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Elevated leptin: consequence or cause of obesity?

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1. ABSTRACT

Leptin is an adipocyte-derived, satiety-regulating hormone that acts within the hypothalamus and other brain sites. Obese humans and animals are largely resistant to central actions of leptin. Rising leptin levels associated with progressing obesity are generally regarded as simply a consequence rather than a causative factor in the leptin resistance and obesity. Several lines of evidence suggest otherwise. Chronic overexpression of central leptin induces a leptin resistance that mimics many of the characteristics associated with diet-induced or adult-onset obesity including reduced leptin receptors, diminished signaling, and impaired responsiveness to exogenous leptin. Moreover, these animals have increased susceptibility to diet-induced obesity. New data with a leptin antagonist demonstrate that blockade of leptin receptors also exaggerates diet-induced obesity. These findings suggest an important role for elevated leptin in the development of leptin resistance and obesity, especially in today's society with an overabundance of readily available high caloric food. Once leptin resistance takes hold, each subsequent exposure to high-density food faces diminished counter-regulatory responses, leading to exacerbated weight gain.

2. INTRODUCTION

Obesity is reaching epidemic proportions across the globe, yet mechanisms underlying obesity as well as novel treatments have eluded scientists. In general, obesity results from an imbalance of food intake over energy expenditure, and both factors are important in long-term weight maintenance (1). White adipose tissue (WAT) was once thought of as merely a storage space for fat, whereas, current knowledge indicates WAT is a vital endocrine organ that is actively involved in whole body homeostasis regulation. WAT secretes a family of hormones collectively known as adipokines, including leptin, adiponectin, tumor necrosis factor (TNF)-alpha, adipsin, and resistin, many of which have neuroendocrine functions in modulating food intake and energy expenditure (2). One of the most enigmatic of the adipokines is leptin. This hormone, secreted by fat cells, circulates in the blood in proportion to whole body adipose tissue mass. Current dogma suggests that the amount of body fat is under tight control via a feedback loop linking peripheral adipose tissue and the central nervous system, particularly, the hypothalamus. Other brain regions are also involved and gaining recognition. Leptin, acting mainly through its

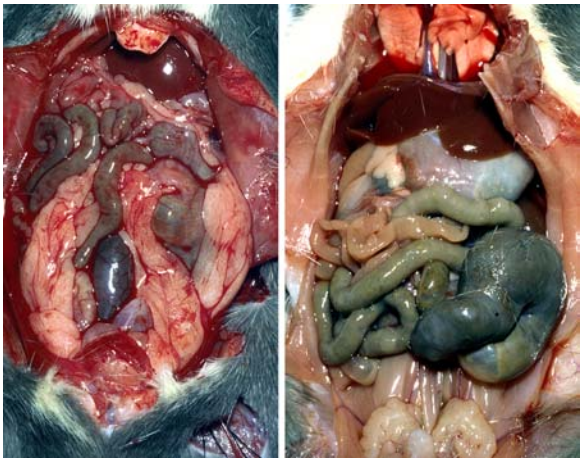

Figure 1. Leptin gene delivery in lean rats results in an almost complete disappearance in body fat by day 11 (not shown) through at least day 46 (right) compared with control vector delivery (left). Reproduced from (15), with permission.

hypothalamic receptors to inhibit feeding and increase thermogenesis, is a key mediator in this feedback regulatory loop. It was once believed to be the cure for obesity and even referred to as the "anti-obesity hormone" (3). In reality, whereas administration of leptin to young, lean rodents produces dramatic fat and weight loss (1, 3-5), obese humans and rodents are only weakly responsive or unresponsive to exogenously administered leptin (6-8). In rodent and human models of diet-induced or adult-onset obesity, leptin levels rise proportionally with adiposity, but the increased leptin fails to curtail the progression of obesity (6-10). This apparent leptin ineffectiveness is identified as leptin resistance. Despite extensive research efforts, the nature of this resistance is yet to be fully delineated. Available data indicate that leptin resistance involves defects both prior to leptin receptor activation as well as at the receptor and post-receptor levels (11-14). The former includes an inability of peripheral leptin to reach the hypothalamus, while the latter involves decreased hypothalamic leptin receptor number and impaired leptin signal transduction (11-14). Although the peripheral defect, attributed to decreased transport of circulating leptin into the CNS with diet-induced or age-related obesity, is an important contributor to leptin resistance, the topic of this review will focus on central leptin resistance. Specifically, we address the question whether elevated central leptin is causal to leptin resistance and obesity or simply a consequence of increasing obesity.

3. LEPTIN IS A POTENT ANOREXIC AGENT IN LEAN ANIMALS

Administration of leptin to lean animals reduces food consumption and body weight, resulting in a dramatic loss of adiposity. We set out to examine whether long-term treatment with leptin via gene delivery prevents or reverses obesity (15). We employed a recombinant adeno-associate virus-mediated (rAAV) gene transfer system encoding the gene for rat leptin (rAAV-leptin) or a control vector encoding green fluorescent protein (rAAV-control). As described in the introduction, the transport of leptin into the central nervous system may be a limiting factor in the response to peripheral leptin. To avoid this complication, we chose to deliver the leptin gene directly into the central nervous system by intracerebroventricular (i.c.v.) injection into lean F344xBN rats, and examine the consequences for periods of 9 and 46 days after gene delivery. Since GFP coding sequence was present in both the control and leptin vectors, flurorescence staining of GFP was used to confirm successful gene delivery in hypothalamic slices of rAAV-control and rAAV-leptin treated rats. Leptin transgene mRNA was measured in the hypothalamus by RT-PCR using a sense primer specific to a region of the transgene that is not present in native rat leptin and an antisense primer specific for leptin. Leptin mRNA was identified in all rAAV-leptin treated rats at 9 days and 46 days with no evidence of abatement (15). The expression of the leptin transgene resulted in a 60-75% elevation in cerebrospinal fluid (CSF) leptin levels. The body weight was substantially reduced in the rAAV-leptin-treated rats. These animals weighed 109 g less than the control group who had steadily gained weight (15). More dramatically, rAAV-leptin induced a near complete disappearance of body fat by day 10 that was sustained through day 46 (Figure 1). In contrast to these responses in lean rats, when the rAAV-leptin vector was delivered to young rats rendered obese by high-fat feeding (diet-induced obese, DIO), there was little effect, suggestive of leptin resistance (Figure 2) (16).

4. DIET-INDUCED LEPTIN RESISTANCE

When provided with a highly palatable diet, such as a high fat diet, rodents usually become obese. This obesity is associated with rising leptin levels but diminished leptin sensitivity. In fact, leptin resistance is the hallmark of diet-induced obesity. The temporal development of diet-induced leptin resistance seems to be dependent on species and strain of the rodent (10, 17, 18). Generally, in DIO rodents, leptin responses are only detectable with high doses of leptin (19). This apparent leptin resistance is reversible if the high-fat diet is withdrawn (6). Moreover, there are two documented defects contributing to the developed leptin resistance in DIO rodents, an impaired ability of peripheral leptin to cross the blood brain barrier and impaired leptin signal transduction in the hypothalamus (11). The inability of leptin to reach the feeding centers in the brain appears to occur more rapidly than the loss of responsiveness to centrally introduced leptin, resulting in an initial resistance to peripheral but not central administration of leptin. Continued high-fat feeding will eventually induce central leptin resistance (17, 18).

5. AGE-RELATED LEPTIN RESISTANCE

Besides diet-induced obesity, another common form of obesity is age-related or adult-onset obesity. Humans demonstrate a steady increase in body weight and adiposity through early senescence followed by a decline in

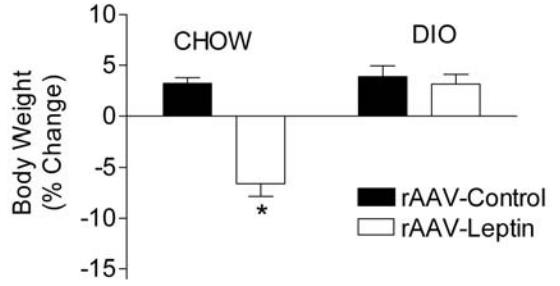

Figure 2. Change in body mass during 29 days following rAAV-leptin or control vector in chow-fed and DIO rats. Data represents mean ± SE of 5 rats per group. $P<0.0001$ for difference between rAAV-leptin and control vector in chow-fed rats. Adapted from (16).

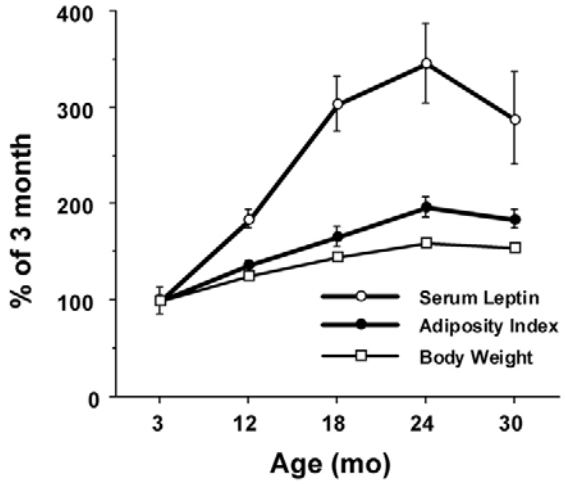

Figure 3. Serum leptin levels, adiposity index, and body weight in rats of 3, 12, 18, 24, and 30 months of age increase significantly between 3 and 24 mo followed by a decline by 30 mo. The rate of increase in serum leptin is greater than the rate of increase in adiposity (regression analysis, $P < 0.001$). Data is expressed as the percent of the 3-month value and represents the mean ± SE of 8 rats per age. Reproduced from (22) with permission.

latter life (20). In our aging rat model, the Fisher 344 cross Brown Norway (F344xBN) rat, body weight and adiposity also follow a steady increase into early senescence (from 3 to 24 months) and then a decline from 24 to 30 months (Figure 3) (9). The gradual gain in body weight with age in F344xBN rats is different from the rapid increase in body weight observed in many other strains of young rats prone to obesity. For example, Sprague Dawley rats gain 330 g between 2.5 months (370 g) and 6 months (700 g) of age (21). In contrast, an F344xBN rat gains only 60 g between 3 and 6 months, and eventually reaches a body weight of 570 g at 24 months of age (9). This weight increase profile mostly mimics that of humans, suggesting that the F344xBN rat strain represents a reasonable animal system for examining the age-related obesity in rodents and perhaps for extrapolating to the human condition (20). These adult-onset obese rats have 3-4 times higher serum leptin levels at 24 mo relative to 3 mo (Figure 3) (9). Despite this hyperleptinemia, which should cause fat loss and promote leanness, obesity persists with the adiposity level of a 24-mo-old rat amounting to ~ 400% of that of a 3-mo-old animal (Figure 3) (9).

Similar to the findings with diet-induced obesity, there appears to be both a peripheral and central component to the age-related leptin resistance (22). Peripheral administration of leptin results in greater suppression in food intake, more weight loss and higher stimulation in energy expenditure in young versus aged rats. But this age-related impairment in leptin responses is 2.5-fold more severe with peripheral than with central leptin administration, indicating age-related leptin resistance and diet-induced leptin resistance share a common feature in that both consist of a peripheral and central component (Figure 5) (22). Focusing on age-related central leptin resistance, we examined responses to *central* leptin treatment by infusion of leptin directly into the brain via an implanted cannula (23). Artificial cerebral spinal fluid or murine leptin (7.8 μg/rat) were infused for 3 days into the lateral ventricle of young and old rats. Groups consisted of ad libitum-fed control, ad libitum-fed leptin and rats pair-fed to the amount of food consumed by the leptin-treated rats. We examined daily food consumption, UCP1 expression in BAT, and hypothalamic NPY mRNA levels. Similar to peripheral administration of leptin, central leptin treatment significantly reduced food intake to a greater extent in the young compared with the aged rats (-42 ± 5% vs. 33 ± 4%). BAT UCP1 mRNA expression was also increased significantly more in young (45 ± 8%) relative to old (10 ± 6%) when compared with age-matched, pair-fed rats, indicating that BAT-mediated induction in energy expenditure by leptin was marred in aged rats. Furthermore, the central leptin treatment suppressed hypothalamic NPY mRNA expression to a greater extent in young (-23 ± 4%) than in old (-8 ± 4%) when compared with age-matched, pair-fed rats.

In addition to F344xBN rats, aged Wistar rats are also associated with both a central and peripheral components of leptin resistance (24). Interestingly, when these aged rat were subjected to three months of caloric restriction, central leptin responsiveness was partially reversed, suggesting that the central leptin resistance was not directly related to aging, but secondary to the increase in obesity (or leptin levels) with age (25). It could be that aging mainly impairs leptin transport across the blood brain barrier, whereas increased obesity or elevated leptin mainly impairs central responses to leptin.

6. HYPOTHALAMIC LEPTIN SIGNALING

To assess leptin signal transduction *in vivo,* we have examined the activation of the Janus tyrosine kinase 2 (JAK2)/cytosolic signal transducer and activator of transcription protein 3 (STAT3) pathway in response to leptin administration. Binding of leptin to the long form of the leptin receptor (Ob-Rb) initiates tyrosine phosphorylation of Ob-Rb by JAK2. Phosphorylated leptin receptor then recruits STAT3 that is activated through

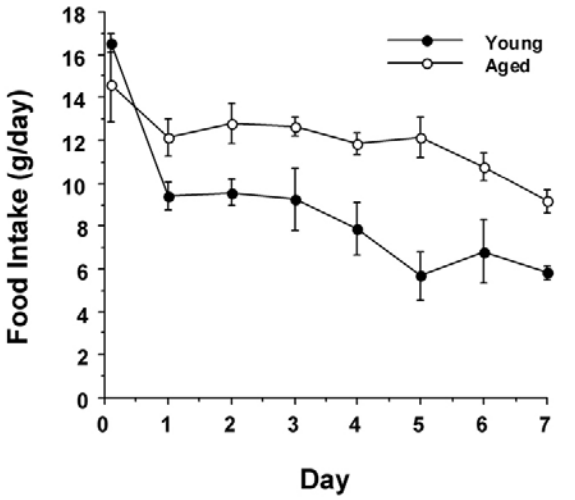

Figure 4. Daily food consumption following leptin administration in young and aged rats. Leptin (1mg/day) was given s.c. via a mini-pump at day 0 and continued for seven days. Data represent the mean ± SE of 8 rats per group. $P < 0.05$ for difference between young and old at each individual day except day 0. Reproduced from (22) with permission.

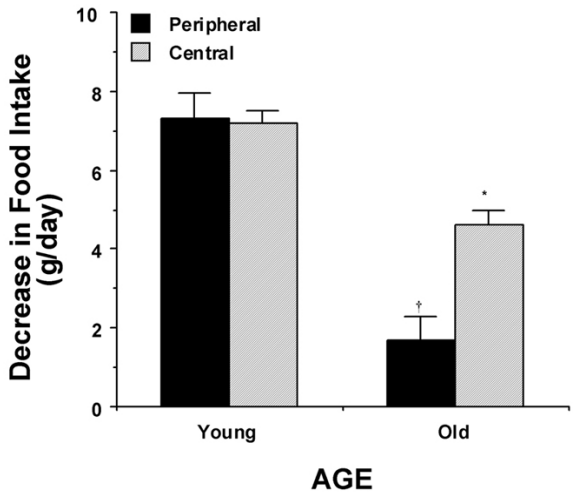

Figure 5. Comparison of the impaired leptin responsiveness following either peripheral or central leptin administration. The doses of peripheral leptin (1 mg/day) and central leptin (15.6 µg/day) evoked similar reductions in food intake in young rats, but different amounts in aged rats. Reproduced from (22) with permission.

phosphorylation by JAK2 (Fig.6) (26). The activated STAT3 proteins dimerize and translocate to the nucleus where they bind DNA and initiate gene transcription (26). Suppresser of cytokine signaling 3 (SOCS-3) and phosphotyrosine phosphotase 1B (PTP1B) are two known negative regulators of leptin signaling following Ob-Rb activation (Figure 6) (26). The JAK2/STAT3 pathway is believed to be essential for mediating leptin's effects on homeostatic energy regulation (27). Beyond STAT3 activation, the JAK2-Phosphotidylinosital 3 kinase (PI3K) pathway has received some recent attention (Fig.6) (28-31).

In one of our earlier studies, we examined both the time- and dose-dependent STAT3 phosphorylation and STAT3 transcription factor binding using hypothalamic homogenates from F344xBN rats of different ages (13, 14). Following a bolus of peripheral (iv) leptin injection, maximal levels of P-STAT3 in the hypothalamus were achieved at 1 h post injection (Figure 7). P-STAT3 levels declined over time in parallel to the decrease in serum leptin, indicating a positive correlation between hypothalamic P-STAT3 and serum leptin over the time period examined (Figure 7). Consistent with STAT3 phosphorylation, the P-STAT3 DNA binding activity was observed to be greater at 1 h than at 25 min post-leptin administration (data not shown). Central icv administration of 2µg leptin also resulted in a similar STAT3 activation time course with maximal P-STAT3 achieved at 1 h (32).

7. LEPTIN SIGNALING WITH CENTRAL LEPTIN RESISTANCE

Both adult-onset obesity and diet-induced obesity are associated with elevated peripheral and central leptin levels and reduced hypothalamic leptin signaling, and this may underlie one mechanism of central leptin resistance.

7.1. Adult-onset obesity

We examined whether leptin-mediated phosphorylation of STAT3 is altered in older rats with adult-onset obesity. The dose response increase in P-STAT3 to increasing amount of exogenous leptin was measured 1 h after icv injection in both young-lean and old-obese rats (Figure 8) (14). Basal P-STAT3 levels between young-lean (0.17 ± 0.03 arbitrary units/mg protein) and adult onset-obese (0.18 ± 0.04 arbitrary units/mg protein) rats were similar. A significant elevation in P-STAT3 at the dose of 20 ng leptin and a maximum response with doses equal to or greater than 250 ng leptin in rats of either age was observed (Figure 8). The dose of leptin required for half-maximal stimulation was similar in both the young-lean (41 ng) and aged-obese (47 ng) animals. However, the maximum phosphorylation of STAT3 was 41% greater in young-lean relative to aged-obese rats (Figure 8). In addition, a higher quantity of leptin of 25 µg did not increase maximum phosphorylation of STAT3 in the older rats (data not shown). The total STAT3 protein levels remained unchanged with increasing doses of leptin or with obesity. In this experiment, the P-STAT3 transcription factor binding was also quantified at 1 h after 1µg leptin icv injection (Table 1). Again, basal levels of P-STAT3 transcription factor binding remained similar between the young-lean and aged-obese rats. In the lean rats, leptin induced a greater than eight-fold increase in transcription factor binding relative to control rats, whereas it produced less than a four-fold increase in the aged-obese animals compared to age-matched counterparts (Table 1). In addition, we determined that hypothalamic leptin receptor protein levels were 50% greater in the young relative to age-obese rats (Table 1). Thus, aged F344xBN rats have fewer leptin receptors and reduced hypothalamic leptin signal transduction. Consistent with these findings, Fernandez-Galaz *et al.* noted the diminished leptin receptor

Table 1. Hypothalamic P-STAT3 transcription factor binding and leptin receptor protein in adult-onset of obesity

	Young (6-month)		Old (24-month)	
	Control	**Leptin (1µg)**	**Control**	**Leptin (1µg)**
P-STAT3 transcription factor binding activity (arbitrary units)	100 ± 13	856 ± 29[1]	110 ± 11	391 ± 111[1]
Leptin receptor protein (arbitrary units)	100 ± 4.4		66 ± 8.0[1]	

Data represent the mean ± SE of 6 rats per group. Levels of P-STAT3 transcription factor binding (assessed by a DNA binding gel shift assay) or leptin receptor protein (measured by Western Blot using hypothalamic homogenate) in the young control rats were set to 100 and SE adjusted proportionally. P-STAT3 transcription factor binding was assessed 1 hr following saline or 1 µg i.v. bolus of leptin. [1]$P < 0.001$ for differences with age. Reproduced from (32) with permission.

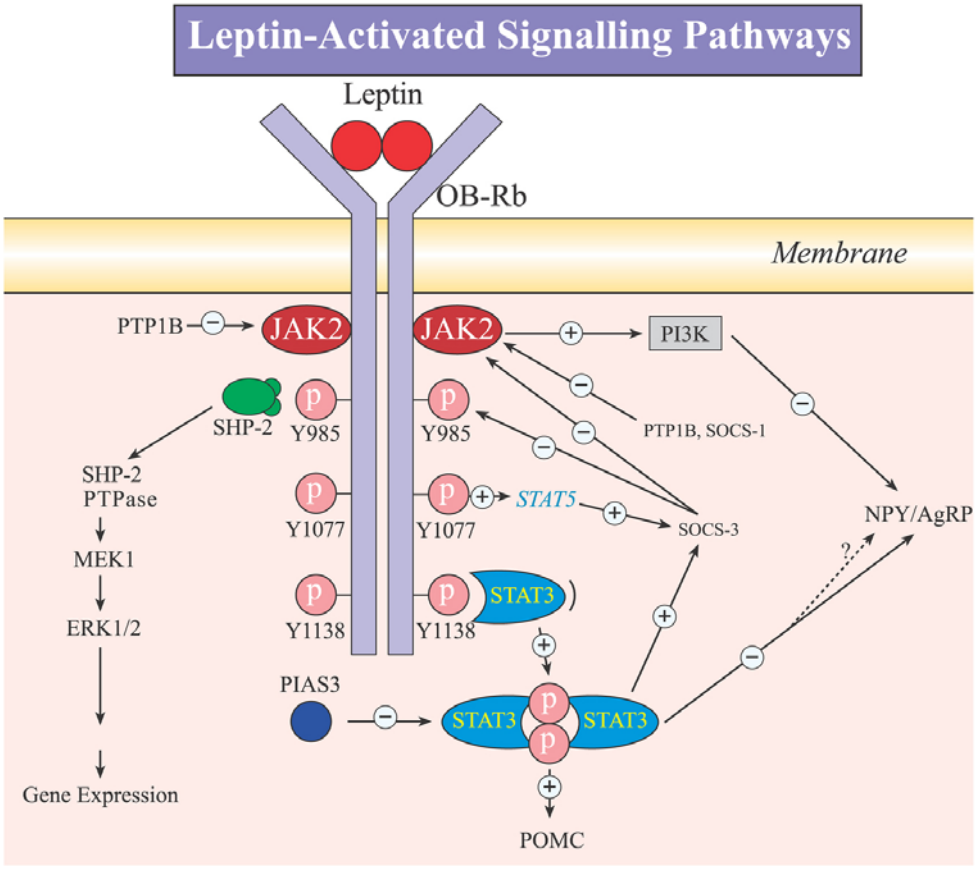

Figure 6. Cartoon of leptin signal transduction pathways in the hypothalamus. STAT3- and PI3K-mediated signaling cascades are two key signal events following Ob-Rb activation by leptin.

mRNA and protein in the hypothalamus in aged Wistar rats (25). The reduction in leptin receptors is likely a result of chronic elevation of central leptin-induced receptor desensitization. Conceivably, diminished functional receptors and the associated defect in hypothalamic leptin signaling underlie one mechanism for the impaired responses to central leptin with age.

7.2. High-fat feeding

The most thoroughly investigated model of leptin resistance is that of diet-induced obesity as a result of high-fat feeding. This model, similar to that of adult-onset obesity, is associated with impaired leptin responses and reduced leptin receptor expression and/or receptor protein levels (6, 7, 16, 33). However, these findings present a paradox in that basal leptin signaling is persistently elevated with high-fat feeding compared to chow fed controls (in general, a two to three fold increase with respect to hypothalamic P-STAT3), albeit leptin receptors are reduced in the high-fat animals (16, 33). The reduced receptors should predict diminished signaling, especially in cases where there is no receptor reserve. By and large, basal signaling is probably a poor indicator of the total leptin signaling capacity. Attempting to resolve the apparent paradox, we evaluated the impacts of high-fat feeding and subsequent caloric restriction (CR) on both leptin receptor expression and maximal leptin signaling. Young-lean F344xBN rats were high-fat fed for over 100 days (33). This treatment reduced leptin receptor expression as well as protein level, but augmented basal leptin signaling by nearly 3-fold. Both chow-fed and DIO rats were then challenged with an acute dose of leptin (2ug) by icv injection. This dose, as mentioned earlier, was

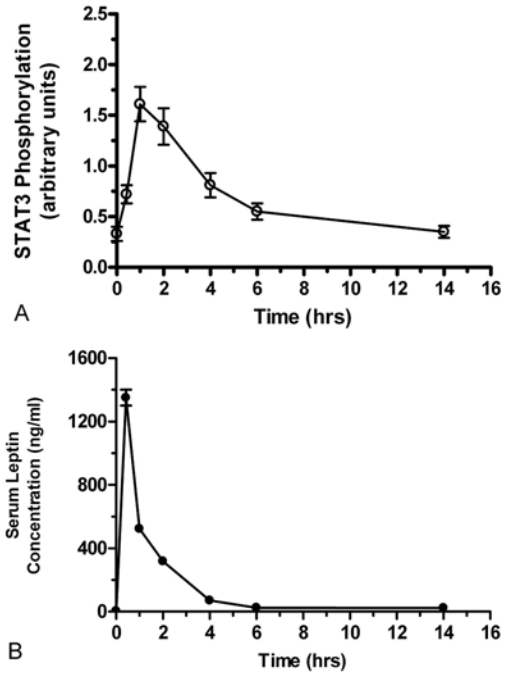

Figure 7. Time course increase and decrease in hypothalamic P-STAT3 (A) and serum leptin levels (B) following a single 1 mg, i.v. dose of leptin. Data represent the mean ± SE of 4–6 rats per time point. $P<0.0001$ for difference in P-STAT3 or serum leptin level with time. $P<0.05$ for difference between individual time points (25 min, 1, 2 and 4 h) and the control value by post hoc analysis. Time points of 6 and 14 h were not significantly different from control. Reproduced from (32) with permission.

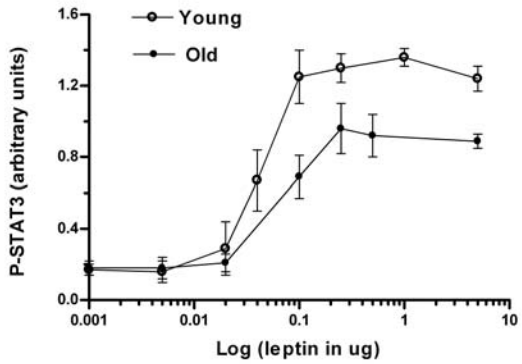

Figure 8. Dose–response increase in P-STAT3 immunoreactivity in the hypothalamus of young (open circles) and old (closed circles) rats following i.c.v. administration of 0, 5, 20, 40 (young only), 100, 250, 500 (old only), 1000 (young only), or 5000 ng leptin. Values at the corresponding zero leptin dose represent ACSF-administered controls, these levels of phosphorylated STAT3 were not different between young and old rats. Rats were killed 1 h post leptin or ACSF administration. Data represent the mean ± S.E.M. of three rats per dose (total of 24 young and 21 old rats). Activation curves were resolved by least squares analysis into a single component with K_{act} of 41 ng for the young and 47 ng for the old rats. Reproduced from (32) with permission.

proven to induce maximal leptin signaling (14). Leptin generated a greater than 6-fold increase in STAT3 phosphorylation in chow-fed rats but less than 2-fold increase in DIO animals, demonstrating an obvious decline in maximal leptin signaling in the DIO rats (Figure 9) (33). A sub-group of DIO rats underwent caloric restriction subsequently for 45 days, and were then challenged with 2ug leptin. Maximal STAT3 phosphorylation after CR was elevated in both CHOW-CR and the DIO-CR rats, reversing completely the diet-induced obesity-associated impairment in signaling capacity (Figure 9). Furthermore, the differences in maximal P-STAT3 were paralleled by changes in leptin receptor mRNA and receptor protein levels following high fat feeding and/or CR. For instance, DIO rats had a 22% reduction in leptin receptor expression in the hypothalamus compared to chow-fed animals. Caloric restriction caused a 43% increase in leptin receptor mRNA in DIO relative to non-restricted chow-fed rats and a 58% increase in DIO compared with the non-restricted DIO rats (Figure 9). Changes in leptin receptor protein mirrored the mRNA data qualitatively (data not shown). Because DIO is associated with elevated serum leptin, and CR decreases serum leptin, our results are consistent with classical pharmacological ligand-mediated up- and down-regulation of receptors. This may be one process by which leptin self-regulates its own responses or at least its receptor and receptor-mediated signal transduction. Our observations suggest that the elevated leptin associated with obesity or age is one factor contributing to the leptin resistance.

8. RELATIONSHIP BETWEEN LEPTIN SIGNALING AND LEPTIN PHYSIOLOGICAL RESPONSES

In search for direct evidence demonstrating a link between chronically elevated central leptin, leptin signaling and physiological outcomes, we employed a unique recombinant adeno-associate virus-mediated (rAAV) gene transfer system that incorporated regulation of transgene expression (34). A tet-inducible promoter was engineered into the viral vector (Tet-Ob-rAAV) which permits activation by the product of an accessory vector, rAAV-rtTA/tTS, expressing mutually exclusive reverse transactivator (rtTA) and transcriptional silencer (tTS) (34). Expression of the product of the accessory vector is under the control of the antibiotic, doxycycline, provided in drinking water. Including doxycycline in the drinking water activates, whereas removal of the antibiotic from the water, terminates leptin transgene expression. With this system, we were able to deliver leptin transgene directly into the hypothalamus and regulate its expression reversibly *in vivo* (34). When the central leptin gene expression was turned on by the presence of doxycycline, we observed increased hypothalamic STAT3 phosphorylation (Figure 10), reduced food consumption, and body weight loss. On the other hand, the removal of doxycycline halted the leptin transgene expression (Figure 10) and resulted in normalization of the food intake, body weight, and adiposity gain. Normalization of adiposity is shown in Figure 10. When we measured leptin receptor expression, first, following activation of leptin gene expression and then again in the subset of rats in which

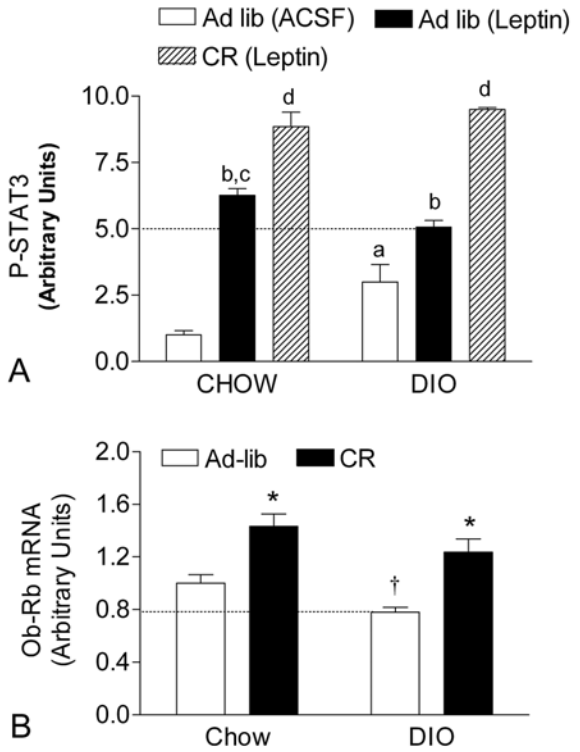

Figure 9. (A) STAT3 phosphorylation 1hr after icv leptin (2ug) or ACSF administration in ad lib-fed and CR animals. Values represent means ± S.E.M. of 5-8 rats per group. By two-way ANOVA with dietary group and leptin as factors, leptin main effect was significant ($F = 84.4$, $p < 0.0001$), as was interaction between dietary group and leptin ($F = 14.1$, $p < 0.001$), but diet main effect was insignificant ($F = 1.75$). By a second two-way ANOVA with diet and CR as factors, significance was found only for the CR main effect ($F = 60.8$, $p < 0.0001$). By host hoc analysis, (a) $p < 0.05$ for difference in basal STAT3 phosphorylation in CHOW vs DIO (open bars); (b) $p < 0.0001$ for effect of leptin in CHOW and $p < 0.01$ for effect of leptin in DIO (solid vs. open bars); (c) $p < 0.05$ for maximally stimulated STAT3 phosphorylation in CHOW-Leptin and DIO-Leptin (solid bars); (d) $p < 0.001$ and $p < 0.001$ respectively, for effect of CR on STAT3 phosphorylation capacity in CHOW and DIO (hatched vs. solid bars). (B) Ob-Rb expression in the hypothalamus. Values represent means ± S.E.M. of 5-17 rats per group. By two-way ANOVA, significance was found for both the CR ($F = 39.9$, $p < 0.0001$) and dietary group ($F = 8.81$, $p < 0.01$) main effects. By post-hoc analysis, basal Ob-Rb expression was significantly reduced in DIO-Ad lib vs CHOW-Ad lib rats, † $p < 0.05$. Effect of CR was significant in both CHOW (* $p < 0.001$) and DIO (* $p < 0.001$) rats. Reproduced from (32) with permission.

leptin expression was turned off, rats with the continuous transgene expression for 2 months tended to have reduced hypothalamic leptin receptor expression compared to control rats. In the subset of rats with leptin transgene activated for 1 month, and then silenced for 1 month, receptor expression was significantly elevated compared to those with continuous leptin transgene expression and equal to the level in control rats (Figure 10). In conclusion, the unique Tet-Ob-rAAV system achieved reversible post-transfection control of leptin transgene expression, with corresponding changes in leptin signaling and associated physiological leptin responses. This study demonstrated a coordinated relationship between hypothalamic leptin signaling and physiological consequences, an inverse relationship between leptin gene expression and leptin receptor expression.

9. LEPTIN-INDUCED LEPTIN RESISTANCE

The above studies provide evidence for the leptin self-regulation of its own receptor and maximal leptin signaling. Next, we asked the question if prolonged exposure to elevated leptin desensitizes physiological responses to leptin, in other words, if leptin induces leptin resistance. In one study, chronic infusion of leptin for 21 days desensitized male Long-Evans rats to the anorexic effect of a subsequent peripheral leptin challenge (35). Because both the leptin infusion and challenge were given peripherally, it was not clear from this study whether the leptin resistance was due to an inability of leptin to cross the blood-brain barrier or truly hypothalamic leptin resistance. To address this issue, we employed central leptin gene therapy to produce chronic elevation of central leptin in lean rats (15, 36-38). The leptin gene was delivered via rAAV transfection directly to the lateral or third ventricle of the brain. Animals initially respond to rAAV-leptin with reduced food intake and elevated oxygen consumption. But over time, both of these leptin responses wane in the leptin-treated rats despite persistent central leptin transgene over-expression in the hypothalamus. For example, in one study, food consumption differed between control and rAAV-leptin-treated rats between days 4 and 140, after which, the difference gradually diminished (Figure 11) (36). Body mass in the rAAV-leptin treated rats was significantly less than that of control rats beginning at day 9 through day 300 (Figure 6B) (36). In addition, oxygen consumption was significantly elevated at day 7 in the rAAV-leptin treated relative to control rats. However, at day 223, during the time period when the suppression in food intake in the rAAV-leptin rats was already diminished, there was no longer a difference in oxygen consumption between the rAAV-leptin and control rats (data not shown). The animals were then challenged by a 7-day leptin icv infusion at day 300. Whereas control rats displayed an expected anorectic response to leptin, the rAAV-leptin-treated rats maintained their regular food intake (Figure 6C) (36). The unresponsiveness to leptin occurred despite lower body mass and lower serum leptin in the leptin-treated animals versus in the controls, confirming a hypothalamic leptin-induced leptin resistant state. This hypothalamic leptin resistance occurs in young rats lacking obesity, indicative of the elevated central leptin as one independent factor causal to leptin resistance.

10. LEPTIN RESISTANCE EXACERBATES DIET-INDUCED OBESITY

We have concluded thus far that elevated central leptin is one factor causal to leptin resistance. Next we set

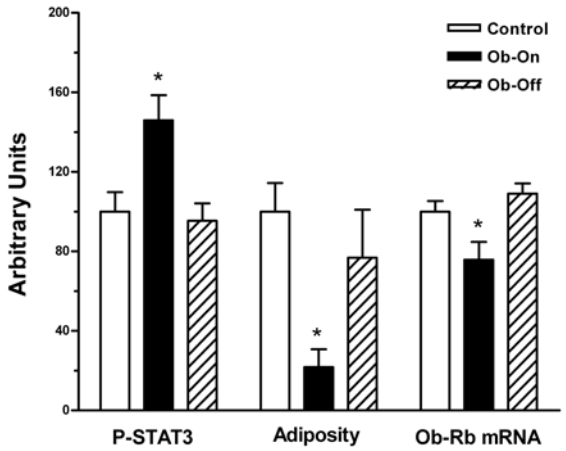

Figure 10. Hypothalamic P-STAT3 levels (measured by immunoblot), visceral adiposity (sum of retroperitoneal and perirenal adipose tissues), and hypothalamic leptin receptor (Ob-Rb) transgene expression (assessed by relative quantitative RT-PCR) in control vector and rAAV-TET-Ob encoding leptin treated animals. Ob-On represents rats in which the leptin transgene expression was active for 66 days and Ob-Off represents the period after which the transgene was inactivated for 32 day following a 34-day period of activation. Values represent the mean ± SE of 6 rats per group. *P < 0.05 for differences between Ob-On and either control or Ob-Off. Adapted from (34).

out to examine the metabolic consequences of high central leptin levels in young, lean animals. Leptin resistance was induced by hypothalamic rAAV-leptin gene therapy in a manner similar to that described in the previous section (see Figs 6A and 6B as examples) (39). In this case, the leptin-mediated reduction in food intake attenuated completely by 46 days post rAAV-leptin delivery. The nadir of body weight decrease in the leptin-treated rats occurred 30 days following leptin gene delivery, and afterwards the rat began to regain the lost weight (Data not shown). By day 94 of the gene therapy, the rAAV-leptin animals had statistically similar body weight relative to the control rats. At this point, we challenged these leptin resistant rats with a high-fat diet (day 94 is set as day zero for the initiation of high-fat feeding, refer to Figure 12, (39). The rats consumed the high-fat diet (60% fat) for 18 days, and caloric intake and body weight recorded. Over the 18-day period, the high-fat fed control rats consumed 43% more calories (Figure 12), gained 175% more weight (Figure 12) and 60% more visceral fat (data not shown) than rats pretreated with control vector and maintained on chow. Moreover, the rAAV-leptin-treated, high-fat fed rat consumed even a 36% greater amount of calories (Figure 12), grew considerably heavier (83 ± 5 vs. 44 ± 3 g; Figure 12), and accumulated 26% more visceral fat (data not shown) relative to high-fat fed controls. The underlying mechanism for the exacerbated weight gain in the leptin resistant rats appears to involve impaired central regulation of energy homeostasis. Typically, high-fat feeding is characterized by an initial increase in energy consumption. A homeostatic response is then initiated that restores caloric intake to pre-treatment or nearly pre-treatment levels (16, 40). Our lean, leptin-induced leptin resistant rats apparently lack this homeostatic response. Consequently, following high-fat feeding, the energy consumption is maintained at an elevated level above that of the control high fat fed animals, leading to greater weight and adiposity gain in these animals. Leptin resistance, therefore, in and of itself, has important functional consequences. It is our contention that leptin resistance is both a consequence and one cause of obesity. An increase in obesity promotes leptin resistance, which in turn worsens the obesity, leading to an ever-escalating cycle of increasing obesity (Figure 13) (39).

11. INSIGHTS FROM A LEPTIN ANTAGONIST

When rodents are provided with a high-fat diet, ad libitum, the immediate response is an increase in caloric intake and a corresponding increase in energy expenditure. In most circumstances, the increase in caloric intake is transitory, with caloric intake returning to control or nearly control levels within several weeks (16). One observation from the above study was the inability of the leptin resistant rats to normalize the elevated caloric intake following high-fat feeding. This suggests that this normalization of caloric intake is a leptin-mediated process. To investigate this possibility, we employed a rat leptin receptor antagonist (41). We first fully characterized the antagonist with respect to *in vivo* action. Simultaneous central administration of leptin and increasing doses of the leptin antagonist revealed a dose-dependent inhibition of leptin-induced hypothalamic STAT3 phosphorylation, and a 7-day infusion of the leptin antagonist produced the predicted increase in food intake and weight gain. When delivered with exogenous leptin in a 7-day infusion, the leptin antagonist blocked leptin-mediated anorexic effects as well as the increase in BAT UCP1 protein and STAT3 phosphorylation (41). Rats were then provided with a high-fat (HF) diet (60% kcal as fat) or chow and simultaneously infused with antagonist (25 μg/d, lateral ventricle) for 7 days and compared with vehicle infused chow-fed rats. Daily caloric intake of both HF groups peaked on day 2. HF feeding elevated caloric intake that nearly normalized by day 7, whereas in the presence of the antagonist, caloric intake remained elevated (Figure 14A). Moreover, the HF-mediated weight gain was exaggerated by the leptin antagonist infusion (Figure 14B). These results indicate that this leptin antagonist is able to block central leptin signaling and leptin-mediated energy expenditure, and demonstrate that leptin is essential for the homeostatic restoration of caloric intake following high-fat feeding.

In aggregate, our data demonstrate the importance of leptin resistance in the development of obesity, especially in today's society with an overabundance of readily available high caloric food. Once leptin resistance takes hold, each subsequent of exposure to high-density food will meet with diminished counter-regulatory responses, leading to exacerbated weight gain.

12. ENHANCING LEPTIN SIGNALING AND RESPONSES WITH VANADIUM

Leptin signaling involves several key phosphorylation steps as described in section 6. One process contributing to the negative regulation of leptin

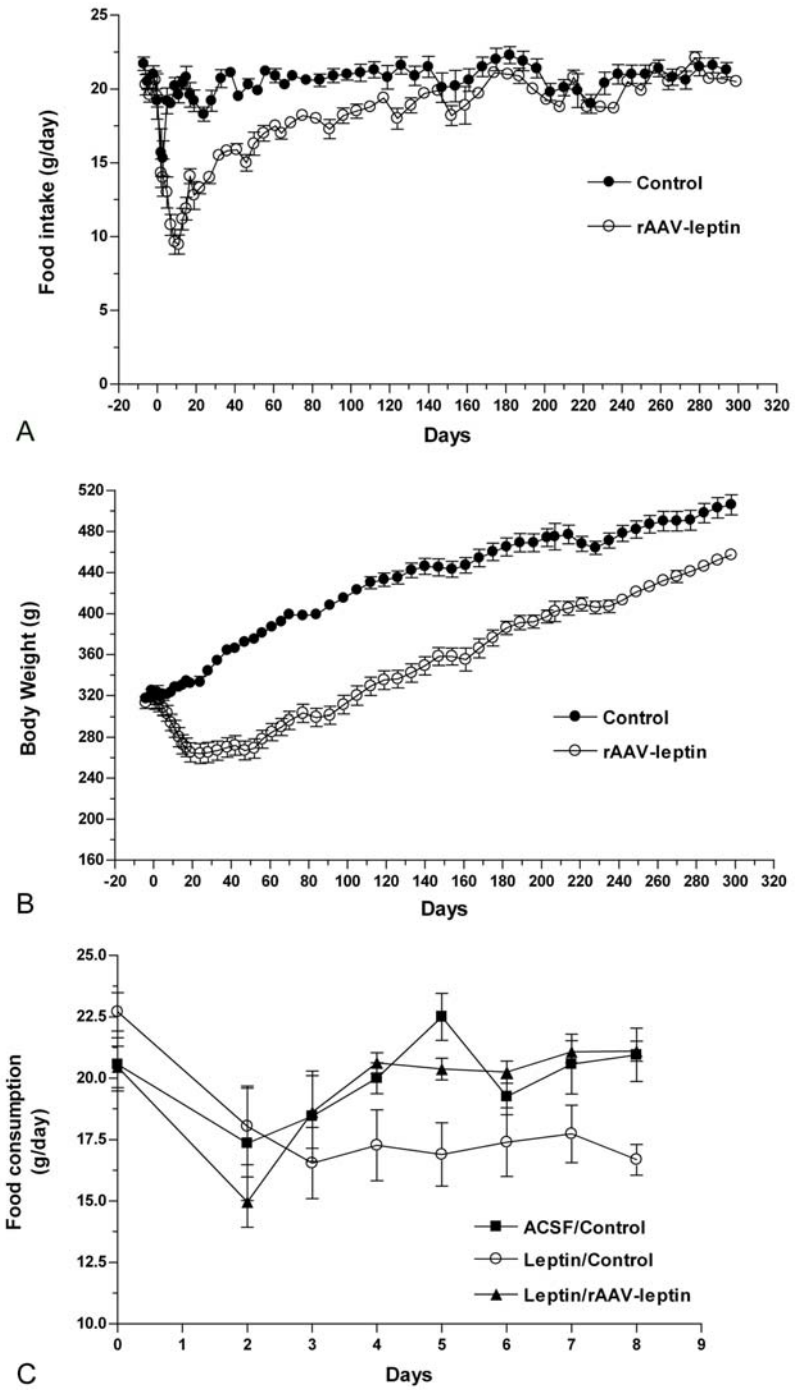

Figure 11. Daily food consumption (A) and body weight (B) in rats following administration of control vector (closed circles) or rAAV-leptin for 300 days (open circles). The rAAV-leptin or control vectors were administered at day 0. Values represent the mean of six rats in each group. Food consumption differs between control and rAAV-leptin-treated rats between days 4 and 140 (P<0.0001 by ANOVA with repeated measures). Body mass in the rAAV-leptin treated rats was significantly different from control rats beginning at day 9 through day 300 (P<0.0001 by ANOVA with repeated measures). (C) Daily food consumption following a 7-day mini-pump infusion into the lateral ventricle with either ACSF or recombinant mouse leptin in rats that were pre-treated with control vector (ACSF/Control, closed squares; Leptin/Control, open circles) or rAAV-leptin (Leptin/rAAV-leptin, closed triangles) for 300 days. Values represent the mean ± SE of 6 rats per group. *P = 0.001 for difference between Control/Leptin and rAAV-leptin/Leptin by one-way ANOVA with repeated measures from days 4 to 6. Reproduced from (32) with permission.

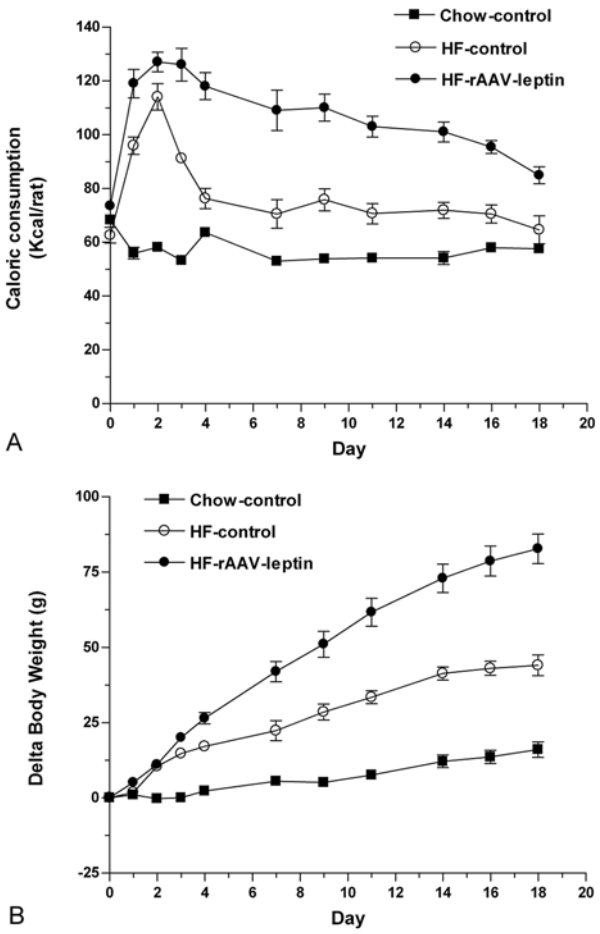

Figure 12. Daily food consumption (A) and body weight gain (B) following a high fat diet in rats pre-treated for 94 days with control vector (open circles) or rAAV-leptin (closed circles) compared with chow fed rats pre-treated with control vector (closed squares). Data in (A) are expressed as caloric consumption per day, based on 3.3 kcal/g of chow and 5.2 kcal/g high fat diet. Values represent the mean ± SE of 8 rats per group. In some cases standard error bars are less than the size of the data point. $P < 0.001$ for difference in cumulative caloric consumption between all pairs (Chow control vs. High-Fat control; Chow control vs. High-Fat rAAV-leptin; and High-Fat control vs. High-Fat rAAV-leptin by one-way ANOVA). Data in (B) are expressed as the change in body weight from initiation of the high fat diet. Values represent the mean ± SE of 8 rats per group. In some cases standard error bar are less than the size of the data point. $P < 0.001$ for difference in cumulative weight gain between all pairs (Chow control vs. High Fat control; Chow control vs. High-Fat rAAV-leptin; and High-Fat control vs. High-Fat rAAV-leptin by one-way ANOVA). Reproduced from (32) with permission.

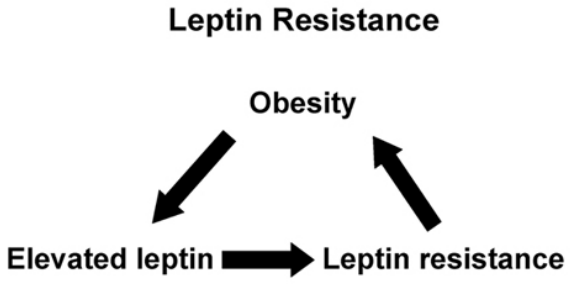

Figure 13. Interconnection between obesity, elevated leptin, and leptin resistance, leading to an ever-escalating susceptibility to further obesity.

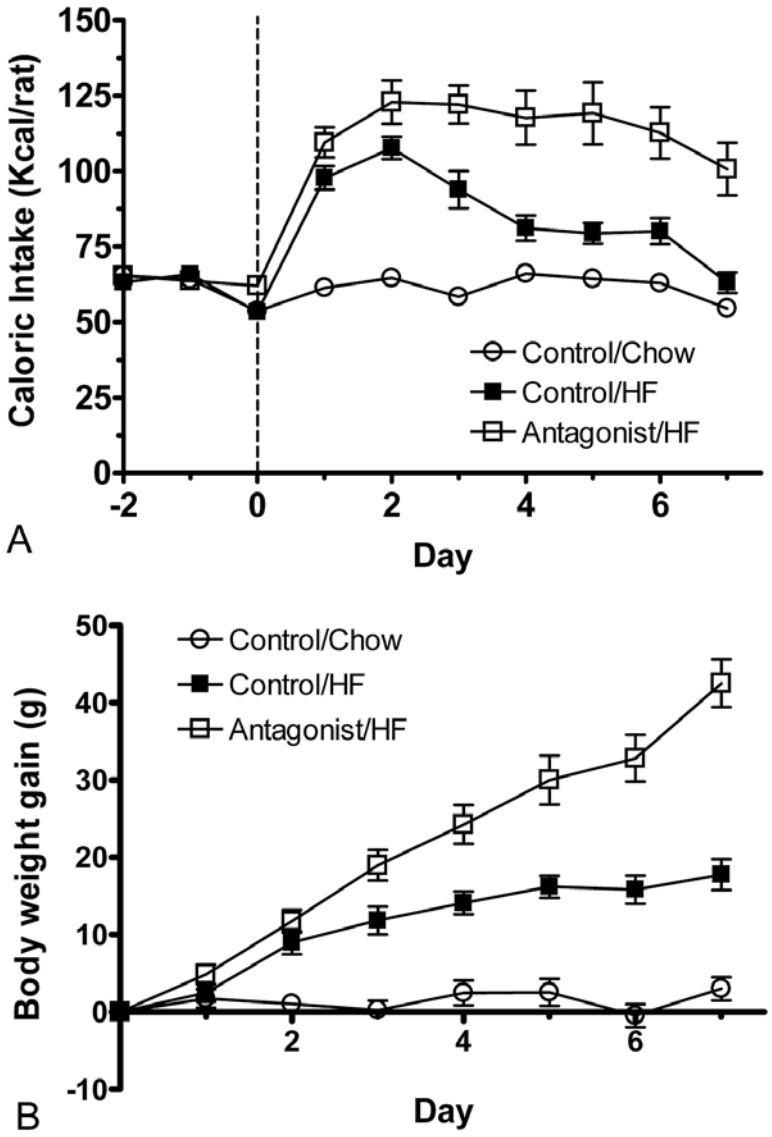

Figure 14. Daily caloric intake (A) and body weight gain (B) in HF-fed rats following a 7-day infusion of antagonist (25 μg/d) or vehicle and in chow-fed rats following vehicle infusion. The antagonist or vehicle infusion and HF feeding started at day 0. Values represent the mean ± SEM of 9 Antagonist/HF, 8 Control/HF and 8 Control/Chow rats. (A): Food intake data are expressed as caloric intake per day, based on 3.10 kcal/g of chow and 5.24 kcal/g of HF diet. Caloric intake and body weight significantly differed between Antagonist/HF and Control/HF beginning at day 1 ($P < 0.01$). Adapted from (41).

signaling is through dephosphorylation by tyrosine phosphatases. In particular, the action of phosphotyrosine phosphotase 1B (PTP1B) is believed to inhibit both leptin and insulin signaling (42-46) (also see Figure 6 for leptin signaling pathway). Compounds of vanadium are general inhibitors of tyrosine phosphatases, and the ability of vanadium to sensitize insulin action has been established in humans (47). We examined whether vanadyl acetoacetonate provided in the drinking water for 2 months would enhance leptin signaling and sensitize leptin action in rodents (48). The vanadium treatment did not significantly change body weight between control and vanadium groups by the end of the 2-month period. However, when leptin responses were gauged by central infusion of leptin (5 ug/day), the leptin infusion resulted in a considerable weight loss in controls, but, more importantly, the leptin-induced weight loss was 43% greater in the rats pretreated with vanadium (Figure 15A). At the end of the 4-week infusion period, leptin signaling was assessed in the hypothalamus. Leptin-mediated STAT3 phosphorylation was 35% greater in the rats pretreated with vanadium compared with controls whereas the vanadium treatment alone had no effect (Figure 15B). These data demonstrate that chronic vanadium administration enhances the weight-reducing effects of centrally administered leptin in lean animals. The underlying

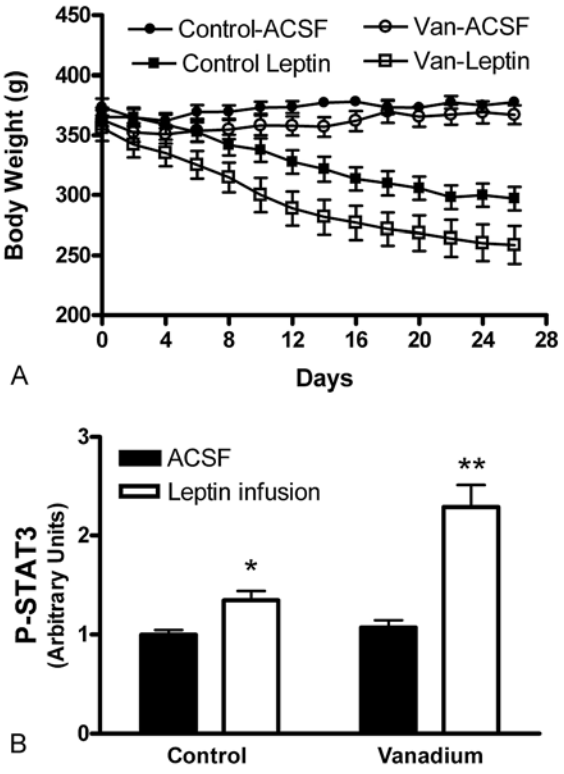

Figure 15. Body weight (A) and hypothalamic STAT3 phosphorylation (B) following a 26-day leptin (5 μg/d) or vehicle (ACSF) infusion. There was a significantly greater loss of body weight in the vanadium plus leptin group compared with leptin infusion alone ($P<0.001$). Similarly, hypothalamic STAT3 phosphorylation was greater in the vanadium plus leptin compared to leptin without treatment with vanadium ($P<0.01$). Adapted from reference (48).

mechanism appears to involve enhanced hypothalamic leptin signal transduction. Whether vanadium treatment can restore responsiveness in aged or diet-induced obese animals with leptin resistance is yet to be determined**.**

13. PERSPECTIVE

This review presents evidence for elevated leptin as a causative factor in leptin resistance and obesity. Elevated central leptin levels result in diminished hypothalamic leptin receptor expression and protein levels as well as impaired leptin signaling. Our rAAV-leptin gene therapy-mediated chronic augmentation of leptin in the hypothalamus initially produces potent anorectic and energy expenditure responses, but the responses wane overtime, and the animals become unresponsive to either the persistent leptin transgene expression or exogenously administered leptin. Additionally, this leptin resistance confers increased susceptibility to diet-induced obesity. In essence, leptin resistance is both a consequence and one cause of obesity. In spite of our progress in understanding leptin resistance and obesity, critical questions remain unanswered. In particular, how does elevated leptin lead to leptin resistance? Although both leptin receptors and leptin signaling are down regulated by chronically elevated leptin, the degree of down-regulation of either component is insufficient to account for largely absent leptin responses. Could selective leptin resistance in the CNS explain this discrepancy? Emerging evidence suggests that leptin signaling is preferentially reduced in the arcuate nucleus of the hypothalamus and not in other regions such as the ventromedial, dorsomedial and/or premammilary nucleus of the hypothalamus that also express leptin receptors (49). By what mechanism(s) is the leptin resistance prevented in these other nuclei? If we can understand the nature of leptin resistance and develop new ways to reverse that resistance, we can unleash the potent fat reducing potential of leptin, and leptin may once again referred to as the "anti-obesity hormone".

14. ACKNOWLEDGEMENTS

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15. REFERENCES

1. R. S. Ahima and J. S. Flier: Leptin. *Annu Rev Physiol* 62413-437 (2000)
2. A. R. Saltiel: You are what you secrete. *Nat Med* 7(8), 887-888 (2001)
3. J. K. Elmquist, E. Maratos-Flier, C. B. Saper and J. S. Flier: Unraveling the central nervous system pathways underlying responses to leptin. *Nat Neurosci* 1(6), 445-450 (1998)
4. M. W. Schwartz, S. C. Woods, D. Porte, Jr., R. J. Seeley and D. G. Baskin: Central nervous system control of food intake. *Nature* 404(6778), 661-671 (2000)
5. J. M. Friedman and J. L. Halaas: Leptin and the regulation of body weight in mammals. *Nature* 395(6704), 763-770 (1998)
6. P. S. Widdowson, R. Upton, R. Buckingham, J. Arch and G. Williams: Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. *Diabetes* 46(11), 1782-1785 (1997)
7. B. E. Levin and A. A. Dunn-Meynell: Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 283(4), R941-948 (2002)
8. S. B. Heymsfield, A. S. Greenberg, K. Fujioka, R. M. Dixon, R. Kushner, T. Hunt, J. A. Lubina, J. Patane, B. Self, P. Hunt and M. McCamish: Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *Jama* 282(16), 1568-1575 (1999)
9. H. Li, M. Matheny, M. Nicolson, N. Tumer and P. J. Scarpace: Leptin gene expressionincreases with age independent of increasing adiposity in rats. *Diabetes* 46(12), 2035-2039(1997)
10. J. L. Halaas, Boozer, C., Blair-West, J., Fidahusein, N., Denton, D. A., Friedman, J. M.,: Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci* 948878-8883 (1997)

11. K. El-Haschimi, D. D. Pierroz, S. M. Hileman, C. Bjorbaek and J. S. Flier: Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 105(12), 1827-1832 (2000)
12. P. J. Scarpace, M. Matheny, R. L. Moore and N. Tumer: Impaired leptin responsiveness in aged rats. *Diabetes* 49(3), 431-435 (2000)
13. P. J. Scarpace, M. Matheny and E. W. Shek: Impaired leptin signal transduction with age-related obesity. *Neuropharmacology* 39(10), 1872-1879 (2000)
14. P. J. Scarpace, M. Matheny and N. Tumer: Hypothalamic leptin resistance is associated with impaired leptin signal transduction in aged obese rats. *Neuroscience* 104(4), 1111-1117 (2001)
15. P. J. Scarpace, M. Matheny, Y. Zhang, N. Tumer, C. D. Frase, E. W. Shek, B. Hong, V. Prima and S. Zolotukhin: 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(4), 548-561 (2002)
16. J. Wilsey, S. Zolotukhin, V. Prima and P. J. Scarpace: Central leptin gene therapy fails to overcome leptin resistance associated with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 285(5), R1011-1020 (2003)
17. S. Lin, T. C. Thomas, L. H. Storlien and X. F. Huang: Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord* 24(5), 639-646 (2000)
18. M. Van Heek, D. S. Compton, C. F. France, R. P. Tedesco, A. B. Fawzi, M. P. Graziano, E. J. Sybertz, C. D. Strader and H. R. Davis, Jr.: Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest* 99(3), 385-390 (1997)
19. M. A. Pelleymounter, M. J. Cullen, D. Healy, R. Hecht, D. Winters and M. McCaleb: Efficacy of exogenous recombinant murine leptin in lean and obese 10- to 12-mo-old female CD-1 mice. *Am J Physiol* 275(4 Pt 2), R950-959 (1998)
20. R. Schwartz. *Obesity in the elderly*. Handbook of obesity, ed. B.C. Bray GA, James WPT. 1998, New York: Marcel Dekker. 103-114.
21. M. M. Klinger, G. D. MacCarter and C. N. Boozer: Body weight and composition in the Sprague Dawley rat: comparison of three outbred sources. *Lab Anim Sci* 46(1), 67-70 (1996)
22. Y. Zhang and P. J. Scarpace: Circumventing central leptin resistance: lessons from central leptin and POMC gene delivery. *Peptides* 27(2), 350-364 (2006)
23. E. W. Shek and P. J. Scarpace: Resistance to the anorexic and thermogenic effects of centrally administrated leptin in obese aged rats. *Regul Pept* 92(1-3), 65-71 (2000)
24. C. Fernandez-Galaz, T. Fernandez-Agullo, F. Campoy, C. Arribas, N. Gallardo, A. Andres, M. Ros and J. M. Carrascosa: Decreased leptin uptake in hypothalamic nuclei with ageing in Wistar rats. *J Endocrinol* 171(1), 23-32 (2001)
25. C. Fernandez-Galaz, T. Fernandez-Agullo, C. Perez, S. Peralta, C. Arribas, A. Andres, J. M. Carrascosa and M. Ros: Long-term food restriction prevents ageing-associated central leptin resistance in wistar rats. *Diabetologia* 45(7), 997-1003 (2002)
26. J. E. Darnell, Jr.: STATs and gene regulation. *Science* 277(5332), 1630-1635 (1997)
27. S. H. Bates, W. H. Stearns, T. A. Dundon, M. Schubert, A. W. Tso, Y. Wang, A. S. Banks, H. J. Lavery, A. K. Haq, E. Maratos-Flier, B. G. Neel, M. W. Schwartz and M. G. Myers, Jr.: STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 421(6925), 856-859 (2003)
28. A. Sahu and A. S. Metlakunta: Hypothalamic phosphatidylinositol 3-kinase-phosphodiesterase 3B-cyclic AMP pathway of leptin signalling is impaired following chronic central leptin infusion. *J Neuroendocrinol* 17(11), 720-726 (2005)
29. G. J. Morton, R. W. Gelling, K. D. Niswender, C. D. Morrison, C. J. Rhodes and M. W. Schwartz: Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. *Cell Metab* 2(6), 411-420 (2005)
30. C. D. Morrison, G. J. Morton, K. D. Niswender, R. W. Gelling and M. W. Schwartz: Leptin inhibits hypothalamic Npy and Agrp gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. *Am J Physiol Endocrinol Metab* 289(6), E1051-1057 (2005)
31. A. W. Xu, C. B. Kaelin, K. Takeda, S. Akira, M. W. Schwartz and G. S. Barsh: PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 115(4), 951-958 (2005)
32. Y. Zhang and P. J. Scarpace: The role of leptin in leptin resistance and obesity. *Physiol Behav* 88(3), 249-256 (2006)
33. J. Wilsey and P. J. Scarpace: 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(2), 297-306 (2004)
34. J. Wilsey, S. Zolotukhin, V. Prima, E. W. Shek, M. K. Matheny and P. J. Scarpace: Hypothalamic delivery of doxycycline-inducible leptin gene allows for reversible transgene expression and physiological responses. *Gene Ther* 9(22), 1492-1499 (2002)
35. R. L. Martin, E. Perez, Y. J. He, R. Dawson, Jr. and W. J. Millard: Leptin resistance is associated with hypothalamic leptin receptor mRNA and protein downregulation. *Metabolism* 49(11), 1479-1484 (2000)
36. P. J. Scarpace, M. Matheny, S. Zolotukhin, N. Tumer and Y. Zhang: Leptin-induced leptin resistant rats exhibit enhanced responses to the melanocortin agonist MT II. *Neuropharmacology* 45(2), 211-219 (2003)
37. P. J. Scarpace, M. Matheny, Y. Zhang, E. W. Shek, V. Prima, S. Zolotukhin and N. Tumer: Leptin-induced leptin resistance reveals separate roles for the anorexic and thermogenic responses in weight maintenance. *Endocrinology* 143(8), 3026-3035 (2002)
38. Y. Zhang, M. Matheny, N. Tumer and P. J. Scarpace: Aged-obese rats exhibit robust responses to a melanocortin agonist and antagonist despite leptin resistance. *Neurobiol Aging* 25(10), 1349-1360 (2004)
39. P. J. Scarpace, M. Matheny, N. Tumer, K. Y. Cheng and Y. Zhang: Leptin resistance exacerbates diet-induced

obesity and is associated with diminished maximal leptin signalling capacity in rats. *Diabetologia* 48(6), 1075-1083 (2005)
40. G. Li, Y. Zhang, J. T. Wilsey and P. J. Scarpace: Unabated anorexic and enhanced thermogenic responses to melanotan II in diet-induced obese rats despite reduced melanocortin 3 and 4 receptor expression. *J Endocrinol* 182(1), 123-132 (2004)
41. J. Zhang, M. K. Matheny, N. Tumer, M. K. Mitchell and P. J. Scarpace: Leptin antagonist reveals that the normalization of caloric intake and the thermic effect of food following high-fat feeding are leptin dependent. *Am J Physiol Regul Integr Comp Physiol* 292, 868-874 (2007)
42. F. G. Haj, J. M. Zabolotny, Y. B. Kim, B. B. Kahn and B. G. Neel: Liver-specific protein-tyrosine phosphatase 1B (PTP1B) re-expression alters glucose homeostasis of PTP1B-/-mice. *J Biol Chem* 280(15), 15038-15046 (2005)
43. S. Dadke and J. Chernoff: Protein-tyrosine phosphatase 1B as a potential drug target for obesity. *Curr Drug Targets Immune Endocr Metabol Disord* 3(4), 299-304 (2003)
44. I. K. Lund, J. A. Hansen, H. S. Andersen, N. P. Moller and N. Billestrup: Mechanism of protein tyrosine phosphatase 1B-mediated inhibition of leptin signalling. *J Mol Endocrinol* 34(2), 339-351 (2005)
45. D. Ren, M. Li, C. Duan and L. Rui: Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metab* 2(2), 95-104 (2005)
46. C. D. Morrison, C. L. White, Z. Wang, S. Y. Lee, D. S. Lawrence, W. T. Cefalu, Z. Y. Zhang and T. W. Gettys: Increased hypothalamic PTP1B contributes to leptin resistance with age. *Endocrinology* (2006)
47. G. Boden, X. Chen, J. Ruiz, G. D. van Rossum and S. Turco: Effects of vanadyl sulfate on carbohydrate and lipid metabolism in patients with non-insulin-dependent diabetes mellitus. *Metabolism* 45(9), 1130-1135 (1996)
48. J. Wilsey, M. K. Matheny and P. J. Scarpace: Oral vanadium enhances the catabolic effects of central leptin in young adult rats. *Endocrinology* 147(1), 493-501 (2006)
49. H. Munzberg, J. S. Flier and C. Bjorbaek: Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145(11), 4880-4889 (2004)

Key Words: Leptin Resistance, Age, STAT3, Leptin Antagonist

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