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THE EFFECT OF *IN UTERO* NUTRIENT RESTRICTION ON GLUCOSE AND INSULIN METABOLISM IN MERINO SHEEP

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SUMMARY

The aim of this experiment was to determine whether the magnitude of seasonal nutrient restrictions experienced by pregnant ewes in typical Australian Mediterranean farming conditions is sufficient to induce changes in glucose or insulin metabolism in the offspring at the age of eight months. Pregnant Merino ewes were fed to either maintain a body condition score of 3 throughout pregnancy or decrease condition score from 3 to 2 by the fourth month of pregnancy. Animals were then grazed at stocking rates calculated to provide either 2500 or 900 kg DM/ha until weaning, and grazed as one flock thereafter. Birth weight was not affected by pre-natal treatment, but liveweight at eight months of age was increased by the high post-natal plane of nutrition. There were no differences between post-natal treatments for any of the other variable studied. There were no effects of pre-natal treatment on the insulin or glucose response to a glucose challenge. Lambs born to ewes fed to lose condition score during pregnancy had lower basal glucose concentrations after the first day of insulin challenges and as a result showed a smaller glucose response to insulin administration at levels of 200, 300 and 500 mU/kg W. It was concluded that a level of nutrition comparable with that experienced by unsupplemented pregnant ewes on many typical Australian farms in the Mediterranean climate zone was sufficient to result in altered glucose metabolism in the offspring at the age of eight months.

Keywords: sheep, glucose, insulin, fetus, pregnancy, nutrition

INTRODUCTION

Since the initial epidemiological study of Barker & Osmond in 1986 which established a link between birth mass and coronary heart disease in humans, it has been established that nutrient restriction during fetal development can alter gene expression in the fetus and that these effects may persist for life (Gallaher *et al.*1995; Greenwood *et al.* 1999*;* Cherif *et al.* 2001). This phenomenon has been termed fetal programming and describes the process whereby a stimulus or insult at a critical period of development has long-term or lifelong effects (Barker 1999). Protein restriction during pregnancy in humans and rats results in changes to insulin sensitivity that can predispose the individual to diabetes as an adult (Ozanne and Holness 1999). Since cattle and sheep are usually pregnant during the period of lowest nutrient availability in some Australian farming systems, it is possible that fetal programming of the insulin axis also takes place in these species. Insulin sensitivity is of substantial practical and economic significance. Insulin regulates the use of fat reserves and muscle protein deposition during periods of low nutrient supply and directs nutrient partitioning during pregnancy and lactation. Oliver *et al.* (2001) have shown that maternal nutritional restriction of sheep during the periconceptual period changes the insulin response to glucose in late gestation fetal lambs. However, it is not known whether the magnitude of seasonal nutrient restriction under practical farming conditions is sufficient to induce fetal programming in the ovine, nor whether these effects persist after birth. This experiment was carried out under field conditions typical of those experienced by farmers in the Mediterranean environment of Australia. The aim of the experiment was to determine whether a decrease in the maternal plane of nutrition sufficient to induce a decrease in condition score from 3 to 2 by the end of pregnancy would change glucose or insulin metabolism in the offspring at the age of eight months.

MATERIAL AND METHODS

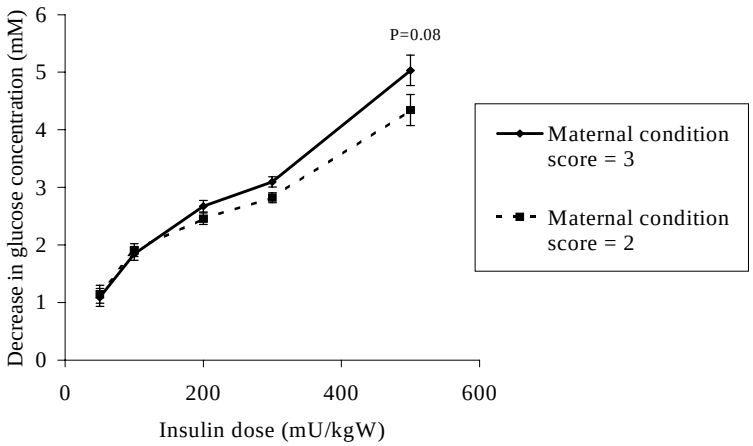
Pregnant Merino ewes were allocated to treatments designed to either maintain a body condition score of 3 throughout pregnancy or decrease condition score from 3 to 2 by the fourth month of pregnancy (high and low pre-natal treatments). They were randomly allocated to one of two different levels of nutrition from one month before lambing until weaning, *viz.* stocking rates calculated to provide either 2500 or 900 kg DM/ha (high and low post-natal treatments). All progeny were run together as one flock after weaning. A total of 24 lambs born as singletons were selected for this experiment, i.e. six

animals for each of the four combinations of pre and postnatal treatments. The lambs used in this experiment were selected from larger groups (n = 8-14) and represented those individuals that had the smallest deviation from the respective group means for body mass at 8 months of age. Insulin sensitivity was measured when the animals were 8 months of age and glucose tolerance was measured when they were 11 months old. Jugular catheters (Portex Ltd, Hythe, UK) were inserted on the day prior to the experiments, and patency mantained using heparin (25 IU/ml sterile saline solution). Body mass on the day prior to the experiments was used to calculate dosages for the insulin and glucose challenges. Insulin (Caninsulin; Intervet Pty Ltd, Australia) was administered as follows: 50 mU/kg bodymass (W) on the morning of day 1; 100 mU/kgW on the afternoon of day 1; 200 mU/kgW on the morning of day 2; 300 mU/kgW on the afternoon of day 2; 500 mU/kgW on the morning of day 3. Blood samples were collected into heparinised tubes immediately before insulin administration and at the following times thereafter (min): 20, 30, 40, 50, 60, 90, 120. Glucose was administered as a 50% (w/v) solution at a rate of 0.3 g/kgW. Blood samples were taken before and at 10, 20, 30, 40, 50, 60, 90, 120 and 180 min after the glucose injection. Blood samples were collected into tubes containing anti-coagulant (Na-fluoride + Na-oxalate for glucose analysis; Li-heparin for insulin analysis tubes). Tubes were stored in ice until centrifuged, after which plasma was aspirated and stored at –20 ^{o}C. Glucose concentrations were analyzed using an end-point enzymatic method with NADH detection (Infinity glucose reagent; Sigma Diagnostics, USA) and an automated analyzer (Cobas Mira, Roche Diagnostics, Switzerland). Quality control samples were included with each batch of analyses, and appropriate corrections applied in cases where the measured concentration differed from the actual concentration. Plasma concentrations of insulin were measured using the assay described by Adams *et al.* (1996). The intra-assay coefficients of variation for four pools with mean concentrations of 0.138, 0.638, 01.56 and 3.72 ng/ml were 2.0, 2.1, 2.0 and 1.5% respectively, and the corresponding inter-assay coefficients of variation were 9.0, 6.5, 2.0 and 5.0% respectively. The minimum detectable concentration was 0.12 ng/ml. Data were analysed statistically by repeated measures ANOVA, using the program Systat (Wilkinson 1998).

RESULTS

Pre-natal treatment had no effect (P>0.05) on birth mass (5.7 kg, s.e. 0.213). At 8 months of age, the mean body weight of animals subject to the 2 500 kg DM /ha post-natal treatment (35.3 kg, s.e. 0.538) was 14% higher (P<0.01) than that of animals subject to the 900 kg DM /ha treatment (31.0 kg, s.e. 0.538). Liveweight at 8 months of age was not affected by pre-natal treatment (P>0.05). Post-natal treatment had no effect on basal plasma glucose concentrations, glucose response to insulin injection or insulin response to glucose challenge (P>0.05). Pre-natal treatment did not affect the insulin response to glucose (P>0.05), but affected glucose response to the insulin challenge. A smaller response was observed in lambs born to ewes with a condition score of 2 during month four of pregnancy than in those born to ewes with a condition score of 3 (Figure 1) at insulin doses of 200 mU/kgW (P=0.08), 300 mU/kgW (P=0.05), 500 mU/kgW (P=0.08).

Figure 1. Effect of pre-natal maternal condition score and insulin dose on the maximum extent of insulin-induced depression of plasma glucose concentration in Merino lambs at eight months of age (basal concentration minus concentration at the point of maximum depression). Standard errors of means are indicated by error bars and significance of contrasts is shown above each pair of means.



The absolute concentration to which glucose concentrations were decreased did not differ between pre-natal treatments (P>0.05), but basal (pre-injection) glucose concentrations differed (Table 1) at times when differences in the extent of depression of glucose concentrations were observed i.e. at dose rates of 200, 300 and 500 mU/kgW.

Table 1. Effect of pre-natal maternal condition score on basal plasma glucose concentrations before insulin administration in Merino lambs at eight months of age

	Insulin dose (mU/kgW)				
	50	100	200	300	500
High	3.67	4.37	4.21	4.33	6.18
Low	3.82	4.36	3.88	4.09	5.43
s.e.	0.156	0.112	0.1	0.089	0.267
P =	ns	ns	0.03	0.07	0.06

There were no effects of either pre-natal or post-natal treatment on the insulin or glucose response to the glucose challenge (P>0.05).

DISCUSSION

Glucose response to insulin administration in eight-month old lambs was decreased by prenatal nutrient restriction. The effects on the extent to which glucose concentrations were depressed by exogenously administered insulin were due to lower basal (pre-injection) plasma glucose concentrations. While this is consistent with reports indicating the maternal undernutrition during various stages of pregnancy increases the insulin content of pancreatic cells and their secretory response to glucose in sheep (Oliver *et* al. 2001), we were unable to detect differences in basal insulin concentrations or insulin response to glucose challenge. The differences in basal glucose concentrations only became evident on day two of the first experiment after two insulin challenge tests had been conducted, and persisted thereafter, becoming progressively larger. This is unusual, as others have successfully applied an identical multiple-dose insulin challenge protocol in lambs (Bellver *et al.* 1995), and suggests that gluconeogenic ability was impaired in the animals subject to *in utero* nutrient restriction. Inhibition of hepatic gluconeogenic ability has been shown to occur in rats, in which *in utero* protein restriction resulted in a reduced response to glucagon (Ozanne *et al*. 1996).

While the reason for the differences in glucose concentration remain unclear, the most important finding of this research is that a level of nutrition comparable with that experienced by unsupplemented pregnant ewes on many typical farms in Australia's Mediterranean climatic zone was sufficient to result in altered glucose metabolism in the offspring at the age of eight months. These data also support the contention that maternal nutrition during pregnancy can alter physiological function in the offspring in the absence of an effect on birth mass. This calls for a re-evaluation of traditional methods of assessing nutritional requirements of the pregnant ruminant.

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