Insufficiency of Janus Kinase 2-Autonomous Leptin Receptor Signals for Most Physiologic Leptin Actions

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

Citation

Published Version
doi://10.2337/db09-1556

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:5978699

Terms of Use
This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
OBJECTIVE—Leptin acts via its receptor (LepRb) to signal the status of body energy stores. Leptin binding to LepRb initiates signaling by activating the associated Janus kinase 2 (Jak2) tyrosine kinase, which promotes the phosphorylation of tyrosine residues on the intracellular tail of LepRb. Two previously examined LepRb phosphorylation sites mediate several, but not all aspects of leptin action, leading us to hypothesize that Jak2 signaling might contribute to leptin action independently of LepRb phosphorylation sites. We therefore determined the potential role of leptin action for signals that are activated by Jak2 independently of LepRb phosphorylation (Jak2-autonomous signals).

RESEARCH DESIGN AND METHODS—We inserted sequences encoding a truncated LepRb mutant (LepRbΔNCc, which activates Jak2 normally, but is devoid of other LepRb intracellular sequences) into the mouse Lepr locus. We examined the leptin-regulated physiology of the resulting ΔΔ mice relative to LepRb-deficient db/db animals.

RESULTS—The ΔΔ animals were similar to db/db animals in terms of energy homeostasis, neuroendocrine and immune function, and regulation of the hypothalamic arcuate nucleus, but demonstrated modest improvements in glucose homeostasis.

CONCLUSIONS—The ability of Jak2-autonomous LepRb signals to modulate glucose homeostasis in ΔΔ animals suggests a role for these signals in leptin action. Because Jak2-autonomous LepRb signals fail to mediate most leptin action, however, signals from other LepRb intracellular sequences predominate.

Diabetes 59:782–790, 2010

A dipose tissue produces the hormone, leptin, in proportion to fat stores to communicate the status of long-term energy reserves to the brain and other organ systems (1–4). In addition to moderating food intake, adequate leptin levels permit the expenditure of energy on myriad processes including reproduction, growth, and immune responses, as well as regulating nutrient partitioning (4–6). Conversely, lack of leptin signaling due to null mutations of leptin (e.g., Lep<sup>ob/ob</sup> mice) or the leptin receptor (LepR) (e.g., Lep<sup>rb/db</sup>) results in increased food intake in combination with reduced energy expenditure (and thus obesity), neuroendocrine dysfunction (including hypothyroidism, decreased growth, infertility), decreased immune function, and hyperglycemia and insulin insensitivity (1,7–9). Many of the effects of leptin are attributable to effects in the central nervous system, particularly in the hypothalamus, but leptin also appears to act directly on some other tissues (2,3).

Alternative splicing generates several integral-membrane LepR isoforms that possess identical extracellular, transmembrane, and membrane-proximal intracellular domains. LepR intracellular domains diverge beyond the first 29 intracellular amino acids, however, with the so-called “short” isoforms (e.g., LepRa) containing an additional 3–10 amino acids, and the single “long” isoforms (LepRb) containing a 300–amino acid intracellular tail (10). Like other type I cytokine receptors (11), LepRb (which is required for physiologic leptin action) contains no intrinsic enzymatic activity, but associates with and activates the Janus kinase 2 (Jak2) tyrosine kinase to mediate leptin signaling. The intracellular domain of LepRb possesses membrane-proximal Box1 and Box2 motifs, both of which are required for association with and regulation of Jak2; although LepRa and other short LepRs contain Box1, they lack Box2 and thus fail to bind and activate Jak2 under physiologic conditions (12).

Leptin stimulation promotes the autophosphorylation and activation of LepRb-associated Jak2, which phosphorylates three LepRb tyrosine residues (Tyr<sub>985</sub>, Tyr<sub>1077</sub>, and Tyr<sub>1138</sub>). Each LepRb tyrosine phosphorylation site recruits specific Src homology 2 (SH2) domain–containing effector proteins: Tyr<sub>985</sub> recruits Src homology phosphatase-2 (SHP-2) and suppressor of cytokine signaling 3 and attenuates LepRb signaling, but does not appear to play other roles in leptin action in vivo (13–15). Tyr<sub>1077</sub> recruits the latent transcription factor, signal transducer, and activator of transcription 5 (STAT5), and Tyr<sub>1138</sub> recruits STAT3 (16–18). Mice in which LepRb<sup>s1138</sup> (mutant for Tyr<sub>1138</sub> and thus specifically unable to recruit STAT3) replaces endogenous LepRb exhibit hyperphagic obesity, with decreased energy expenditure, but increased growth, protection from diabetes, and preservation of several aspects of hypothalamic physiology (19–21). These results thus suggest roles for Tyr<sub>1077</sub> and/or Jak2-dependent signals that are independent of LepRb tyrosine phosphorylation (“Jak2-autonomous signals”) in mediating Tyr<sub>985</sub>/Tyr<sub>1138</sub>-independent leptin actions. Although others have examined the effect of mutating all three LepRb tyrosine phosphorylation sites in mice (22), revealing potential tyrosine phosphorylation–independent roles for LepRb in leptin action, that study did not examine several aspects of leptin action and could not distinguish potential effects of…
nonphosphorylated LepRb motifs from effects due to LepRb/Jak2 interactions specifically.

RESEARCH DESIGN AND METHODS

Cell culture studies. The plasmids pcDNA3LepRbΔ65 and pcDNA3LepRa were generated by mutagenesis of pcDNA3LepRb (23) using the QuikChange kit (Stratagene). The absence of adventitious mutations was confirmed by DNA sequencing for all plasmids. Cell culture, transfection, lysis, and immunoblotting were conducted as reported previously using αJak2(PI1007/8) from Cell Signaling Technology and αJak2 from our own laboratory (24). Leptin was the generous gift of Amylin Pharmaceuticals (La Jolla, CA).

Mouse model generation. The targeting vector encoding LepRbΔ65 was generated by inserting a Stop codon (QuikChange kit) after the 56 intracellular amino acid of LepRb in the 5¢ targeting arm in the pBluescript plasmid; this modified 5¢-arm was subsequently subcloned into the previously described pPNT-derived targeting vector that contained the 3¢ arm (19,25,26). The resulting construct was linearized and transfected into murine embryonic stem (ES) cells with selection of clones by the University of Michigan Transgenic Animal Model Facility. Correctly targeted clones were identified and confirmed with real-time PCR and Southern blotting as performed previously (15,21,26) and were injected into embryos for the generation of chimeras and the establishment of germ-line (ES) cells with selection of clones by the University of Michigan (12), we initially examined signaling by the truncated intracellular domain within the context of LepRbΔ65 in transfected 293 cells (Fig. 1A). Leptin stimulation promoted the phosphorylation of Jak2 on the activating Tyr1007/1008 sites in LepRbΔ65, and LepRb-expressing cells but not in LepRa-expressing or control cells (Fig. 1A), confirming that LepRb and LepRbΔ65, but not LepRa, contain the necessary sequences to mediate Jak2 activation in response to leptin.

To understand the potential roles for Jak2-autonomous LepRb signals in leptin action, we used homologous recombination to replace the genomic Lepr with LepRbΔ65 (henceforth referred to as the Δ allele, encoding LepRbΔ65 in mouse ES cells (Fig. 1B). Correctly targeted ES cell clones were confirmed by Southern blotting (Fig. 1C). This strategy mediates LepRbΔ65 expression from the native Lepr locus, ensuring correct patterns and levels of LepRbΔ65 expression, as previously for other homologously targeted LepRb alleles (15,21,26). Indeed, RT-PCR analysis of hypothalamic mRNA confirmed similar Lepr mRNA expression in homozygous Δ/Δ animals and wild-type animals (Fig. 1D). Prior to subsequent study, we backcrossed heterozygous Δ/+ animals to C57BL/6J mice for six generations to facilitate direct comparison with LepRbΔ/Δ (db/db) animals on this background.

Energy homeostasis in Δ/Δ mice. Because our previous analysis suggested some role for Tyr658/Tyr1138-independent LepRb signals in regulating energy balance, we initially examined parameters of energy homeostasis in Δ compared with db/db animals (15,19,21). We weaned and singly housed Δ/Δ, db/db, and control mice from 4 to 8 weeks of age for the longitudinal determination of body weight and food intake (Fig. 2A–D). Compared with age- and sex-matched db/db animals, Δ/Δ mice displayed similar body weights and food intake over the study period. Furthermore, age- and sex-matched Δ/Δ and db/db mice displayed similar proportions of fat and lean mass (Table 1), revealing that Δ/Δ and db/db mice are similarly obese. Core body temperature in Δ/Δ and db/db animals was also similarly reduced compared with control animals (Table 1). LeptinRbΔ65 fails to alter major parameters of energy homeostasis compared with entirely LepRb-deficient db/db mice, suggesting that Jak2-autonomous LepRb signals are not sufficient to modulate energy balance in mice.

Linear growth and reproductive function in Δ/Δ mice. In addition to modulating metabolic energy expenditure, leptin action permits the use of resources on energy-intensive neuroendocrine processes, such as growth and reproduction. Whereas db/db and ob/ob animals that are devoid of leptin action thus display decreased linear growth and infertility, animals lacking Tyr658 or Tyr1138 of LepRb display normal or enhanced linear growth and preserved reproductive function, suggesting a role for other LepRb signals in mediating these leptin actions (15,19,21). Similar to db/db mice, Δ/Δ males displayed decreased snout-anus length and femur length relative to control animals (Table 1), however, suggesting the inabili...
ROLE OF Jak2 IN LEPTIN ACTION

A

![Diagram of gene-targeting strategy to replace wild-type exon 18b with that encoding the COOH-terminally truncated LepRb
d65.](Image of gene-targeting strategy to replace wild-type exon 18b with that encoding the COOH-terminally truncated LepRb
d65.)

B

![Diagram showing the generation of mice expressing LepRb
d65.](Diagram of gene-targeting strategy to replace wild-type exon 18b with that encoding the COOH-terminally truncated LepRb
d65.)

C

![Image of gel electrophoresis of Lepr-specific RT-PCR products from hypothalamic mRNA of five wild-type and five ΔΔ animals.](Image of gel electrophoresis of Lepr-specific RT-PCR products from hypothalamic mRNA of five wild-type and five ΔΔ animals.)

D

![Image showing the regulation of T-cell function by LepRb signals.](Image showing the regulation of T-cell function by LepRb signals.)

FIG. 1. Generation of mice expressing LepRb
d65. A: HEK293 cells were transfected with plasmids encoding the indicated LepR isoforms, made quiescent overnight, incubated in the absence (−) or presence (+) of leptin (625 ng/ml) for 15 min before lysis, and immunoprecipitated with αJak2 (24). Immunoprecipitated proteins were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with the indicated antibodies. The figures shown are typical of multiple independent experiments. B: Diagram of gene-targeting strategy to replace wild-type exon 18b with that encoding the COOH-terminally truncated LepRb
d65. C: Southern blotting of control (wild-type) and correctly targeted (D/+, C1, C2) Lepr
d65 ES lines, using a Lepr-specific probe. M indicates marker lane. D: Image of gel electrophoresis of Lepr-specific RT-PCR products from hypothalamic mRNA of five wild-type and five ΔΔ animals.

... of Jak2-autonomous LepRb signals to mediate linear growth in the absence of other LepR signals.

To determine the potential role for Jak2-autonomous LepRb signals in the regulation of reproductive function, we monitored estrous cycling from 4 to 8 weeks of age in female mice, along with their ability to deliver pups after housing with wild-type males. In addition to examining ΔΔ, db/db, and wild-type females in these assays, we included mice homozygous for Lepr
dm1mgmj (a.k.a., Lepr
d1138 or s/s mice; mutant for Tyr1138→STAT3 signaling) (21) as a positive control for our ability to detect residual reproductive function in obese mice with altered lep...
examined thymocyte numbers in young s/s mice deficient for LepRb Tyr1138 signaling, suggesting improved immune function in s/s compared with db/db mice (23), many other parameters of immune function in these and other models of altered LepRb signaling remain poorly understood. To better understand the signaling mechanisms by which LepRb modulates the immune system, we thus determined the numbers of total and CD4/11001 splenocytes, as well as the ex vivo proliferative capacity of splenic CD4/11001 cells from a panel of mouse models of altered LepRb signaling. In addition to examining wild-type, ob-ob, /9004/9004, and s/s animals, we also studied mice homozygous for Leprtm2mgmj (a.k.a., Lepry985 or l/l) mutant for LepRb Tyr985 (15) (Fig. 4). Note that ob-ob animals were used as the control for the absence of leptin action in this study, as sufficient numbers of age-matched db/db animals were not available at the time of assay. In addition to revealing the expected decrease in total and CD4/11001 splenocytes and their proliferation in ob-ob animals relative to wild-type controls, we found decreases in these parameters in /9004/9004 animals, and normal parameters of immune function in s/s animals. Interestingly, although l/l animals exhibit increased sensitivity to the anorexic action of leptin (15), these parameters of immune function actually trended down (albeit not significantly) in l/l animals.

### TABLE 1

Phenotypic data for mice expressing mutant LepRb

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild type</th>
<th>db/db</th>
<th>Δ/Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.0 ± 0.7 (10)</td>
<td>47.0 ± 1.1* (8)</td>
<td>47.8 ± 0.9* (8)</td>
</tr>
<tr>
<td>Female</td>
<td>6.4 ± 0.5 (8)</td>
<td>51.0 ± 0.9* (8)</td>
<td>54.1 ± 0.7* (11)</td>
</tr>
<tr>
<td>Lean content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>80.0 ± 0.4 (10)</td>
<td>43.7 ± 1.1* (8)</td>
<td>42.6 ± 0.8* (8)</td>
</tr>
<tr>
<td>Female</td>
<td>76.0 ± 3.0 (8)</td>
<td>39.5 ± 0.7* (8)</td>
<td>37.5 ± 0.6* (10)</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34.6 ± 0.2 (10)</td>
<td>33.0 ± 0.2* (9)</td>
<td>33.4 ± 0.2* (8)</td>
</tr>
<tr>
<td>Female</td>
<td>34.4 ± 0.3 (8)</td>
<td>33.8 ± 0.2 (9)</td>
<td>33.5 ± 0.3 (12)</td>
</tr>
<tr>
<td>Snout-anus length (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>88.5 ± 0.8 (9)</td>
<td>82.6 ± 2.7* (8)</td>
<td>83.0 ± 1.6* (9)</td>
</tr>
<tr>
<td>Femur length (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13.9 ± 0.1 (9)</td>
<td>11.8 ± 0.1* (8)</td>
<td>12.0 ± 0.1* (9)</td>
</tr>
<tr>
<td>Femur mass (mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42.4 ± 0.6 (9)</td>
<td>34.8 ± 0.8* (8)</td>
<td>35.3 ± 0.9* (9)</td>
</tr>
</tbody>
</table>

Fat and lean content were determined at 10 weeks and are expressed as a percentage of body weight. Body temperature was determined at 11 weeks. Snout-anus length, femur length, and femur weight were determined at 9 weeks. Data are means ± SE; *P < 0.05 compared with wild type by Student unpaired, two-tailed t test. Sample size noted in parentheses.
significantly) compared with wild-type animals. Thus, these data suggest that although Tyr985 and Tyr1138 are not required for the promotion of splenic T-cell function by leptin, Jak2-autonomous LepRb signals in mice suffice to delay the onset of diabetes compared with db/db mice, but cannot reverse the later progression to diabetes.

For female mice, there was no difference in serum insulin levels between db/db and ΔΔ mice at any age. In male mice, insulin was also similar between db/db and ΔΔ mice at 4 weeks of age, but older ΔΔ males displayed higher circulating insulin levels compared with age-matched db/db males. To discriminate potential alterations in insulin clearance, we also examined serum C-peptide levels, which mirrored insulin concentrations (supplementary Fig. 1, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1556/DC1). These data suggest that the relative euglycemia of ΔΔ compared with db/db animals at 4 weeks of age is not due to differences in insulin secretion (because insulin and C-peptide levels are similar between genotypes, but ΔΔ animals have decreased blood glucose at this age), but must be secondary to modest improvements in hepatic glucose output and/or insulin sensitivity. This difference is transient, however, as the increased insulin levels in older male ΔΔ animals fail to decrease blood glucose levels relative to those observed in db/db animals. No difference in β-cell mass was detected between 12-week-old ΔΔ and db/db mice (supplementary Fig. 2).

In addition, we performed glucose and insulin tolerance tests in the ΔΔ and db/db animals (supplementary Fig. 3). Although 6-week-old female ΔΔ animals displayed a diminished hyperglycemic response to the glucose tolerance test relative to db/db controls, no other differences between ΔΔ and db/db mice were observed. This suggests that the difference in glucose homeostasis between ΔΔ and db/db animals is small and not sufficient to reveal differences in the face of a substantial glucose load and/or the increased insulin resistance of advancing age.

**TABLE 2**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild type</th>
<th>db/db</th>
<th>ΔΔ</th>
<th>s/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus</td>
<td>8/9</td>
<td>0/9</td>
<td>0/8</td>
<td>3/8</td>
</tr>
<tr>
<td>Litters</td>
<td>9/9</td>
<td>0/7</td>
<td>0/8</td>
<td>5/8</td>
</tr>
</tbody>
</table>

Individually housed females were examined daily from 4 to 8 weeks of age. Cytologic examination of vaginal lavage was used to monitor estrus. Females were paired with one wild-type male and monitored daily for 6 weeks for the production of offspring.
leptin, but emphasize the necessity of signals emanating from the COOH-terminus of LepRb (beyond the Jak2-associating Box1 and Box2 motifs) for most leptin action.

The finding that 4-week-old ∆∆ mice display similar insulin and C-peptide levels as db/db animals, but exhibit improved blood glucose levels, suggests improved glucose disposal or decreased glucose production in the ∆∆ mice independent of insulin production. This is consistent with data suggesting that central nervous system leptin action suppresses hepatic glucose production, and with our previous finding that some portion of this is mediated independently of Tyr_1138→STAT3 signaling (2,20,34). The ∆∆ mice progress rapidly (by 6 weeks of age) to dramatic hyperglycemia and parameters of glucose homeostasis indistinguishable from db/db animals, however. Indeed, even at 5 weeks of age, the response to a glucose bolus is comparably poor in male ∆∆ and db/db animals and barely improved in female ∆∆ compared with db/db animals. Furthermore, the increased insulin production of ∆∆ relative to db/db males at 6 weeks of age and beyond fails to ameliorate their hyperglycemia. Thus, the improvement in glucose homeostasis mediated by Jak2-autonomous LepRb signals in ∆∆ mice compared with db/db animals is very modest, as it is easily overwhelmed by a large glucose load and/or the increasing insulin resistance and diabetes of advancing age. No difference in β-cell mass was detected between ∆∆ and db/db males. The mechanism(s) mediating the increased insulin production of the ∆∆ relative to db/db males is unclear, but could represent an improvement in β-cell function due to the later time at which ∆∆ animals become diabetic or to some residual leptin action in the ∆∆ β-cell (35).

Although the overall similarity of ARC gene expression and physiology between ∆∆ and db/db mice indicates little role for Jak2-autonomous LepRb signals in ARC leptin action, the finding of decreased (worsened) POMC mRNA expression in ∆∆ compared with db/db mice was surprising. One possible explanation for this observation is that some residual signal mediated by LepRb action, the finding of decreased (worsened) POMC mRNA expression in ∆∆ compared with db/db mice is overwhelmed under normal circumstances by the LepRb signals that enhance POMC expression. Unfortunately, the low POMC content of db/db and ∆∆ animals and low activity of these neurons at baseline rendered the examination of POMC c-fos uninformative.

Although the molecular mechanisms underlying Jak2-autonomous LepRb action remain unclear, several pathways could contribute. In cultured cells, the activation of Jak2 by LepRb and similar receptor mutants mediates some activation of the extracellular signal–related kinase pathway (13,36). Indeed, chemical inhibitor studies have suggested a role for extracellular signal–related kinase signaling in the regulation of autonomic nervous system function by leptin, and the autonomic nervous system underlies a major component of the leptin effect on glucose homeostasis (37). Phosphatidylinositol 3-kinase (PI 3-kinase) also plays a role in the regulation of glucose homeostasis by leptin (38–41). As we have been unable to observe the regulation of PI 3-kinase by leptin in cultured cells and the analysis of this pathway in the hypothalami of obese, hyperleptinemic mice remains problematic, the molecular mechanism by which LepRb engages this pathway remains unclear. Although difficult to test directly, it is thus possible that Jak2-autonomous LepRb signals might modulate this pathway in vivo. We have previously demonstrated that the major regulation of hypothalamic mam-

### DISCUSSION

To determine the potential roles for Jak2-autonomous LepRb signals in leptin action in vivo, we generated a mouse model in which LepRb is replaced by a truncation mutant (LepRbΔΔΔ) that contains within its intracellular domain only the sequences required to associate with and activate Jak2. We found that the hyperphagia, obesity, linear growth, ARC physiology, and immune function of these ∆∆ mice closely resembled that of entirely LepRb-deficient db/db mice. ∆∆ and db/db animals did demonstrate some modest differences in glucose homeostasis; however, both male and female ∆∆ mice exhibited a delayed progression to frank hyperglycemia compared with db/db mice. Taken together, these findings demonstrate that Jak2-autonomous LepRb signals may contribute modestly to the modulation of glucose homeostasis by leptin, but emphasize the necessity of signals emanating from the COOH-terminus of LepRb (beyond the Jak2-associating Box1 and Box2 motifs) for most leptin action.

The finding that 4-week-old ∆∆ mice display similar insulin and C-peptide levels as db/db animals, but exhibit improved blood glucose levels, suggests improved glucose disposal or decreased glucose production in the ∆∆ mice independent of insulin production. This is consistent with data suggesting that central nervous system leptin action suppresses hepatic glucose production, and with our previous finding that some portion of this is mediated independently of Tyr_1138→STAT3 signaling (2,20,34). The ∆∆ mice progress rapidly (by 6 weeks of age) to dramatic hyperglycemia and parameters of glucose homeostasis indistinguishable from db/db animals, however. Indeed, even at 5 weeks of age, the response to a glucose bolus is comparably poor in male ∆∆ and db/db animals and barely improved in female ∆∆ compared with db/db animals. Furthermore, the increased insulin production of ∆∆ relative to db/db males at 6 weeks of age and beyond fails to ameliorate their hyperglycemia. Thus, the improvement in glucose homeostasis mediated by Jak2-autonomous LepRb signals in ∆∆ mice compared with db/db animals is very modest, as it is easily overwhelmed by a large glucose load and/or the increasing insulin resistance and diabetes of advancing age. No difference in β-cell mass was detected between ∆∆ and db/db males. The mechanism(s) mediating the increased insulin production of the ∆∆ relative to db/db males is unclear, but could represent an improvement in β-cell function due to the later time at which ∆∆ animals become diabetic or to some residual leptin action in the ∆∆ β-cell (35).

Although the overall similarity of ARC gene expression and physiology between ∆∆ and db/db mice indicates little role for Jak2-autonomous LepRb signals in ARC leptin action, the finding of decreased (worsened) POMC mRNA expression in ∆∆ compared with db/db mice was surprising. One possible explanation for this observation is that some residual signal mediated by LepRb action, the finding of decreased (worsened) POMC mRNA expression in ∆∆ compared with db/db mice is overwhelmed under normal circumstances by the LepRb signals that enhance POMC expression. Unfortunately, the low POMC content of db/db and ∆∆ animals and low activity of these neurons at baseline rendered the examination of POMC c-fos uninformative.

Although the molecular mechanisms underlying Jak2-autonomous LepRb action remain unclear, several pathways could contribute. In cultured cells, the activation of Jak2 by LepRb and similar receptor mutants mediates some activation of the extracellular signal–related kinase pathway (13,36). Indeed, chemical inhibitor studies have suggested a role for extracellular signal–related kinase signaling in the regulation of autonomic nervous system function by leptin, and the autonomic nervous system underlies a major component of the leptin effect on glucose homeostasis (37). Phosphatidylinositol 3-kinase (PI 3-kinase) also plays a role in the regulation of glucose homeostasis by leptin (38–41). As we have been unable to observe the regulation of PI 3-kinase by leptin in cultured cells and the analysis of this pathway in the hypothalami of obese, hyperleptinemic mice remains problematic, the molecular mechanism by which LepRb engages this pathway remains unclear. Although difficult to test directly, it is thus possible that Jak2-autonomous LepRb signals might modulate this pathway in vivo. We have previously demonstrated that the major regulation of hypothalamic mam-

---

**FIG. 4.** Reduced numbers and proliferation of splenic T-cells in ∆∆ and db/db but not s/s or L/L mice. Spleens were isolated from the indicated genotypes of male mice, separated using autoMACS, and counted for (A) total splenocytes and (B) CD4^+^ cells using a flow cytometer (n = 7–22 per genotype). Data are plotted as means ± SEM; *P < 0.05 compared with wild type by one-way ANOVA and Tukey post-test. C: For proliferation assays, CD4^+^CD25^–^ naive T-cells were isolated by autoMACS (n = 4–7 per genotype), incubated in the presence of bone marrow–derived dendritic cells from C57BL/6J mice and stimulated with anti-CD3e. Incorporation of IP^3^-thymidine (1 μCi/well) by proliferating cells was measured during the last 6 h of culture. Proliferation is expressed as a percentage of a paired wild-type sample analyzed concurrently (dashed line) and is plotted as mean ± SEM; *P < 0.05 by one-way ANOVA and Tukey post-test.

---

diabetes.diabetesjournals.org
malian target of rapamycin (mTOR), including in response to nutritional alteration, occurs indirectly, via neuronal activation (42). We examined this pathway (along with the phosphorylation of STAT3) in the hypothalamus of \( ^{+/+} \) animals (supplementary Fig. 4), revealing the expected absence of STAT3 signaling and increased mTOR activity (secondary to the activation of orexigenic neurons) in the mediobasal hypothalamus of \( ^{db/db} \) and \( ^{+/+} \) animals. Thus, the regulation of mTOR is similar in these mouse models.

A potential intermediate linking Jak2 activation to PI 3-kinase activation is SH2B1, a SH2 domain-containing protein that binds phosphorylated Tyr813 on Jak2 (43). Cell culture studies show that SH2B1 binds directly to Jak2, augments its kinase activity, and couples leptin stimulation to insulin receptor substrate activation, a well-known activator of PI 3-kinase (44). Indeed, SH2B1-null mice display hyperphagia, obesity, and diabetes (45). SH2B1 could also mediate other, unknown Jak2-autonomous LepRb signals.

Importantly, the phenotype of mice expressing LepRB\(^{Δ65}\) differs significantly from a mouse model in which LepRB\(^{Y123F}\) (mutated for the three tyrosine phosphorylation sites, but with an otherwise intact intracellular domain) replaces LepRb (22). Unlike \( ^{ΔΔ} \) animals, these LepRB\(^{Y123F}\) mice display improved energy homeostasis and more dramatic improvements in glucose homeostasis (both of which are sustained into adulthood), compared with \( ^{db/db} \) animals. Unfortunately, the C57 genetic background strain used to study the LepRB\(^{Y123F}\) mice not only differs compared with the C57BL/6J background that we used to study our \( ^{+/+} \) and \( ^{db/db} \) animals, but also diverges from that of the \( ^{db/db} \) animals used as comparators for the LepRB\(^{Y123F}\) mice (22). Similarly, it is also possible that minor differences between the incipient C57BL/6J backgrounds of \( ^{db/db} \) and \( ^{+/+} \) mice could contribute to the modest distinctions observed between these two models.

Aside from the background strain, the intriguing possibility arises that the intracellular domain of LepRb may mediate heretofore unsuspected signals independently from tyrosine phosphorylation. As it is, future studies will be needed to carefully compare the phenotype of LepRB\(^{Y123F}\), \( ^{ΔΔ} \), and \( ^{db/db} \) animals within the same facility and on the same genetic background; should these differences remain, further work will be necessary to confirm the importance and determine the identity of the underlying signaling.

FIG. 5. Delayed onset of hyperglycemia in \( ^{ΔΔ} \) compared with \( ^{db/db} \) mice. A–D: Blood glucose was determined for ad libitum–fed (A and B) or fasted (5 h) (C and D) animals of the indicated genotype (wild type [WT], \( ^{db/db} \), \( ^{ΔΔ} \), and \( ^{ΔΔ} \)) and sex at the indicated ages (\( n = 8–12 \) per genotype). E and F: Serum was collected from mice of the indicated genotype and sex at the indicated ages (\( n = 8–10 \) per genotype), and insulin content was determined by ELISA. All panels: Data are plotted as means ± SEM; *\( P < 0.05 \), \( ^{db/db} \) compared with \( ^{ΔΔ} \) at the indicated time points by one-way ANOVA and Tukey post-test.
In summary, our findings reveal the insufficiency of Jak2-autonomous LepRb signals for the bulk of leptin action. These findings do not rule out the possibility that Jak2-autonomous signals may be required to support the action of LepRb phosphorylation, however. Indeed, our present findings suggest a modest role for Jak2-autonomous LepRb signals in the regulation of glucose homeostasis by leptin. Understanding collaborative roles for Jak2-autonomous signals in leptin action and deciphering the mechanisms underlying these signals and potential tyrosine phosphorylation–independent signals mediated by the COOH-terminus of LepRb will represent important directions for future research.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (NIH) DK-57631 (M.G.M.), NIH DK-67536 (R.N.K.), the American Diabetes Association, and the American Heart Association (M.G.M. and S.R.). Core support was provided by The University of Michigan Cancer and Diabetes Centers: NIH CA-46592 and NIH DK-20572.

No potential conflicts of interest relevant to this article were reported.

We thank Amylin Pharmaceuticals for the generous gift of leptin, Mark Sleeman of Regeneron Pharmaceuticals for AgrpLacZ mice, and Diane Fingar, PhD (University of Michigan) for antibodies.

REFERENCES

25. Leshan RL, Bjornholm M, Münzberg H, Myers MG Jr. Leptin receptor signaling and action in the central nervous system. Obesity (Silver Spring) 2006;14(Suppl. 5):212S
32. Berthoud HR. Interactions between the “cognitive” and “metabolic” brain in the control of food intake. Physiol Behav 2007;91:486–498

S. ROBERTSON AND ASSOCIATES

TYPE 2 DIABETES

DIABETES, VOL. 59, APRIL 2010 789