Programming of intermediate metabolism in young lambs affected by late gestational maternal undernourishment

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Husted SM, Nielsen MO, Tygesen MP, Kiani A, Blache D, Ingvartsen KL. Programming of intermediate metabolism in young lambs affected by late gestational maternal undernourishment. Am J Physiol Endocrinol Metab 293: E548–E557, 2007. First published May 15, 2007; doi:10.1152/ajpendo.00441.2006.—Effects of moderate maternal undernourishment during late gestation on the intermediary metabolism and maturational changes in young lambs were investigated. 20 twin-bearing sheep, bred to two different rams, were randomly allocated the last 6 wk of gestation to either a NORM diet [barley, protein supplement, and silage ad libitum ~15 MJ metabolizable energy (ME)/day] or a LOW diet (50% of ME intake in NORM, offered exclusively as silage ~7 MJ ME/day). Post partum, ewes were fed to requirement. After weaning, lambs were fed concentrate and hay ad libitum. At 10 and 19 wk of age, lambs were subjected to an intravenous glucose tolerance test (IGTT) followed by 24 h of fasting. Heat energy (HE) was determined in a respiration chamber at 9 or 20 wk of age. LOW lambs had a lower birth weight and continued to be lighter throughout the experiment. Glucose tolerance did not differ between groups. However, 19-wk-old LOW lambs secreted less insulin during IGTT, released more NEFA, and tended to have lower leptin during fasting than NORM. Surprisingly, several metabolite and hormone responses during IGTT and fasting were greatly influenced by the paternal heritage. In conclusion, when lambs entered adolescence (19 wk) programming effects of late prenatal malnutrition on the glucose-insulin homeostasis and metabolism were manifested: LOW lambs had less insulin-secretory capacity, but this was apparently compensated for by increased target tissue sensitivity for insulin, and adipose lipolytic capacity increased during fasting. Thereby, glucose may be spared through increased lipid oxidation, but overall energetic efficiency is apparently deteriorated rather than improved.

glucose tolerance; insulin sensibility; undernutrition

OVER THE LAST DECADE, retrospective epidemiological studies and extensive animal studies have revealed an inverse relationship between fetal and/or early postnatal development and the risk of developing insulin resistance, hypertension, metabolic syndrome, coronary heart disease, and type 2 diabetes later in life (1, 2, 26, 37).

Epidemiological studies conducted in humans from Hertfordshire and Preston, UK, have established a link between lower birth weight or weight at 1 yr of age and impaired glucose tolerance and subsequent development of type 2 diabetes (24, 44), syndrome X (type 2 diabetes, hypertension, and hyperlipidemia) (2) and higher incidence of death from ischemic heart disease (3). Hales and Barker hypothesized in the “thrifty phenotype hypothesis” that this adverse outcome of low birth weight reflects poor maternal nutrition or placental dysfunction leading to fetal malnutrition, which in turn alters the development of structure and function of a variety of organs i.e., pancreas (23) and liver (14). Studies of both young (~32 yr) and older (~66 yr) twins dissimilar for type 2 diabetes and/or glucose tolerance support the hypothesis of a strong environmental rather than a genetic influence, since birth weights were lower in both the diabetic or glucose-intolerant monozygotic and dizygotic twins compared with their glucose-tolerant or nondiabetic cotwins (6, 45). It is important to keep in mind that a wide range of different insults during and even before pregnancy, like maternal malnutrition, reduced uterine blood flow, impaired function of placenta, or reduced fetal blood flow all are very likely to result in fetal undernourishment and subsequent impaired fetal growth and reduced birth weight.

The outcome of fetal undernourishment later in life appears to depend largely on the timing of the insult. Some of the most convincing data regarding development of different adverse effects and the prenatal timing have come from the retrospective studies of hunger examining the Dutch Winter Famine. Subjects exposed to famine in early gestation had a higher prevalence of coronary heart disease at 50 yr of age compared with nonexposed subjects or subjects exposed to famine during mid and late gestation (49). However, the glucose tolerance at 50 yr of age was decreased most among subjects who were exposed to famine during mid or late gestation compared with nonexposed subjects or subjects exposed during early gestation (47). Furthermore the obesity rate among young men (19 yr of age) showed completely opposite trends. Compared with nonexposed subjects, exposure to undernutrition in late gestation and early postnatal life resulted in a low obesity rate, which was in sharp contrast to a high obesity rate in young men exposed during early gestation (48). Since the different organ systems develop and differentiate in a predetermined manner, it is not surprising that the timing of a nutritional insult may result in more or less distinct phenotypic consequences later in life. For instance, the functional development of the fetal pancreas during mid to late gestation (17) is of great importance for the glucose-insulin homeostasis both in the fetus and in later life.

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A few animal studies have been set up to model these epidemiologic findings and confirmed effects of undernourishment in early gestation on cardiovascular function (18, 27, 33), whereas exposure to prenatal undernourishment during late gestation affected intermediary metabolism, especially glucose-insulin homeostasis (4, 19) and pancreatic β-cell development, as a predisposing factor of impaired glucose tolerance in adult offspring (4). Another study with sheep severely undernourished for either 10 or 20 days during late gestation suggested that birth weight rather than maternal nutrition during late gestation, had an impact on glucose tolerance tested at 5 and 30 mo of age, (36). However, in most other animal studies able to replicate the epidemiological findings and produce risk factors of type 2 diabetes, such as hypertension and insulin resistance in adult offspring, the maternal undernourishment was prolonged throughout gestation and lactation (16, 32, 41, 44). To clarify the impact of prenatal undernourishment confined to only late gestation, on glucose-insulin metabolism and homeostasis in the offspring, further investigation is needed.

With normal maturation and aging, glucose tolerance changes, perhaps in a species-specific manner (21). In both rat and sheep, glucose tolerance has been found to decrease between the early postnatal life and early adulthood due to normal maturational loss of insulin sensitivity (7, 12, 21). The age-dependent change in glucose tolerance, however, appears to be accelerated in offspring that have experienced prenatal undernourishment. In young adult life, prenatally undernourished offspring are demonstrated to have a better glucose tolerance than controls (12, 25, 31, 50), due at least in part to increased insulin sensitivity in target tissues (38, 41, 50). By old age, the glucose tolerance is shown to be significantly worse compared with controls due to insulin deficiency and resistance (25).

It was therefore hypothesized that moderate global maternal undernourishment in late gestation programs the glucose-insulin homeostasis and, hence, intermediary and quantitative metabolism in the offspring and that these programming effects change and become manifested in an age-dependent way during early postnatal life.

To elucidate the above hypotheses, twin-pregnant ewes were subjected to moderate global undernourishment during the last 6 (of 21) wk of gestation, and the impacts on glucose-insulin homeostasis and heat energy production were evaluated in adolescent offspring at two different ages and in the fed and fasted states.

**MATERIALS AND METHODS**

**Experimental Animals and Design**

Forty Shropshire lambs of both sexes, born in April 2003 from 20 twin-bearing ewes in their second or third parity, were used in the experiment at 10 and 19 wk of age. Their mothers were evenly selected from two herds, bred during natural breeding season to two different rams, which differed in the standard breeding index for slaughter quality (34) (index value 93 vs. 117) referred to hereafter as SQ− and SQ+ respectively. On the basis of twin pregnancy and even distribution of body condition score (BCS) 70 days pre partum, the ewes were randomly allocated to feeding treatment (NORM and LOW), for the last 6 wk pre partum, as described below. The characteristics of the experimental animals are presented in Table 1. The experiment was conducted from December 2002 to July 2003 at the large laboratory animal facility, Rørrendegaard, The Royal Veterinary and Agricultural University, Tåstrup, Denmark. All experimental procedures were approved by The National Committee on Animal Experimentation, Denmark.

**Experimental Housing and Feeding**

Prior to the experimental period, the ewes were kept on pasture but allowed a 2-wk period for adaptation to indoor experimental conditions. During the experimental period, ewes and lambs were housed in family pens (1 × 2 m), and later, at weaning, 8 wk post partum, the ewes were returned to pasture and the lambs were left indoors in individual pens (1 × 1 m) with shaving bedding. The last 6 wk pre partum, the ewes were fed the experimental diets. The two diets were composed as an ad libitum diet designed to approximately fulfill daily requirements, according to Danish requirements for protein and energy (46); silage ad libitum [2.64 kg per feed unit (FU) and 7.9% crude protein], 13% of which was long-straw and forage mix (34% crude protein) as a substitute for 7.9% of the silage. Further, the experimental diets were composed as an ad libitum diet designed to approximately fulfill daily requirements, according to Danish requirements for protein and energy (46); silage ad libitum [2.64 kg per feed unit (FU) and 7.9% crude protein], 13% of which was long-straw and forage mix (34% crude protein) as a substitute for 7.9% of the silage.

**Table 1. Experimental animals, design, and diet**

<table>
<thead>
<tr>
<th>Pre Partum Feeding Level (Ewe)</th>
<th>NORM</th>
<th>LOW</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of twin pregnant ewes*</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Weight of ewes 6 wk pre partum, kg</td>
<td>69.9±4.2</td>
<td>70.5±2.3</td>
<td>0.767</td>
</tr>
<tr>
<td>BCS of ewes 6 wk pre partum</td>
<td>4.3±0.1</td>
<td>4.1±0.1</td>
<td>0.283</td>
</tr>
<tr>
<td>Weight of ewes just prior to partum, kg</td>
<td>78.5±3.0</td>
<td>70.5±2.8</td>
<td>0.002</td>
</tr>
<tr>
<td>BCS of ewes just prior to partum</td>
<td>4.2±0.2</td>
<td>3.3±0.2</td>
<td>0.050</td>
</tr>
<tr>
<td>Feed intake (ME) the last 6 wk pre partum, MJ/day</td>
<td>1.4±0.7</td>
<td>1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crude protein intake the last 6 wk pre partum, g/day</td>
<td>229</td>
<td>112</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feed intake (ME) during lactation, MJ/day</td>
<td>21–25</td>
<td>15–24</td>
<td>0.030</td>
</tr>
<tr>
<td>Crude protein intake during lactation, g/day</td>
<td>378</td>
<td>329</td>
<td>0.014</td>
</tr>
<tr>
<td>No. of lambs†</td>
<td>21</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>No. of each sex (M/F)</td>
<td>14/7</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>4.0±0.1</td>
<td>3.1±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Range in birth weight, kg</td>
<td>3.2–4.8</td>
<td>1.6–4.0</td>
<td></td>
</tr>
<tr>
<td>Weight of lambs at 10 wk of age, kg</td>
<td>26.8±1.1</td>
<td>23.2±1.1</td>
<td>0.012</td>
</tr>
<tr>
<td>Weight of lambs at 19 wk of age, kg</td>
<td>47.0±1.2</td>
<td>42.2±1.3</td>
<td>0.010</td>
</tr>
<tr>
<td>DGR from birth to 10 wk of age, g/day</td>
<td>333±13</td>
<td>286±15</td>
<td>0.024</td>
</tr>
<tr>
<td>DGR from 10 to 19 wk age, g/day</td>
<td>283±11</td>
<td>272±12</td>
<td>0.491</td>
</tr>
</tbody>
</table>

Data are presented as least square means ± SE except feed intake and birth weight, which are presented as a range. *Uneven nos. of ewes in the 2 groups were due to exclusion of 2 ewes from the LOW group (fed the restricted diet) because of stillbirth of 1 of the twin lambs. †One male lamb died of urolithiasis just after weaning. BCS, body condition score, scale from 1 to 5 (very lean to very fat); ME, metabolizable energy; DGR, daily growth rate.
E550  FETAL UNDERNUTRITION AND POSTNATAL METABOLISM

Crude protein (CP) plus increasing amounts of an equal mixture of barley (1.0 kg/FU and 10.4% CP) and protein supplement (0.70 kg/FU and 45.5% CP, Lactamin Plus; Skovhus Fåregaard, Stenhusen, Denmark: NORM), up to each 0.4 kg/day, and a restricted diet (silage alone with no feeding of supplements, set to 50% of the net energy intake recorded on a weekly basis in the NORM-fed sheep: LOW). After lambing, all ewes were fed silage ad libitum and adapted to increasing amounts of commercial concentrate [0.98 kg/FU and 14% CP (Färemix, DLG, Denmark)] up to 1.0 kg/day. The average metabolizable energy (ME) intake during the last 6 wk of gestation and during lactation is stated in Table 1. Ewes lambed naturally. Lambs were denied colostrum by covering the udder of the ewe with a specially designed “udder cover” for the first 3 h after birth. To record colostrum production (53), the ewes were then hand milked (the udder emptied) after an intramuscular injection of 100 ml of oxytocin (Novartis, Copenhagen, Denmark), and 200 ml of colostrum was given to the lambs through a stomach tube. All lambs were thereafter allowed to suckle their mother until weaning. From 3 to wk age, lambs were offered the same commercial concentrate as the ewes and silage ad libitum. At weaning, the roughage supply was gradually changed to ad libitum access to artificially dried grass (1.70 kg/FU and 19.9% CP), and the concentrate was changed to commercial concentrate (0.88 kg/FU and 14% CP; Woller, Slangerup Forderstofforening, Denmark) more suitable for growing lambs. All sheep were fed twice a day at approximately 0730 and 1530, one-half of the ration being given at each meal, and provided with fresh drinking water and minerals ad libitum at all times.

### Experimental Procedures

**Glucose challenge, fasting, and refeeding.** At an average age of 72 ± 4 and 133 ± 5 days, all lambs were subjected to an intravenous glucose tolerance test (IGTT) followed by a 24-h fasting period and an 8-h refeeding period. The experiment is outlined in Fig. 1. On day 1, at least 1 h prior to IGTT, all lambs were weighed and fitted with a temporary inserted catheter (Secalon T, 18-gauge; BD Critical Care Systems, Singapore) in a jugular vein. The catheters were locked with a 0.9 ml of isotonic sodium chloride solution containing 100 IU/ml heparin. At time 0 (−10 min), glucose solution containing 100 IU/ml heparin was infused at a rate of 0.45 g glucose/kg body wt. (0.5 g glucose/ml) was infused over 20 s, and the catheters were flushed with 10 ml of isotonic sodium chloride solution. Blood samples were collected in 10-ml syringes at −5, 5, 2.5, 10, 20, 30 and 60 min after the glucose bolus was injected. The first bolus of blood was collected was always discarded. After the IGTT, the catheters were locked and left. The following morning of day 2, at 0700, lambs had their feed removed and were subjected to a 24-h fasting period. Blood samples were drawn at 0 h (fed state) and times 6, 12, 18, and 24 h (fasted state). Right after the 24-h blood sample at 0700, day 3, the lambs were refed, and blood was drawn at 25 h (1 h after refeeding) and 32 h (8 h after refeeding; Fig. 1). All samples were immediately transferred to 4.5-ml tubes coated with EDTA and heparin and kept on ice until centrifugation (3,200 rpm at 4°C for 15 min), which took place within 20 min after sampling. Plasma was transferred to four labeled cryo vials and stored at −20°C until later analyses.

**Respiration experiment.** All lambs were subjected to a 7-day balance and respiration experiment, those sired by the SQ ram (n = 19) at an average age of 138 ± 3 days. The lambs were placed in metabolic cages 59 cm wide, 160 cm long, and 80 cm high. The cage floor was covered with a 90-cm-long rubber mat, where the animals were standing/hanging. After 2 days of adaptation, quantitative collection of feed residues and excreta (urine was collected in 60 ml of 10% sulfuric acid, and a 1% anhydrous citric acid solution was used for final rinsing of the collection device) was carried out once daily between 0900 and 1100 for 5 days, and 10% of the total amount collected from each day was stored at −18°C pending analyses. The respiration experiment took place at the fourth day and lasted 22 h, starting at 1100 and was carried out by means of indirect calorimetry in an open-air circulation system, as outlined by Chwalibog (11). Temperature and relative humidity in the chambers were kept within the range of 15–18°C and 65–75%, respectively, and a 12:12-h (0600–1800) light-dark cycle was maintained. Air from the chambers was analyzed every third minute for concentrations of O2, CO2, and CH4. Ten lambs (6 NORM and 4 LOW) sired by the SQ ram were additionally subjected to a respiration experiment at age 74 ± 1 days during their fasting period.

**Blood and urine analyses.** Glucose concentration in plasma was determined, in all samples obtained, with a commercially available spectrophotometric analytic kit, 17–25 Infinity (Sigma diagnostics, St. Louis, MO). Analyses of nonesterified fatty acid (NEFA), β-hydroxybutyrate (BHB), and acetate concentrations in plasma were undertaken on samples from times 0, 12, 24, 25, and 32 h during fasting and refeeding, using, respectively, a commercially available spectrophotometric analytic kit, Wako NEFA C kit (Wako, Neuss, Germany), a spectrophotometric assay according to Cant (10), and a commercially available enzymatic analytic kit: Enzymtec Acetic Acid Kit combined with a Multi Acid Standard, Enzymtec Multi Acid Standard for manual application (SCIL Diagnostics, Martinsried, Germany). Plasma insulin concentration was determined in samples obtained at times −5, 10, 30, and 60 in the IGTT and time 0, 24, and 32 during the fasting and refeeding and analyzed using a commercially available enzyme immunoassay, DRG Sheep Insulin ELISA (DRG Diagnostics, Marburg, Germany). Leptin and IGF-I analyses were performed at The University of Western Australia, Perth, on selected samples, obtained during fasting and refeeding, with a species-specific RIA using ovine leptin raised against bovine leptin, as described by Blache et al. (5) and by double-antibody RIA with human recombinant IGF-I and antihuman IGF-I antiserum according to Breier et al. (8), respectively. The intra- and interassay coefficients of variation were below 5 and 10%, respectively, for all assays.

The nitrogen content in the urine (NU) was determined by a micro Kjeldal method using a Tecator-Kjeltic system 1026 distilling unit (Tecator, Höganäs, Sweden). By mistake, the dry matter contents of the original feces samples were never determined, which makes the subsequent nitrogen analyses in the feces invaluable.

#### Calculations

The glucose tolerance (GT) was calculated as the area above the basic plasma glucose concentration and under the plasma glucose concentration curve during the IGTT. The absolute insulin secretion (AIS) was calculated as the area above the basic blood insulin concentration and under the plasma insulin concentration curve during the IGTT. And as a measure of the first-phase insulin secretion, the

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**Fig. 1.** Experimental outline for intravenous glucose tolerance test (IGTT), fasting, and refeeding in lambs at 10 and 19 wk of age. X indicates blood sampling point.

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**Table 1.** Fetal undernutrition and postnatal metabolism.
insulin increment (II) (Eq. 1) was calculated as the difference in insulin plasma concentration between times −5 and 10 min of the IGTT:

$$\text{insulin increment (ng/ml)} = [\text{insulin}]_{\text{time} \to 10} - [\text{insulin}]_{\text{time} \to -5} \quad (1)$$

Heat energy (HE) and respiratory quotient (RQ) were calculated from gaseous exchange (CO₂, O₂, CH₄) and nitrogen excretion in urine (UN) according to Brouwer’s equation (9)

$$\text{HE (kJ)} = 16.18 \times O_2(l) + 5.02 \times CO_2(l) - 5.99 \times NU(g)$$

$$- 2.17 \times CH_4(l) \quad (2)$$

$$\text{RQ} = \frac{\text{produced CO}_2(l)}{\text{consumed O}_2(l)} \quad (3)$$

Due to the undetermined dry matter content in the original feces samples, it was impossible to calculate the retained energy.

Statistical Analyses

Blood parameters were analyzed statistically in a mixed model with the MIXED procedure in SAS version V9.1 (SAS Institute, Cary, NC). The model included fixed effects of maternal pre partum feeding level, paternal heritage, sex of the lamb, and time of sampling. Their interactions, limited to three-way interactions at most, were also included in the model, but nonsignificant residuals across all variables were excluded. Sheep within feeding level and the individual lambs were considered random, and samples within lambs were declared as repeated. Normality of residuals was tested using a Shapiro-Wilks test, and homogeneity of variance was evaluated by visual inspection of residuals plots. Log transformations were applied where residuals were not normally distributed. Different covariance structures considering correlation between measurements and nonhomogeneous variances were tested, and the covariance yielding the best fit was chosen for each dependent variable. Estimates are given as least square means ± SE or as geometric means with a SE unless otherwise stated. To rule out any confounding effects, variation of birth weight between twin couples and maternal constraint, birth weight of the lamb, and weight and BCS of the ewe immediately before start of experimental feeding were used as covariables. In addition, milk yield of the ewe during the first 3 wk of lactation, determined by deuterium dilution technique (53), were tested as a covariable, but it was excluded from the final model as it was not statistically significant.

Table 2. Derived values of GT, II, and AIS during IVGTT in lambs at 10 and 19 wk of age of both sexes born from NORM or LOW ewes during the last 6 wk of gestation

<table>
<thead>
<tr>
<th>Measure</th>
<th>Age, wk</th>
<th>Pre Partum Feeding Level (Ewe)</th>
<th>Sex of Lamb</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NORM</td>
<td>LOW</td>
<td>P Value</td>
</tr>
<tr>
<td>GT</td>
<td>10</td>
<td>117±10</td>
<td>126±11</td>
<td>0.540</td>
</tr>
<tr>
<td>GT</td>
<td>19</td>
<td>124±10</td>
<td>126±10</td>
<td>0.906</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>2.5±0.4</td>
<td>2.9±0.4</td>
<td>0.561</td>
</tr>
<tr>
<td>II</td>
<td>19</td>
<td>3.6±0.3</td>
<td>2.8±0.3</td>
<td>0.132</td>
</tr>
<tr>
<td>AIS</td>
<td>10</td>
<td>65±12</td>
<td>80±11</td>
<td>0.371</td>
</tr>
<tr>
<td>AIS</td>
<td>19</td>
<td>117±10</td>
<td>85±11</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Data are presented as least square means ± SE. GT, glucose tolerance (mM·min); II, insulin increment (ng/ml); AIS, absolute insulin secretion (ng·min−1·ml−1); IVGTT, intravenous glucose tolerance test; NORM, ewes fed the control diet; LOW, ewes fed the 50% restricted diet.
insulin concentration compared with LOW (P = 0.046), and only LOW resumed baseline concentrations by 60 min (Fig. 2). As shown in Table 2, there was no effect of pre partum feeding level at any age on the calculated glucose tolerance (AUC) or first-phase insulin secretion; however, in older lambs NORM had a significantly higher absolute insulin secretion compared with LOW lambs. There was no significant effect of paternal heritage of the lambs at 10 or 19 wk of age on either AUC or AIS, however, in the younger, but not the older, lambs, SQ+ lambs had a higher first-phase insulin secretion compared with SQ−. Finally, the glucose tolerance and first-phase insulin secretion were unaffected by age, whereas the AIS almost doubled with age in NORM lambs, as opposed to similar AIS in younger and older LOW lambs.

**Fasting and Refeeding**

**Glucose.** The glucose concentration prior to fasting was similar between treatment groups, and all lambs responded similarly to the fasting with a significant decrease (P < 0.001) and to refeeding with a significant increase (P < 0.001) in glucose at both 10 wk (Fig. 3) and 19 wk of age. The pre partum feeding level did not affect the response to fasting or refeeding at any age. Regardless of the pre partum feeding level, however, the SQ+ lambs maintained significantly higher glucose concentrations compared with SQ− lambs after 18 and 24 h of fasting [4.2 ± 0.3 vs. 3.6 ± 0.2 mM, P = 0.008, and 4.3 ± 0.2 vs. 3.6 ± 0.3 mM, P = 0.002, at 10 wk of age (Fig. 3), and 4.2 ± 0.2 vs. 3.6 ± 0.1 mM, P = 0.010, and 4.3 ± 0.2 vs. 3.6 ± 0.1 mM, P = 0.002, at 19 wk of age respectively].

**Insulin.** All treatment groups responded to fasting with a significant decrease and to refeeding with a significant increase in insulin concentration (P < 0.001). In general, the insulin concentration was much lower in lambs at 10 wk of age [0.37 (0.33–0.43) ng/ml (fed state), 0.09 (0.08–0.10) ng/ml (fasted state), and 0.62 (0.54–0.70) ng/ml (refed state)] compared with lambs at 19 wk of age [1.20 (1.04–1.39) ng/ml (fed state), 0.20 (0.18–0.24) ng/ml (fasted state), and 1.65 (1.43–1.91) ng/ml (re-fed state)]. There was no difference between treatment groups prior to and during fasting or after refeeding at any age, except at 10 wk of age, when SQ+ lambs, regardless of pre partum feeding level, had significantly higher insulin concentration in the fasted state compared with SQ− [0.36 (0.30–0.42) ng/ml vs. 0.21 (0.18–0.24) ng/ml, P < 0.001].

**NEFA.** NEFA concentrations prior to fasting and after refeeding were similar between treatment groups, and all lambs responded to the fasting with a significant increase (P < 0.001) and to refeeding with a significant decrease (P < 0.001) in NEFA concentration. However, the NEFA response after 24 h of fasting was much greater at 10 compared with 19 wk of age (Fig. 4). At 10 wk of age, LOW SQ+ had the significantly highest increase in NEFA concentration (P = 0.004) compared with the other treatment groups. At 19 wk of age, LOW
released significantly more NEFA during fasting \((P = 0.051)\) compared with NORM, and SQ+ released significantly more NEFA compared with SQ− lambs \((P = 0.015; \text{Fig. } 4)\).

**Acetate.** All treatment groups responded to fasting with a significant decrease and to refeeding with a significant increase in acetate concentration \((P < 0.001 \text{ and } P < 0.001, \text{respectively})\). However, the response to fasting was greater in the younger compared with the older animals, and the response to refeeding was greater in the older compared with the younger animals. LOW compared with NORM lambs had a significantly lower acetate concentration after 24 h of fasting at both 10 and 19 wk of age \([0.11 \ (0.10–0.12) \text{ mM vs. } 0.15 \ (0.14–0.16) \text{ mM, } P = 0.007 \text{ and } 0.10 \ (0.09–0.11) \text{ mM vs. } 0.17 \ (0.15–0.19) \text{ mM, } P = 0.015, \text{respectively}]\) and tended to have a higher acetate concentration after refeeding at both 10 and 19 wk of age.

**BOHB.** Concentrations were generally higher in the younger compared with the older animals. BOHB concentrations decreased slightly during the first half of the 24-h fasting period followed by a slight increase during the second half of fasting, irrespective of either pre partum feeding level or paternal heritage. At 10 wk of age, BOHB concentrations decreased slightly in response to refeeding; however, at 19 wk of age a marked increase was observed in response to refeeding. The levels reached 8 h after refeeding were significantly higher in LOW compared with NORM lambs \((0.66 \pm 0.03 \text{ mM vs. } 0.52 \pm 0.02 \text{ mM, } P = 0.001)\) and in SQ− compared with SQ+ \((0.69 \pm 0.03 \text{ mM vs. } 0.50 \pm 0.03 \text{ mM, respectively, } P = 0.001)\).

**IGF-I.** At 10 wk of age, all treatment groups except of NORM SQ+ responded to fasting with a decrease in IGF-I concentration. Prior to fasting, IGF-I concentrations were unaffected by maternal feeding and paternal heritage \((131.0 \pm 13.7, 97.5 \pm 13.9, \text{ and } 99.0 \pm 18.1 \text{ ng/ml and } 90.7 \pm 15.6 \text{ ng/ml, NORM SQ+, NORM SQ−, LOW SQ+, and LOW SQ−, respectively})\). After 24 h of fasting, the IGF-I concentration had declined to 70.2 \pm 13.9, 70.7 \pm 18.1, \text{ and } 47.2 \pm 15.6 \text{ ng/ml, in NORM SQ−, LOW SQ+, and LOW SQ−, respectively; however, NORM SQ+ had unchanged IGF-I levels (125.0 \pm 13.7 \text{ ng/ml}) and ended up having a significantly higher IGF-I concentration \((P = 0.006)\) compared with the other treatment groups. This response to fasting was no longer existing at 19 wk of age; however, NORM generally tended to have higher IGF-I concentrations compared with LOW \((144.7 \pm 10.1 \text{ vs. } 112.9 \pm 11.3 \text{ ng/ml, } P = 0.071)\).

**Respiration**

**HE.** At 9 wk of age, were only lambs born after the SQ+ ram were examined, the LOW-SQ+ lambs tended to have a higher HE/BW \(^{0.75}\) compared with NORM-SQ+ lambs \((P = 0.072)\). Although both groups reduced their HE/BW \(^{0.75}\) significantly during fasting \((P < 0.001 \text{ and } P = 0.010, \text{respectively})\), the LOW-SQ+ group had the smallest reduction, 15.7%, compared with 25.4% in the NORM SQ+ lambs, resulting in a significant higher fasting HE/BW \(^{0.75}\) in LOW SQ+ compared with NORM SQ+ lambs \((P = 0.026; \text{Table } 3)\). No
difference in HE/BW\(^{0.75}\) was establish in the SQ—lams at 20 wk of age (the only type lamb examined at this time); however, at this age the LOW SQ—lams tended to have lower HE/BW\(^{0.75}\) (\(P = 0.105\)) compared with NORM SQ—.

**RQ.** The RQ was reduced by fasting but was not affected by pre partum feeding (Table 3).

**Sex Effects**

The only effects of sex, beside the birth weight-dependent effect on insulin secretion at 10 wk of age already mentioned, were observed for hormone concentrations, where rams had higher IGF-I concentration compared with ewes at both 10 (119.2 ± 8.7 vs. 63.7 ± 10.8 ng/ml, \(P < 0.001\)) and 19 (163.8 ± 9.1 vs. 93.9 ± 11.3 ng/ml, \(P < 0.001\)) wk of age.

**DISCUSSION**

**Late-Gestation Prenatal Undernutrition and Postnatal Glucose Tolerance and Insulin Sensitivity**

Retrospective data from the Dutch Hunger Winter Famine have quite clearly shown that maternal malnutrition in late gestation has permanent negative consequences for glucose-insulin homeostasis, resulting in impaired glucose tolerance and increased risk of type 2 diabetes in later life (47). The present study employing young and adolescent lambs did not demonstrate a prenatal nutritional effect on the postnatal glucose tolerance, but the absolute insulin secretion (AUC\(_{\text{insulin}}\)) was significantly depressed in the LOW lambs. Apart from an epidemiological study in humans (47), only three additional studies have, to our knowledge, examined late-gestation undernourishment as a determinant of glucose-insulin metabolism in postnatal offspring. Similar findings to ours have been reported from a rat study, also using young adolescent (8 wk) offspring born to dams subjected to global feed restriction (50% of the control diet) during late gestation (the last week) (4). Oliver et al. (36) were also unable to demonstrate any effect of 10 or 20 days of severe maternal under nutrition (0.3 ME/day) starting at gestational day 105 (term = 147 days) on the glucose tolerance in the ovine offspring at 5 mo or 3 yr of age. Gardner et al. (19), however, found that global undernutrition (~50% of ME requirements) in sheep for the last 30 days of gestation impacted negatively on glucose tolerance and induced insulin resistance in the 1-yr-old offspring. The results of these studies are thus to some extent conflicting; however, the type and duration of the nutritional insult and the age of the offspring when examined were quite different.

In rat studies it has been demonstrated that a low-protein (LP) diet fed to dams throughout gestation reduced β-cell proliferation, islet size, and islet vascularization in the newborn offspring (52) and that insulin secretion of the pancreatic islets from these offspring during both fetal and young adult life was impaired (13, 28, 35, 54). Reduced β-cell proliferation and lower pancreatic insulin content were also observed when the nutritional insult imposed was as a global feed restriction and confined to only late pregnancy (20). We therefore hypothesize that prenatal undernourishment during late fetal life results in impairment of pancreatic secretion of insulin in the adolescent individual, possibly through impaired β-cell function in the ovine, as it has previously been demonstrated in the rat (20).

The lower pancreatic insulin secretion combined with unaffected glucose tolerance, as observed in both our ovine and in the previous rat study (4), indicates a compensatory upregulation of insulin sensitivity in the young adolescent offspring exposed to prenatal undernourishment. Evidence for such an increased postnatal insulin sensitivity has indeed been provided in several studies with offspring (in the following termed LP) born to rats fed an LP diet during gestation. Muscle strips from LP offspring took up more glucose (41), and they also expressed twice as many insulin receptors in muscle, liver, and adipose tissue (38, 40, 41) compared with controls, suggesting an increased insulin sensitivity in terms of glucose transport. Furthermore alterations in GLUT4 expression and function have been demonstrated in both LP rat offspring and small-for-gestational-age humans (30, 41). In normal muscle fibers (and adipocytes), GLUT4 is recycled between the plasma membrane and intracellular storage vesicles (51), translocation from intracellular pools to the plasma membrane is required for glucose transport to take place, and the process is stimulated by insulin. The muscle plasma membrane of LP offspring have been shown to have an overall increased expression of GLUT4, and insulin increased the GLUT4 membrane content only in controls (41). This implies that LP offspring had a much lower capacity to modulate glucose transport into the muscle, glucose transport being maintained at a high level irrespective of stimulation. We therefore propose that late-gestation undernutrition increases insulin receptor expression and or induces alterations in GLUT4 expression and function, thereby increasing the insulin sensitivity of target tissues in the adolescent ovine offspring.

**Maturational Modifications of Glucose Homeostasis in Young and Adolescent Lambs**

In both rat and sheep, glucose tolerance decreases between early postnatal life and young adulthood as part of normal maturation (7, 21, 22). Individuals exposed to prenatal nutritional insults interestingly appear to have an altered maturational modification of glucose-insulin homeostasis. In several studies in young adolescent LP rat, the glucose tolerance after an intraperitoneal injection of glucose was increased, whereas basal or fasting plasma insulin concentrations were reduced, indicating an increased insulin sensitivity (29, 43, 50). However, later in life, the LP offspring had a lowered glucose tolerance (25) or developed a greater insulin resistance (16, 39).

<table>
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<tr>
<th>Table 3. Estimates of HE/BW and RQ of SQ+ lambs at 9 wk of age and SQ– lambs at 20 wk of age</th>
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<td><strong>Pre Partum Feeding (Ewe)</strong></td>
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<td><strong>Norm</strong></td>
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<td>HE/BW, kJ/kg(^{0.75})</td>
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<td>9 wk of age ((n = 20))</td>
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<td><strong>P value</strong></td>
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Values are means ± SE. HE, heat energy; BW, metabolic body weight; RQ, respiratory quotient; SQ, slaughter quality.
compared with controls. Similarly in a study with twin lambs discordant for birth weight (>25%), it was reported that the lighter lambs were more glucose tolerant than the heavier lambs at both 1 and 6 (but not 3) mo of age. The glucose tolerance was, however, identical for the two birth-weight groups at 12 mo of age (12). They also measured the glucose response to an intravenous insulin challenge and found greater insulin sensitivity (decreased AUC<sub>glucose</sub>) at both 1 and 6 mo of age in the lighter lambs (12). In agreement with these findings, the present study demonstrated that the pattern of maturational changes in pancreatic insulin secretion in response to a glucose load depended on the late fetal nutritional history. The absolute insulin secretion and the glucose tolerance in the LOW lambs were similar to those of NORM lambs at 10 wk of age. But whereas NORM lambs approximately doubled the absolute insulin secretion during the IGTT between 10 and 19 wk of age, without altering glucose tolerance, the LOW lambs did not increase insulin secretion and yet were also able to maintain the same glucose tolerance with advancing age. The results in NORM lambs are in line with other studies, where a maturational loss of peripheral insulin sensitivity and a compensatory increase in insulin secretion was reported in rats and sheep (7, 21, 22). The reason for the lack of maturational upregulation of insulin secretion with age in LOW lambs is not known. Two possible and distinctly different explanations can be proposed. Either the impaired pancreatic development and, hence, inability to upregulate insulin secretion with advancing age may have induced a compensatory up regulation of insulin sensitivity in target tissues, or fetal undernutrition may have encoded for a more efficient insulin signaling and, hence, increased sensitivity in peripheral tissues in early life, whereby the trigger for upregulation of pancreatic insulin secretion with age was abolished. Longitudinal studies where the same animals are followed from early postnatal life into adulthood, are required to shed more light on these important aspects of early nutritional impact on glucose-insulin homeostasis.

Late-Gestation Nutrition and Intermediary Metabolism in Fed and Fasted States

Plasma glucose and insulin levels were of similar magnitude and changed in the same manner in response to fasting in LOW and NORM lambs. However, at 19 (but not 10) wk of age, LOW lambs released more NEFA during the 24-h fasting period from adipose tissue stores compared with NORM lambs. LOW lambs also had lower leptin concentrations during fasting regardless of similar baseline levels. These data are indicative of a higher lipolytic activity and thereby more labile adipose tissue stores during fasting in LOW lambs compared with NORM lambs. Such an increased lipolytic activity is in agreement with findings from an in vitro study of 3-mo-old LP rat offspring, where epididymal adipocytes were shown to be more sensitive toward a synthetic catecholamine (42).

An increased ability to liberate NEFA from adipose tissue stores during fasting in adolescent lambs subjected to a nutritional insult in late fetal life would be indicative of an increased ability to shift oxidative metabolism away from carbohydrate and toward fat oxidation and thus a mechanism whereby glucose potentially could be spared when nutrient availability became limited. It has, however, not been possible to confirm such a change in pattern of nutrient oxidation in a subsequent experiment (A. Kiani, unpublished observations) where the same female animals as used in the present experiment were studied at 2 yr of age. Somewhat to our surprise, we observed that the liberated heat energy in the LOW lambs measured at 10 wk of age (only SQ+ offspring) was higher rather than lower compared with NORM lambs. This was true both in the fed and especially in the fasted state. These findings were supported by the later study in the female animals (across paternal heritage) at 2 yr of age (A. Kiani, unpublished observations).

An alternate explanation of the higher NEFA concentration during fasting in LOW lambs might be impaired hepatic uptake and/or metabolism of NEFA in the LOW lambs. High plasma levels of NEFA would normally lead to an increased production of BOHB in the ruminant liver; however, despite significantly higher NEFA levels in LOW lambs compared with NORM lambs during fasting, the BOHB concentration in the LOW lambs did not increase during the last 12 h of fasting above that observed in NORM lambs. This observation may indicate alterations of the liver metabolism in the lambs exposed to late gestational prenatal undernourishment. In young LP rats (3 and 12 wk), it has further been demonstrated that impaired fetal growth was associated with changed expression of key enzymes involved in the glucose metabolism, i.e., decreased glucokinase and increased phosphoenolpyruvate carboxykinase (PEPCK) expression in the liver, leading to hepatic glucose production rather than glucose utilization (14). Furthermore, the hepatic glucose output of the liver in response to insulin was increased and the response to glucagon decreased in the LP offspring compared with controls (40).

It can therefore be concluded that the programming events induced by late fetal undernutrition make adipose tissue stores more labile and more readily mobilizable during postnatal fasting. This may or may not improve the ability to shift from carbohydrate to fat oxidation and thus spare glucose in oxidative metabolism, but it is definitely not associated with a lowering of overall energy metabolism or overall energy efficiency.

Effect of and Interaction Between Paternal Heritage and Maternal Late Gestation Nutrition on Intermediary Metabolism

The lambs used in this experiment were offspring of two different rams of the same breed, which, however, differed in the standard breeding index for slaughter quality. Our results indicate that the genetic background of the individual apparently interacts with and affects the phenotypic manifestations of the programming effects. In this study, this interaction disappeared with increasing age. The mechanism behind this interaction between prenatal nutrition and paternal heritage or why it was evident only in the very young offspring is not known. We speculate that different maturational patterns or differences in body composition early in life between the two groups of lambs might explain these findings, even though there were no differences in growth patterns or phenotypic body composition i.e., lean body mass or adipose deposition at days 110 and 145 of age (53). The potential influence of the genetic background of the programmed individuals needs further attention and points to the importance of a wide genetic
representation in the material of the experimental animals, especially in light of planning future studies.

In conclusion, when lambs enter adolescence (19 wk), programming effects of late prenatal malnutrition on glucose-insulin homeostasis and metabolism become manifested. Prenatal undernourishment during late fetal life impairs pancreatic secretion of insulin in the adolescent individual, possibly through impaired β-cell function. The lower insulin-secretory capacity is apparently compensated for by increased target tissue sensitivity for insulin. Adipose lipolytic capacity is increased during fasting, whereby glucose may potentially be spared through increased lipid oxidation, but overall energetic efficiency deteriorates rather than being improved. To what extent genetic background of the programmed individuals interacts with late gestation nutrition on the postnatal endocrine signaling and intermediate metabolism needs to be resolved.

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