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Research report

Neonatal leptin deficiency reduces frontal cortex volumes and programs adult hyperactivity in mice

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HIGHLIGHTS

- Perinatal growth restriction increases the risk of adult psychiatric disease.
- Growth restriction decreases circulating leptin, a neurotrophic hormone.
- Neonatal leptin deficiency decreases frontal lobe volume and programs hyperactivity.
- Increased leptin receptor expression suggests a potential therapeutic intervention.

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ABSTRACT

Intrauterine growth restriction and premature delivery decrease circulating levels of the neurotrophic hormone leptin and increase the risk of adult psychiatric disease. In mouse models, neonatal leptin replacement normalizes brain growth and improves the neurodevelopmental outcomes of growth restricted mice, but leptin supplementation of well-grown mice decreases adult locomotor activity. We hypothesized isolated neonatal leptin deficiency is sufficient to reduce adult brain volumes and program behavioral outcomes, including hyperactivity. C57Bl/6 pups were randomized to daily injections of saline or PEG-leptin antagonist (LX, 12.5 mg/kg) from postnatal day 4 to 14. After 4 months, fear conditioning and open field testing were performed followed by carotid radiotelemetry for the measurement of baseline activity and blood pressure. Neonatal LX did not significantly increase cue-based fear or blood pressure, but increased adult locomotor activity during assessment in both the open field (beam breaks: control 930 ± 40, LX 1099 ± 42, $P < 0.01$) and the home cage (radiotelemetry counts: control 4.5 ± 0.3, LX 5.6 ± 0.3, $P = 0.02$). Follow-up MRI revealed significant reductions in adult frontal cortex volumes following neonatal LX administration (control $45.1 \pm 0.4\,mm^3$, LX $43.8 \pm 0.4\,mm^3$, $P = 0.04$). This was associated with a significant increase in cerebral cortex leptin receptor mRNA expression. In conclusion, isolated neonatal leptin deficiency increases cerebral cortex leptin receptor expression and reduces frontal cortex volumes in association with increased adult locomotor activity. We speculate neonatal leptin deficiency may contribute to the adverse neurodevelopmental outcomes associated with perinatal growth restriction, and postnatal leptin therapy may be protective.

1. Introduction

Individuals with a history of low birth weight as a result of intrauterine growth restriction (IUGR) or prematurity are at increased risks of adult psychiatric disease [1–3]. While the origins of adult disease are multifactorial, IUGR and prematurity are both associated with suboptimal nutritional delivery and impaired brain growth during the formative phases of development [2,4,5]. Global nutritional interventions can improve neurodevelopmental outcomes, but accelerated neonatal growth may increase adiposity

Abbreviations: LX, Pegylated super murine leptin antagonist; IUGR, Intrauterine growth restricted.

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and obesity-related morbidities [6–8]. Targeted interventions that support brain growth without excessive somatic growth may thus optimize long-term neurologic and metabolic outcomes.

Leptin, a critical component of adult metabolic and cardiovascular regulation, is neurotrophic factor during perinatal development [9,10]. As a product of the mature adipocyte that readily crosses the placenta, circulating leptin levels correlate with adiposity and are significantly reduced in both IUGR and premature infants [11,12]. In mice and humans with mutations in the leptin gene, exogenous leptin administration improves brain growth in newborns as well as in adults [10,13].

To further clarify the programming effects of leptin, a number of animal models have been employed. In IUGR piglets, neonatal leptin administration elicits neurotrophic effects [14]. Because of their delayed developmental trajectory, neonatal rats and mice are utilized to further model the critical third trimester of human development. In these studies, neonatal growth restriction decreases circulating leptin, impairs neurodevelopment, and elicits adult hypertension [15–17], while neonatal leptin supplementation normalizes blood pressure and brain growth [17]. Likewise, leptin null mice have decreased adult brain weights and impaired hypothalamic projections that improve with neonatal leptin therapy [9,10]. The fact that neonatal leptin administration elicits neurotrophic effects in leptin deficient mice does not provide a causal link between neonatal leptin deficiency and the programmed adult phenotypes. To clarify the importance of neonatal leptin exposure, we tested the hypothesis that isolated neonatal leptin deficiency is sufficient to reduce brain growth and program adult behavior in the absence of neonatal growth restriction.

2. Methods

2.1. Animal model

All procedures were approved by the University of Iowa Animal Care and Use Committee and comply with the guidelines of the Animal Welfare Act, National Institutes of Health guidelines on the care and use of laboratory animals. C57BL/6J mice delivered naturally and offspring were weighed within 24 h of delivery. To avoid the confounding effects of perinatal growth restriction, mice with birth weights below the 10th percentile were excluded, and all litters were culled to 6 pups to facilitate postnatal growth [17]. To assess the programming effects of neonatal pegylated super murine leptin antagonist (LX) exposure, pups within each litter were randomized to daily intraperitoneal injections of either LX (12.5 mg/kg from day 4 to 14) or vehicle alone (10 ml/kg of normal saline) [18,19]. Pup weights were obtained daily from day 4 to 14 and again on weaning day 21. Adult phenotypes, including body weight and feed intake, were evaluated beginning at an attained age of 4 to 6 months. To verify LX's biologic activity, feed intake and body weight were recorded over a 5 day baseline period for 6 adult control mice. Those 6 mice were then randomized to 5 daily injections of either LX (12.5 mg/kg/day) or saline alone (10 ml/kg/day) and feed intake/body weight were again recorded.

2.2. Behavioral phenotypes

Behavioral phenotypes were first assessed during a fear conditioning protocol, as previously described [17]. On the first day, mice were trained to associate a tone (80 dB, 20 s) with a co-terminated electric foot-shock (0.5 mA, 1 s) a total of 5 times at 2 min intervals. The time spent in a characteristic "freezing" posture was digitally captured and recorded. The following day, cue conditioned fear was assessed by recording freezing during 3 min of baseline, 3 min of representation of the learned auditory cue, and 4 min of recovery. The following week, open field testing was performed [17]. Mice were placed into a 40.6 cm by 40.6 cm brightly illuminated field containing an invisible laser beam grid. The frequency of beam breaks was utilized to measure the duration and intensity of locomotor activity.

2.3. Tail cuff blood pressure

Following the behavioral testing, blood pressures and heart rates were recorded by indirect tail-cuff apparatus (BP 2000; Visitech Systems, Apex, NC). Thirty cycles were completed daily for 5 days, as previously detailed [17].

2.4. Brain morphology

Prior to the planned radiotelemetry studies, MRI was completed with a 4.7 T Varian Unity/INOVA system (Varian Inc., Palo Alto, CA) [17]. Anesthesia was provided by isoflurane with titration based upon respiratory status and pedal reflexes. T1 and T2-weighted images were acquired in axial, sagittal, and coronal planes. MRI acquisition and processing were completed by investigators blinded to group assignment.

2.5. Radiotelemetry

At 7–8 months of age, radiotelemetry catheters (PA-C10; Data Sciences International) were implanted in the left carotid artery of male mice [20]. All telemetry implants were performed using isoflurane titrated based upon respiratory status and pedal reflexes. Local anesthesia was provided with 0.5% bupivacaine application along the surgical incision and analgesia was provided with subcutaneous flunixin (2.5 mg/kg once or twice daily for 24 h). After a 7 day recovery period, arterial pressures, heart rate and relative locomotor activity were sampled for 10 s every 5 min for 60 h (encompassing 3 dark cycles and 2 light cycles). During the subsequent light cycle, blood pressure and heart rate were recorded before and after mice were transferred to a metabolic cage (Hatteras Instruments) that restricts movement and evokes a psychological stress response [21]. Following the telemetry studies, mice were euthanized by removal of the heart during isoflurane anesthesia.

2.6. Leptin receptor expression

Given the effects of estrus cycling on blood pressure, female mice were utilized for gene expression analysis, rather than radiotelemetry. Under isoflurane anesthesia titrated to respiratory status, the brain was excised, then segmented into cerebral cortex and midbrain segments, as previously described [22]. The "midbrain" segment included the thalamus and hypothalamus. Quantitative real-time RT-PCR (qPCR) utilized the TaqMan reagent and instrumentation systems (Applied Biosystems, Foster City, CA). Taqman gene expression assay primer/probe sets for mouse leptin receptor (Lepr; assay ID = Mm00440181_m1) and GAPDH were purchased from Applied Biosystems (Foster City, CA).

2.7. Data analysis

All values are presented as mean ± standard error of the mean. Each group was comprised of mice from at least 8 separate litters. Whenever phenotypes were obtained from a single sex (radiotelemetry and RT-PCR), data were compared by unpaired two-tailed Student's *t* test. All other data were compared by 2-way ANOVA, factoring for sex and LX administration. Post hoc analysis (Holm–Sidak method) was performed if statistically significant