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Testosterone interacts with the feedback mechanisms engaged by Tyr985 of the leptin receptor and diet-induced obesity

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ABSTRACT

Inhibitory signaling through Tyr985 of the leptin receptor contributes to the attenuation of anorectic leptin action in obese animals. Leptin receptor (LEPR-B) Tyr985Leu homozygote mutant mice (termed *l/l*) were previously generated to study Tyr985's contributions to inhibition of LEPR-B signaling; young female *l/l* mice display a lean, leptin-sensitive phenotype, while young male *l/l* are not significantly different from wild-type. We report here that testosterone (but not estrogen) determines the sex-specificity of the *l/l* phenotype. This provides additional insight into the cellular mechanism by which gonadal hormones determine central sensitivity to leptin, and may help elucidate the long-noted sex differences in leptin sensitivity. Additionally, we observed that Tyr985 signaling protects against a diet-dependent switch that exacerbates obesity with high fat feeding, such that the enhanced leptin sensitivity of *l/l* mice on a normal diet leads to increased adiposity in the face of chronic high-fat diet.

1. Introduction

The rapid progression of the obesity pandemic in both the developing and developed world carries with it an increased burden of a number of comorbidities, such as cardiovascular disease, type 2 diabetes mellitus, and other chronic conditions, costing an estimated $78.5 billion annually in medical spending in the United States [1–3]. It is estimated that by 2048, all American adults will be obese or overweight [4], further increasing the burden on health care services and expenditures.

The obese state arises from chronic positive energy balance. Leptin, a protein hormone produced by adipocytes, acts as a lipostatic control system to modulate metabolic and energy homeostasis, communicating the status of energy stores from the periphery to the central nervous system [5–7]. Leptin exerts anorectic effects in the hypothalamus to decrease feeding and increase metabolic rate [6,8,9]. In the obese state, the ability of leptin to promote leptin receptor signaling, produce weight loss, and suppress feeding is diminished [10,11].

Leptin signaling occurs through the long form of the leptin receptor (LEPR-B), a cytokine-family receptor, and is necessary for leptin's metabolic effects [12–14]. Upon the binding of leptin to LEPR-B, LEPR-B-associated Janus Kinase 2 transphosphorylates and subsequently phosphorylates LEPR-B cytoplasmic tyrosine residues at amino acid positions 985, 1077, and 1138 [15,16]. Tyr1138 activation allows for phosphorylation of the signal transducer and activator of transcription-3 (STAT3), which is then trans located to the nucleus to initiate its transcriptional effects, promoting satiety in the hypothalamus [15,17–19]. One of the target genes of nuclear activated STAT3 is the suppressor of cytokine signaling-3 (SOCS3), which is upregulated by leptin signaling [20]. Tyrosine 985 of LEPR-B, when phosphorylated, recruits SOCS3 to LEPR-B, causing SOCS3 to act as an intracellular inhibitor of LEPR-B, decreasing LEPR-B signaling [21,22]. This interaction is thought to be key to the impairment of LEPR-B signaling and action on obese animals [20,23,24].

To study the contribution of Tyr985 to LEPR-B signaling, a mouse model containing homologous replacement of tyrosine with leucine at amino acid residue 985 of LEPR-B was previously created (homozygotes termed *l/l*) [25]. As previously hypothesized, *l/l* mice displayed hypophagia, decreased adiposity, decreased insulin,

Abbreviations: LEPR-B, leptin receptor; CAS, castrated; OVX, ovariectomized; E2, estradiol; T, testosterone; V, vehicle; LDLr−/−, low density lipoprotein receptor knockout; ApoE−/−, apolipoprotein E knockout; HFD, high fat diet; AR, androgen receptor; IHC, immunohistochemical; PMv, ventral premammillary nucleus; ARC, arcuate nucleus; VMH, ventromedial hypothalamus; DIO, diet-induced obesity.

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and decreased leptin. However, the resultant *l/l* phenotype was significantly sex-biased, such that males displayed little of the lean, hypophagic phenotype of females; this was unexpected and is the focus of this report.

We have investigated the origin of the sex-specificity of the *l/l* phenotype. We hypothesized that gonadal-derived steroids were responsible for the sex-biased expression of the lean *l/l* phenotype. To this end, *l/l* mice were castrated (CAS), ovariectomized (OVX), or sham-operated and administered testosterone (T) or 17β-estradiol (E2) treatment via mini-osmotic pump. Furthermore, results of the sex hormone experimentation led us to hypothesize and test the possibility that signaling via Tyr985 modulated the response to a chronic high fat diet.

2. Methods

2.1. Animal subjects

LEPR-B Tyr985Leu mice were previously generated [25]. *l/l* mice in this study were generated by intercross of *l/l* parents, except in the case of the longitudinal high fat feeding study, where mice were generated by an *l/+* intercross. All breeder mice were backcrossed greater than 10 generations to a C57BL/6J background. Some mice in the study contained a low density lipoprotein receptor knockout (LDLr −/−) background originally for atherosclerosis studies; however, no difference in phenotypic response to experimentation was observed between LDLr −/− and LDLr +/+ mice in the parameters measured in this study. Additionally, some mice in the longitudinal high fat study contained an Apolipoprotein E knockout (ApoE −/−) background, and are denoted as such. LEPR-B +/+ males for the castration studies were purchased from the Jackson Laboratory (Bar Harbor, Maine). *Lepr*^{EGFP} mice were generated as described previously [26]. Mice were kept on a 12:12-h light:dark cycle in temperature-controlled facilities, with ad libitum access to 5.4% kcal from fat chow (Lab Diet 5053, Lab Diet, Brentwood, MO) (normal chow) and water, unless otherwise specified. The high fat diet (HFD) was composed of 45% kcal from fat (Research Diets D12451 chow, Research Diets, New Brunswick, New Jersey). All animal care and experimentation were overseen and approved by the University of Michigan Committee on Use and Care of Animals.

2.2. Ovariectomy procedure

Female mice were anesthetized with pentobarbital. Bilateral incisions were made to the left and right of the spine and posterior of the lowest rib. A ligature was placed around the fallopian tube near the ovary before excision of the ovary. Following surgery, analgesic (carprofen, 5 mg/kg) was administered, and mice were allowed 2 weeks recovery before pump implantation. Super physiologic and sub physiologic serum estradiol levels were confirmed in a sample of E2-replaced and non-replaced OVX females, respectively.

2.3. Castration procedure

Male mice were anesthetized with pentobarbital. An incision was made between midline and the left leg. Each testicle was isolated, a ligature placed around the *vas deferens*, and the testicle was removed. Following surgery, analgesic (carprofen, 5 mg/kg) was administered, and mice were allowed either 1–2 weeks recovery before mini-osmotic pump implantation or 4 weeks before sacrifice. Sham-operated males underwent incision and loosening of the tunic only; isolation of the testes during sham-surgery was not attempted, to ensure minimal damage of testes and continuation of sex hormone production. Efficacy of castration or hormone replacement treatment on serum testosterone was validated by a testosterone RIA assay on a sample of castrated, sham-operated, and hormone replaced animals.

2.4. Osmotic pump implantation

Mice were anesthetized with short-acting inhaled anesthetic (isoflurane). Mini-osmotic pumps (Alzet Model 1002, Durect Corporation, Cupertino, CA, USA) were implanted subcutaneously in the anterior back, delivering testosterone (Sigma, St. Louis, MO, USA, 1 mg/kg/day) or vehicle (10% ETOH, 90% propylene glycol) for two weeks, and mini-osmotic pumps (Model 1004, Durect Corporation) were implanted subcutaneously in the anterior back delivering 17β-estradiol (Sigma, 0.2 mg/kg/day) or vehicle (3% ETOH, 97% PBS) for four weeks. Estradiol treatment duration was longer than testosterone treatment duration to ensure that sufficient treatment duration would be given to develop an estrogen-dependent phenotype, as a testosterone-dependent phenotype was already evident.

2.5. Adipose depot measurement

Periuterine and epididymal adipose depot wet tissue weights were measured immediately following sacrifice of the animal and excision of the fat pad, either 4-weeks post castration or in the last two days of mini-osmotic pump hormone delivery.

2.6. Serum analyses

Serum testosterone and 17β-estradiol were measured using RIA kits for mouse testosterone and 17β-estradiol (Siemens Healthcare Diagnostics, Deerfield, IL, USA) to ensure removal of gonadal sex hormone production and efficacy of sex hormone replacement. Serum testosterone and 17β-estradiol measurements were completed by the University of Virginia Ligand Assay and Analysis Core (Charlottesville, VA, USA).

2.7. Statistics

Statistical significance of differences between two groups was analyzed by a two-tailed Student's *t* test. A two-way ANOVA was used to analyze the gender by LEPR-B genotype main effects and interactions for measures of adiposity in the long-term feeding studies of ApoE deficient and LDLr deficient mice. Post hoc comparisons, where appropriate, were conducted with Tukey's test. In all statistical tests a value of $p < 0.05$ was accepted as significant. All data are presented as mean ± SEM.

3. Results

3.1. Castration of l/l males

Mice were castrated at 6 weeks of age, and sacrificed at 10 weeks of age. Castration caused a decrease in epididymal fat pad weight of *l/l* males ($p < 0.05$, Fig. 1), but no change of epididymal fat pad weight in +/+ males (sham: 1.16 ± 0.11 as % body weight ($n = 10$), castrated: 1.20 ± 0.12 as % body weight ($n = 9$), $p = 0.7$). The apparent difference between +/+ and *l/l* animals is likely due to a difference in facilities, as +/+ mice were purchased from JAX while *l/l* mice were bred in-house. In a sacrifice of +/+ and *l/l* animals generated in-house, no difference is apparent in adiposity (+/+: 1.05 ± 0.11 as % body weight ($n = 5$), *l/l*: 0.84 ± 0.08 as % body weight ($n = 7$), $p = 0.14$).