0031-3998/00/4806-0808
PEDIATRIC RESEARCH
Vol. 48, No. 6, 2000
Printed in U.S.A.

Placental Insufficiency and Fetal Growth Restriction Lead to Postnatal Hypotension and Altered Postnatal Growth in Sheep

SAMANTHA LOUEY, MEGAN L. COCK, KATHLEEN M. STEVENSON, AND RICHARD HARDING

Fetal and Neonatal Research Group, Department of Physiology, Monash University, Victoria 3800, Australia

ABSTRACT

Low birth weight has been associated with elevated arterial pressure in later life but mechanisms are unknown. Our aim was to determine the effects of low birth weight resulting from intrauterine growth restriction (IUGR) on fetal and postnatal arterial pressures and the potential roles of circulating cortisol and renin. We induced IUGR by umbilico-placental embolization (UPE) in fetal sheep from 120 d of gestation until birth (approximately 147 d); postnatal lambs (8 IUGR, 8 controls) were studied for 8 wk. Fetal and postnatal arterial pressures were measured and blood samples taken for measurement of gas tensions, cortisol concentrations and renin activity. In IUGR fetuses, mean arterial pressure (MAP) initially increased with UPE, but near term was not different to values in controls. IUGR lambs weighed 33% less than controls at birth and remained lighter than controls during the 8 postnatal weeks; their growth pattern was different to that of controls. IUGR lambs had lower MAP than controls, and this relative hypotension (−4 mm Hg) persisted throughout the 8 postnatal weeks. Covariate analysis showed that the relative hypotension of IUGR lambs could have resulted from their smaller size. Plasma cortisol concentrations were not different between IUGR and control animals before or after birth. Plasma renin activity was not different in postnatal IUGR lambs compared with controls. Thus, postnatal cortisol and renin levels were not consistent with the development of hypotension or hypertension. We conclude that late gestational IUGR in sheep leads to relative hypotension in the early postnatal period, probably a result of reduced body size. **(*Pediatr Res* 48: 808–814, 2000)**

Abbreviations:
UPE, umbilico-placental embolization
IUGR, intrauterine growth restriction
MAP, mean arterial pressure

There is increasing evidence that low birth weight resulting from intrauterine growth restriction (IUGR) is associated with an increased risk of later illness. Associations have been demonstrated between low birth weight and an increased risk of adult-onset diseases such as hypertension, coronary heart disease and type II diabetes (1, 2). In adults, an inverse relationship has been found between arterial pressure and birth weight (3, 4) and this relationship has been found to be independent of current body size and lifestyle factors (1). Studies in children have also revealed inverse relationships between birth weight and arterial pressure (5). The relationship in adolescents is less clear with some studies finding inverse relationships between birth weight and arterial pressure (6) and others finding positive relationships (7). Based on epidemiologic findings, it has been proposed that hypertension is initiated *in utero* and is amplified with age (8).

Several studies of fetal growth restriction in animals have also shown relationships between a sub-optimal intrauterine environment and arterial pressure. Mid-gestational uterine artery ligation in guinea pigs has been shown to produce growth restricted offspring with an elevated mean arterial pressure (9). In the rat, a low protein diet during pregnancy also leads to growth restricted offspring with an elevated systolic pressure (10). Using umbilico-placental embolization (UPE) in late gestation to restrict fetal growth in sheep, Murotsuki *et al.* (11) found growth restricted fetuses to have raised arterial pressure and ventricular hypertrophy. However, a similar study using UPE in sheep for 20 d in late gestation found growth restricted fetuses to have lower arterial pressure, with no alteration in heart weight when adjusted for body weight (12).

Owing to uncertainties about the relationship between IUGR and the development of arterial pressure, our aim was to determine the effects of IUGR on arterial pressure in the fetus and during early postnatal life. Our hypothesis was that IUGR

Received December 29, 1999; accepted August 2, 2000.
Correspondence and reprint requests: Samantha Louey, Department of Physiology, Box 13F, Monash University, Clayton, 3800, VIC, Australia.
This study was supported by the National Health and Medical Research Council of Australia.

leads to hypertension in the fetus and early postnatal period. We have used the UPE technique as it is a controllable and reproducible means of restricting placental function that results in IUGR. Furthermore, many of its effects in the sheep have been documented (12–14) and resemble features of growth restricted human fetuses (15). We have measured plasma cortisol and renin as these hormones have been implicated in the regulation of blood pressure (16, 17). An additional aim was to characterize the pattern of early postnatal growth following IUGR induced by late gestational placental insufficiency owing to evidence that postnatal growth patterns may affect the development of hypertension (1). We have used only lambs born at term to avoid the potentially confounding effects of preterm birth.

METHODS

Fetal Studies

Eighteen pregnant Border Leicester X Merino ewes underwent surgery at 116 ± 1 d after mating (term approximately 147 d). Anesthesia was induced by sodium thiopental (1 g, i.v.) and was maintained with halothane (1.5% to 2%) in O_2. Under aseptic conditions, the fetal hindquarters were exposed and catheters implanted into a fetal femoral artery and vein (12). The arterial catheter was inserted such that its tip lay in the abdominal aorta, 1–2 cm below the level of the renal artery. This catheter was later used for blood sampling, the measurement of fetal arterial pressure and to induce UPE. We have previously shown that the catheter tip remains in the desired position for at least 25 d after implantation at 115 d and that no microspheres enter the fetal kidney (12).

Catheters were tracked s.c. to the flank of the fetus for exteriorization through a small incision (1–2 cm) and were sutured to its skin. Another catheter was sutured to the fetal rump for the measurement of amniotic fluid pressure. Before returning the fetus to the uterus, antibiotics (oxytetracycline base 200 mg/mL or procaine penicillin 200 mg/mL, dihydrostreptomycin 250 mg/mL) were administered. EMG electrodes were inserted into the myometrium to monitor labor. After surgery, ewes were housed in rooms with 12-h light (0700–1900) and dark cycles at 18–20°C; ewes had free access to feed and water. Three to four days of recovery were allowed after surgery before UPE commenced. The UPE group ($n = 8$) consisted of 1 singleton and 7 twins (6 males and 2 females). The control group consisted of 8 singleton fetuses (2 males and 6 females). This study was approved by the Monash University Animal Welfare Committee.

Arterial blood samples (0.5 mL) were collected daily for measurement of pH, gas tensions ($Paco_2$, Pao_2, Sao_2, Radiometer ABL 510, Copenhagen, Denmark); values were adjusted for a fetal body temperature of 39°C; blood glucose and lactate concentrations were also measured (2300 STAT analyser; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.).

Umbilico-placental embolization. Fetuses in the IUGR group underwent UPE from 120 d of gestational age (GA) until birth at term. Insoluble microspheres (Sephadex Superfine G-25, 40–70μm, Pharmacia LKB, Uppsala, Sweden) were suspended in 1% wt/vol heparinized saline and 0.02% Tween 80 so that 1 mL of solution contained approximately 10^6 spheres (12). Microspheres were injected daily so as to reduce fetal Sao_2 by 50% or Pao_2 by approximately 8 mm Hg below pre-UPE values. Catheters of control fetuses were flushed daily with heparinized saline containing no microspheres.

Fetal arterial pressure. Recordings of fetal arterial pressure were made for 1 h at 120, 130, and 140 d GA. Arterial pressure was recorded using external pressure transducers (Viggo-Spectramed, Oxnard, CA, U.S.A.) and amniotic sac pressure was electronically subtracted. Fetal heart rate was derived from the arterial pressure signal. Mean arterial pressure and heart rate were logged using a digital data recording system (ADInstruments Pty Ltd, Castle Hill, NSW, Australia).

Plasma cortisol concentration. Blood samples were taken weekly for measurement of fetal plasma cortisol concentrations. For IUGR fetuses, these samples were taken at least two hours after the completion of UPE. Whole blood (2 mL) was placed into a fluoride heparin tube and centrifuged at 4°C for 15 min (3000 rpm). Measurement of cortisol concentration was performed using a RIA (18).

Delivery of lambs. Uterine EMG activity was monitored after 140 d GA and when it indicated the onset of labor, the fetal catheters were blocked and retaining sutures removed, allowing catheters to withdraw as the lamb was born. All lambs included in this study were born vaginally and spontaneously at term (control $n = 8$, IUGR $n = 8$). Animals that were born preterm (<140 d, $n = 6$ lambs from 5 ewes) were excluded from the study. After birth, elasticised netting was placed around the lamb's trunk and catheters were secured to the netting. Lambs were housed with their mothers with access to feed and water.

Postnatal Studies

Postnatal growth. Measurements of body weight and dimensions, including thoracic girth and crown-rump length, were made between birth and 8 wk after birth. Skinfold thickness was measured with calipers at 6 standardized sites over the neck, chest and limbs; measurements from each site were averaged to obtain a mean skinfold thickness score. Ponderal index was calculated as:

$$\text{Ponderal index (kg/cm}^3\text{)} = \text{bodyweight} \div (\text{crown rump length})^3$$

Arterial pressure. Arterial pressure recordings were made in each lamb for 1–1.5 hours at 4 d and at 1, 2, 4 and 8 wk after birth. Lambs lay prone in a sling, in the company of another lamb, in a quiet, dimly lit isolated room at 22°C. Arterial pressure and heart rate were recorded as in the fetus. The behavior of lambs was observed during the recording period and arterial pressure was excluded from the analysis when lambs were active (*e.g.* moving trunk or limbs, bleating). Of the total pressure recordings, an average of 90.1 ± 1.1% (range 64.3% to 100%) was included for analysis. At the completion of the recording sessions, blood samples were taken for the measurement of pH, gas parameters, plasma cortisol concentrations and renin activity.

Plasma renin activity. Whole blood (1.5 mL) was drawn into a chilled syringe and placed into a chilled tube containing 50 μL/ml blood of BAL-EDTA to inhibit the breakdown of renin. Plasma renin activity was measured as the rate of generation of angiotensin I (Ang I) in pg/mL plasma/h by renin acting on endogenous substrate. Angiotensin I concentrations were measured by RIA (19).

Post mortem. At the end of the 8-wk postnatal study period, 6 lambs in each group were painlessly killed by an overdose of sodium pentobarbital (325 mg/mL i.v.) and major organs weighed.

Statistical analysis. Data are presented as mean ± SEM. Unpaired *t*-tests were used to compare growth measurements at birth and 8 postnatal weeks. A repeated measures analysis of variance (ANOVA) was used to compare all other data; factors were treatment and time. An analysis of covariance was performed to assess the effect of current body weight on the postnatal arterial pressure recordings. Significant differences indicated by the ANOVAs were subjected to the Least Significant Difference posthoc test to test for significant differences between individual group means. The level of significance was taken at $p < 0.05$ and only statistically different data are reported unless otherwise stated.

RESULTS

Fetal Studies

Arterial blood data. Before UPE, there were no differences in Sao_2 between the two groups (56.2 ± 2.3%). Between 120 d and birth, fetuses subjected to UPE were hypoxemic (Sao_2 37.2 ± 1.5%, Pao_2 14.7 ± 0.4 mm Hg) compared with controls (Sao_2 55.8 ± 0.9%, Pao_2 20.7 ± 0.4 mm Hg). Before UPE, fetal $Paco_2$ was not different between the two groups (45.5 ± 1.1 mm Hg) but during UPE, it was higher than in controls (53.0 ± 0.7 *versus* 47.8 ± 1.8 mm Hg). Arterial pH was not different between the two groups except at 145 d GA when IUGR fetuses had a lower pH than controls (7.32 ± 0.01 *versus* 7.38 ± 0.01). During the UPE period, fetal blood glucose concentrations were lower in IUGR fetuses than in controls (0.5 ± 0.0 *versus* 0.7 ± 0.0 mmol/L). Blood lactate concentrations did not differ between the two groups during the study period (0.8 ± 0.0 mmol/L) (Fig. 1).

Fetal arterial pressure and heart rate. Before UPE, diastolic, systolic and mean arterial pressures did not differ significantly between IUGR and control fetuses (diastolic: 31 ± 1 mm Hg, systolic: 50 ± 2 mm Hg, MAP: 37 ± 1 mm Hg). One day after the onset of UPE (121 d GA), MAP was higher than in controls (44 ± 2 *versus* 37 ± 2 mm Hg). Regression analysis showed that, in IUGR fetuses, MAP did not change between 121 d and term; in contrast, MAP in controls increased progressively during this time. Near term, there was no significant difference in MAP between the two groups ($p = 0.16$). At 121 d, diastolic pressure was 32 ± 2 mm Hg in controls and 37 ± 3 mm Hg in IUGR fetuses; systolic pressure was 48 ± 3 mm Hg in controls and 56 ± 3 mm Hg in IUGR fetuses. At 140 d, diastolic and systolic pressures were, respectively, 40 ± 3 mm Hg and 59 ± 2 mm Hg in controls, and 38 ± 2 mm Hg and 55 ± 2 mm Hg in IUGR fetuses. Fetal heart rate was not different between the two groups in the pre-UPE period or at 140 d GA (Fig. 2).

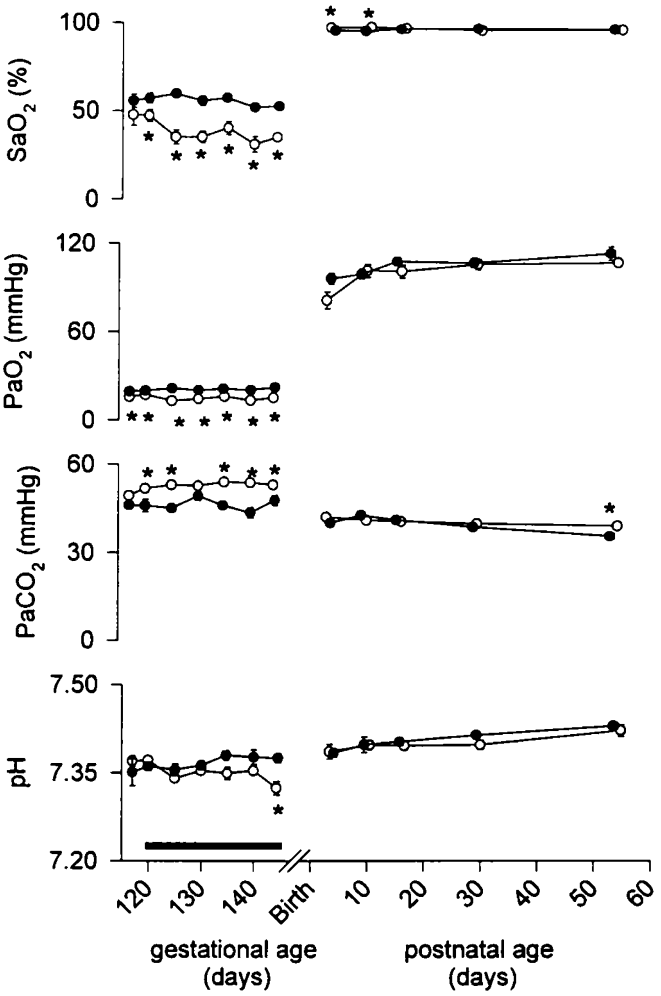

Figure 1. Arterial O_2 saturation (Sao_2), Po_2 (Pao_2), Pco_2 ($Paco_2$) and pH in IUGR (○) and control (●) animals during the pre-embolization period (118 - 119 d of gestation), the prenatal treatment period (120 d until birth), and in the postnatal period up to 8 wk after birth. The bar indicates the period of umbilico-placental embolization. Asterisks (*) indicate values that differ between groups ($p < 0.05$).

Fetal cortisol concentrations. Plasma cortisol concentrations were not different between control and IUGR fetuses. Fetal cortisol concentrations increased with age in control fetuses from 3.2 ± 0.7 ng/mL at 120 d GA to 24.8 ± 6.0 ng/mL at 141 d GA; values increased in IUGR fetuses from 3.5 ± 0.6 ng/mL at 120 d GA to 20.8 ± 3.7 ng/mL at 141 d GA (Fig. 3).

Postnatal Studies

Postnatal growth. All lambs were born at term (IUGR 146 ± 1 d, controls 147 ± 1 d). At birth, IUGR lambs had undergone 26 ± 1 d of UPE. Birth weights of IUGR lambs were lower (2.9 ± 0.2 kg) than those of controls (4.3 ± 0.2 kg), and IUGR lambs remained lighter up to 8 wk after birth (IUGR 12.7 ± 0.9 kg, controls 15.8 ± 1.0 kg). On average, both groups of lambs increased their body weights by 4% each

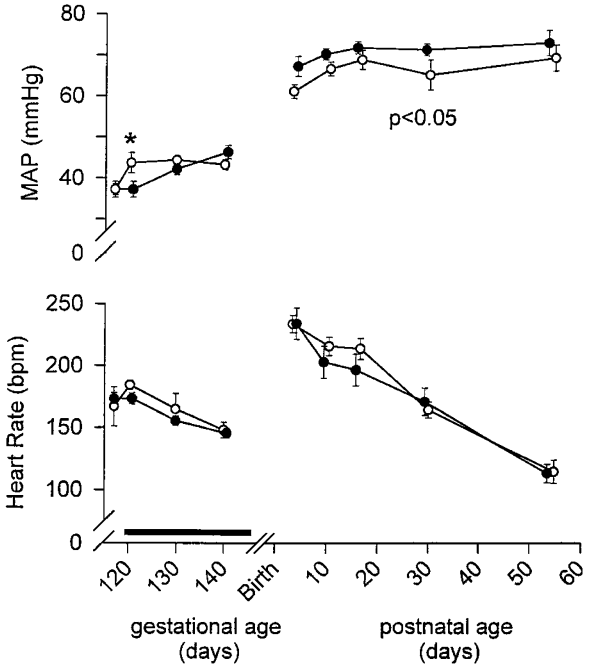

Figure 2. Mean arterial pressure (MAP) and heart rate in IUGR (○) and control (●) fetuses and postnatal lambs up to 8 wk of age. The bar indicates the period of umbilico-placental embolization. p values refer to a significant effect of treatment. Asterisk (*) indicates value that differs between groups ($p < 0.05$).

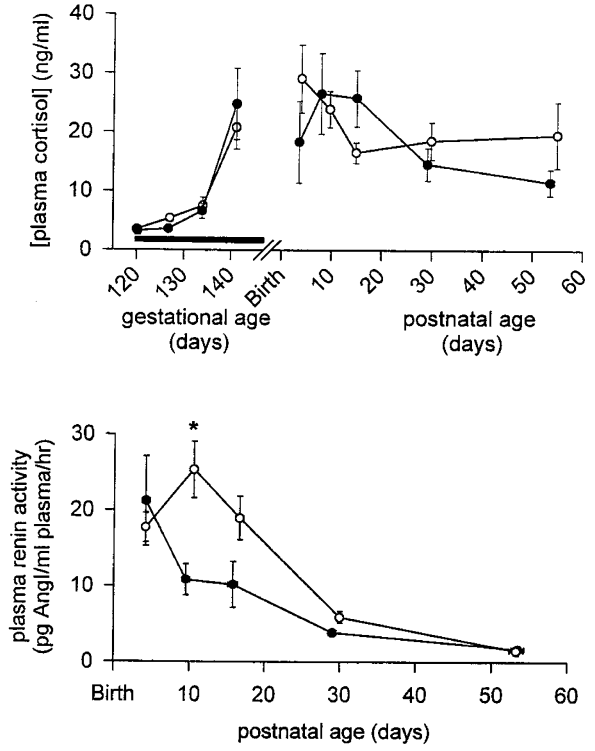

Figure 3. Upper: Plasma cortisol concentration in IUGR (○) and control (●) fetuses and postnatal lambs up to 8 wk of age. The bar indicates the period of umbilico-placental embolization. Lower: Plasma renin activity in IUGR (○) and control (●) postnatal lambs. Asterisk (*) indicates value that differs between groups ($p < 0.05$).

day; control lambs gained 240 ± 22 g per day while IUGR lambs gained 185 ± 19 g per day. By 8 wk, both groups had increased their body weights by similar amounts (9.8 ± 0.7 kg in IUGR *versus* 11.2 ± 0.8 kg in controls) and thus the percentage increase from birth weight of IUGR lambs (441 ± 18%) was significantly greater than that of controls (361 ± 16%) (Table 1, Fig. 4).

Both crown-rump length and thoracic girth were lower in IUGR lambs at birth and remained lower than in controls at 8 wk. At birth, IUGR lambs had lower mean skinfold thicknesses, but values were not different to those of controls at 8 wk. The ponderal index of IUGR lambs at birth was lower than in controls; however, at 8 wk ponderal indexes of the two groups were not different. For both groups, the ponderal index decreased with age, but the percentage decrease was greater in control lambs.

Arterial blood gases and pH. Pa_{O_2} and Sa_{O_2} were similar in postnatal IUGR and control lambs, with the exception that IUGR lambs had elevated Sa_{O_2} during the first 2 wk. Pa_{CO_2} decreased with age but was not different between groups, except at week 8, when IUGR lambs were hypercapnic compared with controls (IUGR 39.0 ± 1.0 mm Hg, controls 35.5 ± 0.9 mm Hg). Arterial pH was not different between groups and increased with age in both groups (Fig. 1).

Postnatal arterial pressure and heart rate. In IUGR lambs, MAP was lower than in controls at 4 d after birth (IUGR 61 ± 2 mm Hg, control 67 ± 2 mm Hg) and remained lower at 8 wk (IUGR 69 ± 3 mm Hg, control 73 ± 3 mm Hg). At 4 d after birth, diastolic pressure was lower in IUGR lambs than controls (IUGR 52 ± 1 mm Hg, control 57 ± 2 mm Hg) and remained lower throughout the 8-wk study period (IUGR 58 ± 3 mm Hg, control 61 ± 3 mm Hg). Systolic pressure in both groups increased with age; it tended ($p = 0.07$) to be lower in IUGR lambs over the postnatal study period. Heart rate was not different between the groups throughout the postnatal study period, but decreased with age in both groups. An analysis of covariance revealed that, when current body weight was taken into consideration, there was no effect of treatment on MAP, that is, the lower arterial pressure of the IUGR lambs could be explained by the lower weight of these animals ($p = 0.128$) (Fig. 2).

Plasma cortisol concentration. After birth, plasma cortisol concentrations varied between animals and were not different between the control and IUGR lambs. Values did not differ

Table 1. *Growth indices of lambs at birth and at 8 weeks of postnatal age*

	Birth		8 Weeks	
	Control	IUGR	Control	IUGR
Crown-rump length (mm)	521 ± 9	486 ± 13*	861 ± 16	807 ± 13*
Thoracic girth (mm)	390 ± 5	339 ± 7*	629 ± 14	565 ± 10*
Mean skinfold thickness (mm)	2.4 ± 0.1	2.1 ± 0.1*	2.8 ± 0.1	2.6 ± 0.1
Ponderal index × 10^5 (kg/cm^3)	3.2 ± 0.2	2.6 ± 0.1*	2.5 ± 0.1	2.4 ± 0.2
% change in ponderal index			−21.3 ± 2.9	−4.7 ± 3.9*

Asterisks (*) indicate values that differ between groups ($p < 0.05$).

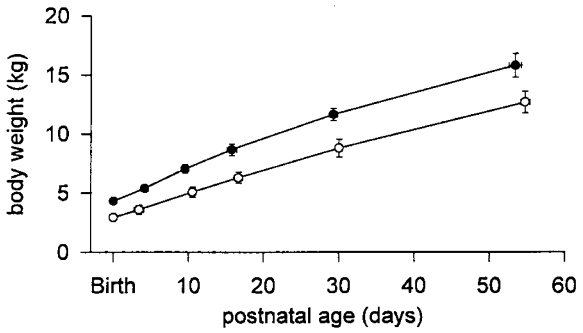

Figure 4. Postnatal body weights from birth to 8 wk in IUGR (○) and control (●) lambs. Throughout the 8-wk period, IUGR lambs weighed less than controls ($p < 0.01$).

significantly between groups and did not change over the 8-wk study period; the mean value was 20.2 ± 1.5 ng/mL (Fig. 3).

Plasma renin activity. Renin activity decreased with age in both control and IUGR lambs. In control lambs it decreased from 21.2 ± 5.9 pg Ang I/mL/h at 4 d to 1.7 ± 0.3 pg Ang I/mL/h at 8 wk and in IUGR lambs, it decreased from 18.3 ± 2.1 pg Ang I/mL/h at 4 d to 1.5 ± 0.5 pg Ang I/mL/h at 8 wk. At 1 wk, plasma renin activity was greater in IUGR lambs (25.3 ± 3.7 pg Ang I/mL/h) than in controls (10.8 ± 2.1 pg AngI/mL/h) (Fig. 3).

Organ weights at 8 wk. IUGR lambs had significantly lower kidney and heart weights; however, when adjusted for body weight, values were not different between groups. Only gastrointestinal and brain weights, in relation to body weight, were greater in IUGR lambs than in controls (Table 2).

DISCUSSION

We have shown that 26 d of fetal hypoxemia and fetal hypoglycemia induced by placental insufficiency can allow birth at term but results in fetal growth restriction, lower postnatal arterial pressure and altered postnatal growth.

During the period of placental insufficiency, alterations in fetal blood gas values were similar to those measured in previous studies in sheep using the UPE technique (11–13), and were similar to values measured by cordocentesis in human fetuses that were small for gestational age (15). We found that UPE altered the normal age-related increase in fetal arterial pressure. The changes we observed after the onset of UPE, namely an initial increase followed by no further change, were similar to those seen in our previous study (12). However, in the previous study (12) we observed a significant reduction in MAP, relative to controls, near to term (140 d GA). A recent study by Hawkins *et al.* (20) has found that fetuses of ewes nutritionally restricted in the first 70 d of pregnancy also have lower arterial pressures (although these fetuses were not growth restricted). Thus, it appears that the relative hypotension observed postnatally in IUGR lambs may develop during fetal life.

Our results are not consistent with measurements of fetal MAP made in two other studies using the UPE technique in sheep. In one study fetal MAP was not altered by 10 d of UPE starting at 122–125 d GA (13), while in another study fetal MAP was increased during 20 d of UPE between 110 and 130 d GA (11). The explanation for the apparent inconsistencies between studies is not clear, but it may be due to differences in the severity and gestational timing of UPE.

As we studied only pregnancies that proceeded to term, our findings on low birth weight postnatal lambs can be attributed to chronic placental insufficiency and IUGR without the potentially confounding effect of preterm birth. Our findings do not support the hypothesis that arterial pressure in the early postnatal period is inversely related to birth weight, when low birth weight is caused by IUGR. In contrast, low birth weight at term was associated with lower arterial pressure in the postnatal period. While the majority of epidemiologic studies (1, 2) have found inverse relationships between birth weight and arterial pressure in adults and children, some studies of infants have found positive relationships as in our study. Studies of human neonates have found that arterial pressure is positively correlated with birth weight up to 8 d after birth (21–23). It is possible that there is a "crossing-over" from relative hypotension to relative hypertension in individuals affected by IUGR some time after the early postnatal period. A study of ovine growth restriction using the carunclectomy technique (reduction of placental mass) has shown growth restricted lambs to have lower postnatal arterial pressure soon after birth, but by 60 d after birth arterial pressure became

Table 2. *Organ weights at 8 weeks of postnatal age in control and IUGR lambs*

	Organ weight (g)		Organ/body weight (g/kg)	
	Control ($n = 6$)	IUGR ($n = 6$)	Control ($n = 6$)	IUGR ($n = 6$)
Heart (total)	82.1 ± 5.1	57.6 ± 3.8*	4.9 ± 0.1	4.7 ± 0.2
Heart (left ventricle)	48.1 ± 3.5	31.4 ± 2.2*	2.9 ± 0.2	2.5 ± 0.1
Heart (right ventricle)	14.1 ± 1.4	9.0 ± 0.6*	0.8 ± 0.7	0.7 ± 0.1
Lungs	224.2 ± 20.3	177.8 ± 24.5	13.3 ± 0.6	14.1 ± 1.1
Kidneys	93.3 ± 6.3	70.2 ± 6.4*	5.6 ± 0.3	5.6 ± 0.3
Adrenal glands	1.28 ± 0.06	1.12 ± 0.09	0.08 ± 0.01	0.09 ± 0.01
Gastrointestinal tract†	2747 ± 257	2781 ± 290	164 ± 12	222 ± 13*
Brain	72.7 ± 1.4	70.3 ± 3.8	4.4 ± 0.2	5.7 ± 0.3*
Liver	305.8 ± 29.8	251.2 ± 16.0	18.2 ± 1.2	20.4 ± 1.3
Brain:liver ratio	0.25 ± 0.03	0.28 ± 0.01		

Asterisks (*) indicate values that differ between groups ($p < 0.05$).
† includes contents.

greater than that of controls and this difference continued into adulthood (24). Although there were no indications of a crossover in our study by 8 wk, we cannot exclude that it may occur after this time.

In our study, all of the control lambs were singletons whereas the majority (7/8) of IUGR lambs were twins. We chose to embolize twin fetuses as our aim was to compare arterial pressures following low birth weight resulting from placental insufficiency and IUGR and we wished to maximize the degree of growth restriction. At the time of initiating this study we were unaware of data relating to differing programming of arterial pressure in twins and singletons (25). However, other studies have shown that birth weight and arterial pressure remain inversely related when the analysis included both singletons and twins (25, 26), and twins alone (26, 27).

In a previous study in which we measured arterial pressure in growth restricted ovine fetuses during UPE over a similar period of gestation (12) we found that MAP of growth restricted fetuses, all of which were singletons, was significantly lower than that of controls at 140 d of gestation. Thus we do not believe that the observed alterations in fetal and postnatal arterial pressures in the present study were due to the use of twins *per se*.

It is possible that the lower arterial pressure observed in our IUGR lambs was simply due to their smaller size, as in children, MAP is related to current size (2). The analysis of covariance showed that the lower arterial pressure of the IUGR lambs could be explained by these lambs having a lower body weight compared with controls. However, their lower body weights at each postnatal age were the result of the IUGR together with their failure to catch-up in absolute body weight in the postnatal period. Therefore, placental insufficiency can still be said to have led to the relative hypotension of these IUGR lambs.

Our measurements of plasma renin activity showed that it decreased in both IUGR and control lambs over the first 8 wk after birth. This was expected as plasma renin activity in fetal sheep has been shown to be higher than maternal levels (28). Based on epidemiologic evidence (1), we hypothesized that IUGR lambs would be hypertensive and a potential mechanism was considered to involve increased activity of the renin-angiotensin system. Higher plasma renin levels have been measured in IUGR infants than in normally grown infants (29). This observation is consistent with our finding of a transient increase in plasma renin activity in the IUGR lambs at 1 wk compared with controls. However, this period of elevated plasma renin activity corresponded to a relative hypotension in the IUGR lambs; this raises the possibility that the increased plasma renin activity was a response to the lower arterial pressure. A recent study (30) found no difference in the MAP of control and IUGR fetuses (following carunclectomy); however, these two groups of animals responded differently to captopril (ACE inhibitor) and angiotensin II infusions. These results (30) suggest that arterial pressure in IUGR fetuses may be regulated by the renin-angiotensin system in a manner different to that in normal fetuses.

Recent studies in rats have implicated exposure to high levels of glucocorticoids *in utero* with hypertension in offspring (31). Dexamethasone treatment of ewes at a critical stage of pregnancy has also been shown to lead to hypertensive offspring (32). In our study, no differences were measured between the plasma cortisol concentrations in the control and IUGR animals before or after birth, suggesting that circulating glucocorticoids are not responsible for the differences in arterial pressure between groups. Owing to high levels of variability in postnatal plasma cortisol levels, it is unlikely that we could have detected small differences between groups.

UPE has been shown to increase the vascular resistance of the placenta (14) by obstructing placental vessels and it may, therefore, increase the total peripheral resistance of the feto-placental circulation. In contrast, arterial pressures in our postnatal IUGR lambs were lower than in controls suggesting that their total peripheral resistance may have been reduced by UPE. We speculate that compensatory vasodilation may have occurred in the fetus (*e.g.* in the brain or heart), possibly in response to chronic hypoxemia (33), and/or elevated plasma concentrations of PGE_2 (34), and that this vasodilation in turn led to persistent changes to vessel wall structure. It is also possible that the hypoxemia and hypoglycemia resulting from the UPE could stimulate angiogenesis and/or affect collagen and elastin content in the fetal vasculature and that these alterations could persist into the postnatal period. A reduced nutrient supply has been shown to inhibit collagen production (35, 36), and hypoxia has been shown to down-regulate the tropoelastin gene (37). Human fetuses considered to be growth restricted have been found to have evidence of impaired ventricular function (38). Thus, it is possible that IUGR lambs had impaired cardiac function and lower cardiac output relative to controls.

Lambs subjected to 26 d of UPE had birth weights that were 33% lower than those of control lambs. Indeed, the birth weights of the IUGR lambs (2.9 kg) suggest that little growth had occurred during the 26 d of UPE; based on data from 17 normal fetal sheep, we estimate that fetal body weight at the onset of UPE (120 d) is 2.2 kg. Although the postnatal growth rate of IUGR lambs was similar to that of normal lambs, there was evidence of relative, but not absolute catch-up in the body weights of the IUGR lambs. Studies of human infants affected by IUGR show that a high percentage of these infants show catch-up growth within the first 2 y (39, 40). While catch-up growth was small in our growth restricted lambs at 8 postnatal weeks of age, it may occur at a faster rate at a later age.

At birth, IUGR lambs were observed to have a "wasted" appearance, confirmed by their lower ponderal index and decreased skinfold thickness compared with controls. In humans, a low ponderal index at birth, indicative of thinness, has been correlated with an increased risk of hypertension in adulthood (3). The percentage reduction in ponderal index during the postnatal period was greater in control lambs than in IUGR lambs, so that by 8 wk, the ponderal index was the same in both groups. This suggests that, in control lambs, body length increased after birth at a relatively greater rate than body weight. In contrast, IUGR lambs had a greater increase in body weight relative to crown rump length; this is consistent with IUGR lambs having a lower thoracic girth but increased weight of the gastrointestinal unit per kg body weight.

While the IUGR lambs had lower body weights and exhibited different patterns of postnatal growth, only the gastrointestinal and brain weights (body weight adjusted) differed between the two groups. Increased relative brain weights in the IUGR lambs suggest the brain sparing effects of growth restriction (12) are still evident at 8 wk of age. Increased fetal heart weight (body weight adjusted) and increased ventricular wall thickness (11) have been associated with increased arterial pressure in growth restricted fetuses. In our study, there were no differences in the heart weights of 8-wk-old lambs after adjustment for body weight. However, as arterial pressure was not increased in the IUGR lambs, ventricular hypertrophy was not expected.

We conclude that UPE in the final 0.18 of ovine gestation can result in the birth of growth restricted lambs at full term. These lambs exhibited evidence of relative catch-up in body weight by 8 wk and developed an altered pattern of body growth. Although the growth restricted fetuses had similar MAP before birth, they had lower MAP for 8 wk after birth. This relative hypotension could be related to their lower body weight. Plasma levels of cortisol and renin in our postnatal animals were not consistent with a role in the development of hypertension or hypotension. It remains to be established whether these lambs will become hypertensive later in life.

Acknowledgments. The authors thank Mr. Alex Satragno for surgical assistance, Ms. Belinda Joyce for assistance in animal experimentation, and Mrs. Jan Loose and Mrs. Katrina Worthy for performing, respectively, the cortisol and renin assays. We are also appreciative of the interest of Dr. Ruth Morley in this study.

REFERENCES

1. Barker DJ 1995 Fetal origins of coronary heart disease. BMJ 311:171–174
2. Law CM, Shiell AW 1996 Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature J Hypertens 14:935–941
3. Barker DJ, Godfrey KM, Osmond C, Bull A 1992 The relation of fetal length, ponderal index and head circumference to blood pressure and the risk of hypertension in adult life. Paediatr Perinat Epidemiol 6:35–44
4. Gennser G, Rymark P, Isberg PE 1988 Low birth weight and risk of high blood pressure in adulthood. BMJ 296:1498–1500
5. Williams S, St George IM, Silva PA 1992 Intrauterine growth retardation and blood pressure at age seven and eighteen. J Clin Epidemiol 45:1257–1263
6. Nilsson PM, Ostergren PO, Nyberg P, Soderstrom M, Allebeck P 1997 Low birth weight is associated with elevated systolic blood pressure in adolescence: a prospective study of a birth cohort of 149 378 Swedish boys. J Hypertens 15:1627–1631
7. Matthes JW, Lewis PA, Davies DP, Bethel JA 1994 Relation between birth weight at term and systolic blood pressure in adolescence. BMJ 308:1074–1077
8. Law CM, de Swiet M, Osmond C, Fayers PM, Barker DJ, Cruddas AM, Fall CH 1993 Initiation of hypertension in utero and its amplification throughout life. BMJ 306:24–27
9. Persson E, Jansson T 1992 Low birth weight is associated with elevated adult blood pressure in the chronically catheterized guinea-pig. Acta Physiol Scand 145:195–196
10. Langley SC, Jackson AA 1994 Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci 86:217–222
11. Murotsuki J, Challis JR, Han VK, Fraher LJ, Gagnon R 1997 Chronic fetal placental embolization and hypoxemia cause hypertension and myocardial hypertrophy in fetal sheep. Am J Physiol 272:R201–207
12. Cock ML, Harding R 1997 Renal and amniotic fluid responses to umbilicoplacental embolization for 20 days in fetal sheep. Am J Physiol 273:R1094–1102
13. Gagnon R, Challis J, Johnston L, Fraher L 1994 Fetal endocrine responses to chronic placental embolization in the late-gestation ovine fetus. Am J Obstet Gynecol 170:929–938
14. Trudinger BJ, Stevens D, Connelly A, Hales JR, Alexander G, Bradley L, Fawcett A, Thompson RS 1987 Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation. Am J Obstet Gynecol 157:1443–1448
15. Nicolaides KH, Economides DL, Soothill PW 1989 Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol 161:996–1001
16. Lumbers ER 1995 Functions of the renin-angiotensin system during development. Clin Exp Pharmacol Physiol 22:499–505
17. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR 1993 Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 341:355–357
18. Bocking AD, McMillen IC, Harding R, Thorburn GD 1986 Effect of reduced uterine blood flow on fetal and maternal cortisol. J Dev Physiol 8:237–245
19. Woods RL, Anderson WP, Korner PI 1986 Renal and systemic effects of enalapril in chronic one-kidney hypertension. Hypertension 8:109–116
20. Hawkins P, Steyn C, Ozaki T, Saito T, Noakes DE, Hanson MA 2000 Effect of maternal undernutrition in early gestation on ovine fetal blood pressure and cardiovascular reflexes. Am J Physiol 279:R340–348
21. Contis G, Lind J 1963 Study of systolic blood pressure, heart rate, body temperature of normal newborn infants through the first week of life. Acta Paediatr Suppl 146:41–47
22. Lee YH, Rosner B, Gould JB, Lowe EW, Kass EH 1976 Familial aggregation of blood pressures of newborn infants and their mother. Pediatrics 58:722–729
23. O'Sullivan MJ, Kearney PJ, Crowley MJ 1996 The influence of some perinatal variables on neonatal blood pressure. Acta Paediatr 85:849–853
24. Robinson JS, McMillen IC, Fielke S, Evans L, Lok F, Owens JA 1998 Role of the placenta: development and function. Equine Vet J 30:456
25. Williams S, Poulton R 1999 Twins and maternal smoking: ordeals for the fetal origins hypothesis? A cohort study BMJ 318:897–900
26. Dwyer T, Blizzard L, Morley R, Ponsonby AL 1999 Within pair association between birth weight and blood pressure at age 8 in twins from a cohort study. BMJ 319:1325–1329
27. Poulter NR, Chang CL, MacGregor AJ, Snieder H, Spector TD 1999 Association between birth weight and adult blood pressure in twins: historical cohort study. BMJ 319:1330–1333
28. Broughton Pipkin F, Lumbers ER, Mott JC 1974 Factors influencing plasma renin and angiotensin II in the conscious pregnant ewe and its foetus. J Physiol 243:619–636
29. Konje JC, Bell SC, Morton JJ, de Chazal R, Taylor DJ 1996 Human fetal kidney morphometry during gestation and the relationship between weight, kidney morphometry and plasma active renin concentration at birth. Clin Sci 91:169–175
30. Edwards LJ, Simonetta G, Owens JA, Robinson JS, McMillen IC 1999 Restriction of placental and fetal growth in sheep alters fetal blood pressure responses to angiotensin II and captopril. J Physiol 515:897–904
31. Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CR 1993 Glucocorticoid exposure in utero: new model for adult hypertension. Lancet 341:339–341
32. Dodic M, May CN, Wintour EM, Coghlan JP 1998 An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin Sci 94:149–155
33. Richardson BS, Bocking AD 1998 Metabolic and circulatory adaptations to chronic hypoxia in the fetus. Comp Biochem Physiol A Mol Integr Physiol 119:717–723
34. Gagnon R, Murotsuki J, Challis JR, Fraher L, Richardson BS 1997 Fetal sheep endocrine responses to sustained hypoxemic stress after chronic fetal placental embolization. Am J Physiol 272:E817–823
35. Deyl Z, Juricova M, Rosmus J, Adam M 1971 The effect of food deprivation on collagen accumulation. Exp Gerontol 6:383–390
36. Spanheimer R, Zlatev T, Umpierrez G, DiGirolamo M 1991 Collagen production in fasted and food-restricted rats: response to duration and severity of food deprivation. J Nutr 121:518–524
37. Berk JL, Massoomi N, Hatch C, Goldstein RH 1999 Hypoxia downregulates tropoelastin gene expression in rat lung fibroblasts by pretranslational mechanisms. Am J Physiol 277:L566–572
38. Rizzo G, Arduini D 1991 Fetal cardiac function in intrauterine growth retardation. Am J Obstet Gynecol 165:876–882
39. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267–271
40. Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S 1993 Longitudinal follow-up of growth in children born small for gestational age. Acta Paediatr 82:438–443