Postnatal ontogeny of glucose homeostasis and insulin action in sheep

K. L. Gatford, M. J. De Blasio, P. Thavaneswaran, J. S. Robinson, I. C. McMillen, and J. A. Owens. Postnatal ontogeny of glucose homeostasis and insulin action in sheep. Am J Physiol Endocrinol Metab 286: E1050–E1059, 2004. First published February 3, 2004; 10.1152/ajpendo.00340.2003.—Glucose tolerance declines with maturation and aging in several species, but the time of onset and extent of changes in insulin sensitivity and insulin secretion and their contribution to changes in glucose tolerance are unclear. We therefore determined the effect of maturation on glucose tolerance, insulin secretion, and insulin sensitivity in a longitudinal study of male and female sheep from preweaning to adulthood, and whether these measures were related across age. Glucose tolerance was assessed by intravenous glucose tolerance test (IVGTT, 0.25 g glucose/kg), insulin secretion as the integrated insulin concentration during IVGTT, and insulin sensitivity by hyperinsulinemic-euglycemic clamp (2 mU insulin·kg\(^{-1}\)·min\(^{-1}\)). Glucose tolerance, relative insulin secretion, and insulin sensitivity each decreased with age (P < 0.001). The disposition index, the product of insulin sensitivity, and various measures of insulin secretion during fasting or IVGTT also decreased with age (P < 0.001). Glucose tolerance in young adult sheep was independently predicted by insulin sensitivity (P = 0.012) and by insulin secretion relative to integrated glucose during IVGTT (P = 0.005). Relative insulin secretion before weaning was correlated positively with that in the adult (P = 0.023), whereas glucose tolerance, insulin sensitivity, and disposition indexes in the adult did not correlate with those at earlier ages. We conclude that glucose tolerance declines between the first month of life and early adulthood in the sheep, reflecting decreasing insulin sensitivity and absence of compensatory insulin secretion. Nevertheless, the capacity for insulin secretion in the adult reflects that early in life, suggesting that it is determined genetically or by persistent influences of the perinatal environment.

INSULIN SENSITIVITY DECREASES with maturation and aging in the human and other species, but the timing and extent of any compensatory hyperinsulinemia and the consequences for glucose tolerance are unclear. Similarly, whether insulin sensitivity and secretion in early life “track” and are predictive of these indexes in later life has not been established in any species to date, despite the implications for diagnosis and prevention of related disorders. In humans, cross-sectional studies have suggested that increased insulin secretion compensates for the decreases in whole body and peripheral insulin sensitivities with increasing age from childhood to puberty, maintaining glucose tolerance (1–3, 9, 35, 42, 45), although decreases in the disposition index (DI; the product of insulin sensitivity and insulin secretion) during puberty were reported in one longitudinal study (26), suggesting that increases in insulin secretion are not sufficient to maintain insulin action at this time. Immediately postpuberty, insulin sensitivity improves and insulin secretion decreases (2, 12). Much later in the older adult human, however, insulin sensitivity and secretion decline with further aging (reviewed in Refs. 13 and 19). In the rat, peripheral and hepatic insulin sensitivities decrease with maturation up to and during puberty, but insulin secretion does not increase sufficiently to compensate, and glucose tolerance decreases (4, 5, 10, 25, 40). Somewhat similarly, direct measures of insulin sensitivity during a hyperinsulinemic-euglycemic clamp decreased with increasing body weight in adolescent sheep over an ~3-mo period (49). It was unclear in this study, however, whether insulin sensitivity was corrected for the circulating levels of insulin achieved during the clamp, which increased with age concurrently with decreasing insulin metabolic clearance rates (49). Nevertheless, insulin sensitivity measured by the insulin tolerance test decreased by 20–30% between 1 and 12 mo of age in another study of twin sheep (14), suggesting that insulin sensitivity does decrease throughout the first year of life in the sheep. It is also likely that changes in insulin secretion fail to adequately compensate for maturational changes in insulin sensitivity in the sheep, since this, together with glucose tolerance measured by intravenous glucose tolerance test (IVGTT), decreased by 20–30% between 1 and 12 mo of age in twin sheep (14). However, neither this nor any other study to date has characterized the ontogeny of insulin secretion either alone or together with insulin action and glucose tolerance and determined whether they are related in the sheep or other species. Collectively, these findings suggest that glucose tolerance and its determinants do vary with development and in a species-specific manner, but some uncertainty remains because of the lack of longitudinal studies from early postnatal life to adulthood, and use of indirect measures of insulin sensitivity.

Whether changes in glucose tolerance and insulin action in an individual with maturation and aging might be predicted by low insulin sensitivity and/or secretion in early life is also unclear. Longitudinal studies in humans over twelve to fifteen years suggest tracking of fasting plasma glucose, insulin secretion, and insulin sensitivity in adults (20, 51) and of the Homeostasis Model Assessment (HOMA) insulin resistance index from childhood to adulthood (36), although the latter finding was dependent on genotype. An individual’s capacity for insulin secretion and
sensitivity to insulin may therefore be stable relative to the population and be established early in life. Genetic variation (22, 36) and the long-term responses to environmental factors acting during perinatal life (22, 38) probably both contribute to tracking of glucose tolerance and its determinants.

To directly test the contributions of insulin sensitivity and secretion to the ontogeny of glucose tolerance in sheep, we have performed a longitudinal study in male and female sheep from before weaning to adulthood to characterize the ontogeny of glucose tolerance and its relationship to two of its major determinants, insulin sensitivity, secretion, and hence insulin action. In addition, the associations between glucose homeostasis and its determinants across ages were examined to assess whether these track with age from early life to young adulthood.

**MATERIALS AND METHODS**

**Animals.** The University of Adelaide Animal Experimentation and Ethics Committee approved all procedures. Timed-pregnant Merino ewes were obtained from the South Australian Research and Development Institute Turrenfield Station. Pregnant ewes were maintained on pasture until 90 days of pregnancy and were subsequently housed in individual pens in animal holding rooms, with a 12:12-h light-dark cycle. For the remainder of pregnancy, and throughout lactation, lucerne chaff and water were available ad libitum, and ewes were also fed 150 g of oats daily. A total of 22 lambs comprising 11 males (10 singleton births, 1 twin birth) and 11 females (7 singleton births, 4 twin births) were used in this study. Weight was measured within 12 h of birth, and lambs were also weighed before each hyperinsulineemic-euglycemic clamp protocol. Lambs were delivered naturally and were housed in pens with their dams with free access to lucerne chaff until weaning at 90 days of age. From weaning until 110 days of age, lambs were housed individually in pens and fed lucerne hay. Feed and water were available ad libitum, when lambs were housed in metabolism crates during study periods, except during surgery, preexperimental fasting, and experimental protocols, as described below.

All experiments were performed on all lambs, except where cather patency was lost, and for two lambs, which were not catheterized until 110 days of age due to housing limitations. A total of nine lambs completed the IVGTT experiments at all four ages, and seven of these lambs also had hyperinsulineemic-euglycemic clamp (which require patency of both catheters) performed at all four ages. A total of 61 IVGTT experiments and 56 hyperinsulineemic-euglycemic clamp experiments were performed in the 22 lambs. Numbers of hyperinsulineemic-euglycemic clamp experiments and IVGTT experiments in male and female animals at each age are detailed in Tables 1 and 2, respectively. Suckling lambs (36 and 65 days old) were fasted for 3 h, and weaned lambs (127 and 372 days old) were fasted overnight, before each experimental procedure. At least 40 h of recovery were allowed between experimental protocols. Samples for plasma collection were taken in heparinized syringes, and catheters were flushed with heparinized saline after each sample. Blood glucose was measured in each sample immediately after collection. Samples were then placed on ice before centrifugation at 1,800 g at 4°C for 10 min. Plasma was harvested and stored at −20°C for later analysis.

**Surgical preparation of animals.** All surgery was performed under aseptic conditions. Catheters were placed in the left femoral artery and vein of each lamb at 4–6 days of age under general anesthesia, which was induced and maintained by inhalation of 3–4% halothane in oxygen. All catheters were filled with heparinized saline, and lambs received a daily 1-ml intramuscular injection of antibiotics (in mg/ml: 250 procaine penicillin, 250 dihydrostreptomycin, and 20 procaine hydrochloride) for 3 days, commencing on the day of surgery. Catheters were removed at ~70 days of age, and the animals were allowed to recover. Approximately 2 wk before commencement of experiments at 127 days of age, catheters were placed in the right femoral artery and vein of each lamb. General anesthesia was induced, after an overnight fast, by an intravenous injection of 0.25–0.5 g thiopentone (Boeringer Ingelheim, NSW, Australia) dissolved in sterile water and maintained by inhalation of 3–4% halothane in oxygen. All catheters were filled with heparinized saline, and lambs received a daily 2-ml intramuscular injection of antibiotics for 3 days, as described above. Catheters were removed at ~130 days of age, and the animals were allowed to recover. Approximately 2 wk before commencement of experiments at 371 days of age, catheters were placed in the right carotid artery and right jugular vein of each lamb, with anesthesia and post-surgery care as described for 127-day-old lambs.

**Analysis of blood glucose and plasma insulin.** Concentrations of glucose in whole blood were rapidly analyzed using a glucometer (HemoCue). Concentrations of insulin in plasma were analyzed by RIA using a commercially available kit (Phadeseph Insulin RIA; Pharmacia, Uppsala, Sweden). The intra-assay coefficient of variation for the insulin assay was 4.4%, and the interassay coefficient of variation was 6.2% (n = 38 assays).

**Measurement of the insulin sensitivity of glucose metabolism in vitro.** The insulin sensitivity of glucose metabolism was assessed by hyperinsulineemic-euglycemic clamp experiment, at ages 36 ± 1, 65 ± 1, 127 ± 1, and 372 ± 1 days. Fasting glucose concentration for the hyperinsulineemic-euglycemic clamp experiment was calculated as the average of blood glucose concentrations in samples taken 10, 5, and 0 min before commencement of the clamp. Human insulin (Actrapid; Novo Nordisk) was infused in the venous catheter at 2 mU/kg·min−1 for 120 min. A single dose of insulin was used at all ages throughout the study to allow comparison of insulin sensitivity between ages. The dose of 2 mU/kg·min−1 chosen for the present study produces steady-state insulin concentrations that are similar to the peak endogenous concentrations observed after an IVGTT. This infusion rate (2 mU/kg·min−1) is also similar to or slightly above the previously measured half-maximal effective dose (ED50 for insulin action in the adult sheep, where dose-response curves have been constructed (7, 39), and produced a sufficient hypoglycemic response to allow blood glucose concentrations to be clamped with a mean coefficient of variation of 5.0% in the 2nd h of the hyperinsulineemic-euglycemic clamp. Blood samples (0.2 ml) were taken at 5-min intervals throughout the experiment, and blood glucose was measured immediately after sample collection. An infusion of glucose (25% wt/vol, 1,390 mmol/l) was commenced 15 min after the insulin infusion, and the glucose infusion rate was adjusted every 5 min according to blood glucose concentration to restore and maintain euglycemia. Steady-state blood glucose (average blood glucose concentration in the 2nd h of the hyperinsulineemic-euglycemic clamp) averaged 98.8 ± 0.3% of fasting blood glucose. Plasma insulin was measured in blood samples (2 ml) that were collected at −10, −5, 0, 60, 75, 90, 105, and 120 min from commencement of the insulin infusion, and steady-state insulin concentration was calculated as the average plasma insulin in the 2nd h of the hyperinsulineemic-euglycemic clamp. The insulin sensitivity of glucose metabolism (Eq. 1) was calculated as the steady-state glucose infusion rate (glucose infusion rate averaged across the 2nd h of the hyperinsulineemic-euglycemic clamp), divided by the steady-state plasma insulin concentration (18)

\[
\text{Insulin sensitivity of glucose metabolism (mg·1·mU}^{-1}·\text{kg}^{-1}·\text{min}^{-1}) = \frac{\text{steady-state glucose infusion rate (mg glucose·kg}^{-1}·\text{min}^{-1})}{\text{steady-state plasma insulin concentration (mU/l)}}
\]

This definition of insulin sensitivity is consistent with the nomenclature used in previous human (18, 31, 32, 48) and animal (24) studies. At a physiological insulin dose similar to the normal ED50 for insulin action, either a leftward shift of the insulin dose-response curve...
metabolic clearance rate of insulin (ml·kg⁻¹·min⁻¹)
\[
= \frac{\text{insulin infusion rate} (2,000 \, \mu\text{U}·\text{kg}^{-1}·\text{min}^{-1})}{\text{steady-state plasma insulin concentration}} - \text{fasting plasma insulin concentration} (\mu\text{U}/ml)
\] (2)

**Measurement of glucose tolerance and insulin secretion in vivo.** Glucose tolerance and insulin secretion were assessed by IVGTT, at average ages of 38 ± 1, 67 ± 1, 130 ± 1, and 374 ± 1 days. Fasting glucose and insulin concentrations were calculated as the average of blood glucose and plasma insulin concentrations, respectively, in blood samples (2 ml) taken at 5, 3, and 0 min before commencement of the IVGTT. A bolus of glucose (0.25 g/kg body wt) was infused over 0.5–2 min, and the start time was taken as the end of the bolus infusion. Blood samples (2 ml) were taken at 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, and 210 min from the start of the experiment. The maximum change in blood glucose and plasma insulin concentrations was calculated as the difference between fasting concentration and maximum concentration after administration of the glucose bolus. The intercepts of the glucose curve with fasting glucose concentration and of the insulin curve with fasting insulin concentration were calculated to obtain times to restore fasting glucose and fasting insulin, respectively. Glucose intolerance (GAUC) was measured as the area above fasting blood glucose under the blood glucose curve. Absolute insulin secretion (IAUC) was measured as the area above fasting plasma insulin under the plasma insulin curve. Relative insulin secretion (Eq. 3) was calculated as IAUC divided by GAUC

relative insulin secretion (mU/mmol)
\[
= \frac{\text{absolute insulin secretion (mU·min}^{-1})}{\text{glucose intolerance (mmol·min}^{-1})}
\] (3)

**Calculations of posthepatic insulin delivery rate and DIs.** Posthepatic insulin delivery rates were calculated for basal (fasting) and glucose-stimulated states. Basal posthepatic insulin delivery rate (Eq. 4) at each age was calculated as the fasting plasma insulin concentration before commencement of the IVGTT, multiplied by the metabolic clearance rate for insulin from the hyperinsulinemic euglycemic clamp (30). Maximal posthepatic insulin delivery rate (Eq. 5) was calculated as the maximum plasma insulin concentration during the IVGTT, multiplied by the metabolic clearance rate for insulin from the hyperinsulinemic euglycemic clamp

basal posthepatic insulin delivery rate (\(\mu\text{U}·\text{kg}^{-1}·\text{min}^{-1}\))
\[
= \text{fasting plasma insulin before IVGTT} (\mu\text{U/ml}) \times \text{metabolic clearance rate of insulin (ml·kg}^{-1}·\text{min}^{-1})
\] (4)

maximal posthepatic insulin delivery rate (\(\mu\text{U}·\text{kg}^{-1}·\text{min}^{-1}\))
\[
= \text{maximum plasma insulin during IVGTT} (\mu\text{U/ml}) \times \text{metabolic clearance rate of insulin (ml·kg}^{-1}·\text{min}^{-1})
\] (5)

DIs were calculated as measures of insulin action (8) by multiplying insulin sensitivity (measured during the hyperinsulinemic euglycemic clamp) by measures of posthepatic insulin delivery rate in the fasting and glucose-stimulated states. The change in plasma insulin during an IVGTT independent of insulin clearance was used as a second measure of glucose-stimulated insulin secretion to allow comparison with similar indexes in human studies where measures of insulin clearance and hence posthepatic insulin secretion are frequently not available. The basal DI (Eq. 6) was calculated as the insulin sensitivity of glucose metabolism multiplied by the basal posthepatic insulin delivery rate. The maximal DI (Eq. 7) was calculated as the insulin sensitivity of glucose metabolism multiplied by maximal posthepatic insulin delivery rate. The change DI (Eq. 8) was calculated as the insulin sensitivity of glucose metabolism multiplied by the increase in plasma insulin from fasting to maximum concentration during the IVGTT.

basal DI (mg·ml·kg⁻²·min⁻¹)
\[
= \text{insulin sensitivity of glucose metabolism} \times \text{basal posthepatic insulin secretion} (\mu\text{U}·\text{kg}^{-1}·\text{min}^{-1})
\] (6)

maximal DI (mg·ml·kg⁻²·min⁻¹)
\[
= \text{insulin sensitivity of glucose metabolism} \times \text{maximal posthepatic insulin secretion} (\mu\text{U}·\text{kg}^{-1}·\text{min}^{-1})
\] (7)

change DI (mg·kg⁻¹·min⁻¹)
\[
= \text{insulin sensitivity of glucose metabolism} \times (\text{maximum plasma insulin concentration in IVGTT} - \text{fasting plasma insulin concentration in IVGTT} \, \text{(mU/l)})
\] (8)

Calculations of DIs required data from both hyperinsulinemic euglycemic clamp and IVGTT experiments in the same animals at the same age, which was available for 7 male and 6 female sheep at 36–38 days of age, 4 male and 4 female sheep at 65–67 days of age, 10 male and 9 female sheep at 127–130 days of age, and 7 male and 8 female sheep at 372–374 days of age.

**Statistical analyses.** All values are presented as means ± SE. Effects of age and gender on measures of glucose homeostasis, insulin secretion, insulin sensitivity, and DIs were analyzed by repeated-measures analysis using the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS, Chicago, IL). Insulin data were log-transformed before analysis. Relationships between pairs of variables were analyzed by Pearson’s correlation, across all animals, and also separately within genders using SPSS version 11.5. The effects of insulin sensitivity and relative insulin secretion or insulin sensitivity and maximal posthepatic insulin delivery rate on glucose intolerance at each age were analyzed by multiple linear regression using SPSS version 11.5. Similarly, the effects of insulin sensitivity and basal posthepatic insulin delivery rate on fasting blood glucose at each age were analyzed by multiple linear regression using SPSS version 11.5. A probability level of 5% (\(P < 0.05\)) was considered significant.

**RESULTS**

**Glucose tolerance and insulin secretion.** Fasted body weights before the IVGTT (Table 1) increased with age and were greater in males than in females. Fasting blood glucose and plasma insulin concentrations before the IVGTT and the ratio of fasting plasma insulin to fasting blood glucose each varied with age but did not differ between genders (Fig. 1). The maximum blood glucose concentration reached during the IVGTT (Table 1) did not change with age and tended to be
greater in females than in males. After administration of the glucose bolus, the time to peak plasma insulin concentrations varied with age (P = 0.030; day 38, 5.9 ± 0.9 min; day 67, 8.3 ± 0.8 min; day 130, 8.4 ± 0.6 min; day 374, 6.3 ± 0.6 min) and was not affected by gender. The maximum plasma insulin concentration reached during the IVGTT (Table 1) decreased with age and tended to be higher in males than in females. Blood glucose and plasma insulin concentrations each took longer to return to fasting values with increasing age, but time to restore fasting values for each was similar in males and females (Fig. 2). The increasing time period of elevated insulin concentrations observed with aging probably partially compensates for a decreasing initial secretory response, since the area under the insulin profile (i.e., insulin secretion uncorrected for the glucose stimulus) did not differ with age or between genders (Table 1). Nevertheless, glucose intolerance increased and relative insulin secretion decreased with age, although neither measure differed between males and females (Fig. 3, A and B). All measures of glucose tolerance and insulin secretion changed similarly with age in both genders.

### Table 1. Animal numbers, body weights, and insulin secretion in male and female sheep, measured during an IVGTT

<table>
<thead>
<tr>
<th></th>
<th>38 Days</th>
<th>67 Days</th>
<th>130 Days</th>
<th>374 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>No. of sheep</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>16.0 ± 0.8</td>
<td>14.0 ± 0.5</td>
<td>23.0 ± 1.2</td>
<td>20.5 ± 0.6</td>
</tr>
<tr>
<td>Maximum blood glucose concentration, mmol/l</td>
<td>10.7 ± 0.2</td>
<td>10.9 ± 0.3</td>
<td>10.8 ± 0.3</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>Maximum plasma insulin concentration, mU/l</td>
<td>146 ± 30</td>
<td>94 ± 17</td>
<td>87 ± 18</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>Area under insulin profile, mU·min⁻¹·l⁻¹</td>
<td>3,080 ± 740</td>
<td>1,540 ± 330</td>
<td>1,790 ± 380</td>
<td>1,110 ± 140</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE, and were analyzed by repeated-measures ANOVA for effects of age, gender, and age-gender interactions (A*G) using the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS). IVGTT, intravenous glucose tolerance test. IVGTTs (0.25 g glucose/kg) were performed after a 3-h (38- and 67-day-old sheep) or overnight (130- and 374-day-old sheep) fast. Body weight of sheep at 38 and 67 days of age was measured before a 3-h fast, and body weight of 130-day- and 374-day-old sheep was measured after an overnight fast. Insulin data were log-transformed before analysis.

---

**Fig. 1.** Ontogeny of fasting blood glucose and plasma insulin concentrations in male (•) and female (○) sheep. Fasting blood glucose and plasma insulin concentrations were calculated as the average of concentrations in 3 samples taken before an intravenous glucose tolerance test (IVGTT) and after a 3-h (38- and 67-day-old sheep) or overnight (130- and 374-day-old sheep) fast. Data are presented as means ± SE for all animals of that age and gender (n = 4–10/group) and were analyzed by repeated-measures ANOVA for effects of age, gender, and age-gender interactions (A*G) with the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS). Insulin data were log transformed before analysis.

**Fig. 2.** Ontogeny of time to restore fasting blood glucose and plasma insulin concentrations in male (•) and female (○) sheep during IVGTT. IVGTTs (0.25 g glucose/kg) were performed after a 3-h (38- and 67-day-old sheep) or overnight (130- and 374-day-old sheep) fast. Data are presented as means ± SE for all animals of that age and gender (n = 4–10/group) and were analyzed by repeated-measures ANOVA for effects of age, gender, and age-gender interactions with the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS). Insulin data were log transformed before analysis.
Insulin sensitivity. Steady-state plasma insulin concentrations (Table 2) tended to increase with age, whereas the steady-state glucose infusion rate required to maintain euglycemia (Table 2) and the insulin sensitivity of glucose metabolism (Fig. 3C) decreased with age, and these measures did not differ between genders (Table 2). The metabolic clearance rate of insulin, measured during the hyperinsulinemic euglycemic clamp, decreased with age such that insulin was cleared most rapidly in 36-day-old lambs and did not differ between genders (Table 2).

Posthepatic insulin delivery rate. Basal posthepatic insulin delivery rate (Fig. 4A) decreased with age and did not differ between males and females. Maximal posthepatic insulin delivery rate (Fig. 4B) also decreased with age but was higher in males than in females. Basal and maximal posthepatic delivery rates were positively correlated at 38 (r = 0.56, P = 0.025, n = 13), 67 (r = 0.730, P = 0.020, n = 8), and 374 (r = 0.599, P = 0.009, n = 15) days of age and tended to correlate positively at 130 days of age (r = 0.340, P = 0.077, n = 19). For a given insulin sensitivity, posthepatic insulin delivery rates were highly variable, although animals with high insulin sensitivity generally had low basal (Fig. 5A) and maximal posthepatic insulin delivery rates (Fig. 5B) at a given age.

DSs. The basal DI decreased with increasing age and did not differ between genders (Fig. 6A). The basal DI fell from that in young lambs (~35 days of age) to lower levels in juveniles (~65 days of age). This impairment in basal insulin action was also apparent by the increasing separation from the regression line (insulin sensitivity vs. basal insulin delivery rate for individual young lambs) with age of the means for males and females (Fig. 5C). This reflects a fall in both basal insulin delivery rate and insulin sensitivity between 35 and 65 days of age. After 65 days of age, however, further age-related decreases in insulin sensitivity were compensated for by increased basal posthepatic insulin delivery rate, and the changes in age means paralleled the response observed in individual young lambs at 35 days of age (Fig. 5C), resulting in no further decrease in basal DI from juveniles to young adults (Fig. 6A). Maximal and change DI (Fig. 6, B and C) also decreased with increasing age but were greater in males than in females. At each age, mean maximal posthepatic insulin delivery for a given insulin sensitivity was greater in males than in females (Fig. 5D). With increasing age, the means of insulin sensitivity vs. maximal posthepatic insulin delivery in both males and females fell progressively away from the regression line derived from individual young lambs, reflecting a failure of
Insulin data were log transformed before analysis. Windows version 11.5 with a first-order autoregressive covariance structure of SPSS for repeated-measures ANOVA for effects of age, gender, and age-gender interactions using the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure of SPSS. Determinants of glucose homeostasis. Neither relative insulin secretion nor insulin sensitivity was independently related to glucose intolerance at 38 days of age. The combination of insulin sensitivity (partial correlation, $r = -0.576, P = 0.176$) and insulin secretion (partial correlation, $r = -0.488, P = 0.267$) explained a substantial proportion of the variation in glucose intolerance at 67 days of age (overall: $r = 0.786, P = 0.090, n = 8$). Similar relationships were seen at 130 days of age (partial correlation, insulin sensitivity: $r = -0.496, P = 0.036$; partial correlation, relative insulin secretion: $r = -0.296, P = 0.232$; overall: $r = 0.522, P = 0.079, n = 19$), when glucose intolerance was negatively related to insulin sensitivity. Both insulin sensitivity (partial correlation, $r = -0.649, P = 0.012$) and insulin secretion (partial correlation, $r = -0.708, P = 0.005$) were independently and negatively related to glucose intolerance at 374 days of age (overall: $r = 0.752, P = 0.007, n = 15$). Multiple linear regression analysis showed that insulin sensitivity and maximal posthepatic insulin delivery rate did not predict glucose intolerance at any age ($P > 0.09$ for overall relationship at all ages). Fasting blood glucose concentrations at 130 days of age were predicted (overall: $r = 0.551, P = 0.055, n = 19$) by the fasting and maximum plasma insulin concentrations from the IVGTT (0.25 g glucose/kg), respectively. IVGTT and hyperinsulinemic euglycemic clamps were performed after a 3-h (35- to 38- and 65- to 67-day-old sheep) or overnight (127- to 130- and 372- to 374-day-old sheep) fast. Data are presented as means ± SE for all animals of that age and gender ($n = 4$ to 10/group) and were analyzed by repeated-measures ANOVA for effects of age, gender, and age-gender interactions using the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS). Insulin data were log transformed before analysis.

Fig. 4. Ontogeny of basal and maximal posthepatic insulin delivery rates in male (•) and female (○) sheep. Basal and maximal posthepatic insulin delivery rates at each age were calculated by multiplying the metabolic clearance rate for insulin from the hyperinsulinemic euglycemic clamp (2 mU·kg⁻¹·min⁻¹) by the fasting and maximum plasma insulin concentrations from the IVGTT (0.25 g glucose/kg), respectively. IVGTT and hyperinsulinemic euglycemic clamps were performed after a 3-h (35- to 38- and 65- to 67-day-old sheep) or overnight (127- to 130- and 372- to 374-day-old sheep) fast. Data are presented as means ± SE for all animals of that age and gender ($n = 4$ to 10/group) and were analyzed by repeated-measures ANOVA for effects of age, gender, and age-gender interactions using the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS). Insulin data were log transformed before analysis.

Basal DI was positively correlated with maximal DI at most ages ($day 38: r = 0.444, P = 0.064, n = 13$; $day 67: r = 0.905, P = 0.001, n = 8$; $day 130: r = 0.478, P = 0.019, n = 19$; $day 374: r = 0.807, P < 0.001, n = 15$). Basal DI was less strongly correlated with change DI at each age, however ($day 38: r = 0.337, P = 0.130, n = 13$; $day 67: r = 0.852, P = 0.004, n = 8$; $day 130: r = 0.387, P = 0.051, n = 19$; $day 374: r = 0.111, P = 0.346, n = 15$). DI$s$ calculated on the basis of maximal posthepatic insulin delivery rate (maximal DI) and the change in insulin during an IVGTT (change DI) were strongly positively correlated at all ages ($day 38: r = 0.960, P < 0.001, n = 13$; $day 67: r = 0.986, P < 0.001, n = 8$; $day 130: r = 0.981, P < 0.001, n = 19$; $day 374: r = 0.591, P = 0.010, n = 15$).

Fig. 5. Insulin sensitivity and basal posthepatic insulin delivery rate ($A$) and maximal posthepatic insulin delivery rate ($B$) in individual male (filled symbols) and female (open symbols) sheep at 36–38 (circles), 65–67 (squares), 127–130 (triangles), and 372–374 (diamonds) days of age. $A$ and $B$: values for individual animals. $C$ and $D$: separate means ± SE for each gender and age for basal posthepatic insulin delivery rate ($C$) and maximal posthepatic insulin delivery rate ($D$). Solid lines show the regression lines for insulin sensitivity vs basal ($A$ and $C$) or maximal ($B$ and $D$) posthepatic insulin delivery rate in combined male and female lambs at 36–38 days of age. Dotted lines ($C$ and $D$) indicate changes in means with age. Basal and maximal posthepatic insulin delivery rates at each age were calculated by multiplying the metabolic clearance rate for insulin from the hyperinsulinemic euglycemic clamp (2 mU·kg⁻¹·min⁻¹) by the fasting and maximum plasma insulin concentrations from the IVGTT (0.25 g glucose/kg), respectively. IVGTT and hyperinsulinemic euglycemic clamps were performed after a 3-h (35- to 38- and 65- to 67-day-old sheep) or overnight (127- to 130- and 372- to 374-day-old sheep) fast.
Tracking of glucose tolerance and its determinants. Glucose intolerance at 374 days of age was unrelated to glucose intolerance at 38, 67, or 130 days of age across all animals or in males and females considered separately. Insulin sensitivity at 372 days of age was also not related to insulin sensitivity at earlier ages, either for all animals or within genders. Relative insulin secretion in adults at 374 days of age was positively correlated with relative insulin secretion at 38 days of age (r = 0.999, n = 3, P = 0.029). The change DI in adults was not related to the change DI at earlier ages overall or in females but was positively correlated with the change DI at 65–67 days of age in males (r = −0.889, n = 7, P = 0.007). Insulin action, assessed as either basal or maximal DI at 372–374 days of age, was not related to the same DI at earlier ages, either overall or in males or females analyzed separately. The change DI in adults was not related to the change DI at earlier ages overall or in females but was positively correlated with the change DI at 65–67 days of age in males (r = −0.889, n = 7, P = 0.007).

DISCUSSION

This study shows that glucose homeostasis, insulin secretion, insulin sensitivity, and insulin action decrease with maturation, from before weaning to early adult life in the sheep. Our results show that impairment of glucose tolerance with aging from the young animal across puberty to the young adult is common to the sheep and the rat (10), in contrast to humans, where glucose tolerance is maintained across this period by compensatory changes in insulin secretion (2, 9, 12, 42, 45). Nevertheless, decreasing insulin sensitivity with maturation or puberty is common across species in sheep, humans (2, 3, 9, 16, 47), and rats (4, 25, 40). The decline in glucose tolerance with maturation in sheep in the present study reflected a progressive decline in glucose-stimulated insulin action because of decreases in both insulin sensitivity and maximal posthepatic insulin delivery rate. This lack of increase in insulin secretion to compensate for decreased insulin sensitivity meant that glucose-stimulated insulin disposition fell progressively with age, at least to puberty. In contrast, although basal insulin action also decreased from that in young lambs (~35 days of age) to lower levels in juveniles (~65 days of age), further age-related decreases in insulin sensitivity were compensated for by increased basal insulin delivery rate, allowing basal insulin action to be maintained from before weaning to early adulthood. White and Leng (50) reported rapid declines in basal glucose entry rates, expressed relative to metabolic body size, from 1 day to 27 days of age in small numbers of fed, suckling lambs and little change in basal glucose entry rates with aging after weaning. The magnitude of the ontogenic change in basal glucose entry rate was markedly blunted by fasting for 16 h in suckling lambs and up to 72 h in adult sheep, to reach a postabsorptive state (50). The shorter fast used in the present study may explain why we still observed marked age-related changes in basal glucose homeostasis before weaning. Before weaning, fasting blood glucose decreased by ~20% between 38 and 67 days of age and then remained relatively stable, implying that, although the response to a glucose challenge deteriorates with age, regulation of glucose in the fasting state is not impaired with maturation up to young adulthood in the sheep.

The decline in fasting blood glucose levels over this time may also reflect the change from a preruminant to a ruminant state, in which the primary nutrients absorbed from the gut would be shifting from carbohydrates to volatile fatty acids (46). Lambs had access to their mothers for suckling and to their mother’s ration of lucerne chaff at both ages, and a shift from milk to ingestion of solid feed was expected during this period, although feed intake of lambs was not character-

Fig. 6. Ontogeny of disposition indexes in male (●) and female (○) sheep. Basal, maximal, and change disposition indexes were calculated by multiplying the insulin sensitivity of glucose metabolism obtained during the hyperinsulinemic euglycemic clamp (2 mU insulin·kg⁻¹·min⁻¹) by basal posthepatic insulin delivery rate, maximal posthepatic insulin delivery rate, and the increase in plasma insulin from basal to maximum concentration during the IVGTT (0.25 g glucose/kg), respectively. IVGTT and hyperinsulinemic euglycemic clamps were performed after a 3-h (35- to 38- and 65- to 67-day-old sheep) or overnight (127- to 130- and 372- to 374-day-old sheep) fast. Data are presented as means ± SE for all animals of that age and gender (n = 4–10/group) and were analyzed by repeated-measures ANOVA for effects of age, gender, and age-gender interactions using the linear mixed-models procedure of SPSS for Windows version 11.5 with a first-order autoregressive covariance structure (SPSS). Insulin data were log transformed before analysis.
ized. In the present study, changes in glucose homeostasis and insulin action with age occurred predominantly between young and adolescent lambs, i.e., between ∼35 and ∼130 days of age, with little subsequent change. Postprandial increases in insulin are believed to be one of the major regulators of neonatal growth, by stimulating glucose and amino acid uptake of peripheral tissues (17), and we have shown that catch-up growth in neonatal lambs is predicted by indexes of insulin action on circulating amino acids and free fatty acids (De Blasio MJ, Gatford KL, Fielke SL, McMillen IC, Robinson JS, and Owens JA, unpublished observation). Interestingly, Bellver et al. (6) report lower insulin sensitivity in milk-fed than in ruminant lambs at 20 kg body weight, implying that the age-related decrease in insulin sensitivity observed in our study is not associated with either the shift from a prernatant to ruminant state, or with weaning of our lambs at 90 days of age. Consistent with this, Hocquette et al. (29) found greater basal and maximally insulin-stimulated glucose transport rates in rectus abdominis muscle of ruminant compared with prernatant calves of the same weight and age, which also suggests that peripheral sensitivity is increased by the change to a ruminant state. The maturational changes in glucose homeostasis and insulin action that we observed in the sheep may be related to puberty, however, as occurs in humans (1–3, 9, 12, 26, 35, 45) and rats (4, 5, 10, 25, 40).

Our observation that fasting glucose decreases with maturational change from the young lamb to adolescence and then remains stable up to young adulthood is broadly consistent with previous studies in sheep (14, 37), although fasting glucose dropped substantially between 1 and 2 mo of age in the present study, in contrast to a later decline between 3 and 6 mo of age in twin female lambs observed in a previous study (14). We also observed a much greater maturational impairment in glucose tolerance and insulin sensitivity than that reported previously in sheep over a similar age range (14). In the latter study, glucose tolerance measured during an IVGTT and insulin sensitivity measured by the plasma glucose response to an insulin bolus, fell by ∼20–30% between 1 and 12 mo of age (14). The decline in the insulin sensitivity of glucose metabolism that we observed with increasing age in the sheep may be continuous from birth to adolescence. Gelardi et al. (24) reported an ∼3.5-fold decrease in insulin responsiveness between 3 and 6 days of age and 31 and 35 days in the lamb, as measured by hyperinsulinemic euglycemic clamp. The glucose infusion rates required to maintain euglycemia at the latter age in that study were similar to those in the present study, despite the fact that the insulin infusion rate was 50-fold higher and the plateau plasma insulin concentrations were ∼230-fold higher in the study by Gelardi et al., implying that either their insulin dose exceeded the threshold for maximum response or that insulin resistance was induced by 72 h of fasting and acute catheterization before the hyperinsulinemic euglycemic clamp. However, these results do imply that at least maximal responses to insulin fall with age before 1 mo of age in the sheep. In the human, insulin sensitivity is about fourfold higher in the preterm neonate than in adults. Farrag et al. (21) reported that, at the same insulin infusion rate, insulin clearance was more rapid in the preterm neonate than in the adult and resulted in steady-state insulin concentrations that were ∼25% of those in adults; also, the glucose infusion rate required to maintain euglycemia was similar in preterm neonates and adults. It therefore seems likely that the decrease in insulin sensitivity with age continues from birth throughout childhood in humans, similar to the pattern suggested in sheep by the results of Gelardi et al. together with the present study.

The mechanisms that regulate maturational changes in insulin secretion and sensitivity have been characterized only to a limited extent in human and rat studies. The pubertal decline in insulin sensitivity and compensatory increase in insulin secretion have been attributed to increased circulating levels of growth hormone and accretion of body fat, and possibly to increased adrenal androgen secretion after adrenarche in humans (2, 9, 12, 26) and rat (4) studies. Whether these contribute to the observed changes in insulin secretion and sensitivity over puberty in the sheep is not known. Exogenous growth hormone impairs insulin sensitivity in the young lamb (6) as in other species, but endogenous growth hormone levels decrease rapidly from high levels in the first 5 days of life and then decrease slowly with increasing live weight up to early adolescence in male lambs (27), suggesting that growth hormone is not the main regulator of onogenic changes in insulin action up to this age. We have previously found that circulating growth hormone patterns were similar in adolescent and adult sheep (23), ages when glucose tolerance and insulin action were also similar. The mechanisms responsible for age-related decreases in insulin sensitivity and secretion in the sheep and other species therefore require further investigation.

In the present study, indexes of fasting glucose homeostasis were similar in males and in females, suggesting that the factors influencing basal glucose metabolism are similar in both genders. Glucose tolerance and insulin sensitivity were also similar in male and female sheep, although the initial rise in blood glucose after the glucose bolus was greater in females than in males. In humans, sex differences in insulin sensitivity are present only in early puberty (16, 47), and it is possible that these may also exist in the sheep, although more frequent testing of glucose tolerance and insulin sensitivity than in the present study, together with characterization of pubertal stages, would be needed to characterize this. Relative insulin secretion was similar in both sexes in the present study, but other measures of insulin secretion in the stimulated state were higher in males than in females, suggesting that their initial response of the pancreas to glucose is greater. Consistent with this, glucose-stimulated insulin secretion from isolated islets of Langerhans is greater in those isolated from adolescent, young, and mature adult male rats than in those from age-matched females (43). Inconsistent sex effects on insulin secretion have been reported after glucose tolerance tests in humans. Smith et al. (45) found no sex difference in acute or total insulin secretion throughout puberty, whereas lower acute insulin secretion in young adult men than in young adult women (15), and conversely, higher total insulin secretion in boys than in girls during puberty (16), have been reported in other studies. Our findings also suggest that there are no sex differences in the age-related changes in glucose homeostasis and their determinants in the sheep from early in life to adulthood.

The results of the present study suggest that the determinants of glucose tolerance change with maturation in the sheep. In young lambs, glucose intolerance was unrelated to insulin sensitivity and tended to be negatively related to insulin secretion, suggesting that glucose effectiveness, the other major
determinant of glucose homeostasis, may be important in the response to a glucose challenge at this age. A low number of animals with complete studies at ~65 days of age limited detection of significant predictors of glucose intolerance at this age, although the magnitude of the negative correlation between glucose intolerance and insulin sensitivity was of a similar strength at ~65, ~130, and ~370 days of age. Glucose intolerance was predicted by insulin sensitivity rather than insulin secretion in adolescent sheep, and both insulin secretion and insulin sensitivity were significant determinants of glucose tolerance in young adults. In the present study, relative insulin secretion, reflecting insulin secretion across the entire IVGTT, was a better predictor of glucose intolerance than maximal posthepatic insulin delivery rate, which reflects acute insulin secretion. Fasting blood glucose was not predicted by insulin sensitivity and was positively related to basal posthepatic insulin delivery rate only in adolescents, suggesting that insulin action is not the main regulator of glucose in the fasted state.

We did not observe “tracking” of glucose intolerance or insulin sensitivity across ages, that is, glucose intolerance or insulin sensitivity at earlier ages did not predict those at later ages. However, insulin secretion in young lambs was positively related to their insulin secretion as young adults, suggesting that the capacity for insulin secretion may be determined by events in early life. Relative insulin secretion in older suckling lambs (67 days of age) or in adolescent lambs (130 days of age) was unrelated to relative insulin secretion in young lambs (data not shown) or in adult lambs, which may reflect the perturbations in insulin-regulated glucose homeostasis around puberty. Previous longitudinal studies have demonstrated tracking for obesity and hypertension, in children and into adulthood (28, 34, 44). Longitudinal studies in humans have reported moderate tracking of fasting glucose concentrations (r ~ 0.3) over a three- or five-year period in young and older children, when initial measures were taken at 5–8 yr of age or 9–14 yr of age, respectively (11). Tracking of plasma insulin concentrations occurred only in the older group of children, however (11). Similarly, correlations between measures of plasma insulin that were taken 6 years apart in a Finnish multicenter trial (41) were stronger in young adults or children initially measured at 18 yr of age (r ~ 0.4) or at 9 yr of age (r ~ 0.4) than in children initially measured at 3 yr of age (r ~ 0.3). For individuals with the nonmutated form of peroxisome proliferator-activated receptor-γ2 (36), ~50% remained in the same quartile for HOMA insulin resistance index as children (4–17 yr of age) and 13 years later as adults (20–38 yr of age), suggesting that some tracking of insulin sensitivity with age may occur in humans.

In summary, we have characterized the ontogeny of glucose tolerance and two of its major determinants, insulin secretion and insulin sensitivity, in a longitudinal study in male and female sheep, from young lambs to young adults. Glucose tolerance, insulin secretion, insulin sensitivity, and insulin action fell with age in the sheep, and most of this decrease occurred in a period between before weaning and adolescence. These changes were similar in males and females. Although glucose homeostasis in response to a glucose challenge is impaired with increasing maturation up to young adulthood, our results suggest that this does not occur for regulation of glucose in the basal state. In addition, insulin secretion in young lambs was predictive of insulin secretion in young adults, suggesting that events in early life may permanently determine the capacity for glucose-stimulated insulin secretion.

ACKNOWLEDGMENTS

We thank Simon Fielke, Melissa Walker, and Arkadi Katsman for assistance with in vivo studies.

GRANTS

A National Health and Medical Research Council of Australia (NHMRC) Project Grant and an Australian Research Council Small Grant funding supported this study. K. L. Gaford was supported by an NHMRC Peter Doherty Postdoctoral Fellowship and now holds the Hilda Farmer Medical Research Associateship at the University of Adelaide.

REFERENCES


