The role of leptin in leptin resistance and obesity

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Abstract

Although the presence of hyperleptinemia with leptin resistance and obesity has long been recognized, a causal role of elevated leptin in these biological states remains unclear. This article summarizes some recent work from our laboratory supporting the concept that leptin, in and of itself, promotes leptin resistance and such resistance compounds the metabolic impact of diet-induced obesity. Results from multiple studies demonstrate that (1) chronically elevated central leptin decreases hypothalamic leptin receptor expression and protein levels and impairs leptin signaling; (2) leptin resistance and obesity are associated with reduced leptin receptors and diminished maximal leptin signaling capacity; and (3) leptin resistance confers increased susceptibility to diet-induced obesity. In essence, the augmented leptin accompanying obesity contributes to leptin resistance, and this leptin resistance promotes further obesity, leading to a vicious cycle of escalating metabolic devastation.
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1. Introduction

The *ob* gene encoding mouse leptin was cloned in 1994 [1]. Subsequently, leptin was identified as an adipocyte-derived hormone that circulates in the blood in proportion to whole body adipose tissue mass. Leptin is a key afferent signal linking adiposity level and nutritional status to neuroendocrine regulation of energy homeostasis mainly through reduction in caloric intake and enhancement in energy expenditure. Administration of leptin to young, lean rodents produces dramatic fat and weight loss [2–5]. Additionally, leptin replacement in obese ob/ob mice corrects metabolic defects and ameliorates obesity [6,7]. However, in rodent and human models of diet-induced or adult-onset obesity, even though leptin levels rise proportionally with adiposity, the increased leptin fails to curtail the progression of obesity [8–12]. Moreover, obese humans and rodents are weakly responsive or unresponsive to exogenously administered leptin [8,10,11]. 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 [13–16]. These include an inability of peripheral leptin to reach the hypothalamus, decreased hypothalamic leptin receptor number and impaired leptin signal transduction [13–16]. The former is attributed to decreased transport of circulating leptin into the CNS associated with diet-induced or age-related obesity [15,16]. Indeed, responsiveness to leptin is more severely blunted in our aged-obese F344xBN rats following peripheral compared with central leptin administration [15], consistent with hampered leptin transport into the brain with adult-onset obesity. Several factors have been identified to influence leptin transport, for example, alpha 1-adrenergics enhances the leptin transporter activity [17], whereas lipopolysaccharide [18], triglycerides [19], obesity [20], and prolonged fasting or starvation [21] largely decrease the rate of transport. Although the peripheral defect is an important contributor to leptin resistance, the topic of this review will focus specifically on central leptin resistance and address one particular question: Is elevated central leptin causal to leptin resistance and obesity or simply a consequence of increasing obesity? We suggest that chronically augmented leptin in the CNS is a negative regulator of central leptin receptor signaling and an important contributor to leptin resistance. We further hypothesize that a leptin resistant state disrupts normal energy homeostasis, favors positive energy storage, and thus leads to even greater obesity. Here, we present some experimental results from our laboratory in support of these arguments.

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2. Hypothalamic leptin signaling

Leptin receptor mediated hypothalamic signaling involves multiple pathways. Of these, the Janus tyrosine kinase 2 (JAK2)/cytosolic signal transducer and activator of transcription protein 3 (STAT3) pathway is the best characterized thus far, although the JAK2-Phosphotidylinosital 3 kinase (PI3K) pathway has received some recent attention (Fig. 1). With respect to the JAK2/STAT3 pathway, leptin binding 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 phosphorylation by JAK2 ([22] and Fig. 1). The activated STAT3 proteins dimerize and translocate to the nucleus where they bind DNA and initiate gene transcription [22]. Suppresser of cytokine signaling 3 (SOCS-3) and phosphotyrosine phosphotase 1B (PTP1B) are two known negative regulators of leptin signaling following Ob-Rb activation (Fig. 1). The JAK2/STAT3 pathway is believed to be essential for mediating leptin's effects on homeostatic energy regulation [23].

3. Leptin signaling in age-related leptin resistance

To assess leptin signal transduction *in vivo*, 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 (Fig. 2A). 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 (Fig. 2B). 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
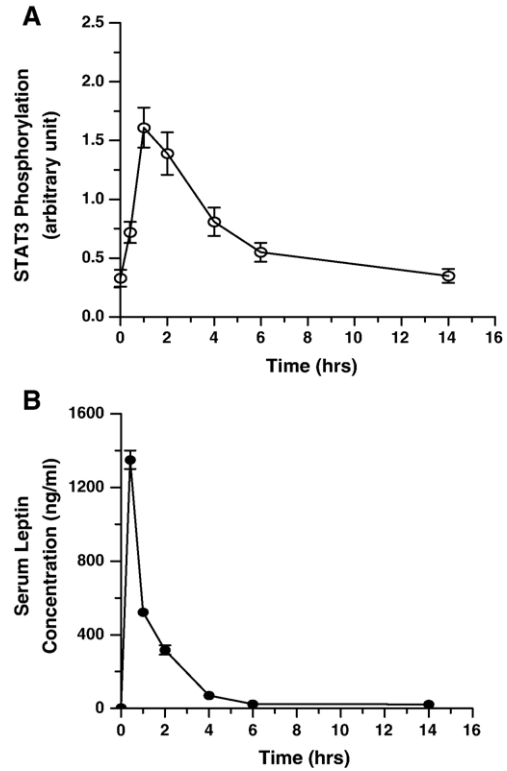
Fig. 2. Time course increase and decrease in hypothalamic P-STAT3 (A) and serum leptin levels (B) following a single 1 mg dose of leptin. Data represent the mean ± S.E. of 4–6 rats per time point. $p < 0.0001$ for difference in P-STAT3 or serum leptin level with time. $p < 0.05$ for difference between 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. Adapted from Ref. [14].

shown). Central i.c.v. administration of 2 µg leptin also resulted in a similar STAT3 activation time course with maximal P-STAT3 achieved at 1 h (unpublished data).
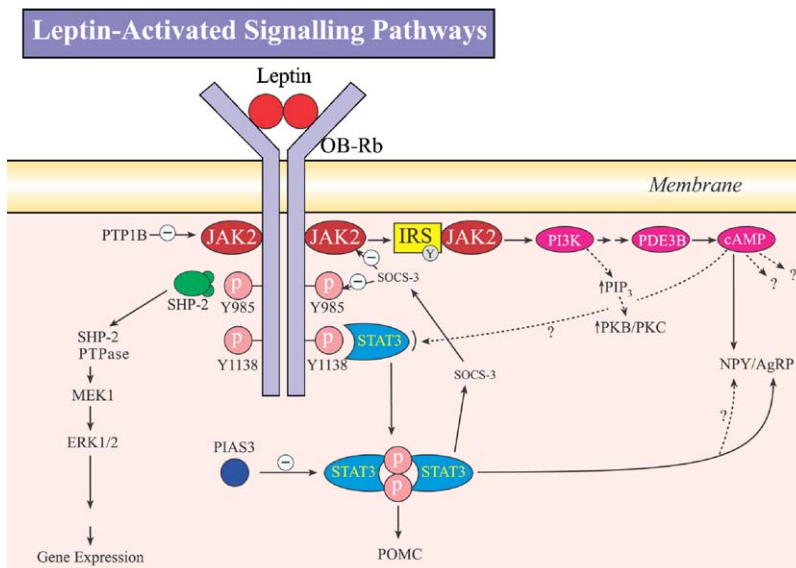
Fig. 1. Cartoon of leptin signal transduction pathways in the hypothalamus. STAT3- and PI3K-mediated signaling cascades are two key signal events following Ob-Rb activation by leptin.

To evaluate leptin signaling in the leptin resistant state, we employed a rat model of adult-onset obesity, F344xBN rats. Body weight and adiposity increase steadily with age in these rats, somewhat mimicking what occurs in humans [9]. These adult-onset obese rats have 3–4 times higher serum leptin levels at 24 months relative to 3 months [9]. Despite this hyperleptinemia, which should cause fat loss and promote leanness, obesity persists with the adiposity level of a 24-month-old rat amounting to ~400% of that of a 3-month-old animal [9]. When given either peripheral or central leptin infusion, the aged-obese rats have markedly blunted food and energy expenditure responses compared with the young-lean rats, and hence are characteristically leptin resistant [15]. To investigate mechanism(s) underlying this age-related leptin resistance, we determined whether leptin-induced phosphorylation of STAT3 is altered in older rats with adult-onset obesity. A dose response of P-STAT3 to increasing amount of exogenous leptin was measured 1 h after i.c.v. leptin injection in both the young and old F344xBN rats [13] (Fig. 3). The dose response displayed similar 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. 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 (Fig. 3). The dose of leptin required for half-maximal stimulation was also 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 (Fig. 3). 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 (data not shown). In this experiment, the P-STAT3 transcription factor binding was

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 (1 µg)	Control	Leptin (1 µg)
P-STAT3 transcription factor binding activity (arbitrary units)	100 ± 13	$856 \pm 29^*$	110 ± 11	$391 \pm 111^*$
Leptin receptor protein (arbitrary units)	100 ± 4.4		$66 \pm 8.0^*$	

Data adapted from [13]. Data represent the mean±S.E. 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 S.E. adjusted proportionally.
P-STAT3 transcription factor binding was assessed 1 h following saline or 1 µg i.v. bolus of leptin.
 * $p < 0.001$ for differences with age [13].

also quantified at 1 h after 1 µg leptin i.c.v. 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). These assessments indicate that STAT3 phosphorylation and P-STAT3 transcription factor binding are reasonable measurements for determining basal and stimulated hypothalamic leptin signal transduction and maximal signaling capacity. The data also reveals diminished leptin signaling in the hypothalamus in rats with adult-onset obesity.

4. Regulation of leptin receptor expression and maximal signaling by high-fat feeding and caloric restriction

In the literature, the most thoroughly investigated model of leptin resistance is diet-induced obesity as a result of high-fat feeding. This model is associated with impaired leptin responses and reduced leptin receptor expression and/or receptor protein levels [8,10,24,25]. However, these findings present a paradox in that basal leptin signaling is persistently elevated with high-fat feeding compared to chow fed controls (a two to three fold difference in general with respect to hypothalamic P-STAT3) albeit leptin receptors are reduced in the high-fat animals [24,25]. 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 [25]. 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 (2 ug) by i.c.v. injection. This dose, as mentioned earlier,
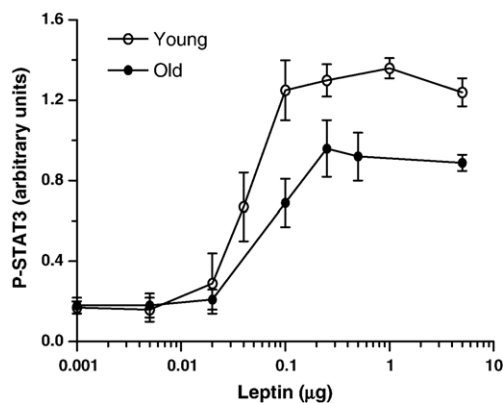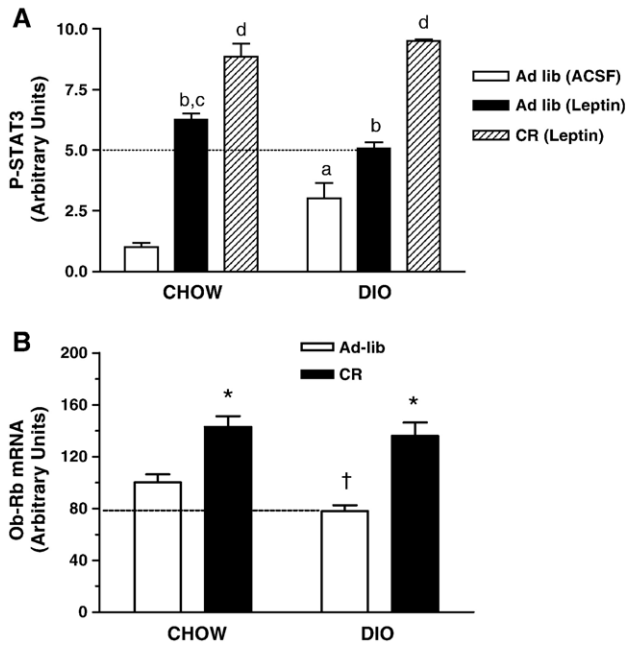

Fig. 3. Dose–response increase in P-STAT3 immunoreactivity in the hypothalamus of young (open circles) and old (closed circles) rats following i.c.v. administration of 0, 5, 20, 40 (young only), 100, 250, 500 (old only), 1000 (young only), or 5000 ng leptin. Values at the corresponding zero leptin dose 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. Adapted from Ref. [13].

Fig. 4. (A) STAT3 phosphorylation 1 hr after i.c.v. leptin (2 ug) or ACSF administration in ad lib-fed and CR animals. Values represent means±S.E.M. of CHOW-Ad Lib, ACSF ($n=5$), CHOW-Ad Lib, leptin ($n=6$), CHOW-CR, leptin ($n=8$), DIO-Ad Lib, ACSF ($n=6$), DIO-Ad Lib, leptin ($n=6$), DIO-CR, leptin ($n=5$). 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-Lep and DIO-Lep (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 CHOW-Ad lib ($n=8$), DIO-Ad lib ($n=17$), CHOW-CR ($n=8$), and DIO-CR ($n=5$). 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. Adapted from Ref. [25].

was proven to induce maximal leptin signaling [13]. 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 (Fig. 4A). A sub-group of DIO rats underwent caloric restriction subsequently for 45 days, and were then challenged with 2 ug 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 (Fig. 4A). 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 (Fig. 4B). Changes in leptin receptor protein mirrored the mRNA data (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 a receptor. This may be one process by which leptin self-regulates its own responses or at least its receptor and receptor-mediated signal transduction. Our earlier finding that the aged-obese and hyperleptinemic F344xBN rats have reduced leptin receptor protein levels (34% less) is also in support of this notion [13].

5. Correlation 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 reversible transgene expression [26]. 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) [26]. Expression of the product of the accessory vector is under the control of the antibiotic, doxycycline, provided in drinking water. Including doxycycline in water activates whereas removal of the antibiotic from 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 [26]. When the central leptin gene expression was turned on by the presence of doxycycline, we observed increased hypothalamic STAT3 phosphorylation (Fig. 5), reduced food consumption and body weight loss. On the other hand, the removal of doxycycline halted the leptin
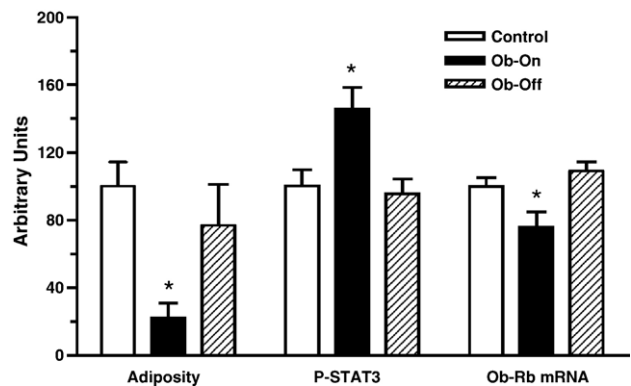

Fig. 5. Visceral adiposity (sum of retroperitoneal and perirenal adipose tissues), hypothalamic P-STAT3 levels (measured by immunoblot) and hypothalamic leptin receptor (Ob-Rb) transgene expression (assessed by relative quantitative RT-PCR) in control vector and rAAV–TET–Ob encoding leptin treated animals. 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±S.E. of 6 rats per group. *$p<0.05$ for differences between Ob-On and either control or Ob-Off [26].

transgene expression (Fig. 5) and resulted in normalization of the food intake and body weight and adiposity gain (Fig. 5). When we measured leptin receptor expression first following activation of leptin gene expression and then again in the subset of rats in which 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 (Fig. 5). In conclusion, the unique Tet–Ob–rAAV system achieved reversible post-transfection control of leptin transgene expression, and in consequence leptin signaling and associated physiological leptin responses.

6. Leptin-induced leptin resistance

The above studies provide evidence for leptin self-regulation of its receptor and maximal signaling. Next, we asked the question if elevated leptin desensitizes physiological responses to leptin, in other words, if leptin induces leptin resistance. Several of our recent studies employing central leptin gene therapy to produce chronic elevation of csf leptin indicated a role for leptin in promoting its own resistance [27–30]. 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 [27–30]. This apparent leptin resistance occurs faster in aged rats that have already impaired leptin sensitivity [28]. In order to elucidate a tentative relationship between high central leptin and leptin resistance, we established a young-lean rat model that avoids the complications of obesity [30]. Particularly, 5-month-old F344xBN male rats were treated with rAAV-leptin via third ventricle gene delivery for over 300 days [30]. Food consumption differed between control and rAAV-leptin-treated rats between days 4 and 140, after which, the difference gradually diminished (Fig. 6A). Body mass in the rAAV-leptin treated rats was significantly less than that of control rats beginning at day 9 through day 300 (Fig. 6B). 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 i.c.v. infusion at day 300. Whereas control rats displayed an expected anorectic response to leptin, the rAAV-leptin-treated rats maintained their regular food intake (Fig. 6C). 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
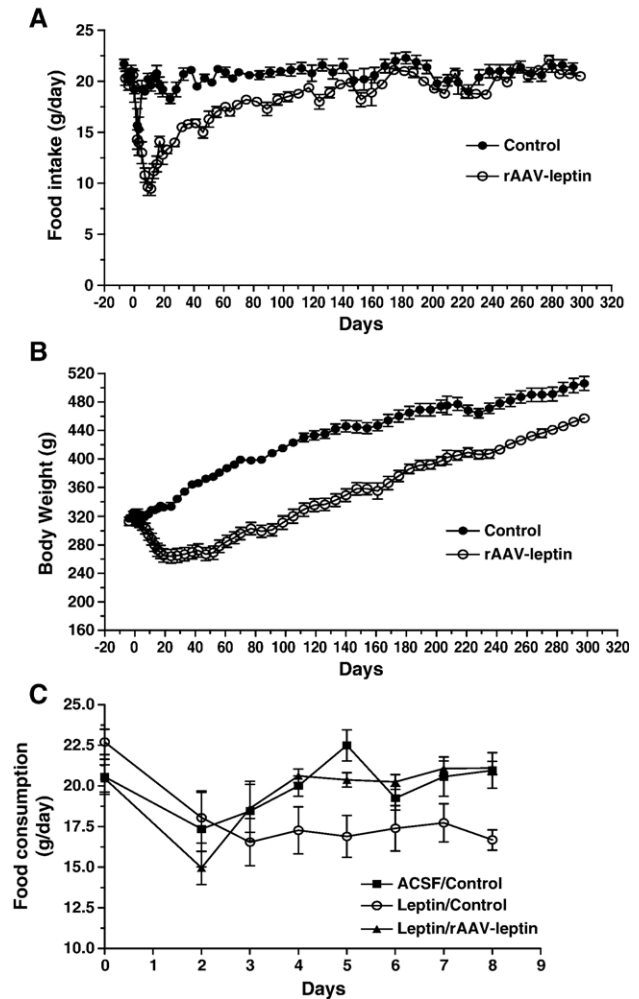

Fig. 6. Daily food consumption (A) and body weight (B) in rats following administration of control vector (closed circles) or rAAV-leptin for 300 days (open circles). The rAAV-leptin or control vectors were administered at day 0. Values represent the mean of six rats in each group. Food consumption differs 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 ± S.E. 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. Adapted from Ref. [30].

elevated central leptin as one independent factor causal to leptin resistance.

7. Leptin resistance compounds diet-induced obesity

We have concluded thus far that elevated central leptin is causal to leptin resistance. Next we set out to examine if such resistance has functional consequences. Leptin resistance was induced by hypothalamic rAAV-leptin gene therapy in a manner similar to that described in the previous section (see Fig. 6A and B as examples). 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 (day 94 is set as day 0 for the initiation of high-fat feeding, refer to Fig. 7A,B), we challenged these leptin resistant rats with a high-fat diet [31]. 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 (Fig. 7A), gained 175% more weight (Fig. 7B) and 60% more visceral fat (data not shown) than rats pretreated with control vector and maintained on chow.
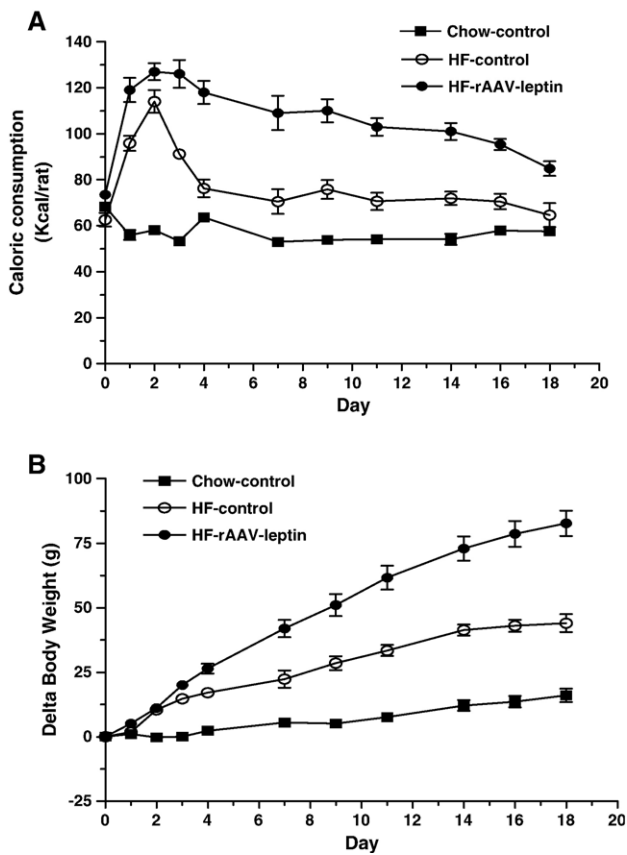

Fig. 7. Daily food consumption (A) and body weight gain (B) following a high fat diet in rats pre-treated for 94 days with control vector (open circles) or rAAV-leptin (closed circles) compared with chow fed rats pre-treated with control vector (closed squares). Day 94 is set as day 0 in (A, B) following which the high-fat feeding was initiated and continued for 18 days. 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±S.E. 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±S.E. 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). Adapted from Ref. [31].

Moreover, the rAAV-leptin-treated, high-fat fed rat consumed even a 36% greater amount of calories (Fig. 7A), grew considerably heavier (83 ± 5 vs. 44 ± 3 g in weight gain, Fig. 7B), 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 involves 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 [24,32]. 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, promoting an ever-escalating cycle of increasing obesity.

8. Overcoming leptin resistance by central melanocortin activation

The central melanocortin system is known to lie directly downstream of leptin receptor activation [33–36]. Leptin increases the hypothalamic expression of the POMC (and CART) whilst it decreases the expression of AGRP (and NPY) [5,37,38]. The precursor neuropeptide POMC gives rise to a number of bioactive products including the principle central melanocortin, alpha-melanocyte stimulating hormone (α-MSH) [39]. Alpha-MSH acts centrally via the melanocortin 3 and 4 receptors (MC3R, MC4R) in the brain to reduce food intake and increase energy expenditure [37–39]. These functions underscore the importance of α-MSH in energy balance regulation.

Collectively, our data indicate that rising central leptin levels lead to reduced hypothalamic leptin receptor gene expression, impaired signaling, and tachyphylaxis of leptin responses. The decreases in leptin receptors or signaling are conceivably causal to the impaired leptin responses associated with leptin resistance. Our findings are also consistent with the idea that leptin resistance lies within the first order neurons containing leptin receptors. Moreover, we have demonstrated defective leptin regulation of POMC levels in the age-related, leptin-induced, and diet-induced leptin resistance models [24,28]. In addition, direct melanocortin activation is shown to circumvent leptin resistance [27,30,32]. For example, the synthetic analog of α-MSH, MTII, when administered through 3rd ventricle infusion, causes dramatic reduction in food intake and loss of body adiposity and body mass in all three of our leptin resistance models including diet-induced obesity, adult-onset obesity, and leptin-induced leptin resistance [27,30,32]. These facts imply that despite profound leptin resistance, central melanocortin system remains intact and fully responsive to exogenous melanocortin activation and that such activation circumvents the leptin resistance.

The success of our short-term pharmacological melanocortin treatment in combating leptin resistance predicts an opportunity for using long-term melanocortin activation as a viable strategy to treat obesity. We incorporated the gene encoding POMC in a rAAV vector (rAAV-POMC) and assessed the effects of central rAAV-POMC gene delivery in both obese Zucker rats and those with adult-onset obesity [40,41]. The POMC-containing vector or control vectors were injected bilaterally into the basal hypothalamus with coordinates targeting the arcuate nucleus. The outcomes in either obese rat model were impressive. In the Zucker rats, there was a sustained reduction in food intake throughout the entire 38-day POMC treatment [41]. Weight gain was attenuated moderately, and visceral adiposity was decreased by 24% in rAAV-POMC rats. POMC treatment enhanced uncoupling protein 1 in brown adipose tissue (BAT) by more than four fold and lowed fasting serum leptin, insulin, and cholesterol levels significantly. In the rats with adult-onset obesity, POMC over-expression decreased food consumption from day 10 after vector injection, but this anorexic effect abated by day 30 [40]. In contrast, there was a persistent steady decrease in body weight. The rAAV-POMC rats lost an average of 9.5% of their initial body weight as compared to only 2.7% in control rats (-56.8 ± 9.9 vs. -14.8 ± 5.0 g of weight change, $p < 0.01$) by the end of the experiment (day 42). POMC gene therapy also decreased adiposity ($\sim 19\%$) and lowered serum levels of leptin (33%), NEFA (30%), and triglyceride (15%). Glucose metabolism and insulin sensitivity were also improved following POMC treatment. These results validate the effectiveness of chronic rAAV-POMC gene therapy in circumventing leptin resistance and combating obesity in genetically obese and adult-onset obese rodents. The central melanocortin system, thus, appears to be a useful drug target for long-term obesity intervention.

9. Conclusion remarks

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 in leptin in the hypothalamus initially produces potent anorectic and energy expenditure responses, but the responses wane eventually, 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. According to our observations, the leptin resistance seems to reside within the first order neurons possessing leptin receptors, and activation of the central melanocortin system downstream of the leptin receptor circumvents this leptin resistance.

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 [42]. Although a decrease in receptor and signaling may indeed account for leptin resistance, new questions still await answers: By what mechanism(s) is the leptin resistance prevented in these other nuclei? Intriguingly, one leptin-mediated response that seems to persist even in the presence of metabolic leptin resistance is the central leptin-induced increase in blood pressure ([43,44] and our preliminary observations). We believe the key to understanding leptin resistance is not only in how it occurs in the arcuate nucleus, but also how it is precluded in these other nuclei.

Acknowledgement

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