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Leptin gene transfer in the hypothalamus enhances longevity in adult monogenic mutant mice in the absence of circulating leptin☆

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Abstract

Leptin, a product of the *ob* gene, is a pleiotropic signal implicated in regulation of multiple physiological functions in the periphery and centrally, including hypothalamic integration of energy homeostasis. Recessive mutations of *ob* gene result in early onset of hyperphagia, morbid obesity, metabolic disorders, early mortality and shortened life-span. Intracerebroventricular injection of recombinant adeno-associated virus vector (rAAV) encoding the leptin gene in adult obese *ob*/*ob* mice enhanced leptin transgene expression only in the hypothalamus, normalized food intake, body weight and more than doubled the life-span as compared to control cohorts and extended it to near that of normal wild type mice. These life-extending benefits were associated with drastic reductions in visceral fat, and blood glucose and insulin levels, but elevated ghrelin levels, the anti-aging biomarkers. Thus, bioavailability of leptin transduced by ectopic gene in the hypothalamus alone is both necessary and sufficient to normalize life-span. Evidently, site-specific ectopic gene expression with rAAV is durable and safe for alleviating neural disorders that stem from missing or functional disruption of a single gene.

Keywords: Leptin gene therapy; Longevity; Fat; Hypothalamus

1. Introduction

Environmental and genetic causes either alone or in a complex interplay are thought to contribute to the current worldwide escalation in the incidence of obesity and obesity-dependent metabolic and neurological afflictions [2,6,14,27,30,36,37,51]. Genetically-based disruptions that tilt energy balance in favor of increased energy consumption, decreased energy expenditure and accelerated rates of fat deposition are diverse and involve complex interactions of neural, hormonal and metabolic factors [1,30,43,45,46,51,61]. Recent delineation of these varied derangements in the internal environment in monogenic and polygenic obesities has uncovered avenues for therapeutic interventions at genetic and molecular levels [6,26,34,45].

Rodent models of monogenic obesity are suitable paradigms to test novel molecular and genetic interventional approaches aimed at retarding fat accretion on a long-term basis [6,26,34,45]. Recessive mutation in *ob* gene results in morbid obesity resulting from an early onset of hyperphagia and decreased energy expenditure [6,28,34,36,55,60]. Leptin, the product of the *ob* gene, is produced by adipocytes and non-adipocyte tissues, including the hypothalamus [3,28,36,37,53,63,64]. Leptin is a pleiotropic signal that regulates multiple physiological functions in the periphery and neural functions in the brain by engaging target specific leptin receptors [1,5,24,28,29]. In the periphery, leptin has been implicated in the regulation of blood pressure, renal function, angiogenesis, wound healing, immune function and bone formation [12,16,18,29,35,42,48,50,57,58]. Among central effects, a primary action of leptin is to integrate energy homeostasis by controlling distinct energy

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intake and expenditure regulating neural pathways in the hypothalamus [1,28,33,36,37]. Absence of leptin engenders intense hyperphagia, excess fat accretion, life-long morbid obesity accompanied by diminished non-shivering thermogenic energy expenditure in leptin-mutant *ob*/*ob* mice and congenital leptin-deficient human subjects [1,20,21,25,26,28,36,37,50,60]. Morbidly obese human patients and obese mice exhibit a variety of life-threatening complications, including metabolic syndrome, early mortality and shortened life-span [2,14,25–27,30,46,51,61,65].

Experimental evidence showing that leptin replacement either systemically or centrally restores energy homeostasis in *ob*/*ob* mice [28,29,33,36], and conditional deletion of leptin receptors in the hypothalamus reproduces the *ob*/*ob*-type phenotype [17,36,40], suggests that leptin action in the hypothalamus alone can reinstate weight homeostasis in *ob*/*ob* mice. However, whether imbalance due to leptin deficiency in the hypothalamus and/or global pathophysiologic complications resulting from leptin deficiency in the periphery accelerate aging and shorten life-span, is not known.

Research in gene transfer strategies for developing interventional therapies for neural disorders has proceeded at rapid pace [15,37,39,40]. Among various gene therapy approaches currently available, insertion of a missing gene in an appropriate site with the aid of a suitable viral vector has the potential to remedy the pathophysiologic consequences of a mutant gene on a long-term basis [15,37,39,40]. Leptin gene transfer into the hypothalamus with a non-pathogenic and non-immunogenic recombinant adeno-associated virus encoding the leptin gene (rAAV-lep), was found to enhance leptin transgene expression in the hypothalamus contemporaneous with weight normalization, decreased adiposity and symptoms of type 2 diabetes in leptin-mutant *ob*/*ob* mice even in the absence of leptin into the periphery [4,5,7,8,11,20–24,37,39,58,59]. A similar central leptin gene therapy retarded the gradual time-related increase in fat accretion and secretion of adipocyte adipokines in wild type (wt) mice in short term experiments [10,58,59]. Since rAAV vectors infect mainly neurons in the nervous system and support expression of targeted gene for the lifetime of cells *in vivo* [15,37,39], we examined the efficacy of bioavailability of leptin by leptin gene transfer in the hypothalamus on mortality and longevity in *ob*/*ob* mice.

2. Research methods and procedures

2.1. Animals

Six week-old (40–50 g) leptin-mutant *ob*/*ob* and wild type (wt) *C57BL/6J* male mice (The Jackson Laboratory, Bar Harbor, ME) were housed individually in temperature and light controlled rooms (lights on 06:00–18:00 h) under specific pathogen-free conditions. Standard chow diet (11 kcal% fat; LM-485 Tecklad, Madison, WI) and water were available *ad libitum* throughout the experiment. All animals were allowed at least 1 week of adaptation to the animal rooms before initiation of experimental procedures. The Institutional Animal Care and Use Committee of University of Florida approved the animal protocols.

2.2. Experimental design

2.2.1. Experiment 1

The objective of this experiment was to evaluate the benefit of alleviating leptin insufficiency selectively in the hypothalamus on mortality and longevity in *ob*/*ob* mice. *ob*/*ob* and wt (controls) mice were anesthetized with sodium pentobarbital (60 mg/kg) and placed in a David Kopf stereotaxic apparatus with a mouse adapter for intracerebroventricular (icv) injection [10,58,59]. The stereotaxic coordinates for icv injections were: 0.3 mm posterior to bregma, 0 mm lateral to midline and 4.2 mm below the dura. One group from each genotype was injected icv with a non-immunogenic, non-pathogenic recombinant adeno-associated virus (rAAV) encoding the green fluorescent protein gene (rAAV-GFP, 9×10^7 particles in 1.5 μl; *ob*/*ob* $n = 12$ and wt $n = 10$), and the other group received rAAV encoding rat leptin gene (rAAV-lep, 9×10^7 particles in 1.5 μl; *ob*/*ob* $n = 12$ and wt $n = 10$). rAAV-lep or rAAV-GFP preparation was slowly infused over a 2 min period and the injector was removed 5 min later. The vectors used in this study were packaged, purified, concentrated, titered and verified for leptin transduction *in vitro*, and *in vivo* after intravenous administration in *ob*/*ob* mice [20,21]. Administration of rAAV-lep vector icv has also been tested in restraining body weight (BW) and in suppressing fat deposition [8,10,11,58,59]. BW and food intake (FI) were monitored weekly for 30 weeks and then biweekly until the end of the experiment. Average daily consumption was derived for data analyses. Since previous studies have shown that the effects of icv rAAV-lep injection on BW and FI are dose-dependent [22] and injection of low doses of rAAV-lep reduced FI in *ob*/*ob* but not in wt mice [58,59], consistent with reported heightened sensitivity of *ob*/*ob* mice to leptin [28,36,37], two additional control groups of *ob*/*ob* mice were monitored in parallel. One group served as an unoperated control (untreated, $n = 8$) and the other group served as a pair-fed (PF, $n = 10$) control. Food was supplied before lights-off to PF mice in amounts adjusted weekly to match the food consumption of rAAV-lep treated *ob*/*ob* mice during the week before [58,59]. All mice were housed one per cage and inspected twice a day on weekdays and once a day on weekends until natural death occurred or were moribund and presented symptoms of an eminent death, when as advised by the veterinarian staff, they were euthanized. In all, eight mice from all experimental groups (*ob*/*ob* untreated; $n = 1$, rAAV-GFP; $n = 1$, *ob*/*ob* PF; $n = 2$ and wt rAAV-GFP; $n = 4$) were euthanized as instructed by the veterinarian. In addition, we did not observe any external signs of malignancy or pathology by these mice during the experiment.