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## Growth Hormone (GH) secretory patterns in genetically lean and fat sheep

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### ABSTRACT

The pattern of GH secretion was studied in Coopworth sheep which have been selected for low (Lean) or high (Fat) ultrasonic backfat depth over 15 years. At each of two developmental stages (February and May) Lean and Fat ram lambs were fed ad libitum or fasted for 48 hours ( $n=6$  per treatment). Animals were blood sampled via an indwelling jugular cannula every 10 minutes for six hours, then slaughtered and the pituitary glands removed and weighed.

Mean GH was greater for Lean than Fat animals (2.82 versus 1.42 ng/ml,  $p<0.001$ ), in February than in May (2.47 versus 1.62 ng/ml,  $p<0.001$ ) and for fasted than fed animals (2.48 versus 1.62 ng/ml,  $p<0.001$ ). Analysis of GH secretory patterns showed that peak frequency for Lean animals was more than twice that for Fat animals (2.64 versus 1.17 peaks/6 hours,  $p<0.001$ ). There was no difference in the peak amplitude. In line with their higher GH concentrations, Lean animals had significantly heavier pituitaries than Fat animals (0.74 versus 0.52 g,  $p<0.001$ ).

Selection for leanness in Coopworth sheep has altered the GH secretory pattern of the animals. The pituitary weight may explain some of these changes in GH secretion.

**Keywords:** growth hormone; pituitary; sheep; lean; fatness

### INTRODUCTION

Lines of Coopworth sheep have been selected on the basis of liveweight adjusted ultrasonic backfat thickness since 1981 (Fennessy *et al.*, 1987). The line of sheep selected for low ultrasonic backfat depth (Lean) now has significantly less subcutaneous and internal fat than the high backfat selection line (Fat), with a randomly selected control line being intermediate in these parameters (McEwan *et al.*, 1989). A number of endocrine studies have been undertaken on these animals to determine what biochemical pathways have been altered by the selection process. Lord and Fennessy (1985) showed that muscle tissue from Lean animals incorporated more [<sup>3</sup>H]-thymidine than Fat animals. Lord (1985) found no significant difference between genotypes in glucose, propionate or acetate tolerance tests. Recent work indicated that the Lean genotype animals have a greater capacity to secrete GH in response to GH-releasing factor (Suttie *et al.*, 1991). In addition, the pattern of GH secretion differs between the Lean and Fat sheep, with Lean animals having greater mean plasma GH levels and less frequent secretory peaks of GH (Suttie *et al.*, 1993). Further data suggest that the amplitude of GH secretory peaks are greater in the Lean genotype sheep (Suttie *et al.*, 1992). During the course of that study it was also found that there is a difference in GH profiles at different times of the year, but this was not affected by photoperiod.

The objective of this work was to measure the GH secretory patterns in the Lean and Fat genotypes under different feeding regimes and in different seasons. The weight of the pituitary was measured to determine if gross anatomical changes are related to the differences in GH secretion.

### MATERIALS AND METHODS

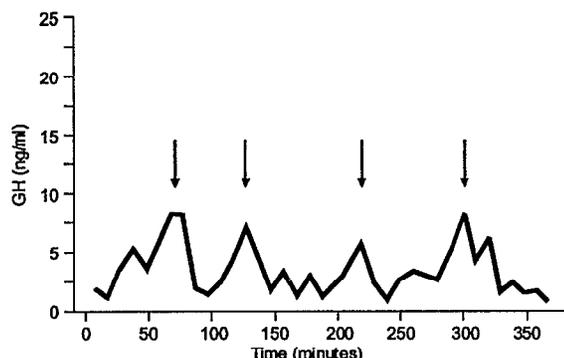
The experiment consisted of a 2x2x2 factorial design using 48 Coopworth ram lambs from 2 different genotypes (Lean or Fat), on 2 levels of nutrition (Fed or Fasted), at 2 times (February or May when the animals were 5 or 8 months of age), with 6 animals per treatment. The first animals (5 months old) were brought off pasture in mid December 1993 and the second group (8 months old) at the end of March 1994. An adaptation period of five weeks was used to accustom animals to the pelleted high roughage diet (10.7 MJME/kg DM). The animals were weighed and ultrasonically scanned to determine fat thickness (GR measurement) before being housed in indoor pens in three groups of eight. Fasting treatments were begun 48 hours prior to start of measurements (2 Lean and 2 Fat from each pen of 8 animals).

The day prior to sampling, a sterile 14 gauge 2½ inch "Insyte" cannula was inserted in the jugular vein. On three consecutive days each group of eight animals was blood sampled (4 ml) every 10 minutes for 6 hours. At the end of each sampling period the animals were immediately slaughtered and the pituitary gland removed and weighed.

Blood samples were centrifuged and plasma was removed and stored at -20°C. GH was measured by radioimmunoassay as described by Suttie *et al.* (1993). The GH secretory patterns were analyzed by the peak detection routine PULSAR (Merriam and Wachter, 1982). The output parameters of mean and basal GH concentration, the number of peaks detected and the average amplitude of the peaks, along with the weight of the pituitary were analyzed by analysis of variance (ANOVA) fitting the full factorial struc-

ture. GR measurements were analyzed by ANOVA with live weight as a covariate. Data are presented as the mean and standard error of the difference (SED).

**FIGURE 1:** GH concentration (ng/ml) measured every 10 minutes over 6 hours for a typical lean, fed animal in February. Arrows indicate peaks detected by PULSAR analysis.



**TABLE 1:** Mean and Basal GH (ng/ml), Frequency of GH peaks (number/6 hours) and Amplitude of peaks detected (ng/ml) for Lean and Fat genotypes.

	Lean	Fat	SED	Significance
Mean	2.81	1.42	0.204	***
Basal	2.14	1.22	0.143	***
Frequency	2.64	1.17	0.355	***
Amplitude	4.52	3.97	0.657	NS

**TABLE 2:** Mean and Basal GH (ng/ml), Frequency of GH peaks (number/6 hours) and Amplitude of peaks detected (ng/ml) for fed and fasted sheep

	Fed	Fasted	SED	Significance
Mean	1.62	2.47	0.203	***
Basal	1.33	1.96	0.142	***
Frequency	1.83	1.98	0.355	NS
Amplitude	4.01	4.47	0.651	NS

**TABLE 3:** Mean and Basal GH (ng/ml), Frequency of GH peaks (number/6 hours) and Amplitude of peaks detected (ng/ml) for sheep of 5 (February) or 8 (May) months of age

	February	May	SED	Significance
Mean	2.46	1.62	0.200	***
Basal	2.09	1.24	0.142	***
Frequency	1.93	1.88	0.355	NS
Amplitude	4.54	3.95	0.653	NS

**TABLE 4:** Comparison of mean pituitary weights (g) between genotypes, feeding level and month of sampling

	Pituitary weight	SED	Significance
Lean	0.74		
Fat	0.52	0.039	***
Fed	0.64		
Fasted	0.61	0.039	NS
5 months	0.70		
8 months	0.55	0.039	**

## RESULTS

The mean live weight of the animals did not differ significantly between the two sampling periods (February 39.0 kg and May 37.2 kg, SED=1.4), but Lean animals were heavier ( $p<0.001$ ) than Fat animals (41.1 kg versus 35.1 kg, SED=1.4). Live weight adjusted ultrasonic GR was less ( $p<0.05$ ) in February than May (7.5 mm versus 9.2 mm, SED=0.6). Lean animals had a smaller ( $p<0.001$ ) GR measurement than Fat animals (5.1 mm versus 11.5 mm, SED=0.7 mm).

The GH secretory pattern for a typical Lean, fed animal in February is shown in Figure 1. From this pattern four major peaks were detected as indicated. The calculated GH secretion parameters for the Lean and Fat genotypes are presented in Table 1. Lean animals had higher ( $p<0.001$ ) mean and basal GH levels than Fat animals, and more ( $p<0.001$ ) GH secretory peaks in the six hour sampling period than the Fat animals. There was no significant difference in the amplitude of the peaks between the genotypes. Table 2 demonstrates the effect of fasting on the GH secretory profiles. Mean and basal GH concentrations were higher ( $p<0.001$ ) for fasted than fed animals but neither the frequency nor amplitude of the secretory peaks were significantly altered. Mean GH concentration was higher ( $p<0.001$ ) in the animals sampled in February than in May (Table 3). Similarly, the basal GH concentration was greater in February than May ( $p<0.001$ ) (Table 3). There was no significant difference in either the frequency or the amplitude of peaks between the two sampling dates.

The weight of the pituitaries removed from the animals after slaughter are presented in Table 4. The pituitaries from Lean animals were heavier ( $p<0.001$ ) than from Fat animals, while pituitaries from animals slaughtered in May were lighter ( $p<0.01$ ) than from the February animals. Feeding had no significant effect on pituitary weight. Pituitary weight was positively correlated with basal GH concentration for the pooled data ( $r=0.53$ ,  $p<0.001$ ).

## DISCUSSION

In agreement with earlier reports (McEwan *et al.*, 1989), the Lean ram lambs had significantly lower GR measurements. There was no significant change in liveweight between the February and May sampling times, although the sheep became fatter as would be expected in a maturing animal. In line with the trends described by Suttie *et al.* (1993) and in other species such as pigs (Althen and Gerrits, 1976; Buonomo and Klindt, 1993), mean GH was greater in Lean than Fat animals. However, in contrast to Suttie *et al.* (1993), the Lean animals had more frequent peaks of GH secretion. While the animals in this trial are from the same flock as those used by Suttie *et al.* (1993), the animals used in this trial were pure-breds from the Lean or Fat lines whereas the Suttie *et al.* (1993) animals were the result of a cross of Lean or Fat rams with unselected ewes. The half-bred status of those animals may have contributed greater variation to the data. Other data (Suttie *et al.*, 1992) indicate that peak frequency is indeed greater in pure-bred Lean than pure-bred Fat animals. In accordance with the published data (Suttie *et al.*, 1993) there was no genotype differences in peak amplitude. The mean and basal levels of GH decreased from February to May. This

is similar to the trend from November to March recorded by Suttie *et al.* (1993) and to the decrease in GH secretion seen during aging of cattle (Trenkle and Topel, 1978) and pigs (Chappel and Dunkin, 1974). Unlike other trials (Klindt *et al.*, 1985; Suttie *et al.*, 1993), no significant change in the frequency or amplitude of GH secretory peaks was seen from February to May. In agreement with the classic work of Driver and Forbes (1981), plasma GH levels were increased with fasting.

Although a number of studies have considered the relationship between body composition and plasma GH concentrations, the weight of the pituitary gland has rarely been recorded. Two studies with mice (Edwards, 1962; Johnson and Eisen, 1975) have shown that selection for larger body size or rapid weight gain produces relatively larger pituitaries. In cattle, Trenkle (1977) found that as cattle increased in size, pituitary weight decreased relative to body weight. In two breeds of pigs, animals selected for low backfat had significantly heavier pituitaries than the fat animals (Althen and Gerrits, 1976). The results presented above show that pituitary weight in Lean sheep was 42% greater than in the Fat animals. This observation would help to explain why Suttie *et al.* (1991) found significantly higher GH secretion after injection of GH-releasing factor in the Lean than Fat sheep. Presumably, the Lean animals have a greater capacity to store and secrete GH due to the larger pituitary size. In cases of acromegaly, pituitary hyperplasia and increased GH secretion are observed (Thorner, 1993). It is not clear however, whether the difference in pituitary weight is due to larger or more numerous GH secreting cells or whether other cell types contribute to the heavier pituitary. It appears that the pituitary weight is related more to the GH secretory pattern than body weight, since there was a 23% decrease in pituitary weight from February to May, while body weight was not different. This also raises the question of when during development the differences in pituitary weight and GH secretion begin to occur.

This work has confirmed that GH secretory patterns are different in Lean and Fat genotypes of sheep. Lean animals have greater mean GH plasma levels and more frequent secretory peaks. This greater GH secretory capacity may be a result of larger pituitaries, but the precise mechanism by which this occurs needs to be elucidated.

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