Bone Reports 21 (2024) 101769

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

Long-duration leptin transgene expression in dorsal vagal complex does not alter bone parameters in female Sprague Dawley rats

Russell T. Turner [a,c], Adam J. Branscum [b], Urszula T. Iwaniec [a,c,*]

[a] Skeletal Biology Laboratory, School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR 97331, USA
[b] Biostatistics Program, School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR 97331, USA
[c] Center for Healthy Aging Research, Oregon State University, Corvallis, OR 97331, USA

ARTICLE INFO

Keywords:
Leptin
Dorsal vagal complex
Hypothalamus
Bone
Microcomputed tomography
Histomorphometry

ABSTRACT

The hypothalamus and dorsal vagal complex (DVC) are both important for integration of signals that regulate energy balance. Increased leptin transgene expression in either the hypothalamus or DVC of female rats was shown to decrease white adipose tissue and circulating levels of leptin and adiponectin. However, in contrast to hypothalamus, leptin transgene expression in the DVC had no effect on food intake, circulating insulin, ghrelin and glucose, nor on thermogenic energy expenditure. These findings imply different roles for hypothalamus and DVC in leptin signaling. Leptin signaling is required for normal bone accrual and turnover. Leptin transgene expression in the hypothalamus normalized the skeletal phenotype of leptin-deficient *ob/ob* mice but had no long-duration (≥10 weeks) effects on the skeleton of leptin-replete rats. The goal of this investigation was to determine the long-duration effects of leptin transgene expression in the DVC on the skeleton of leptin-replete rats. To accomplish this goal, we analyzed bone from three-month-old female rats that were microinjected with recombinant adeno-associated virus encoding either rat leptin (rAAV-Leptin, $n = 6$) or green fluorescent protein (rAAV-GFP, control, $n = 5$) gene. Representative bones from the appendicular (femur) and axial (3rd lumbar vertebra) skeleton were evaluated following 10 weeks of treatment. Selectively increasing leptin transgene expression in the DVC had no effect on femur cortical or cancellous bone microarchitecture. Additionally, increasing leptin transgene expression had no effect on vertebral osteoblast-lined or osteoclast-lined bone perimeter or marrow adiposity. Taken together, the findings suggest that activation of leptin receptors in the DVC has minimal specific effects on the skeleton of leptin-replete female rats.

1. Introduction

Leptin, produced primarily by adipocytes, acts on a wide variety of target cells both in the central nervous system (CNS) and peripheral to the CNS (Bjorbaek and Kahn, 2004). Chronic leptin insufficiency leads to impaired immune function and delayed musculoskeletal maturation (Reid et al., 2018; Salum et al., 2021). Activation of leptin receptors in the CNS regulates appetite and thermoregulation, and influences energy balance, reproduction, and glucose metabolism (Flak and Myers Jr, 2016). These CNS-initiated actions of the hormone are mediated by neurotransmission from brain to peripheral tissues (e.g., sympathetic signaling) and by response hormones, including ones produced in gut, ovaries, pancreas, and pituitary (Flak and Myers Jr, 2016).

Leptin-mediated appetite suppression and increased non-shivering thermogenesis require transport of leptin from peripheral circulation across the blood brain barrier into the brain (Bjorbaek and Kahn, 2004). The biologically active long form of leptin receptor (OB-Rb) is localized in both the hypothalamus and the dorsal vagal complex (DVC) of the caudal brain stem, a neuroaxis important for integration of energy homeostasis by leptin (Mercer et al., 1998; Jacob et al., 1997; Grill et al., 2002; Horvath et al., 2004; Mercer et al., 1996). Anatomical pathways relay sympathetic outflow from hypothalamus to brown adipose tissue (BAT), pancreas, and stomach (Bamshad et al., 1998; Kalra et al., 2003; Kalra et al., 1999; Horvath et al., 2004; Wynne et al., 2005; Bamshad et al., 1999; Bartness and Bamshad, 1998; Bartness et al., 2005; Bowers et al., 2004; Buijs et al., 2001; Chen et al., 1998; Morrison, 2001; Sved et al., 2001; Webber and Macdonald, 2000; Flier, 2004). Targeted leptin transgene expression has identified location-specific effects of the hormone (Beretta et al., 2002; Boghossian et al., 2006b). Whereas leptin transgene expression in the hypothalamus and DVC each suppressed

* Corresponding author at: Skeletal Biology Laboratory, School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR 97331, USA.
E-mail address: urszula.iwaniec@oregonstate.edu (U.T. Iwaniec).

https://doi.org/10.1016/j.bonr.2024.101769
Received 17 March 2024; Received in revised form 21 April 2024; Accepted 23 April 2024
Available online 24 April 2024

weight gain in leptin-replete rats, only activation of leptin signaling in hypothalamus suppressed insulin levels and increased ghrelin, a hormonal regulator of appetite, and *Ucp1* gene expression, a marker for increased non-shivering thermogenesis in BAT (Collins et al., 2010). The previously reported differential effects of leptin transgene expression in hypothalamus and DVC on energy metabolism are summarized in Table 1 (Bagnasco et al., 2002; Bagnasco et al., 2003; Beretta et al., 2002; Boghossian et al., 2006a; Boghossian et al., 2006b; Dhillon et al., 2001; Dube et al., 2002; Torto et al., 2006).

The skeletal phenotype of leptin-deficient *ob/ob* mice has been extensively studied as has the bone response of *ob/ob* mice following leptin administration (Hamrick et al., 2004; Reid et al., 2018). *ob/ob* mice are osteopenic and mildly osteopetrotic due to reduced radial and linear bone growth and defective osteoclast function, respectively (Turner et al., 2013). Most skeletal abnormalities in leptin-deficient *ob/ob* mice were shown to be corrected by leptin replacement. This was accomplished by administration of the peptide using subcutaneously implanted osmotic pumps or by hypothalamic leptin gene therapy (Steppan et al., 2000; Hamrick et al., 2005; Philbrick et al., 2018b; Lindenmaier et al., 2016; Khan et al., 2013; Bartell et al., 2011; Iwaniec et al., 2007). In contrast, the skeletal response to modulating leptin levels in animals able to produce leptin is less well defined. Severe caloric restriction, which lowers body weight and serum leptin levels, results in decreased bone formation, increased bone marrow adiposity (BMAT), and bone loss (Turner and Iwaniec, 2011). In contrast, long-duration leptin transgene expression in the hypothalamus in leptin-replete rats was shown to reduce serum leptin levels, suppress appetite, increase energy expenditure, and prevent weight gain with no effect on bone (Iwaniec et al., 2011). Because increasing leptin levels in the hypothalamus and DVC has differential effects on plasma levels of hormones capable of influencing bone metabolism, it is possible that leptin signaling in different regions of the brain has different effects on bone (Iwaniec et al., 2016; Turner et al., 2022; Nikolopoulos et al., 2010; Leidig-Bruckner and Ziegler, 2001). The goal of the present study was to determine whether long-duration selective increase in leptin levels within the DVC has a specific effect on bone metabolism. To accomplish this, we evaluated archived bone specimens from a study (Boghossian et al., 2006b) which reported the impact of leptin transgene expression in the DVC on energy expenditure and body weight gain, food intake, circulating levels of the white adipose tissue (WAT)-derived leptin and adiponectin, pancreatic-derived insulin, gastric-derived ghrelin, and glucose levels (Table 1).

2. Materials and methods

2.1. Animals

Three-month-old female Sprague Dawley rats ($n = 11$) obtained from Harlan (Indianapolis, IN) were used in the experiment. The rats were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Florida (Gainesville, FL) where the study was conducted. The rats were housed individually in a temperature (21–23 °C)- and light (lights on 8:00–18:00 h)-controlled room at the McKnight Brain Institute under specific pathogen-free conditions. Food and water were available *ad libitum* to all animals.

Table 1
Effects of leptin transgene expression in hypothalamus or dorsal vagal complex (DVC) on endpoints of energy metabolism.

Endpoint	Hypothalamus*	DVC**
Weight gain	Decreased	Decreased
WAT mass	Decreased	Decreased
Blood leptin	Decreased	Decreased
Blood adiponectin	Decreased	Decreased
Blood insulin	Decreased	No change
Blood glucose	Decreased	No change
Blood ghrelin	Decreased	No change
BAT *Ucp1* expression	Increased	No change

* Bagnasco et al., 2002, Bagnasco et al., 2003, Beretta et al., 2002, Boghossian et al., 2006a, Dhillon et al., 2001, Dube et al., 2002, Torto et al., 2006; see Boghossian et al., 2006b for synopsis.
** Boghossian et al., 2006b.

2.2. Experimental protocol

rAAV-Leptin or the control vector coding for green fluorescent protein (GFP; rAAV-GFP) was delivered into the DVC as described (Boghossian et al., 2006b). The rats ($n = 5$ rAAV-GFP and $n = 6$ rAAV-Leptin) were sacrificed at 10 weeks post-vector administration.

2.3. Construction and packaging of rAAV vectors

The rAAV-Leptin and rAAV-GFP vectors were constructed and packaged as described (Boghossian et al., 2006b). In brief, the vector pTR-CBA-Ob *Eco*RI fragment of pCR-rOb containing rat leptin cDNA was subcloned into rAAV vector plasmid pAAVβGEnh after deleting the EcoRI fragment carrying the β-glucoronidase cDNA sequence. The control vector, rAAV-GFP, was similarly constructed to encode the GFP gene.

2.4. rAAV vector administration

Vector administration was performed as described (Boghossian et al., 2006b). Briefly, the rats were anesthetized with ketamine (100 mg/kg) and xylazine (15 mg/kg) and stereotaxically implanted with a permanent cannula (Plastics One, Roanoke, VA) in the DVC. The stereotaxic coordinates for DVC injections were: 4.8 mm from interaural line, 0.5 mm lateral to midline, and 5.0 mm below the dura (Grill et al., 2002).

2.5. Tissue collection and analyses

Femora were excised for μCT analysis and 3rd lumbar vertebrae were excised for histomorphometric evaluation. The bones were placed in formalin for 24-h fixation and subsequently stored in 70 % ethanol at 4 °C until analysis.

2.6. μCT analysis

μCT was used for nondestructive three-dimensional evaluation of bone mass and architecture. Femora were scanned using a Scanco μCT40 scanner (Scanco Medical AG, Basserdorf, Switzerland) at a voxel size of 16 × 16 × 16 μm (55kVp X-ray voltage, 145 mA intensity, and 200 ms integration time). Filtering parameters sigma and support were set to 0.8 and 1 respectively. Bone segmentation was conducted at a threshold of 245 (scale, 0–1000) determined empirically. Cortical bone was evaluated at the femoral midshaft, and cancellous bone was evaluated in the distal femoral metaphysis. For the femoral midshaft, 10 slices (160 μm) were evaluated and total cross-sectional tissue volume (cortical and marrow volume, mm^3), cortical volume (mm^3), marrow volume (mm^3) and cortical thickness (mm) were measured. For the femoral metaphysis, 150 slices (2.4 mm) of bone were measured. Cancellous bone measurements included cancellous bone volume fraction (bone volume/tissue volume, %), connectivity density (mm^{-3}), trabecular number (mm^{-1}), trabecular separation (μm), and trabecular thickness (μm).

2.7. Histomorphometry

For histomorphometric evaluation of cancellous bone, 3rd lumbar vertebrae were dehydrated in a graded series of ethanol and xylene, and embedded undecalcified in modified methyl methacrylate as described

(Iwaniec et al., 2008). Longitudinal sections (5 μm thick) were cut with a vertical bed microtome (Leica 2065) and affixed to slides. One section/animal was stained with toluidine blue and counterstained for acid phosphatase and used for assessing bone area and cell-based measurements. Measurements evaluating the entire cancellous compartment were performed using the OsteoMeasure System (OsteoMetrics, Inc., Atlanta, GA) and included bone area fraction (bone area/tissue area, %) and cell-based measurements. Osteoblast and osteoclast perimeters were measured and expressed as % of total cancellous bone perimeter. Bone marrow adiposity (adipocyte area/tissue area, %), adipocyte density (#/mm^2), and adipocyte size (μm^2) were determined as described (Menagh et al., 2010).

2.8. Statistical analysis

Mean outcomes for the rats receiving rAAV-Leptin or rAAV-GFP (control) were compared using two-sample *t*-tests. Confirmation that the assumption of normality was not violated was made using quantile-quantile plots and the Anderson-Darling test. Levene's test was used to determine equal or unequal variance between the two groups. The Benjamini and Hochberg method (Benjamini and Hochberg, 1995) for maintaining the false discovery rate at 5 % was used to adjust for multiple comparisons. Differences were considered significant at $p \leq 0.05$. All data are presented as mean ± SE with individual animals represented as dots. Data analysis was performed using R version 4.1.2.

3. Results

The effects of leptin transgene expression in DVC on femur microarchitecture are shown in Fig. 1. Differences in cortical bone architecture (cross-sectional volume, $p = 0.956$; cortical volume, $p = 0.713$; medullary volume, p = 0.956; and cortical thickness, p = 0.713) or cancellous bone architecture (bone volume fraction, p = 0.713; connectivity density, p = 0.713; trabecular number, p = 0.713; trabecular spacing, p = 0.713; and trabecular thickness, $p = 0.956$) were not detected between rAAV-Leptin treated rats and rAAV-GFP treated rats.

The effects of leptin transgene expression in DVC on histomorphometry in lumbar vertebra are shown in Fig. 2. Differences in cancellous bone area fraction (p = 0.956), osteoclast-lined bone perimeter (p = 0.956), osteoblast-lined bone perimeter ($p = 0.713$), bone marrow adipocyte area fraction (p = 0.713), and adipocyte density (p = 0.713) and size ($p = 0.645$) were not detected between rAAV-Leptin treated rats and rAAV-GFP treated rats. A representative image depicting the features measured is shown in Fig. 2G.

4. Discussion

Long-duration (10 weeks) leptin transgene expression in the DVC of sexually mature female rats was previously shown to prevent weight gain and lower plasma leptin (Boghossian et al., 2006b). Here we show that neither bone microarchitecture nor indices of bone resorption (osteoclast-lined perimeter) nor bone formation (osteoblast-lined perimeter) were altered by leptin transgene expression in the DVC.

The present study, taken together with our earlier report on the hypothalamus, provides evidence that delivery of leptin transgene into two leptin-responsive regions of the brain (hypothalamus and DVC) having different effects on energy homeostasis have no long-duration effects on bone metabolism. This important finding in leptin-replete rats strongly suggests that increasing leptin levels in the CNS is unlikely to have a major influence on bone in rodents having circulating

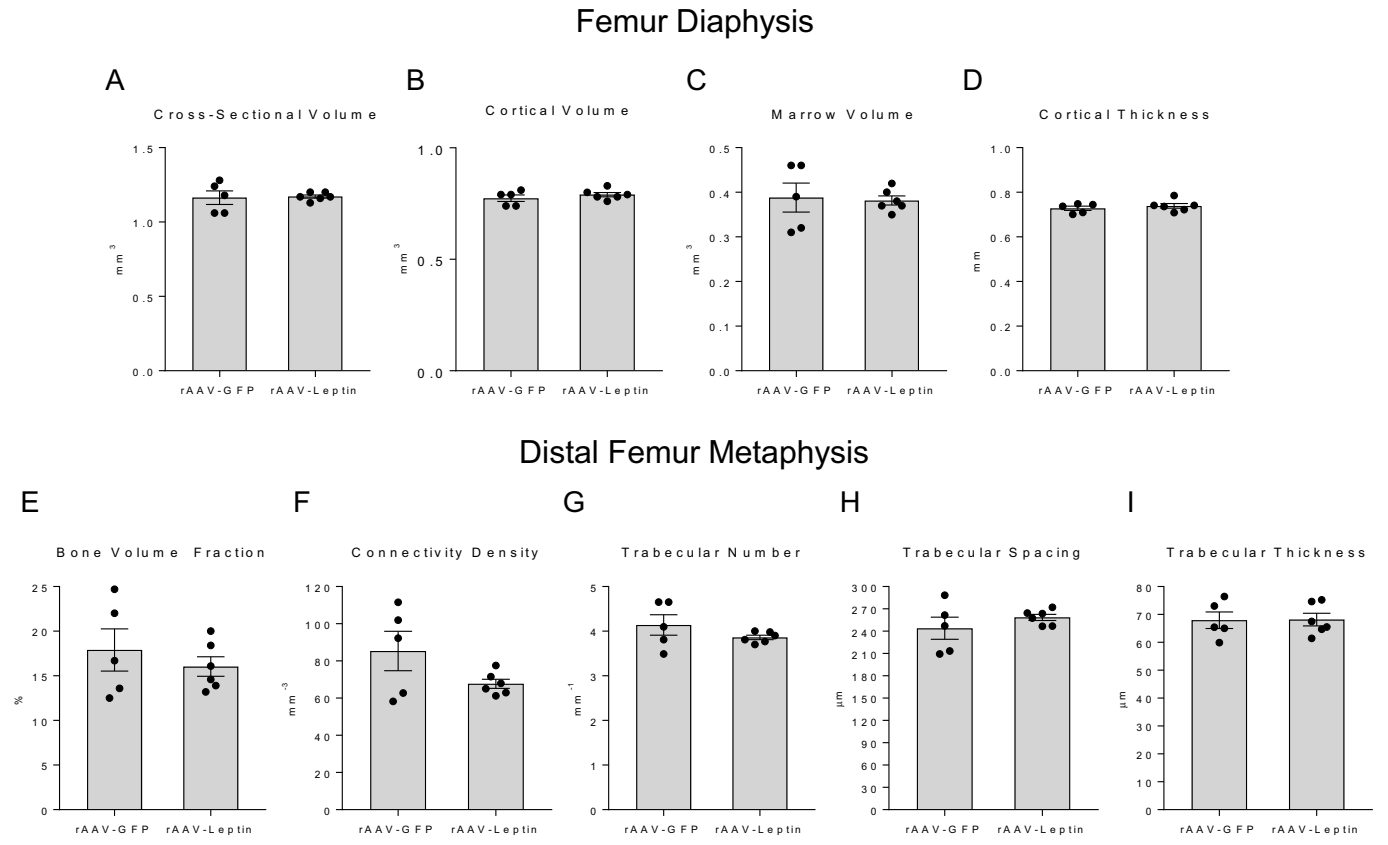

Fig. 1. Effects of 10 weeks of rAAV-Leptin gene therapy in DVC on cross-sectional volume (A), cortical volume (B), marrow volume (C), and cortical thickness (D) in midshaft femur diaphysis and on cancellous bone volume fraction (bone volume/tissue volume) (E), connectivity density (F), trabecular number (G), trabecular spacing (H), and trabecular thickness (I) in distal femur metaphysis of female Sprague-Dawley rats. Data are mean ± SE, with dots representing individual animals. *N* = 5 to 6/group.

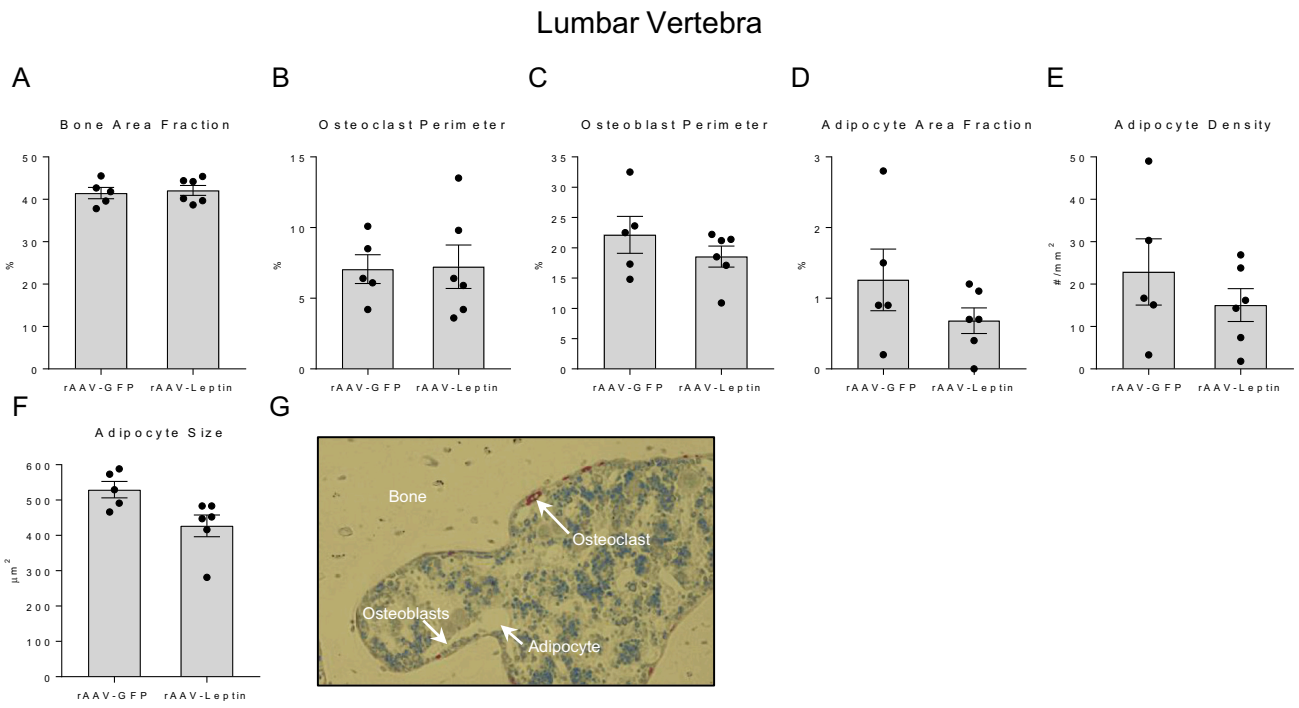

Fig. 2. Effects of 10 weeks of rAAV-Leptin gene therapy in DVC on cancellous bone area fraction (bone area/tissue area) (A), osteoclast perimeter (B), osteoblast perimeter (C), adipocyte area fraction (adipocyte area/tissue area) (D), adipocyte density (E), and adipocyte size (F) in body of 3rd lumbar vertebra of female Sprague-Dawley rats. A representative image of a histological section (stained with toluidine blue and counterstained for acid phosphatase) depicting the features measured is shown in panel G. Data are mean ± SE, with dots representing individual animals. $N = 5$ to 6/group.

levels of the adipokine above a threshold value. Based on a dose-response study performed in growing *ob/ob* mice, the threshold required for optimal bone growth and turnover is below levels of leptin required to suppress appetite or increase adaptive thermogenesis (Philbrick et al., 2017). In addition, the present study provides evidence that the differential metabolic effects of leptin transgene expression in hypothalamus *versus* transgene expression in DVC on gut and pancreatic hormones and on thermogenesis are not paralleled by differential impact on bone metabolism. This is a significant finding as increased *Ucp1* expression in BAT is associated with cancellous bone loss in mice, and glucose, insulin and ghrelin have concentration-dependent effects on bone metabolism (Iwaniec et al., 2016; Turner et al., 2022; Nikolopoulos et al., 2010; Leidig-Bruckner and Ziegler, 2001). Similar to leptin, these findings suggest that the modest magnitude of change of multiple stimulatory and inhibitory factors is insufficient to elicit changes in bone mass and architecture.

Leptin-deficient mice and mice fed a high fat diet (hyperleptinemic mice) have high levels of BMAT (Lindenmaier et al., 2016). Leptin transgene expression in the hypothalamus was shown to normalize BMAT levels in *ob/ob* mice (Lindenmaier et al., 2016). Similarly, intracerebroventricular administration of leptin to rats and *ob/ob* mice resulted in reduced serum leptin and BMAT levels (Ambati et al., 2010; Hamrick et al., 2007). These findings suggest that central leptin plays an important role in regulating BMAT. However, increasing leptin levels in the CNS does not always result in decreases in BMAT. Specifically, increasing leptin transgene expression in hypothalamus (Jackson et al., 2011; Turner et al., 2015; Iwaniec et al., 2011) or DVC (present study) in rats did not influence BMAT levels. However, calorically restricting rats to match body weight of rats expressing leptin transgene in their hypothalamus resulted in higher abdominal WAT mass, higher serum leptin and adiponectin levels, and higher BMAT (Turner et al., 2021). Taken together, these divergent findings emphasize the importance of integration of hypothalamic and peripheral signals in controlling energy partitioning and balance.

Relatively short term (≤4 weeks) administration of leptin to *ob/ob* mice had profound effects on bone mass, density, microarchitecture, growth, and turnover, which differed depending upon the mode of leptin delivery and skeletal sites investigated (Hamrick et al., 2004; Reid et al., 2018). Extended localized CNS production of leptin following delivery of rAAV-Leptin into the 3rd ventricle of the hypothalamus rescued the *ob/ob* skeletal phenotype in growing mice by increasing femoral length and total bone volume, and decreasing femoral and vertebral cancellous bone volume, such that at 15 weeks post transgene introduction the skeleton of *ob/ob* mice no longer differed from WT mice. Further skeletal changes in either the femur or lumbar vertebra were not observed at 30 weeks post-rAAV-Leptin administration (Iwaniec et al., 2007). In contrast to the dramatic effects of leptin on bone metabolism in leptin-deficient *ob/ob* mice, longer duration (10 weeks) hypothalamic leptin gene therapy had little or no effect on bone mass, density, microarchitecture, or bone cell populations in leptin-replete rats (Iwaniec et al., 2011). However, leptin transgene expression lowered body weight gain, WAT mass, and serum leptin levels. Taken together, these findings suggest that the actions of leptin on the skeleton, although important, are largely permissive. Consequently, increasing hormone levels in CNS of animals with normal circulating levels of leptin reduces body weight gain with minimal effects on bone.

Skeletal maturation is impaired in *ob/ob* mice (Burkemper and Garris, 2006; Kishida et al., 2005; Turner et al., 2014). Dose-response studies in *ob/ob* mice reveal that the stimulatory effects of leptin on longitudinal bone growth in long bones and bone formation are initiated at circulating levels of the hormone that are lower than those required for weight loss (Philbrick et al., 2017). The apparent K_m of leptin for its receptor is similar across species (Verkerke et al., 2014; Peelman et al., 2014). Based on this finding, the magnitude of decrease in leptin levels in peripheral circulation following rAAV-Leptin gene therapy is unlikely to have a major effect on bone because the levels remain high enough to maintain a vigorous skeletal response. Adoptive transfer of leptin receptor-deficient *db/db* bone marrow cells into WT mice recapitulated

the low bone formation skeletal phenotype observed in *db/db* mice (Turner et al., 2013). Taken together these findings support the conclusion that the positive effects of leptin on bone growth and maturation are mediated through direct actions of the adipokine on bone target cells.

Not all effects of leptin on the skeleton are positive. Leptin-deficient *ob/ob* mice were shown to be resistant to bone loss induced by polyethylene particles placed over calvaria (von Knoch et al., 2004), a model for inflammation-induced osteolysis. Leptin was likely responsible for the impaired response because normalization of serum leptin levels increased osteolysis in *ob/ob* mice (Philbrick et al., 2018a). Leptin is an immune modulator with properties similar to a proinflammatory cytokine, so it is not surprising that it exaggerates bone loss associated with tissue injury. This may explain, in part, the variable results reported in early studies in which leptin was infused into the hypothalamus using subcutaneously implanted osmotic pumps (Hamrick et al., 2004; Reid et al., 2018). As an example, we observed a lower cancellous bone volume fraction in long bones and lumbar vertebrae of rats 5 weeks following surgery to deliver rAAV-Leptin into the hypothalamus, but bone microarchitecture returned to control values at 10 weeks post-surgery (Iwaniec et al., 2011).

The current study has several limitations. The sample size was relatively small ($n = 5$ to 6/group). However, power analysis revealed that this sample size was sufficient to detect meaningful changes in bone architecture, and bone cells. In support, our prior gene therapy studies with n = 5 to 6/group detected ovariectomy-induced cancellous osteopenia as well as transient leptin transgene-mediated changes in cancellous bone microarchitecture, osteoblast-lined bone perimeter and bone marrow adipocyte area fraction and density (Iwaniec et al., 2011; Jackson et al., 2011). Although larger studies may detect changes, the effect size is likely to be small compared to those elicited by the principle factors influencing bone metabolism in normal growing rats (e.g., age, sex hormones, mechanical loading). Increasing leptin transgene expression in hypothalamus or DVC had no effect on bone but we cannot be certain that simultaneous activation of leptin receptors at both sites could lead to a different result. While the long-duration skeletal effects of leptin transgene expression have been studied in growing and skeletally mature female rats, similar studies have not been performed in males. Thus, future studies should evaluate the skeletal impact of increasing leptin or leptin transgene expression at multiple sites in brains of male and female rats.

In summary, despite major effects on appetite and energy balance, increasing leptin transgene expression in either the DVC (current study) or the hypothalamus (prior research) had no effect on bone mass, density, or architecture in leptin-replete female rats. These null findings do not support the view that increasing leptin signaling in the CNS above normal levels has a major influence on bone metabolism in rats.

CRediT authorship contribution statement

Russell T. Turner: Writing – review & editing, Writing – original draft, Investigation. **Adam J. Branscum:** Writing – review & editing, Formal analysis. **Urszula T. Iwaniec:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Urszula Iwaniec reports financial support was provided by National Institutes of Health. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We gratefully acknowledge the late Dr. Satya P. Kalra for providing the skeletal material for this analysis and Dr. Stéphane Boghossian for conducting the study. The animal work was supported by grants (DK37273 and NS32727) to S.P. Karla.

Funding

This work was supported by National Institutes of Health (AR060913).

References

Ambati, S., Li, Q., Rayalam, S., Hartzell, D.L., Della-Fera, M.A., Hamrick, M.W., Baile, C. A., 2010. Central leptin versus ghrelin: effects on bone marrow adiposity and gene expression. Endocrine 37, 115–123.

Bagnasco, M., Dube, M.G., Kalra, P.S., Kalra, S.P., 2002. Evidence for the existence of distinct central appetite, energy expenditure, and ghrelin stimulation pathways as revealed by hypothalamic site-specific leptin gene therapy. Endocrinology 143, 4409–4421.

Bagnasco, M., Dube, M.G., Katz, A., Kalra, P.S., Kalra, S.P., 2003. Leptin expression in hypothalamic PVN reverses dietary obesity and hyperinsulinemia but stimulates ghrelin. Obes. Res. 11, 1463–1470.

Bamshad, M., Aoki, V.T., Adkison, M.G., Warren, W.S., Bartness, T.J., 1998. Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. Am. J. Phys. 275, R291–R299.

Bamshad, M., Song, C.K., Bartness, T.J., 1999. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. Am. J. Phys. 276, R1569–R1578.

Bartell, S.M., Rayalam, S., Ambati, S., Gaddam, D.R., Hartzell, D.L., Hamrick, M., She, J. X., Della-Fera, M.A., Baile, C.A., 2011. Central (ICV) leptin injection increases bone formation, bone mineral density, muscle mass, serum IGF-1, and the expression of osteogenic genes in leptin-deficient ob/ob mice. J. Bone Miner. Res. 26, 1710–1720.

Bartness, T.J., Bamshad, M., 1998. Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. Am. J. Phys. 275, R1399–R1411.

Bartness, T.J., Kay Song, C., Shi, H., Bowers, R.R., Foster, M.T., 2005. Brain-adipose tissue cross talk. Proc. Nutr. Soc. 64, 53–64.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Beretta, E., Dube, M.G., Kalra, P.S., Kalra, S.P., 2002. 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. Pediatr. Res. 52, 189–198.

Bjorbaek, C., Kahn, B.B., 2004. Leptin signaling in the central nervous system and the periphery. Recent Prog. Horm. Res. 59, 305–331.

Boghossian, S., Dube, M.G., Torto, R., Kalra, P.S., Kalra, S.P., 2006a. Hypothalamic clamp on insulin release by leptin-transgene expression. Peptides 27, 3245–3254.

Boghossian, S., Lecklin, A., Dube, M.G., Kalra, P.S., Kalra, S.P., 2006b. Increased leptin expression in the dorsal vagal complex suppresses adiposity without affecting energy intake and metabolic hormones. Obesity (Silver Spring) 14, 1003–1009.

Bowers, R.R., Festuccia, W.T., Song, C.K., Shi, H., Migliorini, R.H., Bartness, T.J., 2004. Sympathetic innervation of white adipose tissue and its regulation of fat cell number. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286, R1167–R1175.

Buijs, R.M., Chun, S.J., Niijima, A., Romijn, H.J., Nagai, K., 2001. Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. J. Comp. Neurol. 431, 405–423.

Burkemper, K.M., Garris, D.R., 2006. Influences of obese (ob/ob) and diabetes (db/db) genotype mutations on lumber vertebral radiological and morphometric indices: skeletal deformation associated with dysregulated systemic glucometabolism. BMC Musculoskelet. Disord. 7, 10.

Chen, X.M., Hosono, T., Yoda, T., Fukuda, Y., Kanosue, K., 1998. Efferent projection from the preoptic area for the control of non-shivering thermogenesis in rats. J. Physiol. 512 (Pt 3), 883–892.

Collins, S., Yehuda-Shnaidman, E., Wang, H., 2010. Positive and negative control of Ucp1 gene transcription and the role of beta-adrenergic signaling networks. Int. J. Obes. 34 (Suppl. 1), S28–S33.

Dhillon, H., Kalra, S.P., Prima, V., Zolotukhin, S., Scarpace, P.J., Moldawer, L.L., Muzyczka, N., Kalra, P.S., 2001. 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. 99, 69–77.

Dube, M.G., Beretta, E., Dhillon, H., Ueno, N., Kalra, P.S., Kalra, S.P., 2002. Central leptin gene therapy blocks high-fat diet-induced weight gain, hyperleptinemia, and hyperinsulinemia: increase in serum ghrelin levels. Diabetes 51, 1729–1736.

Flak, J.N., Myers Jr., M.G., 2016. Minireview: CNS mechanisms of leptin action. Mol. Endocrinol. 30, 3–12.

Flier, J.S., 2004. Obesity wars: molecular progress confronts an expanding epidemic. Cell 116, 337–350.

Grill, H.J., Schwartz, M.W., Kaplan, J.M., Foxhall, J.S., Breininger, J., Baskin, D.G., 2002. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. Endocrinology 143, 239–246.
Hamrick, M.W., Pennington, C., Newton, D., Xie, D., Isales, C., 2004. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. Bone 34, 376–383.
Hamrick, M.W., Della-Fera, M.A., Choi, Y.H., Pennington, C., Hartzell, D., Baile, C.A., 2005. Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice. J. Bone Miner. Res. 20, 994–1001.
Hamrick, M.W., Della Fera, M.A., Choi, Y.H., Hartzell, D., Pennington, C., Baile, C.A., 2007. Injections of leptin into rat ventromedial hypothalamus increase adipocyte apoptosis in peripheral fat and in bone marrow. Cell Tissue Res. 327, 133–141.
Horvath, T.L., Diano, S., Tschop, M., 2004. Brain circuits regulating energy homeostasis. Neuroscientist 10, 235–246.
Iwaniec, U.T., Boghossian, S., Lapke, P.D., Turner, R.T., Kalra, S.P., 2007. Central leptin gene therapy corrects skeletal abnormalities in leptin-deficient ob/ob mice. Peptides 28, 1012–1019.
Iwaniec, U.T., Wronski, T.J., Turner, R.T., 2008. Histological analysis of bone. Methods Mol. Biol. 447, 325–341.
Iwaniec, U.T., Boghossian, S., Trevisiol, C.H., Wronski, T.J., Turner, R.T., Kalra, S.P., 2011. Hypothalamic leptin gene therapy prevents weight gain without long-term detrimental effects on bone in growing and skeletally mature female rats. J. Bone Miner. Res. 26, 1506–1516.
Iwaniec, U.T., Philbrick, K.A., Wong, C.P., Gordon, J.L., Kahler-Quesada, A.M., Olson, D. A., Branscum, A.J., Sargent, J.L., Demambro, V.E., Rosen, C.J., Turner, R.T., 2016. Room temperature housing results in premature cancellous bone loss in growing female mice: implications for the mouse as a preclinical model for age-related bone loss. Osteoporos. Int. 27, 3091–3101.
Jackson, M.A., Iwaniec, U.T., Turner, R.T., Wronski, T.J., Kalra, S.P., 2011. Effects of increased hypothalamic leptin gene expression on ovariectomy-induced bone loss in rats. Peptides 32, 1575–1580.
Jacob, R.J., Dziura, J., Medwick, M.B., Leone, P., Caprio, S., During, M., Shulman, G.I., Sherwin, R.S., 1997. The effect of leptin is enhanced by microinjection into the ventromedial hypothalamus. Diabetes 46, 150–152.
Kalra, S.P., Dube, M.G., Pu, S., Xu, B., Horvath, T.L., Kalra, P.S., 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr. Rev. 20, 68–100.
Kalra, S.P., Bagnasco, M., Otukonyong, E.E., Dube, M.G., Kalra, P.S., 2003. Rhythmic, reciprocal ghrelin and leptin signaling: new insight in the development of obesity. Regul. Pept. 111, 1–11.
Khan, S.N., Duraine, G., Virk, S.S., Fung, J., Rowland, D.J., Reddi, A.H., Lee, M.A., 2013. The temporal role of leptin within fracture healing and the effect of local application of recombinant leptin on fracture healing. J. Orthop. Trauma 27, 656–662.
Kishida, Y., Hirao, M., Tamai, N., Nampei, A., Fujimoto, T., Nakase, T., Shimizu, N., Yoshikawa, H., Myoui, A., 2005. Leptin regulates chondrocyte differentiation and matrix maturation during endochondral ossification. Bone 37, 607–621.
Leidig-Bruckner, G., Ziegler, R., 2001. Diabetes mellitus a risk for osteoporosis? Exp. Clin. Endocrinol. Diabetes 109 (Suppl. 2), S493–S514.
Lindenmaier, L.B., Philbrick, K.A., Branscum, A.J., Kalra, S.P., Turner, R.T., Iwaniec, U. T., 2016. Hypothalamic leptin gene therapy reduces bone marrow adiposity in ob/ob mice fed regular and high-fat diets. Front. Endocrinol. (Lausanne) 7, 110.
Menagh, P.J., Turner, R.T., Jump, D.B., Wong, C.P., Lowry, M.B., Yakar, S., Rosen, C.J., Iwaniec, U.T., 2010. Growth hormone regulates the balance between bone formation and bone marrow adiposity. J. Bone Miner. Res. 25, 757–768.
Mercer, J.G., Hoggard, N., Williams, L.M., Lawrence, C.B., Hannah, L.T., Trayhurn, P., 1996. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Lett. 387, 113–116.
Mercer, J.G., Moar, K.M., Hoggard, N., 1998. Localization of leptin receptor (Ob-R) messenger ribonucleic acid in the rodent hindbrain. Endocrinology 139, 29–34.
Morrison, S.F., 2001. Differential regulation of brown adipose and splanchnic sympathetic outflows in rat: roles of raphe and rostral ventrolateral medulla neurons. Clin. Exp. Pharmacol. Physiol. 28, 138–143.
Nikolopoulos, D., Theocharis, S., Kouraklis, G., 2010. Ghrelin, another factor affecting bone metabolism. Med. Sci. Monit. 16, RA147-62.
Peelman, F., Zabeau, L., Moharana, K., Savvides, S.N., Tavernier, J., 2014. 20 years of leptin: insights into signaling assemblies of the leptin receptor. J. Endocrinol. 223, T9–23.
Philbrick, K.A., Wong, C.P., Branscum, A.J., Turner, R.T., Iwaniec, U.T., 2017. Leptin stimulates bone formation in ob/ob mice at doses having minimal impact on energy metabolism. J. Endocrinol. 232, 461–474.
Philbrick, K.A., Branscum, A.J., Wong, C.P., Turner, R.T., Iwaniec, U.T., 2018a. Leptin increases particle-induced osteolysis in female ob/ob mice. Sci. Rep. 8, 14790.
Philbrick, K.A., Martin, S.A., Colagiovanni, A.R., Branscum, A.J., Turner, R.T., Iwaniec, U.T., 2018b. Effects of hypothalamic leptin gene therapy on osteopetrosis in leptin-deficient mice. J. Endocrinol. 236, 57–68.
Reid, I.R., Baldock, P.A., Cornish, J., 2018. Effects of leptin on the skeleton. Endocr. Rev. 39, 938–959.
Salum, K.C.R., Rolando, J.M., Zembrzuski, V.M., Carneiro, J.R.I., Mello, C.B., Maya-Monteiro, C.M., Bozza, P.T., Kohlrausch, F.B., Da Fonseca, A.C.P., 2021. When leptin is not there: a review of what nonsyndromic monogenic obesity cases tell us and the benefits of exogenous leptin. Front. Endocrinol. (Lausanne) 12, 722441.
Steppan, C.M., Crawford, D.T., Chidsey-Frink, K.L., Ke, H., Swick, A.G., 2000. Leptin is a potent stimulator of bone growth in ob/ob mice. Regul. Pept. 92, 73–78.
Sved, A.F., Cano, G., Card, J.P., 2001. Neuroanatomical specificity of the circuits controlling sympathetic outflow to different targets. Clin. Exp. Pharmacol. Physiol. 28, 115–119.
Torto, R., Boghossian, S., Dube, M.G., Kalra, P.S., Kalra, S.P., 2006. Central leptin gene therapy blocks ovariectomy-induced adiposity. Obesity (Silver Spring) 14, 1312–1319.
Turner, R.T., Iwaniec, U.T., 2011. Low dose parathyroid hormone maintains normal bone formation in adult male rats during rapid weight loss. Bone 48, 726–732.
Turner, R.T., Kalra, S.P., Wong, C.P., Philbrick, K.A., Lindenmaier, L.B., Boghossian, S., Iwaniec, U.T., 2013. Peripheral leptin regulates bone formation. J. Bone Miner. Res. 28, 22–34.
Turner, R.T., Philbrick, K.A., Wong, C.P., Olson, D.A., Branscum, A.J., Iwaniec, U.T., 2014. Morbid obesity attenuates the skeletal abnormalities associated with leptin deficiency in mice. J. Endocrinol. 223, M1–15.
Turner, R.T., Dube, M., Branscum, A.J., Wong, C.P., Olson, D.A., Zhong, X., Kweh, M.F., Larkin, I.V., Wronski, T.J., Rosen, C.J., Kalra, S.P., Iwaniec, U.T., 2015. Hypothalamic leptin gene therapy reduces body weight without accelerating age-related bone loss. J. Endocrinol. 227, 129–141.
Turner, R.T., Wong, C.P., Fosse, K.M., Branscum, A.J., Iwaniec, U.T., 2021. Caloric restriction and hypothalamic leptin gene therapy have differential effects on energy partitioning in adult female rats. Int. J. Mol. Sci. 22.
Turner, R.T., Nesser, K.L., Philbrick, K.A., Wong, C.P., Olson, D.A., Branscum, A.J., Iwaniec, U.T., 2022. Leptin and environmental temperature as determinants of bone marrow adiposity in female mice. Front. Endocrinol. (Lausanne) 13, 959743.
Verkerke, H., Naylor, C., Zabeau, L., Tavernier, J., Petri Jr., W.A., Marie, C., 2014. Kinetics of leptin binding to the Q223R leptin receptor. PLoS One 9, e94843.
Von Knoch, M., Jewison, D.E., Sibonga, J.D., Turner, R.T., Morrey, B.F., Loer, F., Berry, D.J., Scully, S.P., 2004. Decrease in particle-induced osteolysis in obese (ob/ob) mice. Biomaterials 25, 4675–4681.
Webber, J., Macdonald, I.A., 2000. Signalling in body-weight homeostasis: neuroendocrine efferent signals. Proc. Nutr. Soc. 59, 397–404.
Wynne, K., Stanley, S., McGowan, B., Bloom, S., 2005. Appetite control. J. Endocrinol. 184, 291–318.