CALL FOR PAPERS | Metabolic Syndrome

Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition

D. S. Gardner,1 K. Tingey,2 B. W. M. Van Bon,1 S. E. Ozanne,2 V. Wilson,1 J. Dandrea,3 D. H. Keisler,4 T. Stephenson,1 and M. E. Symonds1

1Centre for Reproduction and Early Life, School of Human Development, University Hospital, Nottingham; 2Department of Clinical Biochemistry, Addenbrookes Hospital, University of Cambridge, Cambridge; 3School of Nursing, University of Nottingham, Mansfield, United Kingdom; and 4Department of Animal Sciences, University of Missouri, Columbia, Missouri

Submitted 17 February 2005; accepted in final form 10 June 2005

Gardner, D. S., K. Tingey, B. W. M. Van Bon, S. E. Ozanne, V. Wilson, J. Dandrea, D. H. Keisler, T. Stephenson, and M. E. Symonds. Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. Am J Physiol Regul Integr Comp Physiol 289: R947–R954, 2005. First published June 16, 2005; doi:10.1152/ajpregu.00120.2005.—The present study examines the effects of late vs. early gestation undernutrition on adult glucose-insulin homeostasis in sheep and investigates whether the lower birth weight of twins alters glucose-insulin handling in adult life. Pregnant sheep were fed to requirement (100% intake) from day 0 of gestation to term (∼147 days of gestation [dGA]), control singles (CS) n = 5; control twins (CT) n = 5) or to 50% requirement from days 0–30 dGA [nutrient restricted during early gestation (NRE); n = 5] or day 110-term [NR during late nutrition (NRL); n = 4]. At all other times, NR sheep received 100% intake. All sheep lambed naturally; offspring were weaned at 10 wk and were reared on pasture until 1 yr of age. At this time, indwelling catheters were inserted, and 2–4 days later, basal metabolic and endocrine status and responses to an intravenous glucose tolerance test (IVGTT) and feeding were assessed. Adipose and skeletal muscle were then sampled after humane euthanasia and were analyzed for expression of insulin-signaling proteins and GLUT4. Between groups, birth weight of singleton twins was similar and increased relative to twins. At 1 yr of age, weights were similar between groups. The areas under the curve for glucose and insulin during the IVGTT were greater in NRE vs. other groups, indicating glucose intolerance. This was associated with reduced adipose, but not muscle, GLUT4, and increased adipose tissue mass. Adult glucose-insulin homeostasis in sheep was unaffected by fetal number. In conclusion, prenatal undernutrition, specifically during late gestation, affects adult offspring intermediary metabolism, and, in particular, glucose-insulin homeostasis.

Type 2 diabetes; maternal nutrition; adipose; glucose tolerance test; leptin

Type 2 diabetes and related risk factors, such as obesity, insulin resistance, and glucose intolerance, have all increased in frequency in Westernized populations over recent years and are predicted to continue increasing unabated (11). The rapid progression of these noncommunicable metabolic disorders within a generation suggests an environmental rather than genetic etiology. In the last 10 yr, it has been suggested, and since corroborated by retrospective human and prospective animal trials, that the risk of developing “metabolic syndrome” in middle age has its origins early in life, specifically during fetal and early postnatal development (5, 22).

Some of the most compelling data regarding the developmental programming of obesity and related metabolic disorders have come from retrospective studies examining a proportion of the Dutch population exposed to famine during World War II—the Dutch Hunger Winter Famine. Very early on, it was realized that the period of famine exposure (3–4 mo) produced different outcomes in the adult offspring, depending on when the famine conditions were endured during pregnancy (36). For example, exposure during the first trimester of pregnancy only, gave rise to offspring with increased risk of coronary heart disease (odds ratio, 8.8%) compared with exposure during midgestation, late gestation, or nonexposed individuals [midgestation, 0.9%; late gestation, 2.5%; nonexposed, 3.0% (37)]. In contrast, famine exposure during late gestation only tended to impact on intermediary metabolism, in particular, glucose-insulin homeostasis (35). Such effects can be readily explained when one considers the chronological development of system/organ growth in the fetus; cardiovascular growth has a priority early in gestation, with adipose and muscle growth (highly insulin-sensitive tissues) occurring relatively late.

Animal studies have, in part, replicated these original hypotheses and shown that undernutrition confined to early gestation may impact on adult cardiovascular function (19, 27), whereas undernutrition throughout gestation and/or lactation produces both hypertensive (26) and insulin-resistant (16, 32) offspring. Surprisingly, to date, no animal study has investigated adult glucose-insulin metabolism after prenatal undernutrition confined to late gestation alone, nor to have directly compared the effect of early vs. late undernutrition on aspects of glucose-insulin intermediary metabolism in the resultant adult offspring. The current study was therefore established to directly address the hypothesis that late, rather than early, gestation undernutrition has the greatest impact on adult glucose-insulin homeostasis.

In addition, one of the many criticisms leveled at the developmental origins of adult disease, hypothesis concerns the incidence of noncommunicable disease in twins that are generally of lower weight at birth (7) but do not seem to exhibit a higher risk for either cardiovascular or metabolic disease (4, 8,
9, 23). Whereas aspects of intrauterine development, predominantly endocrinological (13, 18), are clearly different between singletons and twins, there is little evidence to support a programmed effect on cardiovascular function after maternal undernutrition (19). Follow-up studies in humans have shown that regardless of birth weight, twins have a reduction in insulin sensitivity (24), as well as differences in blood pressure regulation (24). The use of twins as a model for investigating the effect of uterine growth retardation on the developmental origins of later disease is therefore contentious. However, because long-term nutritional programming effects can be observed in the absence of any effect on birth weight (6), twin studies can be useful in determining the relative impact of size at birth as opposed to a reduction in maternal food intake. In this regard, there is very little experimental information on adult intermediary metabolism in comparable singletons and twins, and therefore, in the current study, we include a subgroup of twins born to nutritionally replete mothers to test the hypothesis that programming of adult glucose-insulin handling and sensitivity is independent of fetal number and thus reduced birth weight.

MATERIALS AND METHODS

Animals

All procedures were approved by the local ethics committee of The University of Nottingham and performed under United Kingdom Animals (Scientific Procedures) Act, 1986. The general principles of laboratory animal care were strictly followed (29). Nineteen blue-faced Leicester cross Swaledale (Mule) sheep from the University of Nottingham’s commercial flock were used in the study. Sheep were mated during their natural breeding season without the use of ovarian stimulants. After mating, sheep were individually housed and randomly assigned to receive one of three diets during pregnancy: 1) 100% metabolizable energy (ME) requirements as defined by the Agricultural and Food Research Council (AFRC; Ref. 1) throughout gestation (nutrient restriction–early; NRL, n = 10); 2) 50% AFRC ME requirement from days 1-30 gestation (nutrient restriction–early; NRE, n = 5); or 3) 50% AFRC ME requirement from day 110-term (nutrient restriction–late; NRL, n = 4). All NR sheep received 100% ME requirement at all other times during gestation. Of the 10 control sheep, five bore singletons and five bore twins. Thus four groups were formed in total: 1) control singletons (CS, 46.1 ± 1.8 kg ewe weight at mating), 2) control twins (CT, 44.8 ± 1.5 kg), 3) NRE singles (45.9 ± 1.9 kg), and 4) NRL singles (58.4 ± 2.2 kg). NRE and NRL all bore singletons. The diet for all control and NR sheep contained an adequate amount of vitamins, with minerals provided in the form of mineral blocks and comprised 75% chopped hay [ME, 8.5 MJ/kg dry matter (DM)] and a crude protein content of 85 g/kg DM and 25% concentrate pellets (ME, 8.9 MJ/kg DM; protein, 218 g/kg DM). The nutritional regimen for each ewe was adjusted accordingly throughout gestation to allow for ewe weight gain, fetal number, and conceptus growth. At term (~147 days gestation), lambs were delivered naturally, and birth weights were recorded. Any twin lambs delivered were reared as singletons with the ewe to standardize postnatal nutritional status. The sex ratio for each group was (numbers of male/female): 1) CS (3:2), 2) CT (2:3), 3) NRE (1:4), and 4) NRL (2:2). The offspring were ewe reared until weaning at 3 mo of age and thereafter grass fed until 1 yr of age. During this time the weights and growth of individual lambs were recorded monthly.

At 11 mo of age, all sheep were brought indoors in preparation for the surgical implantation of catheters and experimental studies. For 24 h before surgery all food, but not water, was withdrawn from the animals. Surgical details and postoperative handling of these animals were as described previously (19). In brief, carotid and jugular catheters were inserted under anesthesia (1–2% halothane in 50:50 O2/N2O) with appropriate antibiotic [15 mg/kg im amoxicillin (Duphamox); Fort Dodge Animal Health, Southampton, UK] and analgesic coverage [1 mg/kg flunixin meglumine (Finadyne); Shering-Plough, Kenilworth, UK] postoperatively. Catheters were kept patent by daily flushing with heparinized saline (50 IU heparin/ml).

Experimental Protocols

No experiment was performed until after 2–4 days postoperative recovery. The investigator was blinded to the dietary origin of the sheep before any experiment was performed. In all sheep, two experiments were conducted on separate days.

Experiment 1. METABOLIC RESPONSE TO FEEDING. Both hay and concentrate were withheld from the sheep for a period of 24 h. The following morning, two baseline blood samples (2 ml; −10 and −5 min) were taken before feeding and then subsequently at 5, 15, 30, 45, and 60 min after feed was given at time 0, which coincides with the rapid switch from a dependence on lipid to carbohydrate metabolism in ruminants (38). All concentrate feed for all animals was consumed within 10 min, and blood samples were handled as previously described for experiment 1. The combined 45- and 60-min sample was used for a paired comparison of fasted i.e., mean of baseline samples (−10 and −5 min) vs. fed state (45- and 60-min sample).

Experiment 2. INTRAVENOUS GLUCOSE TOLERANCE TEST. Both hay and concentrate were withheld from the sheep for a period of 24 h. The following morning, two baseline blood samples (2 ml; −10 and −5 min) were collected into chilled heparinized tubes before feeding, and then subsequently at 2, 5, 10, 15, 20, 30, 40, 60, 80, 120, 150, 180, 210, and 240 min after 0.5 g/kg glucose was administered intravenously (in 0.9% NaCl) within 2 min, through the jugular catheter at time 0. All blood samples were centrifuged immediately for 5 min at 4°C and, after decanting of the supernatant plasma, were stored at −20°C for further analysis of plasma glucose, insulin, and nonesterified fatty acids (NEFA) concentrations. In two sheep, a similar volume (~45 ml) of 0.9% NaCl only was administered as a control. Values for glucose, insulin, and NEFA remained unchanged from baseline in these sheep (data not shown).

Biochemical Analyses

Plasma concentrations of glucose and NEFA were measured enzymatically as described by Symonds et al. (39), whereas plasma triglycerides were determined with the use of a commercial colorimetric kit (GPO-PAP TR212; Randox Laboratories, Crumlin, Antrim, UK) adapted for use with 96-well plates. The intra- and interassay coefficients of variation were 6.4 and 9.6% (n = 10 and 8, respectively). Plasma cortisol and leptin were both determined by radioimmunoassay as previously described in detail (6, 10). Insulin (50 µl plasma) was assayed in duplicate using a commercially available coated tube assay (INSI-CTK IRMA; Diasorin, Wokingham, UK). The assay uses human insulin as the reference standard and has a minimum detection limit of 4.3 pmol/l. The intra-assay variation was <5%. All samples for insulin were assayed at the same time with the same standard curve.

Molecular Analyses

At the end of all experimental protocols, the 1-yr-old sheep were humanely euthanized with a lethal dose of pentobarbitol sodium (Euthatal; 100 mg/kg). All major organ weights were recorded, and tissue samples were flash frozen in liquid nitrogen and stored at −80°C. A portion of perirenal fat and skeletal muscle were also collected for Western blot analysis as previously described for the rat (16) but with the following modifications for sheep. The cleared protein lysates from each animal were standardized to a final concentration of 0.5 mg/ml in Laemmli’s sample buffer and equal amounts of protein for each animal (10 µg) were loaded onto 10% SDS
polyacrylamide gels for separation by electrophoresis. The ovine antibodies used in this study were to insulin receptor β-subunit (Irβ; Santa Cruz sc-711, Autogen Biochek, UK); phosphatidylinositol 3-kinase (PI3-kinase) p110 β-subunit (p110β; Santa Cruz); PI3-kinase p85 α-subunit (p85α; Upstate Biotech); and GLUT4 (Abcam, Cambridge, UK). Secondary antibodies and an antibody-binding (enhanced chemiluminescence) kit were both obtained from Amersham.

Calculations

For the intravenous glucose tolerance test (IVGTT) a glucose and insulin curve was plotted. From this plot the rapid increases and gradual decay of glucose, and subsequently insulin, a value for area under the curve (AUC) was derived using the GraphPad Prism software (Version 3; GraphPad). Variables derived were under the curve (AUC) was derived using the GraphPad Prism software (Version 3; GraphPad). Variables derived were

Statistical Analyses

All data are expressed as means ± SE unless otherwise stated. Effects of the nutritional group on measures of postnatal growth rates, glucose homeostasis, insulin secretion, and plasma metabolites or hormones were analyzed by univariate general linear model with group and sex as fixed effects using SPSS version 11.5 (SPSS, Chicago, IL). Relationships between pairs of variables were analyzed by Pearson’s correlation across all animals (SPSS version 11.5). Descriptive data for maternal weight, food intake, and birth weight were analyzed by one-way ANOVA (SPSS version 11.5). For a comparison between hormone concentrations in the fed and fasted state, a paired Student’s t-test was employed. For all comparisons, statistical significance was accepted when P < 0.05.

RESULTS

Gestation and Postnatal Growth

Control sheep consumed from 7.6 ± 0.3 MJ/day at week 1 of pregnancy to 15.8 ± 0.9 control singletons (CS) and 18.9 ± 0.8 control twins (CT) MJ/day at week 21 of pregnancy. NR days 1–30 (NRE) and NR days 110–140 sheep (NRL) consumed 50% of that amount for the first 30 days (3.61 ± 0.11 MJ/day) or days 110–140 (8.67 ± 0.15 MJ/day), respectively. At all other times, the undernourished groups consumed 100% of control intake. Birth weights were similar between singleton lambs in all groups (CS, 4.4 ± 0.4 kg; NRE, 4.2 ± 0.3 kg; NRL, 4.9 ± 0.3 kg) but control-fed twin lambs were significantly smaller at birth (CT, 3.4 ± 0.2 kg). There was no difference in the growth rates (current wt – birth wt/time) of lambs in each group during the first 3 mo (0–3 mo), second 3 mo (4–6 mo), and between 7–12 mo: CS, 1.66 ± 0.31, 1.27 ± 0.15, 0.92 ± 0.07 kg/wk; CT, 1.99 ± 0.16, 1.47 ± 0.09, 0.89 ± 0.11; NRE, 1.62 ± 0.18, 1.32 ± 0.08, 0.88 ± 0.09; NRL, 2.62 ± 0.19, 2.01 ± 0.08, and 1.02 ± 0.09, respectively. Rates of growth in all groups slowed over time (P < 0.0001). At 1 yr of age, when the metabolic studies were undertaken, there was no difference in body weight between the groups of sheep (CS, 47.1 ± 4.0; CT, 50.2 ± 5.9; NRE, 49.8 ± 4.3; NRL, 58 ± 5 kg).

Fed vs. Fasted Metabolite and Leptin Concentrations

The fed and fasted plasma concentrations of metabolites and leptin are given in Table 1. Fasted plasma glucose concentration was similar between groups and values did not change significantly during feeding. In contrast, plasma NEFA significantly decreased from fasted values during feeding in CS (P < 0.01) and NRE (P < 0.002), but no effect was observed in NRL. There was no effect of fasting or feeding on plasma triglycerides or leptin. Fasted values for plasma leptin and triglycerides showed a strong positive correlation with each other (r = 0.74, P < 0.01). Gender had no effect on any metabolite or hormone values as assessed using a univariate general linear model with sex as a fixed influence. Similarly basal plasma cortisol concentration was unaffected by any of the variables assessed (e.g., CS, 31.6 ± 7.5; CT, 29.5 ± 5.5; NRE, 42.7 ± 7.0; NRL, 35.7 ± 12.7 nM).

| Table 1. Fed and fasted plasma concentrations of glucose, nonesterified fatty acids (NEFA), triglycerides, and leptin in control and nutrient-restricted sheep at 1 yr of age |
|-----------------|-------------|-------------|-------------|
|                 | CS          | CT          | NRE         | NRL         |
|                 | 5           | 5           | 4           |
| Glucose, mM     |             |             |             |
| Fed             | 5.59 ± 0.55 | 4.88 ± 0.17 | 5.42 ± 0.54 | 5.25 ± 0.51 |
| NRE             | 4.94 ± 0.60 | 6.08 ± 0.91 | 7.28 ± 1.28 | 7.14 ± 1.82 |
| NEFA, mM        |             |             |             |
| Fed             | 0.63 ± 0.13 | 0.45 ± 0.11 | 0.95 ± 0.12 | 0.45 ± 0.11 |
| NRE             | 0.12 ± 0.01*| 0.15 ± 0.04*| 0.13 ± 0.04*| 0.42 ± 0.19 |
| Triglycerides, mM|             |             |             |
| Fed             | 0.18 ± 0.02 | 0.20 ± 0.04 | 0.23 ± 0.03 | 0.31 ± 0.07 |
| NRE             | 0.21 ± 0.02 | 0.25 ± 0.02 | 0.24 ± 0.03 | 0.26 ± 0.02 |
| Leptin, ng/ml   |             |             |             |
| Fed             | 0.99 ± 0.10 | 1.36 ± 0.28 | 0.93 ± 0.19 | 3.27 ± 1.52 |
| NRE             | 2.60 ± 0.73 | 1.84 ± 0.54 | 2.20 ± 1.23 | 1.49 ± 0.52 |

Values are means ± SE for control single (CS), control twins (CT), early nutrient-restricted single (NRE), and late nutrient-restricted single (NRL) sheep. Fasted concentrations reflect the mean values for 2 baseline samples, and the fed samples are the mean values for 2 samples collected between 45–60 min after feeding. Data were statistically tested using Student’s paired t-test and differences are: *P < 0.05 fasted vs. fed.

IVGTT

Baseline plasma glucose (CS, 5.11 ± 0.43; CT, 5.35 ± 0.35; NRE, 5.42 ± 0.52; NRE, 5.25 ± 0.66 mM) and insulin (CS, 100 ± 18; CT, 113 ± 25; NRE, 76 ± 8.3; NRL, 148 ± 41 nM) concentrations were similar between dietary groups. Infusion of an intravenous glucose load significantly increased plasma glucose (Fig. 1) and insulin (Fig. 2) concentration in all groups of sheep with the peak values achieved being similar (CS, 21.7 ± 1.0 and 1.146 ± 196; CT, 21.7 ± 0.5 and 990 ± 102; NRE, 22.6 ± 0.5 and 803 ± 132; NRL, 24.5 ± 1.8 mM and 1,165 ± 129 nM for glucose and insulin, respectively). There was no significant effect of diet or fetal number on the maximal change in plasma glucose, or insulin, or time to restore plasma glucose to baseline; however, the time taken for plasma insulin to return to baseline after the IVGTT was significantly increased in NRL relative to CS (Table 2, P = 0.02). In addition, glucose tolerance as defined by glucose AUC was decreased
GLUT4 expression \( (r = -0.48, P = 0.049) \) predicted worse glucose tolerance \( (i.e., \text{increased } \text{Glu}_{AUC} \text{ of the offspring}) \). However, when current weight was controlled for in a partial correlation analysis, the relationship with GLUT4 became nonsignificant \( (r = -0.32, P = 0.22) \), and the significance of the relationships with p110β and Irβ were weakened \( (r = 0.56, P = 0.06; r = 0.44, P = 0.07, \text{respectively}) \). The positive relationship between muscle Irβ and Glu_AUC remained after controlling for current weight. Fasting plasma insulin and Ins_AUC correlated negatively with muscle \( (r = -0.56, P = 0.01) \) and adipose GLUT4 expression \( (r = -0.61, P = 0.01) \), respectively. Allowing for current weight weakened the relationship between Ins_AUC and GLUT4 \( (r = -0.38, P = \text{not significant}) \) but had no effect on the relationship between fasting plasma insulin and muscle GLUT4. There was a strong positive correlation between Ins_AUC and p110β expression in adipose tissue \( (r = 0.71, P < 0.001) \), which remained after controlling for current weight.

**Sheep biometry at 1 yr of age.** There were no differences between dietary groups in the weights of any organ measured. However, the proportion of adipose tissue in central (omental) and peripheral (perirenal) depots was significantly \( (P = 0.01) \) greater in NRL relative to other groups of sheep (Table 3).

**DISCUSSION**

In this study, it has been shown that undernutrition confined to late gestation, during the period of maximal fetal growth, impacts negatively on the subsequent young adults’ glucose-insulin homeostasis \( i.e., \) they show evidence of glucose intolerance \( (\text{increased } \text{Glu}_{AUC}) \) and insulin resistance \( (\text{increased } \text{Ins}_{AUC}) \). Importantly, no major change in glucose tolerance and/or insulin sensitivity was observed in the young adult offspring of sheep undernourished for an equivalent period during very early gestation. The underlying mechanism for disturbed glucose-insulin homeostasis in these late gestation undernourished offspring appears to be altered tissue \( (\text{specifically, adipose rather than muscle}) \) glucose uptake, through reduced cellular expression of GLUT4. In addition, the study has clearly shown that the lower birth weight of twins has no

---

**Protein Expression of Insulin Signaling Molecules**

**Perirenal fat.** Western blot analysis indicated a significant increase in the protein for the Irβ (Fig. 4A) and p110β of PI3-kinase (Fig. 4B), but significant decrease in GLUT4 protein expression (Fig. 4C) in NRL relative to the other nutritional groups \( (P < 0.01 \text{ all cases}) \). There was no effect of nutritional group or fetal number on the expression of the p85α of PI3-kinase \( (CS, 20,833 \pm 1,251; CT, 23,089 \pm 1,148; \text{NRE}, 18,755 \pm 2,438; \text{NRL}, 24,074 \pm 1,339 \text{ AU}) \).

**Muscle.** Western blot analysis indicated no differences in protein for the p85α of PI3-kinase \( (CS, 21,157 \pm 2,052; CT, 22,916 \pm 691; \text{NRE}, 22,737 \pm 1,968; \text{NRL}, 21,156 \pm 2,677 \text{ arbitrary units}) \), or GLUT4 \( (CS, 38,002 \pm 3,694; CT, 29,004 \pm 7,304; \text{NRE}, 31,846 \pm 4,499; \text{NRL}, 24,017 \pm 7,619 \text{ AU}) \) protein expression in the muscle. There was, however, a significant effect of nutritional group on Irβ protein expression, with NRE having increased levels compared with the other groups \( (CS, 17,516 \pm 2,488; CT, 13,137 \pm 2,315; \text{NRE}, 25,418 \pm 2,000; \text{NRL}, 19,516 \pm 3,492 \text{ AU}; P = 0.049 \text{ CT vs. NRE}) \). Importantly, the expression of p110β protein in muscle was <10% of the expression in perirenal fat \( (1,072 \pm 593 \text{ vs. } 16,366 \pm 3,388 \text{ AU}; P < 0.0001) \).

**Determinants of glucose-insulin handling.** There was no correlation between birth weight and glucose-insulin handling or insulin signaling proteins at 1 yr of age in the current cohort of sheep. Higher adipose \( (r = 0.56, P = 0.01) \) and muscle \( (r = 0.54, P = 0.01) \) p110β and Irβ \( (r = 0.45, P = 0.057) \) but lower
bearing on their future metabolic competence, at least in terms of their glucose-insulin handling. Retrospective data from the Dutch Hunger Winter Famine have shown quite clearly that specific periods of famine exposure may impact on specific physiological control systems in adult life. For example, exposure during early gestation influenced the cardiovascular system, clinically reflected as an increased risk of coronary heart disease (37); whereas exposure during late gestation tended to affect intermediary metabolism, in particular, glucose-insulin homeostasis, and was clinically reflected as an increased risk of type 2 diabetes (35). Subsequent animal studies established to model these epidemiological data have recognized early programming of the metabolic syndrome and have used a similar degree of undernutrition i.e., 50% normal intake (protein and/or energy) (for reviews see Refs. 3 and 30). However, in general, the diet in these studies has been restricted either throughout gestation or throughout gestation and lactation, thereby covering the entire period of embryonic, fetal, and postnatal growth. This is important when one further considers the relatively greater nutritive demands of the small compared with large animal species used in these studies (28). Nevertheless, in larger farm animal species, programming of cardiovascular and metabolic function has been described, although the magnitude of adaptation is attenuated compared with that described in small laboratory species. These studies have focused on the impact of either birth size (33, 34) or excess glucocorticoid exposure (12) rather than the plane of maternal nutrition. Two recent studies have now directly examined the effect of a specific period of maternal undernutrition (early/early-mid gestation) on adult physiological function in a larger species and have shown programming of cardiovascular control (19, 21) in common with the observations after early famine exposure in the Dutch Hunger Winter Famine. In the current study, these observations are extended to include a further group of late-gestation undernourished animals for comparison and to an examination of metabolic function in the young adult offspring of a large animal species, two hitherto unknown endpoints.

When the current study is considered alongside a contemporaneous study (19), it is clear that late-gestation undernutrition has a more profound impact on intermediary metabolism, whereas early-gestation undernutrition affects cardiovascular control (19), two observations that replicate the Dutch Famine data. Furthermore, we have shown that despite the lower birth weight and relatively increased early growth rate of twins, their intermediary metabolism (current study) and cardiovascular control (19) are not compromised. Thus developmental programming is not related to birth weight per se, which in multiple births is largely due to maternal constraint but is apparent only when the intra/extraterine environment is specifically challenged or inappropriate at that point in time e.g., through nutritional imbalance (under/overnutrition). Twins appear to have specific adaptations to accommodate their compromised prenatal environment (13, 18), but these adaptations appear to confer no further disadvantage in later life. In contrast, brief periods of maternal undernutrition elicit changes

Table 2. Derived values from intravenous glucose tolerance test in control and nutrient-restricted sheep at 1 yr of age

<table>
<thead>
<tr>
<th></th>
<th>CS</th>
<th>CT</th>
<th>NRE</th>
<th>NRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Peak change in glucose, mM</td>
<td>16.6±1.2</td>
<td>16.4±0.7</td>
<td>17.2±0.3</td>
<td>19.3±1.4</td>
</tr>
<tr>
<td>Peak change in insulin, nM</td>
<td>1045±194</td>
<td>876±112</td>
<td>726±131</td>
<td>1134±36</td>
</tr>
<tr>
<td>Time to baseline (glucose), min</td>
<td>175±23</td>
<td>137±7</td>
<td>172±18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>149±20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to baseline (insulin), min</td>
<td>137±7</td>
<td>172±18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>149±20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225±9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose tolerance, (min·mmol⁻¹·l⁻¹)</td>
<td>1111±54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>964±109&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1340±206&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1619±170&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin secretion: (min·mmol⁻¹·l⁻¹)</td>
<td>69.5±9.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.5±6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.0±10.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>115.6±22.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative insulin secretion: (nmol·mmol⁻¹·l⁻¹)</td>
<td>63.2±9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.6±6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.2±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.6±15.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose clearance: (mmol·min⁻¹·l⁻¹)</td>
<td>10.6±1.3</td>
<td>8.1±1.2</td>
<td>8.4±0.6</td>
<td>10.3±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for CS, CT, NRE, and NRL sheep. Values were derived as described in MATERIALS AND METHODS. Values arranged in individual rows with differing superscript letters are statistically different at P < 0.05 (1-way ANOVA).

Fig. 3. Plasma glucose (A) and insulin (B) after an IVGTT in male and female control sheep at 1 yr of age. Values are means ± SE for male (n = 5) and female (n = 5) sheep. Glucose (0.5 g/kg in 0.9% NaCl) was administered as a bolus over 2 min. Blood samples were taken during baseline at −10 and −5 min and subsequently at 2, 5, 10, 15, 20, 30, 40, 60, 80, 120, 150, 180, 210, and 240 min after the glucose bolus.
in the developmental pattern and have further consequences as the individual ages.

The reduced glucose tolerance in NRL vs. other groups in the current study is very clear despite the relatively low number in this group. This glucose intolerance is related to insulin resistance and not insulin deficiency as indicated by the increased insulin AUC during the glucose tolerance test. As in human diabetes, the molecular basis of this insulin resistance appears to lie downstream of the insulin receptor (25), given the increase in expression of the adipose insulin receptor in the NRL group. However, GLUT4, the major insulin-responsive glucose transporter, was substantially reduced in adipose, but not muscle, tissue from the NRL group, suggesting that impaired glucose tolerance was related to the ability of adipose tissue to take up glucose in an insulin-responsive manner. Indeed, expression of adipose-specific GLUT4 is essential for whole body glucose disposal (41), and insulin resistance has been shown to be related to adipose tissue-specific reductions in GLUT4 expression. In the current study, there is no effect of prenatal diet on the p85α of PI3-kinase, and the increase in adipose p110β appears more related to current weight than prenatal diet; however, the interaction between these two subunits may be deficient (16). In contrast to the present study, in low-birth-weight humans (31) and in low-protein-exposed rats (16), p110β is actually reduced, perhaps reflecting the reductions in birth weight in these studies as opposed to the current study where birth weight was unaffected by the dietary regimen.

Although we cannot separate cause or effect in relation to the increased adiposity, alteration in expression of insulin-signaling components, and reduced glucose tolerance of NRL in the current study, it has previously been shown (16) that the subcellular insulin signaling proteins downstream of the receptor may be programmed before development of increased adiposity and glucose intolerance. Thus it is conceivable that late-gestation undernutrition provokes a compensatory increase in adipose insulin receptor in response to the reduction in maternal and/or fetal glucose concentrations (50% reduction with NRL dietary regimen, see Ref. 14). Although the NRL regimen may reduce term fetal adipose mass (40), irreversibly increased adipose insulin receptor may sustain a postnatal anabolic drive toward increased adipose deposition. The underlying mechanism for reduced GLUT4 following late-gestation undernutrition is unclear; however, it has been shown that hyperinsulinemia during late gestation in the ovine fetus produces tissue-specific (i.e., in insulin sensitive tissues such as myocardium, adipose) insulin resistance, and reduced GLUT4 expression (2).

Table 3. Sheep biometry at 1 yr of age

<table>
<thead>
<tr>
<th>Tissue Mass</th>
<th>CS</th>
<th>CT</th>
<th>NRE</th>
<th>NRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain weight</td>
<td>96.7±3.5</td>
<td>99.7±2.8</td>
<td>93.5±1.5</td>
<td>94.3±3.5</td>
</tr>
<tr>
<td>Heart weight</td>
<td>178±17</td>
<td>195±21</td>
<td>213±14</td>
<td>225±22</td>
</tr>
<tr>
<td>Liver weight</td>
<td>633±32</td>
<td>688±107</td>
<td>669±99</td>
<td>672±72</td>
</tr>
<tr>
<td>Kidney weight</td>
<td>11.1±9</td>
<td>116±6</td>
<td>119±6</td>
<td>117±35</td>
</tr>
<tr>
<td>Adrenal weight</td>
<td>2.90±0.64</td>
<td>3.17±0.40</td>
<td>3.17±0.20</td>
<td>2.66±0.66</td>
</tr>
<tr>
<td>Spleen weight</td>
<td>11.1±19</td>
<td>110±10</td>
<td>92±15</td>
<td>145±33</td>
</tr>
<tr>
<td>Lung weight</td>
<td>475±19</td>
<td>476±21</td>
<td>464±22</td>
<td>478±40</td>
</tr>
<tr>
<td>Pancreas weight</td>
<td>42.5±3.5</td>
<td>49.0±10.1</td>
<td>43.3±7.8</td>
<td>29.0±2.4</td>
</tr>
<tr>
<td>Perirenal adipose tissue</td>
<td>104±25a</td>
<td>189±33a</td>
<td>151±27a</td>
<td>361±65b</td>
</tr>
<tr>
<td>Omental adipose tissue</td>
<td>259±96</td>
<td>295±41</td>
<td>273±31</td>
<td>558±132</td>
</tr>
<tr>
<td>Relative fat mass</td>
<td>8.09±1.89</td>
<td>11.24±1.36</td>
<td>9.72±0.60</td>
<td>17.52±4.33c</td>
</tr>
</tbody>
</table>

Values are means ± SE in grams for all weights and tissues, except relative fat mass, which is in grams per kilogram, for (CS; n = 5), CT; (n = 5), NRE and NRL sheep. Values arranged in individual rows with differing superscript letters are significantly different at P < 0.05.
It is clear, therefore, that the period of exposure to a dietary imbalance during gestation may have different effects on the offspring depending on when the imposition occurs. The extent to which these effects may be amplified or ameliorated by the postnatal environment; i.e., the thrifty phenotype cannot be determined in the current study, because all sheep were treated similarly postnatally; but the potential is obvious (15). In addition, it is clear from studies in rats that the age-related decline in glucose tolerance in prenatally protein-restricted offspring is sex specific; i.e., it is delayed in female compared with male rats (16). When included as an independent covariate, the sex of the offspring did not influence any of the outcome measures in the current study; however, it is recognized that we do not have sufficient statistical power to draw firm conclusions regarding sex-specific effects on adult glucose metabolism after prenatal undernutrition in sheep. In addition, future studies should consider longitudinal assessments of glucose tolerance and insulin sensitivity of prenatally programmed male and female sheep to determine whether the observed decline with age (20) is exacerbated by a poor prenatal environment, specifically during functional development of fetal pancreas, i.e., mid-late gestation (17).

ACKNOWLEDGMENTS

The authors acknowledge the Joint Animal Breeding Unit, University of Nottingham for the routine care of the animals used in this study.

GRANTS

This work was supported by the British Heart Foundation Grant BS/03/001, Diabetes United Kingdom Grant RD03/0002677, and a Clinical Endocrinology Trust Medical Student award (to B. W. M. Van Bon).

REFERENCES