was detected in 61 of the 69 oocytes observed. This is consistent with the hypothesis that Staufen and *osk* mRNA form complexes at the anterior cortex that are transported to the posterior pole. In *Khc* mutant oocytes, Staufen protein overaccumulated in the anterior end during stage 8, was not detected in granules, and did not concentrate at the posterior pole (>90 oocytes observed). Normal Staufen distribution patterns were restored in *Khc* null oocytes by the addition of a wild-type *Khc* transgene (*18*).

Thus, KHC, the force-generating component of the plus end–directed microtubule motor kinesin I, is required for the posterior localization of both *osk* mRNA and Staufen protein. The participation of kinesin I in this mRNA motility process could be direct. It might attach specifically to *osk*-Staufen complexes at the anterior pole and transport them toward the posterior pole. However, our initial tests for coimmunoprecipitation of KHC and Staufen from *Drosophila* ovary cytosol have not revealed any robust association, so perhaps the linkage is less direct. It is generally accepted that kinesin I transports membranous organelles toward microtubule plus ends (*12*). Thus, *osk* and Staufen could localize to the posterior pole by virtue of association with mitochondria or other organelles carried by kinesin I. An alternative to these models is derived from the effect of a loss of KHC on the particulate staining pattern of Staufen (Fig. 5). Before stages 7 to 8, while microtubules are still oriented with their plus ends toward the anterior, kinesin I might deliver to the cortex materials necessary for the assembly of transport-competent *osk*-Staufen complexes. Thus, the lack of visible Staufen particles in *Khc* null oocytes may indicate that their assembly or persistence depends on kinesin I activity. New studies, using green fluorescent protein tags to follow the localization dynamics of *osk* mRNA, Staufen, and organelles, may distinguish between these models and provide further insight into the mechanisms that drive the movements of maternal determinants for early developmental patterning.

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18. All defects detected in *Khc* null oocytes and embryos were rescued by a transgenic copy of the wild-type *Khc* gene (*13*). This was determined by generating germ line clones in females carrying the genotype *y w P{hs-FLP}/ w; P{w+, FRT}42B, P{OvoD1}55D / P{w+, FRT}42B c Khc27; P{w+, Khc+}/ +*.
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Role of Brain Insulin Receptor in Control of Body Weight and Reproduction

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Insulin receptors (IRs) and insulin signaling proteins are widely distributed throughout the central nervous system (CNS). To study the physiological role of insulin signaling in the brain, we created mice with a neuron-specific disruption of the IR gene (NIRKO mice). Inactivation of the IR had no impact on brain development or neuronal survival. However, female NIRKO mice showed increased food intake, and both male and female mice developed diet-sensitive obesity with increases in body fat and plasma leptin levels, mild insulin resistance, elevated plasma insulin levels, and hypertriglyceridemia. NIRKO mice also exhibited impaired spermatogenesis and ovarian follicle maturation because of hypothalamic dysregulation of luteinizing hormone. Thus, IR signaling in the CNS plays an important role in regulation of energy disposal, fuel metabolism, and reproduction.

Insulin receptors (IR) are expressed in most tissues of the body, including classic insulin-sensitive tissues (liver, muscle, and fat), as well as "insulin-insensitive" tissue, such as red blood cells and the neuronal tissue of the CNS. In the CNS, the IR displays distinct patterns of expression in the olfactory bulb, the hypothalamus, and the pituitary (*1–3*), although its function in these regions remains largely unknown. Previous experiments have suggested a role for insulin signaling in the regulation of food intake (*4*, *5*) and neuronal growth and differentiation (*6*, *7*). Moreover, insulin has been shown to regulate neurotransmitter release and synaptic plasticity (*8*, *9*), and dysregulation of insulin signaling in the CNS has been linked to the pathogenesis of neurodegenerative dis-

orders such as Alzheimer's and Parkinson's disease (*10*, *11*).

We have used the Cre-loxP system to generate mice with CNS-specific disruption of the IR gene (*12–14*). Mice carrying a "floxed" allele of the IR gene (IR-lox mice) were crossed with mice expressing the Cre-recombinase under control of the rat nestin promoter and enhancer. Nestin is an intermediate filament protein that is expressed in neuroepithelial stem cells (*15*, *16*). The resultant brain-specific IR knockout (NIRKO) mice showed a >95% reduction in the level of brain IR protein (Fig. 1, A and B). In contrast, the abundance of other insulin signaling proteins, such as insulin receptor substrates-1 and -2 (IRS-1 and IRS-2), was unaltered in brain extracts of NIRKO mice (Fig. 1A). Inactivation of the IR gene was specific to the brain, as no change in IR expression was detectable in skeletal muscle, heart, liver, kidney, spleen, and gonads (Fig. 1C).

Because insulin stimulates growth of neurons in culture (*16*, *17*), we investigated the impact of IR deletion on brain development and morphology. Brain weights in NIRKO mice were not significantly different from those in control mice (475 mg compared with 483 mg, $P = 0.18$, $n = 10$ each genotype), and histological analysis revealed no apparent differences in brain development or morphology (*18*). Immunohistochemical analyses of brain sections with antisera against glial fibrillar acidic protein (GFAP), a marker of glial cell activation, also showed no differences between NIRKO and control mice (*18*), suggesting that IR expression is not required for neuronal survival in vivo.

Although the body weights of male NIRKO mice were indistinguishable from those of their control littermates during the first 6 months of life on a normal chow diet, female NIRKO mice exhibited a consistent 10 to 15% increase of body weight in comparison with controls (Fig. 2A). In addition, on this diet, both male and female NIRKO mice demonstrated increased adipose tissue mass with an ~twofold increase in perigonadal white adipose tissue (WAT) in NIRKO females and a 1.5-fold increase in NIRKO males (Fig. 2B). Paralleling the increase in adipose mass, plasma leptin concentrations were elevated 2.5-fold in female NIRKO mice ($P < 0.01$) and 1.5-fold in male NIRKO mice ($P < 0.05$) (Fig. 2C). The increased body weight of NIRKO females also correlated with an ~20% increase in food intake as compared with female controls [121 mg per gram of body weight (BW) per day compared with 100 mg per gram of BW per day; $P < 0.01$]

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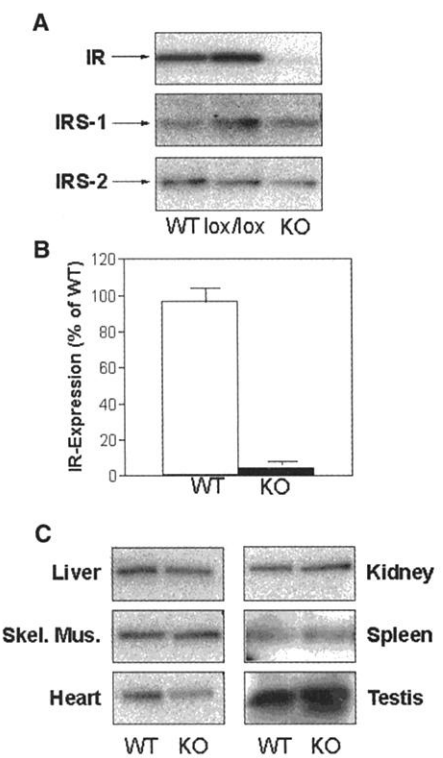

Fig. 1. Insulin receptor expression is specifically disrupted in the brain of NIRKO mice. (**A**) Brain extracts prepared from wild-type (WT), IRlox/lox, and NIRKO (KO) mice were subjected to immunoprecipitation followed by Western blot analyses with antisera specific for the IR-β subunit, IRS-1, and IRS-2 (*30*). (**B**) IR immunoreactivity was quantified by densitometric scanning of blots similar to that in (A). Data represent the mean ± SEM of $n = 8$ of each genotype and are expressed relative to the control mice. (**C**) Western blot analyses of IR-β subunit content in tissues from control and NIRKO (KO) mice.

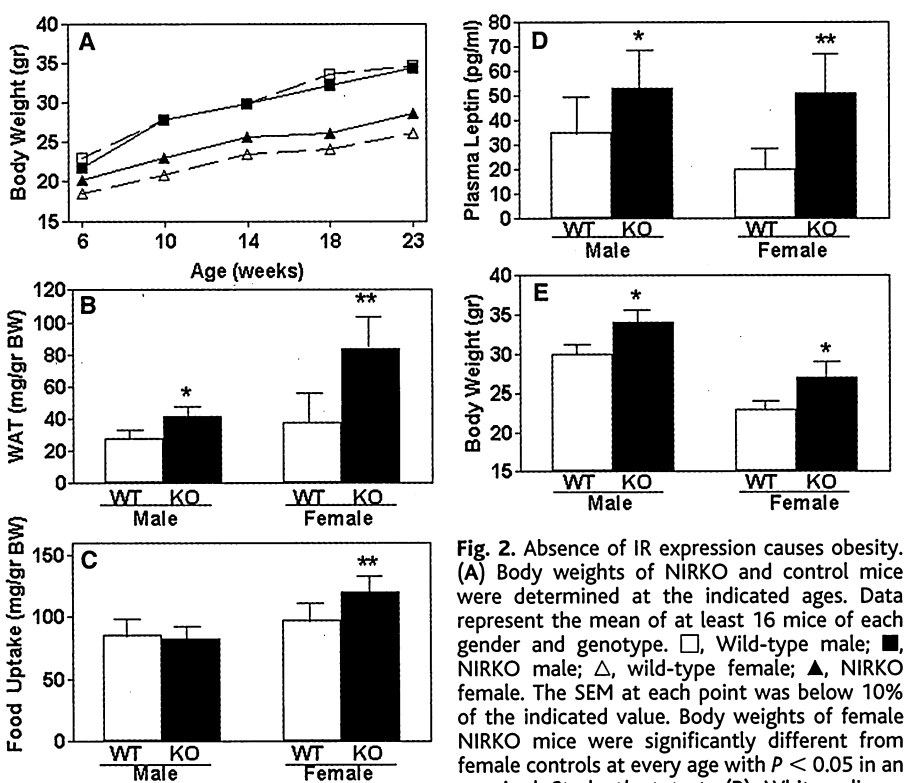

Fig. 2. Absence of IR expression causes obesity. (**A**) Body weights of NIRKO and control mice were determined at the indicated ages. Data represent the mean of at least 16 mice of each gender and genotype. □, Wild-type male; ■, NIRKO male; △, wild-type female; ▲, NIRKO female. The SEM at each point was below 10% of the indicated value. Body weights of female NIRKO mice were significantly different from female controls at every age with $P < 0.05$ in an unpaired Student's t test. (**B**) White adipose tissue (WAT) mass (parametrial fat depots in female mice and epididymal fat depots in male mice) was determined in mice at 8 to 12 months of age. Data represent the mean ± SEM of at least eight animals of each genotype and gender (*, $P < 0.05$; **, $P < 0.005$). (**C**) Plasma leptin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) on blood samples obtained from 6- to 8-month-old mice on a regular chow diet. Data represent the mean ± SEM of at least 10 animals of each genotype and gender (*, $P < 0.05$; **, $P < 0.005$). (**D**) Food intake and body weight of 4- to 6-month-old mice were determined daily over 1 week. Data represent the mean ± SEM of at least eight mice of each genotype and gender (**, $P < 0.01$). (**E**) Body weight of male and female control and NIRKO mice is given at the age of 14 weeks. In these experiments, control and NIRKO mice were put on a high-fat (60%) diet between 5 and 9 weeks of age. Data represent the mean ± SEM of at least six animals of each genotype and gender (*, $P < 0.05$).

(Fig. 2D). In contrast, food intake of male NIRKO mice on the normal chow diet did not differ significantly from that of controls (82 mg per gram of BW per day compared with 87 mg per gram of BW per day; $P = 0.15$, $n = 14$ each genotype). This mild obesity was enhanced when the mice were challenged with a high-fat (60%) diet. Under these conditions, by as little as 14 weeks of age, male NIRKO mice exhibited a 10% elevation of body weight ($P < 0.05$) and female NIRKO mice a 20% increase in body weight ($P < 0.05$) as compared with control mice on the same diet (Fig. 2E).

The obesity in NIRKO mice was associated with insulin resistance and hypertriglyceridemia. At 4 to 6 months of age, the NIRKO mice showed normal fasting blood glucose levels (Fig. 3A), but the circulating plasma insulin levels were elevated by 1.5-fold in males and ~twofold in females (Fig. 3B). Consistent with their obesity phenotype, female NIRKO mice showed a significantly blunted response 15 min after insulin injection and a trend toward elevated blood glucose 30 to 60 min later, whereas after pharmacologic doses of insulin, male NIRKO mice performed similarly to controls (Fig. 3C). Intraperitoneal glucose tolerance tests were normal in both male and female NIRKO mice. Finally, both male and female NIRKO mice showed a 30% increase in circulating triglycerides (Fig. 3D) but had normal plasma cholesterol concentrations (105 mg/dl compared with 109 mg/dl; $P = 0.16$, $n = 9$ each genotype). Thus, brain-specific disruption of the IR gene results in hyperphagia in female mice and causes obesity, hyperleptinemia, insulin resistance, and hypertriglyceridemia in both male and female mice.

Another phenotype of NIRKO mice manifested itself in breeding experiments. Although 76% of the matings established between control mice yielded offspring, breedings of male NIRKO mice with control females produced offspring in only 46% of the cases ($P < 0.05$). Rates were similarly reduced to 42% when NIRKO females ($P < 0.05$) were bred to male controls. The reduction in male fertility was due to impaired spermatogenesis; epididymal sperm content was reduced by 30% ($P < 0.05$) in NIRKO mice as compared with age-matched controls (Fig. 4A). Histological examination of testis sections revealed that, although spermatogenesis was proceeding normally in many seminiferous tubules of NIRKO males, ~20% of tubules lacked a lumen and presented few, if any, maturing spermatogenic cells. Moreover, there was a reduction of the Leydig cell population, and the interstitial stroma did not support organization of seminiferous tubules within the NIRKO testis

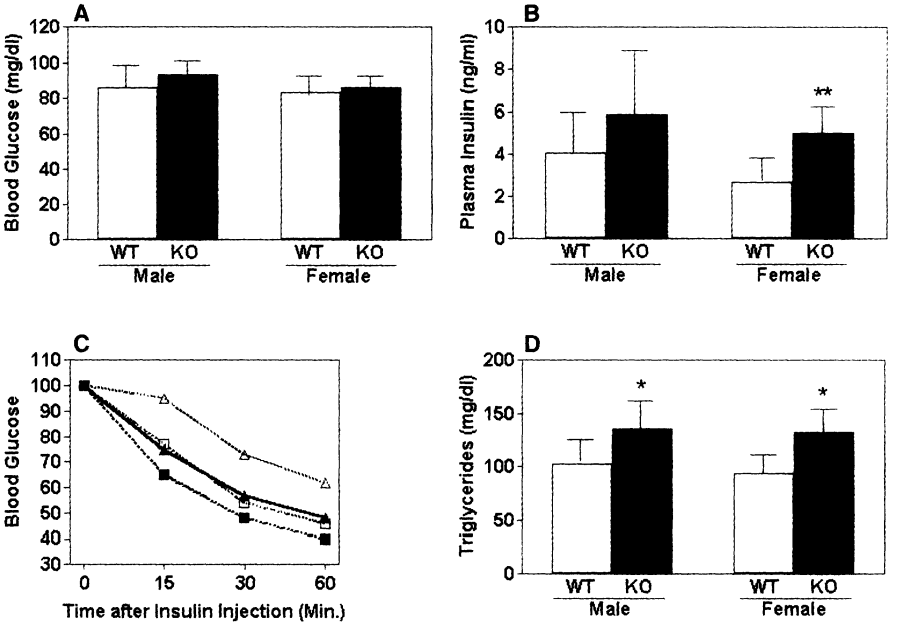

Fig. 3. Obesity in NIRKO mice causes mild insulin resistance and dyslipidemia. (**A**) Blood glucose concentrations were determined on control and NIRKO mice after an overnight fast with a Glucometer Elite. Data represent the mean ± SEM of at least 10 animals of each genotype and gender. (**B**) Plasma insulin concentrations were determined by ELISA on blood samples obtained from 6- to 8-month-old mice on a normal chow diet. Data represent the mean ± SEM of at least 10 animals of each genotype and gender (**, $P < 0.005$). (**C**) Intraperitoneal insulin tolerance tests were performed with 0.75 U of insulin per kg of body weight (*30*). Data represent the mean ± SEM of at least eight animals of each genotype and gender. ■, wild-type male; □, NIRKO male; ▲, wild-type female; △, NIRKO female. (**D**) Plasma triglyceride concentrations were determined on blood drawn from 6- to 8-month-old mice. Data represent the mean ± SEM of at least 10 animals of each genotype and gender (*, $P < 0.05$).

(Fig. 4B). The seminal vesicles, prostate, and epididymis did not appear morphologically altered in NIRKO males (*20*). Histological examination of ovaries from female NIRKO mice also revealed abnormalities. NIRKO ovaries contained reduced numbers of antral follicles (wild type: 2.8 ± 0.46, $n = 8$) and corpora lutea (wild type: 4.0 ± 0.31, $n = 5$, compared with NIRKO: 1.12 ± 0.36, $n = 8$) (Fig. 4B). These observations suggest that NIRKO mice had insufficient gonadotropin input for proper maintenance of ovarian follicle maturation, Leydig cell function, or spermatogenesis.

To assess the role of the hypothalamic-pituitary axis in the gonadal insufficiency, we measured plasma levels of luteinizing hormone (LH) in the NIRKO mice. This assay revealed a 60% reduction of circulating LH concentrations in males ($P < 0.05$) and a 90% reduction in females ($P < 0.01$) (Fig. 4C). This decrease occurred with no alteration in pituitary morphology, as determined by methylene blue staining (*20*), or pituitary LH content, as estimated by immunohistochemical analysis with antisera to LH (Fig. 4D). To test whether the pituitaries of the NIRKO mice respond to LH releasing hormone (LHRH), we injected the mice intraperitoneally with lupron, a GnRH receptor agonist. Male NIRKO mice actually exhibited a normal increase in circulating LH concentrations, whereas female NIRKO mice displayed a twofold enhancement of response compared with controls (Fig. 4E). These data indicate that neuronal expression of the IR is essential for normal regulation of the hypothalamic-pituitary-gonadal axis through its effects on LH secretion.

In summary, this study documents that IR in the CNS plays an important functional role in the regulation of energy homeostasis and reproductive endocrinology. This provides a mechanism for the previous observations that intraventricular injection of insulin inhibits food intake (*21*, *22*) and the evidence that insulin may play a role in regulation of body weight at a central level (*4*, *23*). Thus, insulin acting in the CNS through its receptor appears to provide a negative feedback loop for postprandial inhibition of food uptake. Obesity in NIRKO mice occurs despite elevated circulating plasma leptin concentrations, suggesting that the CNS insulin resistance is also associated with some degree of CNS resistance to leptin action, creating an interesting link between insulin and leptin action in the regulation of body weight. The current data also suggest a mechanism by which insulin resistance in the CNS can modify the metabolic syndrome by leading to hy-

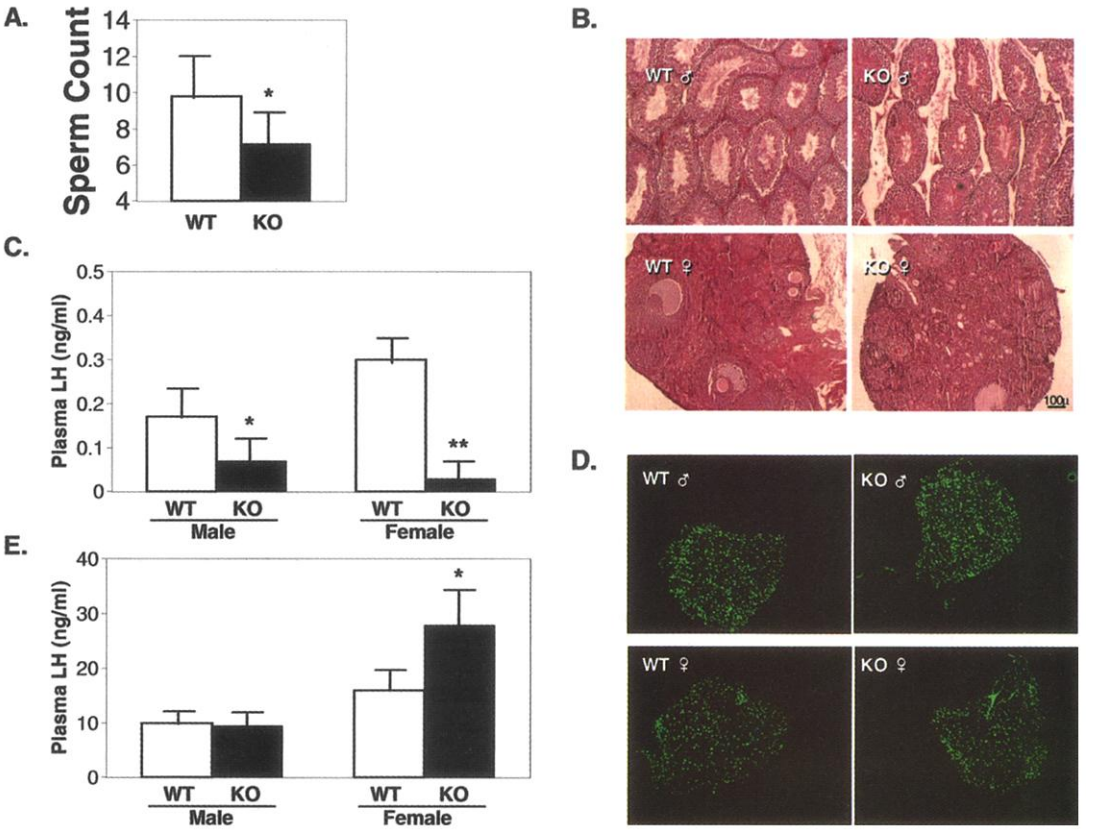

Fig. 4. Absence of brain IR expression results in hypothalamic hypogonadism. (**A**) Epididymi of control and NIRKO mice were removed, and spermatozoa were allowed to diffuse into culture medium. After centrifugation, total epididymal sperm content was determined. Data represent the mean ± SEM of at least 10 animals of each group (*, $P < 0.05$). (**B**) Testes and ovaries were removed from control and NIRKO mice and fixed in 10% formalin. Paraffin-embedded sections were stained with hematoxylin and eosin. The scale bar indicates about 100 μm. (**C**) Plasma LH concentrations were determined by radioimmunoassay on serum samples from 6- to 7-month-old mice. Data represent the mean ± SEM of at least eight animals of each genotype and gender (*, $P < 0.05$; **, $P < 0.01$). (**D**) Pituitaries were dissected from paraformaldehyde-perfused mice. One-micrometer sections were prepared from wild-type and NIRKO mice and stained with polyclonal antibodies to LH. (**E**) Plasma LH concentrations were determined by radioimmunoassay on serum samples obtained 1 hour after intraperitoneal injection of lupron. Data represent the mean ± SEM of at least six animals of each genotype and gender (*, $P < 0.05$).

perphagia, obesity with hyperleptinemia, and hypertriglyceridemia, thereby further aggravating peripheral insulin resistance. Taken together with our previous studies indicating a role for the insulin receptor in β cells for normal glucose sensing (*24*), this study demonstrates that genetically determined insulin resistance in classical insulin target tissues, and nonclassical target tissues such as the brain and beta cell, may act synergistically in the induction of obesity, insulin resistance, glucose intolerance, and dyslipidemia, leading to the complex metabolic syndrome associated with type 2 diabetes (*14*, *24*, *25*).

Our results also reveal an important link between brain insulin signaling and reproduction. There are at least two possible mechanisms by which insulin might regulate the reproductive axis at a central level. First, although leptin concentrations are only mildly elevated, the elevated plasma leptin concentrations may modify LHRH secretion in NIRKO mice. This seems unlikely, however, because the phenotype of the NIRKO mice differs from leptin-overexpressing mice, which exhibit reduced LH secretion in response to exogenous LHRH (*26*). Alternatively, the IR expressed on GnRH-producing neurons or at some even higher center may mediate GnRH synthesis or secretion. Indeed, in cultured hypothalamic neurons, IGF-2, a high-affinity ligand for IR, induces GnRH release (*27*). In a number of severe insulin resistance states, such as the Type A syndrome and lipoatrophic diabetes, hypothalamic-pituitary-gonadal function is perturbed with alterations in menstrual function and even polycystic ovarian disease (*28*, *29*). Thus, the NIRKO mice will provide an important tool for studying insulin action in the CNS and will likely add unexpected aspects to our understanding of genetically determined insulin resistance, obesity, and reproductive function.

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