Perinatal growth and plasma GH profiles in adolescent and adult sheep

K L Gatford, I J Clarke¹, M J De Blasio, I C McMillen, J S Robinson² and J A Owens

Department of Physiology, University of Adelaide, Adelaide, South Australia 5005, Australia

¹Prince Henry's Institute of Medical Research, PO Box 5152, Clayton, Victoria 3168, Australia

²Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia 5005, Australia

(Requests for offprints should be addressed to K L Gatford; Email: kathy.gatford@adelaide.edu.au)

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 indepen-

dently 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.

Journal of Endocrinology (2002) **173**, 151–159

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 $y=24.4(\log x)^2 - 42.7 \log x + 24.1$. 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

Table 1 Size at birth in control, moderate PR and severe PR lambs. Values are means $\pm$ S.E.M.

	Control (n=19)	Moderate PR (n=14)	Severe PR (n=8)
Body weight (kg)	5.27 $\pm$ 0.23 ^a	3.65 $\pm$ 0.32 ^b	4.13 $\pm$ 0.36 ^b
Crown–rump length (cm)	56.9 $\pm$ 0.9 ^a	49.7 $\pm$ 1.1 ^b	50.3 $\pm$ 1.3 ^b
Shoulder height (cm)	39.9 $\pm$ 0.7 ^a	36.7 $\pm$ 0.9 ^b	38.0 $\pm$ 1.1 ^{ab}
Metatarsus length (cm)	12.5 $\pm$ 0.2 ^a	11.4 $\pm$ 0.2 ^b	11.8 $\pm$ 0.3 ^{ab}
Tibia length (cm)	13.3 $\pm$ 0.3 ^a	12.2 $\pm$ 0.3 ^b	12.9 $\pm$ 0.4 ^{ab}
Abdominal circumference (cm)	39.0 $\pm$ 0.9 ^a	32.9 $\pm$ 1.2 ^b	35.8 $\pm$ 1.4 ^{ab}
Weight/length (kg/cm)	0.0934 $\pm$ 0.003 ^a	0.0755 $\pm$ 0.004 ^b	0.0812 $\pm$ 0.005 ^{ab}
Ponderal index (kg/m ³)	28.6 $\pm$ 0.9	29.3 $\pm$ 1.2	31.9 $\pm$ 1.3
FGR _{weight} (%/day) ¹	5.07 $\pm$ 0.40 ^a	6.51 $\pm$ 0.50 ^{ab}	7.91 $\pm$ 0.60 ^b
FGR _{crown–rump length} (%/day)	1.35 $\pm$ 0.11 ^a	1.48 $\pm$ 0.10 ^{ab}	1.79 $\pm$ 0.10 ^b
FGR _{shoulder height} (%/day)	0.736 $\pm$ 0.071	0.827 $\pm$ 0.100	0.841 $\pm$ 0.100
FGR _{abdominal circumference} (%/day)	1.51 $\pm$ 0.11 ^a	1.81 $\pm$ 0.10 ^{ab}	2.08 $\pm$ 0.20 ^b

Significant differences (ANOVA, specific contrasts performed using Tukey's HSD test) between groups are indicated by differing superscripts.

¹FGR (%/day) for a parameter was calculated as the growth rate of that parameter in kg or cm per day, divided by the size of that parameter at birth, and multiplied by 100.

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 $\pm$ 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 moderate 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;

Figure 1 Body weight (means $\pm$ 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.

$P=0.034$). 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

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 indicate plasma GH concentration, dotted lines indicate baseline GH concentration and arrows indicate GH pulses.

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_{shoulder height} (Table 3). At this age, GH pulse

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

	Partial correlations (R, P)		Overall correlation (R, P)
	Size at birth	Neonatal FGR	
GH profile characteristic			
Size measure: weight			
Mean GH conc	−0.711, 0.003	−0.510, 0.038	0.713, 0.010
Mean baseline GH conc	−0.730, 0.003	−0.528, 0.032	0.732, 0.008
Size measure: shoulder height			
Mean GH conc	−0.688, 0.007	−0.408, 0.083	0.700, 0.013
Mean baseline GH conc	−0.610, 0.014	−0.308, 0.153	0.661, 0.021
Pulse amplitude	−0.603, 0.015	−0.417, 0.078	0.616, 0.036
Integrated GH conc/pulse	−0.605, 0.015	−0.441, 0.066	0.612, 0.038
Integrated GH conc/h	−0.601, 0.015	−0.431, 0.071	0.609, 0.039
Size measure: metatarsus length			
Mean GH conc	−0.821, <0.001	−0.719, 0.003	0.822, 0.001
Mean baseline GH conc	−0.793, <0.001	−0.665, 0.007	0.796, 0.002
Pulse amplitude	−0.644, 0.009	−0.526, 0.033	0.645, 0.026
Integrated GH conc/pulse	−0.677, 0.006	−0.585, 0.018	0.677, 0.017
Integrated GH conc/h	−0.667, 0.007	−0.578, 0.019	0.667, 0.020
Size measure: tibia length			
Mean GH conc	−0.626, 0.011	−0.451, 0.061	0.640, 0.028
Pulse amplitude	−0.587, 0.018	−0.580, 0.019	0.654, 0.023
Integrated GH conc/pulse	−0.602, 0.015	−0.608, 0.014	0.675, 0.018
Integrated GH conc/h	−0.564, 0.023	−0.506, 0.039	0.610, 0.039

Conc=concentration.

R=correlation coefficient.

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

	Partial correlations (R, P)		Overall correlation (R, P)
	Size at birth	Neonatal FGR	
GH profile characteristic			
Size measure: weight			
Pulse length	−0.637, 0.013	−0.548, 0.033	0.642, 0.035
Size measure: shoulder height			
Mean GH conc	−0.514, 0.044	−0.535, 0.037	0.549, 0.084
Mean baseline GH conc	−0.580, 0.024	−0.605, 0.019	0.617, 0.046
Pulse length	−0.631, 0.014	−0.438, 0.078	0.645, 0.034
Integrated GH conc/pulse	−0.508, 0.046	−0.515, 0.043	0.535, 0.093
Size measure: tibia length			
Pulse length	−0.643, 0.012	−0.107, 0.371	0.656, 0.030

Conc=concentration.

length correlated independently and negatively with birth weight and FGR_{weight} , and negatively with tibia length at birth, but not with $FGR_{\text{tibia length}}$ (Table 3).

In adolescent female lambs, GH pulse length correlated independently and positively with birth weight and with neonatal FGR_{weight} (Table 4). In young adult females, mean baseline GH concentration was independently and negatively correlated with crown-rump length at birth and $FGR_{\text{crown-rump length}}$ (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).

Postnatal body weight and GH profile characteristics

In adolescent male lambs, mean GH concentration (Fig. 3), mean baseline GH concentration (Fig. 3), GH pulse amplitude ($r = -0.524$, $P = 0.019$, $n = 16$), and integrated

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

GH profile characteristic	Partial correlations (R, P)		Overall correlation (R, P)
	Size at birth	Neonatal FGR	
Size measure: weight			
Adolescents			
Pulse length	0.398, 0.051	0.466, 0.026	0.467, 0.070 (19)
Size measure: crown–rump length			
Adults			
Mean baseline GH conc	−0.435, 0.060	−0.461, 0.049	0.508, 0.083 (15)
Size measure: shoulder height			
Adults			
Mean GH conc	0.499, 0.035	0.546, 0.022	0.561, 0.052 (15)
Inter-pulse interval	−0.657, 0.008	−0.745, 0.002	0.749, 0.006 (14)
Integrated GH conc/h	0.523, 0.034	0.499, 0.041	0.547, 0.071 (14)
Size measure: metatarsus length			
Adults			
Pulse amplitude	0.640, 0.010	0.611, 0.013	0.652, 0.024 (14)
Integrated GH conc/pulse	0.645, 0.009	0.594, 0.016	0.651, 0.024 (14)
Integrated GH conc/h	0.652, 0.008	0.618, 0.012	0.663, 0.021 (14)

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

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, Chatelain *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, Chatelain *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 FGR_{weight} . 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) (Pastoureaux *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

This work was supported by a project grant from the National Health and Medical Research Council of Australia (NH&MRC). K L G was supported by an NH&MRC Peter Doherty Postdoctoral Fellowship. Purified ovine GH and antibody were generously supplied by the NIDDK Pituitary Hormone Program. We thank Simon Fielke, Damian Adams and Melissa Bradbury for

assistance with surgery, maintenance of animals and sample collection, and Julie Mitrevski for performing the GH assays.

References

- Albertsson-Wikland K 1989 Growth hormone secretion and growth hormone treatment in children with intra-uterine growth retardation. *Acta Paediatrica Scandinavica Supplementum* **349** 35–41.
- Albertsson-Wikland K & Karlberg J 1994 Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatrica Scandinavica Supplementum* **399** 64–70.
- Balsamo A, Tassoni P, Cassio A, Colli C, Tassinari D, Cicognani A & Cacciari E 1995 Response to growth therapy in patients with growth hormone deficiency who at birth were small or appropriate in size for gestational age. *Journal of Pediatrics* **126** 474–477.
- Barker DJP 1998 *Mothers, Babies and Health in Later Life*. Edinburgh: Churchill Livingstone.
- Boguszewski M, Rosberg S & Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. *Journal of Clinical Endocrinology and Metabolism* **80** 2599–2606.
- Chatelain PG, Nicolino M, Claris O, Salle B & Chaussain J-L 1998 Multiple hormone resistance in short children born with intrauterine growth retardation? *Hormone Research* **49** (Suppl 2) 20–22.
- Christ ER, Cummings MH & Russell-Jones DL 1998 Dyslipidaemia in adult growth hormone (GH) deficiency and the effect of GH replacement therapy: a review. *Trends in Endocrinology and Metabolism* **9** 200–206.
- Czernichow P 1997 Growth hormone treatment of short children born small for gestational age. *Acta Paediatrica Scandinavica Supplementum* **423** 213–215.
- Dong Q & Handelsman DJ 1990 Hypothalamic opioid mechanisms in experimental uremic hypergonadism. *Endocrinology* **126** 1498–1503.
- Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C & Barker DJP 1999 Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *British Medical Journal* **318** 427–431.
- Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D & Cooper C 1998 Programming of growth hormone secretion and bone mineral density in elderly men: a hypothesis. *Journal of Clinical Endocrinology and Metabolism* **83** 135–139.
- Flanagan DE, Moore VM, Godsland IF, Cockington RA, Robinson JS & Phillips DIW 1999 Reduced foetal growth and growth hormone secretion in adult life. *Clinical Endocrinology* **50** 735–740.
- Flanagan DE, Moore V, Godsland I, Cockington R, Robinson J & Phillips D 2000 Fetal growth and the physiological control of glucose tolerance in adult life: a minimal model analysis. *American Journal of Physiology* **278** E700–E706.
- Forsen T, Eriksson JG, Tuomilehto J, Osmond C & Barker DJP 1999 Growth *in utero* and during childhood among women who develop coronary heart disease: longitudinal study. *British Medical Journal* **319** 1403–1407.
- Gluckman PD, Gunn AJ, Wray A, Cutfield WS, Catelain PG, Guillaud O, Ambler G, Wilton P & Albertsson-Wikland K 1992 Congenital idiopathic growth hormone deficiency associated with prenatal and early postnatal growth failure. *Journal of Pediatrics* **121** 920–923.
- Hew FL, O'Neal D, Kamarudin N, Alford FP & Best JD 1998 Growth hormone deficiency and cardiovascular risk. *Ballière's Clinical Endocrinology and Metabolism* **12** 199–216.
- Hokken-Koelega ACS, De Ridder MAJ, Lemmen RJ, Den Hartog H, De Muink Keizer-Schrama SMPF & Drop SLS 1995 Children born small for gestational age: do they catch up? *Pediatric Research* **38** 267–271.

- Huizinga CT, Oudejans CBM, Steiner RA, Clifton DK & Delemarre-van de Waal HA 2000 Effects of intrauterine and early postnatal growth restriction on hypothalamic somatostatin gene expression in the rat. *Pediatric Research* **48** 815–820.
- Huxley RR, Shiell AW & Law CM 2000 The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *Journal of Hypertension* **18** 815–831.
- Ibanez L, De Zehger F & Potau N 1998 Premature pubarche, ovarian hyperandrogenism, hyperinsulinism and the polycystic ovary syndrome: from a complex constellation to a simple sequence of prenatal onset. *Journal of Endocrinological Investigation* **21** 558–566.
- Jones AP, Simson EL & Friedman MI 1984 Gestational undernutrition and the development of obesity in rats. *Journal of Nutrition* **114** 1484–1492.
- Karlberg J & Albertsson-Wikland K 1995 Growth in full-term small-for-gestational-age infants: from birth to final height. *Pediatric Research* **38** 733–739.
- Langley-Evans SC, Welham SJM, Sherman RC & Jackson AA 1996 Weanling rats exposed to maternal low-protein diets during discrete periods of gestation exhibit differing severity of hypertension. *Clinical Science* **91** 607–615.
- Leon DA, Lithell HO, Vagero D, Koupilova I, Mohsen R, Berglund L, Lithell U-B & McKeigue PM 1998 Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915–1929. *British Medical Journal* **317** 241–245.
- Merriam GR & Wachter KW 1982 Algorithms for the study of episodic hormone secretion. *American Journal of Physiology* **243** E310–E318.
- Nolan LA, Hart EJ, Windle RJ, Wood SA, Hu XW, Levi AJ, Ingram CD & Levy A 2001 Lack of effect of protein deprivation-induced intrauterine growth retardation on behavior and corticosterone and growth hormone secretion in adult male rats: a long-term follow-up study. *Endocrinology* **142** 2996–3005.
- Ong KKL, Ahmed ML, Emmett PM, Preece MA & Dunger DB 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *British Medical Journal* **320** 967–971.
- Pastoureaux P, Charrier J, Blanchard MM, Boivin G, Dulong JP, Theriez M & Barenton B 1989 Effects of a chronic GRF treatment on lambs having low or normal birth weight. *Domestic Animal Endocrinology* **6** 321–329.
- Robinson JS, Kingston EJ, Jones CT & Thorburn GD 1979 Studies on experimental growth retardation in sheep. The effect of removal of endometrial caruncles on fetal size and metabolism. *Journal of Developmental Physiology* **1** 379–398.
- Robinson JS, Chidzanja S, Kind K, Lok F, Owens P & Owens J 1995 Placental control of fetal growth. *Reproduction, Fertility and Development* **7** 333–344.
- Rochiccioli P, Tauber M, Moisan V & Pienkowski C 1989 Investigation of growth hormone secretion in patients with intrauterine growth retardation. *Acta Paediatrica Scandinavica Supplementum* **349** 42–46.
- Rosenbloom AL, Martinez V, Kranzier JH, Bachrach LK, Rosenfeld RG & Guevara-Aguirre J 1999 Natural history of growth hormone receptor deficiency. *Acta Paediatrica Scandinavica Supplementum* **428** 153–156.
- Rosenfeld RG, Rosenbloom AL & Guevara-Aguirre J 1994 Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocrine Reviews* **15** 369–390.
- Stanhope R, Ackland F, Hamill G, Clayton J, Jones J & Preece MA 1989 Physiological growth hormone secretion and response to growth hormone treatment in children with short stature and intrauterine growth retardation. *Acta Paediatrica Scandinavica Supplementum* **349** 47–52.
- Thomas GB, Mercer JE, Karalis T, Rao A, Cummins JT & Clarke IJ 1990 Effect of restricted feeding on the concentrations of growth hormone (GH), gonadotropins, and prolactin (Prl) in plasma, and on the amounts of messenger ribonucleic acid for GH, gonadotropins, and Prl in the pituitary glands of adult ovariectomized ewes. *Endocrinology* **126** 1361–1367.
- de Waal WJ, Hokken-Koelega ACS, Stijnen T & de Muinck Keizer-Schrama SMPF 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. *Clinical Endocrinology* **41** 621–630.
- Woodall SM, Bauer MK, Breier BH, Johnston BM & Gluckman PD 1996a Effects of IGF-1 and GH treatment on postnatal development of intrauterine growth retarded rats. *Proceedings of the 10th International Congress of Endocrinology* **2** 860 (Abstract).
- Woodall SM, Breier BH, Johnston BM & Gluckman PD 1996b A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. *Journal of Endocrinology* **150** 231–242.

Received 28 December 2001

Accepted 9 January 2002