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Interactions between pre- and postnatal diet on metabolic competence in sheep

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Following initial epidemiological research into the correlation between poor fetal growth and predisposition to adult disease(1), many animal models have been established to elucidate the mechanistic origins behind the 'fetal programming' phenomenon(2). An ovine model was used in the present study because of similarities with human reproductive physiology and embryogenesis. The aim of the study was to investigate the effect of maternal nutrient (protein) restriction during early or late gestation on young adult body composition and metabolism.

Nineteen Scottish Blackface ewes were synchronised and mated with Scottish Blackface rams. Three different maternal dietary regimens were established: control, 180 g protein/kg from day 0 (mating date) to term (approximately 145 d gestation; CP; *n* 6 (four males, two females)); low-protein early, 90 g protein/kg from day 0 to day 65 then 180 g protein/kg from day 66 to term (LPE; *n* 7 (two males, five females)); low-protein late, 180 g protein/kg from day 0 to day 65 then 90 g protein/kg from day 66 to term (LPL; *n* 6 (two males, four females). All maternal diets were isoenergetic and were fed as concentrate supplemented with hay. Animals were scanned on day 70 and maternal dietary allowance adjusted according to fetal number and gestational age (according to Agricultural and Food Research Council guidelines(3)). At term lambs delivered naturally were ewe-reared to weaning at 10 weeks and then put to pasture. Aged 1.5 years and relatively 'lean', animals underwent a glucose tolerance test (GTT; GTT1) and body composition analysis (dual-energy X-ray absorptiometry (DXA); DPX-L fast-detail smartscan; GE Lunar, Madison, WI, USA). For the intravenous GTT, glucose (0.5 g/kg) was injected, followed by periodic blood sampling (baseline to 2 h). Blood was analysed using an autoanalyser (ABL805-FLEX; Radiometer, Crawley, West Sussex, UK) and plasma with an RX Imola (Randox, Crumlin, Co. Antrim, UK). Plasma insulin was analysed by an ovine-specific ELISA (Mercodia AB, Uppsala, Sweden). DXA involved sedation (intramuscularly; 0.1 mg xylazine/kg plus 1.5 mg ketamine/kg). Thereafter, all sheep were housed in a barn to decrease physical activity and had increased food availability (150% maintenance intake on 60 g oil concentrate/kg) over approximately 6 months, during this period all animals therefore became 'obese'. All protocols (DXA and GTT; GTT2) were then repeated on all animals and, in addition, an insulin tolerance test (ITT; 0.75 IU insulin/kg; Novorapid®; Novo Nordisk A/S, Bagsvaerd, Denmark) was performed with periodic blood sampling (baseline to 16 min post injection). Animals were then killed by electrocortical stunning and exsanguination. Data are presented as estimated marginal means and were analysed by univariate or repeated measures general linear model with maternal treatment, gender and litter size as fixed effects using SPSS version 14 (SPSS Inc., Chicago, IL, USA). *Post hoc* analysis for treatment was performed using Bonferroni correction. Significance was $P \leq 0.05$.

Birth weight was effected by maternal protein restriction (kg; CP 5.12 (SE 0.21), LPE 5.25 (SE 0.19), LPL 4.37 (SE 0.20); $P = 0.038$), litter size (singletons>twins; $P = 0.002$) and gender (males>females; $P = 0.049$). Over the 6-month weight-gain period animals put on about 150% weight (kg; lean 47.4 (SE 0.84) *v.* obese 74.6 (SE 1.43); $P < 0.001$), associated with an increase in fat mass from 14% (6.85 (SE 0.50) kg) to 28% (15.7 (SE 1.15) kg; $P < 0.001$), with no significant change in lean mass. LPL animals put on less fat than CP and LPE animals (kg; CP 12 (SE 1), LPE 12 (SE 1), LPL 10 (SE 1); $P = 0.037$). The fixed effects accounted for very few observed differences when animals were lean, e.g. GTT1, but with obesity, e.g. GTT2, treatment effects were observed for insulin area under the curve (AUC; mmol/l.2 h; CP 213 (SE 50.5), LPE 418 (SE 47.1), LPL 302 (SE 48.5); $P = 0.038$) and maximal insulin secretion (μg/l; CP 4.3 (SE 1.2), LPE 8.6 (SE 1.1), LPL 4.6 (SE 1.1); $P = 0.036$). However, in response to the glucose bolus *per se* significant changes (between baseline and 2 h post glucose injection) were observed in glucose, lactate, pH, pCO_2, pO_2, Ca^{2+}, Na^+, base excess, HCO_3^- concentration when animals were lean, while only glucose, Ca^{2+} and K^+ changed over the same period when animals were obese. Repeated measures analysis (GTT1 *v.* GTT2) indicated significant effects of adult-onset obesity on the GTT AUC and baseline glucose (AUC (mmol/l.2 h) 731 (SE 53.9) *v.* 1082 (SE 38.4); $P = 0.002$), insulin (AUC (μg/l.2 h) 130 (SE 14.0) *v.* 320 (SE 28.2); $P < 0.001$), NEFA (baseline (mmol/l) 0.86 (SE 0.48) *v.* 0.68 (SE 0.42); $P = 0.016$; AUC (mmol/l.2 h) 63.6 (SE 5.15) *v.* 46.9 (SE 4.09); $P = 0.029$) and TAG (baseline (mmol/l) 0.13 (SE 0.01) *v.* 0.22 (SE 0.01); $P < 0.001$). Maximal insulin secretion also elevated in response to obesity (μg/l; 2.81 (SE 0.31) *v.* 5.99 (SE 0.65); $P < 0.001$). No effects of maternal treatment, litter size or gender were observed after the ITT.

The present study is the first to examine an interaction between timely prenatal protein restriction and postnatal obesity in sheep. When lean, few significant effects of prenatal programming were observed; however, when obese, significant treatment effects arose: elevated glucose intolerance after early protein restriction; reduced gain of fat mass after late gestational protein restriction. When considering the effect of obesity *per se*, significantly blunted metabolic responses were observed after bolus glucose infusion in obese compared with lean animals.

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