Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle


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ABSTRACT: Circulating concentrations of leptin and IGF-I, leptin gene expression, and serum binding of [125I]ovine leptin in cattle during pubertal development, as well as leptin gene expression and circulating concentrations of leptin during the estrous cycle and different calendar seasons, were investigated. Multivariate regression analysis was utilized to evaluate temporal changes in BW, leptin mRNA, and serum concentrations of IGF-I and leptin normalized to the week of puberty (Exp. 1). Body weight accounted for most of the variation associated with the onset of puberty in the full regression model (R² = 0.99; P < 0.01). However, serum leptin was closely related to changes in BW (r = 0.85; P < 0.02) and in the absence of BW was most predictive of pubertal onset (r² = 0.73; P < 0.01). Mean concentrations of leptin increased (P < 0.0001) linearly from 16 wk before until the wk of pubertal ovulation in yearling heifers reaching sexual maturation from early spring to midsummer. Leptin mRNA transformed to a percent of the value at puberty increased (P < 0.02) as puberty approached, but serum leptin and leptin mRNA values were not well correlated. We found no evidence of leptin-binding proteins in serum of developing heifers. Combined mean serum concentrations of IGF-I (ng/mL) during periods III and IV (−9 wk to wk of puberty; 216.6 ± 9) were 21% higher (P < 0.0001) than combined mean concentrations of IGF-I during periods I and II (−19 to wk of puberty; 193 ± 10). In mature heifers and cows (Exp. 2), serum leptin tended to decrease (P = 0.10) during the late luteal/early follicular phase of the estrous cycle, which corresponded to a reduction (P < 0.03) in adipocyte leptin gene expression. In mature ovariectomized cows, serum concentrations of leptin increased (P = 0.001) by 34% from early winter to the summer solstice and remained unchanged throughout the remainder of the year (Exp. 3). Results from these studies indicate that marked increases in both circulating leptin and leptin gene expression occur in developing heifers during pubertal development and are associated with increases in serum IGF-I and BW. Seasonal effects on circulating leptin observed in mature cows from winter to summer could also plausibly account for a portion of the prepubertal rise in serum leptin observed in heifers.

Key Words: Estrous Cycle, Gene Expression, Heifers, Leptin, Puberty, Seasons

Introduction

Sexual maturation involves activation of the hypothalamic-pituitary-gonadal axis, a process that results in ovulation of a viable oocyte in females and spermatogenesis in males (Knobil and Neill, 1988). This transition typically occurs at a genetically predetermined age; however, nongenetic variables such as photoperiod, BW, and adiposity can modify the age at which puberty occurs (Foster and Ryan, 1981; Kinder et al., 1995). Although the precise chemical or hormonal signals that link BW and adiposity to pubertal onset have not been clearly defined, the existence of such signals was proposed over three decades ago (Wiltbank et al., 1966; Frisch, 1984). Recently, leptin, a potent satiety hormone synthesized and secreted by adipocytes that is highly correlated with BW and adiposity, has been proposed as one of those signals (Chehab et al., 1996; Cheung et al., 1997; Strobel et al., 1998).
Recent studies in this laboratory have demonstrated that leptin gene expression and circulating leptin are responsive to short-term nutrient flux and are associated with changes in serum insulin, IGF-I, and LH pulsatility in prepubertal heifers (Amstalden et al., 2000). In addition, central administration of recombinant ovine leptin significantly stimulated pancreatic insulin and pituitary LH secretion in mature, fasted cows (Amstalden et al., 2002). Collectively, these observations suggest that leptin may play an important role as a signal linking nutritional status to the central reproductive axis in cattle.

The current studies examined changes in serum concentrations of leptin, leptin gene expression, and leptin-binding activity of serum (puberty study only) in cattle during pubertal development, different seasons, and the estrous cycle.

Materials and Methods

Animals and Procedures

All animal-related procedures employed in this study were approved by the Institutional Agricultural Animal Care and Use Committee (IAACUC) of The Texas A&M University System.

Experiment 1: Pubertal Development

Forty-two spring-born heifers [Santa Gertrudis, n = 12, 7 to 9 mo of age; Santa Cruz (½ Red Angus × ¼ Santa Gertrudis × ¼ Gelbvieh), n = 12, 7 to 9 mo of age; and Brahman × Hereford F₁, n = 18, 7 to 11 mo of age] were utilized. Heifers were maintained in pens (25.9 m × 9.5 m; n = 8 heifers/pen) and received diets consisting of corn, cottonseed meal, and free choice coastal bermudagrass hay. Diets were formulated to promote a gain of 0.68 kg/d based on NRC recommendations (1984). Dietary DM and composition were adjusted periodically to account for growth-related changes in BW. Blood samples were obtained from the tail by coccygeal venipuncture twice weekly until confirmation of puberty. Serum was harvested and stored at −20°C until analyzed for serum leptin.

Confirmation of puberty was based on serum progesterone concentrations being ≥ 1 ng/mL over three consecutive samples and twice weekly transrectal ultrasonography to visualize the existence of corpora lutea (CL). Body weights and body condition scores (BCS; 1 to 9 scale, 1 = emaciated; 9 = obese) were recorded every 21 d until puberty. A subset of 17 heifers was available for adipose tissue collection. Tissue was collected weekly by coccygeal venipuncture and analyzed for circulating leptin by RIA. Adipose tissue was collected at standing estrus (d 0), during luteal development (d 4) and the midluteal phase (d 10), and 4 and 2 d before the next expected estrus. Tissues were processed as described previously until Northern blot analysis for leptin mRNA. Transrectal ultrasonography was utilized every other day to monitor ovarian follicular and luteal activity relative to different stages of the estrous cycle. Body weights and BCS were recorded at the time of adipose tissue collection. Sampling was performed at approximately the same time each day to reduce potential variability associated with a diurnal rhythm for leptin secretion (Licinio et al., 1998).

Experiment 2: Estrous Cycle

Upon confirmation of puberty, 7 of the 42 heifers were available for daily blood sampling and adipose tissue collection through a complete estrous cycle. In addition, daily blood samples were collected from 5 mature cows through a complete estrous cycle. Serum was harvested and stored at −20°C until analyzed for serum leptin and progesterone concentrations by RIA. Animals were observed twice daily for behavioral estrus following confirmation of at least one normal length estrous cycle before the experimental cycle. Adipose tissue was collected at standing estrus (d 0), during luteal development (d 4) and the midluteal phase (d 10), and 4 and 2 d before the next expected estrus. Tissues were processed as described previously until Northern blot analysis for leptin mRNA. Transrectal ultrasonography was utilized every other day to monitor ovarian follicular and luteal activity relative to different stages of the estrous cycle. Body weights and BCS were recorded at the time of adipose tissue collection. Sampling was performed at approximately the same time each day to reduce potential variability associated with a diurnal rhythm for leptin secretion (Licinio et al., 1998).

Experiment 3. Effects of Season

To determine the effects of season on serum concentrations of leptin and leptin gene expression, mature ovarioectomized, estradiol-implanted cows were utilized (n = 5) to eliminate variability in leptin due to growth and cyclic ovarian activity. Cows had been ovarioectomized for at least 6 mo before the start of the experiment. Estradiol implants were used to produce baseline physiological concentrations of 2 to 4 pg/mL (Gazal et al., 1998). We have found these implants to function at a constant rate for at least 1 yr. However, for the purpose of this experiment, they were changed every 3 to 4 months. Cows were maintained in outdoor pens and fed coastal bermudagrass hay to meet nutritional requirements of mature cows (NRC, 1984). Blood samples were collected weekly by coccygeal venipuncture and analyzed for circulating leptin by RIA. Adipose tissue was collected every 3 wk and stored until Northern analysis for leptin mRNA content. Blood and tissue sampling began on January 21, 2000, and ended on December 19, 2000, the week of the winter solstice. Body weight and BCS were recorded at the time of adipose tissue collection. Sampling was performed at approximately the same time of day each week.

Assays

Concentrations of serum progesterone and leptin were determined by RIA using the commercially available Coat-A-Count assay kit (Diagnostics Product Corporation, Los Angeles, CA) and a highly sensitive ovine specific RIA validated for use in bovine serum (Dela-vaud et al., 2000), respectively. Use of these assays has been reported previously from our laboratory (Fajers-
son et al., 1999; Amstalden et al., 2000). Circulating concentrations of IGF-I were determined by RIA as described previously (Ryan et al., 1995). Intra- and interassay CV were at or below 10% and 20%, respectively.

Estimation of Leptin Binding Activity in Serum

Serum samples collected at the time of adipose tissue collection (every 3 wk) from prepubertal heifers and mature cycling cows were analyzed for serum leptin-binding activity using molecular sieve chromatography as described previously by Sinha et al. (1996) and Gavrilova et al. (1997). Ovine leptin was iodinated as described by Delevaud et al. (2000) and incubated (1 × 10^6 cpm) with 1-mL serum samples for 48 h at 4°C. Samples were eluted on Sephadex G-100 columns (28 × 0.8 cm) at 7°C and collected in 200 μL fractions (Gavrilova et al., 1997). Radioactivity in each fraction was determined utilizing a Packard 5780 Auto-Gamma counter (United Technologies, Downers Grove, IL). Elution profiles of highly purified [125I]ovine leptin were utilized to verify the identification of [125I]ovine leptin peak.

Northern Blot Analysis

Approximately 0.6 g of adipose tissue was used to extract RNA as described previously (Amstalden et al., 2000). Total RNA (10 μg) was separated on a 1.3% agarose denaturing gel, transferred to a Hybond N membrane (Amersham Pharmacia Biotech, Piscataway, NJ) overnight, and baked at 80°C for 2 h. Membranes were prehybridized with hybridization buffer (Ambion, Austin, TX) for 30 min at 65°C. After preincubation, a [32P]UTP (Perkin Elmer, Boston, MA) labeled ovine leptin riboprobe, transcribed from a 350-bp cDNA (GeneBank Accession # U62123) utilizing the Strip EZ kit (Ambion), was added to the hybridization solution (1 × 10^6 cpm/mL) and incubated overnight at 65°C. Following incubation, membranes were washed (3×) with preheated (65°C) 0.1× sodium chloride/sodium citrate with 0.1% sodium dodecyl sulfate at 65°C for 20 min and exposed to x-ray film at –80°C. Blots were stripped of the ovine leptin riboprobe using the Strip EZ kit and rehybridized with mouse β-actin, transcribed from a 1076-bp cDNA (Ambion), to confirm equality in gel loading. Membranes were washed and exposed as described previously. Pixel density of the autoradiographed membranes were quantified using an Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA). Leptin was expressed as a percent of β-actin and transformed as a percent of the week relative to puberty or to the log_{10}.

Statistical Analysis

Experiment 1. Although heifers ranged from 7 to 11 mo of age at onset of the study, variability in age at puberty allowed us to analyze data from 36/42 of the heifers for 19 wk before puberty. Of the six remaining heifers, the period from onset of sampling to puberty was 18 (n = 3), 17 (n = 1), 12 (n = 1), and 11 (n = 1) wk. Leptin mRNA, BW, and serum hormone data were normalized to the week relative to puberty (wk 0) for analysis. Regression analysis, utilizing the REG procedure of SAS (SAS Institute Inc., Cary, NC), was used to evaluate changes in BW, leptin mRNA, serum leptin, and IGF-I relative to wk 0. Least squares means were generated from values grouped by 2-wk intervals. Leptin gene expression, transformed to a percent of the value at wk 0, and circulating concentrations of leptin were analyzed by analysis of variance for repeated measures using the MIXED procedure of SAS. Due to greater variability in serum IGF-I than in leptin, IGF-I values were grouped into 4 arbitrary periods (period I: −19 to −15 wk; period II: −14 to −10 wk; period III: −9 to −5 wk; and period IV: −4 to 0 wk before puberty). Analysis of variance for repeated measures using the MIXED procedure of SAS was utilized to analyze for period effects. Heifer was used as the subject for the MIXED procedure to account for correlated variation within animals. The least squares means procedure was utilized to compare means when a significant difference was detected in the MIXED analyses. Pearson Correlation coefficients were determined among the described variables and serum progesterone using the CORR procedure of SAS.

Experiment 2. Leptin mRNA, BW, and mean serum concentrations of leptin were analyzed by analysis of variance for repeated measures utilizing the MIXED procedure of SAS to determine temporal effects. The least squares means procedure was utilized to compare means when a significant difference was detected in the MIXED analysis. Pearson Correlation coefficients were determined among the described variables and serum progesterone using the CORR procedure of SAS.

Experiment 3. Leptin mRNA, BW, and mean serum concentrations of leptin were analyzed within equinoctial- and solstitial-defined seasons, beginning with winter, when sampling was initiated: winter = January 21 to March 20; spring = March 21 to June 21; summer = July 22 to September 21; autumn = September 22 to December 19. Circulating concentrations of leptin, leptin mRNA, and BW were analyzed by analysis of variance for repeated measures utilizing the MIXED procedure of SAS. Cow was used as the subject for the MIXED procedure to account for correlated variation within animal. Least squares means and Pearson Correlations were determined as described previously.

Results

Experiment 1: Pubertal Development

Simple linear regressions of BW and serum concentrations of leptin relative to onset of puberty are shown in Figure 1. Significant linear increases were observed for both variables. Heifers averaged 292 ± 3.2 kg at the start of the study, gained 1.1 ± 0.1 kg/d thereafter, and reached puberty from March through late August.
Figure 1. Simple linear regressions of BW (panel A; \( y = 9.16x + 277.8 \) \( r = 0.99 \)) and serum leptin (panel B; \( y = 0.177x + 3.79 \), \( r = 0.73 \)) relative to week of pubertal ovulation in developing heifers. Each point represents the least squares means calculated from twice weekly samples pooled over 2-wk intervals. Regressions for each variable were significant \( (P < 0.01) \).

Mean BW at puberty was 370 ± 2.2 kg. Body weight accounted for the greatest amount of variation in the full regression model that included BW, serum leptin, leptin mRNA, and serum IGF-I \( (R^2 = 0.99; P < 0.01) \). However, in the absence of BW, circulating concentrations of leptin, which were closely related to changes in BW \( (P < 0.02; \text{Table 1}) \), were most predictive of pubertal onset \( (r^2 = 0.73; P < 0.01) \). Neither circulating concentrations of IGF-I nor leptin gene expression contributed significantly to the full regression model \( (P > 0.10) \). Beginning 16 wk before puberty, serum leptin concentrations \( (3.8 ± 0.4 \text{ ng/mL}) \) began a linear increase \( (P < 0.0001; \text{Figure 1}) \) and averaged 6.4 ± 0.4 ng/mL during the week of puberty. Circulating leptin and leptin gene expression were not well correlated (Table 1) based on the sampling regimen utilized in this experiment. However, leptin mRNA did increase \( (P < 0.02) \) as puberty approached (Figure 2). The prepubertal increase in serum leptin was observed in all heifers regardless of the month (season) during which puberty was attained (Figure 3). Combined mean concentrations of IGF-I during periods I and II \( (193 ± 10 \text{ ng/mL}) \) increased 21% \( (P < 0.0001) \) to a mean of 216.6 ± 9 ng/mL during periods III and IV (Figure 4).

Figure 5 represents a typical Sephadex G-100 elution profile of radioactivity after incubation with bovine serum for 48 h at 4°C. A single peak of radiolabeled ovine leptin was consistently observed in 3-wk samples collected from onset of the study until puberty, indicating that serum leptin binding proteins may not be present in cattle.

**Table 1.** Correlation coefficients and associated probabilities (in parentheses) among BW, leptin mRNA, and serum concentrations of leptin and IGF-I over a 20-wk period until puberty in heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>BW, kg</th>
<th>IGF-I, ng/mL</th>
<th>Leptin, ng/mL</th>
<th>Leptin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>1.0</td>
<td>0.55</td>
<td>0.85 ( ^a )</td>
<td>0.03 ( ^a )</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>0.55</td>
<td>1.0</td>
<td>0.41</td>
<td>-0.4</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>0.85 ( ^b )</td>
<td>0.55</td>
<td>1.0</td>
<td>-0.9</td>
</tr>
<tr>
<td>Leptin mRNA, Log10AU</td>
<td>0.03</td>
<td>-0.4</td>
<td>-0.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\( ^a \) Indicates a subset of heifers \( (n = 17) \).

\( ^b \) Log10 Arbitrary Units.

Cattle maintained a BCS of 5 throughout the experiment. Mean serum concentrations of leptin did not change \( (P > 0.10, \text{Figure 6}) \) during days of the estrous cycle in either cows or heifers. However, when serum leptin concentrations were pooled by stage of the estrous cycle, stage means tended \( (P = 0.10) \) to differ with a decrease during the late luteal/early follicular phase of the cycle. Similarly, a 61% reduction \( (P < 0.02) \) in leptin gene expression was observed during the late...
Figure 2. Panel A: Northern blot analysis of adipose leptin mRNA from 16 wk before until the week of puberty from a subset of 17 heifers. Arbitrary densitometric (AU) units were transformed to a percentage of the value during the week of puberty. Weeks with different superscripts differ ($P < 0.05$). Panel B: Autoradiograph of leptin mRNA (4.5 kb; top panel) and β-actin mRNA (~2.0 kb; bottom panel) from two representative heifers. Beta actin mRNA was utilized to normalize for quantity of total RNA loaded from each sample. Mouse liver RNA (mLR) was utilized as the negative control. A marker (not shown) was utilized to estimate the relative size of leptin and β-actin mRNA. Numbers above each lane represent week relative to puberty.

Experiment 3: Seasonal Effects

Body condition scores ranged from 5 to 6 in the mature, estradiol-implanted, ovariectomized cows used for this experiment. Mean concentrations of circulating leptin increased 34% from the early winter to the summer (4.7 ± 0.7 ng/mL vs 7.2 ± 0.7 ng/mL, $P < 0.001$, Figure 8). Body weights did not change during this time (494 ± 13.8 kg vs 496 ± 8.5 kg). However BW did increase ($P < 0.05$) from the summer to the winter sol-
Figure 4. Mean serum concentrations of IGF-I (mean ± SEM) in prepubertal heifers (n = 42) normalized to the week of puberty (0) and represented in four arbitrary periods. Period I: −19 to −15 wk; period II: −14 to −10 wk; period III: −9 to −5 wk; period IV: −4 to 0 wk. Period means with different letters differ (P < 0.0001).

Figure 5. Representative elution profile of [125I]ovine leptin in bovine serum after incubation at 4°C for 48 h. Radiolabeled ovine leptin eluted in fractions 28 to 41. Free [125I] eluted in fractions 96 through 108. Based on the elution profile of radiolabeled ovine leptin, leptin-binding proteins in bovine serum were assumed to be absent.

Figure 6. Mean concentrations of leptin in serum during different stages of the estrous cycle (Estrus, E; developing luteal, DL; midluteal, ML; late luteal, LL; and follicular phase, FP) of heifers (A; n = 7) and mature cows (B; n = 5). Leptin tended (P = 0.10) to decline during the late luteal/early follicular phase.

Discussion

Serum leptin has been reported to increase linearly as puberty approaches in rodents and humans (Ahima et al., 1997; Quinton et al., 1999), similar to that observed for heifers in the current study. Interestingly, a more recent study suggests that neither adiposity nor leptin increases before puberty in mice. Steady gains in BW, seasonal changes in day-length, and serum leptin-binding proteins are variables that have been shown to contribute to an increase in circulating leptin (Maffei et al., 1995; Bocquier et al. 1998; Housknecht et al., 1996). However, based on results obtained in Exp. 1,
the increase in serum leptin observed in heifers in the present study was not likely a result of a decrease in serum leptin-binding proteins that has been reported in humans (Quinton et al., 1999). A number of binding proteins for leptin are believed to exist in both rodents and humans (Sinha et al., 1996), one of which has been identified as the extracellular domain of the leptin receptor (Housekneckt et al., 1996) and is hypothesized to play a critical role in transporting leptin across the blood-brain barrier (Golden et al., 1997). The bound form of circulating leptin is reduced in obese subjects (Sinha et al., 1996) and in late-gestating mice (Gavrilova et al., 1997), which leads to an increase in the free form of serum leptin and the development of leptin resistance. In human adolescents, the increase in leptin as puberty approaches is correlated with a decline in serum leptin-binding activity (Quinton et al., 1999).

Based on these studies, we expected to detect lower serum-binding activity for leptin in heifers as puberty approached. In contrast to our previous preliminary report (Garcia et al., 1999) using recombinant human leptin with bovine serum, the increase in circulating leptin does not appear to be the result of serum leptin-binding activity, as none was detected using radiolabeled recombinant ovine leptin in the current studies. In support of this finding, high performance liquid chromatography analysis of bovine serum failed to identify

Figure 7. Panel A: Leptin mRNA expression (mean ± SEM) on d 0 (Estrus; E), 4 (developing CL; EL), and 10 (midluteal; ML) of the estrous cycle, and 4 (late luteal; LL) and 2 d (Follicular Phase; F) before the next expected estrus in heifers. Data are represented as the log10 of arbitrary densitometric units. A decrease (P < 0.03) was detected at LL. Panel B: Autoradiograph of leptin mRNA (4.5 kb; top panel) and β-actin mRNA (mouse 1.9 kb, bovine 2.0 kb; bottom panel) from two representative heifers. Beta actin mRNA was utilized to normalize for quantity of total RNA loaded from each sample. Mouse liver RNA (mLR) was utilized as the negative control for leptin mRNA. Marker (M) was utilized to estimate the relative size of leptin and β-actin mRNA. Definitions of abbreviations are the same as in panel A.

Figure 8. Mean serum concentrations of leptin (mean ± SEM) in mature ovariectomized, estradiol-implanted cows (n = 5) during different seasons. Each cow was implanted subcutaneously with a silastic estradiol implant to maintain serum estradiol at 2 to 4 pg/mL. Circulating leptin increased (P < 0.0001) from winter to spring but remained unchanged from the spring to the winter solstice (last day of autumn and beginning of winter). Means with different subscripts differ (P < 0.05).
Leptin mRNA was not well correlated with circulating leptin in Exp. 1, which may have been due in part to the relatively small number of adipose tissue samples compared to the number of serum samples. However, leptin mRNA did increase as puberty approached, which could be accounted for by increased adipocyte numbers as well as increased leptin gene expression. Leptin gene expression is regulated by many factors, including insulin (Kim et al., 1998), peroxisome proliferator activating receptor gamma (PPAR γ) ligands (De Vos et al., 1996), prolactin (Gualillo et al., 1999), and environmental factors, such as photoperiod (Klingenspor et al., 1996). Also, there are regional differences in the expression of leptin in fat depots. In female rats, leptin gene expression is higher in parametrial and perirenal fat depots than in femoral s.c. fat (Machinal et al., 1999). An increase in the synthesis and secretion of leptin was detected in ewes exposed to long day-lengths (Bocquier et al., 1998). Furthermore, leptin mRNA is reduced during short day-lengths in the Siberian hamster (Atcha et al., 2000). Hence, a change in mean serum concentrations of leptin in response to seasonal changes in day-length was expected and confirmed in this study. Circulating leptin increased in mature ovariectomized, estradiol-implanted cows from January to the summer solstice, which is similar to observations reported in mares (Fitzgerald and McManus, 2000). However, in contrast to mares where a decrease in serum leptin occurred from the summer to the winter solstice, mean serum concentrations of leptin did not change in mature cows. It is possible that the modest increase in BW that occurred during the latter part of the year in our cows contributed to the sustained elevation in circulating leptin. However, preliminary data suggest that moderate changes in cow BW and BC in the mature cow do not result in detectable differences in serum leptin (Ciccioli et al., 2001; Williams et al., unpublished). Changes in serum leptin relative to body condition are only obvious between cows having large differences (e.g., BC 3 vs 8) in BC. The increase in serum leptin detected in the first half of the year in mature cows suggests that seasonal changes in day-length could have contributed to the prepubertal increase in circulating leptin observed in heifers. However, the rate of increase in serum leptin was similar for heifers regardless of whether puberty was reached in the early spring or the mid to late summer months. Day-length has been reported to affect the age at which puberty occurs in beef and dairy heifers (Schillo et al., 1983; Petitclerc et al., 1983). Hansen et al. (1983) reported that spring-born heifers exposed to 18 h of daylight reached puberty earlier than those raised under natural photoperiod. Hence, mechanisms through which seasonal changes in day-length affect puberty appear to involve photoregulated factors that may include leptin.

Based on observed changes in leptin during pubertal development of heifers in the current study, it is plausible to hypothesize that leptin may play a functional role in maturation of the central reproductive axis. Leptin has been reported to be involved in the pubertal process of rodents and humans (Kiess et al., 1999). Children born with a recessive mutation in the genes of the leptin protein or its receptor result in the absence of leptin or its biological action, consequently, these children do not become sexually mature. Normal female mice receiving pharmacological doses of leptin reach puberty earlier than average (Ahima et al., 1997). Furthermore, feed-restricted and/or underweight rodents treated with leptin attain puberty at ages similar to ad libitum-fed controls (Gruaz et al., 1998). The influence of leptin on sexual maturation appears to be driven by its action within the hypothalamic-hypophysial axis. Yu et al. (1997) incubated medial basal hypothalamic and adenohypophyseal explants from mice with leptin and detected an increase in GnRH and LH secretion, respectively. Mean serum concentrations of LH also increased following peripheral and IVC leptin infusion in non-human primates (Finn et al., 1998). A similar observation was made in feed-restricted, ovariectomized, estradiol-implanted cows when leptin was infused into the lateral ventricle (Amstalden et al., 2002). Additionally, a fasting-induced decrease in LH secretion was prevented with leptin treatment in wethers (Foster and Nagatani, 1999). Collectively, these results indicate that leptin may play an important role in the sexual maturation process by signaling energy status to the central reproductive axis. However, the metabolic hormone IGF-I, a primary growth factor involved in somatic development, is also believed to be involved in the pubertal process in heifers (Jones and Clemonns, 1995; Armstrong et al., 1992). Jones et al. (1991) and Yelich et al. (1995) reported an increase in circulating IGF-I in heifers as puberty approached. Furthermore, immunization against growth hormone-releasing factor (GRF) resulted in the reduction of serum IGF-I and an increase in the age at puberty in heifers (Armstrong et al., 1992). An increase in serum IGF-I was observed in the current study as puberty approached, verifying the previous investigations; however, IGF-I did not increase at the same rate as serum leptin.

Similar patterns of circulating leptin were observed throughout the estrous cycle in sexually mature heifers and cows; however, mean concentrations were much lower in cows than in the heifers used for Exp. 2. The difference in concentrations may be attributed to the time of the year in which samples were collected in heifers vs cows or physiological differences, i.e., growth and development. In Exp. 3, mature, ovariectomized cows bearing estradiol implants had serum leptin concentrations during spring and summer that were more comparable to values observed for the heifers in Exp. 2. Despite reports of a diurnal rhythm in leptin secretion (Licinio et al., 1998) and differences in mean serum concentrations in obese vs thin human subjects, circulating leptin did not change appreciably throughout the estrous cycle of rodents or the menstrual cycle of women.
(Bennett et al., 1999; Yamada et al., 2000). In contrast, Ludwig et al. (2000) detected higher mean serum concentrations of leptin during the luteal phase than during the follicular phase of the menstrual cycle of women. We made a similar observation in sexually mature heifers and cows in the current study, which corresponded to a reduction in leptin gene expression in the late luteal/early follicular phase of the estrous cycle. Leptin gene expression during the estrous or menstrual cycle has not, to our knowledge, been reported previously. Moreover, in our study with heifers changes in serum leptin were not correlated with serum progesterone, which agrees with the report by Ludwig et al. (2000). This is in contrast to other studies that reported higher circulating concentrations of leptin in rodents and women receiving estradiol and progesterone replacement therapy (Shimizu et al., 1997; Lavoie et al., 1999) and an estradiol-regulated increase in leptin gene expression in rodent adipocytes (Machinal et al., 1999). The physiological relevance of the declines in serum leptin and leptin mRNA during the late luteal/early follicular phase of the bovine estrous cycle in the current study remains unknown.

Implications

Leptin gene expression and circulating concentrations of leptin and IGF-I increase in heifers as puberty approaches. Changes in circulating leptin observed in mature cows during different seasons indicate that day-length may contribute to this process. The prepubertal rise in serum leptin in heifers is similar to reports in rodents and humans, two species in which leptin is critical for sexual maturation. Hence, results from the present study, coupled with observations that leptin stimulates LH secretion in fasted ruminants, provide a basis for examining further the role of leptin in the central regulation of reproduction in cattle.

References


