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

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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 effective 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.


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See accompanying commentary, p 534.
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 a regular diet (standard diet–raised group). In the second experiment, the rats were raised and maintained on a diet high in fat (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, the 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 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-BOBW (11) was subcloned into pUC7 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 × 1010 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 were 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 150 mg/kg pentobarbital anesthetic. The circulatory system was perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, 0.9% NaCl, and 0.05% glutaraldehyde, 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 electrotransferred 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), 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (Alpha Diagnostic, San Antonio, TX), protein-tyrosine phosphatase-1B, catalase, or CuZn superoxide dismutase (Calbiochem, San Diego, CA).

RT-PCR. Expression levels of hypothalamic leptin, leptin receptor, and suppressor of cytokine signaling (SOCS) 3 were identified by relative quantification using the QIAGEN QuantiTect SYBR Green PCR kit (QIAGEN, Valencia, CA) and the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). The expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the expression of target genes.

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. 1A). 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. 1A), 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. 1B).

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. 1A) (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. 1A). Moreover, body weight gain was negatively correlated with wheel running in both the control (r² = 0.73, P = 0.0145) and rAAV-leptin (r² = 0.70, P = 0.0010) 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 weight that was 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. 1C) 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. 1C). 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. 2A). 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
experiment (Fig. 2A). 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. 2B). 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. 2A). 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).

![Diagram](https://example.com/diagram.png)
TABLE 1
Weight, wheel running, serum leptin, and adiposity in standard diet- and high-fat–raised rats

<table>
<thead>
<tr>
<th></th>
<th>rAAV-control</th>
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<th>rAAV-leptin</th>
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<td>Wheel running</td>
<td>Sedentary</td>
<td>Wheel running</td>
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<tr>
<td>Body mass day 0 (g)</td>
<td>483 ± 5</td>
<td>484 ± 4</td>
<td>486 ± 7</td>
<td>488 ± 6</td>
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<td>Body mass day 51 (g)</td>
<td>617 ± 10</td>
<td>566 ± 13</td>
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<td>487 ± 23</td>
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<td>Wheel running (m/day)</td>
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<td>Leptin day 0 (ng/ml)</td>
<td>3.44 ± 0.53</td>
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<td>3.14 ± 0.33</td>
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<td>Leptin day 51 (ng/ml)</td>
<td>8.47 ± 1.36</td>
<td>4.34 ± 1.37*</td>
<td>4.39 ± 1.25*</td>
<td>0.71 ± 0.21*</td>
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<td>Adiposity (g)</td>
<td>22.3 ± 1.4</td>
<td>13.6 ± 2.5$</td>
<td>13.0 ± 2.4$</td>
<td>2.4 ± 0.74$</td>
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<tr>
<td><strong>High-fat–raised rats</strong></td>
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<td>Body mass day 0 (g)</td>
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<td>Wheel running (m/day)</td>
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<td>26.0 ± 2.9</td>
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<td>Leptin day 51 (ng/ml)</td>
<td>26.1 ± 2.3</td>
<td>25.8 ± 4.4</td>
<td>41.4 ± 3.9‡</td>
<td>25.8 ± 4.1</td>
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<tr>
<td>Adiposity (g)</td>
<td>53.6 ± 5.2</td>
<td>50.6 ± 3.0</td>
<td>73.1 ± 6.9</td>
<td>47.2 ± 7.1$</td>
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</table>

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 0. 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. \( \text{†} P < 0.05 \) 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. \( \text{‡} P < 0.01 \) 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. \( \text{¶} P < 0.05 \) 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,
including IL-6, β-endorphin, and two enzymes related to reducing reactive oxygen species in the hypothalamus, catalase and CuZn superoxide dismutase, 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. 4A).

**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).
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 com-
pared with all other high-fat–raised groups (Fig. 4C). 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. 4C).

DISCUSSION
Leptin was once believed to be the cure for obesity and
even referred to as the antiobesity hormone (19). How-
ever, 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 resis-
tance 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 signifi-
cance 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. Sec-
tioned, this synergy is notably absent in standard diet–raised,
leptin-responsive rats.

Our earlier work demonstrated that chronic hypothal-
amic 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 resis-
tance 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 resis-
tance. 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 dis-
played 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
diated 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
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 dismutase, 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 utilized, 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. Interestingly, 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.

ACKNOWLEDGMENTS

This work was supported by the National Institute on Aging Grant AG-26159, the University of Florida Institute on Aging and the Claude D. Pepper Older Americans Independence Center (NIH P30 AG028740), and the Medical Research Service of the Department of Veterans Affairs. S.Z. was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant RO1 DK62902.

REFERENCES

14. Zolotukhin S, Byrne BJ, Mason E, Zolotukhin I, Potter M, Chesnut K,


