Leptin receptor null mice with reexpression of LepR in GnRHR expressing cells display elevated FSH levels but remain in a prepubertal state

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1Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan; 2Department of Pharmacology and Toxicology, University of Saarland School of Medicine, Homburg, Germany; and 3Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan

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Allen SJ, Garcia-Galiano D, Borges BC, Burger LL, Boehm U, Elias CF. Leptin receptor null mice with reexpression of LepR in GnRHR expressing cells display elevated FSH levels but remain in a prepubertal state. Am J Physiol Regul Integr Comp Physiol 310: R1258–R1266, 2016. First published April 13, 2016; doi:10.1152/ajpregu.00529.2015.—Leptin signals energy sufficiency to the hypothalamo-pituitary-gonadal (HPG) axis. Studies using genetic models have demonstrated that hypothalamic neurons are major players mediating these effects. Leptin receptor (LepR) is also expressed in the pituitary gland and in the gonads, but the physiological effects of leptin in these sites are still unclear. Female mice with selective deletion of LepR in a subset of gonadotropes show normal pubertal development but impaired fertility. Conditional deletion approaches, however, often result in redundancy or developmental adaptations, which may compromise the assessment of leptin’s action in gonadotropes for pubertal maturation. To circumvent these issues, we adopted a complementary genetic approach and assessed if selective reexpression of LepR only in gonadotropes is sufficient to enable puberty and improve fertility of LepR null female mice. We initially assessed the colocalization of gonadotropin-releasing hormone receptor (GnRHR) and LepR in the HPG axis using GnRHR-ires-Cre (GRIC) and LepR-Cre reporter (tdTomato or enhanced green fluorescent protein) mice. We found that GRIC and leptin-induced phosphorylation of STAT3 are expressed in distinct hypothalamic neurons. Whereas LepR-Cre was observed in theca cells, GRIC expression was rarely found in the ovarian parenchyma. In contrast, a subpopulation of gonadotropes expressed the LepR-Cre reporter gene (tdTomato). We then crossed the GRIC mice with the LepR null reactivable (LepRonTB) mice. These mice showed an increase in FSH levels, but they remained in a prepubertal state, and no sexual maturation or reproductive capacity is attained (13). Using the Cre-loxP system, we reexpressed endogenous LepRs selectively in gonadotropes using the gonadotropin-releasing hormone (GnRH) receptor (Gnrhr)-internal ribosome entry site (ires)-Cre (GRIC) mouse model (50). We assessed whether leptin action only in those cells is sufficient to induce puberty and improve fertility in otherwise infertile LepR null female mice.

METHODS

Animals. The GnRHR-ires-Cre (GRIC) (50), the LepR-Cre (JAX mice, stock no. 008320) (17), the LepRonTB (kindly provided by Dr. Elmqquist, UTSW Medical Center, Dallas, TX, available in JAX mice, stock no. 018989) (5), the R26 tdTomato (JAX mice, stock no.
007914) (36), the R26 enhanced green fluorescent protein (eGFP) (JAX mice, stock no. 004077) (25, 26) and db/db (JAX mice, stock no. 000697) mice were kept in the University of Michigan animal facility in a light- (12 h on/off) and temperature-controlled (21–23 °C) environment with free access to water and food. Mice were fed phytoestrogen-reduced Harlan diet 2016 (16% protein/4% fat), except during breeding when mice were fed higher protein and fat phytoestrogen-reduced Harlan diet 2019 (19% protein/8% fat) (Teklad Global Rodent diet, Harlan Laboratories). All procedures and experiments were carried out in accordance with the guidelines established by the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and approved by the University of Michigan Committee on Use and Care of Animals (Animal Protocol no. 04380).

The GRIC mouse is a knock-in strain that coexpresses Cre-recombinase with the 

\[ \text{Gnrhr} \]

gene. The LepR-Cre line is also a knock-in strain with an IRES-Cre sequence inserted immediately 3′ of the stop codon of the \n
\[ \text{Lepr} \]

gene. The \n
\[ \text{LepR}^{\text{loxTB}} \]

mice have a \n
\[ \text{loxP} \]

-flanked transcription-blocking (TB) cassette inserted between \n
\[ \text{exons} 16 \text{ and } 17 \]

of the \n
\[ \text{Lepr} \]

gene, allowing the generation of mice lacking the long isoform of LepR in a conditional Cre recombinase-dependent approach. The ROSA26 (R26)-tdTomato and R26-eGFP mice carry targeted mutations of the R26 locus with a \n
\[ \text{loxP} \]

-flanked TB cassette preventing the expression of CAG promoter-driven tdTomato or eGFP reporters, respectively. Cre-mediated excision of the \n
\[ \text{loxP} \]

-flanked TB cassette allows expression of the red fluorescent protein variant tdTomato or of the eGFP.

All mice were tail-genotyped before and after experiments by extracting DNA (RED Extract-N-Amp Tissue PCR Kit catalog no. XNAT, Sigma, St. Louis, MO) and performing PCR. The primers used for genotyping are described in Table 1. The \n
\[ \text{db/db} \]

mice were directly purchased from JAX mice and were not genotyped.

**Perfusion and histology.** Mice were deeply anesthetized with isoflurane (Fluriso, Vet One, Boise, ID) and perfused intracardially with 10% buffered formalin (Sigma). Brains, pituitary gland, uterus, and ovaries were dissected. Brains were sectioned on a cryostat (14-μm sections) in the frontal plane. Pituitary glands and ovaries were sectioned using a cryostat (14-μm sections, 3 series) or microtome (5-μm sections). The DsRed (tdTomato) was targeted to the nucleus using 0.05% diaminobenzidine and 0.05% nickel sulfate as chromogen. The ovaries were dissected on a freezing microtome (30-μm sections, 5 series) in the frontal plane. Pituitary glands and ovaries were sectioned using a cryostat (14-μm sections, 3 series) or microtome (5-μm sections). The DsRed (tdTomato) was assessed in series of brain, pituitary gland, and ovary sections from GRIC and LepR-Cre reporter mice. The visualization of eGFP was performed after an amplification step using immunohistochemistry.

**Leptin administration.** To evaluate the colocalization of LepR and GnRHR, a group of GRIC-tdTomato female mice (n = 4) was fasted overnight and received intraperitoneal leptin [5 μg/g, purchased from Dr. A. F. Parlow, Harbor-UCLA Medical Center, Torrance, CA, through the National Hormone and Peptide Program (NHP)] to assess leptin-induced phosphorylation of STAT3 immunoreactivity (pSTAT3-ir), as described before (18, 19). Two hours after leptin administration, mice were weighed immediately after euthanasia, placed in 10% formalin, and sectioned and dissected to assess the presence of the \n
\[ \text{Lepr}^{\text{loxTB}} \]

and \n
\[ \text{LepR}^{\text{loxTB}} \]

mice to avoid germline transmission of the \n
\[ \text{LepR}^{\text{loxTB}} \]

strain with an IRES-Cre sequence inserted immediately 3′ of the \n
\[ \text{Gnrhr} \]

gene, allowing the generation of mice lacking the long form of the \n
\[ \text{Gnrhr} \]

gene. The \n
\[ \text{LepR}^{\text{loxTB}} \]

mice have a \n
\[ \text{loxP} \]

-flanked TB cassette flanked TB cassette allowing expression of the red fluorescent protein variant tdTomato or of the eGFP.

**Endogenous reexpression of LepR in GnRHR cells.** Mice with expression of LepR only in GnRHR cells were generated by crossing the \n
\[ \text{LepR}^{\text{loxTB}} \]

with \n
\[ \text{GnRHR} \]

mice. Mice homozygous for \n
\[ \text{LepR}^{\text{loxTB+}} \]

allele (null for LepR long form) are obese, diabetic, and infertile. Hemizygous (\n
\[ \text{LepR}^{\text{loxTB+}}/\text{LepR}^{\text{loxTB+}} \]

) male mice were bred with \n
\[ \text{GnRHR} \]

females to generate our breeders. Female \n
\[ \text{GRIC}^{+/+} \text{LepR}^{\text{loxTB+/+}} \]

mice were crossed with male \n
\[ \text{GRIC}^{+/+} \text{LepR}^{\text{loxTB+/+}} \]

mice to avoid germline activation of Cre recombinase by the male breeder (48). Genotypes of the resulting progeny were produced at the expected Mendelian ratio. Groups were divided into wild-type (control with no \n
\[ \text{GRIC} \]

or \n
\[ \text{LepR}^{\text{loxTB}} \]

allele), \n
\[ \text{LepR}^{\text{loxTB+}} \]

(control homozygous for \n
\[ \text{LepR}^{\text{loxTB}} \]

allele and no \n
\[ \text{GRIC} \]

allele) and \n
\[ \text{GRIC} \text{LepR}^{\text{loxTB+}} \]

experimental mice). At the end of the experiment, genotypes were confirmed, and hypothalamic, pituitary glands, and tail tips were processed with the Sigma kit and \n
\[ \text{LepR}^{\text{loxTB}} \]

primers to assess Cre activity and DNA recombination (defined by using the pDsI Reac primer, 5′ CCC AAG GCC ATA CAA GTG TT 3′, band size = 600 bp).

The ovaries of wild-type, \n
\[ \text{LepR}^{\text{loxTB}} \]

and \n
\[ \text{GRIC} \text{LepR}^{\text{loxTB+}} \]

mice were placed in 10% Formalin (Sigma), stored at 4°C and submitted to the Unit for Laboratory Animal Medicine in vivo animal core of the University of Michigan for the standard paraffin embedding procedure and hematoxylin and eosin staining (5-μm sections). The presence of corpora lutea and follicles in different stages was evaluated. The uteri were weighed immediately after euthanasia, placed in 10% formalin, and photographed without further preparation.

**Body weight and reproductive phenotyping.** Mice were weighed weekly between 5 and 15 wk of age. They were monitored for puberty onset [vaginal opening (VO)] and sexual maturation (until 10 mo of age). Females were further tested for fertility by breeding with sexually experienced males. Three trials of fertility testing were

Table 1. List of primers used for genotyping of mouse models

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Forward</th>
<th>Reverse</th>
<th>Source</th>
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<td>5′CTTTCCCTAAAACCCCTGCCTG3′</td>
<td>5′TGATCTAAAGGAAGTAATGATCC3′</td>
<td>Wen et al. (50)</td>
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<tr>
<td>LepR-Cre</td>
<td>5′TGCTTCTGCTGGCTTGGCTG3′</td>
<td>5′GTGAAAGAGCATGTGTC3′</td>
<td>stock no. 08320*</td>
</tr>
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<td>R26-tdTom</td>
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<td>5′GGCATTAAGGGAGGTAT3′</td>
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<td>R26-eGFP</td>
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<td>LepRloxTB</td>
<td>5′CAAGGTGCAGACAAAGGTTT3′</td>
<td>5′TGGGCGGCAACCCACATTTA3′</td>
<td>stock no. 18989*</td>
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*Commercially available in JAX mice.
performed \((n = 3–4/\text{genotype})\). Each trial was set up to include three to four cages of mice. Each cage housed one \textit{LepR}^{loxTR} female, one \textit{GRIC}-\textit{LepR}^{loxTR} female, and one wild-type female for 4–6 wk. Fertility was assessed by the presence of live pups. The estrous cycles of wild-type females were monitored by vaginal cytology (6), and mice were euthanized during the diestrus phase. At the time of euthanasia, mutant mice were also assessed for uterus size, and ovaries were collected to evaluate the presence of corpora lutea.

**Quantitative PCR.** Adult wild-type (in diestrus), \textit{LepR}^{loxTR} and \textit{GRIC}-\textit{LepR}^{loxTR} female mice were deeply anesthetized with isoflurane and euthanized by decapitation. The hypothalamus and pituitary gland were rapidly dissected and frozen on dry ice. Hypothalamic blocks were limited by an incision 1 mm anterior to the optic chiasm and another immediately posterior to the mammillary bodies. Lateral limits were defined by the optic tract, and superior limits were defined by the dorsal tip of the third ventricle (14). Tissues were stored at \(-80^\circ\text{C}\) until quantitative PCR (qPCR) was performed. Tissue was homogenized using Qiazol, and total RNA was isolated using the mirNeasy minikit (catalog no. 217004, Qiagen, Germantown, MD). Samples were then quantified using an Epoch reader (Biotek, Winooski, GA). Total RNA (500–750 ng) was treated with DNase (Qiagen catalog no. 79254) and reverse-transcribed into cDNA. The qPCR was performed using Integrated DNA Technologies PrimeTime qPCR Assays and TaqMan technology in a CFX96 (C1000, BioRad, Hercules, CA). Primers and references are described in Table 2.

Changes in expression of the following genes were assessed: \textit{Garh}1 and \textit{Garh} in the hypothalamus, and \textit{Garhr}, \textit{Libb}, \textit{Fshb} (follicle stimulating hormone), and \textit{Cga} (glycoprotein hormone α-polypeptide) in the pituitary gland. The ribosomal protein \textit{S29} (\textit{Rps29} gene, a component of the ribosome 40S subunit) and \textit{β}-actin were used as housekeeping genes (29). The qPCR data were analyzed by the \textit{ΔΔCT} method. **Hormone profiles.** The LH and FSH levels were assessed from plasma samples taken from the trunk blood at the time of death. After 45-min incubation at room temperature, the blood samples were centrifuged at 1,000 \(g\) for 20 min at 4°C. Serum samples were collected and stored at \(-20^\circ\text{C}\). Analyses of LH and FSH serum levels were performed at the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core (Charlottesville, VA) using the EMD Millipore mouse/rat LH/FSH multiplex assay. The detection limits were 0.24 ng/ml for LH and 2.4 ng/ml for FSH.

**Table 2. List of primer/probe pairs used for qPCR studies**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Company</th>
<th>Reference</th>
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<tbody>
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<td>β-Actin</td>
<td>F primer: 5’TAT TGC TGC TGG CTC TAG 3’</td>
<td>IDT</td>
<td>Assay no. Mn.PT.39a.22214843.g</td>
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<td></td>
<td>R primer: 5’GGG CGC TGC CTC TGG 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 5’TGG GGT TGC CTC CTC ACG TTG C 3’</td>
<td>IDT</td>
<td>Custom</td>
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<td>GnRH</td>
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<td>Assay no. Mn.PT.45.16240237</td>
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<td></td>
<td>R primer: 5’AGT ACA TGG GAA TCG TGG G 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 5’TGG GGA CAA GGA GGT GGA TCA AAT 3’</td>
<td>IDT</td>
<td>Custom</td>
</tr>
<tr>
<td>GnRH R</td>
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<tr>
<td></td>
<td>R primer: 5’TGA CAC ATT GGA AGA AGA CCG 3’</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 5’TGA TAT CCG TCG CAC GGG GAA 3’</td>
<td>IDT</td>
<td>Assay no. Mn.PT.45.17694677</td>
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<td>RPS29</td>
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<tr>
<td></td>
<td>R primer: 5’AGT CAC CCA CGG AAG TGG G 3’</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 5’TCA TCA AAA AGT GCA GAG GGT G 3’</td>
<td>IDT</td>
<td></td>
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<tr>
<td>LHβ</td>
<td>F primer: 5’CCA GTG TGC ATC ACC TGC 3’</td>
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<tr>
<td></td>
<td>R primer: 5’GAG CCA CAG GGA CAA 3’</td>
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<tr>
<td>FSHβ</td>
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<tr>
<td></td>
<td>R primer: 5’AGC TAC CTG CTG TGC AGT CAG 3’</td>
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<tr>
<td></td>
<td>Probe: 5’TCC ACC ACC AGA ATA AGA TGC 3’</td>
<td>IDT</td>
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<tr>
<td>CGA</td>
<td>F primer: 5’AGG ATG ACC AGA AGA ATC GAC AGA 3’</td>
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<tr>
<td></td>
<td>R primer: 5’CTG GAT ATG CAC GAG CAG CTT 3’</td>
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<tr>
<td></td>
<td>Probe: 5’TCC TCA AAA AGT GCA GAG GGT G 3’</td>
<td>IDT</td>
<td></td>
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F, forward; R, reverse.

Quantification, analyses, and production of digital images.

Sections of brain, pituitary gland, and ovary were analyzed using an Axio Imager M2 microscope (Carl Zeiss, Jena, Germany). The distribution of single-labeled (pSTAT3-ir+) and tdTomato+) and dual-labeled (pSTAT3-ir+ and tdTomato+) neurons was assessed in the entire hypothalamus. Quantification of dual-labeled neurons (LepR-Cre tdTomato+ and LH-β-ir+) and the percentage of colocalization were determined in one representative section of the anterior pituitary gland. Distribution of GFP-ir (GRIC-eGFP mice) and tdTomato (LepR-tdTomato mice) was evaluated in the entire ovary. Statistical analysis was performed using GraphPad Prism 6 software. Comparison among groups was determined by one-way ANOVA, applying Geisser-Greenhouse correction for data variability followed by the Tukey post hoc multiple comparison test. Data are presented as means ± SE and α values \((P < 0.05)\) were considered significant. Photomicrographs were produced by capturing images with a digital camera (AxioCam, Zeiss) mounted directly on the microscope using the Zen software. Adobe Photoshop CS6 image-editing software was used to integrate photomicrographs into plates. Only sharpness, contrast, and brightness were adjusted.

**RESULTS**

**LepR expression in GnRHR expressing cells.** To determine the cell population and degree of colocalization of LepR and GnRHR, we evaluated the distribution of leptin-induced pSTAT3-ir in the hypothalamus and in the pituitary gland of GRIC reporter mice (tdTomato). In the hypothalamus, very few GRIC cells were observed in areas that express LepR or leptin-induced pSTAT3, in agreement with a previous report (49). Small numbers of GRIC-tdTomato neurons were found in the arcuate, ventromedial, and dorsomedial nuclei. Virtually no leptin-induced pSTAT3-ir and GRIC colocalization was observed in the adult female hypothalamus \((n = 3\), Fig. 1). Because we were unable to detect reliable leptin-induced pSTAT3-ir in sections from the pituitary gland, we used the LepR-Cre reporter mice (tdTomato) to map the degree of colocalization of LepR in gonadotropes (LH-β immunoreactive cells). We observed that ~10% of LepR-Cre cells coex-
press LH-β immunoreactivity and ~5% of LH-β immunoreactive cells exhibited LepR-Cre activity (n = 3 males and n = 4 females, Fig. 2, A–D) in both males and females. In addition, ovarian theca cells express LepR (n = 5, Fig. 2E), but GRIC-reporter genes (tdTom or eGFP, n = 3), were sporadically observed in only a few cells of the ovarian parenchyma (Fig. 2F).

**GRIC-LepRloxTB mice show no improvement of the metabolic and the reproductive phenotypes.** To generate mice with endogenous reexpression of LepR selectively in GnRHR cells, we crossed the GRIC mouse with the LepRloxTB previously described (5, 13, 48, 50). For validation of the mouse model and Cre-induced DNA recombination, DNA was extracted from hypothalamus, pituitary gland, and tail tips, and DNA recombination was tested using LepRloxTB primers. Only the pituitary gland showed a detectable band indicating successful genomic recombination (Fig. 3A).

Because lack of leptin signaling in gonadotropes only had a minor impact on male fertility (1) and because of germine transgene activation in GRIC males, we focused our studies on female physiology. Three cohorts of GRIC-LepRloxTB (n = 7) animals were evaluated and compared with LepRloxTB (n = 17) and wild-type (n = 18) littermates. Due to leptin’s role in the regulation of body weight and the deleterious effects of metabolic dysfunction in reproductive control (20, 22, 30, 35), we evaluated changes in body weight of GRIC-LepRloxTB mice compared with LepRloxTB and wild-type control littermates, from 5 to 15 wk of age. No difference between LepRloxTB and GRIC-LepRloxTB mice was observed, but a similar increase in body weight over time for these two models compared with wild-type littermates was apparent (Fig. 3B).

Mice were weaned at 3 wk of age (postnatal day 21) and were monitored for VO as an external estrogen-dependent marker for puberty onset. At 4 wk of age (postnatal day 28), all wild-type littermate controls had shown VOs (n = 18), whereas LepRloxTB (n = 17) and GRIC-LepRloxTB (n = 7) mice never did achieve a developed opening up to 10 mo of age. Wild-type littermate controls displayed normal estrous cycles (data not shown), but we were unable to evaluate any change in vaginal cytology in LepRloxTB or GRIC-LepRloxTB mice due to the lack of VO. Because male odorants can induce sexual maturation in female rodents (47), we further characterized the reproductive phenotype of the females housed with a male of proven fertility. After 4–6 wk of mating, wild types (n = 10) were each able to become pregnant and produce a litter of live pups, but none of the LepRloxTB (n = 10) nor the GRIC-LepRloxTB (n = 7) animals showed any signs of pregnancy.

The ovaries of the LepRloxTB and GRIC-LepRloxTB mice contained follicles in different developmental stages, but no corpora lutea, indicating lack of ovulation. We also noticed higher numbers of atretic follicles in LepRloxTB and GRIC-LepRloxTB ovaries compared with wild type (Fig. 4, A–C). The uteri of LepRloxTB and GRIC-LepRloxTB mice were poorly developed (Fig. 4D), and their weights were significantly

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**Fig. 1.** LepR and GnRHR (GRIC-reporter gene) are expressed in different hypothalamic cell populations. Bright-field and fluorescent images show the distribution of leptin-induced STAT3 phosphorylation (pSTAT3-ir) and GnRHR (GRIC-tdTomato) in the arcuate nucleus (Arc; A–C), in the ventromedial nucleus of the hypothalamus (VMH; D–E), and in the dorsomedial nucleus of the hypothalamus (DMH; G–I). 3V, Third ventricle. Scale bar: 200 μm.
smaller than wild-type littermate controls at similar ages (20–30 wk old, Fig. 4).

**FSH-β expression and FSH levels are increased in GRIC-LepRloxTB mice.** No differences in hypothalamic GnRH and GnRHR mRNA as well as pituitary gland GnRHR and LH-β mRNA expression, were observed among the genotypes (Fig. 5, A–D). However, we found that FSH-β and CGA mRNA were increased in LeprloxTB and in GRIC-LepRloxTB mice compared with wild types (Fig. 5, E and F). No difference was observed between LeprloxTB and GRIC-LepRloxTB mice. The potential effect of the loxTB allele in these findings was evaluated by comparing the expression of gonadotrope subunits between LeprloxTB and db/db female mice. No difference in Lhb, Fshb, and Cga expression was observed between both genotypes (Fig. 5G).

Serum LH levels were very low in all groups (mostly below the detection limit of the multiplex assay, i.e., 0.24 ng/ml), whereas higher circulating levels of FSH were observed in GRIC-LepRloxTB compared with wild-type and LeprloxTB littermates (Fig. 5H).

**DISCUSSION**

In this study, we used a combination of different mouse models to gain insight into the role of leptin action in gonadotropes. Using Cre-mediated recombination as a surrogate marker for LepR expression, we found that a subpopulation of gonadotropes expresses LepR. In ovaries, LepR is expressed in theca cells, whereas GRIC was rarely observed in cells of the ovarian parenchyma. No coexpression of leptin-induced

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**Fig. 2.** Leptin receptor (LepR) is expressed in a subpopulation of cells expressing GnRHR in the pituitary gland. A–C: fluorescent images showing that subsets of LepR-expressing cells (LepR-Cre tdTomato; A) display LH-β immunoreactivity (LH-β-ir; B) in males (arrows indicate dual-labeled cells). C: merged. D: bar graphs showing the percentage of colocalization between LepR-ttdTom and LH-β-ir in the pituitary gland of male and female mice (n = 3 males and n = 5 females). E and F: fluorescent images showing the distribution of LepR-reporter gene (tdTom; E) (arrows mark these cells) and GRIC-reporter gene (eGFP; F) (arrow indicates a cell in the parenchyma) in the mouse ovary. CL, corpus luteum; Gr, granulosa layer. Scale bar: 100 μm in A–C and 200 μm in E and F.

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**Fig. 3.** Reexpression of LepR selectively in GnRHR cells causes no change in body weight of LepRloxTB mice. A: agarose gel demonstrating Cre-induced DNA recombination (higher band) of LepRloxTB in the pituitary (but not in the hypothalamus and tail) of GRIC-LepRloxTB mice (n = 2). B: graph showing the progression of body weight of wild-type, LeprloxTB, and GRIC-LepRloxTB female mice (n = 3–8 for each time point). *P < 0.05 and ****P < 0.0001 per one-way ANOVA, Tukey’s post hoc multiple-comparison test.
pSTAT3 and GRIC was noticed in hypothalamic neurons. We found that endogenous reexpression of LepR selectively in gonadotropes (GRIC-LepRloxTB mice) was not sufficient to induce puberty and improve fertility of female mice. However, interestingly, GRIC-LepRloxTB mice have increased FSH levels.

The use of mouse models to interrogate complex physiological systems has become an important experimental approach in basic sciences. It has been particularly useful in areas in which standard methodologies have come across inherent difficulties. The physiology of the mouse pituitary gland is an example due to its size, anatomical location, and volume of hormone release. Because of these issues, the role of leptin direct action on gonadotropes has been difficult to determine.

Recent studies using mouse genetics have suggested that leptin signaling in gonadotropes is required for normal reproductive function. Conditional deletion of LepR in cells expressing LH-β delayed first pregnancy and decreased the number of pups per litter (1). The mechanisms underlying these effects are not completely understood, but the authors propose that the lack of leptin signaling altered GnRH binding sites and secretion of pituitary hormones. Among these hormones, a significant decrease in FSH levels and Fshb expression in pituitary gland was reported (1). Changes in growth or energy homeostasis were unremarkable. In agreement and as predicted from these findings, our mutant mice showed no change in body weight, discouraging any further evaluation of their metabolic phenotype. Because lack of leptin signaling in gonadotropes caused only minor impact on male fertility (1), and the production of the conditional gene reexpression requires a laborious breeding strategy due to male germline transgene activation in GRIC mouse, we focused our studies on female physiology.

The infertility of the leptin signaling-deficient mice has puzzled investigators for decades. Studies focusing on different reproductive organs indicate that the leptin-deficient ob/ob mice have normal development of the HPG axis until puberty, when sexual maturation is arrested. The ovarian morphology of juvenile ob/ob animals is comparable to that of juvenile wild-type mice, and gametogenesis can be induced following gonadotropin administration (32, 41). Reproductive hormones remain low throughout life, but ob/ob mice can ovulate and become fertile if levels of gonadotropins and sex steroids are maintained in physiological ranges (31). Castration increases gonadotropin secretion, although at a lower magnitude compared with controls, and the negative feedback action of sex steroids is exacerbated, also consistent with a prepubertal condition (33, 43).

Previous studies have shown that the leptin-deficient ob/ob mouse have decreased circulating levels of gonadotropins and reduced pituitary content of LH. However, pituitary FSH content is increased in obese models (4, 43). In our mutant mice, we found that pituitary Lhb expression is not different from control or LepRloxTB mice, but Fshb and Cga expression is increased in LepRloxTB and in GRIC-LepRloxTB females compared with controls in diestrus. Because the difference in Fshb and Cga expression between LepRloxTB and
GRIC-LepRloxTB is not different, the role of restoration of LepR in transcript regulation is unlikely.

A dynamic change in leptin and LepR across the estrous cycle has been demonstrated (27). At midcycle or on estrogen stimulation, leptin rises, and leptin signaling is potentiated (15, 27). This effect appears to be correlated with the metabolic effects of estrogen in female rodents (2, 9, 10, 28). However, it is possible that the increase in leptin signaling at midcycle also has a physiological effect in reproductive function. It may amplify the LH surge and the FSH rise preceding ovulation. Using our genetic approach, we were unable to test this hypothesis due to lack of pubertal development. However, the increase in FSH levels in the GRIC-LepRloxTB mice suggests that leptin action in gonadotropes may potentiate gonadotropin release (1).

Previous studies have shown the majority of isolated gonadotropes express leptin-induced pSTAT3, and that mice with lack of leptin signaling in gonadotropes have decreased FSH levels (1). Using the reporter mice, we found that only a small subset of gonadotropes express LepR. The use of different approaches may explain the differences between the studies. Nevertheless, the results are complementary and reinforce the findings that leptin action in gonadotropes exerts a primary effect in adult, not prepubertal, mice and has a role in FSH secretion (1). Further studies using different models will be necessary to validate our findings and assess the mechanisms associated with the increase in FSH levels.

Little is known about the pituitary gland physiology of leptin signaling-deficient (ob/ob or db/db) mice. Initial studies have indicated that the GnRH action to stimulate FSH secretion is amplified in the pituitary of juvenile ob/ob mice, whereas its effect to induce LH secretion is blunted compared with controls (51). This may be due to the higher FSH content and expression and lower LH content in obese leptin signaling-deficient mice (43). Because previous studies have suggested that leptin action in gonadotropes may increase gonadotropin release by increasing GnRH binding sites, it is possible that the restoration of LepR in gonadotropes of the GRIC-LepRloxTB mice has facilitated FSH release observed as increased FSH.

Fig. 5. Increased FSH levels following endogenous reexpression of LepR in GnRHR cells. A and B: expression of GnRH (A) and GnRHR (B) in the hypothalamus of diestrous control (n = 7), LepRloxTB (n = 6), and GRIC-LepRloxTB (n = 4) mice. C–F: expression of GnRHR (C), LH-β (D), FSH-β (E), and CGA mRNA (F) in the pituitary gland of diestrous wild-type (n = 7), LepRloxTB (n = 6), and GRIC LepRloxTB (n = 4) mice. G: expression of LH-β, FSH-β, and CGA mRNA in the pituitary gland of LepRloxTB (n = 7) and db/db (n = 5) mice. All data were normalized to the housekeeping gene RPS29. H: serum levels of FSH in diestrous wild-type litters (n = 14), LepRloxTB (n = 12), and GRIC-LepRloxTB (n = 6) mice. Increase in FSH levels was observed in GRIC LepRloxTB female mice compared with littermate controls in diestrous and LepRloxTB female mice. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with wild-type control mice per one-way ANOVA, Tukey’s post hoc multiple-comparison test.
levels. Higher FSH, but not LH, secretion was observed, likely due to the low GnRH availability in LepR null mice. Again, additional studies are warranted to test this model.

**Perspectives and Significance**

Our studies indicate that endogenous reexpression of LepR in a subpopulation of gonadotropes increases FSH secretion. However, this increase was isolated and not sufficient to induce puberty and improve the infertility phenotype of the LepR<sup>R00X/B</sup> mice. Together with previous findings, our data indicate that leptin-selective action in gonadotropes has a role in adult reproductive physiology but is not sufficient to allow pubertal maturation in mice. Further studies will be necessary to determine the mechanisms by which leptin can directly modulate gonadotropin secretion.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


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LEPTIN ACTION IN GnRHR CELLS


