Journal of Developmental Origins of Health and Disease (2010), **1**(1), 50–59.

doi:10.1017/S2040174409990201

ORIGINAL ARTICLE

Relationships between early postnatal growth and metabolic function of 16-month-old female offspring born to ewes exposed to different environments during pregnancy

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It was hypothesized that exposure of the fetus to adverse conditions *in utero* due to either maternal constraint or nutrition may result in developmental adaptations altering metabolism and postnatal growth of the offspring. Heavy (H) and light (L) Romney dams (G_0) were allocated to *ad libitum* (A) or maintenance (M) nutritional regimens, from day 21–day 140 of pregnancy. Female twin-born offspring (G_1) born to the dams in the four treatment groups will be referred to as HA-ewes, LA-ewes, HM-ewes and LM-ewes. At 16 months of age, offspring were catheterized and given intravenous insulin tolerance test (ITT), glucose tolerance test (GTT) and epinephrine tolerance test challenges to assess their glucose and fat metabolism in relation to their birth weight and postnatal growth. In HA-ewes, the regression coefficients of growth rates prior to puberty on insulin and glucose curves in response to GTT ($InsAUC_{GTT}$) and ITT ($GluAUC_{ITT}$), respectively, were different from 0 ($P < 0.05$) and were different from the regression coefficients of HM-ewes. This may indicate that HA-ewes may have showed puberty-related insulin resistance at 16 months of age with increasing growth rates prior to puberty compared to HM- or LM-ewes. In HM-ewes, the regression coefficients of growth rates after puberty on $InsAUC_{GTT}$ and $GluAUC_{ITT}$ were different from 0 ($P < 0.05$) and were different from those of HA-ewes. These results may indicate that offspring born to heavy dams fed maintenance during pregnancy and with greater postnatal growth rates after puberty could develop glucose intolerance and insulin resistance in later life.

Received 10 August 2009; Revised 15 October 2009; Accepted 25 November 2009; First published online 22 December 2009

Key words: glucose intolerance, growth, maternal nutrition, maternal progeny, sheep, size.

Introduction

There is increasing support in the literature for the concept that exposure of the fetus to adverse conditions *in utero* may result in developmental adaptations that permanently change the structure, physiology, metabolism and postnatal growth of the offspring.[1] Altered maternal nutrient intake in sheep resulted in offspring with glucose intolerance,[2–4] altered hypothalamus-pituitary-adrenal-axis function,[5–7] increased adiposity[3,4] and altered postnatal growth,[4,8] Dam size could affect fetal growth through the size of the placenta, which influences the nutrient supply for the developing fetus.[9] Embryo transfer and cross-breeding experiments in large and small breeds of sheep,[10,11] horses[12,13] and pigs[14] have shown that fetal growth can be altered from the normal genetic potential by differing dam size.

In addition to the *in utero* environment affecting the offspring's metabolic function in later life, the postnatal growth trajectory has been found to play an important role in the development of metabolic dysfunction.[15,16] For example, postnatal 'catch-up' growth is associated with the development of glucose intolerance in adult life,[17,18] cardiovascular disease[15] and reduced longevity.[19]

Our previous work has shown that maintenance nutrition of the ewe during pregnancy altered the bone mineral content/lean mass ratio of the fetal hindquarters when compared to *ad libitum* feeding, irrespective of dam size.[20] Furthermore, we have shown that dam nutrition affected birth weight in twin-born lambs[21] and that dam nutrition and dam size during pregnancy affected postnatal growth of the female offspring.[22] Therefore, we examined, and report in this study, the relationship between birth weight and early postnatal growth and metabolic function of 16-month-old female offspring born to dams differing in size and diet during pregnancy. We hypothesize that lower birth weight and greater postnatal growth rates until 1 year of age in female offspring born to light dams which were fed maintenance during pregnancy, would negatively affect their metabolic function at 16 months of age. In addition, we hypothesize that the pre-existing maternal body size (heavy *v.* light) would exacerbate

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or reduce the effects of maternal nutrition during pregnancy on the metabolic function of 16-month-old offspring.

Materials and Methods

The study was conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand. The study and all animal-handling procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Dams (G_0)

Approximately 450 heavy (H) (60.8 kg ± 0.18) and 450 light (L) (42.5 kg ± 0.17) Romney dams (G_0) were selected from the extremes in a commercial flock of 2900 ewes, on the basis of size, as determined by live weight, and bred using artificial insemination as previously described by Kenyon *et al.*[21] From day 21 until day 140 post-insemination, the dams were randomly allocated, within size, to *ad libitum* (A) or maintenance (M) nutritional regimens under New Zealand pastoral grazing conditions. The aim of the M nutritional regimen was to ensure that total ewe live weight during pregnancy increased at a level similar to that of the expected conceptus mass. The aim of the A nutritional regimen was to provide dams with unrestricted food intake and, hence, no nutritional restriction to maternal or fetal growth and development (resulting in 78.4 kg ± 0.37 *v.* 65.0 ± 0.35; $P < 0.05$; for H- and M-dams at day 140).[21] Pasture herbage was the only nutritional source and the average pre- and post-grazing pasture covers during the period day 21–day 140 were 1330 ± 140.0 and 804.0 ± 133.4 kg of dry matter per hectare (kg DM/ha), respectively, for the M-feeding regimen and 2304.0 ± 156.8 and 1723.3 ± 149.7 kg DM/ha for the A-feeding regimen.[21] From day 140 of pregnancy through to weaning, all dams and their lambs (G_1) were provided with *ad libitum* feeding. Singleton- and twin-born lambs (female and male lambs combined) born to H-dams ($n = 282$) were heavier at birth (5.51 kg ± 0.05 *v.* 5.37 kg ± 0.05; $P < 0.05$; for lambs born to H- and L-dams, respectively) and weaning (32.7 kg ± 0.36 *v.* 31.2 kg ± 0.33; $P < 0.05$; for lambs born to H- and L-dams, respectively) than lambs born to L-dams ($n = 217$). Twin-born offspring (female and male lambs combined) born to A-dams ($n = 237$) were heavier at birth (5.23 kg ± 0.06 *v.* 4.52 kg ± 0.06; $P < 0.05$; for twin-born lambs born to A- and M-dams) and weaning (30.6 kg ± 0.42 *v.* 28.2 ± 0.41; $P < 0.05$; for twin-born lambs born to A- and M-dams) than lambs born to M-dams ($n = 262$).[21] After weaning, the female offspring (G_1) were managed and fed to nutritional requirements as one group under New Zealand commercial farming practice[22] and the male offspring were slaughtered to obtain carcass information (to be reported elsewhere). The study, therefore, utilized a two by two factorial design: two dam-nutrition treatments (M *v.* A) and two dam-size treatments (H *v.* L). The term dam is used to refer to the G_0 generation of heavy and light ewes that underwent the nutritional treatment during pregnancy. The ewe offspring (G_1) born to the heavy or light dams fed either maintenance or *ad libitum*, will be referred to as HA-, HM-, LA-, or LM-ewes, respectively.

Ewe offspring (G_1)

At 16 months of age, 48 randomly selected twin-born ewe offspring (G_1) were housed indoors in two, random, consecutive batches of 24 ewes ($n = 12$ ewes born to the HA-, HM-, LA- and LM-dam treatment groups, as described above).[21] Each group of 12 ewes (G_1) contained eight ewes from female-female twin sets, born to four dams (G_0), and four ewes from female-male twin sets, born to four dams (G_0); birth weight differences within the twin pairs were <25%.

The selected ewes were housed in a large shed, as one batch for 1 week, followed by housing in individual pens for 2 weeks prior to the metabolic challenges. Ewes had free access to water and were fed to achieve an average liveweight gain of 100 g/day (19 MJ ME/day).[23] The feed was a mixture of pelleted food (500 g of 12 MJ ME/kg) and lucerne chaff (1500 g of 8.6 MJ ME/kg) (average ewe live weight prior to housing was 50 kg (± 4.4 S.D.)). Ewes were fed daily between 1 and 2 pm; feed intake (offered less refusals) was recorded at 8 am each day.

Three days prior to the start of the metabolic challenges, both jugular veins were catheterized with indwelling through the needle (12 g) polyvinyl catheters after administration of local anesthetic (Lopaine, Lignocaine Hydrochloride USP. 20 mg/ml, Ethical Agents Ltd, Auckland, New Zealand); catheters were secured to the neck with tape and secured on the animal's back under a meshed stocking. This was followed by single prophylactic intramuscular (hind leg) administration of antibiotics (Duplocillin® LA, Intervet Ltd, Newmarket, Auckland, 2 ml per 50 kg live weight). One catheter was used for hormone/metabolite administration and the other for blood collection.

After an overnight fast (food was removed between 6 and 7 PM the evening prior to the challenge), ewes were submitted to an insulin tolerance test (ITT) on day 1 (0.15 IU/kg live weight, Humulin R, Eli Lilly, Indianapolis, IN), a glucose tolerance test (GTT) on day 2 (0.17 g/kg live weight, Dextrose 40%, Bomac Laboratories LTD, Auckland, New Zealand), and an epinephrine tolerance test (ETT) on day 3 (1 μg/kg live weight, Sigma-Aldrich Inc. St Louis, MO, USA), between 8 and 9 am. Blood samples were collected in vacutainers containing ethylenediaminetetraacetic acid (BD Vacutainer Systems, UK) (5 ml) at −5, 0, 2, 10, 20, 30, 40, 50, 60 and 120 min from the insulin administration, at −5, 0, 2, 5, 10, 20, 30, 40, 50, 60, 120 min from the glucose administration and at −5, 0, 2, 5, 7, 10, 20, 45 and 60 min from the epinephrine administration. On all 3 days, ewes were re-fed after completion of the sampling. All blood samples were immediately placed on ice until centrifugation

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at 3000 rpm (1006 ***g***) for 15 min. Triplicate plasma aliquots were stored at −20°C until analysis.

Assays

Plasma metabolite concentrations were measured using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) using commercial kits for glucose and cholesterol (Roche, Mannheim, Germany) and non-esterified free fatty acids (NEFAs) and triglyceride (Randox Laboratories Ltd, Ardmore, Crumlin, UK). Insulin was measured by radioimmunoassay (RIA) with ovine insulin as the standard (Sigma, batch no. I9254).[24] The minimal detectable concentration was 0.03 ng/ml; inter- and intra-assay coefficients of variation were 14.3% and 11.5%, respectively.

Plasma cortisol concentrations were measured using mass spectrometry.[25] The internal standard was cortisol-d2. A 100 μl volume of internal standard (20 ng/ml in water) was added to 200 μl plasma. Steroids were extracted using 1 ml ethyl acetate. After removal of the organic supernatant, samples were dried, re-suspended in 100 μl mobile phase (80% methanol and 20% water), and transferred to high performance liquid chromatography (HPLC) injector vials. A 25 μl volume was injected onto an HPLC mass spectrometer system consisting of a Surveyor MS pump and autosampler followed by an Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple-quadrapole mass spectrometer all controlled by Finnigan Xcaliber software (Thermo Electron Corporation, San Jose, CA). The mobile phase was isocratic, flowing at 600 μl/min through a Luna 3 μ C18 (2) 100A 250 × 4.6 column at 40°C (Phenomenex, Auckland, New Zealand). Retention time was 5.9 min. Ionization was in positive mode, and Q2 had 1.2 mTorr of argon for the steroid. The mass transitions, for internal standard and steroid, respectively, were as follows: cortisol-d2, 365.3–122.2 at 28 V, and cortisol, 363.3–121.2 at 28 V. Mean inter- and intra-assay coefficient of variation values were 11.1% and 10.6%, respectively.

Metabolic variables of the offspring (G_1)

Area under the curves for all variables included the area under the baseline.

GTT

Glucose tolerance was measured as the area under the glucose curve ($GluAUC_{GTT}$) and absolute insulin secretion as the area under the plasma insulin curve ($InsAUC_{GTT}$) during the 120 min after the glucose administration.

ITT

Insulin resistance was measured as the area under the glucose curve ($GluAUC_{ITT}$) and absolute cortisol secretion was measured as the area under the plasma cortisol curve ($CortAUC_{ITT}$) during the 120 min after the insulin administration.

ETT

Absolute glucose ($GluAUC_{ETT}$), insulin ($InsAUC_{ETT}$), NEFA ($NefaAUC_{ETT}$), triglycerides ($TrigAUC_{ETT}$) and cholesterol ($CholAUC_{ETT}$) secretion were measured as the area under the curves during the first 20 min after epinephrine administration. Areas under the curves during the first 20 min were used as area under the curves during 60 min showed no relationship with birth weight or growth rates.

Birth weight and growth of the ewe offspring (G_1)

The average day of birth of the lambs (G_1) was 28 August 2005 and the lambs were weighed within 24 h after birth as previously described by Kenyon *et al.*[21] After weaning at the average age of 100 days, the ewe lambs (G_1) were weighed monthly until 1 year of age, as previously described by van der Linden *et al.*[22] Growth rates of the ewe lambs (G_1) were calculated for four periods: $Growth_{wean}$: growth rates from birth to weaning (4 months of age); $Growth_{postwean}$: growth rates post weaning (4–7 months of age); $Growth_{prepub}$: growth rate prior to onset of puberty (7–9 months of age); $Growth_{postpub}$: growth rates post puberty (9–12 months of age).

Statistical analysis

Birth weight and growth rates of the ewe lambs (G_1) were analysed using the MIXED procedure[26] with a linear model that included the fixed effects of dam (G_0) nutrition, dam (G_0) size, the interaction dam (G_0) nutrition by dam (G_0) size and the random effect of batch. Data are presented as least square means and their standard error (±S.E.). Area under the curves were analysed using the same mixed linear model as stated above including the fixed effects of dam nutrition, dam size, and the interaction of dam nutrition by dam size and the random effect of batch. Metabolic variables are presented as least square means and their standard error (±S.E.).

Birth weight and the four growth periods ($Growth_{wean}$, $Growth_{postwean}$, $Growth_{prepub}$ and $Growth_{postpub}$) of the ewe offspring (G_1) were regressed on their metabolic variables at 16 months of age (glucose metabolism: $GluAUC_{GTT}$, $InsAUC_{GTT}$ and $GluAUC_{ITT}$; adrenal function: $CortAUC_{ITT}$; fat metabolism: $GluAUC_{ETT}$, $InsAUC_{ETT}$, $NefaAUC_{ETT}$, $TrigAUC_{ETT}$ and $CholAUC_{ETT}$) for each of the dam (G_0) treatment interaction (dam nutrition by dam size; HM; HA; LM; LA) with the following linear regression model:

$$y_{klm} = \beta_{0k} + \beta_{1k}x_{kl} + \mathrm{M}_m + e_{klm}$$

where y_{klm} is the metabolic variable measured on ewe (G_1) l from dam (G_0) treatment interaction k in batch m, β_{0k} and β_{1k} are regression coefficients describing the regression line in

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Table 1. *The effects of heavy (H) or light (L) dams (G_0) fed ad libitum (A) or maintenance (M) during pregnancy on BW (kg), growth from birth to weaning ($Growth_{wean}$: g/day), growth from weaning 7 months of age ($Growth_{postwean}$: g/day), growth from 7–9 months of age ($Growth_{prepub}$: g/day) and growth from 9–12 months of age ($Growth_{postpub}$: g/day) and glucose-metabolism variables at 16 months of age ($GluAUC_{GTT}$ and $InsAUC_{GTT}$: glucose AUC and insulin AUC in response to GTT, respectively; $GluAUC_{ITT}$: glucose AUC in response to ITT) and fat-metabolism variables at 16 months of age ($InsAUC_{ETT\ 0–20}$ and $Nefa\ AUC_{ETT\ 0–20}$: insulin AUC and NEFA AUC in response to ETT, respectively) of ewe offspring (G_1). Table shows least square means ± s.e.*

	Variables*									
Dam treatment	BW (kg)	$Growth_{wean}$ (g/day)	$Growth_{postwean}$ (g/day)	$Growth_{prepub}$ (g/day)	$Growth_{postpub}$ (g/day)	$GluAUC_{GTT}$ (mmol/min/l)	$InsAUC_{GTT}$ (ng min/ml)	$GluAUC_{ITT}$ (mmol/min/l)	$InsAUC_{ETT}$ (ng min/ml)	$Nefa\ AUC_{ETT}$ (mmol/min/l)
HA ($n = 12$)	4.8 ± 0.19	250^{d} ± 10.0	74 ± 5.9	53^{cd} ± 9.2	44 ± 6.5	553.4 ± 9.46	63.5 ± 5.64	334.9 ± 7.81	4.8^{a} ± 0.70	3.7 ± 0.47
LA ($n = 12$)	4.7 ± 0.19	244^{cd} ± 10.0	74 ± 5.9	45^{c} ± 9.2	51 ± 6.5	568.4 ± 9.34	55.2 ± 5.89	325.5 ± 7.74	5.9^{ab} ± 0.69	3.4 ± 0.49
HM ($n = 12$)	4.9 ± 0.19	236^{cd} ± 10.0	82 ± 5.9	43^{c} ± 9.2	50 ± 6.5	548.9 ± 9.30	57.7 ± 6.18	322.8 ± 7.71	6.7^{ab} ± 0.68	3.4 ± 0.47
LM ($n = 12$)	4.5 ± 0.19	224^{c} ± 10.0	70 ± 5.9	68^{d} ± 9.2	41 ± 6.5	565.9 ± 9.73	65.5 ± 6.18	338.0 ± 8.32	6.8^{b} ± 0.69	4.2 ± 0.47

BW, birth weight; GTT, glucose tolerance test; ITT, insulin tolerance test; ETT, epinephrine tolerance test.
[ab] Significantly different ($P < 0.05$) between dam treatments and within variables;
[cd] Superscripts tend to be different ($P < 0.10$) between dam treatments and within variables.
*Interaction of dam size by maternal nutrition was not significant ($P > 0.10$) for all variables.

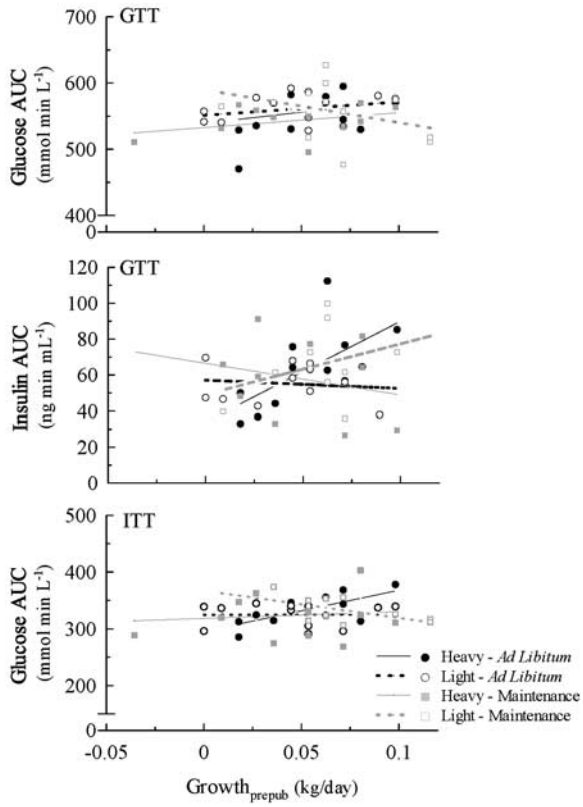

Fig. 1. Linear regressions of pre-puberty growth rates ($Growth_{prepub}$: 7–9 months of age) on glucose-metabolism variables at 16 months of age ($GluAUC_{GTT}$: glucose AUC and $InsAUC_{GTT}$: insulin AUC in response to glucose tolerance test (GTT); $GluAUC_{ITT}$: glucose AUC in response to insulin tolerance test (ITT)) of ewes (G_1) born to heavy or light dams (G_0) fed either maintenance or *ad libitum* during pregnancy. Black solid line and ● heavy – *ad libitum*; black dotted line and ○ light – *ad libitum*; grey solid line and ■ heavy – maintenance; grey dotted line and □ light – maintenance.

dam (G_0) treatment interaction k, M_m is the random effect of batch m and e_{klm} is the residual error corresponding to the observation y_{klm}.

Multiple comparisons were performed and therefore $\alpha(0.05)$ was corrected using the Bonferroni correction for multiple tests[27]:

$$\alpha_{corr} = \alpha \Big/ \sum_{i=1}^{k} \left(\frac{1}{i}\right)$$

Associations were significant at $\alpha_{corr} = 0.02$ and considered a trend $\alpha_{corr} = 0.05$.

Thus, if a relationship within a group is significant, it is represented in a regression coefficient (β_1) that is significantly different from 0. If a relationship within a group is not significant, it is represented in a regression coefficient (β_1) that is not significantly different from 0 (regression line is horizontal).

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Table 2. *Linear regression equations* of pre-puberty growth rates ($Growth_{prepub}$; 7–9 months of age; kg/day) on glucose-metabolism variables at 16 months of age ($GluAUC_{GTT}$: glucose AUC and $InsAUC_{GTT}$: insulin AUC in response to GTT; $GluAUC_{ITT}$: glucose AUC in response to ITT) of ewes (G_1) born to heavy (H) or light (L) dams (G_0) fed either ad libitum (A) or maintenance (M) during pregnancy*

		Independent variable				
Dam treatment	Dependent variable	Intercept (β_0)	*P* value (β_0)	$Growth_{prepub}$ (β_1)	*P* value (β_1)	R^2
HA	$GluAUC_{GTT}$ (mmol/min/l)	539 (±23.2)	0.0001	336 (±383.4)	NS	0.43
LA		551 (±15.4)	0.0001	202 (±276.2)	NS	0.23
HM		533 (±13.9)	0.0001	225 (±239.5)	NS	0.16
LM		590 (±21.2)	0.0001	−496 (±286.4)	0.09	0.19
HA	$InsAUC_{GTT}$ (ng/min/ml)	34 (±12.6)	0.0001	560 (±216.8)[b]	0.01	0.43
LA		57 (±9.9)	0.0001	−46 (±202.0)[ab]	NS	0.03
HM		66 (±11.5)	0.0001	−176 (±198.6)[a]	NS	0.08
LM		49 (±16.3)	0.0001	282 (±261.5)[ab]	NS	0.11
HA	$GluAUC_{ITT}$ (mmol/min/l)	295 (±18.9)	0.0001	725 (±324.8)[b]	0.03	0.51
LA		325 (±14.2)	0.0001	9 (±259.0)[ab]	NS	0.09
HM		318 (±12.5)	0.0001	118 (±222.5)[ab]	NS	0.01
LM		367 (±25.8)	0.0001	−478 (±334.6)[a]	NS	0.38

NS, non significant.
[ab] Significantly different ($P < 0.05$; using Bonferroni correction) between dam treatments and within dependent metabolic variable.
*All regression equation models are significant ($P < 0.01$).

Results

Birth weight, growth rates and metabolic variables of the ewe offspring (G_1)

No dam-nutrition or dam-size effects ($P > 0.10$) were found on birth weight, $Growth_{postwean}$ or $Growth_{postpub}$ of the ewe offspring (Table 1). Growth rates from birth to weaning ($Growth_{wean}$) tended ($P < 0.10$) to be greater in HA-offspring compared to LM-offspring. However, growth rates prior to puberty ($Growth_{prepub}$) tended ($P < 0.10$) to be greater in LM-offspring than in HM- and LA-offspring.

No dam nutrition or dam size effects ($P > 0.10$) were found in area under the glucose ($GluAUC_{GTT}$) and insulin ($InsAUC_{GTT}$) curves in response to the GTT, area under the glucose curve ($GluAUC_{ITT}$) in response to the ITT or area under the NEFA curve ($NefaAUC_{ETT}$) in response to the ETT at 16 months of age (Table 1). Offspring born to LM-dams had a greater ($P < 0.04$) area under the insulin ($InsAUC_{ETT}$) curve in response to ETT than HA-offspring. In addition, HM-offspring tended ($P < 0.10$) to have greater $InsAUC_{ETT}$ than HA-offspring.

Glucose metabolism of the ewe offspring (G_1)

Birth weight, $Growth_{wean}$ and $Growth_{postwean}$ were not related to $GluAUC_{GTT}$, $InsAUC_{GTT}$ and $GluAUC_{ITT}$ in response to the GTT and ITT, respectively, of ewe offspring at 16 months of age (data not shown).

In the period before puberty, the regression coefficient (β_1) of $Growth_{prepub}$ on $InsAUC_{GTT}$ of HA-ewes was significantly ($P = 0.01$) different from 0, indicating that HA-ewes had increased $InsAUC_{GTT}$ with increasing growth rates prior to puberty (Fig. 1 and Table 2). This regression coefficient of $Growth_{prepub}$ on $InsAUC_{GTT}$ of HA-ewes was significantly different ($P = 0.02$) from that of HM-ewes and tended ($P = 0.05$) to be different from that of LA-ewes. This indicates that HA-ewes had greater $InsAUC_{GTT}$ with increasing growth rates prior to puberty than did HM- or LA-ewes.

Thus, for example, if HA- and HM-ewes had growth rates of 0 kg/day prior to puberty, HA-ewes would have a predicted $InsAUC_{GTT}$ of 34 ng min/l ($34 + 560 \times 0$; Table 2) and HM-ewes would have an predicted $InsAUC_{GTT}$ of 66 ng min/l ($66 \pm 176 \times 0$; Table 2). However, if HA- and HM-ewes had growth rates of 0.1 kg/day prior to puberty, HA-ewes would have a predicted $InsAUC_{GTT}$ of 90.0 ng min/l ($34 + 560 \pm 0.1$) and HM-ewes would have an predicted $InsAUC_{GTT}$ of 48.4 ng min/l ($66 \pm 176 \times 0.1$).

The regression coefficient of $Growth_{prepub}$ on $GluAUC_{ITT}$ of HA-ewes was significantly ($P = 0.03$) different from 0, indicating that HA-ewes had increased $GluAUC_{ITT}$ with increasing growth rates prior to puberty. This regression coefficient of $Growth_{prepub}$ on $GluAUC_{ITT}$ of HA-ewes was significantly different ($P = 0.01$) from that of LM-ewes, indicating that HA-ewes had greater $GluAUC_{ITT}$ with increasing growth rates prior to puberty than did LM-ewes.

In the period after puberty, the regression coefficient of $Growth_{postpub}$ on $GluAUC_{GTT}$ of HA-ewes was significantly ($P = 0.03$) different from 0, indicating that HA-ewes had decreased $GluAUC_{GTT}$ with increasing growth rates after puberty (Fig. 2 and Table 3). In addition, the regression

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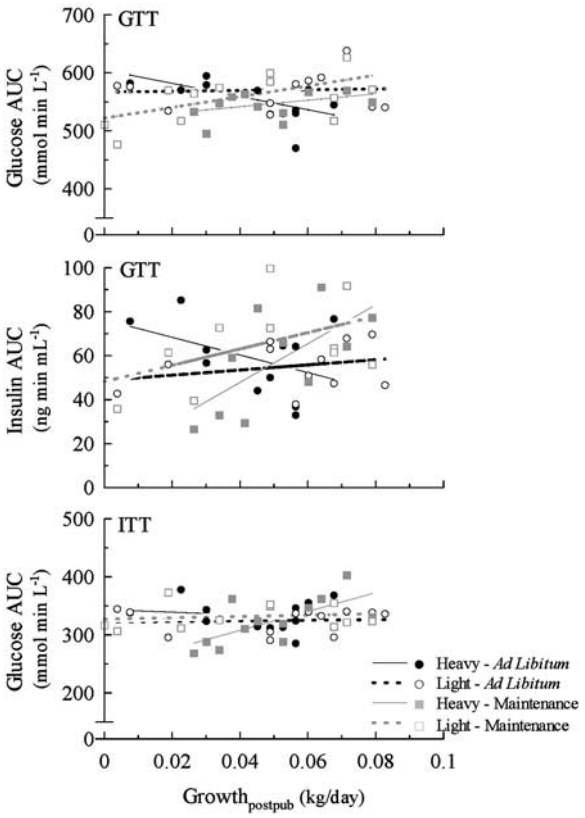

Fig. 2. Linear regressions of post-puberty growth rates ($Growth_{postpub}$: 9–12 months of age) on glucose-metabolism variables at 16 months of age ($GluAUC_{GTT}$: glucose AUC and $InsAUC_{GTT}$: insulin AUC in response to glucose tolerance test (GTT); $GluAUC_{ITT}$: glucose AUC in response to insulin tolerance test (ITT)) of ewes (G_1) born to heavy or light dams (G_0) fed either maintenance or *ad libitum* during pregnancy. Black solid line and ● heavy – *ad libitum*; black dotted line and ○ light – *ad libitum*; grey solid line and ■ heavy – maintenance; grey dotted line and □ light – maintenance.

coefficient of $Growth_{postpub}$ on $GluAUC_{GTT}$ of LM-ewes was significantly ($P = 0.01$) different from 0, indicating that LM-ewes had increased $GluAUC_{GTT}$ with increasing growth rates after puberty. The regression coefficient of $Growth_{postpub}$ on $GluAUC_{GTT}$ of LM-ewes was significantly different ($P = 0.002$) from that of HA-ewes, indicating that LM-ewes had greater $GluAUC_{GTT}$ with increasing growth rates after puberty than did HA-ewes. The regression coefficient of $Growth_{postpub}$ on $GluAUC_{GTT}$ of HM-ewes tended to be different ($P = 0.04$) from that of HA-ewes, indicating that HM-ewes tended to have greater $GluAUC_{GTT}$ with increasing growth rates after puberty than did HA-ewes.

The regression coefficient of $Growth_{postpub}$ on $InsAUC_{GTT}$ of HM-ewes was significantly ($P = 0.02$) different from 0, indicating that HM-ewes had increased $InsAUC_{GTT}$ with increasing growth rates after puberty. The regression coefficient of $Growth_{postpub}$ on $InsAUC_{GTT}$ of HM-ewes was significantly different ($P = 0.005$) from that of HA-ewes, indicating that HM-ewes had greater $InsAUC_{GTT}$ with greater growth rates after puberty than did HA-ewes. The regression coefficient of $Growth_{postpub}$ on $InsAUC_{GTT}$ of HM-ewes tended to be different ($P = 0.05$) from that of LA-ewes, indicating that HM-ewes tended to have greater $InsAUC_{GTT}$ with increasing growth rates after puberty than did LA-ewes.

The regression coefficient of $Growth_{postpub}$ on $GluAUC_{ITT}$ of HM-ewes was significantly ($P = 0.001$) different from 0, indicating that HM-ewes had increased $GluAUC_{ITT}$ with every kg of growth. The regression coefficient of $Growth_{postpub}$ on $GluAUC_{ITT}$ of HM-ewes was significantly different ($P = 0.001$) from that of HA-, LA- and LM-ewes, indicating that HM-ewes had greater $InsAUC_{GTT}$ with increasing growth rates after puberty than did HA-, LA- and LM-ewes.

Adrenal function of the ewe offspring (G_1)

Birth weight, $Growth_{wean}$, $Growth_{postwean}$, $Growth_{prepub}$ and $Growth_{postpub}$ were not related to $CortAUC_{ITT}$ in response to the ITT at 16 months of age (data not shown).

Fat metabolism of the ewe offspring (G_1)

Birth weight, $Growth_{wean}$, $Growth_{postwean}$, $Growth_{prepub}$ and $Growth_{postpub}$ were not related to $GluAUC_{ETT}$, $TrigAUC_{ETT}$ and $CholAUC_{ETT}$ in response to the ETT at 16 months of age.

$Growth_{wean}$, $Growth_{postwean}$, $Growth_{prepub}$ and $Growth_{postpub}$ were not related to $InsAUC_{ETT}$.

The regression coefficients of birth weight on $InsAUC_{ETT}$ of LA-ewes ($P = 0.0001$) and LM-ewes ($P = 0.04$) were significantly different from 0, indicating that LA- and LM-ewes had increased $InsAUC_{ETT}$ with every kg increase of birth weight (Fig. 3 and Table 4). The regression coefficient of birth weight on $InsAUC_{ETT}$ of LA-ewes was significantly different from that of HA-ewes ($P = 0.001$), and HM-ewes ($P = 0.01$), indicating that LA-ewes had greater $InsAUC_{ETT}$ with every kg increase of birth weight than did HA- and HM-ewes.

Birth weight, $Growth_{postwean}$ $Growth_{prepub}$ and $Growth_{postpub}$ were not related to $NefaAUC_{ETT}$ at 16 months of age. The regression coefficient of $Growth_{wean}$ on $NefaAUC_{ETT}$ of LM-ewes was significantly ($P = 0.03$) different from 0, indicating that LM-ewes had decreased $NefaAUC_{ETT}$ with increasing growth rates prior to weaning (Fig. 3 and Table 5). This regression coefficient of $Growth_{wean}$ on $NefaAUC_{ETT}$ of LM-ewes tended to be different ($P = 0.03$) from that of HM-ewes indicating that LM-ewes had smaller $NefaAUC_{ETT}$ with increasing growth rates prior to weaning than did HM-ewes.

Discussion

We hypothesized that low birth weight and greater postnatal growth rates until 1 year of age in female offspring born to light dams which were fed maintenance during pregnancy, would negatively affect their metabolic function at 16 months of age. In addition, we hypothesized that the pre-existing

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Table 3. *Linear regression equations* of post-puberty growth rates ($Growth_{postpub}$; 9–12 months of age; kg/day) on glucose-metabolism variables at 16 months of age ($GluAUC_{GTT}$: glucose AUC and $InsAUC_{GTT}$: insulin AUC in response to GTT $GluAUC_{ITT}$: glucose AUC in response to ITT) of ewes (G_1) born to heavy (H) or light (L) dams (G_0) fed either ad libitum (A) or maintenance (M) during pregnancy*

		Independent variable				
Dam treatment	Depended variable	Intercept (β_0)	*P* value (β_0)	$Growth_{postpub}$ (β_1)	*P* value (β_1)	R^2
HA	$GluAUC_{GTT}$ (mmol/min/l)	605 (±25.2)	0.0001	−1138 (±515.9)[a]	0.03	0.56
LA		567 (±20.8)	0.0001	68 (±345.0)[ab]	NS	0.005
HM		520 (±30.4)	0.0001	551 (±576.5)[ab]	NS	0.28
LM		522 (±17.1)	0.0001	926 (±345.9)[b]	0.01	0.42
HA	$InsAUC_{GTT}$ (ng/min/ml)	76 (±13.0)	0.0006	−400 (±280.8)[a]	NS	0.12
LA		49 (±12.5)	0.002	117 (±209.9)[ab]	NS	0.08
HM		13 (±16.8)	NS	880 (±312.1)[b]	0.02	0.49
LM		48 (±11.1)	0.0001	371 (±212.5)[ab]	NS	0.26
HA	$GluAUC_{ITT}$ (mmol/min/l)	344 (±21.0)	0.0001	−224 (±440.9)[b]	NS	0.16
LA		323 (±16.8)	0.0001	48 (±295.3)[b]	NS	0.09
HM		243 (±24.7)	0.0001	1626 (±474.0)[a]	0.001	0.53
LM		327 (±14.8)	0.0001	114 (±296.7)[b]	NS	0.32

NS, non significant.
[ab] Significantly different ($P < 0.05$; using Bonferroni correction) between dam treatments and within dependent metabolic variable.
*All regression equation models are significant ($P < 0.01$).

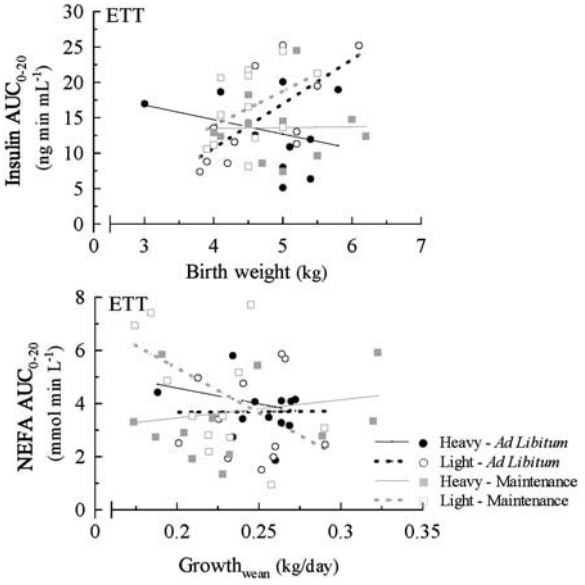

Fig. 3. Linear regressions of birth weight and growth rates to weaning ($Growth_{wean}$: birth – 4 months of age) on fat-metabolism variables at 16 months of age ($InsAUC_{ETT}$: insulin AUC and $NefaAUC_{ETT}$: non-esterified free fatty acids (NEFAs) AUC in response to epinephrine tolerance test (ETT)) of ewes (G_1) born to heavy or light dams (G_0) fed either *ad libitum* or maintenance during pregnancy. Black solid line and ● heavy – *ad libitum*; black dotted line and ○ light – *ad libitum*; grey solid line and ■ heavy – maintenance; grey dotted line and □ light – maintenance.

maternal body size (heavy *v.* light) would exacerbate or reduce the effects of maternal nutrition during pregnancy.

However, no relationship was found between impaired glucose homeostasis at 16 months of age and birth weight or postnatal growth up to 7 months of age in the offspring, which is consistent with other studies.[2,28]

On the other hand, relationships were found between glucose homeostasis at 16 months of age and growth rates prior to puberty ($Growth_{prepub}$) and growth rates after puberty ($Growth_{postpub}$). A shift in metabolism seems to have occurred. Prior to puberty, HA-ewes produced more insulin at 16 months of age (increased predicted $InsAUC_{GTT}$ with increasing growth rates prior to puberty) and were more insulin resistant at 16 months of age (increased predicted $GluAUC_{ITT}$ with increasing growth rates prior to puberty) than the HM- and LM-ewes. However, no such relationship between glucose intolerance and insulin resistance at 16 months of age and growth rates after puberty was observed in HA-ewes. Interestingly, after puberty HM-ewes produced more insulin and were more insulin resistant at 16 months of age with increasing growth rates after puberty compared to the other groups. A possible explanation for the relationship observed prior to puberty in the HA-ewes, is puberty-related insulin resistance, as described in human children.[29,30]

Puberty-related insulin resistance is related to increased growth hormone (GH) concentrations, which stimulates anabolic growth and lipolysis[31] and secretion of insulin-like-growth factor I.[32] Exogenous GH administration is associated

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Table 4. *Linear regression equations* of birth weights (kg) on fat-metabolism variable $InsAUC_{ETT}$ at 16 months of age (insulin AUC in response to ETT) of ewes (G_1) born to heavy (H) or light (L) dams (G_0) fed either ad libitum (A) or maintenance (M) during pregnancy*

		Independent variable				
Dam treatment	Dependent variable	Intercept (β_0)	*P* value (β_0)	Birth weight (β_1)	*P* value (β_1)	R^2
HA	$InsAUC_{ETT}$ (ng/min/ml)	5.2 (±3.3)	NS	−0.4 (±0.7)[b]	NS	0.11
LA		−10.9 (±3.6)	0.004	3.3 (±0.7)[a]	0.0001	0.76
HM		2.0 (±3.7)	NS	0.5 (±0.7)[b]	NS	0.33
LM		−5.6 (±4.8)	NS	2.2 (±1.0)[ab]	0.04	0.24

NS, non significant.
[ab] Significantly different ($P < 0.05$; using Bonferroni correction) between dam treatments and within dependent metabolic variable.
*All regression equation models are significant ($P < 0.01$).

Table 5. *Linear regression equations* of growth rates (from birth until 4 months of age, $Growth_{wean}$: kg/day) on fat-metabolism variable $Nefa\,AUC_{ETT}$ at 16 months of age (NEFA AUC in response to ETT) of ewes (G_1) born to heavy (H) or light (L) dams (G_0) fed either ad libitum (A) or maintenance (M) during pregnancy*

		Independent variable				
Dam treatment	Dependent variable	Intercept (β_0)	*P* value (β_0)	$Growth_{wean}$ (β_1)	*P* value (β_1)	R^2
HA	$Nefa\,AUC_{ETT}$ (mmol/min/l)	7.0 (±5.1)	NS	−11.9 (±20.3)	NS	0.11
LA		3.6 (±4.8)	NS	0.4 (±19.3)	NS	0.02
HM		2.1 (±2.4)	NS	6.8 (±9.6)	NS	0.07
LM		12.1 (±3.5)	0.001	−33.7 (±15.1)	0.03	0.41

NS, non significant.
*All regression equation models are significant ($P < 0.01$).

with both an elevation in circulating free fatty acids (FFAs) and a decrease in insulin sensitivity,[33] as an elevation in FFA is associated with skeletal muscle resistance to insulin-stimulated glucose uptake. Therefore, pubertal metabolism appears to be optimized to permit or promote anabolic growth.[31] However, we cannot explain why the association was only observed in HA-ewes and not in the other groups. After puberty, the relationship between growth rate in early postnatal life and glucose homeostasis at 16 months of age observed in HM- and LM-ewes, is in agreement with the concept that postnatal growth and sub-optimal nutrition during pregnancy are predictors of later development of glucose intolerance and insulin resistance.[34] This could indicate that M-ewes, regardless of dam size, may develop glucose intolerance and insulin resistance later in life. However, the relationship between growth rate after puberty and insulin resistance (greater predicted $InsAUC_{GTT}$ and $GluAUC_{ITT}$) at 16 months was most profound in HM-ewes and significantly different from HA-ewes. This may suggest that the pre-existing dam size (H) exacerbate the effects of nutrition during pregnancy on glucose and insulin metabolism of the offspring, given that the offspring have increased growth rates after puberty.

The absolute insulin secretion after the glucose administration was positively related with growth in the offspring studied in the current study, this may indicate that no dysfunction at pancreatic level had occurred.[35] Thus, it may be more likely that the sub-cellular insulin-signaling proteins downstream of the receptor could be affected[36] especially at adipose tissue level,[3] as mature animals are more likely to accumulate adipose tissue than muscle tissue.

However, size of the dam did affect the area under the insulin curve in response to the ETT. Ewes born to light dams produced more insulin (increased predicted $InsAUC_{ETT}$) with every kg that they were heavier at birth in response to the ETT at 16 months of age. This was not observed in ewes born to heavy dams, which would produce the same amount of $InsAUC_{ETT}$ at 16 months of age irrespective of their birth weight. This may indicate that offspring born to light dams, with greater birth weights, were 'protected' from the lipolytic action of epinephrine as insulin has anti-lipolytic effects,[37] therefore, possibly being more 'thrifty'.[38] However, insulin is a secondary response to an epinephrine challenge, and the role of increased insulin production in response to ETT observed at 16 months of age with increasing birth weight is not fully understood.

An increase in plasma NEFA concentrations in response to catecholamines is most readily explained in terms of changes in the rate of lipolysis (mobilization of adipose tissue to NEFA and glycerol).[39] LM-ewes produced less NEFA (decreased predicted

https://doi.org/10.1017/S2040174409990201 Published online by Cambridge University Press

$NefaAUC_{ETT}$ with increasing growth rates) at 16 months of age with increasing growth rates from birth to weaning ($Growth_{wean}$) than HM-ewes. This may indicate that within the maintenance-fed group, offspring born to light dams are more 'thrifty',[38] as with increasing early postnatal growth rates, the rate of lipolysis at 16 months of age is less (smaller predicted $NefaAUC_{ETT}$), thus 'sparing' their energy reserves, which is in agreement with the relationship found between birth weight and insulin production in offspring born to light dams.

In summary, ewes born to heavy dams fed *ad libitum* during gestation may have showed puberty-related insulin resistance at 16 months of age with increasing growth rates prior to puberty. Post-puberty, ewes born to heavy dams fed maintenance during pregnancy, produced more insulin, and were increasingly insulin resistant at 16 months of age with increasing growth rates after puberty, in response to a glucose and insulin challenge, respectively, compared to ewes born to heavy dams fed *ad libitum*. These results may indicate that offspring born to dams fed maintenance during pregnancy and with greater postnatal growth rates after puberty could develop glucose intolerance and insulin resistance in later life.

Ewes born to light dams were more 'thrifty' at 16 months of age with every kg increase of birth weight and increasing postnatal growth rates until weaning in response to an epinephrine challenge.

Altogether, the observed relationships both between birth weight and (early) postnatal growth and the metabolic response to glucose, insulin and adrenalin challenges at 16 months of age of offspring born to heavy or light dams fed maintenance or *ad libitum* during pregnancy are interesting, and further research will be needed to determine the exact meaning and mechanism(s) of the observed relationships.

Acknowledgements

The authors are grateful to Massey University, Meat and Wool New Zealand and the National Research Centre for Growth and Development for providing funding assistance for this project. The senior author was funded by an AGMARDT doctoral scholarship. The authors would like to thank Florence Delassus, who assisted with all the animal work and data collection, Dr Mark Oliver, Auckland University, for his helpful advice, the team at IVABS for their help with blood collection and Eric Thorstensen, Auckland University, for the blood analyses.

Statement of Interest

None.

References

1. Wu G, Bazer FW, Wallace JM, Spencer TE. Board-invited review: intrauterine growth retardation: implications for the animal sciences. *J Anim Sci*. 2006; 84, 2316–2337.
2. Oliver MH, Breier BH, Gluckman PD, Harding JE. Birth weight rather than maternal nutrition influences glucose tolerance, blood pressure, and IGF-I levels in sheep. *Pediatr Res*. 2002; 52, 516–524.
3. Gardner DS, Tingey K, Van Bon BWM, *et al*. Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. *Am J Physiol Regul Integr Comp Physiol*. 2005; 289, R947–R954.
4. Ford SP, Hess BW, Schwope MM, *et al*. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. *J Anim Sci*. 2007; 85, 1285–1294.
5. Hawkins P, Steyn C, McGarrigle HHG, *et al*. Cardiovascular and hypothalamic-pituitary-adrenal axis development in late gestation fetal sheep and young lambs following modest maternal nutrient restriction in early gestation. *Reprod Fert Develop*. 2000; 12, 443–456.
6. Bloomfield FH, Oliver MH, Giannoulias CD, *et al*. Brief undernutrition in late-gestation sheep programs the hypothalamic-pituitary-adrenal axis in adult offspring. *Endocrinology*. 2003; 144, 2933–2940.
7. Gardner DS, Van Bon BWM, Dandrea J, *et al*. Effect of periconceptional undernutrition and gender on hypothalamic-pituitary-adrenal axis function in young adult sheep. *J Endocrinol*. 2006; 190, 203–212.
8. Greenwood PL, Hunt AS, Hermanson JW, Bell AW. Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency. *J Anim Sci*. 1998; 76, 2354–2367.
9. Mellor DJ. Nutritional and placental determinants of fetal growth-rate in sheep and consequences for the newborn lamb. *Brit Vet J*. 1983; 139, 307–324.
10. Dickinson AG, Hancock JL, Hovell GJR, Taylor SCS, Wiener G. The size of lambs at birth – A study involving egg transfer. *Anim Prod*. 1962; 4, 64–79.
11. Gootwine E, Bor A, Brawtal R, Zenou A. Inheritance of birth-weight and growth traits in crosses between the Booroola Merino and Assaf sheep breeds. *Livest. Prod. Sci.*. 1993; 33, 119–126.
12. Walton A, Hammond J. The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. *Proc Roy Soc Lond B Biol Sci*. 1938; 125, 311–335.
13. Allen WR, Wilsher S, Turnbull C, *et al*. Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero. *Reproduction*. 2002; 123, 445–453.
14. Wilson ME, Biensen NJ, Youngs CR, Ford SP. Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. *Biol Reprod*. 1998; 58, 905–910.
15. Desai M, Hales CN. Role of fetal and infant growth in programming metabolism in later life. *Biol Rev Camb Philos Soc*. 1997; 72, 329–348.
16. Cottrell EC, Ozanne SE. Early life programming of obesity and metabolic disease. *Physiol Behav*. 2008; 94, 17–28.
17. Bloomfield FH, Oliver MH, Harding JE. Effects of twinning, birth size, and postnatal growth on glucose tolerance and hypothalamic-pituitary-adrenal function in postpubertal sheep. *Am J Physiol Endocrinol Metab*. 2007; 292, E231–E237.

18. Eriksson JG, Forsen T, Tuomilehto J, *et al*. Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. *Diabetologia*. 2002; 45, 342–348.
19. Ozanne SE, Hales CN. Poor fetal growth followed by rapid postnatal catch-up growth leads to premature death. *Mech Ageing Dev*. 2005; 126, 852–854.
20. Firth EC, Rogers CW, Vickers M, *et al*. The bone-muscle ratio of fetal lambs is affected more by maternal nutrition during pregnancy than by maternal size. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294, R1890–R1894.
21. Kenyon PR, Blair HT, Jenkinson CMC, *et al*. The effect of ewe size and nutrition regimen beginning in early pregnancy on ewe and lamb performance to weaning. *N Z J Agric Res*. 2009; 52, 203–212.
22. van der Linden DS, Kenyon PR, Jenkinson CMC, *et al*. The effects of ewe size and nutrition during pregnancy on growth and onset of puberty in female progeny. *Proc N Z Soc Anim Prod*. 2007; 67, 126–129.
23. Geenty KG, Rattray PV. The energy requirements of grazing sheep and cattle. In *Livestock Feeding on Pasture* (ed. Nicol AM), 1987; pp. 39–53. Bascands Ltd: Hamilton, New Zealand.
24. Rumball CWH, Harding JE, Oliver MH, Bloomfield FH. Effects of twin pregnancy and periconceptional undernutrition on maternal metabolism, fetal growth and glucose-insulin axis function in ovine pregnancy. *J Physiol Lond*. 2008; 586, 1399–1411.
25. Rumball CWH, Oliver MH, Thorstensen EB, *et al*. Effects of twinning and periconceptional undernutrition on late-gestation hypothalamic-pituitary-adrenal axis function in ovine pregnancy. *Endocrinology*. 2008; 149, 1163–1172.
26. SAS. *Statistical Analysis System. 9.1*. 2006. SAS Institute Inc., Cary, NC, USA.
27. Narum SR. Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conser Gen*. 2006; 7, 783–787.
28. Eriksson JG, Osmond C, Kajantie E, Forsen TJ, Barker DJP. Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia*. 2006; 49, 2853–2858.
29. Amiel SA, Caprio S, Sherwin RS, *et al*. Insulin resistance of puberty – A defect restricted to peripheral glucose-metabolism. *J Clin Physiol Endocrin Metab*. 1991; 72, 277–282.
30. Moran A, Jacobs DR, Steinberger J, *et al*. Insulin resistance during puberty – Results from clamp studies in 357 children. *Diabetes*. 1999; 48, 2039–2044.
31. Gower BA, Caprio S. Puberty, insulin resistance, and type 2 diabetes. In *Handbook of Pediatric Obesity: Etiology, Pathophysiology, and Prevention* (ed. Goran MI), 2006; pp. 175–196. CRC Press-Taylor & Francis Group: Boca Raton, FL, USA.
32. Moran A, Jacobs DR, Steinberger J, *et al*. Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. *J Clin Endocrino Metab*. 2002; 87, 4817–4820.
33. Keller U, Miles JM. Growth-hormone and lipids. In *2nd Symp on hGH (human Growth Hormone). Horm Res*. 1991; 36(Suppl. 1), 36–40.
34. Symonds ME. Integration of physiological and molecular mechanisms of the developmental origins of adult disease: new concepts and insights. *Proc Nutr Soc*. 2007; 66, 442–450.
35. Davies MJ, Rayman G, Grenfell A, *et al*. Loss of the 1st phase insulin-response to intravenous glucose in subjects with persistent impaired glucose-tolerance. *Diabet Med*. 1994; 11, 432–436.
36. Fernandez-Twinn DS, Wayman A, Ekizoglou S, *et al*. Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288, R368–R373.
37. Ozanne SE, Wang CL, Dorling MW, Petry CJ. Dissection of the metabolic actions of insulin in adipocytes from early growth-retarded male rats. *J Endocrinol*. 1999; 162, 313–319.
38. Prentice AM. Early influences on human energy regulation: thrifty genotypes and thrifty phenotypes. *Physiol Behav*. 2005; 86, 640–645.
39. Vernon RG. Lipid metabolism in the adipose tissue of ruminant animals. In *Lipid metabolism in ruminant animals* (ed. Christie WW), 1981; pp. 279–362. Pergamon Press Ltd: Oxford, New York.