Anim. Prod. Aust. 2002 Vol. 24: 33-36

LONG-TERM VARIATIONS IN GROWTH HORMONE LEVELS IN SHEEP EXPRESSING AN INSERTED GENE FOR GROWTH HORMONE

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SUMMARY

Growth hormone (GH) affects important characteristics of animal production, including growth rate and fatness, so sheep transgenic for ovine GH have been produced to test their viability under field conditions. As the gene construct for ovine GH had a non-inducible metallothionein promoter, GH should be expressed uniformly and previous studies in an animal house have shown that secretion is non-pulsatile over a 10-hour period. However, when GH was measured over two years, the transgenic sheep were found to be subject to substantial changes ($P< 0.001$) in their plasma GH concentrations, with a particular rise in August. In two of the transgenic ewes, the GH levels stayed elevated, resulting in insulin-resistant diabetes and cachexia. We conclude that although the gene construct would be expected to secrete GH at a constant rate, plasma concentrations of GH in transgenic sheep were subject to significant long-term variations that may have had serious consequences for the animal.

Keywords: transgenic, sheep, growth hormone

INTRODUCTION

Interest in the development of sheep transgenic for an additional copy of the GH gene was stimulated by the demands of the meat industry for leaner carcasses and increased growth rates. On the premise that if the basal levels of GH are increased, lipolysis is stimulated and there is a reduction in the fat content of the carcass, CSIRO at Prospect produced sheep (Ward and Brown 1998) with an ovine GH gene construct (MTSGH10). As the promoter was found to be non-inducible, i.e. not responsive to zinc, it could be expected that the transgenic sheep would express the GH gene uniformly, regardless of circumstances. The experimental results reported here are from the fourth generation of the original animals described by Ward and Brown (1998). The aim of this study was to determine whether GH transgenesis led to a consistent increase in plasma concentration of GH under field conditions in WA.

MATERIALS AND METHODS

A total of 131 lambs were produced in 1999 from 50 Poll Dorset ewes, 50 Border Leicester ewes and 70 Merino ewes inseminated with transgenic semen. Thirty-four lambs were shown by Southern blot analysis to carry the GH transgene. The lambs were born around June 30 (age 0 days) and were run at Yalanbee Field Station, Bakers Hill, WA. They were weighed fortnightly and single blood samples collected from 60 sheep at the ages of 100, 164, 281, 410, 516 and 667 days. The body condition was scored as described by Russel *et al.* (1969) on the same days as the blood samples were taken. All male lambs were castrated at weaning however 6 (4 transgenic, 2 non-transgenic) were later found to have a retained testis and were slaughtered at 10 months of age.

Commencing in January 2000 (age 198 days), an animal house experiment was conducted to examine the secretion pattern of growth hormone in transgenic wethers. There were 2 groups of 12 (total =24) castrate male lambs that either carried the transgene or did not. The groups were balanced to include equal numbers of Merino and Poll Dorset animals. The sheep were returned to the field in April 2000 and fed a lupin supplement of 150grams/head/day through to August. The sample for age 281 days is a combination of animal house and field samples as statistical analysis showed no effect of environment on GH concentration.

GH is a pulsatile hormone and circulating GH is normally measured at twenty-minute intervals over a 10-hour period. On analysis of GH data from the first animal house study, we found that the transgenic sheep

displayed non-pulsatile secretory patterns (Figure 1a) while the GH patterns in non-transgenic controls were pulsatile as expected (Adams *et al.* 2002). On this evidence, we concluded that it would to be possible to analyse the single blood samples taken from the transgenic sheep over the previous two years to see if the concentration of GH remained stable throughout the life of the animal.

Plasma concentrations of GH were assayed using the method as described in Adams *et al*. (1996). Blood glucose levels were measured using a Precision Blood Glucose Monitor and Precision Plus® blood glucose electrodes (Medisense Inc, Bedford, MA, USA) and an Infinity TM Glucose Reagent Kit (Sigma Diagnostics, St. Louis, MO, USA) on a COBAS Mira Clinical Analyser.

Results were analysed statistically using the ANOVA model of Systat (Wilkinson 1998) by analysis of variance or repeated measure analysis for GH and condition score. The variances of the data in the transgene and the control groups were not homogenous, so the data were converted to logarithms for statistical analysis. The back-transformed means are presented.

During the study it was observed that the high GH levels (up to 120ng/ml) in two transgenic ewes tended to skew the GH data and these ewes later developed chronic disease, marked by loss of condition and depression. Consequentially, these ewes were removed from the analysis of the mean data presented, but are presented separately because of their importance as models of the effects of excessive GH. They were slaughtered at 24 months, their organs and fat deposits weighed and samples taken.

RESULTS

An examination of GH data collected at various times during the first two years of the sheep's life either in animal house trials or in the field has shown that the mean concentration of the hormone changed markedly (Figure 1b). Overall, the transgenic sheep had higher concentrations of GH ($P < 0.001$), and these changed more over time ($P < 0.01$). As shown in Figure 1b, the GH levels in the transgenic sheep remained relatively constant between 100 and 281 days of age, although their levels were higher than the non-transgenic sheep. However, at age 410 days (August 2000), GH levels in the transgenic sheep were significantly increased over the previous sample ($P<0.001$). The mean GH concentration in the non-transgenic sheep was stable over the whole period.

Plasma concentrations of glucose tended to follow the available nutrition, with a substantial increase at the age of 410 days, particularly in the transgenic group (Figure 2c). Mean body condition score also reflected previous nutrition, declining particularly in the transgenic group over autumn and winter (age 164 to 410 days) and then increasing the following spring (Figure 2d).

Plasma concentrations of GH were excessively high in two transgenic ewes (>40 ng/ml) after the age of 410 days, and did not decrease at the subsequent samplings. Their levels stayed above 50 ng/ml for the remainder of the trial. One ewe's GH had begun increasing by the age of 164 days, but the other did not increase until age 410 days. These ewes continually demonstrated gross characteristics of excessive GH, acromegaly (overgrowth of hooves), excessive leanness and a lack of vigour. When the ewes were brought into the animal house in April 2001, their blood glucose levels were found to be 15.5 mmol/L compared with a mean value in the other transgenic sheep of 4.1mmol/L, and 3.7 mmol/L in controls. Treatment twice daily with quick release insulin (Mixtard® 30/70; Novo Nordisk Pharmaceuticals, North Rocks, NSW) and slow release insulin (Humulin®; Eli Lilly Aust., West Ryde, NSW) for three weeks was unable to suppress their glucose levels below 14.0mmol/L, indicating that they were suffering from insulin-resistant diabetes.

While the small number of sheep (2 transgenic and 2 controls) slaughtered at 24 months of age precludes statistical analysis, the two transgenic ewes tended to have distinctly different organ weights. Their omental fat was considerably reduced (a mean value of 202g compared to the control ewes 1450g), as was their uterus weight (a mean value of 8 g compared to control ewes 29g).

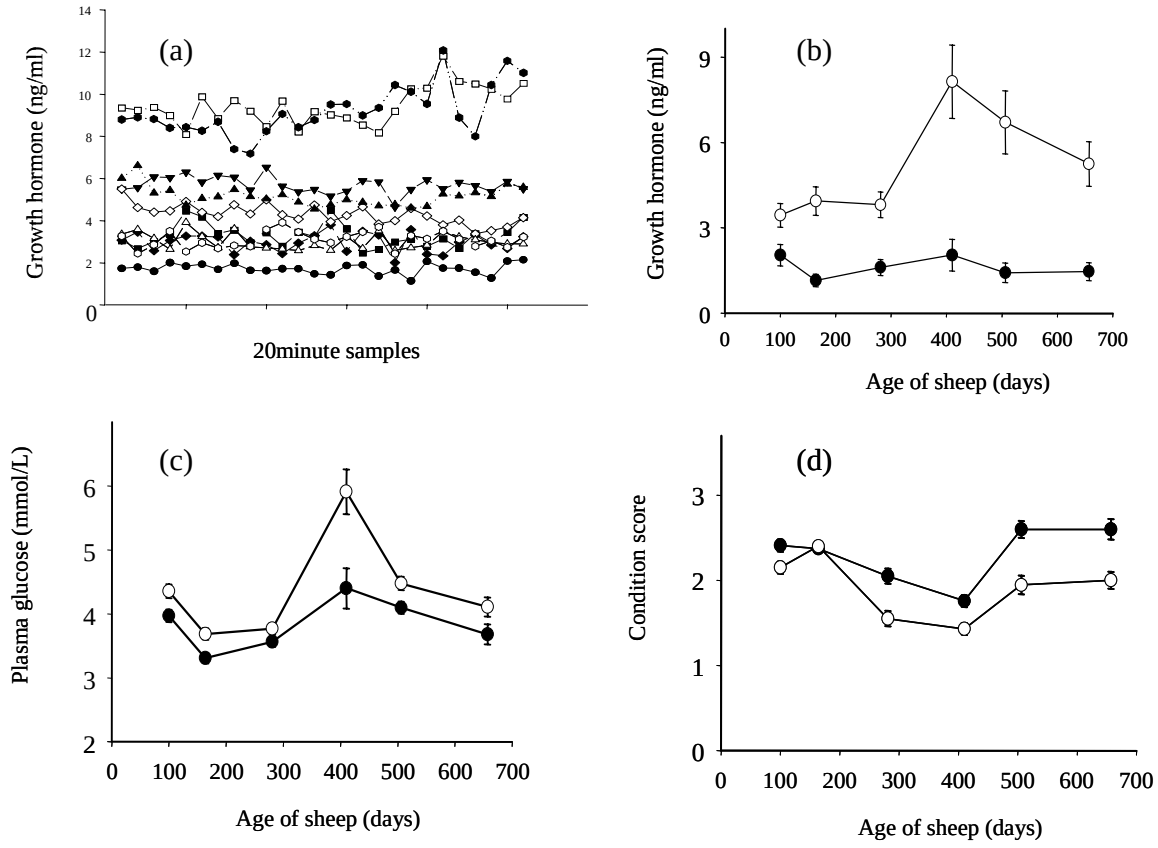

Figure 1. (a) GH secretion in transgenic sheep blood sampled every 20 minutes, indicating lack of pulses. (b)-(d) Mean and s.e.m. bars for transgenic (o) and control (•) sheep from 100 days to 667 days; (b) Plasma concentrations of GH, (c) Plasma concentration of glucose, (d) Condition score

Table 1. **Slaughter data of transgenic and control sheep at 10 months and 24 months**

	Controls	Transgenic	SE	Controls	Transgenic
Number	2	4		2	2
Age of Sheep (months)	10	10		24	24
Carcass Weight (kg)	15.8	16.8	1.27	19.6	16
Perirenal Fat (g)	271.6 [b]	132.3[a]	10.6	387	157
Liver (g)	500 [a]	624 [b]	21.8	533	811
Pancreas (g)	38	42	4.2	37	136
Kidney (g)	91	110*	3.8	93	179
Pituitary (g)	0.53	0.55	.01	.96	.70

[a, b] Values within a row differ significantly at 10 months of age.

DISCUSSION

Transgenic sheep should express GH uniformly throughout their lives because the promoter in the gene construct does not respond to nutritional signals. However, Fig. 2 clearly demonstrates that this was not the case. We have shown in an animal house study that the GH secretory patterns of transgenic sheep were non-pulsatile over short periods, so this does not explain the sudden rise in GH concentration in August. No such increase in GH was observed in the non-transgenic animals. The reason for the increase is unclear. It is tempting to focus solely on the August (age 410 days) result in relation to the seasonal increase in green feed, protein supply and liveweight gain expected on WA pastures at this time of the year. This seasonality is reflected in the substantial increase in plasma glucose concentrations, even in control sheep (Fig. 2b) indicating that GH was having a particular effect, since treatment with GH increases plasma glucose (Ward and Brown, 1998). There was also a subsequent increase in condition

score (Fig. 2c). However it may be that the autumn period between April and August is the key to these results. It is more likely that the increase resulted from the previous restricted nutrient supply that had caused the decline in condition score after the age of 164 days (Fig. 2c). Autumn in WA is a period of low protein feed, undernutrition and weight loss. Adams *et al*. (1996) showed that plasma concentrations of GH were elevated in sheep during under-nutrition as GH facilitates lipolysis and preserves body protein during weight loss. In cases of high GH stimulation, lipolytic products are mobilised to act as an energy source resulting in a reduction in the fat content of the carcass (Ward and Brown 1998). The lack of fat reserves may make it difficult for the transgenic sheep to deal with a low feed supply.

An alternative explanation for the increased plasma concentration of GH at this time is a reduced rate of clearance from plasma, possibly from increased activity of plasma GH binding proteins. Much circulating GH is bound to specific binding proteins, (Gatford *et al.* 1996) the control of which is not fully understood (Bondanelli *et al.* 2001). Low nutrition is also associated with reduced GH binding to liver membranes and high GH concentrations can be associated with a decrease in GH receptor binding (Brier 1988). An increase in circulating GH binding proteins, accompanied by a prolonged half-life of GH, has been observed in mice transgenic for additional copies of GH (Bartke *et al*. 1994).

Two transgenic ewes continued to demonstrate gross characteristics of excessive GH, acromegaly (particularly overgrowth of hooves), excessive leanness and a lack of vigour after this period. The 2 ewes could not control the rise in GH concentration in August 2000 (age 410 days) and their GH levels remained elevated until their death. The increased size of the two transgenic ewes' visceral organs that was found on slaughter (Table 1) has been noted in other publications on GH transgenic animals involving slaughter data (Nancarrow, 1991). Large differences in fat and organ weights explain the leanness of the transgenic ewes' carcass. The great increase in weight of the pancreas is most likely associated with increased activity of this organ in secreting insulin to combat the diabetes.

We conclude that although the gene promoter appeared to be non-responsive, there were changes in plasma GH concentrations that could seriously affected the health of the sheep, and the reasons for these changes could not be simply determined.

ACKNOWLEDGEMENT

Antiserum and standards for the growth hormone radio-immunoassay were kindly provided by Dr AF Parlow, NHPP, NIDDK, NICHHD, USDA.

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