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Characterization of Leptin Levels in Gestating *Callipyge* Ewes*

J. N. Fleming-Waddell[1], D. H. Keisler[2], S. P. Jackson[3] and J. R. Blanton, Jr.[3,] **

[1]Department of Animal Sciences, Purdue University, West Lafayette IN 47907, USA

ABSTRACT : The *callipyge* mutation in sheep is a polar overdominant mutation that results in post-natal muscle hypertrophy in the loin and hindquarters of paternal heterozygotes (+/CLPG). Sheep that are homozygous for the *callipyge* allele (CLPG/CLPG) do not express the muscle hypertrophy phenotype, but serve as carriers for the mutation. *Callipyge* sheep are characterized by improved feed efficiencies and leaner carcasses. Leptin is a protein hormone secreted from adipose tissue and has been found to affect appetite and serve as an indicator of body fat mass. To date, very little knowledge is available as to the effect of the *callipyge* mutation on circulating leptin levels. Due to the interaction of leptin with feed intake and energy availability, and the fact that the majority of fetal growth occurs in late gestation, it is important to understand if the *callipyge* mutation interacts with leptin production in late gestational ewes. Therefore, our objective was to characterize serum concentrations of leptin in late gestational *callipyge* ewes vs. non-*callipyge* ewes. We evaluated genetically verified *callipyge* (n = 6), homozygous (n = 8) and normal (n = 8) ewes weekly during the last eight wks of gestation through one wk post-partum. Weights were taken and body condition scores were assigned by trained personnel weekly. Blood was collected via jugular venipuncture on each sampling date and subjected to an ovine-specific leptin RIA. Genotype influences on peripheral concentrations of leptin were found to be highly significant (p = 0.0005). Total leptin means for *+/CLPG* were 5.41±0.40 ng/ml, *CLPG/CLPG* 8.11±0.70 ng/ml, and *−/−* 9.13±0.93 ng/ml. Sampling date was also significant (p = 0.0098) with all ewes showing a decrease in leptin levels throughout gestation and parturition. Using repeated measures, we were able to detect lower levels of plasma leptin in *callipyge* ewes, which may be indicative of their lower overall body fat content. These results indicate that the *callipyge* phenotype decreases the levels of adipose tissue and leptin production in gestating ewes. (**Key Words :** Adipose, *Callipyge*, Leptin, Sheep)

INTRODUCTION

Leptin is a 16-kDa protein hormone secreted from white adipose tissue, which influences hypothalamic mechanisms regulating appetite and energy balance (Zhang et al., 1994; Halaas et al., 1995; Vega et al., 2004). In species tested thus far, including sheep, plasma leptin levels are highly influenced by the amount of adipose stored, body condition score and physiological status (pregnancy/parturition). High levels of plasma leptin are associated with high levels of adiposity and cause a decrease in appetite and an increase in activity; ideally burning excess fat stores (Friedman and Halaas, 1998; Delavaud et al., 2000; Estienne et al., 2000; Buff et al., 2002; Vega et al., 2004b; Heravi Moussavi et al., 2006). In human pregnancy studies, plasma leptin levels have been shown to peak at 22 to 27 weeks of gestation and decrease thereafter through the third trimester (Sattar et al., 1998; Tamura et al., 1998).

Callipyge sheep are characterized by postnatal muscle hypertrophy and decreased adiposity (Cockett et al., 1994; Jackson et al., 1997). *Callipyge* sheep typically have lower daily feed intakes, higher feed efficiencies and at slaughter weight. *Callipyge* sheep exhibit higher dressing percentages and less subcutaneous fat compared to non-*callipyge* sheep of the same weight (Jackson et al., 1997). Research efforts devoted to elucidating the mechanisms that account for the abundant muscle hypertrophy and decreased adipose levels in *callipyge* sheep have been extensive. There is however, very little data that examines the relationship of *callipyge* sheep in late gestation and early parturition with circulating levels of leptin. Therefore, we sought to characterize serum concentrations of leptin in *callipyge* sheep vs. non-*callipyge*

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** Corresponding Author: J. R. Blanton, Jr. Tel: +1-806-742-2804, Fax: +1-806-742-4003, E-mail: john.blanton@ttu.edu

[2] Department of Animal Sciences, University of Missouri, Columbia MO 65211, USA.

[3] Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA.

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Table 1. Mean serum concentrations of leptin, body condition score (BCS), ewe body weights, and lamb numbers for *callipyge*, homozygous and normal ewes

Genotype	n	Leptin (ng/ml)	BCS (1-5)	Wt (kg)	Lambs (born)
Callipyge	6	5.41±0.40^{A}	2.92±0.03^{A}	76.73±1.27^{B}	1.17±0.06^{A}
Homozygous	8	8.11±0.70^{B}	2.60±0.06^{B}	66.17±1.56^{A}	1.39±0.07^{B}
Normal	8	9.13±0.93^{B}	2.61±0.06^{B}	81.52±1.41^{C}	1.50±0.07^{B}

^{A} Means with common superscripts in each column are not significantly different (p>0.05).

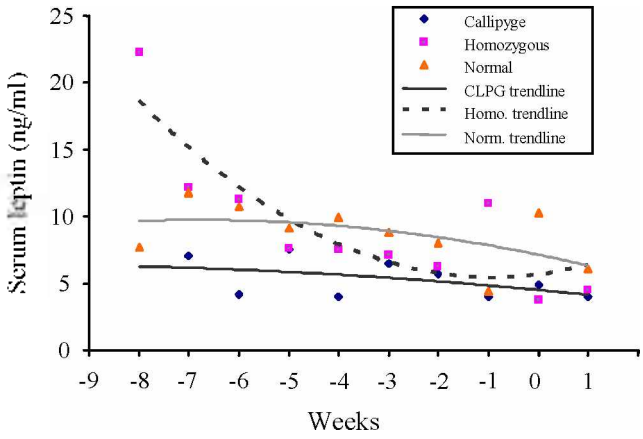

Figure 1. Mean serum concentrations of leptin and trendlines within *callipyge* (+/*CLPG*), homozygous (*CLPG*/*CLPG*) and normal (+/+) ewes during late gestation, lambing and early lactation. The time point "0 weeks" denotes parturition with other time points relative to it.

sheep to test our hypothesis that serum leptin levels will differ with the expression of the mutation.

MATERIALS AND METHODS

Animals

Twenty-four multiparous Rambouillet ewes were selected for this study. All animals were visually and genotypically analyzed to determine *callipyge* categories. Treatment groups consisted of eight paternal heterozygous *callipyge* ewes (+/CLPG), eight homozygous ewes (CLPG/CLPG; these do not exhibit the phenotype), and eight normal ewes (+/+). The maternal heterozygous genotype (CLPG/+) was not used in this study due to a lack of availability. Ewes were housed in partially covered dirt floor pens and maintained on an isocoloric/isonitrogenous ration consisting of 0.45 kg whole corn and 0.23 kg soybean meal per head per day, with access to large round bales of Sudan hay *ad libitum*. This experimental design was reviewed and approved by the Texas Tech University Animal Care and Use Committee (03013-02).

Data collection

Blood samples, body weights and body condition scores (BCS) were collected weekly for 8 wk prior to lambing, within 24 h of lambing and 5 d after lambing. Gestational measurements were adjusted for each ewe according to her actual lambing date. Two *callipyge* ewes were dropped from the study due to a lack of offspring. One normal ewe and one homozygous ewe experienced lamb death due to dystocia, therefore data from these ewes were included in only the gestational portion of the analysis. Sampling of ewes for body weights, blood samples and trained BCS measurements (according to the method of Russel, 1991) were taken without restriction of ewes from feed or water at mid-morning prior to receiving grain supplementation. Blood samples were taken via jugular venipuncture using 10 ml Vacutainers. Upon collection, blood samples were stored at 4°C for transportation to the Texas Tech University Meat Science and Muscle Biology Laboratory and then centrifuged at 1,250×g for 20 min within 4 h of collection. Serum was collected and stored in 1.8 ml microcentrifuge tubes and maintained at -80°C for further analysis. Serum concentrations of leptin were determined in triplicate by the radioimmunoassay procedures as described by Delavaud and coworkers (2000).

Statistical analysis

Statistical analyses were conducted using a completely randomized design with repeated measures. Data were analyzed using the General Linear Model procedure of SAS (SAS Inst., Cary, NC), with genotype and week of gestation as variables and BCS, number of lambs born, and body weight as covariables. Means at each week of gestation were separated using a Students t-test. Animal was designated as the experimental unit. Trendlines were produced across time for each genotype in the study using the least squares fit option in MS Excel (Microsoft Office, 2000).

RESULTS

Genotype significantly affected overall mean serum concentrations of leptin among ewe blood samples for the weekly samplings prior to lambing, the samples within 24 h of lambing and the 5 d after lambing samples (Table 1; $p = 0.0005$). Total serum concentrations of leptin among *callipyge*, homozygous and normal ewes were 5.41±0.40, 8.11±0.70, and 9.13±0.93 ng/ml of serum, respectively (Table 1). Intra-assay coefficient of variation was 0.11.

Gestational status significantly effected leptin levels

across all genotypes (p = 0.0098). In general, mean leptin levels for all ewes decreased during late gestation and continued to decrease through parturition and early lactation (Figure 1).

Body condition scores and number of lambs born also significantly affected the leptin levels in the ewes (p = 0.004 and p = 0.0364, respectively). *Callipyge* ewes exhibited the highest BCS and lowest average number of lambs born in this study (Table 1).

Trendlines were produced for each genotype across the gestational status (Figure 1). Although the regression models for each genotype were significantly different (p = 0.0004) and the intercepts were different (p<0.0001), the slopes for the trendlines were not significant (p = 0.1621). Individually, the slopes of the trendlines for the *callipyge* and homozygous ewes were the most different at p = 0.0746. The normal genotype slope was similar to the other genotypes with normal versus *callipyge* at p = 0.4363, and normal versus homozygous at p = 0.2677 (Figure 1).

DISCUSSION

The *callipyge* mutation is a polar overdominance condition that results in sheep with hypertrophy of the loin and hindquarters while having lower daily feed intakes, higher feed efficiencies and, at slaughter, higher dressing percentages and less fat compared to non-*callipyge* sheep of the same weight (Cockett et al., 1994; Jackson et al., 1997). When evaluating a hormone such as leptin, which is produced by adipocytes, it would be expected to detect variation between sheep that are known to have less fat tissue, as in the case of the *callipyge* mutation (Delavaud et al., 2000). However, very little research has examined plasma leptin levels in sheep affected by the *callipyge* mutation, especially in relation to late gestation and early post-partum ewes that are utilizing large amounts of fat and energy to support a rapidly growing fetus and later produce milk.

In an attempt to minimize animal variation and elucidate the relationship between the *callipyge* mutation and circulating leptin levels, the same group of late gestational ewes were utilized over an extended period of time (8 wk prior to parturition through 5 d post-partum). These time points were chosen to emulate the stages of gestation where leptin has shown to peak (late second trimester in humans) and then decrease until parturition (Sattar et al., 1998; Tamura et al., 1998). By measuring the same group of ewes over an extended period of time, we were able to account for most of the animal variation (associated with individual animal differences), and identify significant genotype effects, thus causing us to accept the hypothesis that the *callipyge* mutation affects circulating leptin levels in ewes physiologically stressed by pregnancy and parturition. *Callipyge* ewes exhibited the lowest mean serum leptin concentrations when compared to normal and homozygous ewes of the same physiological state (Table 1).

Interestingly, the *callipyge* ewes exhibited the highest mean BCS and the lowest number of lambs born, both factors that have been associated with elevated leptin levels in normal sheep (Delavaud et al., 2000; McFadin et al., 2002). Note that amount of muscle is taken into account when measuring BCS in sheep (Russel, 1991) so this most likely accounts for the callipyge ewes having the highest mean BCS in the study. Nonetheless, we interpret these observations as possible evidence that there is an influence of the *callipyge* mutation in late gestational and early post-partum ewes. Since *callipyge* sheep in general have fewer fat stores than normal sheep, it would be feasible that higher feed efficiencies would result in partitioning of more nutrients towards muscle accretion versus fat deposition. It was also noted that *callipyge* ewes exhibited the least change in serum leptin levels from the beginning of the study through five days post-partum. Perhaps the *callipyge* sheep are resistant or less susceptible to changes in body composition relative to their physiological state (i.e pregnancy; Houseknecht et al., 1998).

One possible explanation for the *callipyge* phenotype in relation to low circulating leptin levels may involve the number of receptors found in the hypothalamus. If *callipyge* sheep possessed more leptin receptors, this would cause them to be more sensitive to their plasma leptin, possibly explaining the decrease in appetite and adipose stores (Mercer et al., 1996; Schwartz et al., 1996).

By visually appraising the trendlines (Figure 1), it appears that the *callipyge* and normal lines are most similar in slope, although the intercept is statistically higher for the normal ewes. These curves appear to be almost linear. The homozygous line appears to have more of a quadratic trend, even though the slope is not statistically different from the other two genotypes (p = 0.16). One explanation for this may be that the homozygous ewes as a whole had smaller body size and came from a flock unrelated to the *callipyge* and normal ewes. The *callipyge* and normal ewes were raised from the same flock and share some genetics distinct from the *callipyge* mutation.

IMPLICATIONS

The use of an ovine-specific leptin assay to measure circulating leptin levels in blood samples taken over several weeks during gestation and parturition revealed a genetic influence in relation to the *callipyge* mutation. This can help us understand the physiological stress response of *callipyge*, normal, and homozygous sheep using leptin as an indicator

of body fat composition. *Callipyge* sheep are naturally leaner than the other genotypes. However, the *callipyge* ewes used in this study actually exhibited the highest mean BCS of the study, while still possessing the lowest leptin levels over the gestational period. This may indicate that *callipyge* ewes of equal BCS would exhibit even lower levels of plasma leptin compared to normal phenotypes. We were also able to examine the response to the stress of lambing and early lactation in these sheep. All of this will contribute to a better understanding of the significantly different phenotype seen in sheep possessing the *callipyge* mutation.

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