Astrocytic leptin-receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity

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Jayaram B, Pan W, Wang Y, Hschou H, Mace A, Cornelissen-Guillaume GG, Mishra PK, Koza RA, Kastin AJ. Astrocytic leptin receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity. J Appl Physiol 114: 734–741, 2013. First published January 17, 2013; doi:10.1152/japplphysiol.01499.2012.—To determine how astrocytic leptin signaling regulates the physiological response of mice to diet-induced obesity (DIO), we performed metabolic analyses and hypothalamic leptin signaling assays on astrocytic leptin-receptor knockout (ALKO) mice in which astrocytes lack functional leptin receptor (ObR) signaling. ALKO mice and wild-type (WT) littermate controls were studied at different stages of DIO with measurement of body wt, percent fat, metabolic activity, and biochemical parameters. When fed regular chow, the ALKO mice had similar body wt, percent fat, food intake, heat dissipation, respiratory exchange ratio, and activity as their WT littermates. There was no change in blood concentrations of triglyceride, soluble leptin receptor (sObR), mRNA for leptin and uncoupling protein 1 (UCP1) in adipose tissue, and insulin sensitivity. Unexpectedly, in response to a high-fat diet the ALKO mice had attenuated hyperleptinemia and sObR, a lower level of leptin mRNA in subcutaneous fat, and a paradoxical increase in UCP1 mRNA. Thus, ALKO mice did not show the worsening of obesity that occurs with normal WT mice and the neuronal ObR mutation that results in morbid obesity. The findings are consistent with a competing, counterregulatory model between neuronal and astrocytic leptin signaling.

LEPTIN IS A SO-CALLED LEAN HORMONE that suppresses food intake and curtails weight gain. Predominantly secreted by subcutaneous adipose tissue, this 16-kDa polypeptide crosses the blood-brain barrier (BBB) to reach the hypothalamus and regulate feeding. Both neurons and astrocytes express leptin receptors (ObRs) (11), but the functions of astrocytic leptin signaling in astrocyte-neuron interactions are not clear. When mice receive an intracerebroventricular injection of fluorocitrate, an inhibitor of astrocytic metabolic activity, there is an increase in the amount of leptin transported to the neurons and phosphorylation of Signal Transducer and Activator of Transcription (pSTAT3) in the hypothalamus, indicating greater leptin signaling (23). This suggests that astrocytic and neuronal leptin signaling compete with each other.

The effects of leptin are mediated by ObR isoforms generated by alternative splicing mainly in the regions encoding the cytoplasmic domain. ObRb is the longest receptor isoform that is able to induce pSTAT3 activation; in the absence of ObRb, db/db mice show hyperphagia and severe diabesity. They have hyperleptinemia, hyperinsulinemia, hypercorticosteronemia, infertility, and cold intolerance. Neuronal-specific mutation of ObR impairs hypothalamic functions and mimics the obese phenotype observed in db/db mice (4, 5, 15). The metabolic phenotype of astrocytic ObR mutation, however, has not yet been reported. We have shown that adult-onset obesity specifically increases astrocytic ObR expression, whereas neuronal ObR does not show upregulation. The most pronounced increase in ObR expression with adult-onset obesity is not in neurons but in astrocytes, particularly in the hypothalamus (8, 19, 20). This raises a question of the functional significance of astrocytic leptin signaling in the development of obesity. To test the hypothesis that astrocytic leptin signaling modulates metabolic behavior and the response to diet-induced obesity (DIO), we generated astrocyte-specific ObR-knockout (ALKO) mice and characterized the specificity and efficiency of deletion of astrocytic leptin signaling (10). We show here that the ALKO mice have an improved metabolic phenotype in response to a high-fat diet (HFD) challenge.

MATERIALS AND METHODS

As described previously (10), ALKO mice were generated by cross-breeding ObR-floxed mice (14, 15) with GFAP-cre mice (Jackson Laboratory, Bar Harbor, ME). In the ObR-floxed mice, the two loxP sites flank exon 17, which encodes the cytoplasmic domain. The resulting mutant receptor (Δ17) is membrane-bound, although it does not have a known signaling function because of the absence of Box1 (shared by ObRa, ObRb, and ObRe), Box2, and Box3 (unique to ObRb) cytoplasmic domains. The F1 generation arising from a cross of GFAP-cre/+ heterozygote transgenics with ObR-flox/flox homozygotes was then crossed with ObR-flox/flox homozygotes to obtain F2. Genotyping from tail DNA verified the correct excision of ObR with emergence of the mutant Δ17 polymerase chain reaction (PCR) product in the presence of Cre, and the presence of floxed ObR. The specificity of the astrocytic ObR mutation was confirmed by RT-PCR analyses of isolated astrocytes and other central nervous system cells, and by immunohistochemical staining of brain sections with cre-recombinase and astrocytic markers GFAP and S100B. Because the two genes are on different chromosomes, there is a one-fourth chance of obtaining true ALKO mice that contain PCR products of GFAP-cre, Δ17 sequence, and floxed ObR in genotyping. Weaning and genotyping were performed when the mice were 21 days old. The mice were randomly housed, with ALKO mice maintained in the same cages as wild-type (WT) controls, which contain loxP alleles but show similar metabolic phenotypes (14).

The animal studies for analyses of the metabolic phenotype of ALKO mice by NMR and by TSE calorimetry (TSE Systems, Chesterfield, MO) followed protocols approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee. The mice were housed four to a cage unless being adapted or studied for metabolic activities. The dark span was 18:00–06:00 h. Four groups of mice were studied (n = 5–8/group): WT and ALKO
mice were fed normal rodent chow (control diet, CD) or a 45% HFD (Research Diets, New Brunswick, NJ) beginning at age 5 wk. The HFD (D12451) contains 20 kcal% protein, 35 kcal% carbohydrate, and 45 kcal% fat. The CD has lower caloric content but avoids the issue of high sugar content and has been used more commonly than the isocaloric diet by obesity researchers in the past few years. Only male mice were used for this study because of concerns of significant interactions between leptin and estrogen. The mice were group-housed and monitored daily until they were 7.5 mo old, at which time terminal biochemical assays were performed. Weekly weight and monthly fat proportion were determined until the time of metabolic chamber studies at age 5 mo. The percent fat/body wt (BW) was determined by NMR (Bruker Optics, Billerica, MA). BW was used as the denominator, as previously described (8, 24).

At the age of 5 mo, the four groups of mice were subjected to calorimetry by TSE Phenomaster. The mice were adapted to single housing and special feeding/drinking tubes for 3 days before measurement of oxygen consumption (VO₂), carbon dioxide production (VCO₂), respiratory quotient (RER, equivalent to VCO₂/VO₂), heat dissipation, activity, and feeding. The data were sampled every 35 min. Over the course of 9 days excluding adaptation, 372 data points (217 h) were collected. All data points were included in the graphing and cumulative change analyses.

To replicate the findings from TSE calorimetry, separate batches of ALKO mice were subjected to the Oxymax metabolic chamber study at the age of 4 mo. Oxymax is another calorimetry measurement device from a different manufacturer (Columbus Instruments, Columbus, OH). The effects of overnight fasting were also tested. VO₂, VCO₂, RER, heat dissipation, activity, and feeding were sampled every 18 min, as described previously (18).

Hypothalamic tissue was collected from the four groups of mice described above at age 7.5 mo immediately after anesthesia and decapitation. The tissue was frozen in liquid nitrogen and transferred to −80°C until time of tissue homogenization and protein extraction. Western blotting for the astrocytic marker GFAP (G3893, Sigma), ObR (M18) (sc-1834, Santa Cruz Biotechnology), SOCS-3 (sc-9023), and COX-2 (sc-19999) was performed as described previously (7, 27). Subcutaneous fat and brown adipose tissue were collected for RNA extraction and quantification of leptin and UCP1 mRNA. At the time of the experiment, 1 ml of QIAzol reagent was added to 100 mg of tissue and processed for extraction of RNA with the RNeasy lipid tissue kit (no. 74804, Qiagen). Two reference genes were used: glyceraldehyde-3-phosphate dehydrogenase and cyclophilin. There were no changes in cycling threshold of either reference gene. The target gene expression (leptin or UCP1) was normalized to reference genes by the standard curve method. The primer sequences are shown in Table 1.

Arterial blood was collected into prechilled tubes by dissection of a common carotid artery after mice were anesthetized immediately before decapitation. Serum was obtained by centrifugation of whole blood after overnight storage at 4°C. Insulin was measured by a mouse Ultrasensitive ELISA kit (80-INSMSU-E01, ALPCO Diagnostics, Salem, NH). Leptin and sObR were measured, respectively, by Quantikine Mouse Leptin (MOB00) and Mouse Leptin R DuoSet (DY497) ELISA from R&D Systems (Minneapolis, MN). Triplicates were analyzed following the assay protocol to obtain a mean for each sample. Serum triglyceride was measured as 1-type triglyceride (461–08992, Wako Diagnostics, Richmond, VA). Insulin tolerance tests were performed on 3-h-fasting mice by intraperitoneal injection of 0.75 μg/kg body wt human insulin (Humulins R; NDC 0002–8215–19). Blood glucose was measured at 0, 15, 30, 45, and 60 min after insulin administration in WT and ALKO mice (n = 8; 3 mo old).

For increase in BW and percent fat with age, repeated-measures ANOVA were performed followed by the Bonferroni multiple comparison post hoc test. For metabolic chamber analyses, circadian plots are shown, and the last 12-h light cycle and dark cycle was analyzed by two-way ANOVA to determine the effects of knockout mice and diet in each cycle, and potential interactions of the two independent variables. For biochemical assays among four groups of mice, two-way ANOVA and post hoc Tukey’s tests were performed.

RESULTS

The effects of ALKO and HFD on BW and percent fat. While being fed the CD, the ALKO mice showed a similar lean phenotype as WT mice up to the end of observation at 30 wk of age. After being challenged with a 45% HFD at 5 wk of age up to 7.5 mo, both WT and ALKO mice gained weight. Two-way ANOVA showed that diet had a significant overall effect on weight gain, but there was no significant effect of the

Table 1. Primer sequences

<table>
<thead>
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<th>Gene</th>
<th>Forward primer (5′ → 3′)</th>
<th>Reverse primer (5′ → 3′)</th>
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<tr>
<td>Leptin</td>
<td>TGG CTT TGG TCC TAT CTG TC</td>
<td>ACC ACC TCT GTG GAC TAG AG</td>
</tr>
<tr>
<td>UCP1</td>
<td>CAC TCT CCC CCT GGA CAC T</td>
<td>GCG TAG GAC ACC TTT ATA CTA G</td>
</tr>
<tr>
<td>GPADH</td>
<td>TCT GTC CCT GCT GGA TCT GA</td>
<td>CCT GCT TCA CCA GCT TCT TGA</td>
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Fig. 1. Measurement of body wt (A) and adiposity (B) showed that ALKO and WT mice had similar increases while fed a control diet throughout the study. The high-fat diet expedited weight and fat gain in both ALKO and WT mice without a significant difference between the groups (n = 5–8/group).
knockout or interactions between the diet and knockout variables. BW was comparable in WT and ALKO mice, either on chow or the HFD (Fig. 1A).

The percent of fat (normalized to BW) was not different among the four groups of mice at the beginning of the study. The HFD had a significant effect of increasing adiposity in both ALKO and WT mice. Post hoc analysis showed that there was no difference between the WT and ALKO mice while being fed the HFD at any time (Fig. 1B).

Effects of ALKO and HFD on metabolic activities. The first set of metabolic parameters studied in the TSE calorimetric chambers included VO₂ (Fig. 2A), VCO₂ (Fig. 2B), and RER (Fig. 2C). While being fed the CD, ALKO and WT mice did not show a significant difference in any of these parameters. Both ALKO and WT mice showed a clear 24-h rhythm of VO₂ that was higher in the dark span than the light span. Though the ALKO mice appeared to have a higher oxygen consumption rate than WT mice fed the CD and HFD (data not shown), the difference was not significant. This was verified by cumulative changes in the last 12-h light and dark spans (Fig. 2A).

When fed the HFD, VO₂ was unchanged. Diet and ALKO had an overall effect on VCO₂. There was a reduction in VCO₂ in ALKO mice in the dark cycle (Fig. 2B). RER was reduced by the HFD compared with the CD groups, consistent with an increase in lipid oxidation. However, there was no difference in RER between the ALKO and WT groups fed the HFD (Fig. 2C).

The second set of metabolic parameters included heat dissipation, activity levels, and food intake. The increase in energy expenditure as a result of the ALKO mutation was not significant (Fig. 2D). There was no change in locomotor activity reflected by the light beams crossed in the horizontal plane (Fig. 3A); the rearing activity (z-axis counts) was also not different among groups (not shown). Both WT and ALKO mice consumed more grams of rodent chow (lower caloric counts) than grams of the HFD (higher caloric counts), but there was no difference caused by the mutation with either diet (Fig. 3B).

When separate batches of mice were subjected to Oxymax metabolic chamber measurements, ALKO mice had similar changes as WT mice in response to the HFD and overnight fasting. There was a mild increase in VCO₂, a greater increase in RER (Fig. 3C), and a reduction in food intake with a mild attenuation in heat dissipation of the ALKO mice in response to the HFD (Fig. 3D).

Blood biochemical profile. The ALKO mice had a higher serum insulin level than that in WT mice. A significant increase in response to the HFD was observed in the WT mice but not in the ALKO mice (Fig. 4A). Although WT mice had higher serum triglyceride in response to the HFD, ALKO mice did not show a significant elevation. The triglyceride level of ALKO mice fed the HFD was significantly lower than that in WT mice fed the HFD (Fig. 4B). In the glucose tolerance test, the ALKO mice showed the same insulin sensitivity as the WT mice (Fig. 4C).

Regulation of leptin expression and its signaling by ALKO. ALKO mice fed the CD showed a significant increase in leptin mRNA in subcutaneous fat (Fig. 4D), a major source of leptin production. However, blood leptin concentrations did not show a significant change from those of the WT mice (Fig. 4E). The results suggest that there is a posttranslational block in leptin synthesis in ALKO mice under normal dietary conditions, or that leptin turnover in blood is accelerated in ALKO mice. The latter can be associated with a) leptin transport from blood to tissue, which is shown unchanged, at least across the BBB in normal conditions (10); or b) a reduction in serum binding proteins, particularly sObR, which is the main leptin-binding factor in blood (13). Soluble ObR has high affinity to leptin and inhibits leptin transport into brain (26). An inverse correlation has been established between sObR and leptin concentrations in humans (3). However, sObR levels were similar between WT and ALKO mice fed the CD (Fig. 4F).

WT mice responded to the HFD with elevated leptin mRNA from subcutaneous fat and a higher leptin concentration in
Fig. 3. Effects of ALKO and diet on activity and food intake. A: activity levels, shown in the horizontal planes, were not different between ALKO and WT mice either fed the CD (upper) or HFD (lower). B: food intake showed a circadian pattern with the highest intake in the dark span. Cumulative intake over the course of the study did not differ between ALKO and WT mice fed the CD. The amount of HFD consumed was lower than that of the CD in either ALKO or WT mice, and there was no difference caused by the knockout. C: fasting did not induce significant changes in metabolic behavior. In the Oxymax metabolic chamber study with separate batches of four groups of mice, the results were similar to those shown in the TSE metabolic chamber study. The ALKO groups are shown here. HFD did not change \( V_{O_2} \) (upper), mildly decreased \( V_{CO_2} \) (middle), and significantly reduced RER (lower). Overnight fasting attenuated all these metabolic activities but did not show a sustained effect. D: ALKO mice fed an HFD had reduced consumption of the high-caloric food within Oxymax chambers (upper) but did not differ from mice fed the CD in locomotor activity on the horizontal planes except during overnight fasting (middle). There was somewhat lower heat dissipation but this was not significant (lower).
serum. Surprisingly, when ALKO mice were fed an HFD, there was no further increase in leptin mRNA or elevation in blood leptin concentrations as was observed in WT mice fed the HFD (Fig. 4E).

Because the ALKO mutation did not affect adiposity in either basal (fed CD) or DIO (fed HFD) conditions, one would expect the same level of leptin production. The dissociation between body wt and serum leptin concentrations is unprecedented in mice with intact leptin production by adipose tissue. In parallel, the HFD significantly reduced sObR in the ALKO mice so that sObR levels were lower than those in the WT mice (Fig. 4F). The lack of high sObR in the ALKO mice might partially explain why there was less hyperleptinemia while being fed an HFD.

Leptin production not only correlates with adipose tissue volume under basal conditions, it also is subject to neuroimmunoendocrine regulation. Thus we next determined pertinent changes in the hypothalamus of these mice. We tested whether the ALKO mutation modulates reactive astrogliosis in the hypothalamus, a hallmark of obesity-induced neuroinflammation (8, 20, 21). Consistent with published evidence, expression of hypothalamic GFAP was significantly increased in WT mice on the HFD, no further increase was observed in ALKO mice on the HFD, there was no change in the kinetics of glucose concentrations after insulin challenge, indicating that sensitivity to insulin was preserved in ALKO mice.

We then determined whether a change in UCP1 mRNA plays a role in the paradoxical finding of obesity without hyperleptinemia in ALKO mice fed the HFD. The mitochondrial protein UCP1 acts to reduce adiposity and contributes to nonshivering thermogenesis; it has abundant reciprocal interactions with leptin (12, 17, 25). As shown in Fig. 5F, the ALKO mutation did not affect UCP1 gene expression while mice were fed the CD. However, ALKO mice fed the HFD had a high level of UCP1 mRNA, and this was significantly higher than in ALKO mice fed the CD or WT mice fed the HFD (Fig. 5F). It appears that leptin is still able to induce UCP1 gene expression in ALKO mice in response to an HFD, whereas WT mice lost this capacity, probably because of hyperleptinemia/leptin resistance on prolonged high fat feeding.

**Fig. 4.** Effect of ALKO and HFD on diabetes regulation. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.0001 by two-way ANOVA with a Bonferroni multiple comparison post hoc test. A: insulin level was higher in ALKO mice and increased by the HFD. B: ALKO mice fed the CD had a higher serum triglyceride (TG) concentration (*P < 0.05, Student’s t-test) compared with WT fed the CD. Although TG concentration was increased in WT mice on the HFD, no further increase was observed in ALKO mice on the HFD. C: there was no change in the kinetics of glucose concentrations after insulin challenge, indicating that sensitivity to insulin was preserved in ALKO mice. D: ALKO increased leptin mRNA in subcutaneous fat tissue on the CD; there was no further increase on the HFD. E: blood leptin concentration was not increased by ALKO but increased by the HFD. The HFD increased serum leptin in the WT mice but not in the ALKO mice. F: sObR levels were reduced in ALKO mice on HFD compared with ALKO mice fed the CD or WT mice fed either diet.
DISCUSSION

In these studies we determined how an absence of astrocytic-specific leptin signaling modulates metabolic response. Unlike neuronal ObR knockout mice, the ALKO mice showed the following: 1) no change in basal BW or adiposity; 2) no difference in the development of obesity when fed an HFD; 3) a normal increase in leptin and a corresponding decrease in sObR when fed the HFD; and 4) persistent induction of UCP1 gene expression in response to an HFD. Despite the similarity of ALKO and WT mice in basal metabolic activity and biochemical profile, ALKO mice fed an HFD differed from WT mice fed the HFD. The results are in major contrast to those we recently reported for endothelial leptin receptor knockout (ELKO) mice (18). ELKO mice have an endothelial-specific mutation of ObR resulting from crossing Tie2-cre heterozygote mice with ObR-floxed homozygote mice with loxP sites flanking exon 17; the resulting F1 heterozygote mice contain both cre and floxed genes and were further crossed with ObR-floxed homozygotes. As a result, endothelial cells express a membrane-bound ObR mutant lacking signaling function. Although ELKO mice do not differ from WT mice in body wt or adiposity, they have a striking hyperleptinemia. They respond to an HFD with less gain of body wt and fat, and exhibit higher oxygen consumption, carbon dioxide production, and heat dissipation. There is no increase in feeding and even a reduction in activity level. Apparently, the ELKO mutation is associated with partial resistance to DIO. This points to a facilitatory role of endothelial leptin signaling in obesity development (18).

The ALKO mice were generated by a similar genetic strategy with the GFAP-cre mice to mediate astrocytic-specific deletion of the signaling ObR. Like the ELKO mice, ALKO mice also have preserved leptin transport across the BBB (10). The results from the present study with ALKO mice show that astrocytic leptin signaling has the same direction of change in response to DIO as endothelial leptin signaling in the ELKO mice; however, a milder metabolic phenotype of ALKO than ELKO mice indicates that astrocytic leptin signaling has a
more subtle effect. One possible explanation is that ALKO has a primary effect on CNS leptin signaling, whereas ELKO may affect peripheral organs that express ObR as well as the CNS. By contrast, mice with neuronal ObR knockout show prominent obesity with gains in weight and fat. This is observed in knockout mice generated by neuronal excision of either the entire ObR with a floxed ObR line flanking exon 1 of the gene (4) or the cytoplasmic signaling tails of ObR with a floxed ObR line flanking exon 17, resulting in a mutant but membrane-bound receptor (5, 15). Nonetheless, a small number of neural progenitor cells also use the GFAP promoter, and there can be variability in the efficiency and regional difference of expression (2). However, the specificity of astrocytic ObR mutation in the hypothalamus of the ALKO mice is high, shown by co-localization of cre-recombinase immunoreactivity with S100β but not with neuronal markers (H. Hsuchou et al., unpublished observations). Even if deletion of ObR occurred in some neuronal progenitor cells, ALKO mice show a different phenotype from neuronal ObR knockout mice. Regardless of a minor loss of leptin signaling in GFAP-promoter (+) neuronal cells, the lack of obesity in the ALKO mice fed the CD and the same response as WT mice to the HFD clearly indicate that neuronal leptin signaling remained largely intact. This indicates an antagonistic role of astrocytic and neuronal leptin signaling in obesity.

Although the ALKO mutation by itself did not induce a significant reduction in sObR, ALKO mice responded to the HFD with reduced sObR compared with the WT fed the HFD, or ALKO mice fed the CD. The decrease in sObR in ALKO mice fed the HFD raises the question of whether higher levels of free leptin result in higher brain levels of leptin relative to serum levels. Nonetheless, the reduction in sObR favors leptin sensitivity. Soluble ObR levels are regulated by adiposity and leptin concentrations. Soluble ObR in turn regulates the biological activity of leptin. The reduction in sObR might partially compensate for the loss of astrocytic influence to neurons by leptin signaling.

On the CD, ALKO mice showed obesity-like changes in leptin gene expression. Hyperleptinemia is a common characteristic of DIO in which leptin signaling is aberrant. The ALKO mice show hyperleptinemia in the absence of obesity at baseline, though the elevation is much less than the 15-fold increase observed in the ELKO mice. However, there was a higher basal level in the ALKO mice on an FVB background than in the ELKO mice on a C57 background, probably related to age and strain differences (18).

Although leptin does play a role in neuroregeneration at high concentrations, it can also function as a proinflammatory cytokine. Recent evidence shows that leptin is among the serum factors in obese mice that exacerbates scratch-injury–induced microgliosis and astrogliosis (9). Reactive gliosis during DIO is a sign of neuroinflammation, adding to dysfunctional central leptin signaling. Reactive astrocytes are known to release proinflammatory cytokines (6). Because ALKO mice have baseline hyperleptinemia without obesity, we determined whether these mice show reactive astrogliosis by measurement of GFAP expression in the hypothalamus, and whether this is further exacerbated by DIO. Although the ALKO mutation increased hypothalamic GFAP, DIO did not increase it in either strain; rather, it was reduced. The effect was apparently not mediated by ObRb, which is unchanged, and is consistent with the observation that neurons are the main source of ObRb in the hypothalamus (20, 22). Leptin resistance, reflected by saturation of leptin transport across the BBB and desensitization of intracellular signaling with recruitment of antagonizing signaling elements, could also be lessened in the absence of astrocytic leptin signaling. However, leptin does not seem to act through recruitment of antagonizing pathways such as SOCS-3, a marker for leptin resistance (16), or by induction of COX-2, an indicator of sustained neuroinflammation (1). With better maintained leptin sensitivity, neuronal leptin signaling might serve as a driving force for some of the leptin-induced responses in the ALKO mice. The hypothalamus may provide the structural basis for these changes because it is the head ganglion of the autonomic nervous system for sympathetic output and, among all CNS regions, plays a crucial role in feeding and metabolism. This is supported by a better profile of serum triglyceride and greater elevation of UCP1 when the ALKO mice were fed the HFD, though hyperinsulinemia persisted. Figure 6 repre-

**Fig. 6.** Hypothetical model showing how astrocytic leptin signaling could contribute to impaired central leptin signaling. Under normal dietary conditions, neuron-astrocyte interactions are highly regulated. In the event of a prolonged HFD, astrocytic ObRs become highly reactive to the high circulating concentrations of leptin. Reactive astrocytes are known to release proinflammatory cytokines, which eventually lead to neuroinflammation and thus reduced sensitivity to leptin. In ALKO mice fed an HFD, the number of reactive astrocytes is reduced because of the lack of functional astrocytic leptin signaling. This is turn protects the neurons from inflammatory damage and thus better preserves neuronal leptin signaling.

**Table 1.** Diet-induced obesity in Normal mice

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<th>Central</th>
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<td>Leptin</td>
<td>Astrocytic astrogliosis</td>
<td>ObR, hyperleptinemia</td>
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<tr>
<td>Neuroinflammation</td>
<td>Leptin resistance</td>
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**Table 2.** Diet-induced obesity in ALKO

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<td>Leptin</td>
<td>No astrocytic ObR, normal leptin signaling</td>
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<tr>
<td>Neuroinflammation</td>
<td>Regulation of leptin production</td>
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sents a model of how astrocytic leptin signaling modulates the metabolic response of the mice in basal and DIO states. Astrocytic leptin signaling participates in the interactions of astrocytes with other cells, as shown in mice fed with the CD. An increase in leptin mRNA and hypothalamic GFAP are among the consequences of ALKO. In DIO, astrocytes are reactive, ObR expression is increased, and astrocytic leptin signaling is enhanced in response to hyperleptinemia. In ALKO mice fed an HFD, the elevation in GFAP is attenuated and the micro milieu is modulated. In addition, astrocytic leptin signaling (or its absence in ALKO mice) affects long efferent projections to regulate the production of leptin, sObR, and UCP1 gene expression in response to an HFD.

In general, results from the ALKO mice suggest a biphasic or dual role of astrocytic leptin signaling: its absence in the basal state (ALKO mice fed the CD) results in an obesity-like metabolic change, whereas DIO provides partial protection to preserve the metabolic profile. In obesity, a loss of astrocytic leptin signaling helps to better preserve neuronal leptin signaling, despite a lack of change in body wt or energy expenditure. This may be analogous to improved insulin sensitivity, blood pressure control, or sleep apnea that can occur without weight loss; thus a beneficial effect of ALKO on DIO cannot be dismissed. Overall, results from ALKO mice indicate that astrocytic leptin signaling contributes to obesity-induced hyperleptinemia and impaired neuronal leptin sensing.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

REFERENCES