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TITLE: Central Leptin Gene Therapy to Reduce Breast Cancer Risk Factors

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14. ABSTRACT Obesity is a risk factor for breast cancer, especially in postmenopausal women. Explanations for this association include increased production of estrogenic compounds due to aromatization of androgens to estrone in adipose tissue, and increased production of serum hormones/cytokines identified as promoters of breast tumor formation and growth. The long-term goal of the proposed research is to determine if control of obesity through centrally administered, recombinant adeno-associated virus leptin gene (rAAV-lep) therapy will decrease the incidence of mammary tumor formation, progression, or metastasis in the rat model. Leptin functions as a messenger in a feedback loop between adipose tissue and the hypothalamus and contributes to the regulation of energy intake, energy expenditure, and adaptation to starvation. The objective of this research was to determine whether central rAAV-lep gene therapy will cause a decrease in serum levels of positive risk factors for breast cancer. The results show that central leptin gene therapy is effective in preventing age-related weight gain in adult rats. We also show that the gene therapy is effective in decreasing circulating levels of several breast cancer risk factors, including leptin, insulin, and IGF-I. The results indicate that increasing hypothalamic leptin to control weight has the added benefit of reducing breast cancer risk factors.					
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Introduction

Obesity is a risk factor for breast cancer, especially in postmenopausal women (1). Explanations for this association include increased production of estrogens due to aromatization of androgens to estrone in adipose tissue, and increased production of hormones and cytokines identified as promoters of breast tumor formation. The long-term goal of the proposed research is to determine if control of obesity through centrally administered, recombinant adeno-associated virus leptin gene (rAAV-lep) therapy will decrease the incidence of mammary tumor formation, progression, or metastasis. Leptin functions as a messenger in a feedback loop between adipose tissue and the hypothalamus and contributes to the regulation of energy intake, energy expenditure, and adaptation to starvation (2-5). The use of viral vectors to introduce the leptin gene into the brain for a sustained supply of leptin in the hypothalamus has proven effective in preventing age-associated increases in body weight in rats fed normal diets and in preventing obesity in rats fed high-fat diets (6).

The objective of this research was to determine whether rAAV-lep gene therapy will cause a decrease in serum levels of positive risk factors for breast cancer (IGF-I, leptin, insulin, estradiol, estrone, and 16α -hydroxyestrone) which have been shown to promote breast tumor formation either through stimulation of cell growth, stimulation of cell migration, or enhancement of angiogenesis. This phase of the project was designed to identify potential mediators of breast tumor formation that are altered by reduced weight and adiposity via rAAV-lep gene therapy.

Body

Task 1. Perform animal experiment.

- Randomize rats by weight into 5 treatment groups (baseline, rAAV-lep, rAAV-GFP, pair-fed, and untreated) with 15 rats per group.
- Inject rAAV-lep or rAAV-GFP in the 3rd cerebroventricle of 9-month-old rats and monitor body weight.
- Collect blood at 4 weeks post-treatment initiation and at termination of study (18 weeks post-treatment initiation).

We have completed Task 1. Sustained overexpression of central leptin was induced by adenovirus-mediated leptin gene therapy, a technique shown to be very effective in the transfer of target genes in rats (7). The rats were injected in the 3rd cerebroventricle with rAAV-lep at a dose of 5×10^{10} particles as described (8) and maintained without further treatment for 18 weeks. To date,

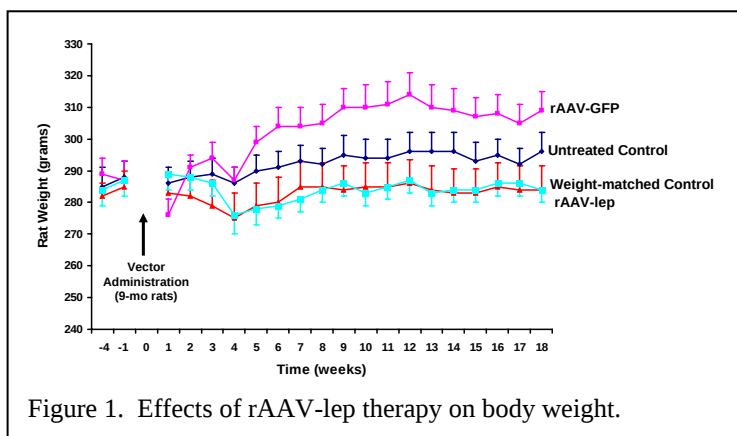


Figure 1. Effects of rAAV-lep therapy on body weight.

single injections of rAAV-lep have been shown to suppress age-associated increases in body weight up to 18 months in rats (6,9). The control vector was rAAV-GFP, encoding green fluorescent protein. Blood was collected from the abdominal aorta at necropsy and stored at -20°C for analysis. Body weight was monitored on a weekly basis and the results are presented in Figure 1. Starting at 6 weeks post-vector administration, body weight in rats treated with rAAV-lep was maintained at 8% below that of the rAAV-GFP control rats ($P<0.05$), indicating the leptin gene therapy was effective in reducing weight gain in adult rats. The decreased body weight in the rAAV-lep animals was associated with over a 50% decrease in abdominal white adipose tissue (WAT) compared to rAAV-GFP and ad-lib control rats (Table 1). The weight-matched controls consumed approximately 15% less food than rAAV-lep rats, indicating that metabolism was increased in the rAAV-lep-treated animals.

Task 2. Collect data, conduct data analysis, and prepare manuscript.

- Conduct serum measurements for IGF-I, leptin, insulin, and estrogens.
- Weigh uteri, embed mammary glands and uteri, prepare thin sections, and conduct histological data collection.
- Conduct data analysis and prepare manuscript.

To date, we have completed the serum analyses for leptin, IGF-I, insulin, and 16 α -hydroxyestrone. Serum IGF-I, leptin, and insulin was measured using radioimmunoassay. 16 α -hydroxyestrone was measured using a monoclonal antibody-based competitive enzyme immunoassay. At 18 weeks post-vector administration, serum leptin levels were significantly lower in rAAV-lep-treated rats in comparison to ad-lib and rAAV-GFP controls (Table 1).

Table 1. Effects of rAAV-lep therapy on body weight, abdominal white adipose tissue (WAT) weight and serum leptin, IGF-1, and insulin levels in female rats.

Endpoint	Ad-lib (n=8)	rAAV-GFP (n=8)	rAAV-lep (n=8)	Weight-matched (n=8)	ANOVA P<
Body Weight (g)	298 $\pm$ 6	309 $\pm$ 6	280 $\pm$ 8 ^b	280 $\pm$ 4 ^b	0.013
Abdominal WAT (g)	6.4 $\pm$ 0.5	8.2 $\pm$ 1.0	3.2 $\pm$ 0.5 ^{a,b}	5.3 $\pm$ 0.4 ^b	0.005
Serum Leptin (ng/ml)	2.7 $\pm$ 0.3	3.0 $\pm$ 0.6	1.0 $\pm$ 0.1 ^{a,b}	2.3 $\pm$ 0.4	0.002
Serum IGF-I (ng/ml)	290 $\pm$ 16	330 $\pm$ 12	261 $\pm$ 21 ^b	275 $\pm$ 10 ^{b*}	0.019
Serum Insulin (ng/ml)	0.23 $\pm$ 0.01	0.22 $\pm$ 0.01	0.18 $\pm$ 0.01 ^a	0.20 $\pm$ 0.02	0.007

Data are Mean $\pm$ SE

^aSignificantly different from Ad-lib, $P<0.05$, ^{a*} $P<0.1$

^bSignificantly different from rAAV-GFP, $P<0.05$, ^{b*} $P<0.1$

Serum IGF-I was lower in rAAV-lep rats than in the rAAV-GFP rats and serum insulin was lower in rAAV-lep rats than in ad-lib rats. Significant differences in serum levels of 16 α -hydroxyestrone were not detected among the treatment groups. We are currently in the process of analyzing serum for levels of estradiol and estrone and processing tissue for histological examination. However, uterine weight, a sensitive bioassay for estrogenic activity, did not differ

among the treatment groups. This finding suggests that leptin gene therapy did not affect estrogen levels.

Hypothalamic administration of leptin has been shown to decrease bone mass in mice (10). As such, osteopenia constitutes a potential adverse side-effect of leptin gene therapy. Because of this potential, we assessed bone mass and architecture in a subsample of the animals (8/group) using a Scanco μ CT40 scanner (Scanco Medical AG, Basserdorf, Switzerland). Cancellous bone in the proximal tibial metaphysis and cortical bone in the tibial diaphysis were evaluated (Figure 2). The results are presented in table 2. Cancellous bone volume in the rAAV-lep-treated rats tended to be lower ($p < 0.1$) than in ad-lib rats. However, significant differences in cancellous bone volume were not detected among rAAV-lep, rAAV-GFP, or weighed-matched animals, indicating that leptin gene therapy is not associated with reduction in bone mass. Differences in cortical bone mass and architecture were not detected among the treatment groups.

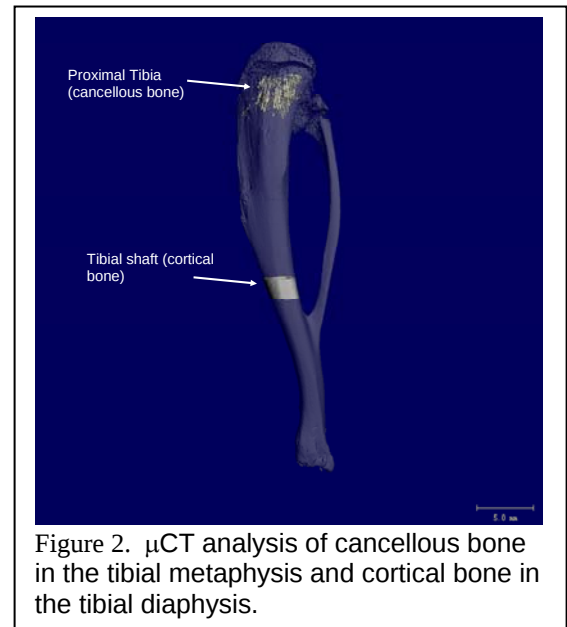


Figure 2. μ CT analysis of cancellous bone in the tibial metaphysis and cortical bone in the tibial diaphysis.

Table 2. Effects of rAAV-lep therapy on cancellous bone volume in the proximal tibia of female rats.

Endpoint	Ad-lib (n=8)	rAAV-GFP (n=8)	rAAV-lep (n=8)	Weight-matched (n=8)	ANOVA P<
Bone volume/Total volume (%)	18.9 $\pm$ 2.1	9.6 $\pm$ 1.8 ^a	12.8 $\pm$ 1.1 ^{a*}	13.9 $\pm$ 1.5	0.005
Trabecular number (1/mm)	3.8 $\pm$ 0.2	3.0 $\pm$ 0.2 ^a	3.5 $\pm$ 0.2 ^{a*}	3.5 $\pm$ 0.1	0.002
Trabecular thickness (μ m)	76.0 $\pm$ 2.5	63.5 $\pm$ 2.4	66.3 $\pm$ 1.5	68.4 $\pm$ 2.9	0.019
Trabecular spacing (μ m)	257 $\pm$ 14	349 $\pm$ 28 ^a	285 $\pm$ 12 ^a	281 $\pm$ 11	0.007

Data are Mean $\pm$ SE

^aSignificantly different from Ad-lib, $P < 0.05$, ^{a*} $P < 0.1$

Key Research Accomplishments

This study demonstrates that:

1. Serum leptin, IGF-I, and insulin are decreased with increased hypothalamic leptin and concomitant weight reduction in adult rats.
2. Increased hypothalamic leptin via rAAV-lep therapy does not cause osteopenia in adult rats.

Reportable Outcomes

1. Iwaniec U.T., Dube M.G., Torto R., Turner R.T., Wronski T.J., and Kalra S.P. (2005) Central leptin gene therapy to reduce breast cancer risk factors. Era of Hope Department of Defense Breast Cancer Research Program meeting. Philadelphia, PA, June 8-11, 2005 (**oral presentation**).
2. This award helped U.T. Iwaniec acquire a tenure-track position at Oregon State University.

Conclusions

We conclude that:

1. Central leptin gene therapy is effective in reducing weight gain for extended intervals even when treatment is initiated in adult rats.
2. Central leptin gene therapy is effective in decreasing circulating levels of several breast cancer risk factors, including leptin, insulin, and IGF-I, even when treatment is initiated in adult rats.
3. Central leptin gene therapy in adult rats does not result in osteopenia.

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