Divergent Effects of Leptin on Luteinizing Hormone and Insulin Secretion Are Dose Dependent

DOROTA A. ZIEBA,*† MARCEL AMSTALDEN,*† MARLON N. MACIEL,*† DUANE H. KEISLER,‡ NINA RAVER,§ ARIEH GERTLER,§ AND GARY L. WILLIAMS*†,[1]

*Animal Reproduction Laboratory, Texas A&M University Agricultural Research Station, Beeville, Texas; †Department of Animal Science, Center for Animal Biotechnology and Genomics, Texas A&M University, College Station, Texas; ‡Department of Animal Science, University of Missouri, Columbia, Missouri; and §Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem, Rehovot, Israel

We have shown recently that fasting permits leptin to modulate both luteinizing hormone (LH) and insulin secretion in cows. In rodents, leptin causes divergent effects on LH and insulin release that are dose dependent. To test the hypothesis that leptin effects on LH and insulin secretion in fasted cows are dose related, we examined the effects of various doses of recombinant ovine leptin (oleptin) in mature cows. Twenty ovariectomized beef cows, each bearing an estradiol implant to maintain basal estradiol concentrations, were used. All cows were fasted for 60 hr with free access to water and were assigned randomly to one of four groups (n = 5/group): 1) saline control; 2) leptin, 0.2 μg/kg; 3) leptin, 2.0 μg/kg; and 4) leptin, 20 μg/kg body wt. Blood samples were collected at 10-min intervals for 6 hr on Days 0 and 2, with saline or oleptin injected intravenously immediately after the first intensive sample on Day 2 (54 hr). Leptin caused a dose-related increase ($P < 0.001$) in mean concentrations of circulating LH. Stimulation of LH release by leptin was significant at the lowest (141% of control) and middle (122% of control) doses used, but no increase was observed for the highest dose. Increased mean concentrations of LH appeared to result from an augmentation of basal secretion, as pulse characteristics were not affected. After 54 hr of fasting, plasma insulin concentrations were lowered ($P < 0.01$) in all treatment groups compared to Day 0. After leptin injections, plasma insulin concentrations increased ($P < 0.01$) and reached highest concentrations during the first hour of sampling. However, this increase was sustained for several hours only in the intermediate (2.0 μg/kg) dose group. Collectively, our results show that leptin has potent positive effects on both LH and insulin secretion in fasted cows, but the anterior pituitary and endocrine pancreas appear to become downregulated in the presence of excess ligand. Exp Biol Med 228:325–330, 2003

Key words: leptin; LH; insulin; fasting; bovine

The discovery of the adipocyte-produced hormone leptin has had a significant influence on research related to energy homeostasis and reproduction in animals. As a result, the body of literature on the role of leptin in the central regulation of luteinizing hormone (LH) release and secretion of peripheral hormones, especially insulin, has developed rapidly. The majority of information in this area has been obtained from research with human subjects (1, 2) and laboratory rodents (3, 4).

In ruminants, the acute link between the central reproductive axis and changes in nutrient intake is less discrete than in monogastrics, and short-term feed restriction does not measurably reduce pulsatile LH release except in sexually immature females and castrate, estradiol-implanted males (5, 6). Nonetheless, our laboratory has shown recently that fasting sensitizes the hypothalamic–pituitary axis to leptin in mature cows, permitting it to modulate basal secretion of LH (7, 8). This effect appears to be primarily at the level of the anterior pituitary (8) and is likely independent of leptin-mediated increases in insulin (6, 8). Moreover, in the rat, potent effects of leptin on gonadotropin secretion have been demonstrated *in vitro* (9). In that study, a dose-related increase in LH and follicle-stimulating hormone (FSH) release was observed using relatively small doses of leptin, with gonadotropin secretion decreasing at higher doses to values not different from controls.

Several observations indicate that leptin can also modulate pancreatic β-cell function. Leptin and insulin are both secreted under the same metabolic circumstances. Each peptide provides short-, as well as long-term signals, related to

This work was supported by TAES H6881.

[1] To whom request for reprints should be addressed at the Animal Reproduction Laboratory, Texas A&M University Agricultural Research Station, Hwy 59E, Beeville, TX 78102-9410. E-mail: glw@fnbnet.net

Received September 19, 2002.
Accepted December 8, 2002.

1535-3702/03/2283-0325$15.00

metabolism and energy balance, and is transported to the central nervous system via receptor-mediated uptake (10, 11). The presence of the leptin receptor (OB-R) has been demonstrated in rat (12) and mice (13) islets of Langerhans, suggesting that leptin can directly regulate the secretion of insulin as part of an adipoinsular endocrine axis (14); however, results have been conflicting, including reports of positive and/or neutral effects using small doses of leptin (12, 15), and negative effects when higher doses were used (13, 14).

Although our recent studies in fasted, mature cows indicated positive responses of both the anterior pituitary and endocrine pancreas to recombinant oleptin (7), inconsistent and contradictory observations across species make it difficult to predict or integrate the physiological consequences of leptin action at these sites. The current experiment tested the hypothesis that divergent pituitary and pancreatic responses to leptin, in addition to being strongly influenced by nutritional state, are exquisitely related to the mass of ligand reaching target tissues over time.

Materials and Methods

All animal-related procedures used in these studies were approved by the Institutional Agricultural Animal Care and Use Committee of the Texas A&M University System.

Animal Model and Delivery of Recombinant Ovine Leptin (oleptin). The animal model used was the ovariectomized, mature beef cow bearing an estradiol implant. Hormonal implants provide a constant level of estradiol negative feedback without the complications associated with ovarian cyclicity. In the present study, mean (±SEM) concentrations of estradiol in implanted cows were of 3.3 ± 0.4 pg/ml. Ovariectomized, steroid-treated females and castrated, steroid-treated males have proven to be good models for studying the effects of nutrition on the neuroendocrine axis (7, 16). In the present experiments, recombinant oleptin was administered intravenously (17).

Procedures. Twenty mature, ovariectomized, estradiol-implanted beef cows were used. All cows were fasted for 60 hr with free access to water. Before onset of fasting, cows were assigned randomly to one of four groups (n = 5/group): 1) saline control; 2) leptin, 0.2 μg/kg; 3) leptin, 2.0 μg/kg; and 4) leptin, 20 μg/kg body wt. Concentrations of the leptin solutions for each dose were 11.525, 129, and 1217 μg/ml, respectively. On the day before the start of dietary treatments (Day −1), all cows were fitted with jugular catheters (polyethylene tubing, 1.4 mm inside diameter, 1.9 mm outside diameter; Becton Dickinson, Parsippany, NJ) for intensive blood sampling. Cows were placed in stanchions and allowed to stand without further restraint during periods of intensive blood sampling on Days 0 and 2.

Blood samples were collected at 10-min intervals for 6 hr on Day 0 and Day 2 of the experiment, with a single saline or oleptin injection administered intravenously immediately after the first intensive sample on Day 2 (54 hr; Fig. 1). Blood samples were dispensed into tubes containing 150 μl of a solution containing heparin (1000 IU/ml) and 5% EDTA and placed immediately on ice. Plasma was harvested by centrifugation and stored at −20°C until radioimmunoassay (RIA) for leptin, LH, insulin, and estradiol.

Hormone Analyses. Circulating concentrations of leptin were determined using a highly specific oleptin RIA validated for use in bovine serum (18). Use of this assay for determining plasma concentrations of leptin in the bovine has been reported previously from our laboratory (7). Determinations of circulating concentrations of leptin were performed in samples collected every 10 min for the first 2 hr and every 30 min for the following 4 hr on Day 2 of the experiment. Plasma concentrations of insulin were determined as validated previously (19) in samples collected every hr on Day 0 and every 30 min on Day 2. Plasma concentrations of LH were determined in all 10-min samples on Day 0 and 2 with a validated RIA (20). Serum estradiol was assayed in selected daily samples as reported previously (21). Intra- and interassay coefficients of variation for LH and insulin assays were 7.6 and 15% and 9.8 and 16%, respectively. The intraassay coefficients of variation of the single leptin and estradiol assays were 5.1 and 3.5%, respectively.

Statistical Analysis. Hormone data were analyzed by analysis of variance for repeated measures using the General Linear Models procedure of the Statistical Analysis System (SAS 8.1; SAS Institute Inc., Cary. NC). Frequencies and amplitude of LH pulses were determined using a pulse detection algorithm, Pulsefit 1.2 (22). Sources of variation were day, treatment, cow (treatment), and appropriate interactions. The least significant mean procedure was used to compare means when a significant F value was obtained. Because of random differences in LH concentrations between groups on day 0, LH values were converted to a percentage of time 0 values. To test the temporal effects of leptin administration on plasma leptin and insulin concentrations, the 6-hr intensive sampling period on Day 2 was

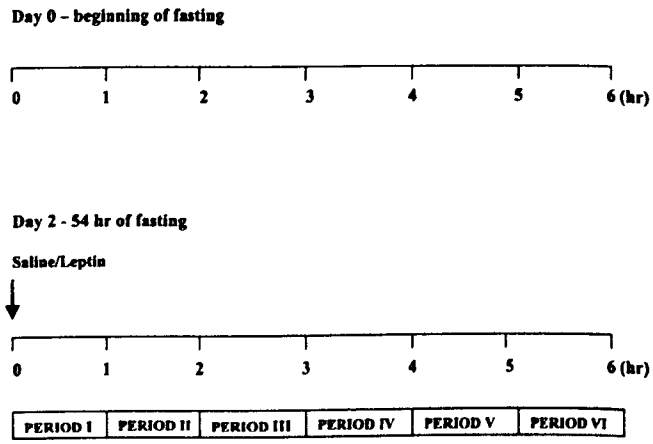

Figure 1. Timeline for experimental procedures. Blood samples were collected at 10-min intervals for 6 hr on Days 0 and 2. The intravenous infusion of oleptin is indicated by the arrow.

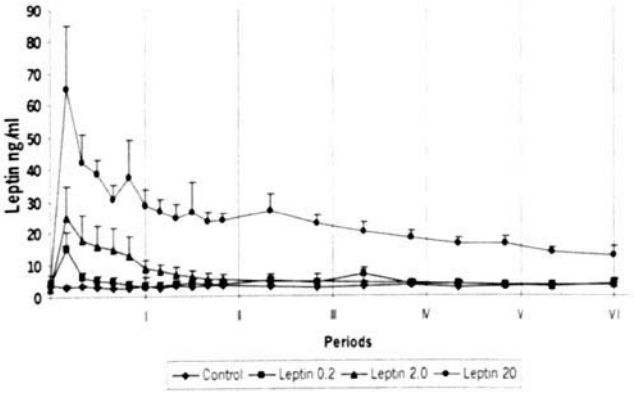

Figure 2. Mean (±SEM) concentrations of leptin on Day 2 of the experiment. Intravenous injection of oleptin at 20 μg/kg increased ($P < 0.01$) mean concentrations of leptin through Period VI compared with the Control group. Doses of 0.2 and 2.0 μg/kg of oleptin increased ($P < 0.05$) circulating concentration of leptin only during Period I.

subdivided into six periods (I–VI). Hormone data were analyzed using analysis of variance for repeated measures. Sources of variations included treatment, period, cow (treatment) and appropriate interactions. The least significant difference procedure was used to compare means when significant F values were obtained.

Results

Plasma Concentrations of Leptin. Single intravenous injections of leptin on Day 2 caused a dose-related increase ($P < 0.001$) in mean concentrations of circulating leptin (Fig. 2). Concentrations of leptin were greater ($P < 0.001$) than controls during all periods (from I to VI) at the highest leptin dose employed. Doses of 0.2 and 2.0 μg/kg of leptin increased ($P < 0.05$) plasma leptin concentrations above controls only during Period I.

Plasma Concentrations of LH and Patterns of Release. As expected, fasting did not suppress LH secretion in the mature cows used in this study. However, leptin caused a dose-related increase ($P < 0.001$) in mean concentrations of circulating LH. Overall, stimulation of LH release by leptin was significant at the lowest (141% of control; $P < 0.01$) and middle (122% of control; $P < 0.001$) doses used (Fig. 3). Secretion of LH increased slightly ($P < 0.05$) during the first hour with the highest dose, but did not differ from controls thereafter or overall (Fig. 3). LH pulse characteristics (frequency, amplitude, and pulse size as determined by the area under each pulse) were not affected ($P > 0.10$). The lack of effect of leptin on pulse characteristics, while affecting the mean baseline, is demonstrated graphically in Figure 4. Patterns of LH secretion on Days 0 (onset of fasting) and 2 (leptin or saline treatments) in two leptin-treated cows (0.2 μg/kg) are shown in which the baselines are clearly elevated but frequencies of pulses are not affected.

Plasma Concentrations of Insulin. On Day 2 of the experiment, mean concentrations of insulin were lower ($P < 0.01$) in all treatment groups compared with Day 0 (Fig. 5). However, after the start of leptin infusions, plasma insulin concentrations increased ($P < 0.01$) in all leptin-treated groups, reached highest concentrations during Period I (1 hr of sampling), and were greater ($P < 0.01$) than the control group (Fig. 6). The stimulation of insulin secretion during the first hour of the experiment (Period I) was greatest ($P < 0.001$) at the intermediate dose (2.0 μg/kg) relative to the control group, and to the rest of the leptin-treated groups (0.2 and 20 μg/kg, respectively). This

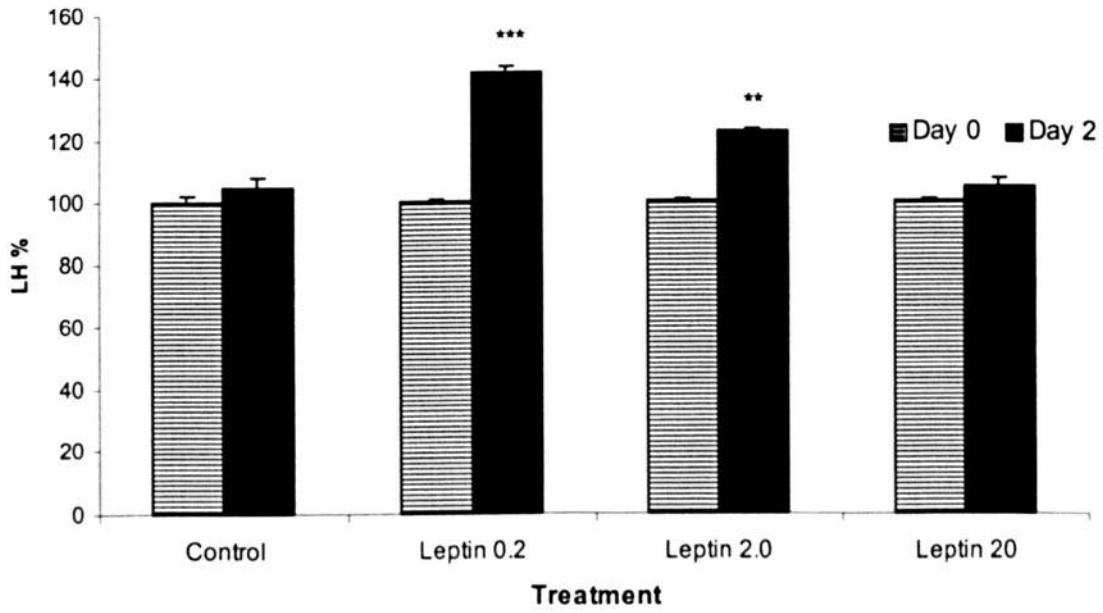

Figure 3. Dose-related effect of recombinant oleptin on mean concentrations of circulating LH. Results are expressed as mean percent (±SEM) of Day 0 for each group. Mean concentrations of LH were proportionally greater than controls at the lowest (141% of control) and middle (122% of control) doses used. ** and *** denote differences from controls ($P < 0.01$ and $P < 0.001$, respectively).

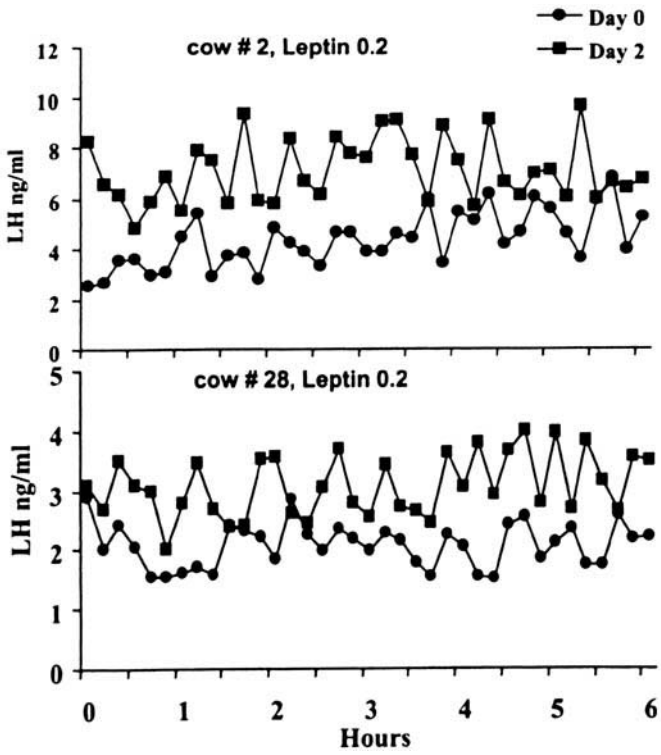

Figure 4. Patterns of LH secretion in two representative cows treated with the lowest dose (0.2 μg/kg) of recombinant oleptin. A proportional increase in the concentration of LH is apparent for each cow on Day 2 (day of leptin treatment) compared with Day 0 in the absence of detectable changes in pulse frequency.

increase persisted through Period III. Increases in insulin caused by the lowest and the highest doses remained above controls ($P < 0.05$) only during Period I of the study (Fig. 5).

Discussion

The stimulation of LH release by the lowest and intermediate doses of leptin in this study appeared to result from an augmentation of basal secretion, as pulse characteristics were not affected. Acute feed restriction (48–72 hr) suppresses plasma concentrations of insulin, but has no effect on circulating glucose or on the pattern or quantity of LH released in intact mature ruminants (7). Nonetheless, fasted, mature cows become hyper-sensitized to leptin and display a marked increase in mean concentrations of LH after acute treatment with the recombinant peptide (7). Although this occurs concomitant with increases in serum insulin, similar to that reported in the current study, direct effects of leptin on LH secretion can be observed *in vitro* with anterior pituitary explants taken from fasted cows (8). Moreover, in ruminant models that exhibit fasting-mediated declines in LH pulse frequency (e.g., estradiol-implanted wethers; prepubertal heifers), leptin attenuates the reduction without measurable effects on plasma insulin (6, 23). Collectively, these observations indicate that the effects of leptin on LH secretion in cattle are independent of changes in insulin release.

It is noteworthy that similar to reports in rodents (9), the high dose of leptin in the current experiment did not increase LH release. Yu *et al.* (9) demonstrated that leptin produced a dose-related increase in FHS and LH release from hemi-anterior pituitaries, with peak responses occurring at 10^{-9} and 10^{-10} M leptin, respectively. Gonadotropin release was lower at higher concentrations of leptin (10^{-7} to 10^{-5} M) and mean concentrations were not different from that of control-treated pituitaries. Various lines of evidence have implicated leptin as a direct regulator of anterior pituitary function (9, 24). The OB-R is expressed in rat anterior pituitary and hypothalamus (25). Whether high doses of leptin can prevent oligomerization of receptors, thus inducing desensitization or leading to a loss of nuclear STAT3 activation (26), needs to be clarified.

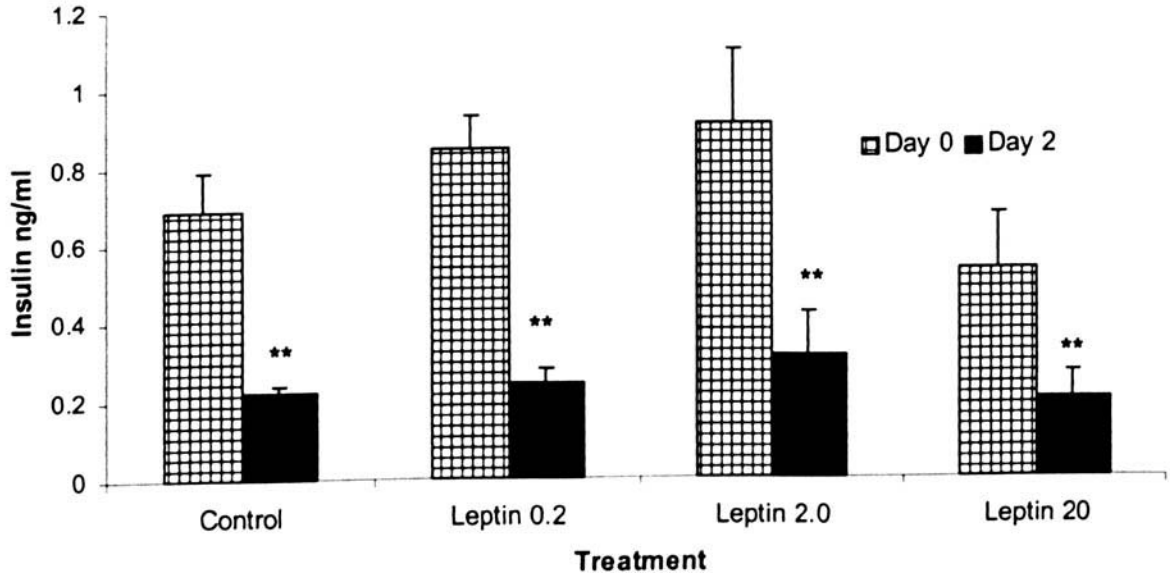

Figure 5. Mean (±SEM) concentrations of insulin on Day 2 of the experiment in control and leptin-treated cows. Mean concentrations of insulin were lower in all treatment groups on Day 2 compared with Day 0. ** denotes differences from controls ($P < 0.01$).

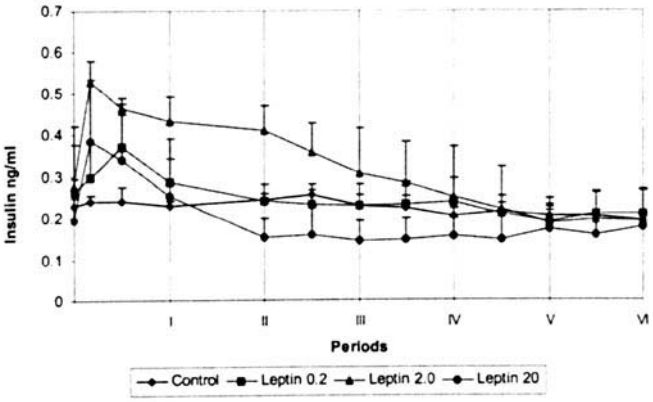

Figure 6. Mean (±SEM) concentrations of insulin on Day 2 of the experiment before and after infusions of saline or recombinant oleptin. Plasma insulin concentrations increased ($P < 0.01$) in all leptin-treated groups and reached highest concentration during Period I. The stimulation of insulin secretion (Period I) was highly significant ($P < 0.001$) at the leptin dose of 2.0 μg/kg relative to both the control and other leptin-treated groups.

We have reported recently (7) that intracerebroventricular infusions of oleptin normalize fasting-mediated declines in circulating insulin and increase secretion of LH in mature cows. Fasting-induced decreases in insulin were accompanied by reductions in plasma leptin, which in cows not treated with leptin, were associated with diminished leptin gene expression in adipose tissue (7). It should be noted that the effects of peripherally administered oleptin on insulin secretion in the present study were strikingly similar to the results that we obtained by administrating leptin centrally, although plasma insulin concentrations did not return to those observed before fasting. Perhaps the most novel observation was that the increase in insulin after leptin treatment was dose dependent. Although, doses of 0.2 and 20 μg/kg of leptin increased circulating insulin briefly, the intermediate dose of leptin (2.0 μg/kg) elevated plasma insulin concentrations for at least 3 hr.

Stimulatory effects of leptin on insulin secretion by pancreatic β-cells support our current and previous observations in fasted cattle, and have been observed in laboratory rodents (12, 27). Concentrations of circulating leptin in cattle range from 2 to 15 pg/ml, depending upon age, adiposity, and season (28). A low dose of leptin (concentration within physiological range or less) has been shown to have either no effect or increased basal release of insulin in isolated rodent islets (12). In contrast, several experiments have failed to find any effect of leptin on either basal or glucose-induced insulin secretion from perfused rat pancreas (15, 29). Other groups, using higher doses of leptin, have noted a suppression of basal (13, 14) or glucose-stimulated (30) insulin secretion from islets isolated from normal or *ob/ob* mice and rats. Emilsson *et al.* (13) and Kieffer *et al.* (14) demonstrated significant effects of leptin on the inhibition of insulin secretion at leptin concentrations of 10 nmol/l and 100 ng/ml (~6 nmol/l), respectively, both of which produced concentrations of leptin that were within the range observed in obese animals. However, they found no effect at 1 nmol/l of leptin (~16 ng/ml), a dose that increased circulating concentrations to within the physiological range of rodents (0.1–5 nmol/l; Ref. 13). In the current study, the highest dose of oleptin, similar to the lowest dose, increased insulin concentrations only during the first period of the experiment (60 min), with insulin then decreasing to concentrations below that in the control group. At least two reports in sheep (6, 31) have reported no effect of leptin on insulin secretion after large doses were injected peripherally.

Morton *et al.* (26) suggested that the high circulating concentrations of leptin that occur in obese individuals may exert an inappropriate inhibitory action on β-cell insulin secretion, and may contribute to the development of non-insulin dependent diabetes mellitus. In humans, many obese subjects with high circulating leptin concentrations are not diabetic (1), and animal studies show that exogenously administered leptin generally improves insulin action in obese animals (13, 14). Moreover, high leptin concentrations could have detrimental effects on leptin receptor number or on signaling molecules. Pancreatic β-cells express leptin receptors and both Janus kinase and a signal transducer and activator of transcription have been reported as intracellular mediators of leptin-receptor interaction (24, 26). Receptors of this class are activated by ligand-induced homo- and hetero-dimerization or oligomerization before activation of receptor associated kinases. Moreover, tissue exposed to relatively large concentrations of leptin, as found in states of obesity, accumulate excessive amounts of suppressors of leptin signaling, which could be a potential mechanism of leptin resistance (32, 33). As a result, downregulation of leptin signaling is observed, which can involve internalization or degradation of leptin receptors (30, 33). However, Kieffer *et al.* (14) proposed that leptin acts on pancreatic cells via hyperpolarization of β-cells that are activated through ATP-sensitive potassium channels. Leptin can also affect pancreatic endocrine functions through the sympathetic nervous system (34).

Taken together, data in the present studies indicate that leptin has a dose-dependent, bimodal influence on the normal sensing mechanism of the pancreatic β-cell, and on basal secretion of LH from the gonadotroph. In both cases low doses of leptin optimized endocrine responses, while the highest doses attenuated them. However, methods of leptin delivery, nutritional status, sex and species also likely contribute to these responses, which in ruminants are permitted only in the presence of negative energy balance.

We acknowledge the National Pituitary Hormone Program for providing LH preparations and Dr. Jerry Reeves for the LH antisera. We also acknowledge with gratitude the careful animal care and assistance of Randle Franke, Melvin Davis, and Justin Kinnemon. We are also grateful to the excellent technical assistance of Marsha Green.

1. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Baurn P. Recombinant mouse OB protein: evidence for a peripheral signal in normal weight and obese humans. N Engl J Med **334**:292–295, 1996.
2. Ahren B, Larsson H, Wilhelmsson C, Nasman B, Olsson T. Regulation of circulating leptin in humans. Endocrine **7**:1–8, 1997.
3. Tannenbaum GS, Gurd W, Lapointe M. Leptin is a potent stimulator of spontaneous pulsatile growth hormone (GH) secretion and the GH response to GH-releasing hormone. Endocrinology **139**:3871–3875, 1998.
4. Chan YY, Steiner RA, Clifton DK. Regulation of hypothalamic neuropeptide-Y neurons by growth hormone in the rat. Endocrinology **137**:1319–1325, 1996.
5. Amstalden M, Garcia MR, Williams SW, Stanko RL, Nizielski SE, Morrison CD, Keisler DH, Williams GL. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: relationships to circulating insulin and insulin-like growth factor I. Biol Reprod **63**: 127–133, 2000.
6. Nagatani S, Zeng Y, Keisler DH, Foster DL, Jaffe CA. Leptin regulates pulsatile luteinizng hormone secretion in the sheep. Endocrinology **141**:3965–3975, 2000.
7. Amstalden M, Garcia MR, Stanko RL, Niezielski SE, Morrison CD, Keisler DH, Williams GL. Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. Biol Reprod **66**:1555–1561, 2002.
8. Amstalden M, Zieba DA, Gallino JL, Morton S, Edwards JF, Harms PG, Welsh TH, Stanko RL, Keisler DH, Williams GL. Leptin modulates basal secretion of LH and enhances gonadotroph responsiveness to GnRH in adenohypophyseal explants from fasted cows [Abs 451]. Biol Reprod **66**(Suppl 1):281, 2002.
9. Yu WH, Walczewska A, Karanth S, McCann SM. Nitric oxide mediated leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. Endocrinology **138**:5055–5058, 1997.
10. Baura GD, Kahn SE, Taborsky GJ Jr, Porte D Jr, Foster D, Bergman RN, Schwartz MW. Evidence for saturable transport of insulin from plasma into cerebrospinal fluid in vivo. Clin Res **40**:55A, 1992.
11. Koinstinen HA, Karonen SL, Iivanainen M, Koivisto VA. Circulating leptin has saturable transport into intrathecal space in humans. Eur J Clin Invest **28**:894–897, 1998.
12. Tanizawa Y, Okuya S, Hisamitsu I, Asano T, Yada T, Oka Y. Direct stimulation of basal insulin secretion by physiological concentrations of leptin in pancreatic β cells. Endocrinology **138**:4513–4518, 1997.
13. Emilsson V, Liu Y-L, Cawthorne MA, Morton NM, Davenport M. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes **46**: 313–316, 1997.
14. Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF. Leptin suppression of insulin secretion by the activation of ATP-sensitive K^+ channels in pancreatic β-cells. Diabetes **46**:1087–1093, 1997.
15. Leclecq-Meyer V, Malaisse WJ. Failure of leptin to counteract the effects of glucose on insulin and glucagon release by the perifused rat pancreas. Med Sci Res **25**:257–259, 1997.
16. Kile JP, Alexander BM, Moss GE, Hallford DM, Nett TM. Gonadotropin-releasing hormone overrides the negative effect of reduced dietary energy on gonadotropin synthesis and secretion in ewes. Endocrinology **128**:843–849, 1991.
17. Gertler A, Simmons JM, Keisler DH. Large-scale preparation of biological-active recombinant ovine obese protein (leptin). FEBS Lett **422**:137–140, 1998.
18. Delavaud D, Bocquier F, Chilliard Y, Keisler DH, Gertler A, Kann G. Plasma leptin in ruminants: Effects of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J Endocrinol **165**:519–526, 2000.
19. Ryan DP, Bao B, Griffith MK, Williams GL. Metabolic and luteal sequelae to heightened dietary fat intake in undernourished, anestrus beef cows induced to ovulate. J Anim Sci **73**:2086–2093, 1995.
20. McVey WR, Williams GL. Mechanical masking of neurosensory pathways at the calf-teat interface: endocrine, reproductive, and lactational features of the suckled anestrous cow. Theriogenology **35**:931–941, 1991.
21. Talavera F, Park CS, Williams GL. Relationships among dietary lipid intake, serum cholesterol, and ovarian function in Holstein heifers. J Anim Sci **60**:1045–1051, 1985.
22. Kushler RH, Brown MB. A model for the identification of hormone pulses. Stat Med **10**:329–340, 1991.
23. Maciel M, Zieba D, Amstalden M, Keisler D, Neves J, Williams G. Recombint oleptin prevents fasting-mediated reductions in pulsatile LH release and stimulates GH secretion in peripubertal heifers. Proc MW Section, Amer Soc Anim Sci, De Moines, IA 2003 (in press).
24. Jin L, Burguera BG, Couce ME, Scheithauer BW, Lamsan J, Eberhardt NL, Kulig E, Llyod RV. Leptin and leptin receptor expression in normal and neoplastic human pituitary: evidence of a regulatory role for leptin on pituitary cell proliferation. J Clin Endocr Metab **84**:2903–2911, 1999.
25. Zamorano PL, Mahesh VB, DeSevilla LM, Chorich LP, Bhat GK, Brann I. Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinology **65**:223–228, 1997.
26. Morton NM, Emilsson V, de Groot RP, Pallett AL, Cawthorne MA. Leptin signaling in pancreatic islets and clonal insulin-secreting cells. J Mol Endocrinol **22**:173–184, 1999.
27. Adashi EY, Hsueh AJ, Yen SSC. Insulin enhancement of luteinizing hormone and follicle stimulating hormone release by cultured pituitary cells. Endocrinology **108**:1441–1449, 1980.
28. Garcia MR, Amstalden M, Williams SW, Stanko RL, Nizielski SE, Morrison CD, Keisler DH, Williams GL. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J Anim Sci **80**:2158–2167, 2002.
29. Poitout V, Ronault C, Guerre-Millo M, Briaud I, Reach G. Inhibition of insulin secretion by leptin in normal rodents islets of Langerhans. Endocrinology **139**:822–826, 1998.
30. Pallett AL, Morton NM, Cawthorne MA, Emilsson V. Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. Biochem Biophys Res Commun **238**:267–270, 1997.
31. Morrison CD, Wood R, McFadin EL, Whitley NC, Keisler DH. Effect of intravenous infusion of recombinant ovine leptin on feed intake and serum concentrations of GH, LH, insulin, IGF-1, cortisol, and thyroxine in growing prepubertal ewe lambs. Dom Animal Endo **22**:103–112, 2002.
32. Emilsson V, Arch JRS, de Groot RP, Lister CA, Cawthorne MA. Leptin treatment increases suppressors of cytokine signaling in central and peripheral tissue. FEBS Letters **455**:170–174, 1999.
33. Bjorbaek C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. J Biol Chem **274**:30059–30065, 1999.
34. Mizuno A, Murakami T, Otani S, Kuwajima M, Shima K. Leptin affects pancreatic endocrine function through the sympathetic nervous system. Endocrinology **139**:3863–3870, 1998.