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Increased leptin expression selectively in the hypothalamus suppresses inflammatory markers CRP and IL-6 in leptin-deficient diabetic obese mice

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Abstract

Low-grade systemic inflammation, as indicated by increased circulating levels of inflammatory markers CRP and IL-6, is linked to increased risks for cardiovascular diseases (CVD) and diabetes mellitus in obese subjects. Whereas hyperleptinemia in obesity are associated with increased CRP and IL-6 release, the hypothalamic versus peripheral site of leptin action has not been ascertained. The effects of increased leptin supply selectively in the hypothalamus by gene therapy on pro-inflammatory CRP and IL-6 levels and on markers of diabetes in the circulation of *ob/ob* mice displaying either age-related or dietary obesity were assessed. A recombinant adeno-associated viral vector encoding either green fluorescent protein (control) or leptin gene was injected intracerebroventricularly. Five weeks later, one-half of each of the vector groups was switched to high-fat diet consumption and the other half continued to consume regular low-fat chow diet. Body weight and visceral white adipose tissue were drastically reduced and hyperinsulinemia and hyperglycemia were abrogated by leptin gene therapy, independent of the dietary fat content. The elevated plasma CRP and IL-6 levels seen in obese *ob/ob* mice receiving the control vector, regardless of the fat content of the diet, were markedly suppressed by increased hypothalamic leptin in both groups. The results show for the first time that leptin deficiency elevates and reinstatement of leptin selectively in the hypothalamus suppresses the release of pro-inflammatory biomarkers, a response likely to alleviate CVD associated with obesity.

Keywords

C-reactive protein; interleukin-6; inflammation; gene therapy; obesity

1. Introduction

Recent epidemiological surveys show that lifestyle modifications and an increase in the consumption of diets rich in calories have contributed to the escalation in the incidence of obesity in children and adults worldwide [2,20,43]. Obese subjects are at high risk of incurring

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metabolic ailments including cardiovascular diseases (CVD) and type 2 diabetes [10,19]. Clinical investigations have also uncovered a strong relationship between obesity and chronic low-grade inflammation [40,45]. In fact, it is generally held that chronic low-grade inflammation is a causative factor in CVD and diabetes mellitus, as well as other metabolic disorders linked to or exacerbated by obesity [36,45]. Elevations in circulating levels of acute-phase proteins, such as C-reactive protein (CRP), and pro-inflammatory cytokines, such as interleukin-6 (IL-6), in obese individuals are consistent with these findings [27,36,40,45]. CRP is a sensitive, though relatively non-specific, biomarker of systemic inflammation and has been identified as a predictor of risks for CVD [27]. In 2003 the Centers for Disease Control and Prevention and the American Heart Association concluded that CRP is currently the best inflammatory biomarker for CVD [39].

IL-6 is a pro-inflammatory cytokine which exerts pleiotropic effects on a variety of tissues and is directly implicated in atherogenesis [18,33]. High levels of IL-6 are responsible for the increase in acute-phase proteins seen in obese patients, particularly CRP, via its action on CRP producing cells in the liver [16,33]. Approximately 30% of circulating IL-6 is derived from white adipose tissue (WAT), with visceral fat producing higher levels of IL-6 than subcutaneous fat [16,35,38]. Increased serum IL-6 is considered as predictive of impending cardiovascular afflictions [30,41] and, thus, it also serves as a reliable biomarker for CVD.

Adipose tissue is now a recognized endocrine organ that secretes a host of adipokines such as leptin, and cytokines such as IL-6 and tumor necrosis factor- α (TNF- α) [1,16,29,35,38]. Leptin is a major secretory product of adipocytes and blood levels are elevated in direct proportion to adiposity [17,24]. Positive correlations between plasma leptin and CRP concentrations with increasing adiposity have been reported. Administration of leptin to humans was also shown to increase blood CRP levels [21,34]. The evidence that leptin can stimulate the production of CRP by cultured human hepatocytes implied that leptin may play a role in the physiological regulation of CRP [11]. Furthermore, since CRP can avidly bind leptin in the blood, it has been suggested that restriction of leptin entry across the blood brain barrier by the CRP-leptin complex may engender leptin insufficiency in the hypothalamus which, in turn, promotes fat accrual [11]. CRP was also shown to block the effects of exogenous leptin on food intake (FI) and body weight (BW) in leptin-deficient *ob/ob* mice [11].

In humans, reduction in BW and adipose tissue mass can evoke beneficial effects on general measures of inflammation, including blood leptin, CRP and IL-6 levels [49]. However, the paucity of information from animal experiments has precluded a clear understanding of the cause and effect relationships among leptin, CRP and IL-6 in obesity and the attendant disease cluster of the metabolic syndrome, such as type 2 diabetes, general inflammation and CVD. In addition, leptin acts primarily in the hypothalamus to play a role in energy homeostasis [17, 24], but the peripheral effects of leptin on a variety of physiological functions in the periphery are also well documented [5]. Thus, whether the positive relationship between leptin and pro-inflammatory cytokines and CRP in the periphery observed clinically with increasing adiposity involve central and/or peripheral action of leptin is unknown.

In a series of investigations, we observed that increasing leptin expression selectively in the hypothalamus with the aid of leptin gene therapy suppressed both the age-related and high-fat diet (HFD)-induced weight gain and greatly reduced abdominal WAT mass in laboratory mice and rats [3,4,6–9,12–15,22,23,25,26,32,37,46,47]. This effect followed either intracerebroventricular (icv) injection or direct microinjection into specific hypothalamic sites of a recombinant adeno-associated viral vector (rAAV) encoding the leptin gene (rAAV-lep). The suppression of weight, primarily due to fat depletion with or without reduction in FI, was concomitant with abolition of hyperinsulinemia and suppression of adipokines – leptin, adiponectin, TNF- α , free fatty acids and triglycerides, increased insulin sensitivity and

euglycemia for the duration of the experiments. Central leptin gene therapy was also efficient in correcting hyperphagia, weight gain and diabetes in obese, hyperinsulinemic and diabetic *ob/ob* mice consuming either regular chow diet (RCD) or HFD. Another pertinent finding of the investigations in these leptin-deficient mice was that varied beneficial effects were conferred in the absence of any detectable circulating leptin [7,9,23,25,46,47]. This experimental paradigm, thus, is suitable for sorting out the contributions of central versus peripheral actions of leptin in the observed relationships among leptin, CRP, pro-inflammatory cytokines and increased adiposity [23]. Therefore, we assessed the effects of dietary obesity and increasing central leptin signaling by leptin transgene expression selectively in the hypothalamus on circulating CRP, IL-6, insulin and glucose levels in *ob/ob* mice consuming either RCD or HFD.

2. Methods

2.1 Animals

Four weeks old male leptin mutant *ob/ob* mice (27–31 g) and wild type (wt) C57 mice (wt 16–18 g) obtained from The Jackson Laboratory (Bar Harbor, ME) were housed individually in a temperature (21° C) and light controlled room (lights-on 06:00–18:00h) under specific pathogen-free conditions. Food and water were available ad libitum. All animals were allowed one week of adaptation to the animal rooms before experimental procedures were begun. The Institutional Animal Care and Use Committee of the University of Florida approved the animal protocols.

2.2 Experimental Design

The experimental design to evaluate the effects of obesity, HFD and rAAV-lep treatment was similar to that described earlier [7]. The *ob/ob* mice were anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and divided into two subgroups; one was injected icv with a non-immunogenic, non-pathogenic rAAV encoding the green fluorescent protein gene (rAAV-GFP, 1.8×10^{10} particles in 1.5 μ l) and the second group received rAAV encoding the rat leptin gene (rAAV-lep, 2.4×10^{10} particles in 1.5 μ l). The stereotaxic coordinates for third ventricle injections were 0.3mm posterior of bregma, on the midline and 4.2mm below the dura. The rAAV-GFP or rAAV-lep solution was slowly infused over a 2 minute period and the injector was left in place for an additional 5 minutes to allow diffusion of the injected solution. The wt mice were left untreated to serve as controls for comparison of various treatments in *ob/ob* mice. The vectors used in this study were packaged, purified, concentrated and titered as previously described [3,4,12,13]. Systemic injection of rAAV-lep in *ob/ob* mice increased circulating leptin levels and normalized body weight and energy intake [4,12,23]. Central rAAV-lep administration consistently increased leptin mRNA expression in the hypothalamus alone [23,25,26]. However, leptin transduced by rAAV-lep injection was in the undetectable range but quite effective in reinstating leptin-induced hypothalamic control on energy homeostasis [4].

Mice were provided with RCD ad libitum (11 Kcal% fat; LM-485 Teklad, Madison, WI). BW and FI were monitored on a weekly basis. At week 5 post-injection, *ob/ob* mice treated with either rAAV-GFP or rAAV-lep were divided into two subgroups: one subgroup continued to consume the RCD and the other was switched to HFD (45 Kcal% fat, primarily from lard, D12451, Research Diets, New Brunswick, NJ). The wt control mice continued to consume the RCD. The caloric density of RCD was 3.4kcal/g and HFD was 4.73 kcal/g.

At week 13 post-injection, animals were anesthetized with sodium pentobarbital for withdrawal of blood by orbital sinus puncture and then sacrificed by decapitation between 10:30 and 15:00

h. Abdominal WAT was dissected out and weighed and plasma was stored at -20°C for analysis of glucose, insulin, CRP and IL-6.

2.3 Analyses

Plasma insulin was measured by RIA kit (Sensitive Rat Insulin RIA kit, Linco Research, Inc., St. Charles, MO) and blood glucose levels were measured with a glucose meter (Glucometer Elite XL; Bayer, Elkhart, IN). CRP and IL-6 were measured with EIA kits specific for mouse (ALPCO Diagnostics, Salem, NH).

2.4 Statistical Analyses

BW was analyzed with repeated measures two-way ANOVA followed by one-way ANOVA and Newman-Keuls multiple comparison *post-hoc* tests. Results of WAT mass, insulin, glucose, CRP and IL-6 levels were analyzed using one-way ANOVA and Newman-Keuls multiple comparison *post-hoc* tests. The significance was set at $p < 0.05$ for all analyses.

3. Results

3.1 Effects of rAAV-lep and HFD on BW and WAT Mass

As shown in Fig. 1A, rAAV-lep treatment not only blocked the time-related increase in BW in *ob/ob* mice consuming RCD (*ob/ob* + rAAV-GFP + RCD) but suppressed the BW (*ob/ob* + rAAV-lep + RCD) to a level well below that observed in wt mice consuming RCD (wt + NT + RCD) within 3 weeks post-injection. As expected, HFD consumption further increased BW in control rAAV-GFP injected mice by 17% from those consuming RCD ($p < 0.01$). In contrast, the rapid rise in weight gain in these *ob/ob* HFD consuming mice (*ob/ob* + rAAV-lep + HFD) was largely prevented by rAAV-lep pretreatment (*ob/ob* + rAAV-lep + HFD). Whereas, the BW profile of *ob/ob* + rAAV-lep + HFD mice remained within the range of mice consuming RCD for up to week 12, it was slightly higher in these mice at the termination of the experiment at week 13 as compared to those rAAV-lep treated mice consuming RCD ($p < .05$).

The WAT mass after either HFD consumption or rAAV-lep treatment was parallel to that of the BW responses (Fig. 1B). HFD consumption significantly augmented WAT mass over that seen in control RCD consuming mice ($p < 0.01$) and rAAV-lep treatment prevented the HFD-induced increases in adiposity (*ob/ob* + rAAV-lep + RCD vs. *ob/ob* + rAAV-lep + HFD, $p > 0.05$, Fig. 1B).

3.2 Effects of HFD and rAAV-lep Treatment on Plasma CRP and IL-6 Levels

As shown in Fig. 2A and B, plasma CRP and IL-6 levels were elevated in obese *ob/ob* control mice consuming RCD (rAAV-GFP) over that seen in wt mice consuming RCD (wt + NT, $p < 0.01$). rAAV-lep treatment normalized the plasma levels of CRP and decreased IL-6 concentrations significantly ($p < 0.01$) but not to the range seen in control wt + NT mice.

Unlike the BW response, consumption of HFD failed to modify the already elevated blood levels of CRP and IL-6 (Fig. 2A & B). However, the suppressive effects of rAAV-lep on these two pro-inflammatory markers persisted. Leptin transgene expression suppressed serum CRP and IL-6 levels in both RCD and HFD consuming mice.

3.3 Effects of HFD and rAAV-lep Treatment on Serum Insulin and Glucose Levels

As expected [7,46] obese *ob/ob* mice displayed severe hyperinsulinemia and hyperglycemia as compared to wt + NT + RCD mice (Fig. 2C & D). This state of hyperinsulinemia and hyperglycemia persisted with HFD consumption. However, rAAV-lep treatment abolished hyperinsulinemia and imposed normoinsulinemia in mice consuming either RCD or HFD.

rAAV-lep treatment was similarly effective in suppressing blood glucose levels to the normoglycemic range in groups of mice consuming either low-calorie or high-calorie diets (Fig. 2D).

4. Discussion

The results of the current investigations show that reinstatement of leptin supply by leptin transgene expression selectively in the hypothalamus of leptin-deficient *ob/ob* mice, which has been previously verified to occur without leakage to the periphery [7,47], suppressed BW gain, depleted fat, abrogated hyperinsulinemia and hyperglycemia in mice consuming either normal low calorie RCD or calorie-enriched HFD. The weekly FI profiles in response to these treatments (not shown) were similar to that reported earlier [7]. A similar outcome of a single central injection of rAAV-lep persisting for longer periods was observed in wt rodents and *ob/ob* mice [6–9,14,32,46,47]. The efficacy of the stable leptin expression, specifically in the hypothalamus in minute amounts, to curb accumulation of fat for the lifetime of rodents is important because excessive long-term secretion of several adipokines contributes to a state of chronic, systemic and local vascular inflammation as the volume of adipose tissue, especially visceral adipose tissue, expands [19,21,33,34,36,38,42,45]. The co-morbidities of obesity, in particular CVD and diabetes, are hypothesized to be due, in part, to persistence of chronic low-grade, subclinical inflammation. In obese individuals, enlarged, activated adipocytes recruit macrophages and release various adipokines that promote both inflammation and insulin resistance. Consequently, elevated CRP and IL-6 levels serve as biomarkers for the risk of CVD in clinically obese patients [27,30,39,41].

There are several new findings of the current study that aimed at evaluating the effects of morbid obesity and marked depletion of visceral fat on CRP and IL-6 in the complete absence of the hyperleptinemia of obesity. First, we observed that plasma CRP levels were elevated in obese *ob/ob* mice over those in wt controls, an effect which was independent of both the amount of fat in the diet and peripheral leptin. CRP is synthesized by hepatocytes under the transcriptional control of several cytokines, with IL-6 being a primary stimulus [30]. We observed also that CRP levels were normalized when leptin signaling was instituted selectively in the hypothalamus. These findings argue for a hypothalamic restraining role of leptin on CRP secretion by hepatocytes. *ob/ob* mice exhibit a fatty liver response, but similarly, central leptin therapy normalized liver weights in these mice [7]. Together these observations, therefore, demonstrate that efferent neural pathways from hypothalamus to liver [31,44,48] relay inhibitory messages propagated by leptin. It is also reasonable to propose that high blood CRP levels found in *ob/ob* mice result from a lack of leptin-induced restraint from hypothalamus on hepatocyte CRP efflux. This is consistent with our previous reports that a similar lack of leptin restraint in the hypothalamus on pancreatic insulin secretion engenders hyperinsulinemia [7,13,15,23,32,37]. However, our results do not exclude the alternate possibility that diminution in CRP levels may be a secondary response linked to a depletion in fat depots, altered secretion of adipokines and abrogation of diabetes, as reflected by normoinsulinemia and euglycemia in the rAAV-lep treated *ob/ob* mice [7,47].

The observation of elevated IL-6 levels in *ob/ob* mice as compared to wt controls and a fall in IL-6 levels in response to rAAV-lep injection is the second new finding of this study. It is possible that reduction in IL-6 levels is due to diminished WAT, a significant source of blood IL-6 [16,35,38]. In fact, the 30% drop in IL-6 levels in these mice is within the range reported to arise from adipocytes [16,35,38]. The third new finding relates to parallel fluctuations in IL-6 and CRP responses in *ob/ob* mice. An important function of IL-6 is amplification of the inflammatory cascade, including promoting synthesis of several acute-phase reactants, such as CRP [16,30,33,35,38]. Thus, it is highly possible that diminution in IL-6 levels underlies

the reduced CRP secretion by hepatocytes [16,30,33,35,38] and this cause and effect relationship is not influenced by HFD consumption.

The fourth new finding of this investigation relates to the role of hyperleptinemia in causing chronic low-grade inflammation and secretions of pro-inflammatory CVD markers [21,28, 34,42]. Although a positive relationship between hyperleptinemia and high levels of IL-6 and CRP have been observed in obese patients, experimental evidence that peripheral actions of leptin on targets in the periphery engenders these relationships is scarce. Since rAAV-lep treated *ob/ob* mice lack leptin in the periphery [7,47], the current results are inconsistent with the notion of long-term hyperleptinemia as a contributor in the positive relationships among these biomarkers of CVD.

Finally, previous studies have shown that both the enhanced leptin expression in wt rodents and its reinstatement in the hypothalamus of leptin-deficient *ob/ob* mice confer a broad range of health benefits [25,26]. These include maintenance of reduced BW and adiposity, abolition of dyslipidemia, normalization of the pancreatic-glucose axis, suppression of TNF- α and insulin growth factor-1, and bone remodeling [7,9,22,37,46,47]. Recently, we reported that a similar reinstatement of bioavailability of leptin transduced by ectopic leptin gene in the hypothalamus of *ob/ob* mice reduced the mortality rate and doubled the lifespan to the range of wt mice [9]. The results of the current study strongly suggest that suppression of systemic inflammation, as suggested by diminution of biomarkers by increased activation of leptin responsive pathways in the hypothalamus, may complement these varied life-prolonging benefits of central leptin gene therapy. Thus, central leptin gene therapy or leptin mimetics capable of crossing the blood brain barrier have the potential to correct metabolic disease cluster, including cardiovascular ailments.

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References

1. Ailhaud G. Adipose tissue as a secretory organ: from adipogenesis to the metabolic syndrome. *C R Biol* 2006;329:570–577. [PubMed: 16860275]discussion 653–575
2. Anderson PM, Butcher KE. Childhood obesity: trends and potential causes. *Future Child* 2006;16:19–45. [PubMed: 16532657]
3. Bagnasco M, Dube MG, Kalra PS, Kalra SP. Evidence for the existence of distinct central appetite and energy expenditure pathways and stimulation of ghrelin as revealed by hypothalamic site-specific leptin gene therapy. *Endocrinology* 2002;143:4409–4421. [PubMed: 12399438]
4. Bagnasco M, Dube MG, Katz A, Kalra PS, Kalra SP. Leptin expression in hypothalamic PVN reverses dietary obesity and hyperinsulinemia but stimulates ghrelin. *Obesity Research* 2003;11:1463–1470. [PubMed: 14694210]
5. Baratta M. Leptin--from a signal of adiposity to a hormonal mediator in peripheral tissues. *Med Sci Monit* 2002;8:RA282–RA292. [PubMed: 12503048]
6. Beretta E, Dube MG, Kalra PS, Kalra SP. Long-term suppression of weight gain, adiposity, and serum insulin by central leptin gene therapy in prepubertal rats: Effects on serum ghrelin and appetite-regulating genes. *Ped Res* 2002;52:189–198.
7. Boghossian S, Dube MG, Torto R, Kalra PS, Kalra SP. Hypothalamic clamp on insulin release by leptin-transgene expression. *Peptides* 2006;27:3245–3254. [PubMed: 16962683]
8. Boghossian S, Lecklin AH, Torto R, Kalra PS, Kalra SP. Suppression of fat deposition for the life time of rodents with gene therapy. *Peptides* 2005;26:1512–1519. [PubMed: 16042991]
9. Boghossian S, Ueno N, Dube MG, Kalra P, Kalra S. Leptin gene transfer in the hypothalamus enhances longevity in adult monogenic mutant mice in the absence of circulating leptin. *Neurobiol Aging* 2007;28:1594–1604. [PubMed: 17011078]

10. Carr MC, Brunzell JD. Abdominal obesity and dyslipidemia in the metabolic syndrome: importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk. *J Clin Endocrinol Metab* 2004;89:2601–2607. [PubMed: 15181030]
11. Chen K, Li F, Li J, Cai H, Strom S, Bisello A, et al. Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nat Med* 2006;12:425–432. [PubMed: 16582918]
12. Dhillon H, Ge Y, Minter RM, Prima V, Moldawer LL, Muzyczka N, et al. Long-term differential modulation of genes encoding orexigenic and anorexigenic peptides by leptin delivered by rAAV vector in ob/ob mice. Relationship with body weight change. *Regul Pept* 2000;92:97–105. [PubMed: 11024571]
13. Dhillon H, Kalra SP, Kalra PS. Dose-Dependent Effects of Central Leptin Gene Therapy on Genes That Regulate Body Weight and Appetite in the Hypothalamus. *Mol Ther* 2001;4:139–145. [PubMed: 11482985]
14. Dhillon H, Kalra SP, Prima V, Zolotukhin S, Scarpace PJ, Moldawer LL, et al. Central Leptin Gene Therapy Suppresses Body Weight Gain, Adiposity and Serum Insulin Without Affecting Food Consumption in Normal Rats: A Long-Term Study. *Regul Pept* 2001;99:69–77. [PubMed: 11384767]
15. Dube MG, Beretta E, Dhillon H, Ueno N, Kalra PS, Kalra SP. Central leptin gene therapy blocks high fat diet-induced weight gain, hyperleptinemia and hyperinsulinemia: effects on serum ghrelin levels. *Diabetes* 2002;51:1729–1736. [PubMed: 12031959]
16. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115:911–919. [PubMed: 15867843]
17. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763–770. [PubMed: 9796811]
18. Fruhbeck G, Salvador J. Role of adipocytokines in metabolism and disease. *Nutrition Research* 2004;24:803–826.
19. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab* 2004;89:2595–2600. [PubMed: 15181029]
20. Hill JO, Peters JC. Environmental contributions to the obesity epidemic. *Science* 1998;280:1371–1374. [PubMed: 9603719]
21. Hukshorn CJ, Lindeman JH, Toet KH, Saris WH, Eilers PH, Westerterp-Plantenga MS, et al. Leptin and the proinflammatory state associated with human obesity. *J Clin Endocrinol Metab* 2004;89:1773–1778. [PubMed: 15070944]
22. Iwaniec UT, Boghossian S, Lapke PD, Turner RT, Kalra SP. Central leptin gene therapy corrects skeletal abnormalities in leptin-deficient ob/ob mice. *Peptides* 2007;28:1012–1019. [PubMed: 17346852]
23. Kalra SP. Central leptin insufficiency syndrome: An interactive etiology for obesity, metabolic and neural diseases and for designing new therapeutic interventions. *Peptides* 2008;29:127–138. [PubMed: 18053615]
24. Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocr Rev* 1999;20:68–100. [PubMed: 10047974]
25. Kalra SP, Kalra PS. Gene transfer technology: a preventive neurotherapy to curb obesity, ameliorate metabolic syndrome and extend life-expectancy. *Trends Pharmacol Sci* 2005;26:488–495. [PubMed: 16125798]
26. Kalra, SP.; Kalra, PS. Keeping obesity and metabolic syndrome at bay with central leptin and cytokine gene therapy. In: Hamilton, I., editor. *Current Medicinal Chemistry - Central Nervous System Agent*. San Francisco, CA: Bentham Science Publishers Ltd; 2003. p. 189-199.
27. Kao PC, Shiesh SC, Wu TJ. Serum C-reactive protein as a marker for wellness assessment. *Ann Clin Lab Sci* 2006;36:163–169. [PubMed: 16682512]
28. Kazumi T, Kawaguchi A, Hirano T, Yoshino G. C-reactive protein in young, apparently healthy men: associations with serum leptin, QTc interval, and high-density lipoprotein-cholesterol. *Metabolism* 2003;52:1113–1116. [PubMed: 14506615]
29. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;89:2548–2556. [PubMed: 15181022]
30. Khuseynova N, Koenig W. Biomarkers of outcome from cardiovascular disease. *Curr Opin Crit Care* 2006;12:412–419. [PubMed: 16943718]

31. Kreier F, Kap YS, Mettenleiter TC, van Heijningen C, van der Vliet J, Kalsbeek A, et al. Tracing from fat tissue, liver, and pancreas: a neuroanatomical framework for the role of the brain in type 2 diabetes. *Endocrinology* 2006;147:1140–1147. [PubMed: 16339209]
32. Lecklin AH, Dube MG, Torto R, Kalra PS, Kalra SP. Perigestational suppression of weight gain with central leptin gene therapy results in lower weight F1 generation. *Peptides* 2005;26:1176–1187. [PubMed: 15949636]
33. Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* 2003;144:2195–2200. [PubMed: 12746274]
34. Mackintosh RM, Hirsch J. The effects of leptin administration in non-obese human subjects. *Obes Res* 2001;9:462–469. [PubMed: 11500526]
35. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *J Clin Endocrinol Metab* 1997;82:4196–4200. [PubMed: 9398739]
36. Oren H, Erbay AR, Balci M, Cehreli S. Role of novel biomarkers of inflammation in patients with stable coronary heart disease. *Angiology* 2007;58:148–155. [PubMed: 17495262]
37. Otukonyong EE, Dube MG, Torto R, Kalra PS, Kalra SP. Central Leptin Differentially Modulates Ultradian Secretory Patterns of Insulin, Leptin and Ghrelin Independent of Effects on Food Intake and Body Weight. *Peptides* 2005;26:2559–2566. [PubMed: 15936848]
38. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF- α and IL-6. *Diabetes Res Clin Pract* 2005;69:29–35. [PubMed: 15955385]
39. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499–511. [PubMed: 12551878]
40. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. *Arterioscler Thromb Vasc Biol* 2006;26:968–976. [PubMed: 16627822]
41. Ruan H, Lodish HF. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor- α . *Cytokine Growth Factor Rev* 2003;14:447–455. [PubMed: 12948526]
42. Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, et al. Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation* 2004;109:2181–2185. [PubMed: 15117839]
43. Stein CJ, Colditz GA. The epidemic of obesity. *J Clin Endocrinol Metab* 2004;89:2522–2525. [PubMed: 15181019]
44. Sved AF, Cano G, Card JP. Neuroanatomical specificity of the circuits controlling sympathetic outflow to different targets. *Clin Exp Pharmacol Physiol* 2001;28:115–119. [PubMed: 11153526]
45. Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans* 2005;33:1078–1081. [PubMed: 16246049]
46. Ueno N, Dube MG, Inui A, Kalra PS, Kalra SP. Leptin modulates orexigenic effects of ghrelin and attenuates adiponectin and insulin levels and selectively the dark-phase feeding as revealed by central leptin gene therapy. *Endocrinology* 2004;145:4176–4184. [PubMed: 15155574]
47. Ueno N, Inui A, Kalra SP, Kalra PS. Leptin transgene expression in the hypothalamus enforces euglycemia in diabetic, insulin-deficient nonobese Akita mice and leptin-deficient obese ob/ob mice. *Peptides* 2006;27:2332–2342. [PubMed: 16621153]
48. Uyama N, Geerts A, Reynaert H. Neural connections between the hypothalamus and the liver. *Anat Rec A Discov Mol Cell Evol Biol* 2004;280:808–820. [PubMed: 15382020]
49. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 2002;105:804–809. [PubMed: 11854119]

Figure 1.

Effects of icv rAAV-lep injection on BW during the 13 weeks duration of the experiment (A) and on abdominal WAT mass at the termination of the experiment (B). Note that the scale of the y-axis has been adjusted in graph A to accommodate the high weight of the rAAV-GFP vs rAAV-lep *ob/ob* mice. Arrows indicate time of vector injection at week 0 and introduction of the HFD at week 5. Similar superscripts on bars (Fig. 1B) are not significantly different from each other $p > 0.05$. In this and figure 2 numbers in parentheses indicate number of animals and the abbreviations are: wt = wild type, NT = no treatment, RCD = regular chow diet, HFD = high-fat diet.

Figure 2. Effects of icv rAAV-lep injection on blood levels of CRP (A), IL-6 (B), insulin (C) and glucose (D) at week 13 post-vector injection (see figure 1 for details). Bars with similar superscripts are not significantly different from each other $p > 0.05$.