

Central Leptin Gene Therapy Blocks High-Fat Diet–Induced Weight Gain, Hyperleptinemia, and Hyperinsulinemia

Increase in Serum Ghrelin Levels

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Recombinant adeno-associated virus (rAAV), encoding either rat leptin (rAAV-lep) or green fluorescent protein (rAAV-GFP, control), was injected intracerebroventricularly in rats consuming a high-fat diet (HFD; 45 kcal%). Caloric consumption and body weight were monitored weekly until the rats were killed at 9 weeks. Untreated control rats consuming regular rat diet (RCD; 11 kcal%) were monitored in parallel. Body weight gain was accelerated in rAAV-GFP + HFD control rats relative to those consuming RCD, despite equivalent kcal consumption. At 9 weeks, serum leptin, free fatty acids, triglycerides, and insulin were elevated in HFD control rats. In contrast, rAAV-lep treatment reduced intake and blocked the HFD-induced increase in weight, adiposity, and metabolic variables. Blood glucose was slightly reduced but within the normal range, and serum ghrelin levels were significantly elevated in rAAV-lep + HFD rats. Uncoupling protein-1 (UCP1) mRNA in brown adipose tissue (BAT), an index of energy expenditure through nonshivering thermogenesis, was decreased in rats consuming HFD. Treatment with rAAV-lep significantly augmented BAT UCP1 mRNA expression, indicating increased thermogenic energy expenditure. These findings demonstrate that central leptin gene therapy efficiently prevents weight gain, increased adiposity, and hyperinsulinemia in rats consuming an HFD by decreasing energy intake and increasing thermogenic energy expenditure. *Diabetes* 51:1729–1736, 2002

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AAV, adeno-associated virus; AAV-lep, AAV encoding leptin; BAT, brown adipose tissue; BW, body weight; FFA, free fatty acid; FI, food intake; GFP, green fluorescent protein; HFD, high-fat diet; ICV, intracerebroventricular; PF, pair-fed; rAAV, recombinant AAV; RCD, regular control diet; RIA, radioimmunoassay; UCP, uncoupling protein.

Obesity, defined as an increase in mass of adipose tissue, confers a higher risk for metabolic diseases such as non–insulin-dependent diabetes, cardiovascular disease, and stroke and an increased incidence of morbidity (1). Human obesity is rarely genetic but has environmentally based causes, such as voluntary consumption of excess calories or diets with a higher percentage of calories from fat and/or a sedentary lifestyle with reduced energy expenditure (2,3). Leptin, an adipocyte hormone, regulates weight by reducing food intake (FI) and increasing energy expenditure by actions primarily within the hypothalamus (3,4). However, blood leptin levels increase with increasing adiposity and are directly proportional to fat mass (5–7). This loss of effectiveness of endogenous leptin or exogenous leptin therapy on weight maintenance is attributed to the development of leptin resistance (2–8). Potential explanations to account for leptin resistance include insufficiency of leptin at central target sites caused by either defective leptin transport across the blood-brain barrier or reduced production of leptin in the hypothalamus (9–12), or disruption of leptin signal transduction and postreceptor signaling in hypothalamic targets (2–4).

As with humans, laboratory rodents maintained on regular rat diet (RCD) also gain weight and display hyperleptinemia gradually over time (13–16), and the onset of these is hastened by consumption of a high-fat diet (HFD). Many of the same metabolic disorders, such as insulin resistance, hyperinsulinemia, and perturbed lipid metabolism, as reflected by elevated free fatty acids (FFAs) and triglycerides, are seen in these rats (1,2,6,15).

Gene therapy represents an unprecedented opportunity not only to understand the underlying causes but also to treat or cure human pathologies (17). Adeno-associated virus (AAV) vectors are rapidly becoming one of the most extensively used systems to transduce transgenes in the central nervous system (17,18). These vectors can be produced in relatively high titers and display no immunological side effects. We reported recently that increased endogenous expression of leptin in the hypothalamus, after the intracerebroventricular (ICV) administration of a replication-defective recombinant AAV (rAAV) encoding leptin c-DNA (rAAV-lep), suppressed the age-related weight gain in rats

maintained on RCD ad libitum (15,19). This sustained weight suppression was accompanied by a marked reduction in adiposity and serum leptin and insulin levels and was a result of increased energy expenditure through nonshivering thermogenesis in brown adipose tissue (BAT) with or without a decrease in food consumption (15,19–21). These observations led us to hypothesize that a similar increase in ectopic expression of leptin in the hypothalamus may overcome the central leptin insufficiency that develops in rats fed HFD. We report the beneficial effects of a single ICV injection of rAAV-lep in preventing HFD-induced weight gain, adiposity, and associated metabolic and hormonal disturbances. Furthermore, either central or peripheral administration of ghrelin, a hormone produced by cells in the oxyntic glands of the stomach and intestine (22,23) and released into the peripheral circulation, has recently been shown to stimulate feeding and promote adiposity (23,24). Stimulation of feeding by ghrelin is presumably mediated by the same hypothalamic appetite-stimulating signals that are suppressed by leptin (22,25). Therefore, we also evaluated the effects of central rAAV-lep on serum ghrelin levels in these investigations.

RESEARCH DESIGN AND METHODS

Animals and diets. Adult male Sprague Dawley rats that weighed 225–250 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). Rats were housed one per cage and maintained in a temperature- (21°C) and light-controlled (14-h light/10-h dark) specific pathogen-free environment with food available ad libitum in a hanging feeder, which allowed for ready measurement of daily FI. The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Florida.

Irradiated standard RCD (11 kcal% fat #LM-485, Teklad, Madison, WI) was used as the maintenance and control diet. A purified ingredient HFD with 45 kcal% fat primarily from lard (#D12451, Research Diets, New Brunswick, NJ) was used to induce a rapid increase in body weight (BW) and obesity (26). The caloric density of the control diet was 3.4 kcal/g; that of the HFD was 4.73 kcal/g, resulting in lower daily food consumption in grams for the rats fed the HFD.

Construction and packaging of rAAV vectors. rAAV vector pTR-CBA-leptin, the *EcoRI* fragment of pCR-leptin (a gift of Dr. Roger H. Unger, Southwestern Medical School, Dallas, TX) containing rat leptin cDNA, was subcloned into rAAV vector plasmid pAAVβGEnh after deleting the *EcoRI* fragment carrying β-glucuronidase cDNA sequence (15,18,27). Vectors were packaged, purified, concentrated, and titred in the Vector Core laboratory at the University of Florida as described earlier and used in our previous studies (15,19,27). The ratio of physical-to-infectious particles was <100. Because mini-Ad helper plasmid pDG (27) was used to produce vectors, there was no Ad or wtAAV contamination in rAAV stocks used in this study. rAAV vectors, purified using iodixanol gradient/heparin-affinity chromatography, were 99% pure as judged by PAAG/silver-stained gel electrophoresis. The control virus, rAAV-GFP, was similarly constructed to encode the green fluorescent protein (GFP) gene.

Experiment 1. A pilot experiment was performed to test the efficacy of using central rAAV-lep in preventing rapid weight gain and obesity in rats fed HFD. Permanent cannulae were implanted in rats in the third cerebrotentri- cle under sodium pentobarbital (60 mg/kg body wt) anesthesia as described (15,19). After a 7-day recovery period, rats received 5 μl of either rAAV-GFP (5.0×10^{10} particles, $n = 6$) or rAAV-lep (1.5×10^{11} particles, $n = 6$) intracerebroventricularly. The rAAV-lep dose is based on our previous results (15,19). Rats were then allowed to consume HFD. Twenty-four-hour FI and BW were monitored before and at weekly intervals after the injection. A group of control rats ($n = 9$) did not undergo stereotaxic surgery and was maintained on RCD. Weekly FI and BW were similarly monitored. At the end of 9 weeks, a blood sample (1 ml) was withdrawn from the jugular vein of each rat under sodium pentobarbital anesthesia in the middle of the lights-on phase. Plasma samples were stored frozen at –20°C for determination of leptin as described below.

Experiment 2. Because the results of experiment 1 demonstrated the efficacy of rAAV-lep treatment on HFD-induced obesity, a second experiment was undertaken to evaluate the effects of leptin gene therapy on leptin, ghrelin, and

other metabolic hormones and nonshivering thermogenesis by analysis of UCP1 mRNA expression in BAT. Before stereotaxic surgery to implant the ICV cannulae, rats were fasted for 4 h and a blood sample was withdrawn from the tail vein to provide a pretreatment sample. Two days later, rats underwent stereotaxic surgery as described in experiment 1. After 7 days, these rats were divided into two groups: one group received rAAV-GFP ($n = 5$, 1.9×10^{11} particles), and the other received rAAV-lep ($n = 8$, 3.6×10^{10} particles). These rats were provided the HFD after injection. FI and BW were monitored as described in experiment 1. The third group of rats did not undergo stereotaxic surgery and continued to receive RCD to serve as an additional control. On week 9, the rats were fasted for 4 h before being killed by decapitation in the middle of the lights-on phase. Interscapular BAT was dissected and frozen for subsequent analysis of UCP-1 mRNA (15,19). Trunk blood was collected for analyses of leptin, insulin, glucose, triglycerides, FFA, and ghrelin as described below.

Experiment 3. Because the rAAV-lep-treated rats of experiment 2 had a lower energy intake than the controls, an experiment was undertaken to determine the effects of lower food consumption on the variables measured. A group of rats that were not operated on were adapted to the laboratory, and baseline measurements were made of BW and RCD intake. These rats were then switched to HFD and divided into two groups, one with ad libitum access to the diet and the other pair-fed (PF) to the amount consumed by the rAAV-lep-treated rats at the corresponding time so that cumulative FI in these rats was equal to that of the rAAV-lep group in experiment 2. Rats were maintained for 9 weeks, fasted for 4 h, and killed in the middle of the lights-on phase. Blood samples were collected for analyses as described in experiment 2.

Analysis of UCP1 gene expression. UCP1 mRNA expression in BAT was measured as described previously (15,19). Total RNA from BAT was isolated, and a dot-blot hybridization analysis of UCP1 mRNA levels was performed.

Analyses of serum leptin, insulin, triglycerides, FFAs, glucose, and ghrelin. Serum insulin was measured using a sensitive rat insulin radioimmunoassay (RIA) kit from Linco Research (St. Charles, MO). Serum leptin was measured using a RIA kit from American Laboratory Products (Wyndham, NH); the ghrelin RIA kit was purchased from Phoenix Pharmaceuticals (Belmont, CA). Serum triglycerides and FFAs were measured by an in vitro enzymatic colorimetric method using kits from Wako Chemicals USA (Richmond, VA). Serum glucose was measured using a blood glucose meter (Glucometer Elite XL; Bayer, Elkhart, IN).

Statistical analyses. Results were analyzed using two-way repeated-measures ANOVA with time and treatment as variables. One-way ANOVA was used to analyze cumulative FI, UCP1 mRNA, and plasma leptin values. Post hoc tests were performed using Newman-Keuls multiple comparison test or Student's *t* test as appropriate. Student's *t* test was used to determine significance for cumulative FI in experiment 3. Significance was set at $P < 0.05$ for all analyses.

RESULTS

Effects of rAAV-lep on HFD-induced weight gain and plasma leptin levels. The results of the pilot study show that the rate of BW gain was accelerated in rAAV-GFP + HFD controls over the group of untreated rats maintained on RCD during the 9 weeks of the experiment; rAAV-GFP + HFD rats gained 32.7%, as compared with 17.8% gained by the untreated + RCD group of rats (Fig. 1A). In contrast, rAAV-lep treatment blocked the HFD-induced rapid weight gain as these rats gained only 9.1% over the initial BW. BW suppression was first evident at week 2 ($P < 0.05$ vs. rAAV-GFP + HFD), followed by an extremely slow rate of weight gain. Analysis of 24-h energy consumption (kcal) on a weekly basis showed that the two control groups consumed a similar amount of kilocalories, whereas the rAAV-lep-treated rats maintained on HFD consumed slightly fewer kilocalories than controls. Cumulative kilocalories consumption over 9 weeks was reduced by 9.3% ($P < 0.05$) relative to untreated controls and by 5.1% ($P > 0.05$) relative to rAAV-GFP + HFD rats (Fig. 1B inset). Plasma leptin levels were increased by 64% in rAAV-GFP + HFD as compared with the untreated + RCD group (Fig. 1C). In the rAAV-lep-treated rats, not only was the HFD-induced rise in plasma leptin blocked, but also

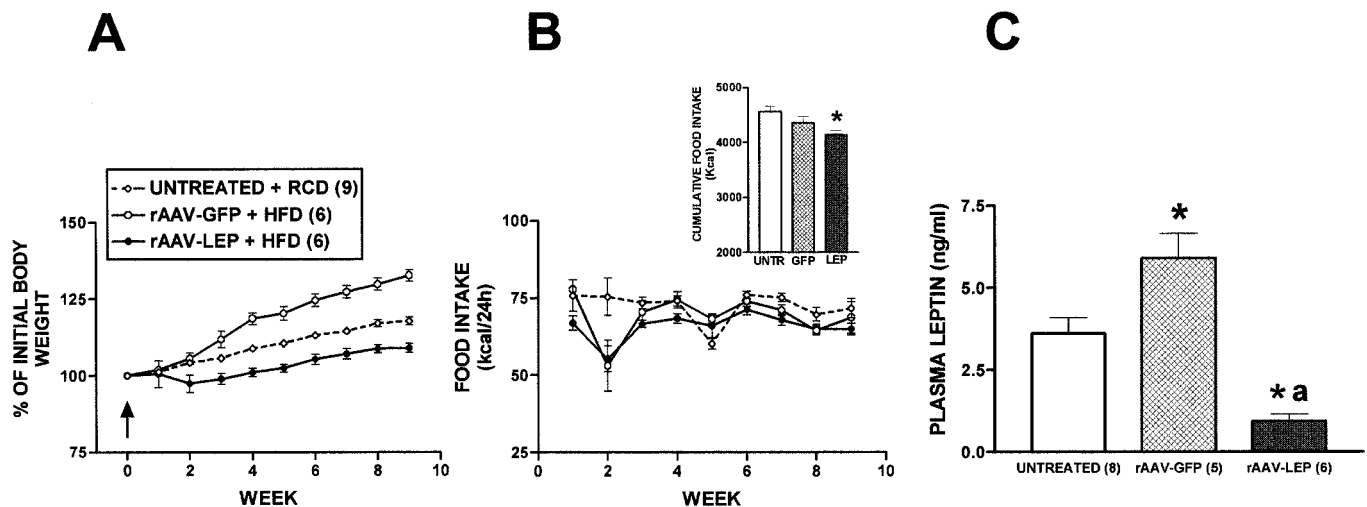


FIG. 1. The effects of ICV rAAV-GFP (control) or rAAV-lep vector (arrow, experiment 1) on HFD-induced BW (A), FI (B), and plasma leptin levels (C). * $P < 0.05$ vs. untreated + RCD; a, $P < 0.05$ vs. rAAV-GFP + HFD. Numbers in parentheses denote number of animals.

levels were drastically reduced by 74% relative to untreated + RCD rats and by 84% relative to the rAAV-GFP + HFD rats.

Effects of rAAV-lep on HFD-induced weight gain, energy consumption, serum hormone, and BAT-UCP1 mRNA responses. The control rats in the second experiment showed similar patterns of weight gain (Fig. 2A) and energy consumption (Fig. 2B) as in experiment 1. rAAV-lep treatment completely suppressed the time-related HFD-induced weight gain (4.8 vs. 42.3% rAAV-GFP + HFD and 27.8% untreated + RCD group) in association with significantly reduced 24-h kilocalorie consumption analyzed at weekly intervals ($P < 0.01$). The cumulative kilocalorie consumption for the 9-week duration of the experiment was also suppressed (14.7%; $P < 0.01$) (Fig. 2B inset).

Consistent with previous reports (6,7,15,28,29), serum leptin levels increased at 9 weeks from initial values in untreated + RCD and rAAV-GFP + HFD control groups ($P < 0.01$) (Fig. 3A). However, the magnitude of this leptin rise was higher in rats consuming HFD (491%) as compared with those consuming RCD (262%; $P < 0.01$). In marked contrast in rAAV-lep + HFD rats, not only was the time-related RCD- and HFD-induced rise in serum leptin prevented, but also leptin levels at week 9 were 92% below the initial values ($P < 0.01$). This marked diminution in serum leptin resulted from reduced adiposity evidenced by the virtual disappearance of abdominal fat (Fig. 4A) and by markedly reduced serum triglycerides and FFAs in the rAAV-lep + HFD group relative to the levels in the two control groups (Fig. 3B and C).

In contrast to the effects on serum leptin, serum ghrelin levels were unaffected by either RCD or HFD consumption at week 9 posttreatment (Fig. 3D). However, rAAV-lep therapy markedly enhanced serum ghrelin levels at this time from initial values as well as from the 9-week values in the control groups ($P < 0.01$) (Fig. 3D).

The effects of HFD on serum insulin were different at week 9 (Fig. 3E). Whereas RCD consumption did not alter serum insulin, HFD consumption evoked hyperinsulinemia with significantly higher levels than the initial values and the values of the untreated + RCD group ($P < 0.05$). However, rAAV-lep treatment completely blocked the

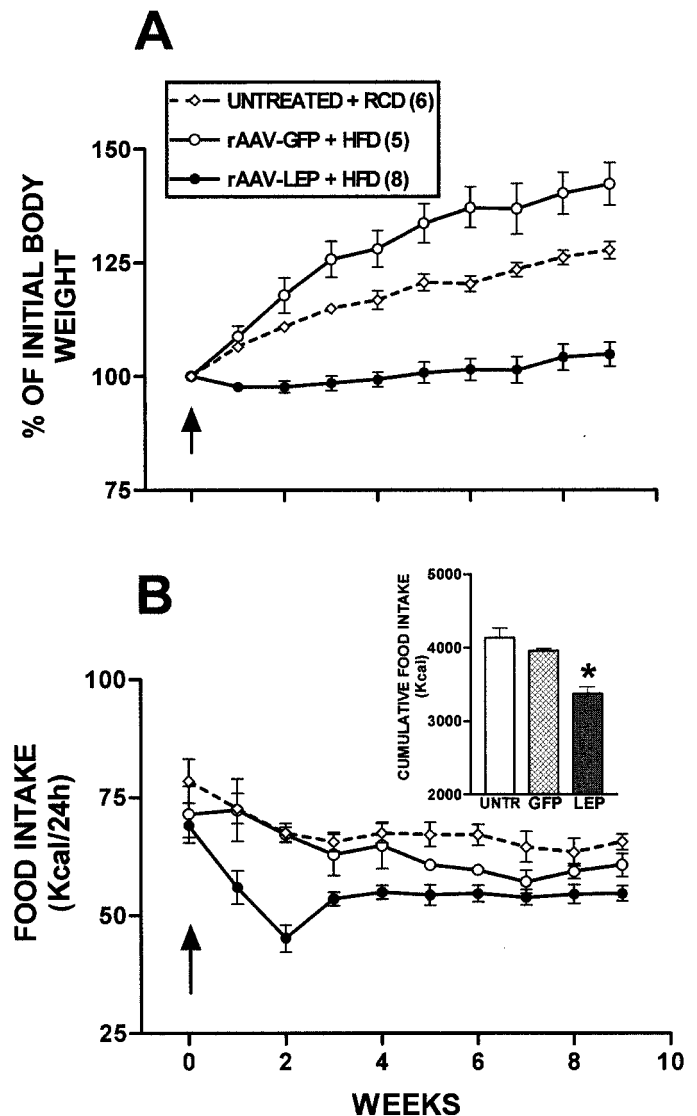


FIG. 2. The effects of rAAV-GFP or rAAV-lep administration (arrow, experiment 2) on weight gain (A) and FI (B) in rats maintained on HFD. * $P < 0.01$ vs. other groups.

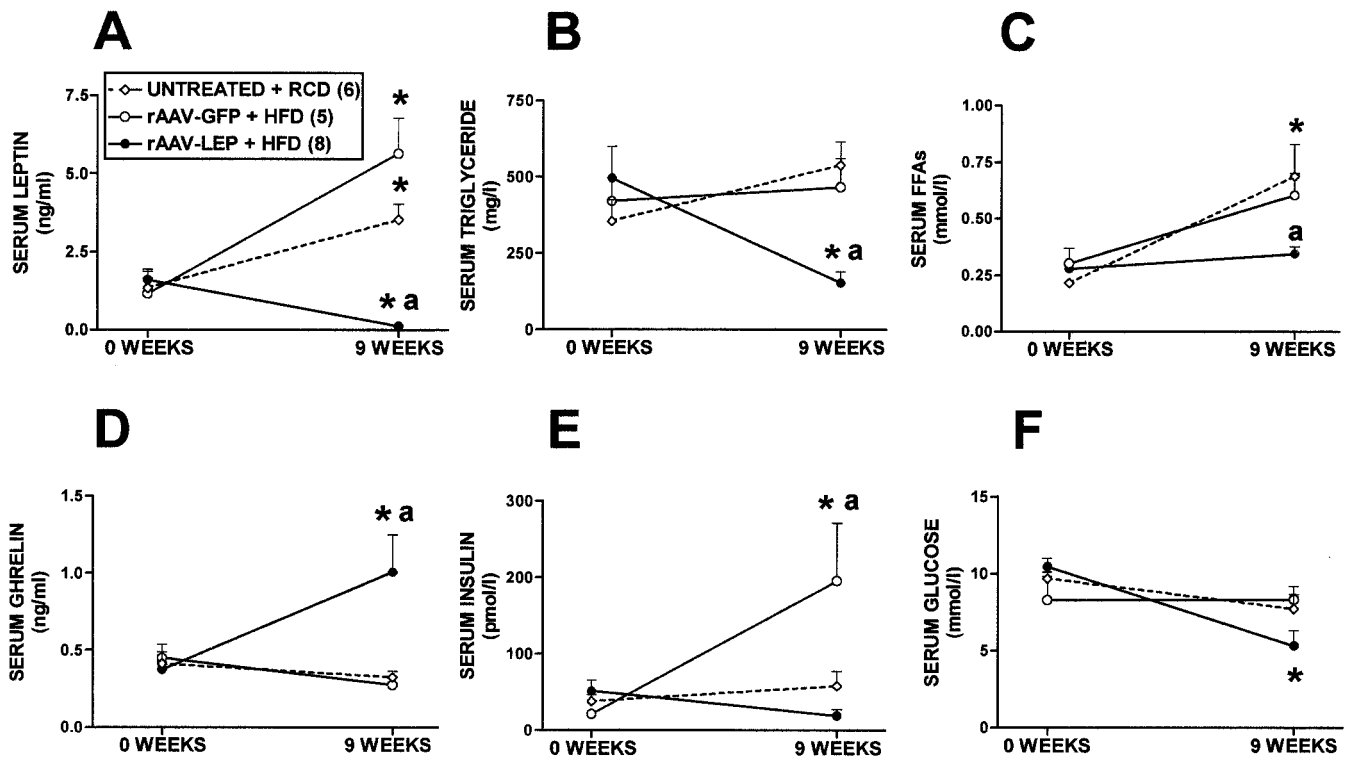


FIG. 3. Serum levels of leptin (A), triglycerides (B), FFAs (C), ghrelin (D), insulin (E), and glucose (F) in the three treatment groups in experiment 2 shown in Fig. 2. **P* < 0.01 vs. week 0 values; a, *P* < 0.05 vs. other groups. Numbers in parentheses denote number of animals.

HFD-induced hyperinsulinemia, and levels remained in the preinjection range. Despite hyperinsulinemia at week 9, serum glucose concentrations were unchanged from the initial range in the rAAV-GFP + HFD group. However, in rAAV-lep + HFD rats, glucose levels were slightly reduced but still within the normoglycemic range (*P* < 0.05) (Fig. 3F).

The physical appearance of BAT in rAAV-lep-treated rats showed it to be highly vascularized as compared with that in control rats consuming either HFD or RCD (Fig. 4B), an indication of increased thermogenesis (20). This was coincident with highly significantly increased BAT UCP1 mRNA in response to rAAV-lep therapy in rats consuming HFD (*P* < 0.01), even though HFD consumption without rAAV-lep treatment decreased BAT UCP1 mRNA (*P* < 0.01 vs. RCD rats) (Fig. 5).

Effects of pair feeding on HFD-induced weight gain and serum hormone and BAT-UCP1 mRNA responses.

To investigate whether the effects on serum hormones and other metabolic parameters resulted from reduced caloric intake and BW imposed by rAAV-lep therapy in HFD rats, we fed one group of rats the same amount of HFD consumed by the rAAV-lep-treated rats (Fig. 6A). This PF + HFD group maintained BW in the range of the rAAV-lep group (Fig. 6B); however, serum hormones and metabolic parameters were different (Fig. 7). Serum leptin (Fig. 7A), triglycerides (Fig. 7B), and FFAs (Fig. 7C) rose in an age-related manner in PF rats as compared with the drastic suppression seen in rAAV-lep rats (Fig. 3). Ghrelin levels in PF + HFD rats rose slightly at week 9 from the initial range (*P* < 0.05), but these levels were not different from those in ad libitum + HFD controls (*P* > 0.05). As seen in experiment 2 (Fig. 3E), serum insulin levels

increased concomitant with unchanged blood glucose levels in response to HFD consumption. Serum insulin also rose in the PF + HFD group, but the increment was less than in the ad libitum + HFD group. Thus, unlike rAAV-lep treatment, pair feeding failed to prevent HFD-induced hyperinsulinemia and the decrease in BAT UCP1 mRNA (1.38×10^5 PF + HFD vs. 1.41×10^5 ad libitum + RCD, arbitrary units, *P* > 0.05).

DISCUSSION

As documented previously (2,5–7,28–30), these results show that despite consuming equivalent kilocalories, weight gain is higher in rats consuming HFD than in those consuming regular RCD, and the excessive weight gain is primarily due to fat deposition, as reflected by increased serum leptin levels that rise in direct proportion to fat mass (2–7). Evidently, HFD consumption rapidly confers leptin resistance because rising serum leptin levels neither arrested the ascending weight nor curbed kilocalorie consumption. The new finding of this investigation is that a single central injection of rAAV vector encoding leptin almost completely blocked the HFD-induced weight gain and reduced kilocalorie consumption during the entire 9 weeks of observation. Previous studies have reported only transient effects, lasting 2 weeks, on FI after central administration of either leptin in mice (31) or immunogenic adenovirus encoding leptin in rats (32). We show further that long-term blockade of weight gain was concomitant with a marked reduction in serum leptin relative to that seen in control groups and from the preinjection range. Suppression of the basal and time-related rise in serum leptin together with marked reduction in visible

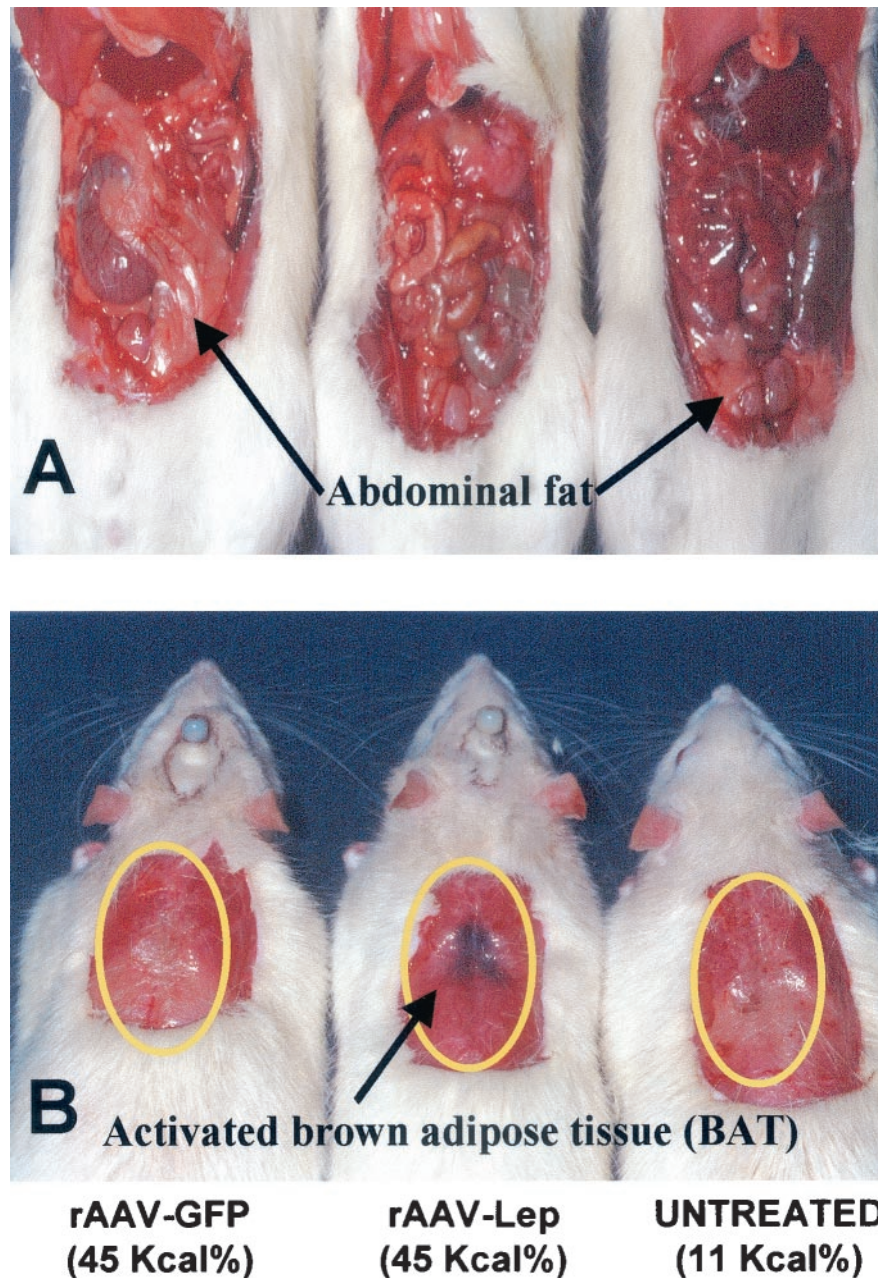


FIG. 4. The effects of rAAV-GFP (left) or rAAV-lep (middle) on abdominal fat deposits (A) and BAT (B) appearance in rats maintained on HFD (45 kcal%). Although not quantified, abdominal fat deposits were completely depleted and BAT was highly vascularized, shown previously to reflect increased thermogenesis (20), in rAAV-lep-treated rats. Control untreated rats (right) were maintained on RCD (11 kcal%).

abdominal fat and serum FFAs and triglycerides clearly suggest fat depletion in rAAV-lep-treated rats. Although not examined in the current study, body composition analysis of rAAV-lep-treated rats in a previous study showed depletion of fat depots without affecting lean mass (15). Leptin treatment also selectively depleted fat in *ob/ob* mice and rats (33–35). This sustained suppression of weight, adiposity, and hyperleptinemia is attributed to increased ectopic production of leptin in the hypothalamus acting in a paracrine/autocrine manner. This is supported by our previous study showing that GFP was localized in cells with neuron-like morphology in hypothalamic nuclei implicated in weight homeostasis in rAAV-GFP-injected rats, and hypothalamic leptin mRNA expression, as analyzed by RT-PCR, was upregulated two-

to threefold in rAAV-lep-injected rats (15). Also, this leptin overexpression appropriately affected neuropeptidergic signaling involved in weight control (3,4,19). The ineffectiveness of pair feeding to reproduce the suppressive effects of central rAAV-lep therapy on leptin, FFAs, and triglycerides also lends credence to the notion of a selective central action of leptin.

The sympathetic nervous system is of prime importance in the hypothalamic control of energy expenditure through BAT thermogenesis (20,21). UCP1, a mitochondrial inner membrane protein, uncouples proton entry from ATP synthesis and enhances thermogenesis. In several models of obesity, including long-term HFD-induced obesity, sympathetic nervous system activity is reduced along with diminution of UCP1 mRNA and protein in BAT (20,21).

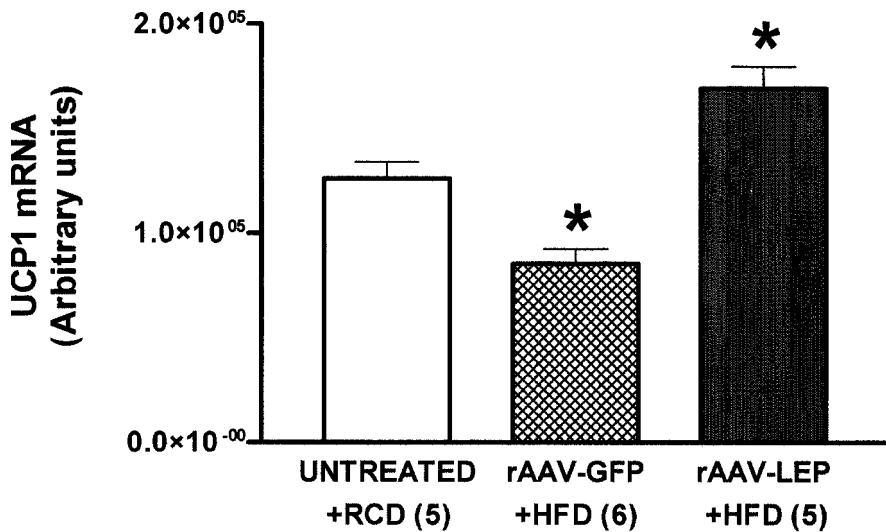


FIG. 5. UCP1 mRNA in BAT of rats in treatment groups shown in Fig. 2 (experiment 2). **P* < 0.01 vs. other groups. Numbers in parentheses denote number of animals.

Leptin administration increases sympathetic nerve activity and BAT-UCP1 mRNA (21). We observed that rAAV-lep therapy not only reversed HFD-induced suppression but also further augmented UCP1 mRNA expression. Thus, along with reduced kcal intake, increased energy expenditure contributed substantially to weight maintenance and preventing fat deposition in these rats. Increased ectopic leptin expression in the hypothalamus similarly enhanced energy expenditure for the 6-month duration of the experiment in rats consuming ad libitum RCD (15). Apparently, leptin overexpression in the hypothalamus is capable of reducing appetite and concomitantly enhancing energy expenditure on a long-term basis in rats maintained on either RCD or HFD ad libitum. Peripheral hyperleptinemia is reported to deplete fat by an action directly at the level of adipose tissue (36). However, our current and previous findings showing that serum leptin levels are reduced drastically after central rAAV-lep treatment and that the fat-depleting effects of peripheral hyperleptinemia are blocked in rats that bear hypothalamic lesions (37) argue for a central site of leptin action for fat depletion through BAT thermogenesis and, possibly, increased fat oxidation and adipocytes apoptosis.

Our results also provide strong evidence in support of the prevailing view (2–7) that rats consuming HFD develop rapid resistance to peripheral leptin because an experimentally induced increase in leptin availability in the hypothalamus efficiently arrested weight gain. These results and previous reports that peripheral leptin injections failed to control BW in rodents (2–4,26) and humans (8) and that leptin transport to brain target sites is impaired in obesity (9–11,38) are in accordance with the view that insufficiency of central leptin, rather than impaired leptin receptor or postreceptor signal transduction, contributes to the loss of weight regulation. Thus, one can assume that by experimentally increasing leptin expression in the hypothalamus, it is possible to reinstate weight control for extended periods, even when rats consume HFD.

Increased adiposity and hyperinsulinemia resulting from increased tissue insulin insensitivity are widely known (39). In rAAV-lep-treated rats, HFD-induced increase in circulating insulin levels was suppressed along with fat deposition. Seemingly, the absence of fat depots abrogated

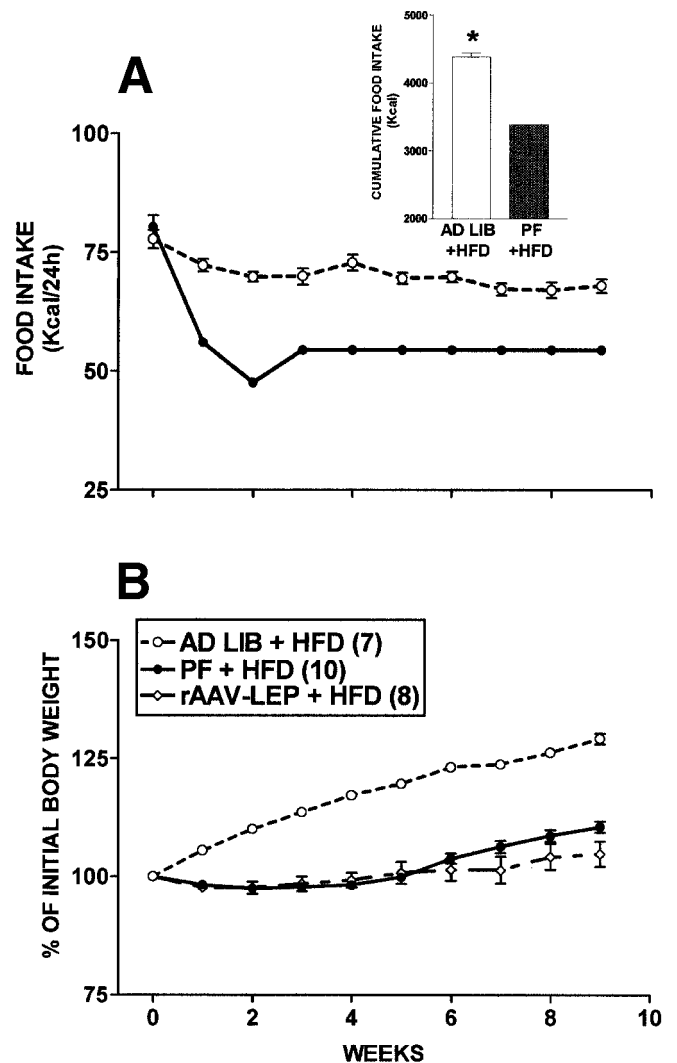


FIG. 6. Effect of pair feeding (PF) on FI (A) and BW (B). For comparison, the BW profile of the rAAV-lep + HFD group from experiment 2 (Fig. 2) is also depicted. FI of the AD LIB + HFD group (A) is significantly elevated above the amount of food given the PF + HFD group from week 1 onward (*P* < 0.05). Cumulative FI over the 9-week period is shown in the inset. **P* < 0.05 vs. PF + HFD. Numbers in parentheses denote number of animals.

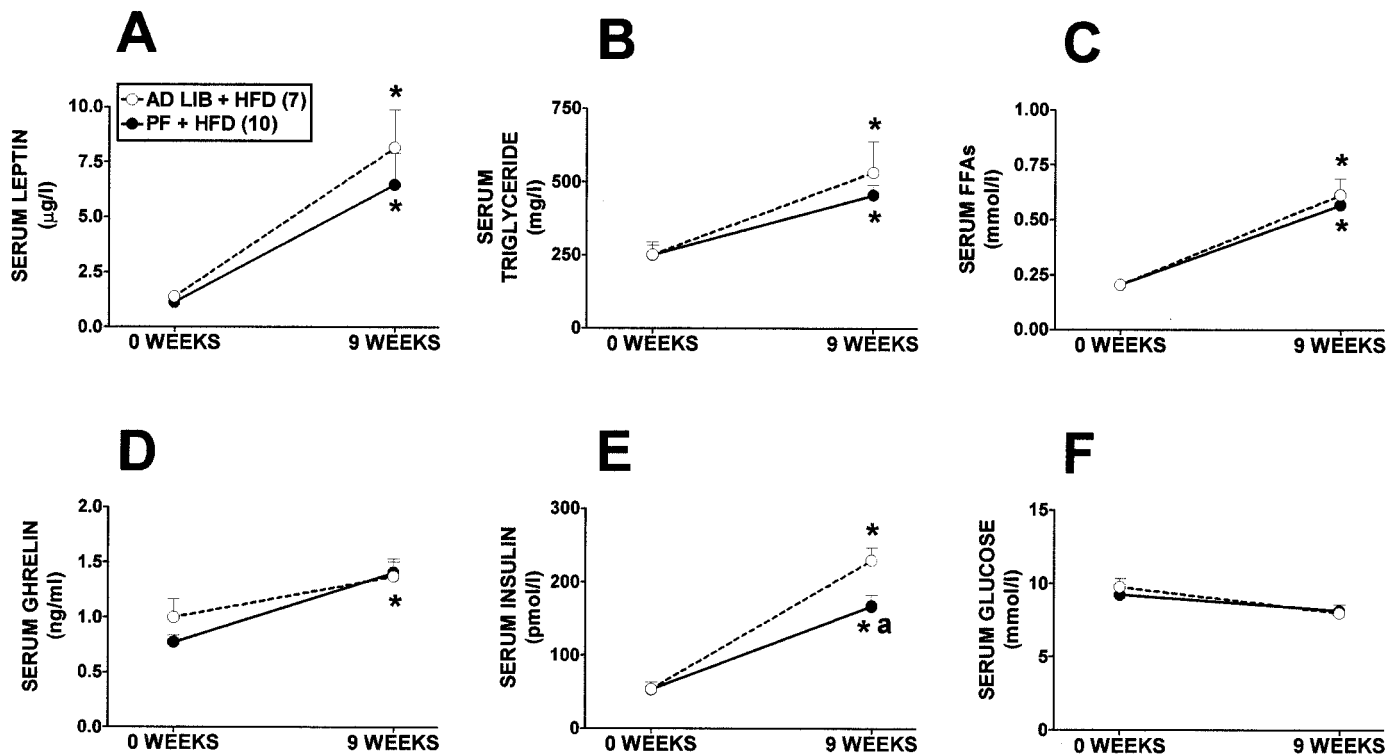


FIG. 7. The effects of pair feeding (PF) on serum leptin (A), triglycerides (B), FFA (C), ghrelin (D), insulin (E), and glucose (F) levels. * $P < 0.05$ vs. week 0 values; a, $P < 0.05$ vs. AD LIB + HFD group. Numbers in parentheses denote number of animals.

hyperinsulinemia because equivalent kilocalorie consumption and BW in PF rats did not prevent the HFD-induced rise in circulating insulin. Clinically, a severe reduction in energy consumption and/or an increase in energy expenditure leads to decreased adiposity, which is beneficial in reducing hyperinsulinemia and improving insulin sensitivity (40). Our observation of blood glucose levels in the normal range despite low circulating insulin levels in rAAV-lep + HFD rats strongly indicates that both increased energy expenditure and voluntary reduction in kilocalorie consumption induced by central leptin overexpression played a role in blocking HFD-induced hyperinsulinemia and hint at improved insulin sensitivity in these rats. Investigations are under way to characterize in detail the level of insulin sensitivity and glucose tolerance in response to central rAAV-lep treatment.

Finally, ghrelin has been shown to stimulate FI in rodents, and circulating ghrelin levels rise after fasting (22–25). A surprising observation of this study is that serum ghrelin levels increased markedly in conjunction with significantly reduced kilocalorie consumption in rAAV-lep + HFD but not in PF rats. The reason for either increased ghrelin levels or the failure of these high serum levels to stimulate FI in rAAV-lep rats is unknown. It may be that the increased restraint exerted by central leptin counteracted the appetite-stimulating effects of peripheral ghrelin. If this is the case, then it raises the intriguing possibility that leptin and ghrelin interact in an opposing manner at the hypothalamic appetite-stimulating neuropeptide Y neurons that co-express leptin and ghrelin receptors (2,25). Additional studies are warranted to delineate the role of various interacting central effector pathways mobilized by leptin and ghrelin in hypothalamic integration of energy homeostasis (3,4,25).

In summary, a major finding of this investigation is that rapid weight gain, adiposity, and adiposity-related hyperinsulinemia, the consequences of a diet rich in calories from fat, can be prevented by central leptin gene therapy. The long-term beneficial effects result from reinstatement of hypothalamic control on weight homeostasis by imposing decreased intake and increased energy expenditure through thermogenesis. The current results, taken together with similar demonstrations in rats consuming a normal rat diet (15,19), and the fact that rAAV vectors are nonpathogenic, are nonimmunogenic, and can be used for long-term expression of therapeutic genes virtually through the life of the cell (17,18), lend credence to the view that central leptin gene therapy is a viable therapeutic strategy to control weight and provide protection from adiposity-related metabolic disorders.

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