

# Central Pro-opiomelanocortin Gene Delivery Results in Hypophagia, Reduced Visceral Adiposity, and Improved Insulin Sensitivity in Genetically Obese Zucker Rats

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**Zucker (*fa/fa*) rats with defective leptin receptors are obese, hyperphagic, and hyperinsulinemic. For testing whether chronic activation of the central melanocortin pathway can bypass the defective leptin signaling and normalize altered energy homeostasis in these rats, recombinant adeno-associated virus encoding pro-opiomelanocortin (rAAV-POMC) or control vector was delivered bilaterally into the basal hypothalamus with coordinates targeting the arcuate nucleus. Thirty-eight days after POMC gene delivery, hypothalamic POMC expression increased fourfold and melanocortin signaling (indicated by phosphorylation of CREB) increased by 62% with respect to controls. There was a sustained reduction in food intake, a moderate but significant attenuation of weight gain, and a 24% decrease in visceral adiposity in rAAV-POMC rats. POMC gene delivery enhanced uncoupling protein 1 in brown adipose tissue (BAT) by more than fourfold. Fasting serum leptin, insulin, and cholesterol levels were also significantly reduced by rAAV-POMC treatment. This study demonstrates that targeted POMC gene delivery in the hypothalamus suppresses food intake and weight gain and reduces visceral adiposity and hyperinsulinemia in leptin-resistant obese Zucker rats. The mechanisms may involve the sustained hypophagia and the augmentation of thermogenesis in BAT. *Diabetes* 52:1951–1957, 2003**

**T**he central melanocortin system plays a critical role in the homeostatic regulation of body weight (1–5). Melanocortins (MCs) are bioactive peptides derived from a common prohormone, pro-opiomelanocortin (POMC), one of which,  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), is a major regulator of

feeding and body weight homeostasis. Reduced expression of hypothalamic POMC is associated with obesity syndromes caused by mutations in the leptin receptor (6,7) or other genes (*tubby*, *Nhlh2*, etc.) (8,9), by hypothalamic damage (10), and, perhaps most common, by aging (11). That reduced hypothalamic POMC mRNA could contribute to the obese phenotypes in these models is suggested by the observation that mutations in the POMC gene cause obesity in mice (12) and humans (13). However, it is still unclear whether normalization of central POMC tone can reverse obese phenotypes. The present study aims to address this question.

Leptin, an adipocyte-derived hormone, acts on satiety and appetite centers in the hypothalamus to both reduce food consumption and increase energy expenditure (14–16). Recent evidence suggests that the MC system may be located downstream of the hypothalamic leptin-signaling pathway. Leptin activates POMC- and agouti-related protein (AgRP)-containing neurons of the ventrolateral and ventromedial arcuate nucleus, respectively, resulting in an increase in the expression of POMC and a reduction in AgRP (15,17–20). In addition, leptin-induced suppression of food intake is effectively blocked by an MC 3/4 receptor antagonist (21), and the leptin-mediated induction of uncoupling protein 1 (UCP1), an important thermogenic protein in the brown adipose tissue (BAT), is also attenuated by central MC receptor antagonism (22).

Genetically obese *fa/fa* Zucker rats with a recessive mutation of the leptin receptor gene develop severe, early-onset obesity associated with hyperphagia, hyperleptinemia, and hyperinsulinemia (23,24). Using this leptin- and insulin-resistant rodent model, we examined whether overproduction of POMC in the hypothalamus would reduce body mass and adiposity and improve glucose metabolism in obese Zucker rats. In addition, some evidence suggests that tachyphylaxis to the MC-mediated reduction in food intake develops after chronic pharmacologic treatment of MC agonists in rodents (25,26). Thus, the second purpose of this study was to examine the consequences of long-term targeted overexpression of POMC on the MC-mediated anorexic response.

Recent successes in using recombinant adeno-associated virus (rAAV) to obtain long-term expression of transgenes provide an opportunity to test our hypotheses (27). There are many advantages of using rAAV, including nonpathogenicity, nonimmunogenicity, high viability of the virion, and, most important, long-term expression of the delivered transgene. The rAAV type 2 vector has been

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AgRP, agouti-related protein; BAT, brown adipose tissue; CBA, chicken  $\beta$ -actin; CREB, cAMP response element binding protein; FFA, free fatty acid; MC, melanocortin;  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulating hormone; P-CREB, phosphorylated cAMP response element binding protein; POMC, pro-opiomelanocortin; PWAT, perirenal white adipose tissue; rAAV, recombinant adeno-associated virus; RTWAT, retroperitoneal white adipose tissue; UCP1, uncoupling protein 1; WAT, white adipose tissue.

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uniquely successful as a gene transfer vector into the CNS (28).

In the present study, we used a rAAV type 2 vector encoding murine POMC (rAAV-POMC) to assess the long-term consequences of POMC gene delivery on energy balance, BAT thermogenesis, and hypothalamic MC signal transduction in obese Zucker rats. To this end, we administered rAAV-POMC or control vectors bilaterally into the arcuate nucleus of the hypothalamus of obese Zucker rats for 38 days and assessed food intake, body weight, adiposity, serum hormone and metabolite levels, BAT UCP1 protein, hypothalamic POMC, AgRP mRNA levels, and hypothalamic phosphorylation of the cAMP response element binding protein (CREB) (29).

## RESEARCH DESIGN AND METHODS

**Construction of rAAV vector plasmids.** pTR-CBA-POMC encodes murine POMC cDNA (a gift from Dr. James Roberts) (30) under the control of the hybrid cytomegalovirus immediate early enhancer/chicken  $\beta$ -actin (CBA) promoter (31). The woodchuck hepatitis virus posttranscriptional regulatory element is placed downstream of the POMC transgene to enhance its expression (32). The control plasmid, termed pTR-control, is similar to pTR-CBA-POMC except for the incorporation of the cDNA encoding an enhanced form of green fluorescent protein instead of POMC cDNA. The control vector was described previously (33).

**In vitro analysis of pTR-CBA-POMC plasmid.** The pTR-CBA-POMC construct was tested for in vitro expression of POMC by transfecting HEK 293 cells using FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) according to the protocol provided by the manufacturer. One day after the transfection, cells were harvested and total RNA was isolated. POMC mRNA levels were analyzed by RT-PCR.

**Packaging of rAAV vectors.** Vectors were packaged, purified, concentrated, and titered as previously described (34). The titers for both rAAV-POMC and rAAV-control vectors used in this study were  $4.26 \times 10^{11}$  physical particles/ml, and the ratios of physical-to-infectious particles for both vectors were  $<30$ .

**Animals.** Nine-week-old male obese Zucker (*fa/fa*) rats were obtained from Charles River (Wilmington, MA). Upon arrival, rats were examined and remained in quarantine for 2 weeks. Animals were cared for in accordance with the principles of the National Institutes of Health Guide to the Care and Use of Experimental Animals. Rats were housed individually with a 12:12-h light:dark cycle (0700–1900 h). Standard Purina 5001 rodent diet and water were provided ad libitum.

**rAAV vector administration.** Under 6 mg/kg xylazine (Phoenix Pharmaceutical, St. Joseph, MO) and 60 mg/kg ketamine (Monarch Pharmaceuticals, Bristol, TN) anesthesia, rats were administered bilaterally ( $1.28 \times 10^9$  particles/injection in 3  $\mu$ l) rAAV-POMC ( $n = 6$ ) or rAAV-control ( $n = 6$ ) into the basal hypothalamus with coordinates targeting the arcuate nucleus. The coordinates for injection into the hypothalamus were 3.14 mm posterior to bregma,  $\pm 0.4$  mm lateral to the midsagittal suture, and 10 mm ventral from the skull surface. On each side, a small hole was drilled through the skull and a 23-G stainless steel cannula was inserted followed by an injection cannula. With the use of a 10- $\mu$ l Hamilton syringe, a 3- $\mu$ l volume of virus stocks was delivered over 5 min to each site. The needles remained in place at the injection site for an additional 5 min. At the time of surgery, rats received an injection of the analgesic Buprenex (0.05 mg/kg; Reckitt and Colman, Richmond, VA).

**Tissue harvesting and preparation.** Rats were fasted overnight and killed by cervical dislocation under 85 mg/kg pentobarbital anesthesia. Blood samples were collected by heart puncture, and serum was harvested by a 15-min centrifugation in serum separator tubes. The circulatory system was perfused with 30 ml of cold saline, and BAT, perirenal white adipose tissue (PWAT), and retroperitoneal white adipose tissue (RTWAT) were excised. The hypothalamus was removed by making an incision medial to piriform lobes, caudal to the optic chiasm and anterior to the cerebral crus to a depth of 2–3 mm. Tissues were stored at  $-80^\circ\text{C}$  until analysis. For Western analysis, hypothalamus and BAT were homogenized in 0.3 ml of 10 mmol/l Tris-HCl (pH 6.8), 2% SDS, and 0.08  $\mu$ g/ml okadaic acid. Protease inhibitors, 1 mmol/l phenylmethylsulfonyl fluoride, 0.1 mmol/l benzamide, and 2  $\mu$ mol/l leupeptin were also present. Homogenates were immediately boiled for 2 min, cooled on ice, and stored frozen at  $-80^\circ\text{C}$ . Protein was determined using the DC protein assay kit (Bio-Rad, Hercules, CA). BAT samples were filtered through a 0.45- $\mu$ m syringe filter (Whatman, Clifton, NJ) to remove lipid particles before protein measurements.

**Serum leptin, insulin, glucose, free fatty acids, and cholesterol.** Serum leptin and insulin were measured using rat radioimmunoassay kits (Linco Research, St. Charles, MO). Serum free fatty acids (FFAs) and total cholesterol were determined by enzymatic colorimetric kits from WAKO Chemicals (Neuss, Germany). Serum glucose was via a colorimetric reaction with Trinder, the Sigma Diagnostics Glucose reagent (St. Louis, MO).

**RT-PCR.** POMC and AgRP expression in the hypothalamus were identified by relative quantitative RT-PCR using QuantumRNA 18S Internal Standards kit (Ambion, Austin, TX). Total cellular RNA was extracted as described (33) and treated with RNase-free DNase (Ambion). First-strand cDNA synthesis was generated from 2  $\mu$ g of RNA in a 20- $\mu$ l volume using random primers (Life Technologies, Rockville, MD) containing 200 units of M-MLV reverse transcriptase (Life Technologies). Relative quantitative PCR was performed by multiplexing corresponding primers (POMC sense 5'-GCTTGCAAACCTCGACC TCTC-3', antisense 5'-CTTGATGATGGCGTCTTGA-3'; AgRP sense 5'-AGGG CATCAGAAGGCCTGACCA-3', antisense 5'-CTTGAAGAAGCGGCAGTAGCA C-3'), 18S primers, and competitors and coamplifying. Linearity for the POMC and AgRP amplicons was determined to be between 20 and 29 cycles. The optimum ratio of 18S primer to competitor was 1:4 for POMC and 1:9 for AgRP. PCR was performed at  $94^\circ\text{C}$  denaturation for 60 s,  $59^\circ\text{C}$  annealing temperature for 45 s, and  $72^\circ\text{C}$  elongation temperature for 60 s for 26 (POMC) or 24 (AgRP) cycles. The PCR product was electrophoresed on acrylamide gel and stained with SYBR green (Molecular Probes, Eugene, OR). Gels were scanned using a STORM fluorescent scanner, and data were analyzed using ImageQuant (Molecular Dynamics). The relative values of POMC and AgRP mRNA were derived from dividing the signal obtained for corresponding amplicon by that for 18S amplicon.

**CREB and phosphorylated CREB assay.** Immunoreactive CREB and phosphorylated (P-CREB) were determined with a PhosphoPlus CREB (Ser 133) antibody kit (New England Biolabs, Beverly, MA). Hypothalamic samples (40  $\mu$ g) prepared as described above were separated on an SDS-PAGE gel and electrotransferred to nitrocellulose membrane. Immunoreactivity was assessed on separate membranes with antibodies specific to CREB (phosphorylated and unphosphorylated) and antibodies specific to serine 133 P-CREB. Immunoreactivity was visualized by enhanced chemiluminescent detection (Amersham Pharmacia Biotech) and quantified by video densitometry (Bio-Rad).

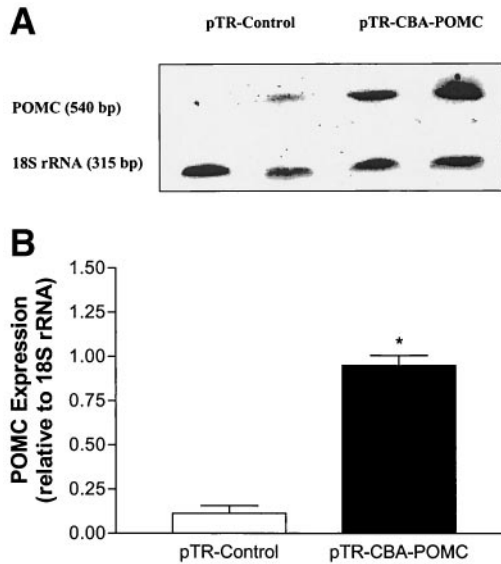
**UCP1 Protein.** Immunoreactive UCP1 in BAT homogenates (20  $\mu$ g) was determined as described for CREB, except that an antibody specific to rat UCP1 (Linco Research) was used.

**Statistical analysis.** Results are presented as mean  $\pm$  SE. Repeated measures ANOVA was used for analyses of body weight and food intake. When the main effect was significant, a post hoc *t* test was applied to determine individual differences between means. For all other data, unpaired two-tailed Student's *t* test was used.  $P < 0.05$  was considered significant.

## RESULTS

**Transient expression of POMC in HEK 293 cells.** HEK 293 cells were transfected with pTR-CBA-POMC or pTR-control plasmids (Fig. 1). Twenty-four hours after the transfection, total POMC mRNA expression was measured by relative quantitative RT-PCR. The primers used do not discriminate among mouse, rat, and human POMC; thus, the measured POMC mRNA represents both transgene mouse POMC and endogenous HEK 293 human POMC mRNA. The pTR-CBA-POMC transfection increased POMC expression in HEK 293 cells by more than eightfold as compared with pTR-control ( $P < 0.001$ ).

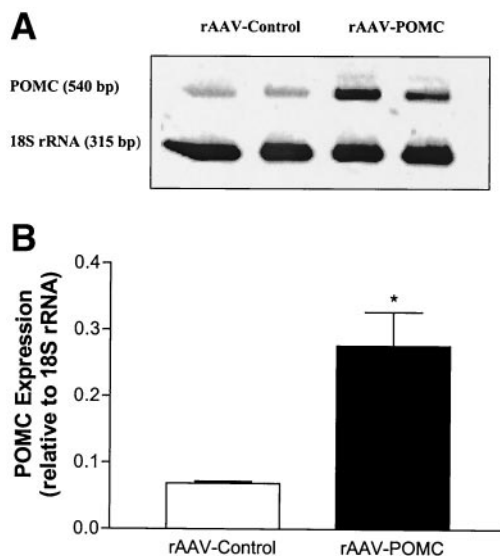
**POMC expression in the hypothalamus of obese Zucker rats.** To verify the overexpression of the POMC transgene after central viral delivery, POMC mRNA was measured in the hypothalamus by RT-PCR (Fig. 2). Thirty-eight days after vector delivery, hypothalamic POMC mRNA levels were elevated by fourfold in obese Zucker rats that were given rAAV-POMC as compared with those that were given rAAV-control ( $P < 0.02$ ). The ratio of hypothalamic POMC to 18S rRNA expression ranged from 0.061 to 0.073 for control animals and from 0.186 to 0.435 for rAAV-POMC-treated rats. Therefore, the POMC mRNA levels were at least twofold higher in all of the rAAV-



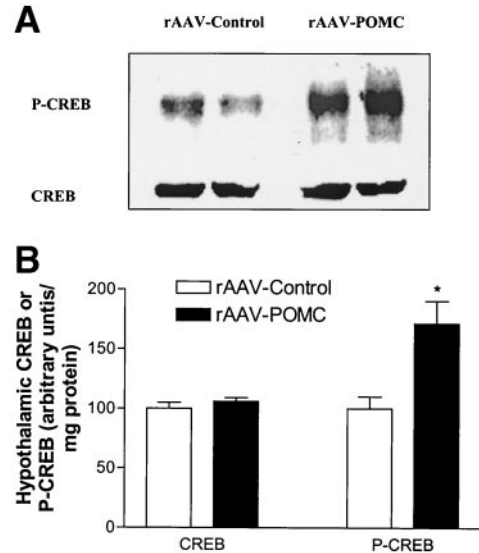
**FIG. 1.** POMC expression in HEK 293 cells 24 h after pTR-CBA-POMC or pTR-control transfection. **A:** Representative image of relative quantitative RT-PCR analysis of POMC mRNA expression with 18S rRNA as an internal standard. **B:** Quantification of POMC mRNA normalized to 18S rRNA. Data represent mean  $\pm$  SE from three experiments. \* $P < 0.001$  vs. control by unpaired  $t$  test.

POMC rats as compared with the mean levels in the control animals.

**MC signal transduction and AgRP mRNA levels.**  $\alpha$ -MSH, one of the cleavage products of POMC, is reported to exert its anorexic and thermogenic effects via activation of hypothalamic melanocortin receptors and subsequent phosphorylation of the transcription factor CREB (P-CREB) (35). We thus assessed hypothalamic CREB and P-CREB immunoreactivities 38 days after POMC vector delivery. Phosphorylation of CREB was elevated by 62% in the hypothalami of rats with rAAV-POMC treatment, whereas total CREB levels were unchanged (Fig. 3).



**FIG. 2.** Hypothalamic POMC expression 38 days after rAAV-POMC or control vector delivery in obese Zucker rats. **A:** Representative image of relative quantitative RT-PCR analysis of hypothalamic POMC mRNA with 18S rRNA as an internal standard. **B:** Quantification of POMC mRNA normalized to 18S rRNA. Data represent mean  $\pm$  SE from six rats per group. \* $P < 0.02$  vs. control by unpaired  $t$  test.



**FIG. 3.** Total CREB and P-CREB in the hypothalamus of obese Zucker rats 38 days after rAAV-POMC or rAAV-control delivery. **A:** Representative Western immunoblots using antibodies specific for CREB or P-CREB. **B:** Quantification of average CREB and P-CREB in six control and six rAAV-POMC rats. Levels in rAAV-control rats are set to 100 and SE adjusted proportionally. \* $P < 0.05$  vs. control by unpaired  $t$  test.

The expression of AgRP, an endogenous antagonist of MC receptors, is presumably regulated by leptin in normal animals (15,17–20). RT-PCR revealed that hypothalamic AgRP expression levels were unchanged in rats that were given rAAV-POMC as compared with control rats at the point at which they were killed ( $0.927 \pm 0.092$  vs.  $0.995 \pm 0.053$  relative to 18S rRNA;  $P > 0.5$ ).

**Body weight and food intake.** Bilateral delivery of rAAV-POMC into the basal hypothalamus reduced weight gain and food intake in obese Zucker rats (Fig. 4). Before and on the day of vector delivery, average body weight of rAAV-POMC-treated rats was comparable to that of control rats ( $428 \pm 18$  vs.  $403 \pm 23$  g at day 0). After vector delivery, the rats that were given rAAV-POMC consistently gained less weight, and the difference in body mass between the two groups gradually increased over the 38 days (Fig. 4A). Because daily food consumption of obese Zucker rats varied noticeably between individual rats (Fig. 4B), daily food intake after vector delivery was also expressed as percentage of individual baseline levels, represented by the average daily food intake 1 week before vector administration (Fig. 4C). Hypothalamic delivery of rAAV-POMC induced a sustained anorexic response in these obese rats. The inhibition of food intake became statistically significant starting at day 7 after POMC vector delivery and lasted for the duration of the experiment (Fig. 4B and C).

**Visceral adiposity and serum leptin levels.** Because POMC gene therapy reduced the weight gain of the obese Zucker rats, body adiposity levels were assessed. Thirty-eight days after central POMC gene delivery, there were significant reductions in the visceral adiposity, as reflected by a 24% reduction in the sum of the PWAT and RTWAT ( $P < 0.05$ ) in rAAV-POMC-treated compared with control rats (Fig. 5A). Fasting serum leptin levels, known to be highly correlated with body fat mass (36), were 43.5% lower in the rAAV-POMC group compared with the control group (Fig. 5B).

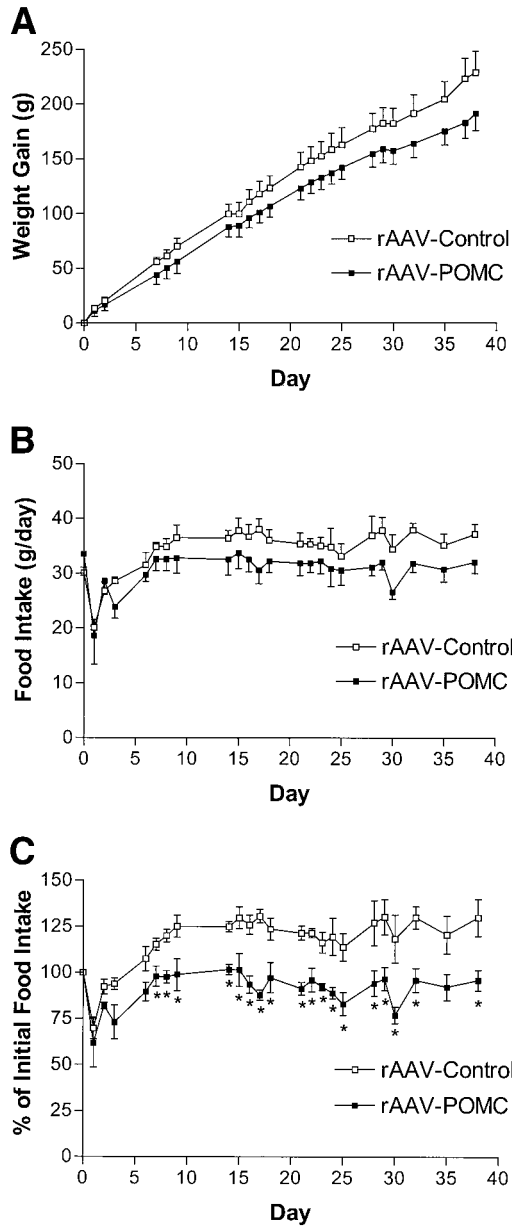


FIG. 4. Body weight gain (A), daily food consumption (B), and the percentage of initial food intake (C) after rAAV-POMC or rAAV-control administration in obese Zucker rats. The vectors were injected at day 0. Data represent mean  $\pm$  SE of six rats per group. Initial food intake is the average daily food consumption 1 week before vector delivery.  $P < 0.0001$  for difference in weight gain (A), food intake (B), or percentage of initial food intake (C) with treatment by repeated measures ANOVA.  $*P < 0.05$  for difference in food intake between rAAV-POMC and control rats (C) at day 7 through day 38 except day 35.

**Fasting serum insulin, glucose, FFAs, and cholesterol.** At the point at which rats were killed, serum insulin was significantly reduced by rAAV-POMC as compared with rAAV-control, and serum glucose tended toward a decrease (Fig. 6A and B). The rAAV-POMC delivery also reduced serum total cholesterol levels by 34.5% and increased FFA levels by 33% compared with rAAV-control (Fig. 6C and D).

**BAT.** Induction of UCP1 in BAT is an important marker for enhanced thermogenesis and, thus, energy expenditure in rodents (16). In the present study, we examined the

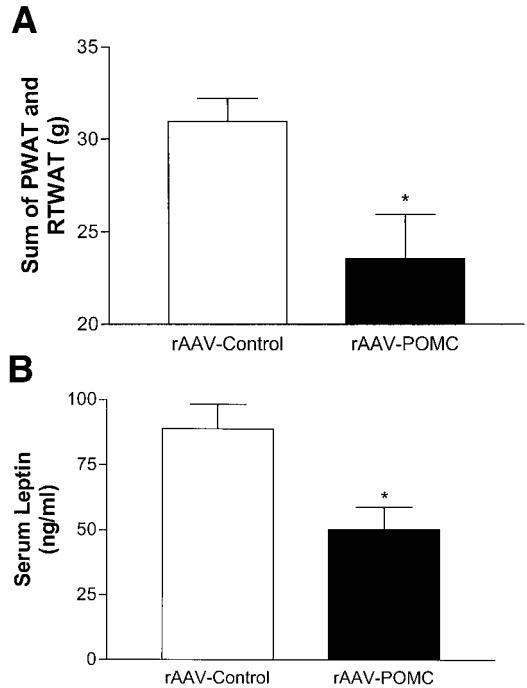


FIG. 5. Visceral adiposity (A) and fasting serum leptin (B) 38 days after rAAV-POMC or rAAV-control administration in obese Zucker rats. Data represent mean  $\pm$  SE of six rats per group.  $*P < 0.05$  vs. control by unpaired *t* test.

UCP1 protein levels 38 days after POMC gene delivery. Total BAT weight markedly declined with rAAV-POMC treatment, whereas the protein concentration (per unit of BAT) increased slightly, suggesting that the reduction in BAT mass was due to the lipolysis associated with the activation of BAT. This was further supported by a four-fold increase in BAT UCP1 protein levels in the rAAV-POMC-treated compared with control rats (Table 1).

**DISCUSSION**

The present study examined the long-term consequences of central rAAV-POMC gene therapy in obese Zucker rats with inherent defective leptin receptors and deficient

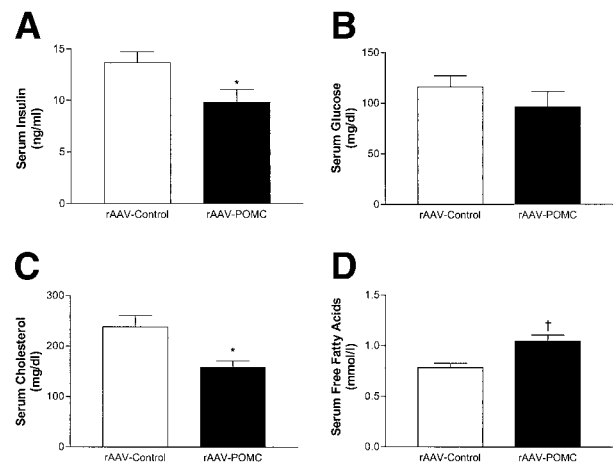


FIG. 6. Fasting serum insulin (A), glucose (B), cholesterol (C), and FFA (D) levels 38 days after rAAV-POMC or rAAV-control delivery in obese Zucker rats. Data represent mean  $\pm$  SE of six rats per group.  $*P < 0.05$  and  $\dagger P < 0.01$  vs. control by unpaired *t* test.

TABLE 1  
UCP1 and BAT parameters 38 days after rAAV-POMC or rAAV-control delivery

	Treatment	
	rAAV-control	rAAV-POMC
BAT weight (mg)	2,193 ± 275	1,366 ± 189*
BAT protein (mg/g BAT)	23.4 ± 1.4	27.2 ± 1.8
BAT protein (mg/total BAT)	50.2 ± 5.2	38.3 ± 7.2
UCP1 protein (arbitrary units/g BAT)	100 ± 17	662 ± 114†
UCP1 protein (arbitrary units/total BAT)	100 ± 33	412 ± 104*

For UCP1 protein, the levels in rAAV-control rats are set to 100 and SE adjusted proportionally. Data represent the mean ± SE of six rats per group. \* $P < 0.05$  and † $P < 0.01$  vs. control by unpaired  $t$  test.

endogenous POMC expression. Our findings are in agreement with several previous pharmacologic studies indicating that direct activation of the central MC system is effective in partially reversing the hyperphagia and obesity in obese Zucker rats (37,38). Our approach, in particular, resulted in long-term overexpression of POMC in the basal hypothalamus, and these obese Zucker rats responded with significant reductions in both food intake and weight gain. The level of hypothalamic P-CREB, the putative active form of the transcriptional factor mediating MC3/MC4 receptor signaling (29,35), was significantly elevated in the obese Zucker rats, whereas hypothalamic AgRP mRNA was unaffected at day 38 after POMC vector delivery. Thus, it seems that long-term POMC gene therapy attenuates weight gain primarily through activation of the central MC system characterized by increased hypothalamic POMC mRNA levels, CREB phosphorylation, and unchanged AgRP expression. Because POMC vector delivery was aimed at the arcuate nucleus, where POMC-expressing neurons are located, it is most likely that POMC overexpression in these neurons is mainly responsible for the observed physiologic consequences. However, given the proximity of the third ventricle to the arcuate nucleus, the possibility exists that some of the viral vectors entered the third ventricle. The vectors could also diffuse out of the arcuate nucleus, resulting in the transduction of neurons outside the arcuate nucleus. The potential ectopic expression of POMC in the brain might account for some of the responses observed. In such cases, the responses would be expected to be more pharmacologic rather than physiologic in nature.

The present study provides several distinct sets of salient findings. First, our data suggest that an increase in energy expenditure might contribute to the reduced weight gain and visceral adiposity after POMC gene delivery. It is known that pharmacologic activation of the MC system augments energy expenditure in rodents. For example, normal animals treated with the  $\alpha$ -MSH analog MTII have elevated levels of BAT UCP1 expression compared with pair-fed controls (38). The unique UCP1-mediated nonshivering thermogenesis in BAT represents an essential element in adaptive energy expenditure in rodents. An increase in UCP1 protein is indicative of increased BAT-facilitated energy expenditure. Obese Zucker rats, however, have impaired BAT thermogenesis because

of the genetically defective leptin receptors (39). Despite this inherent problem, we observed a fourfold increase in BAT UCP1 at 38 days after POMC vector delivery in the obese Zucker rats, indicating markedly stimulated BAT thermogenesis. Although lacking direct measurement of whole body energy expenditure, we speculate on the basis of our observation that, in addition to the hypophagia, an increase in energy expenditure plays a part in mediating the fat- and weight-trimming effects of central POMC gene therapy.

Second, rAAV-mediated POMC gene delivery produces an impressive reduction in adiposity. For example, by the end of the 38-day POMC gene therapy, PWAT and RTWAT combined were decreased by 24% when compared with controls. In addition, a key indicator of whole body mass (36), fasting serum leptin levels, was also reduced by 44%. Central chronic infusion of  $\alpha$ -MSH has been shown by one study to preferentially reduce visceral fat mass as opposed to lean body mass in lean rats (40). If such a preference in action is proved to exist, then strategies based on MC activation to treat obesity will offer an apparent advantage over some other weight control remedies that indiscriminately reduce both lean and fat body mass through suppression in food consumption.

Third, rAAV-POMC gene delivery seems to improve glucose metabolism. The obese Zucker rats are insulin-resistant as indicated by remarkably high levels of serum insulin. Central POMC gene therapy decreased fasting serum insulin, and it also generated a downward trend in serum glucose levels. These data suggest improved glucose metabolism and insulin sensitivity by POMC gene delivery. This observation is consistent with previous findings that central MC receptor activation reduces insulin release from the pancreas and enhances glucose metabolism (2,40). However, without a pair-fed group to control for the effect of food intake, we are not certain whether the improvement in glucose metabolism is directly related to the increased central POMC expression or a consequence of the decreased food consumption and body weight. In addition to its impact on insulin and glucose, POMC gene delivery lowered total serum cholesterol levels in obese Zucker rats. Thus, central activation of the MC system seems to have a cholesterol-reducing effect in these obese animals that possess both leptin and insulin resistance. The mechanism underlying the reduction in cholesterol is currently unknown, and one explanation could be that insulin-mediated stimulation of cholesterol synthesis is impeded after a fall in circulating insulin levels (41).

Most surprising, the present study revealed a sustained anorexic response to rAAV-POMC in the obese Zucker rats throughout the entire 38-day experimental period. This is in sharp contrast to all previous reports in which there was a rapid attenuation of the anorexic response after pharmacologic infusions of MCs in both normal rats and rats with diet-induced obesity and in mice (25,26). The suppression in food intake lasted no longer than 4 days in any of these studies. In the case of obese Zucker rats, one study noted a 3-day anorexic response to chronic pharmacologic administration of MTII (38). Therefore, the sustained anorexic response that we observed over 38 days may be unique to central POMC gene delivery. With this

procedure, the presumed overproduction of  $\alpha$ -MSH is derived from POMC with assistance from a variety of endogenous enzymes such as prohormone convertases, carboxypeptidase E, and peptidyl  $\alpha$ -amidating mono-oxygenase (42). This endogenously regulated production of  $\alpha$ -MSH may help to prevent the rapid desensitization witnessed in previous pharmacologic studies. In addition, obese Zucker rats, in comparison with their lean counterparts, are associated with reduced POMC expression in the arcuate nucleus, lower amount of  $\alpha$ -MSH peptide in the paraventricular nucleus, and higher MC4 receptor densities in several hypothalamic regions pivotal to energy regulation (7,43). These factors may also contribute to the prolonged responsiveness to POMC gene delivery in obese Zucker rats. Central MC signaling has been implicated in the development of cachexia (44), and we cannot rule out the possibility that the sustained anorexic response by central POMC gene delivery might be in part a mimic of inflammatory-like activities of POMC products, although in the present study, POMC gene delivery seemed to reduce visceral adiposity to a greater extent as compared with the decrease in whole body mass, an observation inconsistent with cachexigenic action.

In summary, the present study demonstrates that targeted POMC gene delivery to the hypothalamus suppresses food intake and weight gain and reduces visceral adiposity in genetically obese Zucker rats. This treatment also seems to improve glucose and cholesterol metabolism and insulin sensitivity. The sustained hypophagia and augmentation of thermogenesis in BAT are the likely mechanisms underlying these improvements.

#### ACKNOWLEDGMENTS

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