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## Metabolic changes in sheep divergently selected for plasma IGF-1

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### ABSTRACT

Treatment of sheep, mice and humans with exogenous recombinantly-derived insulin-like growth factor-1 (IGF-1) has been reported to produce a hyperglycaemic state. In mice this is associated with the abolition of maternal constraint during pregnancy. Exogenous IGF-1 also alters renal clearance in sheep and humans, decreasing circulating concentrations of urea and creatinine. The objective of this study was to determine whether similar changes are apparent in sheep divergently selected for plasma IGF-1 concentrations.

Ewe hoggets (10 months old), 16 each from the Massey University Low (L), Control (C) and High (H) IGF-1 selection lines, were blood sampled by jugular venipuncture over a 5 hour period after removal from pasture. Plasma metabolite concentrations were assayed and adjusted for the effects of sampling time relative to removal from pasture.

There were no differences between the lines (L vs C vs H) in plasma concentrations of urea (7.34 vs 7.35 vs 7.32 mM, Pooled SE = 0.25 mM) and, although there was a significant line effect for creatinine ( $P < 0.05$ ), none of the between-line comparisons was significant (0.073 vs 0.075 vs 0.075 mM, Pooled SE = 0.002 mM). However, glucose concentrations were greatest in the H line (3.72 mM), followed by the C line (3.56 mM) and the L line (3.45 mM) (PSE = 0.07 mM,  $P < 0.05$ ).

Whereas glucose and urea concentrations declined with time off pasture in the H line ( $b = -0.133 \pm 0.040$  mM hr<sup>-1</sup>,  $P < 0.01$  and  $b = -0.333 \pm 0.062$  mM hr<sup>-1</sup>,  $P < 0.001$  respectively), there was no such relationship in the L line ( $b = 0.017 \pm 0.031$  mM hr<sup>-1</sup> and  $b = -0.004 \pm 0.061$  mM hr<sup>-1</sup> respectively). Furthermore while L line plasma concentrations of creatinine increased with time off pasture ( $b = 4.864 \pm 0.398$   $\mu$ M hr<sup>-1</sup>,  $P < 0.0001$ ), H line creatinine concentrations did not ( $b = 0.796 \pm 0.892$   $\mu$ M hr<sup>-1</sup>). The C line regression coefficients were ( $b = -0.120 \pm 0.018$  mM hr<sup>-1</sup>,  $b = -0.030 \pm 0.057$  mM hr<sup>-1</sup>, and  $b = 2.166 \pm 1.072$   $\mu$ M hr<sup>-1</sup> for glucose, urea and creatinine respectively).

Results suggest that just 1.5 generations of divergent selection for IGF-1, has generated in the H line a mildly hyperglycaemic state similar to that observed in IGF-1 treated animals. However, the effect of divergent selection for plasma IGF-1 levels on metabolite concentrations would appear to be dependent on the nutritional status of the animals as indicated by the heterogeneous regressions of metabolite concentrations on time off pasture.

**Keywords:** Insulin-like growth factor-1, selection, sheep, glucose, urea, creatinine.

### INTRODUCTION

The hormone insulin-like growth factor-1 (IGF-1) has been identified as having many of the characteristics desired of a physiological marker (Roberts *et al.*, 1990). High IGF-1 levels are associated with increased growth rate or liveweight (Ringberg Lund-Larsen *et al.*, 1977; Merimee *et al.*, 1982; Eigenmann *et al.*, 1984; Blair *et al.*, 1987; Roberts *et al.*, 1990), and increased milk production (Ahlborn-Breier *et al.*, 1987). Selection for IGF-1 in mice has resulted in increased liveweight at 42 days (Blair *et al.*, 1988) and the abolition of maternal constraint (Gluckman *et al.*, 1992).

Although IGF-1 seems to have potential as a physiological predictor of genetic merit for some production traits, the role it plays in the control of metabolism and growth is far from being fully characterised. There is a need to better understand the physiological role of IGF-1 so that selection for IGF-1, while positively influencing selection gains in one trait, does not have unforeseen repercussions on others.

Previous work has established that the chronic administration of recombinantly derived human IGF-1 (rhIGF-1) to

sheep (Cottam *et al.*, 1992) and humans (Guler *et al.*, 1989a; Walker *et al.*, 1991) increases circulating concentrations of glucose while decreasing circulating concentrations of insulin, urea and creatinine. Exogenous IGF-1 thus produces a state of hyperglycaemia and hypoinsulinaemia, and alters glomerular filtration rate in the kidney (Guler *et al.*, 1989a; Hirschberg & Kopple 1989; Cottam *et al.*, 1992). However, these effects have not been confirmed using animals whose endogenous IGF-1 levels have been altered.

Lowered concentrations of urea and creatinine have also been identified in animals of superior genetic merit for fleeceweight (McCUTCHEON *et al.*, 1987; Clark *et al.*, 1989; Thomson *et al.*, 1989) and low backfat depth (Hanrahan, 1986; Bremmers *et al.*, 1988; Carter *et al.*, 1989; van Maanen *et al.*, 1989), suggesting that the administration of IGF-1 and selection for superior productive performance results in similar metabolic changes.

The present study was undertaken to examine the effects of selecting for divergent plasma IGF-1 levels, in sheep, on some aspects of metabolism. The key issue was whether metabolic changes observed in animals treated with exogenous

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IGF-1 (particularly in respect to glucose, urea and creatinine metabolism) would be also observed in animals selected for high plasma IGF-1 levels. If so, this would point to IGF-1 having parallel actions (presumably of an endocrine nature) irrespective of whether its levels in circulation were elevated by exogenous administration or by selection for high endogenous levels.

## MATERIALS AND METHODS

### Experimental procedure

Sixteen 10-month-old ewe hoggets were chosen from each of the Massey University High (H), Low (L), and Control (C) plasma IGF-1 selection lines of sheep (Morel *et al.*, 1991). The lines were established in 1987 by screening a population of 111 Romney ram lambs for animals with high and low plasma concentrations of IGF-1. C line rams were selected randomly from the 111 lambs. The selected sires were each mated to 25 randomly selected Romney ewes of unknown IGF-1 levels in 1988 and 1989. Subsequent selection of replacements has been within-line and is based on high or low plasma IGF-1 concentrations, as appropriate, taken 3-4 weeks after weaning (H or L lines) or selection at random (C line). After 1.5 generations of selection (4 years) the H line have circulating IGF-1 levels significantly greater than those of the L line ( $160 \pm 5$  versus  $125 \pm 5$  ng/ml,  $P < 0.01$ ) and tend to have higher weaning weights. The C line have a plasma IGF-1 concentration of  $131 \pm 4$  ng/ml (Blair, H.T., *pers. comm.*).

For this study four animals were randomly selected from each sire-within-line group. The animals were subjected to normal grazing management at pasture on the Massey University Sheep and Beef Cattle Research Unit until the morning of the experiment on 14 July 1992.

Mean plasma metabolite concentrations were compared between lines, over a 5 hour period after removal from pasture. Each animal was blood sampled once and the sampling time relative to the time of removal from pasture recorded. Blood samples were collected by jugular venipuncture into vacutainer tubes containing EDTA as the anticoagulant and chilled on ice, before being centrifuged at 3000g and 4°C for 20 minutes. Plasma was harvested and frozen in duplicate tubes at -20°C until subsequent analysis. Plasma concentrations of glucose (Trinder 1969), urea (Tiffany *et al.*, 1972), and creatinine (Larson 1972) were measured using a Cobas Fara II autoanalyser (Hoffmann-La Roche, Basel, Switzerland). Intra- and inter-assay coefficients of variation for glucose, urea, and creatinine were: 1.9, 4.6; 2.1, 3.4; 1.0 and 1.4%, respectively.

### Statistical analysis

Analyses of variance were performed using the ordinary least-squares procedure (SAS 1988). The plasma concentrations of glucose, urea, and creatinine were fitted to a model that included the effects of line and time off pasture within-line. The heterogeneity of the within-line regressions of metabolite concentration on time off pasture was tested by the method of Searle (1971). Statistical comparisons of the

least square means between lines were undertaken using Student's *t*-test.

## RESULTS

Table 1 gives the time adjusted plasma concentrations of glucose, urea, and creatinine for the high (H), control (C), and low (L) lines of IGF-1 selected sheep. High line animals exhibited significantly greater baseline concentrations of glucose than L line animals ( $P < 0.05$ ) but, although glucose concentrations were also higher in the H line than in the control line, this difference was not significant ( $P = 0.14$ ). Although the line effect controlled a significant proportion of the total variation in plasma creatinine concentrations ( $P < 0.05$ ), none of the between-line comparisons were significant. There was no significant line effect for plasma urea concentrations.

**TABLE 1:** Plasma concentrations of glucose, urea, and creatinine corrected for time off pasture in 16 High, 16 Control, and 16 Low IGF-1 line ewe hoggets.

Metabolite	IGF-1 Selection Line			PSE <sup>1</sup>
	High	Control	Low	
Glucose (mM)	3.72 <sup>ab</sup>	3.56 <sup>ab</sup>	3.45 <sup>b</sup>	0.07
Urea (mM)	7.32	7.35	7.34	0.25
Creatinine (mM)	0.075	0.075	0.073	0.002

<sup>ab</sup> means with superscripts in common are not significantly different ( $P > 0.05$ )

<sup>1</sup> Pooled standard error

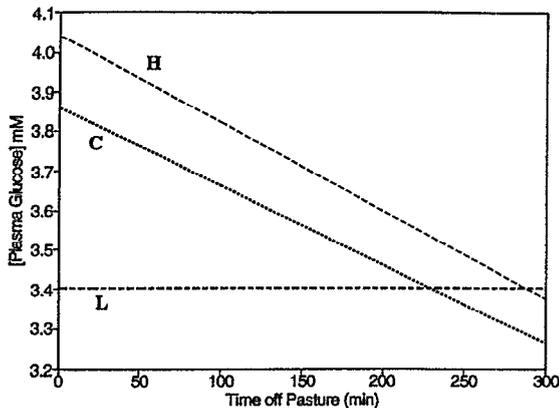
The within-line regressions of plasma glucose on time off pasture were heterogenous ( $P < 0.10$ ), coefficients for the H and C lines being negative while that of the L line was not significantly different from zero (Table 2 and Figure 1). Although there was no significant heterogeneity in the within-line regressions of plasma urea concentration on time off pasture (Figure 2), the H line regression coefficient for plasma urea concentration on time off pasture was negative while those of the C and L lines were not different from zero (Table 2).

The within-line regressions of plasma creatinine on time off pasture were also heterogenous ( $P < 0.05$ ), the L line coefficient for plasma creatinine being significantly higher than those of the H and C lines (Table 2).

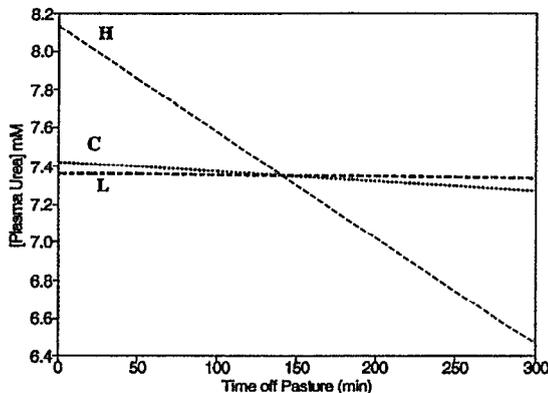
**TABLE 2:** Within-line regression coefficients ( $\pm$  S.E.) of metabolite concentrations on time off pasture for the High, Control, and Low IGF-1 selected lines of sheep

Metabolite	IGF-1 Selection Line		
	High	Control	Low
Glucose (mM hr <sup>-1</sup> )	-0.133 $\pm$ 0.040	-0.120 $\pm$ 0.018	0.017 $\pm$ 0.031
Urea (mM hr <sup>-1</sup> )	-0.333 $\pm$ 0.062	-0.030 $\pm$ 0.057	-0.004 $\pm$ 0.060
Creatinine ( $\mu$ M hr <sup>-1</sup> )	0.796 $\pm$ 0.892	2.166 $\pm$ 1.072	4.860 $\pm$ 0.398

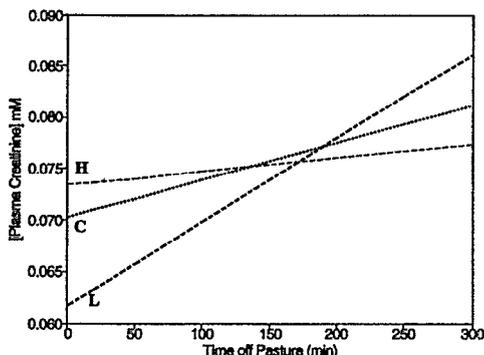
**FIGURE 1:** The within-line regression of plasma glucose concentrations on time off pasture for the High (H), Control (C) and Low (L) IGF-1 selection lines



**FIGURE 2:** The within-line regression of plasma urea concentrations on time off pasture for the High (H), Control (C) and Low (L) IGF-1 selection lines



**FIGURE 3:** The within-line regression of plasma creatinine concentrations on time off pasture for the High (H), Control (C) and Low (L) IGF-1 selection lines.



**DISCUSSION**

The results of this experiment suggest that divergent selection on the basis of circulating IGF-1 levels does not exert a consistent effect on metabolite concentrations but rather that this effect varies with time off pasture (presumably a nutritional effect). For up to about 5 hours off pasture, basal glucose concentrations were significantly higher in the H line than in the L line sheep, and tended to be higher than in the

C line. This is consistent with other studies showing that increased circulating IGF-1 levels within normal physiological limits, achieved either by using exogenous administration of rhIGF-1 in humans and sheep (Walker *et al.*, 1991 and Cottam *et al.*, 1992 respectively) or by using mice divergently selected for endogenous levels of plasma IGF-1 (S.H.Zhang, unpub. data), are associated with a mild hyperglycaemic state.

Guler *et al.*, (1989a) and Cottam *et al.*, (1992) found that the administration of rhIGF-1 decreased plasma concentrations of urea and creatinine. This was attributed to the ability of IGF-1 to increase glomerular filtration rate and renal plasma flow (Guler *et al.*, 1989b; Hirschberg and Kopple 1989). The current study found no significant between-line differences in either circulating urea or creatinine concentrations. This suggests that divergent selection for endogenous IGF-1 (at least to the extent that has occurred with these lines) has little or no effect on renal function, in contrast to the apparent effect on glucose homeostasis.

The heterogeneous within-line regressions of plasma glucose, urea and creatinine concentrations on time off pasture indicate that expression of genetic merit (for circulating IGF-1 concentrations) in terms of circulating metabolite concentrations is strongly influenced by nutritional status. A greater rate of decline in glucose and urea concentrations was observed in H than in L sheep and may reflect line differences, over time, in the entry rate of gluconeogenic precursors and ammonia from the rumen. Such differences could reflect a greater food intake in the H sheep immediately prior to the study, although only small effects of exogenous IGF-1 on feed intake have been demonstrated in controlled studies (Cottam *et al.*, 1992). The more rapid rise in plasma creatinine concentrations in L sheep is consistent with a greater entry rate of creatinine into the plasma pool and hence with the known protein-sparing effects of IGF-1 (Jacob *et al.*, 1989; Douglas *et al.*, 1991; Koea *et al.*, 1992). Whatever the mechanisms involved, these results clearly indicate that more comprehensive assessments of selection effects on metabolic status will need to be performed under controlled conditions.

In conclusion, the present study attempted to establish whether selection for high endogenous plasma IGF-1 levels leads to the same responses in metabolite concentrations seen with exogenous administration of rhIGF-1. The results show that selection for high levels of plasma IGF-1 has a hyperglycaemic effect, which agrees with findings from studies using exogenous IGF-1. However, differences between the selection lines in metabolite concentrations are also strongly dependent on time off pasture.

**ACKNOWLEDGEMENTS**

The authors wish to thank Margaret Scott for the laboratory analysis, all those who took part in the experimental work and the staff of the Massey University Sheep and Beef Research Unit.

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