Synergy between leptin therapy and a seemingly negligible amount of voluntary wheel running prevents progression of dietary obesity in leptin resistant rats

A. Shapiro¹ PhD, M. Matheny¹ BS, Y. Zhang¹,4 PhD, N. Tümer¹,²,4 PhD, K. Y. Cheng¹ BS, E. Rodrigues¹ MD, S. Zolotukhin³ PhD, and P. J. Scarpace¹,² PhD

¹Department of Pharmacology and Therapeutics, ²Department of Aging and Geriatrics, ³Department of Pediatrics, University of Florida College of Medicine, Gainesville, Florida 32610; ⁴Department of Veterans Affairs Medical Center, Gainesville, Florida 32608-1197.

Running title: Wheel running and leptin synergy

Corresponding author:
Philip J. Scarpace, Ph.D.
Department of Pharmacology and Therapeutics
Box 100267
University of Florida
Gainesville, Florida 32610
scarpace@ufl.edu

Received for publication 26 June 2007 and accepted in revised form 10 December 2007.
ABSTRACT

Objective: We examined whether chronic leptin treatment of diet-induced obese (DIO) rats promotes or alleviates the susceptibility to continued high-fat (HF) feeding. Secondly, we examined if voluntary wheel running (WR) is beneficial in reducing the trajectory of weight gain in HF-raised leptin resistant rats.

Research Design and Methods: Sprague-Dawley rats were chow or HF fed for five months, and then hypothalamic leptin overexpression was induced through central administration of rAAV-leptin while continuing either chow or HF diet. Two weeks later, half of the rats in each group were provided access to running wheels for 38 days, whilst maintained on either chow or HF.

Results: In chow-raised rats, either WR or leptin reduced the trajectory of weight gain, and the combined effect of both treatments was additive. In HF-raised leptin-resistant rats, leptin overexpression first transiently reduced weight gain, but then accelerated the weight gain 2-fold over controls. WR in HF-raised was 6-fold less than in chow raised rats and did not affect the weight gain. Surprisingly, WR plus leptin completely prevented the weight gain. This synergy was associated with enhanced hypothalamic signal transducer and activator of transcription 3 (STAT3) phosphorylation and suppressor of cytokine signaling 3 (SOCS3) expression in the WR plus leptin compared with leptin-treated sedentary HF counterparts. This enhanced STAT3 signaling associated with the combination treatment occurred only in HF-raised, leptin-resistant, and not in chow-raised, leptin-responsive rats.

Conclusions: Chronic leptin treatment in diet-induced obese rats accelerates dietary obesity. However, leptin combined with WR prevents further dietary weight gain. Thus, this combination therapy may be a viable anti-obesity treatment.

KEYWORDS. Central leptin gene therapy, diet-induced obesity, leptin resistance, STAT3 phosphorylation, voluntary wheel running.
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 are dietary obese due to high fat (HF) feeding and consequently, 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 indicated that a single bout of exercise enhances brain leptin signaling and short-term leptin responses in leptin sensitive rats (9). 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 WR as well as any impact of WR on leptin resistance by examining whether WR alone or in combination with leptin treatment can reduce the weight gain in rats already obese and leptin-resistant due to HF feeding.

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

Research Design and Methods

Experimental animals. Male Sprague Dawley rats obtained from Charles River (Wilmington, MA) were fed either a standard rodent chow (4% fat, 3.41kcal/g, diet 7912, Harlan Teklad; Madison, WI), or a HF diet (32% kcal from fat and 25% kcal from sucrose, 4.41kcal/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 (07:00 to 19:00 hr).

Experimental design. This study was divided into two experiments with identical design differing only in diet. In first experiment, the rats were raised and maintained on regular chow (Chow-raised), whereas, in the second, they were raised and maintained on the 32% HF diet throughout the study (HF-raised). After 5 months on the respective diets, the rats were divided into two groups, those administered control vector and those administered rAAV-leptin by i.c.v. 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 extent of WR was recorded daily. Each experiment was terminated 38 days after access to the running wheels. There were 8 or 7 animals per group except for the HF-raised sedentary and WR groups provided the control vector, where there were 6.

Production of rAAV vectors. Rat leptin cDNA under the control of a chicken beta-actin promoter from pTR-betaObW (11) was subcloned into pUCDM transfer plasmid. 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 2.5x10^10 physical particles/rat in 5µl of either control vector or rAAV-leptin was delivered by i.c.v. injection into the third cerebral ventricle as previously described (11). The coordinates for injection are 1.3mm anterior to Bregma, 9.6mm ventral from the skull surface, at an angle of 20 degrees anterior to posterior.

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

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

**Western analysis.** Protein homogenate (50µg) was separated on a SDS-PAGE gel and electro-transferred to PVDF membranes (16). Immunoreactivity was assessed with antibodies specific to phospho-tyrosine 705 of STAT3 or either phosphorylated or unphosphorylated STAT3 (Cell Signaling, Danvers, MA), IL6 (Biotechnology, Santa Cruz, CA), 11-beta-HSD1 (Alpha Diagnostic, San Antonio, TX), PTP1B, Catalase, or CuZnSOD (Calbiochem, San Diego, CA).

**RT-PCR.** Expression levels of hypothalamic leptin, leptin receptor and SOCS3 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 beta-endorphin.** Radioimmunoassay was used to determine serum leptin (Millipore, Billerica, MA), hypothalamic beta-endorphin (Phoenix Pharmaceuticals, Burlingame, CA) and serum corticosterone (EIA 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 two-fold increase in leptin levels in the cerebral spinal fluid (CSF) (2). This increase is less than the 3 to 4 fold increase in CSF leptin following high fat feeding (17).

**Body weight and food consumption.** Chow-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 (-18.1±3.38 vs. -26±1.75g, 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 34g 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 pre-treatment food consumption, whereas with leptin treatment, food intake remained significantly reduced. The cumulative food intake between days 3-13 diminished by 22% relative to controls (Fig. 1B).

Chow-raised rats rAAV-mediated leptin gene delivery combined with WR (day 14 to day 51). At this point, half the animals in each
The distances ran by the control and rAAV-leptin-treated WR rats were similar (Table 1). Interestingly, WR 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 WR, followed by a trajectory of weight gain that paralleled their corresponding sedentary counterpart (Fig. 1A). Moreover, body weight gain was negatively correlated with WR in both the control \( (r^2=.73, p=0.0145) \) and rAAV-leptin \( (r^2=.70, p=0.0101) \) groups. The overall reduction in body weight gain with WR 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 WR. By day 51, the sedentary control rats gained twice that of WR control or sedentary leptin-treated rats, whereas leptin in conjunction with WR prevented nearly all weight gain (Table 1).

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

HF-raised rats: rAAV-mediated leptin gene delivery combined with WR (day 14 to day 51). At day 14, at the time when the body weight and food consumption were similar between the two HF-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 6 times less than the distances ran by the chow-raised rats. Among the control rats, WR had no measurable effect on body weight. In stark contrast, WR 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 2-fold greater than in the control sedentary rats, indicating an acceleration of diet-induced obesity with leptin treatment. Second, voluntary WR in leptin-treated rats not only precluded this exacerbated weight gain, but also prevented the expected HF feeding-mediated weight gain displayed in the control rats (Fig. 2A). In contrast to the chow-raised rats, there was no correlation between the amount of WR and body weight change in the HF-raised WR rAAV-leptin rats. By day 51 (day 38 of WR), the control rats with or without WR 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 WR group gained virtually no weight (Table 1).
The pattern of food consumption during the first part of WR 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 to either control groups (Fig 2C). WR did not alter food intake in the control animals, whereas it reduced cumulative food consumption by 22% in the WR leptin-treated rats compared with the respective sedentary counterparts (Fig. 2C). Afterwards, from days 38-51, food intake was similar between all groups (data not shown).

**Serum leptin and adiposity levels.** Serum leptin level, one marker of adiposity, was examined at day zero, prior to vector administration, and at death. The HF-raised rats were consuming the HF diet for five months prior to day zero, and leptin levels were elevated in these compared with chow-raised rats (Table 1).

**Chow-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 WR or leptin treatment prevented this increase in serum leptin (Table 1). Notably, in the WR plus leptin-treated rats, serum leptin was 4-fold 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 WR control rats, and lowest in the WR plus rAAV-leptin rats whose adiposity was at least 80% lower than any other groups (Table 1). Moreover, we were unable to recover any PWAT or RTWAT in three out of eight rats from this latter group.

**HF-raised rats.** In contrast to chow-raised rats, WR 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 WR plus leptin-treated rats or either the WR or sedentary controls. On the other hand, despite the lower body weight in the WR leptin-treated rats, leptin levels were not significantly different from either the sedentary or WR 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 HF-raised rats in response to leptin plus WR 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 PTP1B. As expected, rAAV-leptin elevated P-STAT3 in chow-raised, sedentary and WR animals by 60%, but WR did not affect P-STAT3 in either group (Fig. 3A). In the HF-raised rats, rAAV-leptin also elevated P-STAT3 in the sedentary animals by 30%. Although WR did not impact P-STAT3 in the control rats, WR 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 chow-raised animals and unchanged in sedentary rAAV-leptin or WR control HF-raised rats (Fig 3C, D). However, SOCS3 expression was increased in the leptin plus WR HF-raised rats (Fig. 3D). In contrast, both leptin receptor message and PTP1B levels were unchanged across groups (data not shown). Additionally, a number of hypothalamic factors often associated with exercise-induced changes including IL-6, beta-endorphin, and two enzymes related to reducing reactive oxygen species in the hypothalamus, catalase and CuZn superoxide dismutaze (CuZnSOD) were also unaltered among treatments in the HF-raised rats (data not shown).

**Hormone sensitive lipase.** Phosphorylation of hormone-sensitive lipase (HSL) at Ser-563 in PWAT was examined as one measurement of fat mobilization. Treatment with rAAV-leptin in the chow-raised rats resulted in a nearly two-fold increase in HSL-phosphorylation.
Wheel running and leptin synergy relative to control rats. WR, however, did not cause additional change in P-HSL (Fig. 4A). Similar results were obtained in the HF-raised rats with a two-fold elevation in P-HSL in response to rAAV-leptin but no further response with WR (Fig 4B).

**Serum corticosterone and adipose tissue 11β-HSD1.** Each intervention in our protocol, exercise, HF feeding, and leptin therapy, could potentially change circulating as well as locally regulated tissue glucocorticoid levels. In the HF-raised rats, WR non-significantly raised serum corticosterone levels in both control (57.6±16.3 versus 96.7±23.1ng/ml) and leptin-treated groups (60.4±12.4 versus 93.6±16.3ng/ml), but leptin treatment made no difference. An important determinant in glucocorticoid activity is the enzyme, 11β-hydroxysteroid dehydrogenase type 1 (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-WR group compared with all other HF-raised groups (Fig. 4D). On the contrary, 11β-HSD1 levels in PWAT were unchanged with leptin, WR or the combination treatment in the chow-raised rats (Fig. 4C).

**DISCUSSION**

Leptin was once believed to be the cure for obesity and even referred to as the “anti-obesity 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 the 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 HF-raised rats. Although neither leptin therapy nor WR by itself alters the course of dietary weight gain in the HF-raised rats, the combination of the two impedes the progression of the weight gain in these otherwise leptin-resistant rats. The significance of this remarkable leptin/WR 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 chow-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 HF diet compared to their respective HF-fed controls (3; 4). Hence, leptin resistance promotes dietary obesity in lean rodents. In the present study, the five-month HF-raised rats already had elevated serum leptin and diminished anorectic response to rAAV-leptin gene delivery versus the chow-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 HF 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 WR were equally effective in reducing normal body weight gain in the chow-raised rats, and the combination therapy elicited additive effects. Moreover, the extent of WR was directly correlated with reduced weight gain in the WR 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 chow-raised, leptin-responsive rats.

WR alone is ineffective in curbing the weight gain in HF-raised rats. These rats ran only 1/6th of the distance the chow-raised animals ran and this amount of daily WR appears to be too miniscule to directly impact energy balance. The lack of a correlation
between WR and weight change among the HF-raised rats supports this assumption. This suggests that the act of WR rather than the distance ran, is more important in mediating the leptin/WR synergy resulting in the reduced weight gain in the HF-raised rats. Indeed, several hypothalamic factors often altered with vigorous exercise such as beta-endorphin, IL6, catalase, and CuZnSOD were all unchanged with WR 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 HF-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 chow- and HF-raised animals, whereas WR alone did not change STAT3 signaling. However, WR/leptin increased STAT3 phosphorylation beyond the level evoked by the leptin treatment alone, which occurred only in the HF-raised and not in the chow-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 HF-raised WR/leptin group. Because the synergy was evident immediately after the initiation of WR in the HF-raised animals already treated with rAAV-leptin for 13 days, it is rather tempting to hypothesize that WR restored leptin responsiveness. For instance, the magnitude of reduced weight gain in the HF-raised animals due to WR/leptin (90g) was comparable to the response to leptin alone in the chow-raised rats (65g). However, because the increase in P-STAT3 was an end-point measurement, we cannot rule out the possibility that the elevated P-STAT3 was secondary rather than a primary cause for the reduced dietary body weight gain. It also remains unclear at present time if the elevated STAT3 in HF-leptin/WR rats is the result of leptin stimulation. However, a recent study indicated that an acute bout of exercise enhances leptin signaling (9), consistent with the concept that WR could potentially restore leptin responsiveness. Future studies are needed to address these issues.

It is not readily apparent why the WR-mediated synergy is absent in the chow-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 WR we employed, 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 chow-raised animals to either leptin or WR may mask any subtle or modest synergy between the two interventions, whereas, such synergy is readily evident in HF-raised rats lacking responses to either WR or leptin.

The fact that the leptin/WR weight gain reduction synergy only surfaced in the presence of exogenous leptin but not with the high endogenous leptin already present in HF-raised animals is also perplexing. Diet-induced obesity is associated with leptin receptor down-regulation (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 HF-raised rats was the result of a gradual increase in leptin over the 5 months of HF feeding, which provides ample time for adaptation (or brain rewiring) to occur. The
Wheel running and leptin synergy, however, could result from an interaction between WR and the sudden elevation in leptin (due to 13 days of rAAV-leptin gene therapy before the initiation of WR). It is also interesting to note that this synergy occurred immediately following WR, suggesting 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 hormone-sensitive lipase (HSL) at Ser-563 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 chow- and HF-raised animals independent of WR. Interestingly, HSL appears to be activated despite the present 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 WR combination therapy but not with individual treatment in the HF-raised rats. This evidence suggests an additional potential mechanism underlying the weight loss synergy between WR and leptin and resonates with studies in which selective decreases in 11β-HSD1 alleviates obesity-related metabolic complications (34; 35).

In conclusion, neither WR nor leptin curbs dietary weight gain in HF-raised rats, and moreover, leptin treatment actually worsens the obesity. Remarkably, these two otherwise ineffective therapies act synergistically to prevent the HF-induced dietary weight gain. This weight-reduction synergy is unique to the full leptin-resistant state and does not occur in the chow-raised rats displaying leptin sensitivity or HF-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 WR and leptin will synergistically reduce dietary weight gain in obese humans is a tantalizing prospect.

ACKNOWLEDGEMENTS

Supported by the National Institute on Aging Grant AG-26159, 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. Sergei Zolotukhin was supported by NIH NIDDK Grant RO1 DK62302.
REFERENCES

TABLE 1. Weight, wheel running, serum leptin, adiposity in chow and high-fat raised rats

<table>
<thead>
<tr>
<th></th>
<th>rAAV-Control</th>
<th>rAAV-Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Wheel Running</td>
</tr>
<tr>
<td><strong>Chow Raised Rats</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass day 0, g</td>
<td>483 ± 5</td>
<td>484 ± 4</td>
</tr>
<tr>
<td>Body Mass, day 51</td>
<td>617 ± 10</td>
<td>566 ± 13</td>
</tr>
<tr>
<td>WR, m/day</td>
<td></td>
<td>4167 ± 1091</td>
</tr>
<tr>
<td>Leptin, day 0, ng/ml</td>
<td>3.44 ± 0.53</td>
<td>3.94 ± 0.88</td>
</tr>
<tr>
<td>Leptin, day 51, ng/ml</td>
<td>8.47 ± 1.36</td>
<td>4.34 ± 1.37*</td>
</tr>
<tr>
<td>Adiposity, g</td>
<td>22.3 ± 1.4</td>
<td>13.6 ± 2.5*</td>
</tr>
<tr>
<td><strong>HF- Raised Rats</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass d 0, g</td>
<td>702 ± 13</td>
<td>701 ± 35</td>
</tr>
<tr>
<td>Body Mass d 51, g</td>
<td>743 ± 14</td>
<td>738 ± 22</td>
</tr>
<tr>
<td>WR, m/day</td>
<td></td>
<td>865 ± 265</td>
</tr>
<tr>
<td>Leptin, day 0, ng/ml</td>
<td>24.4 ± 3.0</td>
<td>27.9 ± 4.5</td>
</tr>
<tr>
<td>Leptin, day 51, ng/ml</td>
<td>26.1 ± 2.3</td>
<td>25.8 ± 4.4</td>
</tr>
<tr>
<td>Adiposity, g</td>
<td>53.6 ± 5.2</td>
<td>50.6 ± 3.0</td>
</tr>
</tbody>
</table>

Data represent the mean ± SE of 6-8 rats per group. Adiposity represents the sum of PWAT, EWAT, and RTWAT. In three of eight chow-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: Chow Raised: $P = 0.003$ for difference with leptin or WR 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: Chow raised: $P = 0.001$ for difference with leptin or WR 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 WR by two-way ANOVA. *$P < 0.05$ for difference from corresponding sedentary group by post-hoc analysis.
FIGURE LEGENDS

Fig 1. A: Body mass changes in chow-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 chow diet for 5 months and continued on the chow diet throughout the experiment. Values represent the mean ± SE of 8 sedentary/control, 7 wheel-running/control, 7 sedentary/AAV-leptin and 8 wheel-running rats per group. P < 0.05 (WR vs. respective sedentary groups; Sedentary/Control vs. Sedentary/AAV-leptin) for difference in slopes following division of groups into sedentary and WR. 
B: Daily food consumption following control vector (open squares) or rAAV-leptin (closed squares) prior to 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 14-37 during the first part of the sedentary and wheel running period. P = 0.004 (WR), P = 0.007 (rAAV-leptin), and P = 0.03 (interaction) by two-way ANOVA; *P < 0.01 for difference with WR among rAAV-GFP groups and for difference from either rAAV-leptin groups by post-hoc analysis.

Fig 2. A: Body mass changes in HF-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 6 sedentary and 8 wheel-running rats per group. P < 0.05 (Control vs. corresponding AAV-leptin; Sedentary/AAV-leptin vs WR/AAV-leptin) for difference in slopes following division of groups into sedentary and WR.
B: Daily food consumption following control vector (open squares) or rAAV-leptin (closed squares) prior to division into sedentary or wheel running groups. Food consumption is significantly less (P<0.05) between days 7-10, but cumulative food consumption during entire period is not significantly different.
C: Cumulative food consumption between days 14-37 during the first part of the sedentary and wheel running period. P = 0.015 (WR) and P = 0.008 (interaction) by two-way ANOVA; *P < 0.001 for difference with WR among rAAV-leptin groups by post-hoc analysis, and **P < 0.05 for difference between Sedentary rAAV-leptin and Sedentary rAAV-GFP. After day 37, food consumption was not different between groups.

Fig 3. Hypothalamic STAT3 phosphorylation and SOCS3 mRNA in chow-raised (A, C) and HF-raised (B, D) rats following administration of control vector or rAAV-leptin in sedentary rats (open bars) or rats given access to running wheels beginning at day 15 (solid bars). Values represent the mean ± SE of 6-8 rats per group.
PSTAT3: Chow 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. HF-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: HF-raised; P = 0.04 for interaction by two-way ANOVA. *P < 0.05 for difference from corresponding sedentary group and for difference from control WR group by post-hoc analysis.

Fig 4. Top: Phosphorylation at tyrosine 563 of hormone sensitive lipase in PWAT in chow raised (A) and high-fat raised (B) and following administration of control vector or rAAV-leptin in
sedentary rats (open bars) or rats given access to running wheels beginning at day 14 (solid bars). Bottom: Eleven-beta hydroxysteroid dehydrogenase-1 protein level in PWAT in chow raised (C) high-fat raised (D) following administration of control vector or rAAV-leptin in sedentary rats (open bars) or rats given access to running wheels beginning at day 14 (solid bars). Values represent the mean ± SE of 6-8 rats per group.

P(563)-HSL: $P = 0.0005$ (Chow raised) or $P = 0.0031$ (HF-raised) for difference with leptin by two-way ANOVA. *$P < 0.01$ for difference from control vector by post-hoc analysis.
11-Beta HSD1: HF-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.
FIGURE 1

A Pretreatment period: 5 months of chow diet
- Sedentary/Control Vector
- Sedentary/AAV-Leptin
- Wheel Running/Control Vector
- Wheel Running/AAV-Leptin

Days
Delta Body Weight (g)

0 10 20 30 40 50 -50

WR

B
Food Consumption (Kcal/day)

0 2 4 6 8 10 12

rAAV-GFP rAAV-Leptin

C
Cumulative Food (Kcal)

Days 14-37

rAAV-GFP rAAV-Leptin

*
FIGURE 2

A

Pretreatment period: 5 months of 32% fat diet

- Sedentary/Control Vector
- Sedentary/AAV-Leptin
- Wheel Running/Control Vector
- Wheel Running/AAV-Leptin

B

Food Consumption (Kcal/day)

0  2  4  6  8  10  12

Day

0  2  4  6  8  10  12

C

Cumulative Food (Kcal)

Days 14 - 37

0  1000  2000  3000

rAAV-GFP  rAAV-Leptin
FIGURE 3

A B C D

Wheel running and leptin synergy

CHOW RAISED

P-STAT3
(Arbitrary units)

SOCS3
(Arbitrary units)

High Fat Raised

rAAV-GFP rAAV-Leptin

Sedentary WR

Sedentary WR

Sedentary WR

Sedentary WR

0 25 50 75 100 125 150 175 200

0 25 50 75 100 125 150 175 200

0 25 50 75 100 125 150 175 200

0 25 50 75 100 125 150 175 200

* * **

*
FIGURE 4

A  Chow Raised

B  High Fat Raised

C  Chow Raised

D  High Fat Raised

Wheel running and leptin synergy