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Does ewe nutrition during pregnancy affect the metabolism of twin-born lambs?

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ABSTRACT

An insulin tolerance test was conducted on twin-born ewes born to dams offered sward heights of either 2 or 6 cm between day 64 and 132 of pregnancy in order to identify any alterations in the lamb's metabolism. Lambs were also categorised into groups according to birth weight (< 4.0 kg, light and > 4.0 kg, heavy). Changes in metabolism may have long term effects on growth and productivity. An insulin challenge (0.2 U/kg) was administered to ewe lambs at 8 months of age and their plasma insulin, glucose and cortisol responses were monitored. The plasma cortisol concentration of ewe lambs born to ewes offered 2cm sward height was significantly ($P < 0.05$) higher than ewe lambs born to ewes offered 6 cm sward height 50 minutes after the insulin challenge. No difference was found in the insulin or glucose response to the insulin challenge between dam sward height group (2 vs 6 cm). Birth weight had no effect on the insulin, glucose or cortisol response of the ewe lambs to the insulin challenge. These results show that both dam nutrition and lamb birth weight had little to no effect on the metabolic responses measured. These results suggest that lambs born to ewes offered a herbage sward height of only 2 cm or lambs with low birth weights may not show altered metabolism.

Keywords: lamb; insulin tolerance test; twins; ewe nutrition; metabolism.

INTRODUCTION

Currently in New Zealand there is a trend towards greater lambing percentages through an increase in the number of multiple births (Geenty, 1997). It is possible that twin and triplet lambs may be more susceptible to nutritional restriction during gestation than singletons (Geenty, 1997). Maternal undernutrition and impaired fetal growth have been linked with postnatal glucose intolerance, insulin resistance, hyperinsulinaemia and increased blood pressure in humans and sheep (Oliver *et al*., 2002; Kind *et al*., 2003). To date the effect of ewe nutrition during pregnancy on the metabolism of twin-born lambs has not been examined in a pasture based farming system.

The insulin tolerance test (ITT) has been used to examine differences in glucose homeostasis of singleton (Oliver *et al*., 2002) and twin lambs (Clarke *et al*., 2000). At 30 months of age, singleton lambs born to ewes underfed for 20 days in late pregnancy display higher plasma insulin concentrations than lambs born to ewes underfed for 10 days during the same period (Oliver *et al*., 2002). Clarke *et al*. (2000) reported that low birth weight twin lambs had a lower area under the insulin tolerance curve than high birth weight lambs indicating enhanced insulin tolerance at 1 year of age. Oliver *et al*. (2002) concluded that birth weight is more important than dam nutrition during pregnancy on postnatal glucose tolerance. These studies suggest that both dam nutrition during pregnancy and birth size can have long term effects on the metabolism of the offspring.

This study was designed to examine the effects of maternal nutrition and birth size on ewe lamb glucose and cortisol response to an ITT. The ewe lambs were born to ewes offered an average sward height of either 2 or 6 cm between day 64 and 132 of pregnancy.

METHODS AND MATERIALS

This study was conducted at Massey University's Keebles farm, 5 km south of Palmerston North, New Zealand. In the study by Morris and Kenyon (2004) twin- and triplet-born lambs born to ewes offered 2 cm sward heights were 0.3 kg lighter at birth than those born to ewes offered 6 cm. In addition the increase in total dam live weight was 7.3 kg vs 18.7 kg from day 64 to day 132 of pregnancy for ewes offered either 2 or 6 cm sward heights respectively. These results suggest that these multiple bearing ewes offered sward heights of 2 cm were undernourished during the mid- to late-pregnancy period in comparison with those offered 6 cm.

An ITT was conducted to determine the effect of herbage sward height on some aspects of metabolism of twin-born ewe lambs at 8 months of age. In addition the effect of birth weight irrespective of dam nutrition was examined. Lambs weighing less than 4 kg at birth were classified as 'light' or those greater than 4 kg as 'heavy'.

The day before the ITT each ewe lamb was weighed (range 48 to 57.5 kg) and an indwelling catheter was inserted into the jugular vein (for methods see Oliver *et al*., 2002). The ewe lambs were fasted overnight and given an intravenous injection of 8 U insulin (≈ 0.2 U/kg Humulin R, Eli Lilly, Indianapolis, Indiana, USA). Heparinised blood samples were

TABLE 1: The effect of sward height fed to dam (2 vs 6 cm) and ewe lamb birth weight (light or heavy) on the mean ± SEM (and untransformed mean) plasma insulin concentration (ug/ml) with time (0, 10, 20, 30, 40, 50, 60 and 120 minutes after insulin challenge).

		Time from insulin challenge (minutes)							
	n	0	10	20	30	40	50	60	120
Dam sward surface height									
2	14	0.10[1]±0.15 (0.10[2])	3.63±0.33 (169.8)	1.97±0.12 (6.5)	1.20±0.12 (2.5)	0.70±0.05 (1.0)	0.42±0.13 (0.6)	0.30±0.02 (0.4)	0.12±0.02 (0.13)
6	11	0.10±0.17 (0.11)	3.37±0.37 (49.8)	1.98±0.13 (7.5)	1.32±0.14 (3.8)	0.67±0.05 (1.0)	0.57±0.15 (1.5)	0.25±0.02 (0.3)	0.12±0.02 (0.13)
Birth weight group									
light	12	0.12±0.16 (0.13)	3.45±0.34 (79.2)	1.97±0.12 (7.3)	1.28±0.13 (3.5)	0.68±0.05 (1.0)	0.54±0.13 (1.4)	0.27±0.02 (0.3)	0.11±0.02 (0.12)
heavy	13	0.08±0.16 (0.09)	3.55±0.37 (140.3)	1.98±0.13 (6.7)	1.25±0.13 (2.8)	0.70±0.05 (1.0)	0.44±0.14 (0.7)	0.28±0.02 (0.3)	0.13±0.02 (0.14)

1 Mean of natural log of insulin (+1) concentration

2 Un-transformed insulin mean

TABLE 2: The effect of sward height fed to dam (2 vs 6 cm) and ewe lamb birth weight (light or heavy) on mean ± SEM (and untransformed mean) plasma glucose concentration (mM) with time (0, 10, 20, 30, 40, 50, 60 and 120 minutes after insulin challenge).

		Time from insulin challenge (minutes)							
	n	0	10	20	30	40	50	60	120
Dam sward surface height									
2	14	1.57[1]±0.04 (3.8[2])	1.44±0.04 (3.2)	1.23±0.05 (2.4)	1.06±0.04 (1.9)	1.01±0.04 (1.8)	1.03±0.04 (1.8)	1.06±0.04 (1.9)	1.40±0.04 (3.1)
6	11	1.61±0.04 (4.1)	1.50±0.05 (3.6)	1.30±0.05 (2.7)	1.16±0.05 (2.3)	1.10±0.04 (2.0)	1.09±0.04 (2.0)	1.13±0.04 (2.1)	1.36±0.04 (2.9)
Birth weight group									
light	12	1.62±0.04 (3.8)	1.48±0.04 (3.3)	1.28±0.05 (2.5)	1.12±0.05 (2.0)	1.08±0.04 (1.8)	1.09±0.04 (1.8)	1.10±0.04 (2.0)	1.40±0.04 (2.9)
heavy	13	1.57±0.04 (4.1)	1.46±0.04 (2.7)	1.25±0.05 (2.7)	1.10±0.05 (2.1)	1.03±0.04 (2.0)	1.03±0.04 (2.0)	1.09±0.04 (2.0)	1.36±0.04 (3.1)

1 Mean of natural log of glucose (+1) concentration

2 Un-transformed glucose mean

TABLE 3: The effect of sward height fed to dam (2 vs 6 cm) and ewe lamb birth weight (light or heavy) on mean ± SEM (and untransformed mean) plasma cortisol concentration (mM) with time (0, 10, 20, 30, 40, 50, 60 and 120 minutes after insulin challenge). Means within treatments with different superscripts are significantly different (P < 0.05).

		Time from insulin challenge (minutes)							
	n	0	10	20	30	40	50	60	120
Dam sward surface height									
2	14	11.89[1]±0.43 (147.8[2])	10.49±0.68 (117.3)	8.69±0.53 (80.5)	10.06±0.49 (104.2)	14.29±0.35 (205.8)	15.85[b]±0.37 (252.4)	16.34±0.46 (268.4)	10.92±0.68 (123.6)
6	11	11.65±0.50 (143.8)	10.11±0.78 (106.4)	8.22±0.61 (76.5)	9.91±0.56 (101.6)	13.40±0.40 (181.1)	14.57[a]±0.42 (214.4)	15.33±0.53 (239.2)	10.15±0.77 (110.5)
Birth weight group									
light	12	12.27±0.48 (160.7)	10.76±0.75 (121.8)	8.45±0.60 (80.8)	9.48±0.54 (93.7)	13.45±0.39 (183.0)	15.16±0.41 (232.8)	15.91±0.51 (257.4)	10.80±0.75 (123.4)
heavy	13	11.28±0.44 (130.9)	9.84±0.70 (102.0)	8.45±0.54 (76.3)	10.48±0.51 (112.0)	14.23±0.37 (203.9)	15.25±0.38 (234.0)	15.76±0.48 (250.3)	10.27±0.70 (110.7)
Dam sward surface height X Birth weight group									
2 x light	8	13.20[b]±0.56 (177.4)							
2 x heavy	6	10.57[a]±0.64 (113.3)							
6 x light	4	11.33[ab]±0.79 (129.3)							
6 x heavy	7	12.0[ab]±0.60 (145.7)							

1 Mean of square root of cortisol concentration

2 Un-transformed cortisol mean

collected at 0, 10, 20, 30, 40, 50, 60 and 120 minutes after i.v. insulin injection. Blood samples were placed on ice and, once chilled, centrifuged for 10 minutes at 4000 rpm. The resulting plasma was harvested and stored frozen at -20 °C.

Samples were analysed for insulin, glucose and cortisol. Plasma glucose concentration was analysed using standard enzymatic micro-methods modified for 96-well micro-plates (Kunst *et al*., 1984; Ashour *et al*., 1987). Plasma insulin concentration was measured by radioimmunoassay (RIA) (Oliver *et al*., 1993). Plasma cortisol concentration was measured by RIA using diagnostic kits (Clinical Assays GammaCoat Cortisol ^{125}I RIA Kit, DiaSorin Inc., Minnesota, USA).

Statistical analysis

Plasma concentrations of insulin, glucose and cortisol were not normally distributed. The natural log of 1 + insulin and 1 + glucose concentration and the square root of cortisol were calculated. The effect of sward height (2 cm vs 6 cm) and the birth weight group (light vs heavy) were tested using the analysis of variance for repeated measures (SPSS, 2003). Individual time points were tested using generalised linear model (GLM, Minitab, 2003).

RESULTS

The insulin challenge produced an increase in plasma insulin concentration at 10 minutes which then returned to baseline levels by 20 minutes (Table 1). The insulin challenge failed to produce a significant difference in the plasma insulin concentrations between the ewe lambs of ewes offered either 2 or 6 cm sward heights over the sampling period or between light and heavy birth weight lambs.

Glucose concentrations in plasma decreased steadily after the insulin challenge for 50 minutes and began to rise from 60 to 120 minutes (Table 2). There were no significant differences in the plasma glucose concentrations at any time after the insulin challenge between the progeny of ewes offered either 2 or 6 cm sward heights or between light and heavy birth weight ewe lambs.

Plasma cortisol concentration decreased for the first 20 minutes after the insulin challenge and then increased until 60 minutes post challenge. It then returned to baseline levels at 120 minutes (Table 3). There was a significant ($P < 0.05$) dam sward height group x birth weight group interaction of the baseline cortisol concentration. Light birth weight lambs born to ewes offered 2 cm sward heights had a higher cortisol concentration than their heavy counterparts. However this relationship was not observed in lambs born to ewes offered 6 cm sward heights. Progeny born to ewes fed 2 cm sward heights had a significantly ($P < 0.05$) higher cortisol concentration at 50 minutes after the insulin challenge than the progeny born to ewes fed 6 cm sward heights.

DISCUSSION

This study was conducted to investigate the effects of dam nutrition during mid- to late-pregnancy on the twin-born ewe progenys' metabolic response to an ITT. Maternal undernutrition during pregnancy has been linked to impaired fetal growth and postnatal metabolism (Oliver *et al*., 2002). The ITT provides information on the ewe lambs' sensitivity to insulin in terms of the degree and rate of glucose depletion and stimulation of the hypothalamic-pituitary-adrenal axis. Therefore an ITT will provide some information on the potential long-term effects of maternal undernutrition on lamb productivity.

In general, dam nutritional treatment had no effect on the metabolic response of the female progeny at 8 months of age except for an effect on plasma cortisol concentration at one time point, such that ewes born to dams offered 2 cm sward heights displayed a greater cortisol response at 50 minutes after the insulin challenge. This suggests that dam nutrition in mid- to late-pregnancy has little effect on cortisol production in the resulting progeny.

Birth weight (light vs heavy) had no effect on the insulin, glucose and cortisol response to an ITT. This finding is contrary to Clarke *et al*. (2000) who found a significant difference between light and heavy lambs. However, Clarke *et al*. (2000) compared the insulin tolerance within sets of twins whose birth weights differed by more than 25%. In this trial the birth weights of unrelated twin lambs classified as light and heavy differed by 45%. The mean weights of both the light and heavy groups were lower than those reported by Clarke *et al*. (2000). In addition, Clarke *et al*. (2000) did not have a ewe nutritional treatment thereby examining the effect of in utero nutrition on postnatal metabolism. Therefore these trials can not be compared due to differences in trial design.

There was no difference in plasma insulin concentration at any time after insulin challenge between dam nutrition or birth weight groups. In contrast Oliver *et al*. (2002) found plasma insulin concentrations were higher in lambs born to dams severely underfed for 20 days in late pregnancy which also increased with increasing birth weight. However Oliver *et al*. (2002) employed severe underfeeding (underfed = 0.3 MJ/day vs ad libitum = 15 MJ/day) for a short period while in this trial a longer period (70 days) of moderate underfeeding was utilised. Therefore these studies can not be directly compared.

CONCLUSIONS

These data suggest that maternal undernutrition in mid- to late-pregnancy and birth weight of twin-born ewes may only have a minor affect on their offspring's subsequent long-term metabolism. In biological terms these differences are small and may have no effect on the growth potential of the progeny.

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