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The insulin status of sheep with genetic differences in glucose tolerance and carcass composition.

S.M. FRANCIS, R. BICKERSTAFFE¹ AND D. O'CONNELL

MAF Technology, Canterbury Agricultural and Science Centre, Lincoln

ABSTRACT

The first two years of a breeding trial have shown that sires with slow clearance of glucose after a glucose tolerance test will produce progeny with similar slow clearance and leaner carcasses (Line 1), than progeny from sires with fast glucose clearance (Line 2).

Six extreme animals from each Line were selected to study the biochemical mechanisms controlling the effects seen in the breeding trial. An euglycaemic clamp experiment involved infusing insulin at three levels, 0.63, 3.46 and 6.29 mU/kg liveweight^{0.75}/min, each for four hours. At the same time glucose was infused with adjustments to maintain plasma glucose at basal levels. The amount of glucose infused is a measure of the sensitivity of the peripheral tissues to insulin.

Line 1 animals required significantly ($P < 0.05$) more glucose to maintain euglycaemia during all three levels of insulin infusion. Because there were differences in basal plasma glucose (Line 1: 71, Line 2: 61 mg/100 ml, $P < 0.05$), glucose utilization was corrected for basal glucose. Line 1 animals still had greater glucose utilization. Another measure of insulin sensitivity is the change in glucose utilization relative to the change in plasma insulin as insulin infusion increases. Line 1 animals had a significantly greater insulin sensitivity index than Line 2 animals (0.505 versus 0.193, $P < 0.01$) for the change from level 1 to level 2. When corrected for differences in basal glucose, Line 1 animals were still significantly more sensitive to the increase in insulin (0.680 versus 0.346, $P < 0.01$).

Selecting animals for breeding by glucose tolerance produces sheep with differences in glucose utilization by peripheral tissues. These biochemical differences are important in partitioning nutrients for deposition of lean or fat tissue.

Keywords Sheep; insulin; carcass fatness; euglycaemic clamp; glucose utilization.

INTRODUCTION

Traditional methods of selection for leanness in sheep have relied on direct measurement of sire fatness using ultrasonic scanning techniques (Bennett *et al.*, 1988; Fennessy *et al.*, 1987; Solis-Ramirez *et al.*, 1989). While these breeding trials have been successful in the past, it would be more desirable to select directly for a biochemical parameter that is genetically correlated with fat deposition, rather than on the phenotype itself. Ultimately the genes controlling these biochemical pathways may be identified and selection for breeding based on the presence or absence of a particular genotype.

A breeding trial was initiated in 1987 to look at the effect of selection for glucose tolerance on carcass fatness. The first two years have shown that breeding from sires with slow clearance of glucose after a glucose tolerance test (GTT) will yield progeny with

similar slow clearance and leaner carcasses, than progeny from sires with fast glucose clearance (Francis *et al.*, 1988; 1990).

This paper outlines a euglycaemic clamp experiment which measures glucose utilization by animals with extreme differences in glucose tolerance and discusses how this may relate to differences in carcass composition.

METHOD

The breeding trial involved mating seven Coopworth rams with high (Line 1) T-half (slow clearance of glucose after a GTT) or low (Line 2) T-half to a randomly selected group of ewes. Six first generation ram progeny from each Line with consistently high or low T-half over three GTTs were selected for detailed study of their insulin status. One such experiment is the hyperinsulinaemic euglycaemic clamp developed by

¹ Department of Biochemistry and Microbiology, Lincoln University, Canterbury

Sherwin *et al.* (1974) and Insel *et al.* (1975). The animals, with a mean liveweight of 60 kg, had previously been fed pasture and had free access to food and water throughout the experiment. Full details of the experiment are given in Francis (1990).

Ovine insulin dissolved in sterile saline was infused via jugular catheters at three levels, 0.63, 3.46 and 6.29 mU/kg liveweight(lwt)^{0.75}/min, each for four hours. Throughout the experiment, blood samples were taken at five minute intervals and analyzed immediately for glucose concentration. When plasma glucose began to decline in response to insulin infusion, additional glucose was infused through a variable speed pump. The aim was to maintain animals at their basal glucose levels throughout the entire experiment. Plasma samples, taken at half hourly intervals, were analyzed for immunoreactive insulin.

The experiment was carried out on two animals per day - one from each Line paired for liveweight. Calculations are based on infusion rates and plasma insulin levels during the period when plasma glucose was stable for each level of insulin infusion. Statistical analyses corrected for variations between the days of infusion. Least significant difference (Lsd) values ($P < 0.05$) are given for the difference between Lines at each level of insulin infusion.

RESULTS AND DISCUSSION

Prior to the commencement of infusion, basal glucose was significantly ($P < 0.05$) greater in Line 1 (71 mg/100 ml) than Line 2 (61 mg/100 ml) animals. These differences were maintained throughout the experiment and did not change as the level of insulin infusion increased (Table 1). Plasma insulin levels were the same for both Lines at the first and second levels of insulin infusion and increased significantly from level one to level two ($P < 0.05$), but not from level two to three, where Line 2 animals showed little change in plasma insulin (Table 1). The reason for this anomaly is unknown and so more emphasis is placed on the changes from the first to the second level of insulin infusion.

A measure of insulin sensitivity is the amount of glucose that must be administered to maintain glucose at basal levels while insulin is being infused at a constant rate (Bergman *et al.*, 1985). This is independent of pancreatic secretion which should be inhibited by

the infusion of exogenous insulin. Table 2 shows that Line 1 required significantly ($P < 0.05$) more glucose at all levels of insulin infusion than Line 2 animals. This means that at the same level of insulin, Line 1 animals utilize glucose more rapidly.

TABLE 1 Concentration of plasma (mg/100 ml) and insulin (μ U/ml) during the period when plasma levels were stable.

Level	Glucose			Insulin		
	Line 1	Line 2	Lsd	Line 1	Line 2	Lsd
1	75.2	66.1	3.0	22.7	24.1	8.8
2	75.1	57.3	12.4	41.8	42.3	10.8
3	73.6	58.6	14.4	53.8	43.2	28.4

One problem with clamping the animals at basal glucose is that this differed between the two groups. Glucose utilization may be affected by plasma glucose levels (Doberne *et al.*, 1982). This dilemma can be avoided by dividing glucose utilization by actual plasma glucose level to calculate the glucose clearance rate. Even after this correction, Line 1 still required significantly ($P < 0.05$) more glucose infused per unit of plasma glucose than Line 2 animals (Table 2).

TABLE 2 Rate of glucose infused (mg/kg lwt^{0.75}/min) and glucose metabolic clearance rate (min/kg lwt^{0.75}) during the period when plasma glucose levels were stable.

Level	Glucose infusion			Glucose clearance		
	Line 1	Line 2	Lsd	Line 1	Line 2	Lsd
1	3.01	1.05	1.31	0.043	0.017	0.014
2	8.92	5.22	2.68	0.132	0.096	0.017
3	10.29	6.07	3.66	0.147	0.116	0.041

Another way to compare insulin sensitivity is to look at the change in glucose utilization as insulin infusion increases. These parameters are shown in Table 3. The increase in glucose to maintain euglycaemia as insulin infusion increased from level 1 to level 2, was not statistically different between the Lines. However, when this increase was assessed relative to the actual increase in plasma insulin caused by the change in

infusion rate, the insulin sensitivity index was significantly greater ($P < 0.01$) for Line 1 than Line 2 animals. As well, the correction of this index for differences in glucose levels showed Line 1 animals to have significantly ($P < 0.01$) greater insulin sensitivity. The changes in insulin sensitivity from level 2 to level 3 have some anomalies such as negative values for Line 2 caused by the decrease in plasma insulin levels for some animals as discussed above.

TABLE 3 Change in insulin sensitivity parameters with the increase in insulin infusion levels.

Parameter	Level 1 to 2			Level 2 to 3		
	Line 1	Line 2	lsd	Line 1	Line 2	lsd
Infusion rate ¹	5.91	4.18	2.29	1.37	0.85	2.80
Plasma insulin ²	19.2	18.2	9.9	12.0	1.0	24.8
Insulin sensitivity ³	0.505	0.193	0.156	0.039	-0.011	1.615
Plasma glucose ⁴	75.2	61.7	6.5	74.3	58.0	11.2
CIS ⁵	0.680	0.346	0.187	0.094	-0.323	2.72

¹ Difference in glucose infusion rate at plateau between levels of insulin infusion

² Difference in plasma insulin at plateau between levels of insulin infusion

³ Insulin sensitivity index : parameter 1 / parameter 2

⁴ Mean plasma glucose level during measurement period

⁵ Corrected insulin sensitivity - an index corrected for plasma glucose level (Parameter 3 / Parameter 4)

Clearly, for a given amount of exogenous insulin, Line 1 animals have greater glucose utilization. This apparently contrasts with the whole basis of the trial, where Line 1 animals are genetically selected for slow glucose clearance after a GTT. This implies that two mechanisms are acting in these animals. The first is that peripheral tissues of Line 1 animals have enhanced insulin sensitivity relative to Line 2. This agrees with work on obese and normal humans (Olefsky, 1981), since obesity is associated with reduced insulin sensitivity, as is found in Line 2 animals, which have been shown to have greater carcass fatness. However a second mechanism must operate at the pancreatic level. Because the peripheral tissues of Line 1 animals have greater glucose utilization, the slow clearance of glucose after a GTT can not be explained by a slow glucose

uptake. The reason must be a smaller release of insulin in response to a glucose challenge - that is reduced pancreatic sensitivity. In fact unpublished data (Francis, 1990) has shown that, while basal plasma insulin levels did not differ, mean plasma insulin level per unit time during a GTT was significantly less ($P < 0.05$) in Line 1 than Line 2 (8.4 versus 15.7 uU/ml/min). This would suggest lower insulin release in response to the glucose challenge for Line 1 compared to Line 2 animals.

There is insufficient information to tell which of these mechanisms has the primary effect on insulin status - that is - does a change in peripheral insulin sensitivity through the cellular insulin receptor protein affect pancreatic insulin secretion or vice versa; or are there genetic differences in both mechanisms?

The reason for differences in leanness between the breeding Lines of animals (Francis *et al.*, 1990) is also unclear. It seems that the lean group utilize glucose in the peripheral tissues more rapidly so one could assume that the differences exist in the insulin receptor on muscle as opposed to adipose tissue, thereby partitioning nutrients into protein not fat deposition.

Work on the binding activity of isolated insulin receptors will continue to try to understand the basis for the measured differences in phenotype between the two Lines of sheep. Other research is looking for differences in the insulin receptor gene to explain these observations.

It is concluded that selecting animals on the basis of their glucose tolerance produces Lines of sheep with differences in the action of insulin on peripheral tissues as well as the release of insulin from the pancreas. These biochemical differences have an important role in partitioning nutrients for deposition of lean or fat tissue.

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