Insulin regulates hepatic leptin receptor expression in early lactating dairy cows

Stephanie R. Thorn, Richard A. Ehrhardt, W. Ronald Butler, Susan M. Quirk, and Yves R. Boisclair

Department of Animal Science, Cornell University, Ithaca, New York

Submitted 29 June 2008; accepted in final form 19 September 2008

Thorn SR, Ehrhardt RA, Butler WR, Quirk SM, Boisclair YR. Insulin regulates hepatic leptin receptor expression in early lactating dairy cows. Am J Physiol Regul Integr Comp Physiol 295: R1455–R1462, 2008. First published September 24, 2008; doi:10.1152/ajpregu.90546.2008.—Energy balance controls the expression of the leptin receptor (Lepr) in the ruminant hypothalamus but whether similar regulation occurs in peripheral tissues is unknown. To address this issue, we measured Lepr expression in the liver and adipose tissue of dairy cows during the transition from late pregnancy (LP) to early lactation (EL). This period is characterized by the development of a profound state of energy insufficiency and is associated with reduced plasma insulin and leptin and with increased plasma growth hormone. Hepatic expression of the short (Lepr-a) and long (Lepr-b) isoforms was 40% higher during EL (8 days postpartum) than LP (30 days prepartum). A similar effect was observed when negative energy balance was induced in nonpregnant, late-lactation dairy cows by food restriction, implicating energy insufficiency as a specific cause in EL. The stimulation of hepatic Lepr expression was reversed after a 48-h period of hyperinsulinemic euglycemia in EL. Changes in hepatic Lepr expression during chronic elevation of plasma leptin in EL or plasma growth hormone in nonpregnant, late-lactation cows did not support a role for these hormones in mediating the effects of energy insufficiency on hepatic Lepr expression. In adipose tissue, Lepr expression was increased 10-fold during the transition from LP to EL. Overall, these data indicate that hypoinsulinemia is partly responsible for the induction of Lepr expression in the liver, and perhaps adipose tissue, of energy-deficient dairy cows.

bovine; energy balance; liver; adipose

TRANSCRIPTION OF THE LEPTIN receptor gene (Lepr) gives rise to transcripts encoding the full-length receptor (Lepr-b) and a family of truncated receptors with limited signaling capabilities (Lepr-a, Lepr-c, Lepr-d, Lepr-e, Lepr-f) (30, 40). Regulation of Lepr expression has been studied extensively in rodents. Lepr-b is most abundant in the hypothalamus (19, 40), where its expression is stimulated by caloric restriction, dietary fat, and ovariectomy (4, 12, 20, 43, 46). Lepr is also expressed in peripheral tissues, with the truncated Lepr-a isoform accounting for nearly all of Lepr transcripts in the uterus and mammary adipose tissue, where it reduced leptin concentrations play a causative role. Studies were performed in the lactating dairy cow, the ruminant animal experiencing the greatest degree of energy insufficiency during the periparturient period (3, 7, 45). We focused on the liver, the most abundant site of Lepr expression (41), consistent with the spatial distribution of Lepr isoforms reported by others (27). We also found that estrogen had no effect on peripheral Lepr expression, except in the uterus and mammary adipose tissue, where it reduced Lepr-a and Lepr-b transcript abundance (41). There is, however, no information on the effect of metabolic factors, such as energy insufficiency, on the expression of Lepr transcripts in peripheral tissues of ruminants.

The most dynamic naturally occurring change in energy metabolism in ruminants occurs during the transition from pregnancy to lactation (3, 7, 45). This period is characterized by the onset of negative energy balance due to the high metabolic demands of the mammary gland. The transition from pregnancy to lactation involves coordination among tissues, including the mammary gland and metabolically active tissues like liver, skeletal muscle, and adipose tissue (2, 7). Examples of this include the development of growth hormone resistance in the liver and insulin resistance in skeletal muscle and adipose tissue to spare glucose for utilization by the mammary gland (3, 7).

Accordingly, our objectives were to determine whether Lepr expression is altered in peripheral tissues during the transition from pregnancy to lactation and, if this is the case, whether periparturient changes in plasma insulin, growth hormone, and leptin concentrations play a causative role. Studies were performed in the lactating dairy cow, the ruminant animal experiencing the greatest degree of energy insufficiency during the periparturient period (3, 7, 45). We focused on the liver, the most abundant site of Lepr expression (41), and on adipose tissue, a site of lesser expression that plays an equally important role in maintaining metabolic homeostasis during the periparturient period. Our data indicate that Lepr expression in liver and adipose tissue is increased in early lactating dairy cows and that hypoinsulinemia contributes to this effect.

MATERIALS AND METHODS

Animal experiments. All experiments were performed on multiparous Holstein dairy cows. Experimental procedures were conducted with the approval of the Cornell University Institutional Animal Care and Use Committee. Cows were held in stalls during all experiments.

In species such as sheep and cattle, regulation of Lepr expression has been studied almost exclusively in the hypothalamus. In sheep, hypothalamic Lepr-b expression is increased during fasting, food restriction, and lactation (1, 16, 29, 39). In prepubertal dairy heifers, we showed that Lepr-b is the most abundant isoform expressed in the hypothalamus, whereas Lepr-a accounted for nearly all of Lepr transcripts in peripheral tissues (41), unlike what was observed in the mammary gland (3, 7).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
to allow for measurement of individual food intake. Tissue biopsy samples were obtained under local anesthesia as previously described (8, 31). For liver biopsies, an incision was made through the skin, and a liver sample was obtained using a biopsy instrument. Adipose tissue samples were obtained from the tail head region by an incision through the skin and collection of adipose tissue. Samples were immediately frozen in liquid nitrogen and stored at −80°C for subsequent use in RNA analyses.

Periparturient period and leptin infusion. The objectives of this study were to evaluate the effect of energy insufficiency during the transition from late pregnancy (LP) to early lactation (EL) and the effect of leptin treatment during early lactation. Ten cows were studied during the transition from LP to EL, as previously described (18). In brief, cows were fed ad libitum amounts of a total mixed ration (TMR) containing 1.5 Mcal net energy of lactation (NE\textsubscript{L}) and 140 g crude protein per kg dry matter (DM). After calving, cows were fed a lactation TMR containing 1.5 Mcal NE\textsubscript{L} and 180 g crude protein per kg DM. Individual blood samples were obtained at 2-h intervals between 0800 and 1600 in LP (day 0) and again in EL 8 days after parturition (day +8). Liver and adipose tissue biopsies were obtained immediately after blood sampling. Following biopsies on day +8, cows were randomly allocated to receive a continuous jugular vein infusion for 4 days of either saline or recombinant human leptin (42.4 ng·min\textsuperscript{−1}·kg body wt\textsuperscript{−1}; Lilly, Indianapolis, IN) (n = 5 per treatment). Liver and adipose tissue biopsies were obtained again on day +12, immediately prior to the end of the infusion period. Individual net energy balance was estimated as the difference between energy intake and energy expenditure (maintenance and pregnancy during LP and maintenance and milk energy during EL) essentially as described by Block et al. (5). The energy intake was estimated from intake and chemical composition of feeds (34). Maintenance energy requirement was estimated from body weight, and milk energy output was calculated from daily milk yield and composition (34).

Food restriction and growth hormone treatment. The objectives of this study were to evaluate the effect of energy insufficiency in nonpregnant, late-lactation dairy cows and the effect of growth hormone treatment. Five nonpregnant, late-lactation cows were assigned to a 14-day period of adequate feeding (AF), a 3-day intervening period, and a 14-day period of food restriction (FR) as previously described (38). The AF period was set to 120% of predicted energy requirements and FR to 30% of maintenance energy requirements. Each plane of nutrition was achieved by feeding appropriate amounts of a TMR (1.5 Mcal NE\textsubscript{L} and 157 g of crude protein/kg DM). During the last 10 days of each feeding regime, cows were assigned in a single reversal design with 4-day treatment periods and a 2-day interval. Treatments were daily intramuscular injections at 0900 of saline or recombinant bovine growth hormone (GH) (40 mg; Monsanto, St. Louis, MO). The treatment sequence was randomly assigned, with some cows receiving saline first and GH second, and with others receiving treatments in the reciprocal order. Liver and adipose tissue biopsies were obtained from each cow at the end of each 4-day treatment during both the AF and FR periods. Biopsies were taken 6 h after intramuscular injections when GH-treated AF and FR cows had similarly elevated plasma GH concentrations (34.6 ng/ml) that were higher than saline-treated AF and FR cows (4.5 ng/ml during the AF period and 6.5 ng/ml during the FR period, P < 0.001 for both comparisons). Net energy balance was estimated as described above and reported (38).

Hyperinsulinemic-euglycemic clamp. The objective of these studies was to evaluate the effect of hyperinsulinemia during LP and EL. Two hyperinsulinemic-euglycemic clamp studies were used to obtain liver or adipose tissue. In the first experiment, 6 cows were studied during LP (−31 ± 1.5 days, relative to parturition) and EL (+7 ± 1.6 days, relative to parturition) (31). Cows were fed a TMR containing 1.56 Mcal NE\textsubscript{L} and 140 g crude protein/kg DM during LP and a TMR containing 1.58 Mcal NE\textsubscript{L} and 198 g of crude protein/kg DM during EL. Basal conditions were characterized by taking blood samples over 66 h (−66, −43, −30 and 0 h relative to initiation of the clamp). Immediately after the last basal blood sample, a liver biopsy was obtained followed by intravenous administration of bovine insulin at the rate of 1 μg·kg body wt\textsuperscript{−1}·h\textsuperscript{−1} for 96 h during LP and 48 h during EL. The concentration of blood glucose was monitored hourly and maintained to the concentration of blood glucose observed during the basal period by varying the rate of intravenous infusion of a glucose solution (50% wt/vol dextrose solution). A second liver biopsy was obtained just before the end of the insulin infusion.

A second study was performed in EL with 12 lactating cows. Cows were fed a TMR containing 1.63 Mcal NE\textsubscript{L}/kg DM. On day +10 (relative to parturition), cows were randomly allocated to a 96-h period of saline infusion or hyperinsulinemic-euglycemic clamp (8). The hyperinsulinemic-euglycemic clamp was performed as described above. Immediately prior to the end of the infusion period, an adipose tissue biopsy sample was obtained from each cow. For both clamp studies, net energy balance was estimated as described above and reported (8, 31).

Analytical metabolites and hormones. Plasma glucose was measured by the glucose oxidase method and nonesterified fatty acids (NEFA) by the acyl-CoA synthetase/oxidase method (5, 31). The plasma concentrations of bovine hormones were assayed by double antibody RIA validated in our laboratory using bovine proteins for iodination and standards. The IGF-I RIA was described previously (5). The insulin RIA was performed with a guinea pig antihuman insulin primary antibody (Sigma; lot 062K4829; 1:10,000 dilution) and a goat anti-guinea pig IgG secondary antibody (Equitech-Bio, Kerrville, TX; Lot GAGP80-426, 1:30 dilution). Bovine leptin was measured by a RIA that we developed that does not recognize human leptin (17). Human leptin was measured using a RIA with no cross-reactivity with bovine leptin (Linco Research, St. Charles, MO). Interassay and intra-assay coefficients of variation for all assays averaged less than 7% and 6%, respectively.

Gene expression analyses. Representative tissue samples (50 to 80 mg of liver and 200 mg of adipose tissue) were homogenized with 1 ml Qiazol (Qiagen, Valencia, CA). Total RNA was isolated and purified using RNeasy Mini columns and on-column RNase-Free DNase treatment (Qiagen). Quantity and integrity of RNA were determined using the RNA Nano Lab Chip Kit and BioAnalyzer (Agilent, Palo Alto, CA). Reverse-transcription reactions were performed with 2 μg of RNA, 500 ng of random primers (Invitrogen, Carlsbad, CA), and ImPromII reverse transcriptase (Promega, Madison, WI) in a 20-μl volume. Real-time PCR assays were used to measure all leptin receptor isoforms (Lepr-Total), one of the short isoforms (Lepr-a), the long isoform (Lepr-b), and leptin as previously described (41). Abundance of 18S ribosomal RNA was measured with a commercial kit (Applied Biosystems, Foster City, CA) in liver samples and a SYBR Green assay in adipose tissue samples (25). Reactions were performed in duplicate in a 25-μl volume using Perfect Real Time 2× Premix with supplied ROX dye for probe-based assays (Takara, Madison, WI) or ABI Power SYBR Mix (Applied Biosystems). Reactions contained appropriate amounts of diluted cDNA (4 to 10 ng for 18S assays, 25 ng for leptin assays, and 100 ng for Lepr assays) and optimal concentrations of primers and probes (41, 42). For the Lepr assays, data were analyzed using the standard curve method with a single plasmid, containing sequences homologous to the amplification products for Lepr-Total, Lepr-a, and Lepr-b, as previously described and validated (41). The standard curve was prepared by serial dilution of the plasmid (6 standards, ranging from 10 to 1,000,000 copies) and used to calculate transcript copy number for each Lepr assay. For leptin and 18S, a relative standard curve was prepared using pooled cDNA. Data were normalized to 18S abundance.

Statistical analyses. Data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Institute, Raleigh, NC). Data from the periparturient period were analyzed with a model accounting for
stage (LP or EL) as a fixed effect and cow as a random effect. For the
leptin infusion experiment, the data at the end of the infusion (day
+12) were analyzed with the fixed effect of treatment (saline or
leptin) and data prior to the start of the infusion (day +8) were used
as a covariate. Data from the nutrition and GH experiment were
analyzed with a model accounting for physiological stage (LP or EL),
clamp (basal or insulin), their interaction as fixed effects, and cow as
a random effect. Data from the second insulin clamp experiment were
analyzed with fixed effect of treatment (saline or insulin). The level of
statistical significance was set at \( P < 0.05 \).

RESULTS

Leptin receptor expression is increased in liver during
energy insufficiency. Estimated net energy balance was 12.1
Mcal/day in LP and decreased to –15.2 Mcal/day in EL (18). The
energy insufficiency state of EL was associated with lower plasma
concentrations of glucose, insulin, and leptin and higher plasma
NEFA concentrations compared with LP (Table 1, \( P < 0.01 \) for
all). Plasma IGF-I was also reduced in EL \( (P < 0.01) \). We and
others have shown that reduced plasma IGF-I in early lactating
dairy cows is associated with increased plasma GH (5, 28, 36–38).

Copy number for the truncated Lepr-a and long Lepr-b
isoform increased by 40% and 50%, respectively, during the
transition from LP to EL (Fig. 1A). The abundance of all Lepr
transcripts (Lepr-Total), measured by an independent assay
detecting all isoforms, changed in a manner similar to that of
Lepr-a \( (r = 0.94, P < 0.001) \). This similarity reflects that
Lepr-a accounts for more than 75% of Lepr-Total abundance,
whereas Lepr-b accounts for less than 1% of Lepr-Total, ir-
respective of physiological state (Fig. 1A).

To determine whether the energy deficit of EL was responsible
for increased Lepr abundance, without confounding effects
associated with parturition, nonpregnant, late-lactation cows
were studied during periods of AF or FR. FR reduced estimated
net energy balance from 6.1 Mcal/day to –12.4
Mcal/day (38). Induction of energy insufficiency in this model
was also associated with reductions in the plasma concentra-
tions of insulin \( (1.6 \text{ vs. } 0.4 \text{ ng/ml}, P < 0.01) \) and leptin \( (3.0 \text{ vs.}
2.3 \text{ ng/ml}, P < 0.01) \) and an increase in the plasma concen-
tration of GH \( (4.5 \text{ vs. } 6.5 \text{ ng/ml}, P < 0.01) \) \( (6, 38) \). FR
increased hepatic Lepr-Total and Lepr-a copy number by
nearly 40% but had no significant effect on Lepr-b copy
number \( (Fig. 1B \text{ and data not shown for Lepr-Total}) \). Together,
these data indicate that energy insufficiency is responsible for
increased hepatic Lepr expression in EL.

Leptin receptor expression is reduced by insulin and
increased by leptin. Because negative energy balance is associated
with increased plasma GH and reduced plasma insulin
concentrations, we sought to evaluate whether these changes
mediated the effects of energy balance on Lepr abundance. To
evaluate the role of GH, nonpregnant, late-lactation cows were
 treated with GH during the periods of AF and FR. GH treat-
ment had no effect on net energy balance (38), and more
importantly, had no effect on hepatic Lepr-a or Lepr-b expres-
sion during either feeding period \( (Fig. 1B) \).

The effect of insulin on Lepr expression was assessed by
performing hyperinsulinemic euglycemic clamps during LP
and EL. During the clamp, estimated net energy balance
increased from a basal value of 9.0 Mcal/day to 14.1 Mcal/day
in LP and from a basal value of –14.6 Mcal/day to –11.5
Mcal/d during EL \( (31) \). Plasma insulin concentrations in-
creased twofold during the clamp in LP \( (1.8 \text{ to } 4.0 \text{ ng/ml}) \) and
nearly four-fold during EL \( (0.7 \text{ to } 2.5 \text{ ng/ml}) \) \( (31) \). Consistent
with the results presented in Fig. 1, hepatic abundance of all

Table 1. Response in plasma hormone and metabolite
centrations during the transition from late pregnancy
and early lactation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Physiological State</th>
<th>LP</th>
<th>EL</th>
<th>SE</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, ng/dl</td>
<td></td>
<td>66</td>
<td>54</td>
<td>1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>NEFA, ( \mu M )</td>
<td></td>
<td>107</td>
<td>355</td>
<td>36</td>
<td>0.01</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td></td>
<td>3.1</td>
<td>2.3</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td></td>
<td>0.26</td>
<td>0.08</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td></td>
<td>193</td>
<td>56</td>
<td>11</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Dairy cows were studied during late pregnancy (LP) and early lactation
(EL), as described in MATERIALS AND METHODS. Mean values and standard error
are shown, and significance level \( (P) \) is indicated. NEFA, nonesterified fatty
acids.
three Lepr transcript categories increased during the transition from pregnancy to lactation (Fig. 2A and data not shown for Lepr-Total). Chronic insulin infusion had no effect on hepatic Lepr expression during LP, but reduced Lepr-a, Lepr-b, and Lepr-Total expression during EL to levels below those observed in LP (Fig. 2A and data not shown for Lepr-Total).

In the clamp study, chronic hyperinsulinemia increased plasma leptin levels from 3.2 to 5.6 ng/ml in LP but only from 2.7 to 3.1 ng/ml in EL (31). In rodents, leptin treatment induced hepatic Lepr expression (12). A similar effect in dairy cows could explain the lack of insulin inhibition on hepatic Lepr during LP because the greater increase in plasma leptin may counteract the effect of insulin. To determine the effect of leptin on expression of its receptor, dairy cows received a 96-h intravenous infusion of saline or recombinant human leptin during EL. At the end of the infusion period, circulating concentrations of human leptin averaged 15.1 ng/ml in leptin-treated cows, and plasma concentrations of endogenous bovine leptin were unchanged in saline and leptin-treated cows (2.4 and 2.5 ng/ml, respectively) (18). There was no change in estimated net energy balance (−12.3 and −15.4 Mcal/day, P > 0.3) or plasma insulin (0.15 and 0.10 ng/ml, P > 0.3) between the saline- and leptin-treated cows at the time of biopsy (18). Leptin treatment increased hepatic expression of Lepr-a and Lepr-Total transcripts by more than 40% and Lepr-b expression by 30% (Fig. 2B and data not shown for Lepr-Total). These data indicate that insulin inhibits, whereas leptin induces hepatic Lepr expression.

Energy insufficiency increased leptin receptor expression in adipose tissue. Next, adipose tissue biopsy samples were analyzed to evaluate whether energy balance, GH, leptin, and insulin had effects on Lepr expression in adipose tissue similar to those observed in the liver. Consistent with reduced plasma leptin in EL (Table 1), leptin expression in adipose tissue was lower in EL than in LP (Fig. 3A).

Expression of Lepr-Total, Lepr-a, and Lepr-b was substantially lower in adipose tissue than in liver, averaging less than 7% of the levels found in the liver during LP and EL (compare Figs. 1A and 3A). The overall effect of the transition from LP to EL was similar to that seen in liver, with Lepr-a and Lepr-Total increasing by over 10-fold and Lepr-b by nearly eight-fold (Fig. 3A). As was the case for liver, Lepr-a accounted for the majority of Lepr-Total expression (nearly 80%), whereas Lepr-b accounted for less than 3%. Thus, Lepr-a abundance is representative of Lepr-Total in both the liver and adipose tissue.

FR also reduced leptin expression in the adipose tissue of nonpregnant, late-lactation cows (P < 0.001, Fig. 3B), but in contrast to the energy insufficiency of EL, FR did not cause significant increases in the expression of Lepr-a or Lepr-b. Irrespective of the plane of nutrition, GH treatment had no significant effects on Lepr-a or Lepr-b copy number.

The effects of insulin in EL were evaluated in a second hyperinsulinemic-euglycemic clamp experiment. In this experiment, cows were infused with saline or subjected to a hyperinsulinemic-euglycemic clamp in EL for 96 h (8). Insulin infusion increased leptin expression (P < 0.05, Fig. 4A) but only tended to reduce Lepr expression in adipose tissue (P = 0.1 for Lepr-a and P = 0.13 for Lepr-b, Fig. 4A). Peripheral infusion of human leptin in EL did not alter Lepr expression in adipose tissue (Fig. 4B). Human leptin infusion also had no effect on leptin expression (results not shown), consistent with its lack of effects on endogenous plasma leptin (18).

**DISCUSSION**

A quantitative determination of Lepr isoforms has not been performed in any tissue of the lactating dairy cow. In this study, we focused on the liver and subcutaneous adipose tissue because both tissues experience major shifts in metabolism to accommodate the negative energy balance that occurs at the onset of lactation (2, 3, 7). Expression of Lepr isoforms in dairy cows was 50- to 100-fold higher in the liver than in subcutaneous adipose tissue, consistent with data in rodents and our findings in prepubertal dairy heifers (12, 41). In both tissues, Lepr-a accounts for greater than 70% of Lepr-Total, whereas Lepr-b accounts for less than 5%. This also agrees with our data obtained in prepubertal dairy heifers, showing that Lepr-a predominates in all tissues, except the hypothalamus, whereas Lepr-b is the major isoform (41). The transition from pregnancy to lactation caused an increase in Lepr expression in both liver and adipose tissue but did not alter the relative contribution of Lepr-a and Lepr-b to the total popul-
tion of Lepr transcripts. In our studies, all conditions tested affected Lepr-a and Lepr-Total in a similar manner.

To dissociate the effects of energy insufficiency from par-turition-related events, we studied nonpregnant, late-lactating dairy cows first when well fed and then during a period of severe food restriction. This sequential design caused shifts in net energy balance similar to those described previously between late pregnancy and the first week of lactation (5, 31, 37). We recently showed that this similarity extends to blood variables indicative of energy status (glucose, NEFA, insulin, leptin, GH, and IGF-I) in terms of both the magnitude of their change and the steady-state concentration reached during the energy-deficient period (6, 38). The results that we obtained in this model indicate that energy insufficiency is primarily re-sponsible for increased Lepr expression in EL in liver. These findings are in agreement with data in rodents that show increased Lepr expression in liver and adipose tissue in re-sponse to caloric restriction (12, 20). Caloric restriction has also been shown to increase Lepr-b expression in the hypothalamus of rats (46). In the sheep, hypothalamic Lepr-b expression is increased during fasting, food restriction, and lactation (1, 16, 29, 39). The effect of negative energy balance on hypothalamic Lepr abundance has yet to be evaluated in cattle.

The mechanisms, whereby energy insufficiency increases Lepr expression, are not known but could include hormonal changes associated with EL and FR (reduction in IGF-I, increase in GH, reduction in insulin, or increase in leptin) (5, 28, 36–38). We did not consider IGF-I because it is well known that both liver and adipose tissue do not have IGF-I receptors (11). In the case of GH, if an increase were responsible for stimulating Lepr expression in the liver and adipose tissue, we would expect that treatment of cows with exogenous GH would increase Lepr expression. Our data show that irrespective of energy balance, GH treatment did not increase hepatic and adipose tissue Lepr expression. Cohen et al. (12) hypothesized that increased Lepr expression in the liver during fasting may reflect the loss of an inhibitory effect of insulin on transcript abundance (12). This is further supported by recent data showing that mice lacking the insulin receptor in the liver have increased expression of all Lepr transcripts in the liver and that insulin treatment reduces Lepr-a expression in normal isolated mouse hepatocytes (13). Our results provide direct support in vivo for this by showing that raising plasma insulin to levels prevailing in LP is sufficient to reduce Lepr expres-sion in the liver of dairy cows in EL.

Chronic elevation of insulin did not, however, reduce Lepr expression during pregnancy. Previously, we reported that chronic hyperinsulinemia caused a twofold increase in plasma leptin in LP but had no effect in EL (31). These findings together with the differential expression of Lepr in response to insulin during LP and EL suggested that leptin may regulate expression of its receptor. We hypothesized that insulin and leptin had opposite effects on hepatic Lepr transcription, with insulin decreasing and leptin increasing Lepr expression. This appears to be a potential mechanism because leptin treatment in EL increased Lepr in liver. This result is similar to data obtained in mice showing that leptin treatment increased

![Fig. 3. Effect of the transition from pregnancy to lactation, food restriction, and growth hormone on adipose tissue leptin and leptin receptor abundance. Total RNA was isolated from adipose biopsy samples, and relative abundance for leptin and transcript copy number for Lepr-a, Lepr-b, and Lepr-Total were determined by quantitative real-time PCR. A: adipose tissue biopsy samples were obtained from dairy cows \((n = 10)\) on day \(-30\) (relative to parturition) during late pregnancy (LP) and on day \(+8\) in EL. Means \pm SE are shown for each treatment. *\(P < 0.05\). B: nonpregnant, late-lactation dairy cows \((n = 5)\) were studied during periods of AF and FR. During each feeding period, cows were treated for 4 days with saline or GH and adipose tissue biopsies were obtained. Means \pm SE are shown for each treatment. Significant effect of nutrition is shown.](http://ajpregu.physiology.org/)
Leptin a continuous infusion of either saline or were subjected to a hyper-

As discussed earlier, nearly all of Lepr transcripts in both liver

derived from the central nervous system. For example, a soluble leptin receptor (SLR) is produced in rodents by shedding of membrane-bound Lepr-a in liver, and to a lesser degree in adipose tissue (12, 20, 24). An SLR protein derived from ectodomain shedding of membrane-bound receptors also exists in humans (22). SLR levels are increased by food restriction, age, and leptin in rodents (12, 20, 24). SLR inhibits leptin action by binding leptin and forming a complex that is unable to activate Lepr-b in target tissues (24, 47). Interestingly, the fraction of plasma leptin circulating in a bound form appears negligible in cattle (7, 21, 48). Alternatively, truncated Lepr isoforms could provide a mechanism for receptor-mediated endocytosis of leptin (44). Circulating leptin could bind membrane-bound Lepr in peripheral tissues and be internalized. Both mechanisms would serve to reduce the amount of bioactive leptin available for Lepr-b binding in the hypothalamus, allowing the activation of central pathways stimulating appetite and energy conservation.

Perspectives and Significance

Overall, our results demonstrate that Lepr expression is increased in liver and adipose tissue of early lactating dairy cows, and this is likely caused by the onset of negative energy balance. Our data also indicate that changes in insulin levels may mediate the stimulatory effects of negative energy balance on peripheral Lepr expression. Increased peripheral Lepr expression during energy insufficiency may be a mechanism to attenuate central leptin action. Additional studies using cattle are needed to assess this model. In particular, it will be necessary to demonstrate that increased Lepr expression leads to increased Lepr protein levels and to determine whether Lepr signaling in adipose tissue is increased hepatic Lepr expression.

The functional significance of increased Lepr expression in peripheral tissues in the early lactating dairy cow is unknown. As discussed earlier, nearly all of Lepr transcripts in both liver and adipose tissue are accounted for by Lepr-a. Lepr-a and the other truncated leptin receptors lack most or all of the intracellular residues that are required for leptin signaling ability (33). The db/db mouse, which lacks Lepr-b but retains expres-

sion of truncated Lepr receptors, is a phenocopy of the ob/ob mouse (10). The db/db phenotype can be reversed by transgenic complementation of Lepr-b into neural tissues or the hypothalamic arcuate nucleus (14, 15). Furthermore, mice lacking Lepr expression specifically in the liver have no change in body weight, suggesting that hepatic leptin signaling is dispensable for whole body energy homeostasis (24). In contrast to the liver, leptin signaling in adipose tissue may have a functional impact. A reduction in Lepr expression in adipose tissue of mice increased body weight, adipose tissue mass, plasma insulin and leptin, and tissue triglyceride content (26). Mice lacking Lepr signaling in adipose tissue and small intestine, in addition to liver, have increased leptin production due to disruption of a negative feedback loop in the adipose tissue (24). The ability of leptin to deplete lipids in vivo requires its action not only on the hypothalamus but also on adipocytes (35). Finally, adipose-specific ablation of STAT3, which mediates a major portion of Lepr-b signaling, increases adiposity and impairs leptin-induced lipolysis (9).

It is possible that Lepr plays a role in peripheral tissues that is completely different from its role in the central nervous system. Lepr-a, Lepr-b, and Lepr-c expression in the liver (12). Lepr induction in mice, however, was caused, in part, by the anorexic effect of leptin. In contrast, the positive effects of leptin infusion observed in the present study occurred in the absence of any reduction in food intake or energy balance (18) and thus can be attributed completely to leptin. Overall, however, plasma insulin fell considerably more than leptin between LP and EL in this and other experiments in which both hormones were measured (18, 31, 37), and, therefore, we speculate that the sum effect in EL is increased hepatic Lepr expression.

The functional significance of increased Lepr expression in peripheral tissues in the early lactating dairy cow is unknown. As discussed earlier, nearly all of Lepr transcripts in both liver and adipose tissue are accounted for by Lepr-a. Lepr-a and the other truncated leptin receptors lack most or all of the intracellular residues that are required for leptin signaling ability (33). The db/db mouse, which lacks Lepr-b but retains expres-

Fig. 4. Effect of leptin and insulin on adipose tissue leptin and leptin receptor abundance. Total RNA was isolated from adipose tissue biopsy samples, and relative abundance for leptin and transcript copy number for Lepr-a and Lepr-b was determined by quantitative real-time PCR. A: early lactating dairy cows received a continuous infusion of either saline or were subjected to a hyper-insulinemic-euglycemic clamp. Infusions began on day +10 and continued for 96 h. Adipose tissue biopsies were obtained from each cow at the end of the infusion. Means ± SE is shown for each treatment. P < 0.05. B: early lactating dairy cows received a continuous intragraftual infusion of either saline or recombinant human leptin (n = 5 per treatment). Infusions began on day +8 and continued for 96 h. Adipose tissue biopsies were obtained from each cow immediately before the start (used as covariate) and again at the end of the infusion period. Means ± SE are shown for each treatment.
This project was supported by the National Research Initiative Competitive Grant 2003-35203-12832 and 2007-35206-17844 from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service.

REFERENCES


27. Kobayashi Y, Boyd CK, Bracken CJ, Lamberson WR, Keisler DH, Lucy MC. Reduced growth hormone receptor (GHR) messenger ribonucleic acid in liver of periparturient cattle is caused by a specific down-regulation of GHR 1A that is associated with decreased insulin-like growth factor I. Endocrinology 140: 3947–3954, 1999.


36. Radcliff RP, McCormack BL, Crooker BA, Lucy MC. Plasma hormones and expression of growth hormone receptor and insulin-like growth


