Synergy Between Leptin Therapy and a Seemingly Negligible Amount of Voluntary Wheel Running Prevents Progression of Dietary Obesity in Leptin-Resistant Rats

Alexandra Shapiro,[1] Michael Matheny,[1] Yi Zhang,[1,2] Nihal Tümer,[1,2,3] Kit-Yan Cheng,[1] Enda Rogrigues,[1] Sergei Zolotukhin,[4] and Philip J. Scarpace[1,3]

OBJECTIVE—We examined whether chronic leptin treatment of diet-induced obese rats promotes or alleviates the susceptibility to continued high-fat feeding. Second, we examined if voluntary wheel running is beneficial in reducing the trajectory of weight gain in high-fat–raised leptin-resistant rats.

RESEARCH DESIGN AND METHODS—Sprague-Dawley rats were fed a standard diet or a high-fat diet for 5 months, and then hypothalamic leptin overexpression was induced through central administration of adeno-associated virus–encoding leptin while continuing either the standard or high-fat diet. Two weeks later, half of the rats in each group were provided access to running wheels for 38 days while being maintained on either a standard or high-fat diet.

RESULTS—In standard diet–raised rats, either wheel running or leptin reduced the trajectory of weight gain, and the combined effect of both treatments was additive. In high-fat–raised leptin-resistant rats, leptin overexpression first transiently reduced weight gain but then accelerated the weight gain twofold over controls. Wheel running in high-fat–raised rats was sixfold less than in standard diet–raised rats and did not affect weight gain. Surprisingly, wheel running plus leptin completely prevented weight gain. This synergy was associated with enhanced hypothalamic signal transducer and activator of transcription (STAT) 3 phosphorylation and suppressor of cytokine signaling 3 expression in wheel running plus leptin compared with leptin-treated sedentary high-fat counterparts. This enhanced STAT3 signaling associated with the combination treatment occurred only in high-fat–raised, leptin-resistant rats and not in standard diet–raised, leptin-responsive rats.

CONCLUSIONS—Chronic leptin treatment in diet-induced obese rats accelerates dietary obesity. However, leptin combined with wheel running prevents further dietary weight gain. Thus, this combination therapy may be a viable antiobesity treatment. *Diabetes* 57:614–622, 2008

The adipocyte-derived hormone leptin acts in the brain to reduce food intake and stimulate energy expenditure (1). Leptin treatment exerts potent responses in lean rodents, producing impressive weight and fat loss (2) but is generally ineffective in rats that have diet-induced obesity due to high-fat feeding and, consequently, that are leptin resistant (3–5). The presence of this leptin resistance constitutes a major obstacle in curtailing diet-induced weight gain (6,7), and one approach to treat obesity would be to restore leptin actions or circumvent the leptin resistance in dietary obese animals.

Exercise, one time-honored treatment for obesity, has proven beneficial in humans. Exercise reduces body weight and decreases visceral adiposity in overweight patients (8) who presumably possess some degree of leptin resistance. The beneficial effects of exercise are generally believed to be derived mostly through increased energy expenditure. A recent study (9) indicated that a single bout of exercise enhances brain leptin signaling and short-term leptin responses in leptin-sensitive rats. Although chronic exercise exerts a positive impact on obese rodents in terms of weight regulation (10), this issue has not been addressed from the perspective of leptin resistance and/or leptin responsiveness. Thus, we hypothesized that chronic exercise may be beneficial in restoring leptin responses even in a leptin-resistant state, such as with diet-induced obesity. The goal of the present study was to explore the potential synergism between leptin and wheel running as well as any impact of wheel running on leptin resistance by examining whether wheel running alone or in combination with leptin treatment can reduce weight gain in rats already obese and leptin resistant due to high-fat feeding.

To these ends, we raised rats on standard or high-fat food for 5 months and then examined leptin responsiveness by administration of recombinant adeno-associated virus–encoding leptin (rAAV-leptin) via intracerebroventricular injection while continuing either the standard or high-fat diet. Two weeks later, half of the rats in each group were provided access to running wheels while being maintained on either the standard or high-fat diet, and the trajectory of weight gain was assessed along with biochemical parameters of energy homeostasis at death.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats obtained from Charles River (Wilmington, MA) were fed either a standard rodent diet (4% fat, 3.41 kcal/g, diet 7912; Harlan Teklad, Madison, WI) or a high-fat diet (32% kcal from fat and 25% kcal from sucrose, 4.41 kcal/g, diet 12266B; Research Diets, New Brunswick, NJ) from 4

weeks of age. Animals were cared for in accordance with the principles of the *Guide to the Care and Use of Experimental Animals*. Rats were housed individually with a 12:12 h light:dark cycle (0700–1900 h).

This study was divided into two experiments with identical design, differing only in diet. In the first experiment, the rats were raised and maintained on regular diet (standard diet–raised group). In the second experiment, the rats were raised and maintained on the 32% high-fat diet throughout the study (high-fat–raised group). After 5 months on the respective diets, the rats were divided into two groups, those administered control vector and those administered rAAV-leptin by intracerebroventricular injection. All rats were allowed access to food and water ad libitum, and food consumption and body weight were recorded daily. Two weeks after vector administration, half the rats in each group were allowed free access to running wheels, and the extent of wheel running was recorded daily. Each experiment was terminated 38 days after access to the running wheels. There were seven or eight animals per group, except for in the high-fat–raised sedentary and wheel-running groups provided the control vector, where there were six rats.

Production of rAAV vectors. Rat leptin cDNA under the control of a chicken β-actin promoter from pTR-βObW (11) was subcloned into pUCDM transfer plasmid (12). Recombinant baculoviruses were constructed using the MultiBac Expression System (12). Serum-free medium-adapted Sf9 cells were used for large-scale rAAV preparations (13). Vectors were purified and concentrated and physical rAAV particle titers determined, as described previously (14,15).

rAAV-leptin administration. A single dose of 2.5×10^{10} physical particles per rat in 5 μl of either control vector or rAAV-leptin was delivered by intracerebroventricular injection into the third cerebral ventricle, as previously described (11). The coordinates for injection are 1.3 mm anterior to Bregma, 9.6 mm ventral from the skull surface, at an angle of 20° anterior to posterior.

Wheel running. Rats were housed in cages equipped with Nalgene Activity Wheels (1.081-m circumference; Fisher Scientific, Pittsburgh, PA) and allowed free access to the wheel. Each wheel was equipped with a magnetic switch and counter. The number of revolutions was recorded daily.

Tissue harvesting and preparation. Rats were killed by thoractomy under 150 mg/kg pentobarbital anesthetic. The circulatory system was perfused with 20 ml cold saline, and the epididimal white adipose tissue (EWAT), perirenal white adipose tissue (PWAT), and retroperitoneal white adipose tissue (RTWAT) and hypothalami were excised. Protein concentrations were determined using the DC (detergent compatible) protein assay kit (Bio-Rad, Hercules, CA).

Western analysis. Protein homogenate (50 μg) was separated on an SDS-PAGE and electrotransfered to polyvinylidene fluoride membranes (16). Immunoreactivity was assessed with antibodies specific to phospho-tyrosine 705 of signal transducer and activator of transcription (STAT) 3 or STAT3 (either phosphorylated or unphosphorylated) (Cell Signaling, Danvers, MA), interleukin (IL)-6 (Biotechnology, Santa Cruz, CA), 11-β-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) (Alpha Diagnostic, San Antonio, TX), protein-tyrosine phosphatase-1B, catalase, or CuZn superoxide dismutaze (Calbiochem, San Diego, CA).

RT-PCR. Expression levels of hypothalamic leptin, leptin receptor, and suppressor of cytokine signaling (SOCS) 3 were identified by relative quantitative RT-PCR using the QuantumRNA 18S Internal Standards kit (Ambion, Austin, TX), as described previously (3).

Serum leptin, serum corticosterone, and β-endorphin. Radioimmunoassay was used to determine serum leptin (Millipore, Billerica, MA), hypothalamic β-endorphin (Phoenix Pharmaceuticals, Burlingame, CA), and serum corticosterone (enzyme immunoassay kit; Caymon, Ann Arbor, MI).

Statistical analysis. Data were analyzed by two-way ANOVA, with repeated measures when appropriate. A post hoc test (Bonferroni) was applied to determine individual differences between means.

RESULTS

Leptin overexpression. Leptin overexpression was confirmed by examination of leptin mRNA levels in the hypothalamus. Every rAAV-leptin–treated animal displayed strong expression, whereas no expression was detected in those administered rAAV-control vector (data not shown). We have previously demonstrated that a similar level of leptin overexpression results in an approximately twofold increase in leptin levels in the cerebral spinal fluid (2). This increase is less than the three- to fourfold increase in cerebral spinal fluid leptin following high-fat feeding (17).

Body weight and food consumption

Standard diet–raised rats: rAAV-mediated leptin gene delivery (day 0 to day 12). The rAAV-leptin rats initially lost body mass until day 3 then maintained the reduced weight until day 11, at which point they started gaining weight (Fig. 1*A*). The control rats also lost weight initially due to the surgical procedure, but this loss was significantly less than with rAAV-leptin (-18.13 ± 3.38 vs. -26 ± 1.75 g, $P = 0.048$). After day 3, the controls gained weight rapidly throughout the remainder of the experiment (Fig. 1*A*), and by day 13, they were nearly 34 g heavier than the rAAV-leptin–treated rats. Food intake was also initially diminished in both the control and rAAV-leptin–treated animals. The control group rapidly rebounded to pretreatment food consumption, whereas with leptin treatment, food intake remained significantly reduced. The cumulative food intake between days 3 and 13 diminished by 22% relative to controls (Fig. 1*B*).

Standard diet–raised rats: rAAV-mediated leptin gene delivery combined with wheel running (day 14 to day 51). At this point, half the animals in each group were allowed free access to running wheels, and the other half remained sedentary. The sedentary control rats continued to gain weight, while the trajectory of weight gain in the sedentary rAAV-leptin–treated rats was significantly diminished with weight gains of less than half that of sedentary controls by day 51 (Fig. 1*A*) (Table 1).

The distances run by the control and rAAV-leptin–treated wheel-running rats were similar (Table 1). Interestingly, wheel running had a similar effect on body weight among the control and rAAV-leptin–treated rats. Both groups displayed an initial decrease in body weight in the first 4 days of wheel running, followed by a trajectory of weight gain that paralleled their corresponding sedentary counterpart (Fig. 1*A*). Moreover, body weight gain was negatively correlated with wheel running in both the control ($r^2 = 0.73$, $P = 0.0145$) and rAAV-leptin ($r^2 = 0.70$, $P = 0.0101$) groups. The overall reduction in body weight gain with wheel running in the control-vector group paralleled that in the sedentary rAAV-leptin group, and these effects were additive in the rats that experienced leptin plus wheel running. By day 51, the sedentary control rats gained twice that of wheel-running control or sedentary leptin-treated rats, whereas leptin in conjunction with wheel running prevented nearly all weight gain (Table 1).

With respect to food intake in control rats, during the first part of wheel-running phase (days 14–37), cumulative food consumption was 20% less in the wheel-running compared with sedentary rats (Fig. 1*C*) but was unchanged between these groups afterward (data not shown). Food intake among the rAAV-leptin–treated rats was similar during days 14–37 but was significantly less than sedentary control rats (Fig. 1*C*). From day 38 to the end of the experiment, the sedentary rAAV-leptin rats consumed significantly less food than either control group or the wheel-running rAAV leptin–treated rats (data not shown),

High-fat–raised rats: rAAV-mediated leptin gene delivery (day 0 to day 12). The rAAV-leptin–treated rats initially lost body mass until day 5. After which, the rats began to steadily regain the lost body weight over the next 10 days (Fig. 2*A*). The control rats also lost weight initially due to the surgical procedure, but this amount was significantly less than with rAAV-leptin rats (-18.95 ± 4.25 vs. -31.18 ± 1.79, $P = 0.006$). After day 5, the control rats steadily gained weight throughout the remainder of the
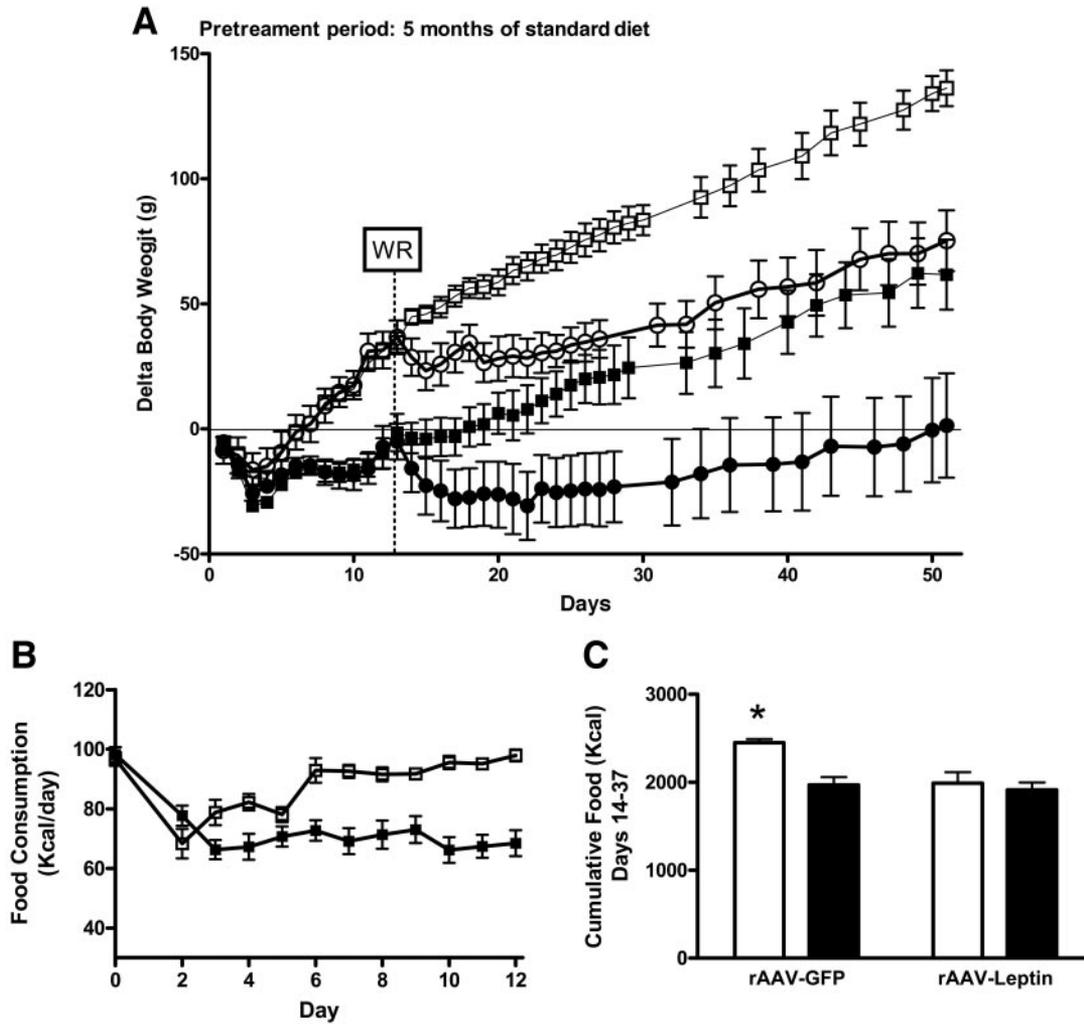
FIG. 1. *A*: Body mass changes in standard diet–raised rats following administration of control vector (open symbols) or rAAV-leptin (closed symbols) in sedentary rats (squares) or rats provided access to running wheels (circles) beginning at day 14. The rAAV-leptin or control vectors were administered at day 0 in rats maintained on a standard diet for 5 months and continued on the standard diet throughout the experiment. Values represent the mean ± SE of eight sedentary/control, seven wheel-running/control, seven sedentary/AAV-leptin, and eight wheel-running rats per group. *P* < 0.05 (wheel-running vs. respective sedentary groups; sedentary/control vs. sedentary/AAV-leptin) for difference in slops following division of groups into sedentary and wheel running. □, sedentary/control vector; ■, sedentary/AAV-leptin; ○, wheel running/control vector; ●, wheel running/AAV-leptin. *B*: Daily food consumption following control vector (□) or rAAV-leptin (■) before division into sedentary or wheel-running groups. Food consumption is significantly less (*P* < 0.01) beginning at day 3. Note: *P* < 0.0001 for cumulative food consumption during this period. *C*: Cumulative food consumption between days 14 and 37 during the first part of the sedentary (□) and wheel running (■) period. *P* = 0.004 (wheel running); *P* = 0.007 (rAAV-leptin); *P* = 0.03 (interaction) by two-way ANOVA; **P* < 0.01 for difference with wheel running among rAAV-GFP groups and for difference from either rAAV-leptin groups by post hoc analysis.

experiment (Fig. 2*A*). Food consumption, like body weight, initially decreased in both groups, and there was a brief period between days 7 and 10 when food consumption was significantly diminished in the rAAV-leptin–treated group (Fig. 2*B*). However, this anorexic response was significantly less in the high-fat–raised rats (difference in cumulative food intake between control and leptin, 78 ± 13 kcal) than in the standard diet–raised rats (162 ± 1 kcal decrease, *P* < 0.001).

High-fat-raised rats: rAAV-mediated leptin gene delivery combined with wheel running (day 14 to day 51). At day 14, at the time when the body weight and food consumption were similar between the two high-fat–raised groups, half the animals in each group were allowed free access to running wheels. Both the control and rAAV-leptin–treated ran to similar extents (Table 1) but six times less than the distances run by the standard diet–raised rats. Among the control rats, wheel running had no measurable effect on body weight. In stark contrast, wheel

running resulted in a dramatic reduction in weight gain in the rAAV-leptin–treated animals. First, in the rAAV-leptin–treated sedentary rats, the trajectory of weight gain was more than twofold greater than in the control sedentary rats, indicating an acceleration of diet-induced obesity with leptin treatment. Second, voluntary wheel running in leptin-treated rats not only precluded this exacerbated weight gain but also prevented the expected high-fat feeding–mediated weight gain displayed in the control rats (Fig. 2*A*). In contrast to the standard diet–raised rats, there was no correlation between the amount of wheel running and body weight change in the high-fat–raised wheel-running rAAV-leptin rats. By day 51 (day 38 of wheel running), the control rats with or without wheel running gained similar amounts of weight, whereas, the sedentary rAAV-leptin rats gained two times more weight than the either of two control groups. In comparison, the leptin plus wheel-running group gained virtually no weight (Table 1).

TABLE 1
Weight, wheel running, serum leptin, and adiposity in standard diet– and high-fat–raised rats

| | rAAV-control | | rAAV-leptin | |
	Sedentary	Wheel running	Sedentary	Wheel running
Standard diet–raised rats				
Body mass day 0 (g)	483 ± 5	484 ± 4	486 ± 7	488 ± 6
Body mass day 51 (g)	617 ± 10	566 ± 13	552 ± 16	487 ± 23
Wheel running (m/day)		4167 ± 1091		4267 ± 1377
Leptin day 0 (ng/ml)	3.44 ± 0.53	3.94 ± 0.88	3.30 ± 0.46	3.14 ± 0.33
Leptin day 51 (ng/ml)	8.47 ± 1.36	4.34 ± 1.37*	4.39 ± 1.25†	0.71 ± 0.21*
Adiposity (g)	22.3 ± 1.4	13.6 ± 2.5§	13.0 ± 2.4&	2.4 ± 0.74§
High-fat–raised rats				
Body mass day 0 (g)	702 ± 13	701 ± 35	708 ± 23	695 ± 32
Body mass day 51 (g)	743 ± 14	738 ± 22	800 ± 32	697 ± 27
Wheel running (m/day)		865 ± 265		629 ± 212
Leptin day 0 (ng/ml)	24.4 ± 3.0	27.9 ± 4.5	28.4 ± 2.1	26.0 ± 2.9
Leptin day 51 (ng/ml)	26.1 ± 2.3	25.8 ± 4.4	41.4 ± 3.9‡	25.8 ± 4.1
Adiposity (g)	53.6 ± 5.2	50.6 ± 3.0	73.1 ± 6.9	47.2 ± 7.1¶

Data are means ± SE of six to eight rats per group. Adiposity represents the sum of PWAT, EWAT, and RTWAT. In three of eight standard diet–raised rats, we were unable to recover any PWAT or RTWAT; thus, in these rats, the adiposity is represented by EWAT only. Leptin day 51: standard diet raised: $P = 0.003$ for difference with leptin or wheel running by two-way ANOVA. *$P < 0.05$ for difference from corresponding sedentary group by post-hoc analysis. †$P < 0.05$ for difference with leptin among sedentary groups by post-hoc analysis. High-fat raised: $P < 0.05$ for difference with leptin by two-way ANOVA. ‡$P < 0.05$ for difference from all other groups by post-hoc analysis. Adiposity: Standard diet raised: $P = 0.001$ for difference with leptin or wheel running by two-way ANOVA. §$P < 0.01$ for difference from corresponding sedentary group by post-hoc analysis. &$P < 0.01$ for difference with leptin among sedentary groups by post hoc analysis. High-fat raised: $P < 0.05$ for difference with wheel running by two-way ANOVA. ¶$P < 0.05$ for difference from corresponding sedentary group by post hoc analysis.

The pattern of food consumption during the first part of wheel-running phase (days 14–37) was qualitatively similar to the changes in body weight; that is, cumulative food consumption was greater in the sedentary leptin-treated rats compared with either control groups (Fig. 2C). Wheel running did not alter food intake in the control animals, whereas it reduced cumulative food consumption by 22% in the wheel-running leptin-treated rats compared with the respective sedentary counterparts (Fig. 2C). Afterward, from days 38 to 51, food intake was similar among all groups (data not shown).

Serum leptin and adiposity levels. Serum leptin level, one marker of adiposity, was examined at day 0, before vector administration, and at death. The high-fat–raised rats were consuming the high-fat diet for 5 months before day 0, and leptin levels were elevated in these compared with standard diet–raised rats (Table 1).

Standard diet–raised rats. As expected, the sedentary control rats experienced the normal growth-related increase (2.5-fold) in serum leptin by day 51 compared with day 0. However, either wheel running or leptin treatment prevented this increase in serum leptin (Table 1). Notably, in the wheel-running plus leptin-treated rats, serum leptin was fourfold less than the initial level and was significantly lower than any other group (Table 1). End point adiposity levels coincided with those of serum leptin. The sum of three adiposity tissues, PWAT, RTWAT, and EWAT were highest in sedentary control rats, lower (by nearly 40%) and similar in the sedentary leptin-treated and wheel-running control rats, and lowest in the wheel-running plus rAAV-leptin rats whose adiposity was at least 80% lower than any other group (Table 1). Moreover, we were unable to recover any PWAT or RTWAT in three of eight rats from this latter group.

High-fat–raised rats. In contrast to standard diet–raised rats, wheel running was without effect on serum leptin levels among the control rats. Moreover, parallel to body weight pattern, leptin levels in the sedentary leptin-treated rats were greater than the corresponding wheel-running plus leptin-treated rats or either the wheel-running or sedentary controls. On the other hand, despite the lower body weight in the wheel-running leptin-treated rats, leptin levels were not significantly different from either the sedentary or wheel-running control-vector groups (Table 1). End point adiposity levels correlated with those of serum leptin. The sum of three adiposity tissues, PWAT, RTWAT, and EWAT were similar in all groups, except for a nearly 50% increase in the sedentary leptin-treated rats (Table 1).

Hypothalamic signaling factors. The diminished dietary weight gain in the high-fat–raised rats in response to leptin plus wheel running implies a possible connection to leptin function, which prompted us to examine hypothalamic factors associated with leptin signaling, including STAT3 phosphorylation (P-STAT3), SOCS3, leptin receptor message, and protein-tyrosine phosphatase-1B. As expected, rAAV-leptin elevated P-STAT3 in standard diet–raised, sedentary, and wheel-running animals by 60%, but wheel running did not affect P-STAT3 in either group (Fig. 3A). In the high-fat–raised rats, rAAV-leptin also elevated P-STAT3 in the sedentary animals by 30%. Although wheel running did not impact P-STAT3 in the control rats, wheel running enhanced P-STAT3 levels by 75% in rAAV-leptin–treated rats (Fig. 3B). Total STAT3 levels remained unchanged across groups (data not shown). SOCS3 expression levels were unchanged in all standard diet–raised animals and unchanged in sedentary rAAV-leptin or wheel-running control high-fat–raised rats (Fig. 3C and D). However, SOCS3 expression was increased in the leptin plus wheel-running high-fat–raised rats (Fig. 3D). In contrast, both leptin receptor message and protein-tyrosine phosphatase-1B levels were unchanged across groups (data not shown). Additionally, a number of hypothalamic factors often associated with exercise-induced changes,
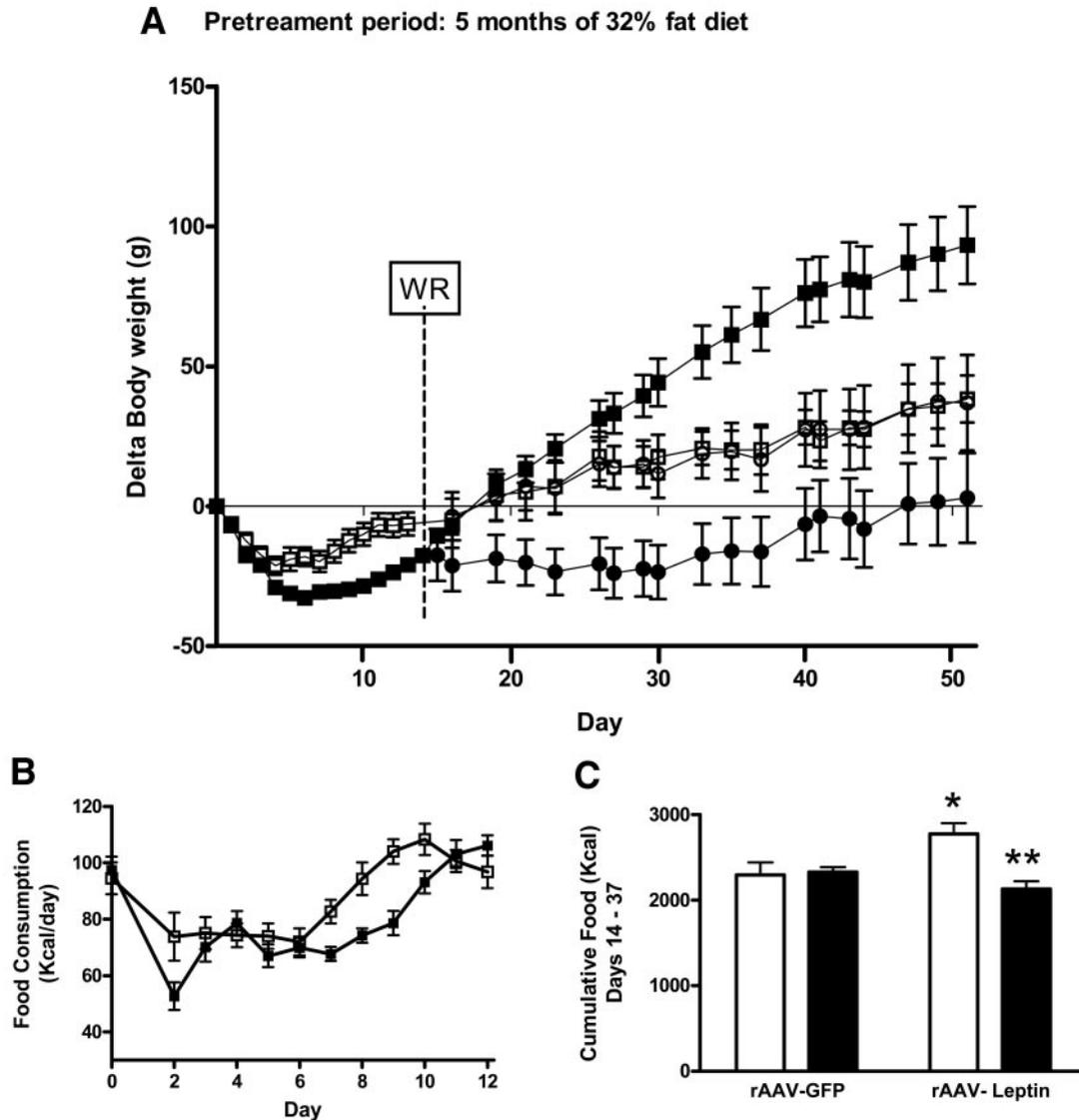
A Pretreament period: 5 months of 32% fat diet

FIG. 2. *A*: Body mass changes in high-fat–raised rats following administration of control vector (open symbols) or rAAV-leptin (closed symbols) in sedentary rats (squares) or rats provided access to running wheels (circles) beginning at day 14. The rAAV-leptin or control vectors were administered at day 0 in rats raised on a high-fat diet for 5 months and continued on the high-fat diet throughout the experiment. Values represent the mean ± SE of six sedentary and eight wheel-running rats per group. $P < 0.05$ (control versus corresponding AAV-leptin; sedentary/AAV-leptin vs. wheel running/AAV-leptin) for difference in slops following division of groups into sedentary and wheel running. □, sedentary/control vector; ■, sedentary/AAV-leptin; ○, wheel running/control vector; ●, wheel running/AAV-leptin. *B*: Daily food consumption following control vector (□) or rAAV-leptin (■) before division into sedentary or wheel-running groups. Food consumption is significantly less ($P < 0.05$) between days 7 and 10, but cumulative food consumption during entire period is not significantly different. *C*: Cumulative food consumption between days 14 and 37 during the first part of the sedentary (○) and wheel-running (●) period. $P = 0.015$ (wheel running) and $P = 0.008$ (interaction) by two-way ANOVA; *$P < 0.001$ for difference with wheel running among rAAV-leptin groups by post hoc analysis; **$P < 0.05$ for difference between sedentary rAAV-leptin and sedentary rAAV-GFP. After day 37, food consumption was not different among groups.

including IL-6, β-endorphin, and two enzymes related to reducing reactive oxygen species in the hypothalamus, catalase and CuZn superoxide dismutaze, were also unaltered among treatments in the high-fat–raised rats (data not shown).

Hormone-sensitive lipase. Phosphorylation of hormone-sensitive lipase (HSL) at Ser563 in PWAT was examined as one measurement of fat mobilization. Treatment with rAAV-leptin in the standard diet–raised rats resulted in a nearly twofold increase in HSL phosphorylation relative to control rats. Wheel running, however, did not cause additional change in HSL phosphorylation (Fig. 4*A*). Similar results were obtained in the high-fat–raised rats with a twofold elevation in HSL phosphorylation in response to

rAAV-leptin but no further response with wheel running (Fig. 4*B*).

Serum corticosterone and adipose tissue 11β-HSD1. Each intervention in our protocol, exercise, high-fat feeding, and leptin therapy, could potentially change circulating as well as locally regulated tissue glucocorticoid levels. In the high-fat–raised rats, wheel running nonsignificantly raised serum corticosterone levels in both control (57.6 ± 16.3 vs. 96.7 ± 23.1 ng/ml) and leptin-treated (60.4 ± 12.4 vs. 93.6 ± 16.3 ng/ml) groups, but leptin treatment made no difference. An important determinate in glucocorticoid activity is the enzyme 11β-HSD1, which catalyzes the cellular activation of circulating inert 11-dehydrocorticosterone (cortisone in humans) to corticosterone (cortisol
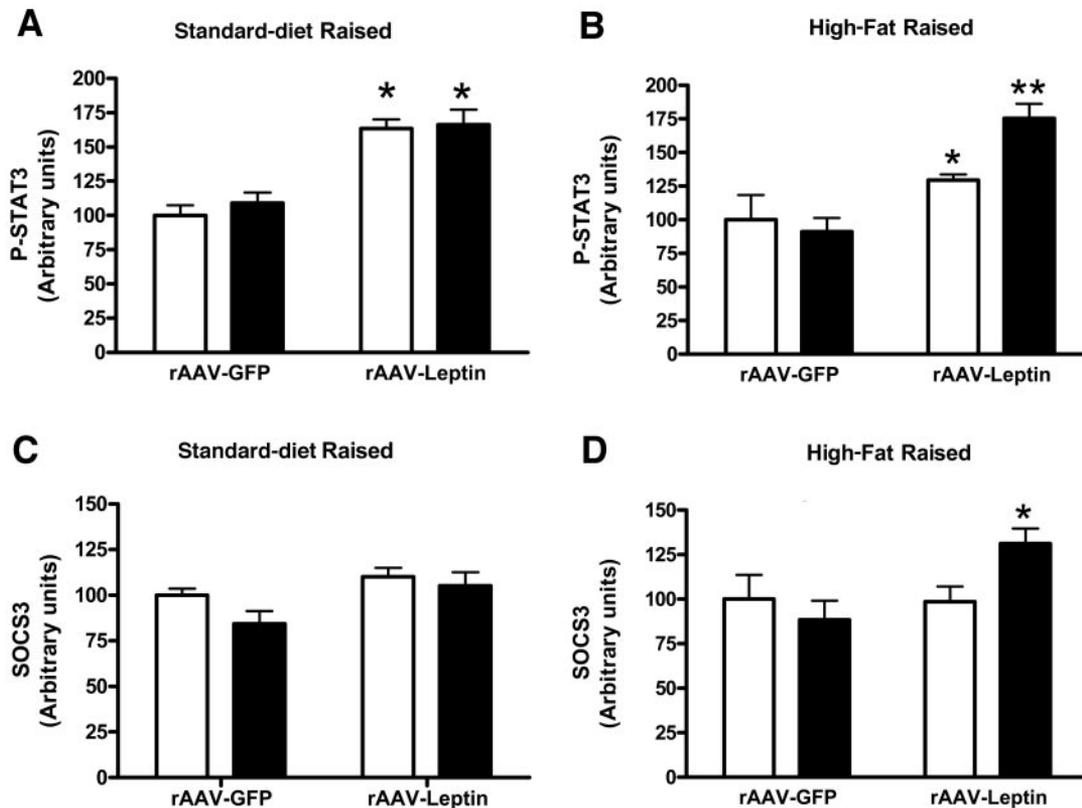
FIG. 3. Hypothalamic STAT3 phosphorylation and SOCS3 mRNA in standard diet–raised (*A* and *C*) and high-fat–raised (*B* and *D*) rats following administration of control vector or rAAV-leptin in sedentary rats (○) or rats given access to running wheels beginning at day 14 (●). Values represent the mean ± SE of six to eight rats per group. PSTAT3: standard diet raised; $P < 0.0001$ for difference with leptin by two-way ANOVA. *$P < 0.001$ for difference from corresponding rAAV-GFP group by post hoc analysis. High-fat raised; $P = 0.015$ for interaction and $P < 0.0001$ for difference with leptin by two-way ANOVA. *$P < 0.05$ for difference from corresponding rAAV-GFP group by post hoc analysis. **$P < 0.001$ for difference from all other groups by post hoc analysis. SOCS3: high-fat raised; $P = 0.04$ for interaction by two-way ANOVA. *$P < 0.05$ for difference from corresponding sedentary group and for difference from control wheel-running group by post hoc analysis.

in humans) independent of circulating corticosterone (18). Levels of 11β-HSD1 in PWAT were significantly reduced by nearly 75% only in the leptin wheel-running group compared with all other high-fat–raised groups (Fig. 4*D*). On the contrary, 11β-HSD1 levels in PWAT were unchanged with leptin, wheel running, or the combination treatment in the standard diet–raised rats (Fig. 4*C*).

DISCUSSION

Leptin was once believed to be the cure for obesity and even referred to as the antiobesity hormone (19). However, the pronounced leptin resistance associated with obesity has rendered leptin therapy in humans futile, and this approach has been mostly abandoned. New strategies that either revitalize leptin function or bypass leptin resistance may hold a promise in today's battle against the obesity epidemic.

The present study explores one such strategy to combat dietary obesity in high-fat–raised rats. Although neither leptin therapy nor wheel running by itself alters the course of dietary weight gain in the high-fat–raised rats, the combination of the two impedes the progression of weight gain in these otherwise leptin-resistant rats. The significance of this remarkable leptin/wheel-running synergy should be considered in light of two other intriguing observations. First, leptin therapy alone in the dietary obese rats worsens rather than ameliorates obesity. Second, this synergy is notably absent in standard diet–raised, leptin-responsive rats.

Our earlier work demonstrated that chronic hypothalamic leptin overexpression induces leptin resistance in young, lean rats (2,11,20). Such leptin-induced, leptin-resistant lean rats exhibited an exacerbated weight and adiposity gain on a high-fat diet compared with their respective high-fat–fed controls (3,4). Hence, leptin resistance promotes dietary obesity in lean rodents. In the present study, the 5-month high-fat–raised rats already had elevated serum leptin and diminished anorectic response to rAAV-leptin gene delivery versus the standard diet–raised rats, reflecting a diet-induced partial leptin resistance. Additionally, the anorectic response attenuated completely at day 11 after rAAV-leptin administration, indicative of a leptin-induced full leptin resistance. Beyond this point, these leptin-resistant dietary obese rats displayed greatly aggravated weight gain with continued high-fat feeding. Therefore, leptin resistance accelerates dietary obesity in obese rats. This new evidence again supports our notion that leptin resistance is causal to and compounds obesity.

Both leptin and voluntary wheel running were equally effective in reducing normal body weight gain in the standard diet–raised rats, and the combination therapy elicited additive effects. Moreover, the extent of wheel running was directly correlated with reduced weight gain in the wheel-running groups. This pattern of individual and additive effects was also reflected by the decreases in adiposity and serum leptin. Therefore, each treatment
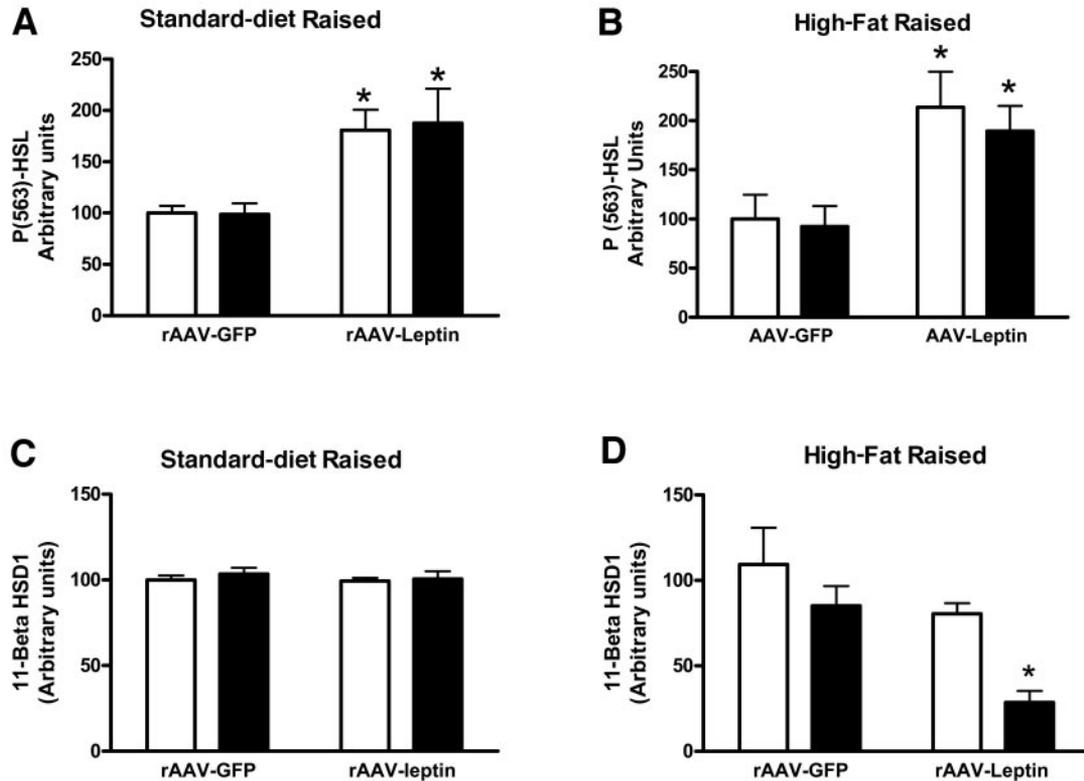
FIG. 4. *Top*: Phosphorylation at tyrosine 563 of HSL in PWAT in standard diet–raised (*A*) and high-fat–raised (*B*) rats and following administration of control vector or rAAV-leptin in sedentary rats (□) or rats given access to running wheels beginning at day 14 (■). *Bottom*: 11-β-HSD1 protein level in PWAT in standard diet–raised (*C*) and high-fat–raised (*D*) rats following administration of control vector or rAAV-leptin in sedentary rats (□) or rats given access to running wheels beginning at day 14 (■). Values represent the mean ± SE of six to eight rats per group. P(563)-HSL: $P = 0.0005$ (standard-diet raised) or $P = 0.0031$ (high-fat raised) for difference with leptin by two-way ANOVA. *$P < 0.01$ for difference from control vector by post hoc analysis. 11-β-HSD1: high-fat raised; $P = 0.0005$ for difference with leptin and $P = 0.0014$ for difference with wheel running by two-way ANOVA. *$P < 0.001$ for difference from all other groups by post hoc analysis.

appears to act independently to deter normal weight gain in the standard diet–raised, leptin-responsive rats.

Wheel running alone is ineffective in curbing the weight gain in high-fat–raised rats. These rats ran only one-sixth of the distance the standard diet–raised animals ran, and this amount of daily wheel running appears to be too miniscule to directly impact energy balance. The lack of a correlation between wheel running and weight change among the high-fat–raised rats supports this assumption. This suggests that the act of wheel running, rather than the distance run, is more important in mediating the leptin/wheel-running synergy resulting in the reduced weight gain in the high-fat–raised rats. Indeed, several hypothalamic factors often altered with vigorous exercise, such as β-endorphin, IL-6, catalase, and CuZn superoxide dismutaze, were all unchanged with wheel running in this case. The mechanism underlying the importance of the act of wheel running is intriguing but unknown at this point and warrants vigorous investigation.

The mechanisms underlying the weight loss synergy in the high-fat–raised rats are likely complex but may involve enhanced STAT3 signaling. Leptin resistance is characterized by impaired leptin signaling in the hypothalamus (16,21–24). Treatments that enhance leptin signaling, such as food restriction or inhibition of tyrosine phosphatases, also increase leptin responsiveness (25,26). Chronic central rAAV-leptin gene delivery, as expected, augmented hypothalamic P-STAT3 in both the standard diet– and high-fat–raised animals, whereas wheel running alone did not change STAT3 signaling. However, wheel running/

leptin increased STAT3 phosphorylation beyond the level evoked by the leptin treatment alone, which occurred only in the high-fat–raised and not in the standard diet–raised animals. SOCS3, a negative regulatory signaling molecule normally induced following leptin-like cytokine receptor activation (27), is a tracer of leptin signaling. Resonant with the synergistic increase in P-STAT3, hypothalamic SOCS3 is also elevated only in the high-fat–raised wheel-running/leptin group. Because the synergy was evident immediately after the initiation of wheel running in the high-fat–raised animals already treated with rAAV-leptin for 13 days, it is rather tempting to hypothesize that wheel running restored leptin responsiveness. For instance, the magnitude of reduced weight gain in the high-fat–raised animals due to wheel running/leptin (90 g) was comparable with the response to leptin alone in the standard diet–raised rats (65 g). However, because the increase in P-STAT3 was an end point measurement, we cannot rule out the possibility that elevated P-STAT3 was a secondary rather than a primary cause for the reduced dietary body weight gain. It also remains unclear at the present time whether the elevated STAT3 in high-fat leptin/wheel-running rats is the result of leptin stimulation. However, a recent study (9) indicated that an acute bout of exercise enhances leptin signaling, consistent with the concept that wheel running could potentially restore leptin responsiveness. Future studies are needed to address these issues.

It is not readily apparent why the wheel-running–mediated synergy is absent in the standard diet–raised, leptin-responsive animals. Acute exercise was documented to

enhance leptin signaling in such animals (9). However, in that study, the protocol involved acute, forced, strenuous exercise as opposed to the unforced and mild wheel running we utlized, and leptin signaling was assessed immediately following the exercise. Any acute leptin signaling events would have been difficult to detect in our experimental design. Additionally, the robust responses in standard diet–raised animals to either leptin or wheel running may mask any subtle or modest synergy between the two interventions, whereas such synergy is readily evident in high-fat–raised rats lacking responses to either wheel running or leptin.

The fact that the leptin/wheel-running weight gain reduction synergy only surfaced in the presence of exogenous leptin but not with the high endogenous leptin already present in high-fat–raised animals is also perplexing. Diet-induced obesity is associated with leptin receptor downregulation (25); thus, the elevated endogenous leptin in this case might still be insufficient to trigger the synergistic response. However, in the present study, leptin receptor message was not significantly changed, suggesting against this supposition. Alternatively, endogenous and exogenous leptin may evoke differential responses. For instance, a recent study reported involvement of IL-1 signaling only in response to exogenous leptin but not endogenous leptin (28). Furthermore, the elevated leptin in the high-fat–raised rats was the result of a gradual increase in leptin over the 5 months of high-fat feeding, which provides ample time for adaptation (or brain rewiring) to occur. The wheel-running/leptin synergy, however, could result from an interaction between wheel running and the sudden elevation in leptin (due to 13 days of rAAV-leptin gene therapy before the initiation of wheel running). It is also interesting to note that this synergy occurred immediately following wheel running, suggesting that the synergy is the primary cause for the reduction in body weight gain.

Leptin elevates white fat catabolism, potentially through a centrally mediated sympathetic mechanism (29). Phosphorylation of HSL at Ser563 by protein kinase A is one important regulator of HSL activity and lipolysis in white fat (30). Central leptin augmented P-HSL, implicating enhanced WAT lipolysis in PWAT in both the standard diet– and high-fat–raised animals independent of wheel running. Interesting, HSL appears to be activated despite the presence of leptin resistance with respect to the control of energy intake. This observation may be explained by the concept of selective leptin resistance (i.e., even though the satiety effect of leptin diminishes, central regulation of sympathetic activity by leptin persists) (31).

Glucocorticoids participate in the regulation of fuel metabolism, energy partitioning, and body fat distribution. Elevated 11β-HSD1 activity in adipose tissue leads to increased glucocorticoid receptor activation and, hence, promotes obesity (32,33). We discovered that 11β-HSD1 protein levels were decreased only with the leptin and wheel-running combination therapy but not with individual treatment in the high-fat–raised rats. This evidence suggests an additional potential mechanism underlying the weight loss synergy between wheel running and leptin and resonates with studies in which selective decreases in 11β-HSD1 alleviates obesity-related metabolic complications (34,35).

In conclusion, neither wheel running nor leptin curbs dietary weight gain in high-fat–raised rats, and, moreover, leptin treatment actually worsens the obesity. Remarkably, these two otherwise ineffective therapies act synergistically to prevent high-fat–induced dietary weight gain. This weight reduction synergy is unique to the full leptin-resistant state and does not occur in the standard diet–raised rats displaying leptin sensitivity or high-fat–raised animals with partial leptin resistance and no exogenous leptin treatment. It is possible that changes in leptin signaling and/or responsiveness contribute to this outcome, but additional work is necessary to test this postulate. To date, leptin resistance has limited the value of leptin as a therapeutic agent for treating obesity. Procedures that mitigate or bypass leptin resistance may provide a viable strategy to treat dietary obesity. The present study explored this idea in an animal model of dietary obesity. Whether this combination therapy of wheel running and leptin will synergistically reduce dietary weight gain in obese humans is a tantalizing prospect.

REFERENCES

1. Ahima RS, Flier JS: Leptin. *Annu Rev Physiol* 62:413–437, 2000
2. Scarpace PJ, Matheny M, Zhang Y, Tumer N, Frase CD, Shek EW, Hong B, Prima V, Zolotukhin S: Central leptin gene delivery evokes persistent leptin signal transduction in young and aged-obese rats but physiological responses become attenuated over time in aged-obese rats. *Neuropharmacology* 42:548–561, 2002
3. Wilsey J, Zolotukhin S, Prima V, Scarpace PJ: Central leptin gene therapy fails to overcome leptin resistance associated with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 285:R1011–R1020, 2003
4. Scarpace PJ, Matheny M, Tumer N, Cheng KY, Zhang Y: Leptin resistance exacerbates diet-induced obesity and is associated with diminished maximal leptin signalling capacity in rats. *Diabetologia* 48:1075–1083, 2005
5. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS: Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1:1311–1314, 1995
6. Widdowson PS, Upton R, Buckingham R, Arch J, Williams G: Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. *Diabetes* 46:1782–1785, 1997
7. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM: Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci U S A* 94:8878–8883, 1997
8. Slentz CA, Duscha BD, Johnson JL, Ketchum K, Aiken LB, Samsa GP, Houmard JA, Bales CW, Kraus WE: Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE: a randomized controlled study. *Arch Intern Med* 164:31–39, 2004
9. Flores MB, Fernandes MF, Ropelle ER, Faria MC, Ueno M, Velloso LA, Saad MJ, Carvalheira JB: Exercise improves insulin and leptin sensitivity in hypothalamus of Wistar rats. *Diabetes* 55:2554–2561, 2006
10. Levin BE, Dunn-Meynell AA: Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 286:R771–R778, 2004
11. Scarpace PJ, Matheny M, Zhang Y, Shek EW, Prima V, Zolotukhin S, Tumer N: Leptin-induced leptin resistance reveals separate roles for the anorexic and thermogenic responses in weight maintenance. *Endocrinology* 143:3026–3035, 2002
12. Berger I, Fitzgerald DJ, Richmond TJ: Baculovirus expression system for heterologous multiprotein complexes. *Nat Biotechnol* 22:1583–1587, 2004
13. Urabe M, Nakakura T, Xin KQ, Obara Y, Mizukami H, Kume A, Kotin RM, Ozawa K: Scalable generation of high-titer recombinant adeno-associated virus type 5 in insect cells. *J Virol* 80:1874–1885, 2006
14. Zolotukhin S, Byrne BJ, Mason E, Zolotukhin I, Potter M, Chesnut K,

Summerford C, Samulski RJ, Muzyczka N: Recombinant adeno-associated virus purification using novel methods improves infectious titer and yield. *Gene Ther* 6:973–985, 1999

15. Zolotukhin S, Potter M, Zolotukhin I, Sakai Y, Loiler S, Fraites TJ, Jr, Chiodo VA, Phillipsberg T, Muzyczka N, Hauswirth WW, Flotte TR, Byrne BJ, Snyder RO: Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods* 28:158–167, 2002

16. Scarpace PJ, Matheny M, Tumer N: Hypothalamic leptin resistance is associated with impaired leptin signal transduction in aged obese rats. *Neuroscience* 104:1111–1117, 2001

17. Unger RH: Leptin physiology: a second look. *Regul Pept* 92:87–95, 2000

18. Wake DJ, Walker BR: Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 in obesity. *Endocrine* 29:101–108, 2006

19. Elmquist JK, Maratos-Flier E, Saper CB, Flier JS: Unraveling the central nervous system pathways underlying responses to leptin. *Nat Neurosci* 1:445–450, 1998

20. Scarpace PJ, Matheny M, Zolotukhin S, Tumer N, Zhang Y: Leptin-induced leptin resistant rats exhibit enhanced responses to the melanocortin agonist MT II. *Neuropharmacology* 45:211–219, 2003

21. Scarpace PJ, Matheny M, Moore RL, Tumer N: Impaired leptin responsiveness in aged rats. *Diabetes* 49:431–435, 2000

22. Scarpace PJ, Matheny M, Shek EW: Impaired leptin signal transduction with age-related obesity. *Neuropharmacology* 39:1872–1879, 2000

23. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS: Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 105:1827–1832, 2000

24. Martin RL, Perez E, He YJ, Dawson R Jr, Millard WJ: Leptin resistance is associated with hypothalamic leptin receptor mRNA and protein down-regulation. *Metabolism* 49:1479–1484, 2000

25. Wilsey J, Scarpace PJ: Caloric restriction reverses the deficits in leptin receptor protein and leptin signaling capacity associated with diet-induced obesity: role of leptin in the regulation of hypothalamic long-form leptin receptor expression. *J Endocrinol* 181:297–306, 2004

26. Wilsey J, Matheny MK, Scarpace PJ: Oral vanadium enhances the catabolic effects of central leptin in young adult rats. *Endocrinology* 147:493–501, 2006

27. Krebs DL, Hilton DJ: SOCS proteins: negative regulators of cytokine signaling. *Stem Cells* 19:378–387, 2001

28. Wisse BE, Ogimoto K, Morton GJ, Williams DL, Schwartz MW: Central interleukin-1 (IL1) signaling is required for pharmacological, but not physiological, effects of leptin on energy balance. *Brain Res* 1144:101–106, 2007

29. Nonogaki K: New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia* 43:533–549, 2000

30. Carmen GY, Victor SM: Signalling mechanisms regulating lipolysis. *Cell Signal* 18:401–408, 2006

31. Mark AL, Correia ML, Rahmouni K, Haynes WG: Selective leptin resistance: a new concept in leptin physiology with cardiovascular implications. *J Hypertens* 20:1245–1250, 2002

32. Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, Shinyama H, Sharp MG, Fleming S, Mullins JJ, Seckl JR, Flier JS: Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *J Clin Invest* 112:83–90, 2003

33. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294:2166–2170, 2001

34. Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ, Seckl JR: Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes* 53:931–938, 2004

35. Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ: 11beta-hydroxy-steroid dehydrogenase type 1 knockout mice show attenuated glucocorti-coid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A* 94:14924–14929, 1997