Transgenic Neuronal Expression of Proopiomelanocortin Attenuates Hyperphagic Response to Fasting and Reverses Metabolic Impairments in Leptin-Deficient Obese Mice

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Hypothalamic proopiomelanocortin (POMC) gene expression is reduced in many forms of obesity and diabetes, particularly in those attributable to deficiencies in leptin or its receptor. To assess the functional significance of POMC in mediating metabolic phenotypes associated with leptin deficiency, leptin-deficient mice bearing a transgene expressing the POMC gene under control of the neuron-specific enolase promoter were produced. The POMC transgene attenuated fasting-induced hyperphagia in wild-type mice. Furthermore, the POMC transgene partially reversed obesity, hyperphagia, and hypothermia and effectively normalized hyperglycemia, glucosuria, glucose intolerance, and insulin resistance in leptin-deficient mice. Effects of the POMC transgene on glucose homeostasis were independent of the partial correction of hyperphagia and obesity. Furthermore, the POMC transgene normalized the profile of hepatic and adipose gene expression associated with gluconeogenesis, glucose output, and insulin sensitivity. These results indicate that central POMC is a key modulator of glucose homeostasis and that agonists of POMC products may provide effective therapy in treating impairments in glucose homeostasis when hypothalamic POMC expression is reduced, as occurs with leptin deficiency, hypothalamic damage, and aging. *Diabetes* 52:2675–2683, 2003

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AGRP, agouti-related peptide; FAT, fatty acid transporter; FFA, free fatty acid; GTG, gold-thio-glucose; MC4-R, melanocortin 4 receptor; MSH, melanocyte-stimulating hormone; NPY, neuropeptide Y; Nse, neuron specific enolase; PGC-1, peroxisome proliferator−activated receptor-γ coactivator 1; POMC, proopiomelanocortin; PTP-1B, protein tyrosine phosphatase 1B; SCD-1, stearoyl-CoA desaturase 1; SOCS-3, suppressor of cytokine signaling 3.

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Many forms of obesity and diabetes are associated with impairments in the production of (1–5), processing of (6,7), or sensitivity to (8,9) products of the proopiomelanocortin (*Pomc*) gene. In particular, ablation of leptin or its receptor causes reduced hypothalamic expression of POMC as well as obesity and impairments in glucose metabolism (1–3). Fasting also leads to reduced leptin and reduced expression of hypothalamic POMC (3). Similarly, hypothalamic POMC expression is reduced in other conditions involving impairments in glucose metabolism caused by hypothalamic lesions (10), mutations in the *tubby* and *nhlh2* genes (11,12), aging (13), or insulin deficiency (14). The suggestion that reduced POMC may actually be a cause of the metabolic impairments comes from the observation that mutations in the *Pomc* gene lead to metabolic impairments in humans (4) and mice (5).

It is plausible that the POMC-derived peptide α-melanocyte-stimulating hormone (MSH) mediates many of the effects of hypothalamic POMC on metabolic function, as central injection of α-MSH or an α-MSH agonist decreases food intake and increases energy expenditure (8,15,16), whereas central injection of a melanocortin receptor antagonist increases food intake and decreases energy expenditure (17–19). Because the blockade of α-MSH signaling blocks the acute effects of leptin on feeding behavior (20), α-MSH may mediate some of leptin's metabolic effects and therefore reduced hypothalamic POMC may mediate effects of leptin deficiency on metabolic and neuroendocrine impairments. Accumulating genetic data also support a role for the melanocortin system in the regulation of glucose metabolism. Agouti mice develop obesity, hyperphagia and diabetes with hyperinsulinemia, and glucose intolerance because of the ectopic expression of the agouti peptide, which acts as an antagonist to α-MSH (21–24). Mice with disruption of the α-MSH receptor melanocortin 4 receptor (MC4-R) exhibit a phenotype similar to that observed in agouti mice, including obesity, hyperphagia, and hyperinsulinemia (9). However, whether perturbed glucose homeostasis is primarily caused by decreased melanocortin tone or secondary to obesity is unknown at present. That impairments in the POMC system might directly cause insulin resistance is suggested

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by the observation that mice with impaired sensitivity to α-MSH are highly insulin resistant, possibly independent of adiposity (25,26). These data suggest that α-MSH agonists might be effective in improving diabetic phenotypes such as insulin resistance and hyperglycemia. On the other hand, infusion of α-MSH agonists directly into the brain causes marked impairments in glucose tolerance while reducing insulin secretion (26), suggesting that α-MSH agonists might worsen diabetic symptoms, at least acutely.

Although considerable evidence suggests that reduced hypothalamic POMC might contribute to metabolic impairments in many forms of obesity and diabetes, these metabolic impairments involve altered expression of many genes. Therefore, a key question is whether elevation of POMC gene expression can reverse the metabolic impairments in conditions otherwise associated with reduced hypothalamic POMC gene expression. Thus the present study assessed whether chronic enhancement of central POMC expression attenuates metabolic impairments associated with leptin deficiency. The results demonstrated that elevation of POMC gene expression in neurons markedly attenuates fasting-induced hyperphagia in wild-type mice, obesity and hyperphagia in leptin-deficient $Lep^{ob/ob}$ mice, and impairments in glucose metabolism in $Lep^{ob/ob}$ mice independent of effects on food intake or body weight.

RESEARCH DESIGN AND METHODS

Animals. Mice were weaned 3–4 weeks after birth and fed regular rodent diet (4.02 kcal/g, 11.9% kcal from fat; #5,053, Purina Mills, St. Louis, MO) and water thereafter. Mice were individually housed under a 12:12 light/dark cycle (lights on at 0700). All studies were approved by the Institutional Animal Care and Use Committee of Mount Sinai School of Medicine.

Generation of POMC transgenic $Lep^{ob/ob}$ mice. A 950-bp mouse POMC cDNA (27) was cloned into the *Hin*dIII site of the pNSE-Ex4 vector (28) and a 6-kb *Sal*I fragment spanning the neuron specific enolase (*Nse*) promoter and POMC cDNA along with the SV40 polyA signal was excised, purified, and used for the microinjection. Transgenic mice were produced on the C57BL/6J X C3H F1 background and bred as heterozygotes onto the C75BL/6J background. Genotypes were confirmed by Southern blot analysis. Genomic DNA (5 μg) extracted from the tail tip was digested with *Eco*RI and *Hin*dIII, after which it was electrophoresed and transferred. Transferred membrane was hybridized with ^{32}P-labeled random-primed DNA probe for POMC (spanning exons 1–3). A 1-kb band was obtained from DNA of POMC transgenic mice, but not from wild-type mice. Heterozygous $Lep^{ob/+}$ mice (C57BL/6J background; Jackson Laboratory, Bar Harbor, ME) were crossed with heterozygous $Pomc^{+/tg}$ mice to obtain mice heterozygous for both the *ob* gene and the POMC transgene. Heterozygous $Pomc^{+/tg}$ $Lep^{ob/+}$ mice were then crossed with heterozygous $Pomc^{+/+}Lep^{ob/+}$ mice to generate all genotypes ($Pomc^{+/+}$ $Lep^{+/+}$, $Pomc^{+/tg}$ $Lep^{+/+}$, $Pomc^{+/+}$ $Lep^{ob/ob}$, and $Pomc^{+/tg}$ $Lep^{ob/ob}$) required for the experiment. Wild-type ($Lep^{+/+}$), rather than heterozygous $Lep^{ob/+}$ mice, were used as controls for $Lep^{ob/ob}$ mice in the present study. To detect the mutated *ob* gene, PCR combined with *DdeI* digestion was performed. A 142-bp leptin fragment spanning the *ob* mutation was amplified from genomic DNA (0.1 μg) by using the NH_2-terminal primer 5′-TGTCCAAGATGGACCA GACTC-3′ and the COOH-terminal primer 5′-AGGGAGCAGCTCTTGGAGAA-3′. The resultant PCR products were digested with *DdeI* and analyzed by agarose gel electrophoresis. Wild-type, heterozygous, and homozygous *ob* mutations produced a single 142-bp fragment, three fragments (142, 100, and 42 bp), and two fragments (100 and 42 bp), respectively.

$Pomc^{+/tg}$ $Lep^{ob/ob}$ mice. All mice were weaned 3–4 weeks after birth and individually housed thereafter. Body weight and blood glucose were monitored weekly starting at ages 3 and 6 weeks, respectively. Metabolic rates were measured between ages 16 and 18 weeks. Mice were killed by decapitation after being briefly exposed to carbon dioxide at the end of the experiment (between ages 17 and 19 weeks). After they were killed, blood samples were collected, serum was separated and stored at –20°C, and the liver and white adipose tissue were quickly removed, weighed, frozen on dry ice, and stored at –70°C until RNA extraction.

Food intake and fasting-induced hyperphagia. Food intake over 24 h was measured daily over 1 week between ages 13 and 14 weeks. Fasting-induced hyperphagia was assessed in $Pomc^{+/+}$ and $Pomc^{+/tg}$ mice at age 7 months. Mice were fasted for 24 h by removing food immediately before lights out (at 1900), and 24 h later, mice were allowed free access to food by reintroducing preweighed food. Food intake was measured after access to the food for 1 h after lights out. As a control, food intake was also measured for 1 h after lights out in mice of both genotypes fed ad libitum.

Pair-feeding. $Lep^{ob/ob}$ mice were pair-fed to the level of food intake of age-matched $Pomc^{+/tg}$ $Lep^{ob/ob}$ mice for 2 weeks (between ages 20 and 22 weeks). Half the amount of food consumed during the previous 24 h by $Pomc^{+/tg}$ $Lep^{ob/ob}$ was presented to $Pomc^{+/+}$ $Lep^{ob/ob}$ mice at 0900 and the other half at 1600 to prevent effects of prolonged fasting on blood glucose levels. Blood glucose was measured at 1800. At the end of the pair-feeding period, mice were fasted overnight and a glucose tolerance test was performed as described below.

Measurement of metabolic rate. The metabolic rate was determined using an indirect calorimetry system (29). In the morning, mice were placed into metabolic cages (Accuscan, Columbus, OH) for 6 h to determine metabolic rate and activity. Oxygen consumption (V_{O_2}) and heat production was calculated per 5-min period. Data during the last 2 h of measurement were used for the analysis to avoid responses attributable to a novel environment. Because $Lep^{ob/ob}$ mice are substantially heavier than $Lep^{+/+}$ mice, V_{O_2} may reflect differences in the mass of nonmetabolically active tissue and differences in the metabolic rate of metabolically active tissue. To address this issue, total oxygen consumption during the 2-h experimental period was also calculated.

Glucose tolerance and insulin tolerance tests. Glucose tolerance tests were performed at age 12 weeks. Insulin tolerance tests were performed between ages 21 and 25 weeks. For the glucose tolerance test, mice were fasted overnight (15 h) by removing their food immediately before lights out (1900) and injecting them intraperitoneally with glucose (2 mg/g body wt) at 1000. A drop of blood was taken from the tail vein immediately before glucose injection and 15, 30, 60, 120, and 180 min after injection. Plasma insulin concentrations during the glucose tolerance test were also measured. Mice were fasted overnight and injected intraperitoneally with glucose (2 mg/g body wt). Blood samples were collected from the tail vein every 15 min for 1 h. For insulin tolerance tests, human insulin (Novolin; Novo Nordisk, Princeton, NJ) was injected (2 mU/g body weight, i.p.) after an overnight fast and blood glucose concentrations were measured with samples obtained from tail vein at 0, 15, 30, 45, and 60 min after injection.

RNA analysis. mRNA for protein tyrosine phosphatase 1B (PTP-1B), peroxisome proliferator−activated receptor-γ coactivator 1 (PGC-1), stearoyl-CoA desaturase 1 (SCD-1), fatty acid transporter (FAT), suppressor of cytokine signaling 3 (SOCS-3), and adiponectin was quantified by Northern blot analysis. Total RNA was extracted in TRIzol (GIBCO BRL, Rockville, MD) and Northern blot analysis was performed without pooling individual samples by using a single-stranded, internally labeled DNA probe, as previously described (25). Template DNA for the probe was prepared from mouse liver or adipose RNA by RT-PCR with specific primers (Table 1). Membranes were reprobed and hybridized with ^{32}P-labeled probe encoding 18S ribosomal RNA (rRNA). The total integrated densities of hybridization signals were determined by phosphoimager (STORM 860; Molecular Dynamics, Sunnyvale, CA) and all signals were normalized to the 18S rRNA signal.

Assays. Blood glucose was measured by a Glucometer Elite kit (Bayer, Elkhart, IN). Serum or plasma insulin and leptin were assayed by enzyme-linked immunosorbent assay with commercial kits (Crystal Chem, Chicago, IL). The presence of glucose in urine was tested using Keto-Diastix strips (Bayer). Brain α-MSH and β-endorphins were assayed by radioimmunoassay with commercial kits (Peninsula Laboratories, San Carlos, CA). Serum free fatty acid (FFA) concentrations were measured by an enzymatic colorimetric method (Waco Chemicals, Richmond, VA).

Statistical analysis. Data are presented as means ± SE. One- or two-way ANOVA followed by Tukey-Kramer test was used, except for comparisons between two groups, which were done by unpaired Student's *t* test. When the variances across groups were not equal, the Wilcoxon (nonparametric) test was used to assess statistical significance. In all cases, differences were taken to be significant at $P < 0.05$.

RESULTS

Effect of POMC transgene in wild-type mice. Using the *Nse* promoter (28) to generate mice with an increased expression of the *Pomc* gene in neurons, we chose one of two founders (Fig. 1*A*) for further study. This promoter construct has previously been shown to produce ubiquitous expression of the target gene in neurons throughout

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TABLE 1
Primer sequences of genes used for RT-PCR to generate probes for Northern blot analysis

Gene	Sequence of forward (upper) and reverse (lower) primers	GenBank accession no.
PTP-1B	5′-GAACTGGGCGGCTATTTACCAG-3′ 5′-GGGTCTTTCCTCTTGTCCATCAG-3′	NM_011201
PGC-1	5′-CTCCCACAACTCCTCCTCATAAAG-3′ 5′-CATTACTGAAGTCGCCATCCCTTAG-3′	NM_008904
SCD-1	5′-GTCAAAGAGAAGGGCGGAAAAC-3′ 5′-AAGGTGTGGTGGTAGTTGTGGAAG-3′	NM_009127
FAT	5′-GCAGGTCTATCTACGCTGTGTTCG-3′ 5′-TGGTCCCAGTCTCATTTAGCCAC-3′	NM_007643
SOCS-3	5′-CCACTTCTTCACGTTGAGCGTC-3′ 5′-CACTTTCTCATAGGAGTCCAGGTGG-3′	U88328
Adiponectin	5′-GCAAGCTCTCCTGTTCCTCTTAATC-3′ 5′-TGCATCTCCTTTCTCTCCCTTCTC-3′	AF304466
18S rRNA	5′-TACCTGGTTGATCCTGCC-3′ 5′-GCCATTCGCAGTTTCACTG-3′	X00686

the central nervous system (28). POMC mRNA was expressed in brain, including hypothalamus, thalamus, hippocampus, and cortex, but not other tissues, including liver, adrenal gland, kidney, spleen, heart, white adipose tissue, stomach, and skeletal muscle (Fig. 1*B*). The expression pattern of POMC mRNA was corroborated by in situ hybridization and immunocytochemistry (data not shown). The POMC transgene led to a sixfold increased expression of hypothalamic POMC mRNA (combined endogenous and transgene) (Fig. 1*C* and *D*). Consistent with the elevated level of POMC mRNA, the POMC transgene increased hypothalamic α-MSH ~10-fold higher in $Pomc^{+/tg}$ mice (Fig. 1*E*). The POMC transgene also led to an elevation of hypothalamic β-endorphin levels (175.8 ± 50.1 ng/mg protein; $P < 0.05$) compared with $Pomc^{+/+}$ mice (10.2 ± 3.1 ng/mg protein).

In female wild-type mice, the POMC transgene did not influence food intake (Figs. 1*F* and 2*E*) or gonadal fat pad weight (Fig. 2*D*), although body weight decreased slightly (Fig. 2*C*). The POMC transgene produced similar but slightly greater effects on body weight in male wild-type mice (Fig. 2*B*). To facilitate interpretation of these results, most of the data presented are data derived from female mice (in which the POMC transgene produced minimal effects in wild-type ad libitum−fed mice that otherwise expressed normal levels of POMC), although the effects of the POMC transgene on phenotypes in obese animal models were qualitatively identical in both sexes (e.g., body weight) (Fig. 2*B*). Although the POMC transgene did not influence metabolic rate in wild-type mice as indicated by oxygen consumption (V_{O_2}) (Fig. 2*F*), the POMC transgene did reduce total oxygen consumption during the 2-h experimental period very slightly, but significantly (Fig. 2*G*), apparently secondary to the slight decrease in body weight. The POMC transgene on the wild-type background did not influence blood glucose, serum insulin, or serum leptin (data not shown). The POMC transgene did not influence spontaneous food intake in ad libitum−fed wild-type mice, but did significantly reduce hyperphagia produced by a 24-h fast (Fig. 1*F*).

POMC transgene partially corrected obese phenotypes in leptin-deficient mice. To assess if increased POMC gene expression could reverse obese and diabetic phenotypes, we expressed the POMC transgene in leptin-deficient mice. The POMC transgene robustly reduced body size, body weight, and gonadal fat pad weight in leptin-deficient mice to levels intermediate between those of $Lep^{+/+}$ and $Lep^{ob/ob}$ mice (Fig. 2*A*– *D*). The POMC transgene also largely, but not completely, corrected the robust increase in liver weight and serum FFA levels in leptin-deficient mice (Table 2).

The POMC transgene also partially, but significantly, reversed the effect of leptin deficiency on food intake and body temperature (Fig. 2*E* and *H*), but did not influence the activity level (data not shown). The POMC transgene partially reversed the reduced V_{O_2} (oxygen consumption normalized to body weight) in leptin-deficient mice (Fig. 2*F*). Total oxygen consumption during the 2-h period examined was not reduced in $Lep^{ob/ob}$ mice compared with $Lep^{+/+}$ mice, but the POMC transgene decreased total oxygen consumption in both genotypes (Fig. 2*G*), apparently secondary to the reduction in body weight.

POMC effectively normalized glucose metabolism in leptin-deficient mice. The POMC transgene effectively normalized ad libitum−fed blood (Fig. 3*A*) and urine glucose levels in leptin-deficient mice. Thus, 8 of 15 $Pomc^{+/+}$ $Lep^{ob/ob}$ mice showed glucosuria, whereas none of the $Pomc^{+/+}$ $Lep^{+/+}$ ($n = 18$) or $Pomc^{+/tg}$ $Lep^{ob/ob}$ mice ($n = 12$) exhibited detectable levels of glucose in the urine. Similarly, the POMC transgene effectively normalized impaired glucose tolerance in leptin-deficient mice (Fig. 3*B*), an effect we have observed up to age 1 year (data not shown). The POMC transgene also almost completely normalized serum insulin levels (Fig. 3*C*) and sensitivity to insulin (Fig. 3*D*), as assessed by insulin-induced hypoglycemia, in leptin-deficient mice. Finally, the POMC transgene enhanced glucose-induced insulin secretion, which was otherwise completely impaired in leptin-deficient mice (Fig. 3*E*).

To assess if the effect of the POMC transgene on improving glucose metabolism in leptin-deficient mice was attributable to reduced food intake and body weight, we compared glucose tolerance in $Pomc^{+/tg}$ $Lep^{ob/ob}$, age-matched $Pomc^{+/+}$ $Lep^{ob/ob}$, pair-fed $Pomc^{+/+}$ $Lep^{ob/ob}$, and weight-matched $Pomc^{+/+}$ $Lep^{ob/ob}$ mice. Pair-feeding $Pomc^{+/+}$ $Lep^{ob/ob}$ mice to match the food intake of $Pomc^{+/tg}$ $Lep^{ob/ob}$ mice did not reduce body weight (67.4 ± 4.4 vs. 66.7 ± 3.9 g, before and after pair-feeding, respec-

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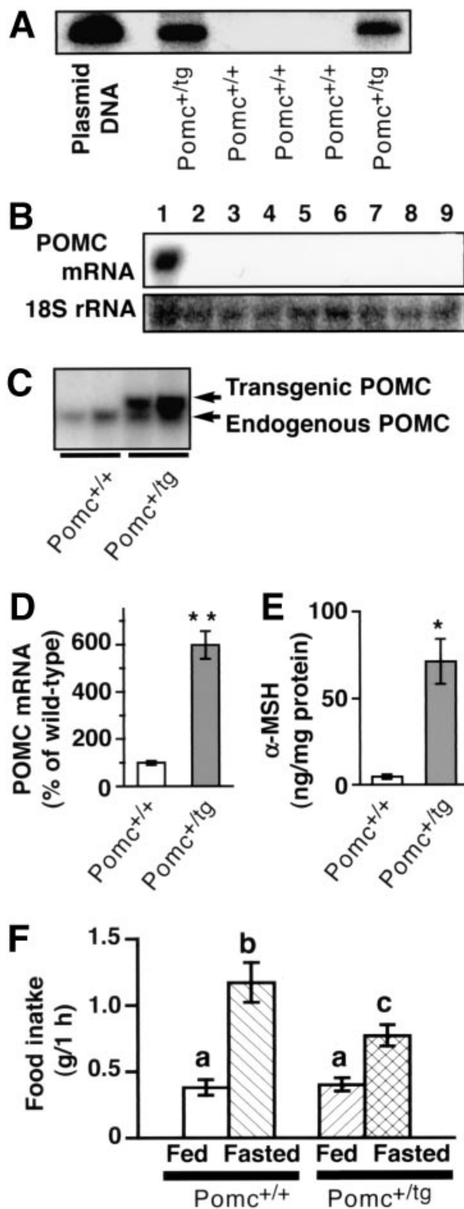

FIG. 1. Increased expression of *Pomc* gene in the brain of POMC transgenic mice. *A*: Representative Southern blot analysis of genomic DNA for genotyping. *B*: Expression of POMC mRNA assessed by Northern blot analysis in total RNA (7 μg) from various tissues (brain, *lane 1*; liver, *lane 2*; heart, *lane 3*; spleen, *lane 4*; kidney, *lane 5*; white adipose tissue, *lane 6*; stomach, *lane 7*; skeletal muscle, *lane 8*; adrenals, *lane 9*) from transgenic mouse. The membrane was reprobed with the probe encoding 18S rRNA. *C*: Northern blot analysis on hypothalamic tissue of $Pomc^{+/+}$ (*lanes 1* and *2*) and $Pomc^{+/tg}$ mice (*lanes 3* and *4*). Hypothalamic RNA from transgenic mice revealed two bands; lower (~0.9-kb) and upper (~1.5-kb) bands represent endogenous POMC mRNA and transgene-derived mRNA, respectively. *D*: Quantification of hypothalamic POMC mRNA in $Pomc^{+/+}$ ($n = 11$) and $Pomc^{+/tg}$ mice ($n = 8$) as assessed by Northern blot analysis. Data are presented as percent of $Pomc^{+/+}$ mice. Data are means ± SE. **$P < 0.0001$ vs. $Pomc^{+/+}$ mice by unpaired Student's t test. *E*: Hypothalamic content of α-MSH in $Pomc^{+/+}$ ($n = 3$) and $Pomc^{+/tg}$ mice ($n = 3$). Data are means ± SE. *$P < 0.05$ vs. $Pomc^{+/+}$ mice by unpaired Student's t test. *F*: Fasting-induced hyperphagic response. Animals' 1-h food intake was measured during 1st h after lights out in ad libitum–fed mice and 24-h fasted mice at age 27 weeks. Data are means ± SE for $Pomc^{+/+}$ ($n = 10$) and $Pomc^{+/tg}$ ($n = 9$). Groups with different letters were statistically different ($P < 0.05$ by Tukey-Kramer test).

tively), nonfasting blood glucose (263.6 ± 74.3 vs. 298.6 ± 75.4 mg/dl, before and after pair-feeding, respectively) (Fig. 3*A*), fasting blood glucose levels (Fig. 4*A*), or glucose tolerance in $Pomc^{+/+}$ $Lep^{ob/ob}$ mice (Fig. 4*A*). Matching body weight to $Pomc^{+/tg}$ $Lep^{ob/ob}$ mice also failed to normalize glucose tolerance in $Pomc^{+/+}$ $Lep^{ob/ob}$ mice (Fig. 4*B*). Consistent with these observations, an impaired glucose tolerance was observed in $Pomc^{+/+}$ $Lep^{ob/ob}$ mice as early as age 4 weeks, before the effect of leptin deficiency on body weight was significant.

POMC transgene corrected metabolic gene expression in leptin-deficient mice. To identify possible molecular mechanisms through which the POMC transgene corrects glucose metabolism in leptin-deficient mice, we measured the expression of genes involved in the regulation of glucose homeostasis in the liver and adipose tissue (Table 3). In the liver, the POMC transgene completely reversed the elevated expression of PTP-1B and PGC-1, but only partially reversed the elevated expression of SCD-1 and FAT mRNA in leptin-deficient mice (Table 3). In adipose tissue, the POMC transgene completely reversed the elevated expression of SOCS-3 and partially reversed the reduced expression of adiponectin in leptin-deficient mice (Table 3).

DISCUSSION

The results of the present study demonstrated that the POMC transgene attenuates fasting-induced hyperphagia without having significant effects on spontaneous food intake or adiposity in wild-type mice. Because hypothalamic POMC mRNA is reduced by fasting (3), the current observations suggest that enhanced POMC tone may be effective in reducing food intake only when endogenous POMC expression is reduced. Because fasting is associated with elevated hypothalamic neuropeptide Y (*Npy*) (30,31) and agouti-related peptide (*Agrp*) gene expression (32), these and other genes may also contribute to fasting-induced hyperphagia. However, ablation of AGRP does not appear to impair fasting-induced hyperphagia, and the effects of ablating the *Npy* gene on fasting-induced hyperphagia appears to depend on genetic background and the timing of fasting (33–36). Thus, reduction in *Pomc* gene expression appears to be required for the complete compensatory hyperphagic response to fasting, although other factors, possibly including an elevation of NPY under some circumstances, also contribute to this response.

Because leptin-deficient mice exhibited decreased hypothalamic POMC mRNA and POMC neurons are responsive to leptin (3,37), we hypothesized that increased central *Pomc* gene expression might correct metabolic impairments associated with leptin deficiency. In the present studies, the POMC transgene significantly, but not completely, reversed effects of leptin deficiency on body weight, food intake, adiposity, free fatty acids, V_{O_2}, and body temperature. Pair-feeding $Pomc^{+/+}$ $Lep^{ob/ob}$ mice to levels of food consumed by $Pomc^{+/tg}$ $Lep^{ob/ob}$ mice did not significantly reduce body weight in the absence of the POMC transgene, suggesting that the effect of the POMC transgene on reducing body weight in leptin-deficient mice was not mediated by reduction of food intake. Although V_{O_2} and total oxygen consumption (over a 2-h period) suggested somewhat conflicting conclusions regarding the effect of the POMC transgene and leptin deficiency on metabolic rate, the POMC transgene clearly increased body temperature in leptin-deficient mice, strongly indicating that the POMC transgene did in fact increase the

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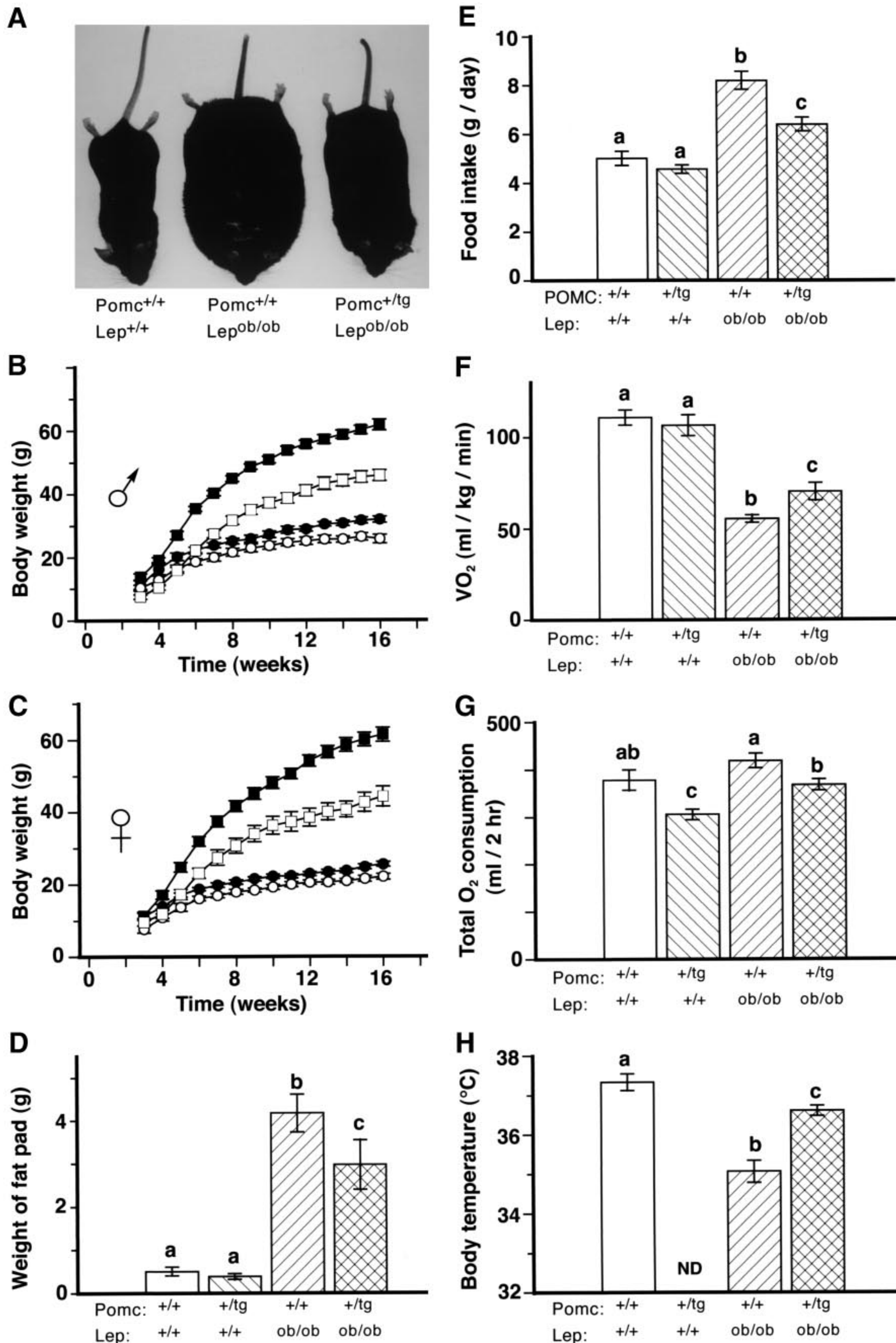

FIG. 2. Effects of the POMC transgene on physical appearance (*A*), body weight (*B* and *C*), gonadal white adipose tissue mass (*D*), food intake (*E*), V_{O_2} (*F*), total oxygen consumption (*G*), and body temperature (*H*) in *Lep*$^{ob/ob}$ mice. Body temperature was not determined in *Pomc*$^{+/tg}$ *Lep*$^{+/+}$ mice. Data are means ± SE (n = 6–17), and were obtained from the entire cohort in *B* and *C*. All curves were significantly different in *B* and *C* (P < 0.0001, two-way ANOVA). Groups with different letters were statistically different (P < 0.05, Tukey-Kramer or Wilcoxon test). *B* and *C*: ●, *Pomc*$^{+/+}$ *Lep*$^{+/+}$; ○, *Pomc*$^{+/tg}$ *Lep*$^{+/+}$; ■, *Pomc*$^{+/+}$ *Lep*$^{ob/ob}$; □, *Pomc*$^{+/tg}$ *Lep*$^{ob/ob}$.

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TABLE 2
Effects of the POMC transgene on liver weights and serum FFA levels in $Lep^{ob/ob}$ mice

	$Pomc^{+/+}$ $Lep^{+/+}$	$Pomc^{+/tg}$ $Lep^{+/+}$	$Pomc^{+/+}$ $Lep^{ob/ob}$	$Pomc^{+/tg}$ $Lep^{ob/ob}$
Liver (g)	1.44 ± 0.07[a]	1.17 ± 0.04[a]	5.72 ± 0.42[b]	2.28 ± 0.35[c]
Serum FFA (mEq/l)	0.74 ± 0.04[a]	0.82 ± 0.06[ab]	1.46 ± 0.20[c]	0.94 ± 0.11[b]

Data are means ± SE (n = 6–22). Groups with different letters were statistically different (P < 0.05, Tukey-Kramer or Wilcoxon test).

metabolic rate in leptin-deficient mice. Consistent with this conclusion, central administration of α-MSH or MTII, a melanocortin receptor agonist, decreases body weight gain independent of food intake (38,39). Taken together, these data suggest that the POMC transgene reduced body weight largely by enhancing thermogenesis.

In contrast to its partial effect in many obese phenotypes, the POMC transgene effectively normalized impairments in glucose metabolism in leptin-deficient mice. This effect appeared to be mediated in large part by enhancement of peripheral insulin sensitivity, as the POMC transgene profoundly reduced serum insulin and improved responsiveness to insulin in leptin-deficient mice. Nevertheless, the POMC transgene also enhanced glucose-induced insulin secretion in leptin-deficient mice, which otherwise was almost completely impaired (although leptin-deficient mice without the transgene were of course already hyperinsulinemic). The effect of the POMC transgene on glucose homeostasis appeared to be independent of the effects on food intake and body weight, as glucose homeostasis in leptin-deficient mice without the POMC transgene was not significantly improved by matching food intake or body weight levels observed in leptin-deficient mice with the POMC transgene. These data suggest that the effects of the POMC transgene on glucose homeostasis may be at least partially independent of effects on energy homeostasis. This conclusion is consistent with several observations: *1*) central administration of MTII results in decreased basal insulin secretion within 1 h after injection in both leptin-deficient and wild-type mice (26); *2*) young mice with a disrupted *Mc4r* gene exhibit elevated plasma insulin levels before the onset of hyperphagia and abnormal weight gain (26); *3*) in goldthioglucose (GTG)-treated mice, another animal model of obesity and type 2 diabetes associated with reduced hypothalamic POMC (10), hyperinsulinemia is also evident within 1 week after GTG treatment preceding the progressive increase in adiposity (40); and *4*) insulin resistance is

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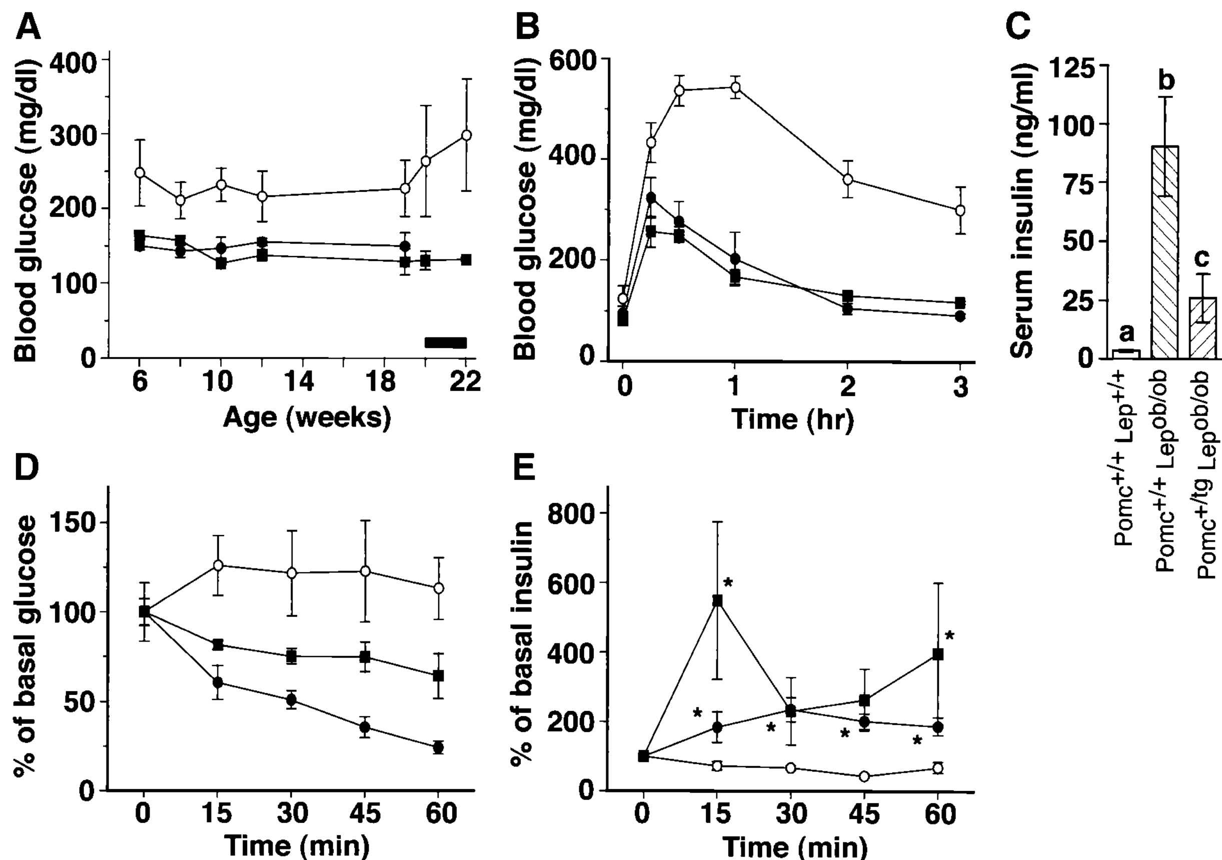

FIG. 3. Effects of the POMC transgene on blood glucose (*A*), glucose tolerance (*B*), serum insulin (*C*), insulin tolerance (*D*), and plasma insulin levels during the glucose tolerance test (*E*) in $Lep^{ob/ob}$ mice. The bar represents the period of pair-feeding in *A*. Data are means ± SE (n = 4–12). Groups with different letters were statistically different in *C* (P < 0.05, Wilcoxon test). Curves were significantly different in *A*, *B*, and *D* with the exception of $Pomc^{+/+}$ $Lep^{+/+}$ versus $Pomc^{+/tg}$ $Lep^{ob/ob}$ in *A* and *B* (P < 0.005, two-way ANOVA). *P < 0.05 vs. $Pomc^{+/+}$ $Lep^{ob/ob}$ mice at same time point in *E* (Tukey-Kramer or Wilcoxon test). *A–D*: ●, $Pomc^{+/+}$ $Lep^{+/+}$; ○, $Pomc^{+/+}$ $Lep^{ob/ob}$; ■, $Pomc^{+/tg}$ $Lep^{ob/ob}$.

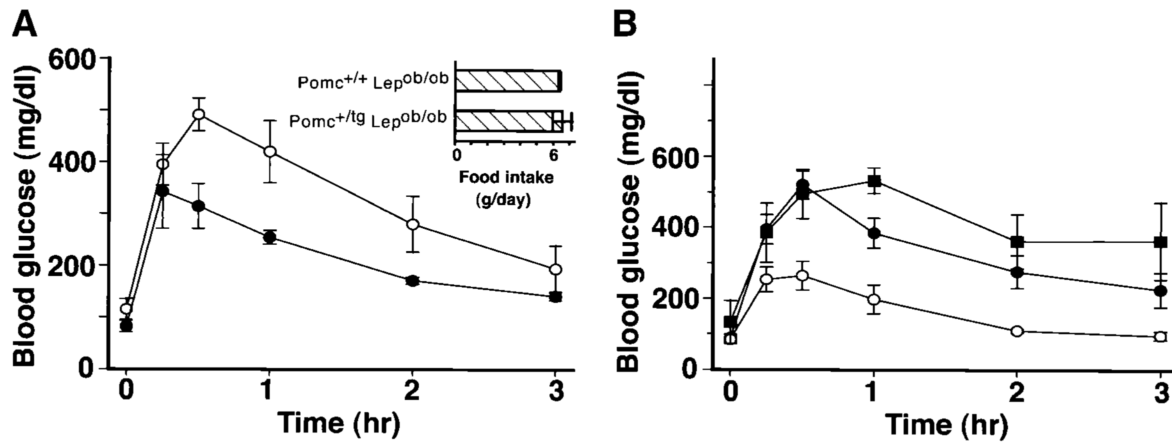

FIG. 4. Effects of pair-feeding (*A*) and body weight (*B*) on glucose tolerance in *Pomc$^{+/+}$ Lep$^{ob/ob}$* mice. *Pomc$^{+/+}$ Lep$^{ob/ob}$* mice were pair-fed to the amount of food intake of age-matched *Pomc$^{+/tg}$ Lep$^{ob/ob}$* mice for 2 weeks. Glucose tolerance tests were performed in *Pomc$^{+/tg}$ Lep$^{ob/ob}$* mice (age 18 weeks) and age-matched *Pomc$^{+/+}$ Lep$^{ob/ob}$* and weight-matched *Pomc$^{+/+}$ Lep$^{ob/ob}$* mice (age 12 weeks). *A*, *inset*: Average food intake (g/day) of ad libitum−fed *Pomc$^{+/tg}$ Lep$^{ob/ob}$* and pair-fed *Pomc$^{+/+}$ Lep$^{ob/ob}$* mice. Data are means ± SE (n = 4–6). Curves are significantly different (*A*: P < 0.0001, *B*: P < 0.05, two-way ANOVA). *A*: ○, *Pomc$^{+/+}$ Lep$^{ob/ob}$*; ●, *Pomc$^{+/tg}$ Lep$^{ob/ob}$*. *B*: ○, *Pomc$^{+/tg}$ Lep$^{ob/ob}$* (45.0 ± 2.4 g); ●, *Pomc$^{+/+}$ Lep$^{ob/ob}$* (66.8 ± 3.5 g); ■, *Pomc$^{+/+}$ Lep$^{ob/ob}$* (46.1 ± 1.0 g).

increased in rats treated with SHU9119, a melanocortin receptor antagonist, independent of food intake (38).

Because the POMC transgene led to the elevation of α-MSH as well as β-endorphins and probably other peptides normally derived from the POMC precursor, the present study did not allow direct assessment of which POMC products mediate the effects of the POMC transgene on obese and diabetic phenotypes. Thus, although α-MSH clearly plays an essential role in regulating metabolic phenotype, recent studies have also supported a similar role for β-endorphins (41). Other recent studies have also suggested an important role for another product of the POMC precursor, β-MSH, in regulating food intake (42). It will therefore be of interest to assess if specific pharmacological blockers of POMC-derived peptides can block the effects of the POMC transgene on metabolic function, and if transgenes expressing only specific POMC products such as α-MSH will be able to mimic the metabolic effects of the POMC transgene. These studies are currently in progress.

The precise mechanism by which enhanced neuronal POMC expression reverses obese and diabetic phenotypes in leptin-deficient mice is unknown at present. Elevated hepatic PTP-1B and adipose SOCS-3 are associated with insulin resistance, and expression of these genes is elevated in leptin-deficient mice (43,44). The transcriptional coactivator PGC-1 activates gluconeogenesis and increases hepatic glucose output (45) and is also elevated in leptin-deficient mice. Because the POMC transgene effectively normalized the expression of genes involved in carbohydrate metabolism, these gene products may mediate some of the effects of neuronal POMC on glucose metabolism. We hypothesize that central POMC regulates the expression of these genes in the liver through modulation of the sympathetic nervous system, but this hypothesis remains to be assessed.

Although it remains unclear if POMC is a necessary mediator of metabolic effects of leptin, our data suggest that the reduced expression of hypothalamic POMC observed in leptin-deficiency (1–3) may largely account for the impaired glucose homeostasis associated with leptin deficiency and may partially mediate effects of leptin deficiency on abnormal weight gain and hyperphagia. These results suggest that centrally acting agonists of POMC products might be effective in treating impairments in glucose metabolism associated with reduced hypothalamic POMC caused, for example, by mutations in several genes (e.g., *obese*, *tubby*, *nhlh2*) (11,12), hypothalamic damage (10), or, perhaps most commonly, aging (13).

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TABLE 3
Effects of the POMC transgene on hepatic and adipose gene expressions in Lep$^{ob/ob}$ mice

Gene expression	*Pomc$^{+/+}$ Lep$^{+/+}$*	*Pomc$^{+/+}$ Lep$^{ob/ob}$*	*Pomc$^{+/tg}$ Lep$^{ob/ob}$*
Liver			
PTP-1B mRNA	100.0 ± 6.7^{a}	157.6 ± 7.2^{b}	111.6 ± 7.3^{a}
PGC-1 mRNA	100.0 ± 11.4^{a}	142.7 ± 5.2^{b}	99.2 ± 25.1^{a}
SCD-1 mRNA	100.0 ± 15.8^{a}	288.4 ± 26.7^{b}	172.5 ± 35.9^{c}
FAT mRNA	100.0 ± 26.4^{a}	491.9 ± 36.5^{b}	342.8 ± 53.0^{c}
White adipose tissue			
SOCS-3 mRNA	100.0 ± 21.9^{a}	275.8 ± 28.9^{b}	150.7 ± 23.8^{a}
Adiponectin mRNA	100.0 ± 5.9^{a}	39.7 ± 3.7^{b}	56.1 ± 4.3^{c}

Data are means ± SE (n = 3–12). Values are expressed as a percentage of those in *Pomc$^{+/+}$ Lep$^{+/+}$* mice. Groups with different letters were statistically different ($P < 0.05$, Tukey-Kramer or Wilcoxon test).

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