Perinatal growth and plasma GH profiles in adolescent and adult sheep

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Abstract

Poor prenatal growth is associated with limited evidence of GH deficiency in adult humans, which may contribute to their increased risk of cardiovascular and metabolic disease. We therefore examined the effects of placental restriction of fetal growth (PR) on size at birth, neonatal fractional growth rate (FGR) and the circulating GH profile in adolescent and young adult sheep of both sexes. Moderate or severe PR decreased birth size and increased neonatal FGR of weight, crown–rump length and abdominal circumference. In adolescent males, mean and baseline GH concentrations correlated negatively and independently with birth weight and FGR of weight, and mean GH concentrations correlated negatively with current weight. In young adult males, mean GH concentrations correlated negatively and independently with birth shoulder height and FGR of shoulder height whilst, in young adult females, these correlations were positive. This suggests that restricted fetal growth and reduced neonatal growth rate in sheep are followed by elevated circulating GH in adolescent and adult males, but GH deficiency or increased GH clearance in adult females.

Introduction

The somatotrophic axis is important in postnatal growth and for metabolic and cardiovascular homeostasis (Gluckman et al. 1992, Rosenfeld et al. 1994, Hew et al. 1998). Small size at birth is associated with reduced growth hormone (GH) secretion in young men and women (Flanagan et al. 1999), and with increased risk of developing adult-onset metabolic diseases including hypertension, diabetes and cardiovascular disease (Barker 1998). In the rat, maternal feed restriction during pregnancy induces GH resistance of growth in adolescent, but not adult, progeny (Woodall et al. 1996a). These results suggest that restricted fetal growth programmes alter GH synthesis, secretion and action postnatally, and that these effects may vary with age. Small size at birth is also associated with accelerated neonatal growth in most infants (Albertsson-Wikland & Karlberg 1994, Hokken-Koelega et al. 1995, Karlberg & Albertsson-Wikland 1995), and it has been suggested that such catch-up growth is an independent risk factor for hypertension and other adverse health outcomes in later life (Eriksson et al. 1999, Huxley et al. 2000, Ong et al. 2000). The effect of restricted fetal growth on-- and relationships of its associated neonatal catch-up growth with-- subsequent altered GH synthesis, secretion and action have not been examined in the human or in other species.

Placental size and function are major determinants of fetal growth in mammalian species and can be experimentally restricted in the sheep (Robinson et al. 1979, 1995). Surgical restriction of placental implantation in sheep reduces subsequent placental growth and substrate delivery to the fetus (Robinson et al. 1979). We have therefore investigated the effect of placental restriction of fetal growth (PR) on circulating GH profiles in adolescent and adult sheep. We also determined if indices of fetal growth and of neonatal growth separately predicted circulating GH profiles, and whether there were sex differences in the effect of PR and/or nature of these relationships.

Materials and Methods

Animals

All procedures were approved by the University of Adelaide Animal Experimentation and Ethics Committee. Placental growth was restricted by removal of the majority of endometrial caruncles from the uterus of non-pregnant ewes prior to mating (Robinson et al. 1979), leaving either six to seven (moderately placentally restricted group,
moderate PR) or three to four (severely placentally restricted group, severe PR) visible caruncles in each uterine horn. A recovery period of at least 10 weeks was allowed prior to mating. A total of 41 Australian Merino lambs (19 control, 14 moderately placentally restricted, 8 severely placentally restricted) were used in this study. Lambs were delivered naturally, and were housed in pens with their dams until weaning at 90 days of age. Throughout lactation, lucerne chaff and water were available ad libitum, and ewes were also fed 150 g oats daily. From birth until weaning, lambs suckled their dams and had free access to lucerne chaff. From weaning until 110 days of age, lambs were housed individually in pens, then subsequently during studies in metabolism crates with lucerne chaff and water available ad libitum. Between studies (140–355 days of age), male and female lambs were housed separately on pasture and fed lucerne hay. Size parameters (weight, crown–rump length, shoulder height, hind limb circumference, skull width and length, abdominal circumference) were measured within 12 h of birth, and at 5- to 10-day intervals until 45 days of age. Absolute growth rates (AGR) for size parameters from birth until 45 days of age were calculated by linear regression analysis. Fractional growth rate (FGR) for each size parameter was calculated as AGR, as a fraction of the size parameter at birth, as these more closely reflect the anabolic effort of growth relative to size. Lambs were also weighed, following an overnight fast as adolescents and as adults, 2 days prior to collection of blood samples for analysis of GH.

**Catheterization of lambs**

Catheters were placed in the right femoral artery and vein of each lamb at ∼115 days of age. All surgery was performed under aseptic conditions. General anaesthesia was induced following an overnight fast, by an i.v. injection of thiopentone (0·5–1·g; Boehringer, Ingelheim, NSW, Australia) and maintained by inhalation of 3–4% halothane in oxygen. All catheters were filled with heparinized saline (500 IU/ml), and lambs received a daily 2 ml intramuscular injection of antibiotics (procaine penicillin, 250 mg/ml; dihydrostreptomycin, 250 mg/ml; procaine hydrochloride, 20 mg/ml; Ilium Penstrep, Troy Laboratories Pty Ltd, Smithfield, NSW, Australia) for 3 days commencing on the day of surgery. Catheters were removed at ∼135 days of age and the animals were allowed to recover. At ∼360 days of age, catheters were placed in the right carotid artery and jugular vein of each lamb, with anaesthesia and post-surgery care as described above.

**Sample collection**

Blood samples for GH were collected from 37 lambs (16 male, 21 female) at 132 ± 1 days of age (termed adolescent sheep), and 33 lambs (15 male, 18 female) at 378 ± 1 days of age (termed young adult sheep). Blood samples for analysis of GH profiles were collected at both ages in 29 lambs (14 male, 15 female), but could not be collected in the remaining lambs due to loss of catheter patency. Blood samples (2 ml) were withdrawn from the venous catheter at 10-min intervals over an 8-h sampling period, commencing at 0900 h. Samples were taken into heparinized syringes and placed on ice, and catheters were flushed with heparinized saline following each sample. Plasma was separated by centrifugation at 1800 g at 4 °C for 10 min, and stored at −20 °C prior to analysis. Lambs were fed lucerne chaff 30 min before collection of the first plasma sample, and at 4 h after collection of the first plasma sample, to ensure availability of feed throughout the protocol.

**Analysis of plasma GH**

Concentrations of GH in plasma were analysed with a double-antibody radioimmunoassay (RIA) (Thomas et al. 1990), using NIDDK ovine GH-I-4 as standard and [125I]NIDDK ovine GH-I-4 as tracer. Intra- and inter-assay coefficients of variation were 9·9% and 13·0% respectively, for a quality control sample containing 16 ng/ml GH (n=16 assays). Mean assay sensitivity was 0·82 ng GH/ml. Plasma GH profile characteristics (mean GH concentration, mean baseline GH concentration, GH pulse amplitude (height of pulse above baseline), GH pulse length (minutes from start to end of pulse), inter-pulse interval (minutes between each peak), GH pulse frequency (pulses per 8-h profile) and integrated GH concentration per pulse and per hour (area above baseline per pulse or averaged per hour)) were determined with the pulse analysis programme TurboPulsar (Dong & Handelsman 1990), which is a modification of the algorithm devised by Merriam & Wachter (1982). The values assigned to the programme parameters were chosen to fit the secretion characteristics of GH in sheep as derived by Thomas et al. (1990). Briefly, the G parameter settings were G(1)=4·4, G(2)=2·6, G(3)=1·92, G(4)=1·46 and G(5)=1·13 co-variance (CV) values, and the assay CV distributed across the GH RIA standard curve was expressed as $y = 24·4 \log x + 24·1$. This algorithm defines a GH pulse as occurring when concurrent GH concentrations in the series contained 1 point which was ≥4·4 CV above the baseline concentration, or 2 points which were ≥2·6 CV above the baseline concentration, etc. The pulse splitting cut-off was 3·0 CV values and the smoothing time was 240 min.

**Statistical analysis**

Effects of PR (none, moderate or severe) and sex (male or female) on size at birth, neonatal growth rates and plasma GH were determined by between factor analysis of variance (ANOVA). Hormone data were log-transformed.
where necessary to ensure equal variance in each group. Specific contrasts between PR groups were performed using Tukey’s HSD test. Effects of PR and sex (between factors), together with that of age (within factor), on current size and plasma GH profile characteristics were determined by repeated measures ANOVA for those animals studied at both ages (n=29). Relationships between variables were assessed separately for each sex by correlation analysis. The independent effects of size at birth and neonatal FGR on characteristics of GH secretion at each age were analysed separately for each sex by partial correlation and multiple linear regression analysis. Statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as estimated means ± S.E.M. from ANOVA, with P<0.05 taken as significant.

Results

Effect of PR and sex on size at birth and neonatal growth

Size at birth did not differ between sexes for any parameter of size at birth, and data for males and females are therefore pooled in Table 1. Degree of PR altered birth weight, crown–rump length, appendicular bone length (shoulder height, metatarsus length and tibia length), abdominal circumference and weight/length ratio (P<0.05), but not ponderal index (Table 1). Parameters of size at birth were lower in the surviving lambs of the severe PR group when compared with control lambs (Table 1). Whilst weight and crown–rump length at birth were lower in surviving lambs of the severe PR group than in control lambs, other parameters of size at birth did not differ between these two groups (Table 1). Neonatal FGR in terms of body weight (P=0.001), crown–rump length (P=0.045) and abdominal circumference (P=0.017) were also affected by degree of PR. These measures of neonatal growth rate were highest in severe PR lambs, intermediate in the moderate PR group, and lowest in control lambs (Table 1).

Effect of PR and sex on body weight and GH profile characteristics

Body weight was lower in females compared with males, in both adolescent (P=0.006) and young adult sheep (P=0.001) (Fig. 1). In adolescent sheep, the effect of degree of PR on body weight differed between males and females (P=0.013) (Fig. 1a). PR altered body weight in adolescent males (P=0.024), but not in adolescent females. In adolescent males, body weight was lower in the moderate PR lambs than in severe PR lambs (P=0.022), and tended to be reduced in moderate PR lambs compared with control lambs (P=0.069). The effect of degree of PR on body weight also differed between sexes in young adult sheep (P=0.018) (Fig. 1b). Body weight did not differ between PR groups in adult males (P=0.345), but was affected by degree of PR in females (P=0.024). In adult female sheep, body weight was reduced in severe PR sheep compared with control sheep (P=0.020) (Fig. 1b).

Representative GH profiles for a control, moderate PR and severe PR male and female sheep at each age are presented in Fig. 2. In adolescent sheep, degree of PR altered mean GH concentration (control 5.14±1.53 ng/ml, moderate PR 6.21±2.28 ng/ml, severe PR 2.12±2.79 ng/ml; P=0.037) and mean baseline GH concentration (control 2.23±0.57 ng/ml, moderate PR 5.23±0.77 ng/ml, severe PR 1.79±0.89 ng/ml;
Degree of PR did not alter GH pulse amplitude, GH pulse length, inter-pulse interval, integrated GH concentration per pulse or integrated GH concentration per hour. GH pulse characteristics were not affected by sex in adolescent sheep. In young adult sheep, PR did not alter plasma GH profile characteristics. Mean GH concentration (males 6.61 ± 2.07 ng/ml, females 2.37 ± 1.58 ng/ml; P=0.008), and number of GH pulses per 8 h (males 13.3 ± 1.0, females 10.6 ± 0.8; P=0.038) were higher in male than in female young adult sheep. Mean baseline GH concentration tended to be higher in males than in females (males 3.59 ± 1.16 ng/ml, females 1.38 ± 0.88 ng/ml; P=0.059), but other characteristics of the circulating GH profile in young adult sheep did not differ between males and females. There were no interactions between the effects of sex and PR on plasma GH profile characteristics at either age.

In those animals which were studied at both ages, there were no effects of PR on body weight or plasma GH profile characteristics. In this cohort, body weight increased with age (P<0.001), was greater in males than in females (P=0.001), and increased to a greater extent with age in males than in females (P=0.024). Plasma GH profile characteristics were also unaffected by sex and age. There were no interactions between the effects of PR and age on any plasma GH profile characteristics.

**GH profile characteristics, size at birth and neonatal growth**

In adolescent male lambs, several characteristics of the plasma GH profile, including mean and baseline GH concentrations, were correlated independently and negatively with weight, shoulder height, metatarsus length and tibia length at birth and their corresponding neonatal FGR (Table 2). In young adult males, mean GH concentration, mean baseline GH concentration, pulse length, and integrated GH concentration per pulse correlated independently and negatively with birth shoulder height and neonatal FGR  

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*Figure 1* Body weight (means ± S.E.M.) of male and female sheep in which GH was measured as (a) adolescents (132 days of age) or (b) adults (378 days of age). Data are shown separately for control, moderate PR and severe PR sheep.

*Figure 2* Representative GH profiles from a male and female (a) control, (b) moderate PR and (c) severe PR sheep at 125–138 (left-hand panels) and 371–385 days (d) of age (right-hand panels). Solid line indicates plasma GH concentration, dotted lines indicate baseline GH concentration and arrows indicate GH pulses.
length correlated independently and negatively with birth weight and FGRweight, and negatively with tibia length at birth, but not with FGRtibia length (Table 3).

In adolescent female lambs, GH pulse length correlated independently and positively with birth weight and with neonatal FGRweight (Table 4). In young adult females, mean baseline GH concentration was independently and negatively correlated with crown–rump length at birth and FGRcrown–rump length (Table 4). Other indications of GH secretion (mean GH concentration, integrated GH concentrations and pulse amplitude) were correlated independently and positively with size at birth and neonatal FGR in terms of shoulder height and/or metatarsus length (Table 4).

Table 2 Plasma GH profile characteristics in adolescent male lambs, size at birth and neonatal growth rate (n=14)

<table>
<thead>
<tr>
<th>GH profile characteristic</th>
<th>Size measure: weight</th>
<th>Size measure: shoulder height</th>
<th>Size measure: metatarsus length</th>
<th>Size measure: tibia length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean GH conc</td>
<td>−0.711, 0.003</td>
<td>−0.688, 0.007</td>
<td>−0.821, &lt;0.001</td>
<td>−0.626, 0.011</td>
</tr>
<tr>
<td>Mean baseline GH conc</td>
<td>−0.730, 0.003</td>
<td>−0.610, 0.014</td>
<td>−0.793, &lt;0.001</td>
<td>−0.793, &lt;0.001</td>
</tr>
<tr>
<td>Pulse amplitude</td>
<td>−0.603, 0.015</td>
<td>−0.417, 0.078</td>
<td>−0.603, 0.015</td>
<td>−0.603, 0.015</td>
</tr>
<tr>
<td>Integrated GH conc/pulse</td>
<td>−0.605, 0.015</td>
<td>−0.441, 0.066</td>
<td>−0.605, 0.015</td>
<td>−0.605, 0.015</td>
</tr>
<tr>
<td>Integrated GH conc/h</td>
<td>−0.601, 0.015</td>
<td>−0.431, 0.071</td>
<td>−0.601, 0.015</td>
<td>−0.601, 0.015</td>
</tr>
</tbody>
</table>

Conc=concentration.

Table 3 Plasma GH profile characteristics in adult male lambs, size at birth and neonatal growth rate (n=13)

<table>
<thead>
<tr>
<th>GH profile characteristic</th>
<th>Size measure: weight</th>
<th>Size measure: shoulder height</th>
<th>Size measure: tibia length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse length</td>
<td>−0.637, 0.013</td>
<td>−0.514, 0.044</td>
<td>−0.643, 0.012</td>
</tr>
<tr>
<td>Mean GH conc</td>
<td>−0.514, 0.044</td>
<td>−0.535, 0.037</td>
<td>−0.564, 0.023</td>
</tr>
<tr>
<td>Mean baseline GH conc</td>
<td>−0.580, 0.024</td>
<td>−0.605, 0.019</td>
<td>−0.578, 0.019</td>
</tr>
<tr>
<td>Pulse length</td>
<td>−0.631, 0.014</td>
<td>−0.438, 0.078</td>
<td>−0.608, 0.014</td>
</tr>
<tr>
<td>Integrated GH conc/pulse</td>
<td>−0.508, 0.046</td>
<td>−0.515, 0.043</td>
<td>−0.506, 0.039</td>
</tr>
<tr>
<td>Integrated GH conc/h</td>
<td>−0.564, 0.023</td>
<td>−0.506, 0.039</td>
<td>−0.610, 0.039</td>
</tr>
</tbody>
</table>

Conc=concentration.
GH concentration per pulse ($r = -0.519, P = 0.020, n = 16$) and per hour ($r = -0.508, P = 0.023, n = 16$) correlated negatively with concurrent weight, whilst GH pulse frequency, length and interval were unrelated to current weight ($P > 0.1$). In adolescent female lambs, GH pulse length correlated negatively with concurrent weight ($r = -0.439, P = 0.024, n = 21$), whilst all other characteristics of the circulating GH profile were unrelated to concurrent weight ($P > 0.1$). In adult male and adult female lambs, characteristics of the circulating GH profile were unrelated to concurrent weight ($P > 0.1$).

**Discussion**

This study has shown for the first time in any species that variations in prenatal and neonatal growth each independently predict alterations in the dynamic circulating GH profile in later life, and that the nature of these alterations varies with age and sex. Restriction of placental growth altered mean and baseline plasma GH concentrations in adolescent sheep, but the consequences appeared to differ between the moderate PR and severe PR groups, and were consistent with the effects on size at birth. Perinatal survival of lambs born to severe PR ewes was low compared with that of lambs born to control or to moderate PR ewes (data not presented), and the birth size of the lambs from severe PR ewes which survived and were studied as adolescents and adults was intermediate between lambs born from moderate PR and control ewes. This could explain why circulating GH concentrations were not elevated in severe PR lambs compared with control lambs. Moderate PR sheep tended to have elevated baseline GH as adolescents, consistent with their smaller size at birth, and the relationships which were observed between birth size and circulating GH in adolescent male sheep. Hence the consequences of variable PR for circulating GH in the adolescent reflect the outcomes in terms of size at birth, not the degree of restriction of implantation sites in the ewe. This may reflect differences in perinatal survival between lambs of moderate and severe PR ewes. Group differences may also be masked by variability in size at birth within each group due to twinning, variable degrees of compensation by accelerated maturation of the placenta and differences in the actual number of placental implantation sites remaining after surgery, since implantation sites in the tip of the uterine horn are not visible at surgery.

The associations between poor prenatal growth and elevated circulating GH in adolescent and adult male

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**Table 4** Plasma GH profile characteristics in adolescent and adult female lambs, size at birth and neonatal growth rate. Numbers of animals are shown in parentheses

<table>
<thead>
<tr>
<th>GH profile characteristic</th>
<th>Size measure: weight</th>
<th>Adolescents</th>
<th>Adults</th>
<th>Overall correlation (R, P)</th>
<th>Size at birth</th>
<th>Neatnol FGR</th>
<th>Size at birth</th>
<th>Neatnol FGR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adolectens</td>
<td>Adults</td>
<td></td>
<td>0.398, 0.051</td>
<td>0.466, 0.026</td>
<td>0.467, 0.070 (19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean baseline GH conc</td>
<td>-0.435, 0.060</td>
<td>-0.461, 0.049</td>
<td>-0.508, 0.083 (15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean GH conc</td>
<td>0.499, 0.035</td>
<td>0.546, 0.022</td>
<td>0.561, 0.052 (15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inter-pulse interval</td>
<td>-0.637, 0.008</td>
<td>-0.745, 0.002</td>
<td>0.749, 0.006 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Integrated GH conc/h</td>
<td>0.523, 0.034</td>
<td>0.499, 0.041</td>
<td>0.547, 0.071 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean GH conc</td>
<td>0.640, 0.010</td>
<td>0.611, 0.013</td>
<td>0.652, 0.024 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Integreted GH conc/h</td>
<td>0.645, 0.009</td>
<td>0.594, 0.016</td>
<td>0.652, 0.024 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Integrated GH conc/h</td>
<td>0.652, 0.008</td>
<td>0.618, 0.012</td>
<td>0.663, 0.021 (14)</td>
<td></td>
</tr>
</tbody>
</table>

Conc = concentration.

**Figure 3** Body weight, mean and mean baseline GH concentrations in male adolescent sheep (132 days of age).
sheep, together with the negative association between circulating GH and concurrent size in adolescent males suggests that intrauterine growth restriction may induce GH resistance in adolescent and possibly adult males. Measurements of circulating insulin-like growth factor-I (IGF-I), rates of GH secretion and clearance or of GH sensitivity are needed to confirm this, however. Consistent with this suggestion, small size at birth due to maternal food restriction in the rat (30% throughout pregnancy) induces GH resistance in adolescent males in terms of growth responses to GH (Woodall et al. 1996a,b). Maternal protein restriction and reduced fetal growth in the rat did not alter circulating GH profiles in adolescent or adult males however, suggesting that GH secretion and possibly GH sensitivity were not altered (Nolan et al. 2001). In humans, small size at birth is associated with reduced GH excretion in young men (Flanagan et al. 1999), and no changes in circulating GH profiles in elderly men (Fall et al. 1998). This range of outcomes suggests differences between species and in the effects of different prenatal perturbations in prenatal programming of the GH axis. To date, however, circulating GH profiles or more direct indices of the GH axis are yet to be characterized in relation to size at birth in adolescent or young adult humans. Furthermore, in children with intrauterine growth retardation (IUGR) who do not catch up, there is evidence of GH resistance (Balsamo et al. 1995, Czernichow 1997, Chatelan et al. 1998). Therefore it remains possible that IUGR does induce GH resistance during adolescence in humans as in other species.

In this study, the male sheep that was small at birth and failed to catch up as a neonate exhibited the highest circulating GH levels in adolescence. Elevated mean GH in adolescent male sheep reflected increases in their baseline GH concentrations and GH pulse amplitudes, rather than an increase in GH pulse frequency. The subset of IUGR children who do not catch up have evidence of both impaired GH secretion (Albertsson-Wikland 1989, Rochiccioli et al. 1989, Stanhope et al. 1989, de Waal et al. 1994, Boguszewski et al. 1995) and GH resistance (Balsamo et al. 1995, Czernichow 1997, Chatelan et al. 1998). Failure of both prenatal and neonatal growth thus appears to be associated with alterations in the postnatal GH axis in both prepubertal children and the male adolescent sheep. The effects of restricted prenatal growth with normal or accelerated neonatal growth on the postnatal GH axis are less clear. The majority of IUGR children do undergo catch-up growth in the neonatal period (Albertsson-Wikland & Karlberg 1994, Hokken-Koelega et al. 1995, Karlberg & Albertsson-Wikland 1995), but the GH axis has not been characterized in these individuals.

Variation in size at birth, reflecting prenatal growth, accounted for a larger proportion of the variation in characteristics of the circulating GH profiles in adolescent male sheep than did neonatal growth. For adolescent males, a 10% decrease in birth weight increased mean plasma GH 1.7 times more than did a 10% decrease in neonatal FGRweight. Similarly, a 10% decrease in tibia or metatarsus length at birth increased mean plasma GH by four to ten times more than did a similar decrease in neonatal FGR for the same bone length.

The mechanisms which alter circulating GH profiles following poor prenatal or neonatal growth are mostly unknown. It seems likely that factors which determine prenatal and/or neonatal growth rate, such as oxygen and/or nutrient supply, programme the GH axis at one or more sites in cells of the hypothalamus, pituitary or in GH-target tissues. In rats, restriction of fetal growth increases somatostatin gene expression in the periventricular nucleus of the hypothalamus in juveniles and adults, although the consequences for circulating GH were not determined (Huizinga et al. 2000). In male sheep, reduced birth weight increases GH secretion responses to GH-releasing hormone (GHRH) from neonatal life to adolescence, which might reflect programming at either the hypothalamus (decreased somatostatin secretion) or pituitary (increased sensitivity to GHRH) (Pastoureau et al. 1989). GH secretion may also be increased by GH resistance at the hypothalamus and pituitary via decreased negative feedback of GH and/or IGF-I at these sites (reviewed by Rosenfeld et al. 1994). GH responsiveness of target tissues could also be programmed by poor perinatal growth and its causes. Adolescent rats, which were growth restricted in utero by maternal feed restriction, have impaired growth responses to GH, in conjunction with reduced hepatic GH receptor numbers (Woodall et al. 1996a,b), suggesting that prenatally induced GH resistance is, at least in part, hepatic. This suggests that variable perinatal growth programmes the postnatal GH axis at multiple sites.

Such changes in the GH axis may have important consequences for the individual, in addition to those for growth and its regulation. Since GH deficiency or resistance is associated with impaired metabolic and cardiovascular function and obesity in adults (Christ et al. 1998, Hew et al. 1998, Rosenbloom et al. 1999), programming of GH synthesis, secretion or sensitivity may be mechanisms by which poor prenatal growth contributes to the increased risk of adult-onset diseases including hypertension, diabetes and cardiovascular disease which are observed in individuals who were small at birth (Barker 1998).

This study has also demonstrated for the first time sex differences in the relationships of the dynamic GH profile in postnatal life with prenatal and neonatal growth. In contrast to males, characteristics of the circulating GH profile in adolescent female lambs were largely unrelated to size at birth or neonatal growth. Furthermore, the observed associations between mean GH and size at birth and neonatal growth rate in terms of shoulder height in young adult female sheep were the opposite of those in
males, such that impaired prenatal or neonatal growth was characterized by reduced circulating GH in young adult female sheep. Limited evidence of sex differences in relationships between perinatal growth and subsequent function of the somatotrophic axis has been reported in human studies. Across the normal range of birthweight, low birthweight was associated with reduced urinary GH excretion in both men and women as young adults (Flanagan et al. 1999), whilst the effects of size at birth and neonatal growth on dynamic GH profiles in the elderly have been reported only for men (Fall et al. 1998). An early report did find that differences in mean GH levels and overnight urinary GH excretion between short IUGR children who fail to catch up and controls were significant only for boys, although circulating GH profiles were similar in IUGR boys and girls (de Waal et al. 1994). Sex differences in relationships between circulating GH and body composition have also been reported in these children (Boguszewski et al. 1995), although these may reflect the impact of gonadal steroids on growth. There is, however, good evidence that other postnatal consequences of impaired perinatal growth, including hypertension, obesity, insulin resistance and risk of coronary heart disease, differ between males and females (Jones et al. 1984, Langley-Evans et al. 1996, Leon et al. 1998, Forsen et al. 1999, Flanagan et al. 2000). It is possible that sex differences in fetal and neonatal growth rates and of maturation between males and females may affect susceptibility to programming by an adverse environment. Sex differences in programming might also reflect postnatal interactions with gonadal steroid secretion since, in humans, IUGR is associated with accelerated puberty in females, but not males (Ibanez et al. 1998).

In conclusion, poor fetal growth, indicated by small size at birth, and the patterns of neonatal growth, are each associated with altered circulating GH profiles in adolescent and adult sheep, and the outcomes vary with age and sex. Restricted fetal growth and failure of neonatal catch-up growth are independently associated with elevated circulating GH in pubertal and adult males, but low circulating GH in adult females. The contributions of altered GH secretion, clearance and sensitivity and their determinants to these major alterations in circulating GH profiles following restriction of prenatal growth and their sex-specific basis remain to be determined.

Acknowledgements

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