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Sex effects on plasma leptin concentrations in newborn and postnatal beef calves

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ABSTRACT

Changes in neonatal plasma leptin play a central role in regulating development of the hypothalamic appetite control centers in rodents. In lambs, a postnatal leptin surge has just been reported, and maternal obesity induced by overfeeding initiated before conception and continued throughout gestation blocks this leptin surge in newborn lambs. To investigate the presence, timing, and duration of neonatal leptin profiles, nulliparous cows carrying bull (n = 6) and heifer (n = 6) calves were selected. Cows exhibited no complications during parturition and received no assistance during delivery were chosen for this experiment. A blood sample from the jugular vein was obtained within 2 h of birth, then daily until d 8, and then every other day until d 18 of age at 0700 h. Plasma was collected and analyzed for glucose, insulin, cortisol, and leptin concentrations via validated colorimetric and radioimmunoassay procedures. Plasma hormone and metabolite values were analyzed as repeated measures. Bull calves exhibited elevated plasma leptin concentrations compared with heifers (P = 0.01). Furthermore, plasma leptin concentrations increased from birth until d 2 and then decreased until d 16 of age (P < 0.01). Plasma cortisol was elevated (P < 0.01) at birth and then decreased over the next 5 d. Bull calves had greater (P = 0.04) plasma insulin than did heifer calves. We conclude that there is a postnatal change in plasma leptin with differences due to calf sex and may affect the appetite centers of the hypothalamus influencing appetite and BW gain.

Key words: cortisol, plasma leptin, postnatal calf

INTRODUCTION

Leptin is produced by adipose tissue and acts on hypothalamic appetitive centers (Yura et al., 2005), and correct regulation of leptin feedback is central to maintenance of normal postnatal BW and its composition. In neonatal altricial rodents, leptin has a characteristic peak that occurs between postnatal d 8 to 21 (Elias et al., 1998; Elmquist et al., 1998; Proulx et al., 2001; Delahaye et al., 2008) and varies between studies, strains, and species. This leptin peak programs the activity balance of orexigenic and anorexigenic appetitive centers of the hypothalamus and influences leptin sensitivity (Yura et al., 2005). The leptin peak is amplified and prolonged in offspring of obese rats (Kirk et al., 2009), and alterations in postnatal leptin concentrations in control offspring leads to an increase in feed intake and BW gain (Yura et al., 2005; Toste et al., 2006). The presence of a postnatal leptin surge from postnatal d 5 to 9 in control neonatal lambs has recently been reported (Long et al., 2011). In lambs born from obese mothers, no leptin surge occurred during the first 11 d of life, and these offspring consumed more feed during an ad libitum feeding challenge (Long et al., 2010). It has also been shown that this lack of leptin surge persists into the F2 generation, independent of further maternal obesity or differences in maternal nutrient intake (Ford et al., 2011). Furthermore, the administration of synthetic glucocorticoids to ewes at d 103 to 104 of gestation results in newborn F2 lambs without a postnatal leptin surge, who consume more feed during an ad libitum feed challenge (Long et al., 2013). Multiple hormones may affect the secretion and production of leptin, but only cortisol and insulin have been shown to be altered preceding or during the postnatal leptin surge in sheep (Ford et al., 2011; Long et al., 2013). Therefore, we hypothesize that postnatal beef calves have changes in plasma leptin concentrations during the first 18 d of life. Also, plasma insulin and cortisol changes may be associated with plasma leptin changes, and calf

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sex may affect these early hormone changes.

MATERIALS AND METHODS

Animals

All procedures were approved and conducted under standard operating procedures of the V-V Ranch by the University of Arizona Animal Care and Use Committee. Twelve nulliparous ~2-yr-old heifers [3 Polled Herefords, 9 Angus crossbreeds (50% Angus with Tuli, Wagyu, and Gelbvieh as the remainder)] that had unassisted parturition and that had 6 bulls and 6 heifer calves were chosen for this experiment. All cows used for this experiment had been maintained under range conditions as a group and had been supplemented for the last 6 wk of gestation with alfalfa and Sudan hay. There were 3 sires of the calves, all Polled Hereford calves originating from a common sire, 1 Angus × Tuli crossbred sired 6 calves, and a Wagyu × Tuli crossbred sired 3 calves. The resulting calves were classified in the following breed classifications: 2 were <50% Angus crossbreed; 3 Polled Hereford; and 7 crossbreed with <31% Wagyu, Tuli, or both. Calf birth weight and sex were determined on all calves along with dam BCS (Wagner et al., 1988) determined immediately after parturition. An initial blood sample (~6 mL of whole blood) was collected by jugular venipuncture into a blood-collection syringe (Starstedt, Newton, NC) containing sodium heparin (16 IU/mL of whole blood) within 2 h of parturition, and then calves were bled daily up to d 8 of age and then on d 10, 12, 14, 16, and 18 of age at 0700 h. Blood samples were centrifuged within 30 min of collection at 1,000 × *g* for 15 min at ~7°C, and plasma was collected and stored at −20°C until analysis. Calves remained with their mothers in a drylot and were fed alfalfa (1.37 Mcal/kg NE_m, 26.3% ADF, and 16.8% CP) and Sudan hay (1.21 Mcal/kg NE_m, 35.8% ADF, and 8.2% CP) ad libitum during the sampling period.

Hormone Quantification

Calves were blocked by sex for laboratory assays so that at least a pair of calves was in all assay blocks. Plasma glucose was measured colorimetrically in triplicate (Liquid Glucose Hexokinase Reagent, Pointe Scientific Inc., Canton, MI) with a mean intraassay CV of 1.9% and an interassay CV of 2.4%. To validate this assay, 8 randomly chosen samples of cattle plasma containing 48.9 to 115.02 mg/dL of glucose was diluted (1-, 2-, and 3-fold dilutions) and assayed. The recovered values were plotted; the slopes of the inhibition curves were similar to that of the standard curve ($P = 0.89$). In addition, these plasma samples had 25 mg of glucose added and assayed. This resulted in a 98.6 ± 0.6% recovery of added glucose. Plasma leptin was measured in a single assay by RIA (Multispecies leptin RIA, Linco Research, St. Charles, MO) with an intraassay CV of 3.8%. To validate this assay, 6 randomly chosen samples of cattle plasma containing 1.2 to 15.2 ng/mL of leptin had 1, 2, and 5 ng of leptin (Alphadiagnostic International, San Antonio, TX) added. The overall recovery of added leptin was 97.5 ± 2.6, 95.3 ± 2.9, and 91.5 ± 2.9%, respectively. Additionally, when 3 different dilutions (1-, 2-, and 3-fold dilutions) of each of 6 randomly chosen plasma samples were assayed and the recovered values were plotted, the slopes of the inhibition curves were similar to that of the standard curve ($P = 0.69$). Insulin was measured in duplicate in a single assay by commercial RIA (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with a mean intraassay CV of 9.5% and a sensitivity of 2.40 μIU/mL. To validate this assay, 6 randomly chosen samples of cattle plasma containing 4.5 to 35.44 μIU/mL of insulin had 2, 5, and 10 μIU of bovine insulin (Sigma-Aldrich, St. Louis, MO) added. The overall recovery of added insulin was 94.1 ± 1.9, 91.9 ± 2.1, and 94.5 ± 2.3%, respectively. Additionally, when 3 different dilutions (1-, 2-, and 3-fold dilutions) of each of 6 randomly chosen plasma samples were assayed and the recovered values were plotted, the slopes of the inhibition curves were similar to that of the standard curve ($P = 0.79$). Concentrations of cortisol were determined using Coat-A-Count cortisol RIA with a sensitivity of 5 μg/dL (Siemens Medical Solutions Diagnostics) with an intraassay CV of 7.5% and an interassay CV of 8.6%. To validate this assay, 6 randomly chosen samples of calf plasma containing 1.10 to 62.34 μg/dL of cortisol had 5, 10, and 25 μg of cortisol added. The overall recovery of added cortisol was 96.52 ± 1.3, 98.6 ± 1.4, and 102.3 ± 1.5%, respectively. Additionally, when 3 different dilutions (1-, 2-, and 3-fold dilutions) of each of 6 randomly chosen plasma samples were assayed and the recovered values were plotted, the slopes of the inhibition curves were similar to that of the standard curve ($P = 0.74$).

Statistical Analysis

Calf birth weight and dam BCS at parturition were analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC) with calf sex in the model as a random effect. Plasma glucose, insulin, cortisol, and leptin during the postnatal period were analyzed as repeated measures using PROC MIXED procedure of SAS with sex of calf (random effect) and day (fixed effect) and their interaction in the model statement. Data are presented as least squares means ± SEM. Calf breed was initially included in the models but found to have no effect ($P < 0.18$).

RESULTS AND DISCUSSION

Dam BCS at calving was similar ($P = 0.15$) between heifers that had bull and heifer calves (4.7 ± 0.1 vs. 4.9 ± 0.1). Calf birth weight was unaffected by calf sex ($P = 0.46$), with bull calves weighing 31.7 ± 1.4 kg and heifer calves weighting 31.4 ± 1.4 kg.

Plasma hormone and metabolites are shown in Figure 1. Plasma leptin was greater ($P = 0.01$) in bull calves compared with heifer calves. Plasma