to Albright hereditary osteodystrophy (AHO), which presents with skeletal and neurobehavioral abnormalities. AHO patients who inherit the mutation maternally or who have a de novo mutation on the maternal allele also develop multihormone resistance, including insulin resistance, as well as early-onset obesity, whereas these features are absent in patients with mutations on the paternal allele (10–12). Similarly, mice with germ line Gsα deletion on the maternal allele develop obesity and insulin-resistant diabetes, whereas these features are absent in mice with paternal Gsα deletion (13–15). This parent-of-origin–specific metabolic phenotype is due to Gsα being imprinted in a tissue-specific manner (16). Gsα imprinting in

This work was supported in whole by the Intramural Research Program of NIDDK, National Institutes of Health. The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

This article contains Figs. S1 and S2.

5 The abbreviations used are: MC4R, melanocortin 4 receptor; CNS, central nervous system; AHO, Albright hereditary osteodystrophy; MC4RGsKO, mMC4RGsKO, and pMC4RGsKO, mice with homozygous, maternal-, and paternal-specific loss of Gsα in MC4R-expressing cells, respectively; DMH, dorsomedial hypothalamus; mBrGsKO, mice with CNS-specific mutation of the Gsα maternal allele; PVN, paraventricular nucleus of the hypothalamus; BAT and WAT, brown and white adipose tissue, respectively; eWAT and iWAT, epididymal and inguinal WAT, respectively; IBAT, inguinal BAT; REE and TEE, resting and total energy expenditure, respectively; SNS, sympathetic nervous system; PYY, peptide YY; GLP-1, glucagon-like peptide 1; MTII, melanotan-II; i.p., intraperitoneal; H&E, hematoxylin and eosin; GTT, glucose tolerance test; AUC, area under the curve; ITT, insulin tolerance test; RT, room temperature.
**Results**

**Gsα deficiency in MC4R-expressing cells leads to severe obesity**

Mice with homozygous deletion of Gsα in MC4R-expressing cells (Mc4RGsKO mice) were generated by breeding Gsα-floxed mice (E1\[^{+/−}\]) with Mc4R-cre mice. To confirm proper targeting of the MC4R-cre line and specific loss of Gsα in targeted cells, Mc4R-cre:E1\[^{+/−}\] mice were serially mated with Ai14 reporter mice with a Cre-dependent tdTomato transgene. No tdTomato staining was observed in the heart muscle, quadriceps muscle, or BAT of Mc4RGsKO mice (data not shown). The present study investigated the consequences of Gsα deficiency in MC4R-expressing cells and provided evidence that Gsα signaling in MC4R-expressing cells plays a critical role in the control of food intake and energy expenditure, thermogenesis, and peripheral glucose metabolism. In addition, we show that Gsα imprinting within these cells contributes to the parent-of-origin–specific metabolic phenotype observed with Gsα mutations.

Mc4RGsKO offspring were born and weaned at expected Mendelian ratios. Mc4RGsKO mice steadily gained more weight than their littermate controls starting after 5 weeks of age, and by 12 weeks of age, male Mc4RGsKO mice were 123% and female Mc4RGsKO mice were 143% heavier than their littermate controls. This increased weight gain was due to a marked increase in fat mass, with a lesser increase in lean mass (Fig. 2B). Mc4RGsKO mice also had a small, but significant, increase in body length (Fig. 2C). Consistent with their increased fat mass, Mc4RGsKO mice had enlarged adipocytes in both brown and white adipose tissue (BAT and WAT, respectively) with greater intracellular lipid accumulation as compared with controls (Fig. 2J) and had markedly increased serum leptin levels (Table 1). Mc4RGsKO mice also developed obesity with increased fat mass and a lesser increase in lean mass when raised at thermoneutrality (30 °C), where sympathetic nervous system (SNS) activity is at a minimum (Fig. S1, A and B).

Mice with a heterozygous deletion within the Gsα maternal allele in MC4R-expressing cells (mMc4RGsKO) also gained significantly more weight than controls (Fig. 2, D and E), although the differences were much less than those observed with homozygous MC4RGsKO mice (Fig. 2A). Both male and female mMc4RGsKO mice had significantly greater fat mass and body length, whereas lean mass was only slightly greater in male mMc4RGsKO mice (Fig. 2, E and F). VAT adipocytes tended to be slightly enlarged, although the differences in cell size did not reach statistical significance (Fig. 2K). Similar small increases in body weight and fat mass were also observed in mMc4RGsKO mice maintained at thermoneutrality (Fig. S1, C).
and D). In contrast, G_α deficiency within the paternal allele in MC4R-expressing cells in pMC4RGsKO mice showed no changes in body weight, composition, or length or histological appearance of WAT or BAT (Fig. 2, G–I and K). Consistent with a parent-of-origin effect of G_α mutation on fat mass, serum leptin levels were increased in mMC4RGsKO mice but were unaffected in pMC4RGsKO mice (Table 1). These results indicate that G_α in MC4R-expressing cells is essential for the control of energy homeostasis and that there is a parent-of-origin effect of G_α deletion in heterozygotes consistent with G_α imprinting within a population of MC4R-expressing cells that are important for metabolic regulation.

**MC4RGsKO mice are hyperphagic**

To avoid the impact of the large differences in body weight and adiposity that developed between the mutants and controls, we determined whether the observed differences were due to increased food intake and energy expenditure. To do this, we measured body weight and body composition curves in male and female mice on a Chow diet for 12 weeks (Fig. 2A–C). While mMC4RGsKO mice showed significant increases in body weight curves (A) and body composition (B), body length (C) of mMC4RGsKO and their control littermates (n = 7–12/group). Shown are body weight curves (D), body composition (E), and body length (F) of pMC4RGsKO and their control littermates (n = 6–7/group). H&E-stained sections of BAT, eWAT, and iWAT from MC4RGsKO mice and their control littermates (J) and mMC4RGsKO and pMC4RGsKO mice and their respective control littermates (K) are shown. Shown below each panel is quantification of cell areas of adipocytes relative to controls (n = 3/group). Scale bar, 100 μm. Data are expressed as mean ± S.E. (error bars). *, p < 0.05; **, p < 0.01 versus controls.

**Table 1**

| Serum chemistries in male MC4RGsKO, mMC4RGsKO, pMC4RGsKO, and control mice in the fed state |
|---|
| Control | mMC4RGsKO | pControl |
| Glucose (mg/dl) | 126 ± 7 | 421 ± 25* | 92 ± 5 |
| Insulin (ng/ml) | 19.0 ± 0.5 | 194 ± 80.3* | 3.0 ± 0.5 |
| Leptin (ng/ml) | 11 ± 2 | 100 ± 12* | 6 ± 2 |
| FFA (mg/dl) | 0.70 ± 0.10 | 0.74 ± 0.10 | 0.72 ± 0.10 |
| TG (mg/dl) | 169 ± 37 | 342 ± 108 | 131 ± 41 |
| Cholesterol (mg/dl) | 111 ± 10 | 144 ± 18 | 94 ± 10 |
| GLP-1 (pg/ml) | 41 ± 3 | 94 ± 8* | 43 ± 4 |

Data are presented as mean ± S.E., n = 5–8/group. FFA, free fatty acids; TG, triglycerides. *, p < 0.05 versus control.
**Figure 3. Energy balance in MC4RgsKO mice.** A and B, body weight curves (A) and daily food intake averaged by week (B) measured in male MC4RgsKO mice and control littermates housed at 22 °C from 7 to 10 weeks of age (n = 6/group; week 0 is at 7 weeks of age). C, plots of mean daily total energy expenditure versus mean body weight for individual mice from A and B with linear regression lines. D and E, body weight curves (D) and daily food intake averaged by week (E) measured in male MC4RgsKO mice and control littermates housed at thermoneutrality (30 °C) from 5 to 7 weeks of age (n = 7–9/group; week 0 in D is at 5 weeks of age). F, plots of mean daily total energy expenditure versus mean body weight for individual mice from D and E with linear regression lines. G, respiratory exchange ratios (RER; vCO2/vO2) measured at 22 °C in 3–4-month-old male MC4RgsKO mice and control littermates (n = 5/group). H, total and ambulatory (Amb) activity levels measured by beam break interruption were significantly reduced in MC4RgsKO mice at 22 °C (Fig. 4, C). TEE measured by indirect calorimetry (normalized to lean mass) was significantly reduced, whereas resting energy expenditure (REE) tended to be reduced in MC4RgsKO mice at 22 °C (Fig. 4, A). No differences in RER or TEE were observed in these mice (Fig. 4, B and C). No differences in food intake, energy expenditure, or activity levels were observed in pMC4RgsKO mice (Fig. 4, D–F). Therefore, the mild obesity observed in mMC4RgsKO mice is primarily the result of a small decrease in energy expenditure, consistent with what has been observed in mice with heterozygous disruption of the maternal Gnas allele in other mouse models (17, 18).
MC4RGsKO and mMC4RGsKO mice have impaired responses to an MC3/4R agonist

We previously showed that whole brain–specific loss of \( G_\alpha \) expression from the maternal allele (mBrGsKO) leads to an impaired energy expenditure response to the MC3/4R agonist melanotan-II (MTII), whereas the food intake response to MTII is unaffected (17). To determine the extent to which \( G_\alpha \) deficiency in MC4R-expressing cells alters responses to a melanocortin agonist, we measured the energy expenditure and food intake responses to MTII in homozygous and heterozygous MC4RGsKO mice. MTII-stimulated oxygen consumption (a measure of energy expenditure) was markedly reduced in MC4RGsKO and mMC4RGsKO, but not in pMC4RGsKO, mice (Fig. 5, A and D). This is consistent with impaired melanocortin stimulation of energy expenditure as the underlying cause of the obesity associated with maternal \( G_\alpha \) mutation and confirms that \( G_\alpha \) imprinting in a population of MC4R-expressing cells plays a role in the parent-of-origin–specific effects of \( G_\alpha \) deletion on energy expenditure observed in mBrGsKO and pBrGsKO mice, respectively (17).

Similar to what was observed previously in mBrGsKO and pBrGsKO mice (17), the food intake response to MTII was unaffected in both mMC4RGsKO and pMC4RGsKO mice (Fig. 5E), which is consistent with the obesity in mMC4RGsKO mice being primarily due to lower energy expenditure. Interestingly, the ability of MTII to inhibit food intake in homozygous MC4RGsKO mice was impaired (Fig. 5B), suggesting that resistance to the effects of melanocortins on food intake may contribute to the hyperphagia observed in these mice.

MC4Rs are expressed in cells outside of the CNS, including the enteroendocrine L cells in the gastrointestinal tract (25), where their activation stimulates secretion of peptide YY (PYY), a circulating peptide that acts to reduce appetite, and this response is mediated by MC4R activation of \( G_\alpha \) (26). Loss of PYY secretion from L cells in response to MTII due to \( G_\alpha \) deficiency in these cells could be one potential explanation for the impaired food intake response to MTII in MC4RGsKO mice. Whereas circulating PYY levels significantly increased in response to MTII in control mice, there was no significant response to MTII in MC4RGsKO mice, although this lack of response may reflect the fact that baseline PYY levels were significantly increased in MC4RGsKO and control littersmates (n = 4–8/group). To determine the extent to which \( G_\alpha \) deficiency in MC4R-expressing cells leads to obesity (27), the food intake response to MTII in male MC4RGsKO and control littersmates (n = 4–7/group) was markedly reduced in MC4RGsKO and mMC4RGsKO, but not in pMC4RGsKO, mice (Fig. 5B, D, and E). This is consistent with impaired melanocortin stimulation of energy expenditure as the underlying cause of the obesity associated with maternal \( G_\alpha \) mutation and confirms that \( G_\alpha \) imprinting in a population of MC4R-expressing cells plays a role in the parent-of-origin–specific effects of \( G_\alpha \) deletion on energy expenditure observed in mBrGsKO and pBrGsKO mice, respectively (17).

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Loss of Gs/H9251 in MC4R-expressing cells alters glucose metabolism

Homozygous MC4RGsKO mice had significantly increased baseline glucose, insulin, and leptin levels (Table 1) and severe glucose intolerance and insulin resistance (Fig. 6 A and B and Fig. S2 A and B)) when measured at 12–20 weeks of age, well after the establishment of obesity. In contrast, these mice showed no significant differences in serum-free fatty acid, triglyceride, or cholesterol levels (Table 1). To determine whether loss of Gs/α expression in MC4R-expressing cells directly alters glucose metabolism, we studied MC4RGsKO mice at 5–6 weeks of age, before the onset of obesity. At this age, when body weight was still normal (Fig. 6 G), MC4RGsKO mice had impaired glucose tolerance (Fig. 6 H), whereas insulin levels during the glucose tolerance test tended to be higher in the mutants (Fig. 6 I). These results suggest that Gs/α signaling in MC4R-expressing cells directly affects glucose tolerance and insulin sensitivity independently of its effects on energy balance.

Both male and female mMC4RGsKO mice had mild glucose intolerance (Fig. 6 C and Fig. S2 C). Insulin sensitivity tended to be reduced in male mMC4RGsKO mice, although the areas below baseline calculated based on the insulin tolerance tests were not significantly different (Fig. 6 D). Male pMC4RGsKO mice also tended to have higher insulin and leptin levels compared with control mice (Table 1). Female mMC4RGsKO mice did not show evidence of insulin resistance based upon insulin tolerance tests (Fig. S2 D). Male pMC4RGsKO mice displayed slightly but significantly impaired glucose tolerance (Fig. 6 E), despite having normal body weight, serum glucose, and insulin levels (Fig. 2 G and Table 1). Insulin sensitivity tended to be lower in these mice, although the areas below baseline were not significantly different (Fig. 6 F). Female pMC4RGsKO mice had normal glucose tolerance and insulin sensitivity (Fig. S2, E and F).
MC4RGsKO mice, but not mMC4RGsKO or pMC4RGsKO mice, have impaired cold tolerance

Reduced energy expenditure in MC4RGsKO and mMC4RGsKO mice at ambient temperature but not at thermoneutral temperature (Figs. 3 (C and F) and 4B), at which the SNS activity is minimal, suggests an impairment of SNS activity in these mice. We measured tolerance to an acute cold environment, which requires SNS-mediated BAT thermogenesis. Body temperature of MC4RGsKO mice rapidly declined within 1 h of cold exposure, and 3 of 10 mice had to be removed from the cold environment prematurely, as their body temperature had dropped below 25 °C (Fig. 7A) before the end of the experiment. BAT temperature measured with an implanted probe was also significantly decreased in MC4RGsKO mice that were placed in the cold, indicating a defect in BAT thermogenesis (Fig. 7B).

These mice had significantly lower basal BAT Ucp1 (uncoupling protein 1) mRNA levels, a marker of thermogenic capacity that is stimulated by SNS activity, at room temperature compared with controls (Fig. 7C), consistent with inactive brown adipocyte morphology (Fig. 2J). Cold exposure significantly stimulated Ucp1 gene expression in control but not in MC4RGsKO mice (Fig. 7C), indicating that these mice have impaired BAT activation in response to acute cold challenge.

MC4RGsKO mice were able to maintain their body temperature for up to 6 h at 6 °C (Fig. 7D) and had normal baseline BAT Ucp1 mRNA levels, although the response of BAT Ucp1 mRNA to cold was impaired (Fig. 7F). BAT temperature also remained similar to controls except for a slight decrease at time points 1 and 3 h after being placed in the cold (Fig. 7E).

Figure 7. Acute responses to cold tolerance in MC4RGsKO mouse lines. Shown are rectal temperature (Tb) (A) and iBAT temperature (E) measured in 2-month-old male MC4RGsKO mice and controls maintained on standard diet at room temperature (RT, time 0) and hourly after being placed at 6 °C (n = 4–5/group). Shown are rectal (D) and iBAT temperature (E), respectively, in similar experiments performed in 2-month-old male mMC4RGsKO (4–5/group). G, rectal temperature in 3–4-month-old pMC4RGsKO (11–13/group) after acute cold exposure. C, F, and G, BAT Ucp1 mRNA levels (normalized to control mice at RT) in male MC4RGsKO (C), mMC4RGsKO (F), and pMC4RGsKO (H) and their respective controls at room temperature (22 °C, RT) and after 6 h at 4 °C (n = 4–6/group). Data are expressed as mean ± S.E. (error bars). *, p < 0.05 versus controls. #, p < 0.05 versus RT.

pMC4RGsKO mice maintained their body temperature during cold exposure, and baseline and cold-induced levels of BAT Ucp1 mRNA were similar to those of controls (Fig. 7, G and H).

We next examined the response of the mutants to chronic cold adaptation. In these experiments, environmental temperature was lowered by 2 °C each day down to 6 °C, and then the mice were maintained at 6 °C for 5 days. MC4RGsKO mice maintained their body temperature ~2 °C lower than that of controls throughout most of the experiment (Fig. 8A). Immunostaining of inguinal WAT (iWAT) for UCP1 in MC4RGsKO mice showed areas of increased UCP1 staining but little formation of obvious beige-appearing cells (Fig. 8C). Whether this represents a failure of beiging or is a reflection of the increased lipid accumulation in iWAT cells is unclear. It is also unclear whether the small decrease in body temperature throughout this experiment is due to insufficient cold-induced thermogenesis or rather is due to a change in the central temperature set point at lower environmental temperatures. Interestingly, after longer cold exposure, there was now significant induction of Ucp1 mRNA expression in interscapular BAT (Fig. 8E) in these mutants.

Similar chronic cold adaptation experiments in mMC4RGsKO mice showed that these mice were able to maintain normal body temperature in the cold (Fig. 8B). Like MC4RGsKO mice, these mice showed increased UCP1 staining in iWAT but no clear-cut beige-appearing cells (Fig. 8D). Induction of Ucp1 mRNA in interscapular BAT was normal (Fig. 8F).
Reduced expression of adipogenesis and lipogenesis-related genes in adipose tissue from MC4RGsKO mice

Examination of gene expression in BAT and epididymal WAT (eWAT) samples from MC4RGsKO mice that were maintained at 22 °C showed that expression of virtually all of the genes examined that are involved in beiging/browning, adipogenesis, or lipogenesis was either significantly reduced or tended to be reduced in MC4RGsKO mice (Fig. 9, A and B). These changes are consistent with the reduced levels of Ucp1 mRNA that we observed in BAT from MC4RGsKO mice (Figs. 7C and 8E). We previously showed that loss of Gs/H9251 expression in adipose tissue, which results in reduced sensitivity of adipose tissue to SNS activity, also led to significantly reduced expression of adipogenesis, browning, and lipogenesis genes (28). Together with the adipose tissue histology and cold intolerance, the present data are consistent with reduced effent SNS activity to adipose tissue in MC4RGsKO mice. Similar analysis in adipose tissue from mMC4RGsKO mice showed no significant differences in gene expression except for reduced Pgc1a expression in BAT, although the expression of many of the genes tended to be lower in eWAT from mMC4RGsKO mice (Fig. 9, C and D).

MC4RGsKO mice have reduced heart rate

MC4RGsKO mice had significant decreases in heart rate, whereas blood pressure was unaffected (Fig. 10, A and B), indicating that Gsα deficiency in MC4R-expressing neurons leads to a reduction of cardiac SNS activity. Heart rate and blood pressure were unaffected in both mMC4RGsKO (Fig. 10, C and D) and pMC4RGsKO mice (Fig. 10, E and F).

Discussion

MC4R mutations are the most common cause of severe monogenic obesity and are associated with hyperphagia, reduced energy expenditure, increased body length, impaired BAT thermogenesis, insulin resistance, and decreased heart rate and blood pressure (4, 7, 8). The present study investigated to what extent Gsα signaling might play a role in these diverse physiologic effects of MC4R mutations. Our results show that mice with homozygous Gsα mutation in MC4R-expressing cells were severely obese primarily due to increased food intake, although reduced energy expenditure most likely contributes to this phenotype as well.

Consistent with increased food intake observed in MC4RGsKO mice, the ability of the MC4R agonist MTII to reduce food intake was impaired in these mice, suggesting resistance to the effects of MC4R on food intake. Although we have shown that MC4R mediates its effects on food intake within the PVN via Gq/11α signaling, loss of Gq/11α signaling in PVN did not completely abolish the effect of MTII on food intake (19), suggesting that MC4R may also mediate effects on food intake via Gsα in other brain regions, most likely outside of the PVN. The fact that we saw no effect on food intake in mMC4RGsKO mice or in more global deletion of the maternal Gnas allele...
within the CNS (17) indicates that the loss of MC4R action on food intake requires complete loss of G\(_{\alpha}\) expression and its signaling and is probably occurring in one or more areas where G\(_{\alpha}\) does not undergo imprinting. However, it should be stressed that other anorexigenic hormones and neurotransmitters, such as GLP-1, mediate their actions to lower food intake via G\(_{\alpha}\) (29) and that melanocortins may alter the sensitivity to other satiety factors (30), so that defects in signaling pathways besides MC4R also probably contribute to the hyperphagia observed in MC4RGsKO mice.

Intestinal endocrine L cells are one potential site of action outside of the CNS where loss of MC4R–G\(_{\alpha}\) signaling might mitigate the acute anorectic response to MTII, as MC4R–G\(_{\alpha}\) signaling in these cells stimulates the secretion of various anorexigenic peptides, including PYY and GLP-1 (26). In contrast to controls, MC4RGsKO mice showed no increase in serum PYY levels in response to MTII, which may partly account for the impaired ability of MTII to reduce food intake in these mice. However, it should be noted that PYY levels (as well as GLP-1 levels) were elevated in MC4RGsKO mice at baseline. Circulating PYY levels are known to be low in the fasting state and increase postprandially. Obesity in humans is associated with elevated fasting PYY levels (31) and attenuated PYY release in response to feeding (32, 33). It is unclear whether the elevated fasting PYY levels observed in MC4RGsKO mice is secondary to obesity or to a direct perturbation in enteroendocrine L cells due to loss of G\(_{\alpha}\). It is possible that the elevation in PYY is a response to hyperphagia, but this effect would be secondary, as PYY does not mediate its actions primarily by G\(_{\alpha}\). In contrast, GLP-1 does mediate its actions via G\(_{\alpha}\) so that G\(_{\alpha}\) deficiency in MC4R-expressing cells may directly contribute to elevated GLP-1 levels in MC4RGsKO mice.

MC4RGsKO mice also had a very small but significant increase in body length, which was not observed in mouse models with CNS-specific maternal G\(_{\alpha}\) mutations (17, 34). In fact, a greater increase in body length was observed in mice with PVN-specific loss of G\(_{q/11}\) signaling, and similar increases in linear growth are observed in humans and mice with loss of SIM1 (35, 36), a relatively PVN-specific transcription factor that is regulated by PVN MC4R–G\(_{q/11}\) signaling (19). The extent to which MC4R–G\(_{\alpha}\) signaling contributes to the effect of MC4R mutations on linear growth appears to be small, and further studies will be required to determine whether the small changes in linear growth observed in MC4RGsKO mice represent a primary effect due to loss of MC4R–G\(_{\alpha}\) signaling as opposed to a secondary effect due to other metabolic perturbations or disturbance of other G\(_{\alpha}\)-mediated signaling pathways in MC4RGsKO cells.
**G\(_\alpha\) deficiency in MC4R-expressing cells leads to obesity**

Existing evidence shows that activation of central MC4R signaling inhibits basal plasma insulin secretion and improves glucose tolerance and insulin sensitivity (4) and that MC4Rs expressed in cholinergic neurons are required for their inhibitory effect on plasma insulin levels (37, 38). Our data revealed that young MC4RGsKO mice with normal body weight exhibited impaired glucose tolerance, which is probably due to a primary defect in insulin sensitivity, as insulin levels during the glucose tolerance test showed no evidence of insulin deficiency. The more severe effects observed in older MC4RGsKO mice probably reflect the additional effects of severe obesity on glucose metabolism. Moreover, female mMC4RGsKO mice had mild glucose intolerance, whereas pMC4RGsKO mice did not. Loss of response to insulin was also observed on glucose metabolism.

The more severe effects observed in older MC4RGsKO mice probably reflect the additional effects of severe obesity on glucose metabolism. Moreover, female mMC4RGsKO mice had mild glucose intolerance, whereas pMC4RGsKO mice did not. Loss of response to insulin was also observed on glucose metabolism.

**Experimental procedures**

### Animals

MC4RGsKO mice were generated by breeding G\(_\alpha\)-floxed mice with flanking loxP sites around G\(_\alpha\) exon 1 (E1\(^{fl/fl}\), Black Swiss genetic background) (21) with MC4R-cre mice (gift from B. Lowell; C57BL/6 genetic background), which express Cre recombinase from the endogenous MC4R promoter (22). mMC4RGsKO (E1\(^{fl/+}\):MC4R-cre\(^+\)) mice were generated by mating female E1\(^{fl/+}\) mice with male MC4R-cre mice, whereas pMC4RGsKO mice (E1\(^{+/+}\):MC4R-cre\(^+\)) were generated by reciprocal crosses. Homozygotes (MC4RGsKO; E1\(^{fl/+}\):MC4R-cre\(^+\)) were generated by breeding between heterozygotes. Genotyping was performed as described previously (21). E1\(^{+/+}\) or MC4R-cre\(^+\) littermates were used as controls. Animals were maintained on a 12-h light/12-h dark cycle with unimpeded access to water and food (standard chow diet, 5% fat by weight). All experiments were performed on 12–20-week-old mice unless otherwise noted. Studies were approved by the NINDS, National Institutes of Health, Animal Care and Use Committee.

To examine the expression pattern of the MC4R-cre transgene and the loss of expression of G\(_\alpha\) in MC4R-expressing cells, mice heterozygous for the G\(_\alpha\)-floxed (E1\(^{fl/+}\)) allele and simultaneously carrying the Mc4r-Cre transgene were crossed with B6;129S6-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J mice (Ai14 mice, Jackson Laboratories, Stock no. 007908) (23). The resulting Mc4r-cre: E1\(^{fl/+}\):tdTom mice were then crossed with one another to generate homozygous MC4RGsKO:tdTomato mice (Mc4r-cre: E1\(^{fl/+}\):tdTomato) mice. Littermates for the G\(_\alpha\) WT allele (Mc4r-cre: E1\(^{+/+}\):tdTomato) were used as controls. Because Ai14 mice harbor a genetic locus containing a loxP-flanked STOP cassette upstream of a CAG promoter that drives expression of the red fluorescent protein variant, tdTomato, Mc4r-Cre expression can be directly visualized using fluorescence microscopy.
**Body weight, composition, food intake, and metabolism**

Body composition was measured using an Echo 3-in-1 NMR analyzer (Echo Medical Systems, Houston, TX). Food intake was performed on individually housed male mice that were acclimated for 5 days, followed by food and body weight measurements every other day for 7 days. REEs and TEEs were measured over a 24-h period by indirect calorimetry using a 12-chamber CLAMS/Oxymax system (Columbus Instruments, Columbus, OH) as described previously (17). Total and ambulatory activity levels were measured using IR beam interruption (OptoVarimex mini, Columbus Instruments). Day (light cycle) was 0600 – 1800 h, and night (dark cycle) was 1800 – 0600 h. For energy balance studies, mice were acclimated to a room temperature (22 °C). Body composition was measured weekly, and TEE was calculated by subtracting the gain in total body energy stores from the total energy intake over the measurement interval (24).

**Glucose and insulin tolerance tests**

Blood glucose or insulin tolerance tests were performed on overnight fasted mice with intraperitoneal injection of glucose (2 mg/g) or insulin (Humulin, 0.75 mIU/g) as described (21). Blood glucose was obtained from tail blood and measured using a Contour glucometer (Bayer) at the indicated times.

**Responses to MTII**

For measurement of food intake response to MTII, single-caged mice were fasted for 24 h before receiving vehicle (saline, 100 µl i.p.) at 30 min before lights out, and food intake was measured during the first 3.5 h after injection. Mice were allowed to recover for 2 days before a second 24-h fast, followed by administration of MTII (200 µg i.p.) and measurement of food intake. For measurement of the energy expenditure response to MTII, mice were given MTII (10 µg/g i.p.) or saline on separate days, and total oxygen consumption was measured at 30 °C before and between 1 and 3 h after injection of MTII or saline.

**Responses to acute and chronic cold exposure**

Rectal temperature was measured with a TH-5 rectal probe (Thermalet, TX) inserted 1 cm deep, and interscapular BAT (iBAT) temperature was directly assessed using a telemetry temperature probe (PII-300, Bio Medic Data Systems Inc., Sea ford, DE) that was surgically implanted into the iBAT of mice under isoflurane anesthesia and detected by a receiver (DAS-7007S, Bio Medic Data Systems). Before acute cold tolerance testing, mice were acclimatized to experimental conditions at room temperature for 3 days with daily measurement of rectal and iBAT temperatures. During the acute cold tolerance test, mice were individually housed without bedding and provided ad libitum access to food and water. Rectal and iBAT temperatures were measured before (time 0) and at the indicated time points after exposure to 6 °C. For chronic cold adaptation, single-caged mice were housed in a temperature-controlled chamber (Memmert 750 LIFE Chamber) with bedding and were provided with food and water ad libitum. The ambient temperature of the chamber decreased in 2 °C/day increments from 22 to 6 °C, and then mice were housed at 6 °C for the remainder of the experiment. Rectal temperatures were measured at the indicated time points.

**PYY measurements**

Male mice were injected with saline (100 µl i.p.) and MTII (200 µg i.p.) on separate days following a 24-h fast to reduce postprandial hormones to basal levels. Mice were allowed to recover for 1 week between administration of saline or MTII. Blood samples were collected 10 min after injection of saline or MTII by submandibular bleeding. Serum PYY levels were measured using the Milliplex MAP mouse metabolic hormone magnetic bead panel (EMD-Millipore, MMHMAG-44K), following the manufacturer’s instructions. The PYY values were read on a Bio-Plex Magpix multiplex reader (Bio-Rad) and analyzed with xPONENT software (Luminex).

**Biochemical assays**

Serum glucose levels were measured with an Elite glucometer (Bayer). Insulin and leptin levels were measured using an ELISA kit (obtained from Crystal Chem and R&D Systems, respectively). Free fatty acids were measured using reagents from Roche Applied Sciences, and triglycerides and cholesterol levels were measured using reagents from Thermo (Middletown, VA). Serum GLP-1 levels were measured by ELISA following the manufacturer’s protocol (EMD Millipore, Darmstadt, Germany).

**Blood pressure and heart rate measurements**

Blood pressure and heart rate were measured using a BP-2000 specimen platform (Visitech).

**Gene expression**

Gene expression in adipose tissue was assessed by quantitative RT-PCR as described previously (28).

**Adipocyte size quantification**

BAT, eWAT, and iWAT fat pads were fixed in 10% formalin. Tissue sections (5 µm thick for BAT; 8 µm thick for eWAT and iWAT) were subsequently H&E-stained and imaged at ×20 (BAT) or ×10 (eWAT and iWAT) magnifications with a Hamamatsu NanoZoomer 2.0RS slide scanner (Meyer Instruments). Adipocyte size was measured for each tissue section using the FIJI plug-in Adiposoft, an open source software for the quantification of adipose tissue cellularity in histological sections (45).

**UCP1 immunohistochemistry**

iWAT fat pads were fixed in 10% formalin and then cut into 8-µm-thick sections. Tissue sections were treated in 10 mM sodium citrate with 0.05% Tween 20 at 85 °C for 20 min, followed by 3% hydrogen peroxide treatment for 10 min. After blocking for 20 min in 5% BSA, tissue sections were incubated with anti-UCP1 antibody (Abcam; catalogue no. Ab10983; BioMedica No. 910101).
**Gα deficiency in MC4R-expressing cells leads to obesity**

1:1000 dilution) at 4 °C overnight. The sections were then washed with PBS and subsequently incubated with biotinylated goat anti-rabbit IgG secondary antibody (Agilent DAKO; catalogue no. E043201-6; 1:500 dilution) at room temperature for 1 h. UCP1 signal was detected with streptavidin horseradish peroxidase (Vector Laboratories) and visualized with diaminobenzidine tetrahydrochloride (Sigma). Tissue sections were counterstained with hematoxylin.

**Gα immunohistochemistry**

Mice were anesthetized with avertin and transcardially perfused with cold PBS followed by cold 4% paraformaldehyde. After removal, brains were post-fixed in 4% paraformaldehyde overnight at 4 °C and subsequently cryoprotected in 30% sucrose in PBS at 4 °C for 3 days. Free-floating brain sections (40 μm) were prepared using a microtome, and sections were stored at −80 °C. For immunohistochemistry, brain sections were blocked in 2.5% normal horse serum (Vector Laboratories) plus 0.3% Triton X-100 at room temperature for 2 h and then incubated with anti-Gα antibody (46) in blocking solution overnight at 4 °C. After washing with PBS, brain sections were incubated with an Alexa Fluor–conjugated secondary antibody (Alexa Fluor 488, Life Technologies) for 1 h at room temperature. The signals in MC4R+ neurons in the PVN and DMV were captured and visualized using confocal microscopy (Carl Zeiss).

**Statistical analysis**

Data are presented as mean ± S.E. Statistical significance was determined using unpaired t tests or linear regressions by analysis of covariance. Differences were considered significant if p < 0.05.

**Author contributions**—B. P., M. C., O. G., E. A. W., E. P., B. C., and H. S. designed and performed the experiments and analyzed the data. M. C. and L. S. W. conceived the idea for the project. B. P., M. C., and L. S. W. wrote the paper.

**Acknowledgments**—We thank Shalini Jain and Yinyan Ma for technical assistance and Brad Lowell (Boston, MA) for providing MC4R−/− mice lines.

**References**

1. Mountjoy, K. G., Mortrud, M. T., Low, M. J., Simerly, R. B., and Cone, R. D. (1994) Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol. Endocrinol.* 8, 1298–1308 CrossRef Medline

2. Balthasar, N., Dalgaard, L. T., Lee, C. E., Yu, J., Funahashi, H., Williams, T., Ferreira, M., Tang, V., McGovern, R. A., Kenny, C. D., Christiansen, L. M., Edelstein, E., Choi, B., Boss, O., Aschkenasi, C., Zhang, C. Y., Mountjoy, K., Kishi, T., Elmqist, J. K., and Lowell, B. B. (2005) Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 123, 493–505 CrossRef Medline

3. Fan, W., Morrison, S. F., Cao, W. H., and Yu, P. (2007) Thermogenesis activated by central melanocortin signaling is dependent on neurons in the rostral raphe pallidus (rRPa) area. *Brain Res.* 1179, 61–69 CrossRef Medline

4. Fan, W., Dinulescu, D. M., Butler, A. A., Zhou, J., Marks, D. L., and Cone, R. D. (2000) The central melanocortin system can directly regulate serum insulin levels. *Endocrinology* 141, 3072–3079 CrossRef Medline

5. Rossi, J., Balthasar, N., Olson, D., Scott, M., Berglund, E., Lee, C. E., Choi, M. J., Lauzon, D., Lowell, B. B., and Elmqquist, J. K. (2011) Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. *Cell Metab.* 13, 195–204 CrossRef Medline

6. O’Rahilly, S., Farooqi, I. S., Yeo, G. S., and Challis, B. G. (2003) Minireview: human obesity-lessons from monogenic disorders. *Endocrinology* 144, 3757–3764 CrossRef Medline

7. Farooqi, I. S., Keogh, J. M., Yeo, G. S., Lank, E. J., Cheetham, T., and O’Rahilly, S. (2003) Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N. Engl. J. Med.* 348, 1085–1095 CrossRef Medline

8. Huszar, D., Lynch, C. A., Fairchild-Huntress, V., Dunmore, J. H., Fang, Q., Berkemeier, L. R., Gu, W., Kesterson, R. A., Boston, B. A., Cone, R. D., Smith, F. J., Campfield, L. A., Burn, P., and Lee, F. (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88, 131–141 CrossRef Medline

9. Ste. Marie, L., Miura, G. I., Marsh, J. D., Yagaloff, K., and Palmiter, R. D. (2000) A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12339–12344 CrossRef Medline

10. Weinstein, L. S., Chen, M., Xie, T., and Liu, J. (2006) Genetic diseases associated with heterotrimERIC G proteins. *Trends Pharmacol. Sci.* 27, 260–266 CrossRef Medline

11. Long, D. N., McGuire, S., Levine, M. A., Weinstein, L. S., and Germain-Lee, E. L. (2007) Body mass index differences in pseudohypoparathyroidism type 1a versus pseudopseudohypoparathyroidism may implicate paternally imprinted Gαs in the development of human obesity. *J. Clin. Endocrinol. Metab.* 92, 1073–1079 CrossRef Medline

12. Muniyappa, R., Warren, M. A., Zhao, X., Aney, S. C., Courville, A. B., Chen, K. Y., Brychta, R. J., Germain-Lee, E. L., Weinstein, L. S., and Skarulis, M. C. (2013) Reduced insulin sensitivity in adults with pseudohypoparathyroidism type 1a. *J. Clin. Endocrinol. Metab.* 98, E1796–E1801 CrossRef Medline

13. Chen, M., Gavrilova, O., Liu, J., Xie, T., Deng, C., Nguyen, A. T., Nackers, M. M., Lorenzo, J., Shen, L., and Weinstein, L. S. (2005) Alternative Gnas gene products have opposite effects on glucose and lipid metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7386–7391 CrossRef Medline

14. Germain-Lee, E. L., Schwindinger, W., Crane, J. L., Zewdu, R., Zweifel, L. S., Wand, G., Huso, D. L., Saij, M., Ringel, M. D., and Levine, M. A. (2005) A mouse model of Albright hereditary osteodystrophy generated by targeted disruption of exon 1 of the Gnas gene. *Endocrinology* 146, 4697–4709 CrossRef Medline

15. Xie, T., Chen, M., Gavrilova, O., Lai, E. W., Liu, J., and Weinstein, L. S. (2008) Severe obesity and insulin resistance due to deletion of the maternal Gαs allele is reversed by paternal deletion of the Gαs imprint control region. *Endocrinology* 149, 2443–2450 CrossRef Medline

16. Yu, S., Yu, D., Lee, E., Eckhaus, M., Lee, R., Corria, Z., Accili, D., Westphal, H., and Weinstein, L. S. (1998) Variable and tissue-specific hormone resistance in heterotrimERIC G protein α-subunits (Gαs) knockout mice is due to tissue-specific imprinting of the Gαs gene. *Endocrinology* 146, 4697–4709 CrossRef Medline

17. Chen, M., Wang, J., Dickerson, K. E., Kelleher, J., Xie, T., Gupta, D., Lai, E. W., Pacak, K., Gavrilova, O., and Weinstein, L. S. (2009) Central nervous system imprinting of the Gαs protein and its role in metabolic regulation. *Cell Metab.* 9, 548–555 CrossRef Medline

18. Chen, M., Shrestha, Y. B., Podyма, B., Cui, Z., Naglieri, B., Sun, H., Ho, T., Wilson, E. A., Li, Y. Q., Gavrilova, O., and Weinstein, L. S. (2017) Gαs deficiency in the dorsomedial hypothalamus underlies obesity associated with Gαs mutations. *J. Clin. Invest.* 127, 500–510 Medline

19. Li, Y. Q., Shrestha, Y., Pandey, M., Chen, M., Kablan, A., Gavrilova, O., Offermanns, S., and Weinstein, L. S. (2016) Gαs1 and Gαs mediate distinct physiological responses to central melanocortins. *J. Clin. Invest.* 126, 40–49 CrossRef Medline

20. Hinney, A., Hohmann, S., Geller, F., Vogel, C., Hess, C., Wermter, A. K., Brokamp, B., Goldschmidt, H., Siegfried, W., Remschmidt, H., Schäfer, H., Gudermann, T., and Hebebrand, J. (2003) Melanocortin-4 receptor gene: case-control study and transmission disequilibrium test confirm that functionally relevant mutations are compatible with a major gene effect
for extreme obesity. J. Clin. Endocrinol. Metab. 88, 4258–4267 CrossRef Medline

21. Chen, M., Gavrilova, O., Zhao, W. Q., Nguyen, A., Lorenzo, J., Shen, L., Nackers, L., Pack, S., Jou, W., and Weinstein, L. S. (2005) Increased glucose tolerance and reduced adiposity in the absence of fasting hypoglycemia in mice with liver-specific Gα deficiency. J. Clin. Invest. 115, 3217–3227 CrossRef Medline

22. Garfield, A. S., Li, C., Madara, J. C., Shah, B. P., Webber, E., Steger, J. S., Campbell, J. N., Gavrilova, O., Lee, C. E., Olson, D. P., Elmqist, J. K., Tannous, B. A., Krashes, M. J., and Lowell, B. B. (2015) A neutral basis for melanocortin-4 receptor-regulated appetite. Nat. Neurosci. 18, 863–871 CrossRef Medline

23. Madisen, L., Zwingman, T. A., Sunkin, S. M., Oh, S. W., Zarivala, H. A., Gu, H., Ng, L. L., Palmer, R. D., Hawrylycz, M. J., Jones, A. R., Lein, E. S., and Zeng, H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. 13, 133–140 CrossRef Medline

24. Ravussin, Y., Gutman, R., LeDuc, C. A., and Leibel, R. L. (2013) Estimating energy expenditure in mice using an energy balance technique. Int. J. Obes. 37, 399–403 CrossRef Medline

25. Gautron, L., Lee, C., Funahashi, H., Friedman, J., Lee, S., and Elmqquist, J. (2010) Melanocortin-4 receptor expression in a vago-vagal circuit involved in postprandial functions. J. Comp. Neurol. 518, 6–24 CrossRef Medline

26. Panaro, B. L., Tough, I. R., Engelstoft, M. S., Matthews, R. T., Digby, G. J., Moller, C. L., Svendsen, B., Gribble, F., Reimann, F., Holst, J. J., Holst, B., Schwartz, T. W., Cox, H. M., and Cone, R. D. (2014) The melanocortin-4 receptor is expressed in enterodocrine L cells and regulates the release of peptide YY and glucagon-like peptide 1 in vivo. Cell Metab. 20, 1018–1029 CrossRef Medline

27. Al-Sabah, S. (2016) Molecular pharmacology of the incretin receptors. Med. Prin. Pract. 25, 15–21 CrossRef Medline

28. Li, Y. Q., Shrestha, Y. B., Chen, M., Chanturiya, T., Gavrilova, O., and Weinstein, L. S. (2016) Gα deficiency in adipose tissue improves glucose metabolism and insulin sensitivity without an effect on body weight. Proc. Natl. Acad. Sci. U.S.A. 113, 446–451 CrossRef Medline

29. Hayes, M. R., Leichner, T. M., Zhao, S., Lee, G. S., Chanturiya, T., Meng, D., De Jonghe, B. C., Kanosi, S. E., Grill, H. J., and Bence, K. K. (2011) Intracellular signals mediating the food intake-suppressive effects of hindbrain glucagon-like peptide-1 receptor activation. Cell Metab. 13, 320–330 CrossRef Medline

30. Blevins, J. E., Morton, G. J., Williams, D. L., Caldwell, D. W., Bastian, L. S., Wisse, B. E., Schwartz, M. W., and Baskin, D. G. (2009) Forebrain melanocortin signaling enhances the hindbrain satiety response to CCK-8. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R476–R484 CrossRef Medline

31. Cahill, F., Ji, Y., Wadden, D., Amini, P., Randell, E., Vasdev, S., Gulliver, H., and Sun, G. (2014) The association of serum total peptide YY (PYY) with obesity and body fat measures in the CODING Study. PLoS One 9, e95235 CrossRef Medline

32. Le Roux, C. W., Batterham, R. L., Aylwin, S. J., Patterson, M., Borg, C. M., Wynne, K. J., Kent, A., Vincent, R. P., Gardiner, J., Ghatei, M. A., and Bloom, S. R. (2006) Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology 147, 3–8 CrossRef Medline

33. Karra, E., Chandarana, K., and Batterham, R. L. (2009) The role of peptide YY in appetite regulation and obesity. J. Physiol. 587, 19–25 CrossRef Medline

34. Chen, M., Berger, A., Kablan, A., Zhang, J., Gavrilova, O., and Weinstein, L. S. (2012) Gα deficiency in the paraventricular nucleus of the hypothalamic partially contributes to obesity associated with Gα mutations. Endocrinology 153, 4266–4265 CrossRef Medline

35. Holder, J. L., Jr., Butte, N. F., and Zinn, A. R. (2000) Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. Hum. Mol. Genet. 9, 101–108 CrossRef Medline

36. Kublaoui, B. M., Holder, J. L., Jr, Gemelli, T., and Zinn, A. R. (2006) Sim1 haploinsufficiency impairs melanocortin-mediated anorexia and activation of paraventricular nucleus neurons. Mol. Endocrinol. 20, 2483–2492 CrossRef Medline

37. Sohn, J. W., Harris, L. E., Berglund, E. D., Liu, T., Vong, L., Lowell, B. B., Balghasas, N., Williams, K. W., and Elmqquist, J. K. (2013) Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. Cell 152, 612–619 CrossRef Medline

38. Berglund, E. D., Liu, T., Kong, X., Sohn, J. W., Yong, L., Deng, Z., Lee, C. E., Lee, S., Williams, K. W., Olson, D. P., Scherer, P. E., Lowell, B. B., and Elmqquist, J. K. (2014) Melanocortin 4 receptors in autonomic neurons regulate thermogenesis and glycemia. Nat. Neurosci. 17, 911–913 CrossRef Medline

39. Voss-Andreae, A., Murphy, J. G., Ellacott, K. L., Stuart, R. C., Nillni, E. A., Cone, R. D., and Fan, W. (2007) Role of the central melanocortin circuitry in adaptive thermogenesis of brown adipose tissue. Endocrinology 148, 1550–1560 CrossRef Medline

40. Vaughan, C. H., Shrestha, Y. B., and Bartness, T. J. (2011) Characterization of a novel melanocortin receptor-containing node in the SNS outflow circuitry to brown adipose tissue involved in thermogenesis. Brain Res. 1411, 17–27 CrossRef Medline

41. Satoh, N., Ogawa, Y., Katsuura, G., Numata, Y., Masuzaki, H., Yoshimas, Y., and Nakao, K. (1998) Satiety effect and sympathetic activation of leptin are mediated by hypothalamic melanocortin system. Neurosci. Lett. 249, 107–110 CrossRef Medline

42. Williams, D. L., Bowers, R. R., Bartness, T. J., Kaplan, J. M., and Grill, H. J. (2003) Brainstem melanocortin 3/4 receptor stimulation increases uncoupling protein gene expression in brown fat. Endocrinology 144, 4692–4697 CrossRef Medline

43. Butler, A. A., Marks, D. L., Fan, W., Kuhn, C. M., Bartolome, M., and Cone, R. D. (2001) Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat. Neurosci. 4, 605–611 CrossRef Medline

44. Liu, H., Kishi, T., Roseberry, A. G., Cai, X., Lee, C. E., Montez, J. M., Friedman, J. M., and Elmqquist, J. K. (2003) Transgenic mice expressing green fluorescent protein under the control of the melanocortin-4 receptor promoter. J. Neurosci. 23, 7143–7154 CrossRef Medline

45. Galarraga, M., Campión, J., Muñoz-Barrutia, A., Boqué, N., Moreno, H., Martínez, J. A., Milagro, F., and Ortiz-de-Solórzano, C. (2012) Adiposoft: automated software for the analysis of white adipose tissue cellularity in histological sections. J. Lipid Res. 53, 2791–2796 CrossRef Medline

46. Simonds, W. F., Goldsmith, P. K., Woodard, C. J., Unson, C. G., and Spiegel, A. M. (1989) Receptor and effector interactions of Gα; functional studies with antibodies to the α subunit carboxyl-terminal decapetide. FEBS Lett. 249, 189–194 CrossRef Medline