ADRENAL GLANDS ARE ESSENTIAL FOR ACTIVATION OF GLUCOGENESIS 
DURING UNDERNUTRITION IN FETAL SHEEP NEAR TERM

A.L. Fowden and A.J. Forhead

Department of Physiology, Development and Neuroscience, University of Cambridge, 
Downing Street, Cambridge CB2 3EG

Key words: Adrenal glands, glucogenesis, fetus

Short Title: Fetal adrenal glands and glucogenesis

Address for correspondence: Abigail L. Fowden
Dept. of Physiology, Development and Neuroscience, 
University of Cambridge 
Downing Street 
Cambridge, 
CB2 3EG 
UK 
Tel: 44 (0)1223 333855 
Fax: 44 90)1223 333840 
E-mail: alf1000@cam.ac.uk
ABSTRACT

In adults, the adrenal glands are essential for the metabolic response to stress but little is known about their role in fetal metabolism. This study examined the effects of adrenalectomizing fetal sheep on glucose and oxygen metabolism in utero in fed conditions and after maternal fasting for 48h near term. Fetal adrenalectomy (AX) had little effect on the rates of glucose and oxygen metabolism by the fetus or uteroplacental tissues in fed conditions. Endogenous glucose production was negligible in both AX and intact, sham operated fetuses in fed conditions. Maternal fasting reduced fetal glucose levels and umbilical glucose uptake in both groups of fetuses to a similar extent but activated glucose production only in the intact fetuses. The lack of fasting-induced glucogenesis in AX fetuses was accompanied by falls in fetal glucose utilization and oxygen consumption not seen in intact controls. The circulating concentrations of cortisol and total catecholamines, and the hepatic glycogen content and activities of key gluconeogenic enzymes were also less in AX than intact fetuses in fasted animals. Insulin concentrations were also lower in AX than intact fetuses in both nutritional states. Maternal glucose utilization and its distribution between the fetal, uteroplacental and non-uterine maternal tissues were unaffected by fetal adrenalectomy in both nutritional states. Ovine fetal adrenal glands, therefore, have little effect on basal rates of fetal glucose and oxygen metabolism but are essential for activating fetal glucogenesis in response to maternal fasting. They may also be involved in regulating insulin sensitivity in utero.
Adrenal hormones are essential for survival during stressful conditions after birth (20,21). They regulate multiple physiological systems and have a key role in glucose homeostasis and blood pressure control, even in normal, basal conditions (21). Postnatal deficiency of adreno-cortical and adreno-medullary hormones induced by adrenalectomy leads to profound hypoglycaemia and hypotension in several species including rats and sheep (1,6,28,31,39). Adrenal insufficiency also impairs the response to common physiological challenges, such as cold exposure, fasting, and exercise, with adverse consequences for morbidity and mortality in the long term (1,31,39). However, the role of the adrenal glands in homeostasis before birth, particularly during conditions like undernutrition, is less well established.

Adrenalectomy of the sheep fetus is known to alter development of several fetal tissues during late gestation (29). It prevents the normal decline in fetal growth rate towards term and abolishes several of the morphological and functional changes in tissues, such as the lungs, liver and gut, that are essential for neonatal survival (11,14,18,38). It also prevents the onset of labour in the ewe (8). Adrenalectomized fetuses, therefore, tend to weigh more at term and are significantly heavier 10-15 days after normal term than intact, term controls (3,4,18). In addition, the normal ontogenic increase in fetal blood pressure is abolished in adrenalectomized (AX) sheep fetuses with the result that fetal blood pressure is lower in AX than intact controls during the 5-10 days before normal term (36,43). In contrast, fetal metabolite concentrations during the normal and extended periods of gestation appear to be unaffected by adrenalectomy of the sheep fetus (3). There are no significant differences in fetal blood pO₂ or plasma concentrations of glucose, lactate, fructose, urea or α-amino nitrogen between AX and control fetuses during late gestation (3,4,18). However, prepartum deposition of glycogen in ovine fetal tissues, such as the liver, heart and skeletal muscle, is adversely affected by fetal adrenalectomy and is known to depend on the adreno-cortical hormone, cortisol (3,4). Similarly, activation of glucogenesis in normal well nourished sheep fetuses during the period immediately before birth is related to the circulating concentrations of both adreno-cortical and adreno-medullary hormones (16).
However, little is known about the role of adrenal hormones in regulating basal metabolism or the metabolic responses of the fetus to challenges, such as maternal undernutrition, during late gestation. Therefore, this study investigated the effects of adrenalectomizing the sheep fetus on the metabolism of glucose and oxygen by the fetal and uteroplacental tissues in late gestation and during a short period of maternal food deprivation close to normal term.

METHODS

Animals
A total of 10 Welsh Mountain ewes carrying single fetuses were used in this study. During the experimental period, the ewes were housed individually and maintained on concentrate (200 g/day, Beart Ltd., Stowbridge, Suffolk, UK) and hay and water ad libitum. Half the daily ration of concentrates was fed at 08.00h while the remainder was given at 17.00h. Food but not water was withheld for 18-24h before surgery. All procedures were carried out under the Animals (Scientific Procedures) Act 1986.

Surgical procedures
Under general anaesthesia (1.5% halothane in a 5:1 mixture of O₂ and N₂O₂), fetuses were either adrenalectomized (AX, n = 5) or sham operated (Intact controls, n = 5) at 115-118 days of gestation (normal term 145 ± 2 days) and then catheterized at a second operation 10-12 days later using the same anesthetic regime. Catheters were inserted into the uterine vein, umbilical vein, fetal dorsal aorta and caudal vena cava and into the maternal aorta via a femoral artery of all animals as described previously (17). At the end of the experimental period, ewes and fetuses were given a lethal dose of anaesthetic (200mg/kg sodium pentobarbitone iv) before collection of fetal tissues in the fed state.
Experimental procedures

Blood samples (0.5-1.0ml) were taken from the fetus and mother daily between 09.00-10.00h to monitor well-being. At least 6 days after vascular catheterization (136-138 days), measurements of fetal glucose and oxygen metabolism were made in the fed state in all 10 animals. Following this study, food but not water was withdrawn from the ewes and a second set of measurements were made in the fasted state at 139-141 days of gestation when the animals had been without food for 48h. In both fed and fasted animals, tritiated water (8μCi/ml, Amersham International, Bucks, UK) and universally labelled [14C]glucose (10μCi/ml in 0.09% NaCl w/v, ICN Biochemicals, High Wycombe, Bucks, UK) were infused together into the fetal caudal vena cava for 2-4h at known rates between 0.08-0.09 ml/min after an initial priming dose (3-4ml). Blood samples (3.5ml) were taken simultaneously from the umbilical vein, fetal dorsal aorta, uterine vein and maternal dorsal aorta before (0min) and, when steady state had been established, at known times approximately 120, 140, 160 and 180min after beginning the infusion.

The simultaneous blood samples were analysed immediately for blood pH, gas tensions, packed cell volume and O2 content (0.5ml) and for labelled carbon dioxide (14CO2) where appropriate (1.0ml). The remainder of the sample (2ml) was added to a chilled tube containing EDTA for subsequent analyses. An aliquot (0.5ml) of the EDTA treated blood was deproteinised with zinc sulphate (0.3M) and barium hydroxide (0.3M) and the supernatant used for determination of both labelled and total concentrations of glucose. The remaining EDTA sample was centrifuged at 4°C and the plasma stored at -20°C until required for 3H2O and hormone measurements. An additional aliquot of fetal arterial blood (1ml) was taken at 0min and placed in a chilled heparinized tube containing EGTA (5.0μmol/ml blood) and glutathione (40μmol/ml blood) for catecholamine assay.

At the end of the second study, the ewes and their fetuses were euthanized and the fetuses and uteroplacental tissues weighed. Samples of liver and kidney (5-10g) was collected from all fetuses in the fasted state and frozen immediately in liquid nitrogen before storage at -80°C for the subsequent analyses of glycogen content and key gluconeogenic enzyme activities. The
adrenal glands were weighed in the intact, control animals while the completeness of 
adrenalectomy was assessed in the AX fetuses. The position of all catheters was also verified at 
autopsy. Tissue and plasma samples were also obtained in the fed state from 11 additional 
fetuses (5 adrenalectomized and 6 intact), which were euthanized as part of another study (15,18) 
at the same gestational ages as the current cohort of fasted animals (139-141 days). No adrenal 
remnants were found in any of the AX fetuses.

**Biochemical analyses**

The blood gas tensions, packed cell volume, O₂ content and whole blood concentrations of 
glucose, [¹⁴C]-glucose, ³H₂O and ¹⁴CO₂ were measured in all five sets of simultaneous samples 
in the fed and fasted states. Blood O₂ content was calculated from the percentage O₂ saturation 
and the haemoglobin concentrations measured using an OSM2 Hemoximeter (Radiometer, 
Copenhagen, Denmark) that had been calibrated for ovine blood. Blood pH and partial pressures 
of O₂ and CO₂ were measured using an ABL5 Radiometer and corrected for a fetal body 
temperature of 39ºC.

Glucose concentrations were determined enzymatically in whole blood and plasma using a 
colorimetry assay (17) and an automated analyser, respectively (2300 StatPlus, Yellow Springs, 
OH, USA). Plasma ³H₂O concentrations were measured using scintillation counting and 
converted to blood concentrations using the packed cell volume as described previously (17, 22). 
Labelled glucose and CO₂ were determined using chemical methods published previously (22). 
Labelled glucose was separated from all other ¹⁴C labelled products by anion exchange 
chromatography following preincubation with and without glucose oxidase (17,23). The mean 
recovery of [¹⁴C]-glucose from the anion exchange column was 99.7 ± 1.3% (n = 32). No 
corrections for glucose recovery were therefore made. In contrast, the mean recovery of ¹⁴CO₂ 
was 71.9 ± 0.6% (n = 22) and, hence, all blood ¹⁴CO₂ values have been corrected for recovery.

Plasma catecholamine concentrations were determined by high pressure liquid chromatography 
using electrochemical detection (17). Recovery of isoprenaline added to the samples ranged from 
63-97% and, hence, all samples have been corrected for their respective recoveries. The limits of
sensitivity of the method were 50pg/ml for epinephrine and 30pg/ml for norepinephrine. The
interassay coefficients of variation for epinephrine and norepinephrine were 7.3 and 6.2%,
respectively. Total catecholamine concentrations were calculated as the sum of the
concentrations of epinephrine and norepinephrine in each sample. Plasma concentrations of
insulin and cortisol were measured by radioimmunoassay validated for use with ovine plasma
(9,37). The inter-assay coefficients of variation for these two assays were 13.7 % and 10.0%,
respectively, while the minimum detectable quantity of hormone was 1.5ng/ml for cortisol and
5.0µU/ml for insulin. Only plasma cortisol concentrations were measured in the additional
cohort of animals delivered in the fed state at 139-141 days.

Hepatic glycogen content and the activities of glucose-6-phosphatase (G6Pase, EC 3.1.3.9) and
phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) in liver and kidney were assayed
using established methods described in detail elsewhere (15,19). Hormone concentrations and
enzyme activities were measured in duplicate while all other biochemical analyses were
measured in triplicate.

Calculations
All calculations were made using equations derived for steady-state kinetics (13,23). Umbilical
blood flow was measured using the $^3$H$_2$O steady-state diffusion technique and calculated using
equations 1 and 2 in Table 1. Net umbilical uptake of glucose and oxygen and net umbilical
excretion rates of $[^{14}$C]-glucose and $^{14}$CO$_2$ were calculated by Fick principle as the product of
umbilical blood flow (ml/min) and the umbilical venous-arterial (uptake) or arterio-venous
(excretion) concentration difference across the umbilical circulation (µmol/l). The net uterine
uptake of glucose and oxygen, and the uterine output of tracer glucose were measured in a
similar manner using uterine blood flow and the corresponding concentration differences across
the uterine circulation. Net uteroplacental consumption of glucose and oxygen was calculated as
the differences between the uterine and umbilical uptakes.
The fetal rates of utilization and production of glucose, CO₂ production from glucose carbon and the fraction of the net umbilical O₂ uptake used for glucose carbon oxidation by the fetus were calculated as shown in Table 1 (13,17,23). Oxidation of glucose carbon was measured as the rate of \(^{14}\text{CO}_2\) production (Table 1). The glucose carbon oxidation fraction and the fraction of O₂ consumption used to oxidize glucose carbon in the fetus were then calculated where the amount of O₂ used to oxidize fetal glucose carbon is equal to the amount of CO₂ produced by this oxidative process (Table 1). Endogenous glucose production by the fetus was calculated from the rates of umbilical uptake and fetal utilization of glucose (Table 1). Finally, glucose utilization by non-uterine maternal tissues was calculated in the ewes by measuring the labeled and unlabelled glucose concentrations in maternal arterial and uterine venous blood (Equations 9 & 10, Table 1). Since glucose uptake from the gastrointestinal tract is negligible in sheep (5), the total rate of glucose utilization by the maternal tissues is equivalent to the rate of glucose production by the ewe. Because studies were carried out in both the fed and fasted states, the values for \(^{14}\text{C}\)-glucose and \(^{14}\text{CO}_2\) in the 0min arterial, uterine and umbilical venous samples of the fasted study were subtracted from the subsequent samples before calculation of the glucose metabolic rates. All fetal metabolic rates have been expressed per kg fetal bodyweight. No increase in fetal bodyweight was assumed to occur during the 48h period of maternal food withdrawal.

**Statistical analyses**

Steady state was defined as <10% variation of values around the mean for each sampling period with no consistent trend for the absolute values to increase or decrease with time. Mean values ± SEM have been used throughout. Statistical analyses were made using Sigmasstat (Jandel Scientific, Chicago, IL, USA). Comparison of metabolic rates between treatments and nutritional states were made using two ANOVA and unpaired and paired t-tests, as appropriate. For all statistical analyses, significance was accepted when P<0.05.
RESULTS

The effects of fetal adrenalectomy on tissue biometry
Fetal adrenalectomy had no effect on the body weight, crown rump length or ponderal index of the fetuses at delivery at 139-141 days of gestation (Table 2). There was also no significant difference in the total weight of the utero-placental tissues or of the placentomes alone between AX and intact, sham operated fetuses at delivery (Table 2). No adrenal remnants were found in any of the AX fetuses (Table 2). Adrenal weight of the sham operated controls (Table 1) was similar to that seen in previously in intact fetuses at the same gestational age (3).

The effects of fetal adrenalectomy on metabolite and hormone concentrations
Fetal adrenalectomy also had no effect on the blood gas status of the fetus in either the fed or fasted state: mean values of blood pH, pO\textsubscript{2}, pCO\textsubscript{2} O\textsubscript{2} saturation and O\textsubscript{2} content were not significantly different between AX and intact fetuses in either nutritional state (Table 3). In addition, there was no change in fetal blood gas status with maternal food withdrawal for 48h in either group of animals (Table 3). Glucose concentrations in maternal and fetal blood were not significantly different between AX and intact animals in either the fed or fasted states and decreased to a similar extent during the 48h period of fasting (Table 4). The transplacental concentration gradient in plasma glucose was similar in AX and intact fetuses irrespective of nutritional state and fell to the same extent during maternal fasting in the two groups of animals (Table 3).

Plasma cortisol concentrations were not significantly different between AX and intact fetuses in the fed state at 136-138 days but were significantly lower in AX than intact fetuses at 139-141 days after maternal fasting for 48h (Table 4). The plasma cortisol concentration in the additional cohort of intact fetuses delivered in the fed state at 139-141 days (25.5 ± 4.6ng/ml, n = 6) was lower than the value found in the current cohort of animals sampled in the fasted state at the same gestational age, but not significantly so (Table 4, P>0.05). There was also no significant difference between the plasma cortisol concentration in the AX fetuses of the fed (6.8 ± 0.7ng/ml, n = 5) and fasted cohorts at 139-141 days (Table 4, P>0.05).
In the current cohort of animals, plasma insulin concentrations were significantly lower in AX than intact fetuses in both the fed and fasted states and were reduced significantly by maternal fasting in both groups of animals (Table 4). The mean decrement in fetal plasma insulin during maternal fasting was similar in AX (-3.7±1.0µU/ml, n = 5) and intact fetuses (-7.1±2.1µU/ml, n = 5, P>0.05). There were no significant differences in the plasma concentrations of epinephrine and norepinephrine between AX and intact fetuses in either nutritional state (Table 4). Plasma epinephrine concentrations were unaffected by maternal fasting in both groups of fetuses whereas plasma norepinephrine concentrations were higher in the fasted than fed state in intact but not AX fetuses (Table 4). Total catecholamine concentrations were similar in the two groups of fetuses in the fed state and, although higher after 48h of fasting in both groups, mean values in the fasted state were significantly lower in AX than intact fetuses (Table 4).

The effects of fetal adrenalectomy on glucose and oxygen metabolism by the fetus

**Fed state:** Fetal adrenalectomy had no significant effect on the basal rates of umbilical blood flow and O₂ uptake; mean values were similar in the two groups of fetuses in fed conditions (Table 5). There were also no significant differences in the rates of umbilical glucose uptake, glucose utilization or CO₂ production from glucose carbon between AX and intact fetuses in the fed state (Figure 1). Neither was the glucose oxidation fraction nor were the fraction of umbilical O₂ uptake used for glucose carbon oxidation significantly different between the two groups of fetuses (Table 5). Endogenous glucose production was negligible in both groups of fetuses in the fed state (Figure 1C).

**Fasted state:** Maternal fasting for 48h significantly reduced the rate of umbilical glucose uptake in both AX and intact fetuses; mean decrements were similar in the two groups (Figure 1A). In AX but not intact fetuses, this occurred in parallel with a significant reduction in the rate of glucose utilization (Figure 1B). The rate of endogenous glucose production, therefore, remained negligible in AX fetuses but occurred at a significant rate in intact fetuses after 48h of maternal fasting (Figure 1C). The rate of CO₂ production from glucose carbon decreased to a similar extent during fasting in both groups of fetuses (Figure 1D). This was accompanied by a reduction in the fraction of the umbilical O₂ uptake used for oxidation of glucose carbon in both groups of
fetuses and by a decreased rate of umbilical O₂ uptake in the AX but not intact fetuses (Table 5). During fasting, the fall in the weight specific rate of oxygen consumption was significant in AX fetuses (−48±16 µmol/min/kg, n = 5, P<0.05) but not in intact controls (−11±28 µmol/min/kg, n=5, P>0.05). In neither group of fetuses was the glucose oxidation fraction affected by maternal fasting (Table 5). Nor did umbilical blood flow alter with maternal food withdrawal (Table 5). Hepatic glycogen content and hepatic, but not renal, activities of G6Pase and PEPCK were significantly lower in AX than intact fetuses in the fasted state at delivery at 139-141 days of gestation (Figure 2). Hepatic glycogen content was lower while hepatic but not renal activities of G6Pase and PEPCK were higher in the fasted than fed state in both groups of fetuses at 139-141 days (Figure 2).

The effects of fetal adrenalectomy on oxygen and glucose metabolism by the utero-placental tissues and non-uterine maternal tissues.

Compared to intact controls, fetal adrenalectomy had no apparent effect on uterine blood flow or the uptake of oxygen by the uterus and utero-placental tissues; mean rates were similar in the two groups of animals, irrespective of nutritional state (Table 5). There were also no significant differences in the rates of glucose uptake by the uterus and utero-placental tissues between AX and intact fetuses in either nutritional state (Figure 3). Total maternal glucose utilization and its percentage distribution between the fetal, utero-placental and non-uterine maternal tissues were also similar in ewes with AX and intact fetuses in both nutritional states (Figure 3). Fasting had no effect on uterine blood flow or oxygen uptake in either group of animals (Table 5). There was a trend for increased utero-placental O₂ consumption in AX but not intact fetuses in response to maternal fasting but this did not reach statistical significance (Table 5). In contrast, fasting reduced total maternal glucose utilization and glucose uptake by the non-uterine maternal tissues to a similar extent in the two groups of animals (Figure 3). Uteroplacental glucose consumption also decreased significantly with fasting in the intact but not AX fetuses (Figure 3). There was no change in the percentage distribution of maternal glucose production between the fetal, utero-placental and non-uterine maternal tissues with maternal food withdrawal for 48h in either group of animals (Figure 3).
DISCUSSION

The results demonstrate that the fetal adrenal glands have little apparent effect on the basal rates of fetal glucose and oxygen metabolism in well nourished ewes but are essential for activation of fetal glucogenesis in response to short term maternal fasting close to term. This inability of AX sheep fetuses to induce glucogenesis during undernutrition was associated with lower circulating concentrations of cortisol and total catecholamines and with reductions in the hepatic glycogen content and activities of key gluconeogenic enzymes, relative to intact, sham-operated controls. It was also accompanied by a reduced rate of oxygen consumption. Insulin concentrations were also lower in AX than intact fetuses in both the fed and fasted states, despite the similarity in fetal glycaemia in the two groups of animals. These observations show that, like the adult adrenals, the fetal adrenal glands have an important role in the metabolic response to stressful conditions in utero. In addition, they suggest that adrenal secretions may be involved in regulating fetal insulin sensitivity, even in well nourished conditions during late gestation.

The lack of glucogenesis in response to maternal fasting in AX fetuses may be due to the combined deficiencies of adreno-cortical and adreno-medullary hormones as both cortisol and total catecholamine concentrations were low in AX relative to intact fetuses after maternal fasting for 48h. Cortisol is known to activate glucose production by the liver in intact fetuses near term (42). It also increases the circulating concentration of gluconeogenic amino acids, such as alanine, in fetal sheep during short term infusion (33,41). In the longer term, cortisol has also been shown to enhance glycogen deposition in several tissues of intact and AX fetuses during late gestation and to increase the activities of G6Pase and PEPCK in the liver and kidney of intact fetuses during the last 10-15 days of gestation (4,15). Certainly, the low hepatic activities of G6Pase and PEPCK are likely to be an important contributory factor to the lack of endogenous glucose production by the AX fetus after maternal fasting for 48h. Similar defects in fasting-induced glucogenesis associated with low hepatic glycogen and gluconeogenic enzyme activities have been observed in late gestation in sheep fetuses made cortisol deficient by fetal hypophysectomy (12). However, the finding that hepatic G6Pase and PEPCK activities were higher in the fasted than fed state in both AX and intact fetuses suggests that the increasing level of cortisol is not the only factor activating gluconeogenic enzymes in the ovine fetal liver.
Like cortisol, epinephrine and norepinephrine have been shown to stimulate hepatic glucose output in intact sheep fetuses during short term infusions close to term (2). At high doses, catecholamine infusions also cause fetal hyperglycaemia, although this only occurs at catecholamine concentrations higher than those seen in the current study (35). Basal levels of catecholamines were unaffected by fetal adrenalectomy and were within the range of values reported previously for AX and intact fetuses (7,16,35,40). Similarly, basal catecholamine concentrations were unaffected by short term chemical sympathectomy and by more long term adrenal denervation or demedullation of fetal sheep (25,34). Collectively, these observations suggest that the circulating catcholamine and norepinephrine, in particular, can also be derived from the peripheral nervous system and/or the para-aortic and other extra-adrenal chromaffin tissue. Fetal adrenalectomy reduced but did not completely abolish the catecholaminergic response to maternal fasting in the present study, a finding in common with the response of AX sheep fetuses to hypoxia reported previously (40). In intact fetuses of well fed ewes during late gestation, significant glucogenesis is observed at total fetal catecholamine concentrations similar to those seen in the AX fetuses after maternal fasting for 48h (16). Fetal cortisol concentrations, therefore, appear critical in determining fetal glucogenesis. Indeed, previous studies of intact fetuses during fed conditions have shown that endogenous glucose production only rises with physiological increases in the total catecholamine concentration when fetal cortisol concentrations exceed 18ng/ml (16), a value well above that seen in the AX fetuses in the present study in either the fed or fasted state. In part, the dependence of catecholamine-induced glucogenesis on the cortisol level is due to the cortisol induced up-regulation of hepatic gluconeogenic enzyme activities but it may also reflect the action of cortisol in increasing β-adrenoreceptor abundance in the fetal liver near term (2,10). Taken together, these observations suggest that cortisol deficiency may be the primary cause of the absence of glucogenesis in response to maternal fasting after adrenalectomy of the sheep fetus.

This lack of fasting-induced glucogenesis in the AX fetus led to a significant reduction in its rate of glucose utilization as its umbilical supply of glucose declined. In contrast, in intact fetuses, the decreased glucose supply during maternal fasting was ameliorated, in part, by the endogenous production of glucose and, hence, there was no significant reduction in the rate of fetal glucose utilization. Oxidation of glucose carbon decreased during fasting to the same extent in both AX
and intact fetuses as did fetal glycaemia, consistent with previous observations that the glucose oxidation is determined primarily by the fetal glucose level (22). Despite the decrease in glucose oxidation, oxygen consumption by the intact fetuses was unaffected by maternal fasting so other substrates must be oxidised in increased amounts to account for the normal rate of oxidative metabolism. A number of previous studies have shown that amino acids act as alternative oxidative substrates when glucose availability is limited in the sheep fetus (26,30). In the AX fetuses, oxidative metabolism fell by 15-10% with the decreases in glucose utilization and oxidation during maternal fasting. Consequently, recruitment of alternative substrates for oxidative metabolism during fetal hypoglycaemia may be compromised by deficiency of adrenal hormones in keeping with the known role of cortisol in stimulating protein catabolism and leucine oxidation in fetal sheep during late gestation (32). Thus, the low cortisol levels in AX fetuses after 48h of maternal fasting may not only limit upregulation of the glucogenic pathways but also impair the provision of amino acids for fetal gluconeogenesis and oxidative metabolism. The maintenance of glucose uptake and the tendency for increased O2 consumption by the uteroplacental tissues of AX fetuses during fasting may also reflect the low fetal cortisol levels or, alternatively, the differences in frequency distribution of the different placentome types between AX and intact fetuses at this stage of gestation (44).

Insulin concentrations were low in AX compared to intact fetuses in both the fed and fasted states, despite no significant differences in fetal glycaemia. Low insulin levels have also been observed in sheep fetuses after specific demedullation of the adrenal, which suggests that deficiency of the adreno-medullary secretions rather than cortisol may be the more important factor in determining the hypoinsulinaemia seen after complete fetal adrenalectomy (25). These secretions are unlikely to be the catecholamines as epinephrine and norepinephrine suppress, not enhance, fetal insulin secretion and their basal concentrations were unaltered in the AX fetuses (2,9,27). Despite their low insulin levels, AX fetuses maintained rates of glucose metabolism within the normal range in fed conditions. Since insulin is known to regulate glucose utilization in fetal sheep by enhancing glucose uptake into fetal tissues (13), the current findings suggest that either tissue sensitivity to insulin must be increased or there are other insulin-independent mechanisms of stimulating glucose utilization in the AX fetus. Preliminary observations of the insulin signalling pathways in skeletal muscle from AX fetuses in the fed state indicate that there
are no changes in the abundance of the insulin-sensitive glucose transporter, GLUT-4, or of the receptors for insulin and insulin-like growth factor-I, relative to the values in intact fetuses (24). Further studies are, therefore, needed to determine whether the insulin sensitivity of fetal glucose metabolism is altered during late gestation by removal of the fetal adrenal glands.

In summary, fetal adrenalectomy altered the fetal metabolic response to maternal fasting but had no apparent effect on the basal rates of fetal glucose and oxygen metabolism in the fed state, despite concomitant hypoinsulinaemia. The changes in the fetal metabolic response to fasting induced by fetal adrenalectomy were closely related to the low cortisol and catecholamine levels and were not accompanied by any major differences in utero-placental metabolism of glucose and oxygen or in the distribution of the uterine uptake of these substances between the fetal and utero-placental tissues. The poor glucogenic capacity of the AX fetus will limit its ability to withstand hypoglycaemia and other stressful challenges during late gestation. Adrenal hormones are, therefore, essential for the adaptive changes in fetal metabolism that ensure survival during adverse nutritional and other conditions in utero.

ACKNOWLEDGEMENTS

We would like to thank Nuala Daw for her assistance with the biochemical analyses and both Sue Nicholls and Scott gentle for their care of the animals. We are indebted to the Wellcome Trust and the BBSRC for their financial support.
REFERENCES


FIGURE LEGENDS

Figure 1: Mean (±SE) rates of A) umbilical glucose uptake, B) glucose utilization, C) endogenous glucose production and D) CO₂ production from glucose carbon in fed conditions (open columns) and after maternal fasting for 48h (grey columns) and the mean change in rate between the fed and fasted states (black columns) in intact and adrenalectomized (AX) fetuses in late gestation (n = 5 in each group).

† Significant change between the fed and fasted states (P<0.05, paired t-test).
* Significant rate of endogenous glucose production (P<0.05, t-test).

Figure 2: Mean (±SE) values of the glycogen content and activities of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) in the liver and kidney of intact (open columns) and adrenalectomized (AX) fetuses (stippled columns) in the fed state (Intact, n = 6 and AX = 5) and after 48h of maternal fasting at 139-141 days of gestation (n = 5 in both groups).

* Significantly different from the corresponding value in the intact fetuses (P<0.05, Student’s t-test).
† Significantly different from the corresponding value in the same group of fetuses in the fed state (P<0.05, Student’s t-test).

Figure 3: Mean (±SE) rates of total maternal glucose utilization (whole column) and its distribution between the non-uterine maternal tissues (grey column), the uteroplacental tissues (stippled column) and the fetal tissues (open column) in ewes with intact and adrenalectomized fetuses in fed conditions and after fasting for 48h. The figures within the columns show the mean percentage (±SE) distribution of the total rate of maternal utilization set at 100% between the different tissues. * Significant difference in the absolute rate of glucose utilization by the whole
ewe (above column) or the specific tissue (to the right of the column) from the values seen in the fed state (P<0.05, paired t-test).
Table 1: Equations

(1) Umbilical blood flow (ml/min) =
Rate of loss of $^3$H$_2$O by the umbilical circulation (dpm/min) ÷ Umbilical arterio-venous concentration difference in blood $^3$H$_2$O (dpm/ml).

(2) Uterine blood flow (ml/min) =
Rate of $^3$H$_2$O uptake by the uterine circulation (dpm/min) ÷ Uterine venous-arterial concentration difference in blood $^3$H$_2$O (dpm/ml).

(3) Fetal glucose utilization ($\mu$mol/min) =
Net fetal tracer glucose uptake (dpm/min) ÷ Fetal arterial glucose specific activity (dpm/$\mu$mol glucose)

(4) Net fetal tracer glucose uptake (dpm/min) =
Tracer glucose infusion rate (dpm/min) – net umbilical tracer glucose excretion rate (dpm/min).

(5) $^{14}$CO$_2$ production from the oxidation of glucose carbon ($\mu$mol/min) =
Net umbilical $^{14}$CO$_2$ excretion rate (dpm/min) ÷ Fetal arterial glucose specific activity (dpm/$\mu$mol glucose carbon).

(6) Glucose carbon oxidation fraction (fraction of fetal glucose carbon utilization used for oxidation) =
Net umbilical $^{14}$CO$_2$ excretion rate (dpm/min) ÷ Net fetal tracer glucose infusion uptake (dpm/min).

(7) Fraction of $O_2$ uptake used for oxidation of glucose carbon =
Amount of $O_2$ used to oxidize fetal glucose carbon ($\mu$mol/min) ÷ Net umbilical $O_2$ uptake rate ($\mu$mol/min).

(8) Endogenous glucose production ($\mu$mol/min) =
Fetal glucose utilization ($\mu$mol/min) – Umbilical glucose uptake ($\mu$mol/min).

(9) Glucose utilization by maternal non-uterine tissues ($\mu$mol/min) =
Net maternal tracer glucose uptake (dpm/min) ÷ maternal arterial specific activity (dpm/ $\mu$mol).

(10) Total maternal glucose utilization ($\mu$mol/min) =
Net uterine glucose uptake ($\mu$mol/min) + Glucose utilization by maternal non-uterine tissues ($\mu$mol/min).
Table 2: Mean (±SE) crown rump length (CRL), ponderal index and weights of the total uteroplacental tissues, placentomes, fetus and adrenals of intact and adrenalectomized sheep fetuses at delivery at 139-141 days of gestation (n = 5 in each group).

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>CRL cm</th>
<th>Ponderal index kg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uteroplacental tissues g‡</td>
<td>Placentomes g</td>
<td>Fetus g</td>
<td>Adrenals mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>1641 ± 199</td>
<td>262 ± 23</td>
<td>3435 ± 242</td>
<td>425 ± 49</td>
<td>50.6 ± 0.7</td>
<td>26.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>1341 ± 135</td>
<td>310 ± 30</td>
<td>3532 ± 277</td>
<td>ND</td>
<td>50.8 ± 1.8</td>
<td>27.0 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

‡ Combined weight of uterus, placentomes and fetal membranes. ND = Not detected.
Table 3: Blood gas status. Mean (±SE) values of pH, pO₂, pCO₂, O₂ saturation, O₂ content, haemoglobin content and packed cell volume (PCV) in arterial blood of intact and adrenalectomized sheep fetuses (n = 5 in each group) in fed conditions at 136-138 days and in the fasted state after maternal food withdrawal for 48h at 139-141 days in the same animals.

<table>
<thead>
<tr>
<th></th>
<th>Intact Fed</th>
<th>Intact Fasted</th>
<th>Adrenalectomized Fed</th>
<th>Adrenalectomized Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.374 ± 0.017</td>
<td>7.366 ± 0.018</td>
<td>7.340 ± 0.013</td>
<td>7.332 ± 0.008</td>
</tr>
<tr>
<td>pO₂ mmHg</td>
<td>21.7 ± 1.6</td>
<td>20.4 ± 0.3</td>
<td>20.2 ± 0.7</td>
<td>21.9 ± 0.7</td>
</tr>
<tr>
<td>pCO₂ mmHg</td>
<td>50.7 ± 1.9</td>
<td>47.1 ± 1.4</td>
<td>53.9 ± 2.3</td>
<td>51.8 ± 1.1</td>
</tr>
<tr>
<td>O₂ saturation</td>
<td>60.1 ± 2.2</td>
<td>62.0 ± 2.5</td>
<td>54.6 ± 2.9</td>
<td>55.6 ± 1.5</td>
</tr>
<tr>
<td>O₂ content mmol/l</td>
<td>3.75 ± 0.22</td>
<td>3.92 ± 0.20</td>
<td>3.32 ± 0.20</td>
<td>3.62 ± 0.24</td>
</tr>
<tr>
<td>Haemoglobin g%</td>
<td>10.1 ± 0.5</td>
<td>10.2 ± 0.4</td>
<td>10.0 ± 0.5</td>
<td>10.5 ± 0.5</td>
</tr>
<tr>
<td>PCV %</td>
<td>33.5 ± 1.4</td>
<td>34.0 ± 1.8</td>
<td>31.1 ± 1.3</td>
<td>34.1 ± 2.0</td>
</tr>
</tbody>
</table>
Table 4: Metabolite and hormone concentrations. Mean (±SE) concentrations of glucose in maternal and fetal arterial blood and plasma and of cortisol, insulin, epinephrine, norepinephrine and total catecholamines in fetal arterial plasma together with the transplacental plasma glucose concentration gradient in intact and adrenalectomized fetuses (n = 5 in each group) in fed conditions at 136-138 days and after maternal fasting for 48h at 139-141 days in the same animals.

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Adrenalectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fasted</td>
</tr>
<tr>
<td>Blood glucose mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.86 ± 0.08</td>
<td>0.53 ± 0.04*</td>
</tr>
<tr>
<td>Mother</td>
<td>2.34 ± 0.13</td>
<td>1.18 ± 0.12*</td>
</tr>
<tr>
<td>Plasma glucose mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.99 ± 0.09</td>
<td>0.64 ± 0.05*</td>
</tr>
<tr>
<td>Mother</td>
<td>3.07 ± 0.12</td>
<td>1.83 ± 0.13*</td>
</tr>
<tr>
<td>Transplacental</td>
<td>2.08 ± 0.16</td>
<td>1.19 ± 0.10*</td>
</tr>
<tr>
<td>Cortisol ng/ml</td>
<td>12.8 ± 2.7</td>
<td>41.0 ± 9.9*</td>
</tr>
<tr>
<td>Insulin µU/ml</td>
<td>22.6 ± 2.4</td>
<td>14.0 ± 1.6*</td>
</tr>
<tr>
<td>Epinephrine pg/ml</td>
<td>42 ± 24</td>
<td>62 ± 32</td>
</tr>
<tr>
<td>Norepinephrine pg/ml</td>
<td>196 ± 54</td>
<td>864 ± 211*</td>
</tr>
<tr>
<td>Total catecholamines pg/ml</td>
<td>258 ± 74</td>
<td>928 ± 185*</td>
</tr>
</tbody>
</table>

*Significantly different from the value in the fed state in the same group of fetuses (P<0.05, paired t-test). †Significantly different from the value in intact fetuses in the same nutritional state (P<0.05, ANOVA).
Table 5: Oxygen metabolism. Mean (±SE) values of absolute and weight specific umbilical and uterine blood flow, weight specific umbilical oxygen uptake, the fraction of umbilical oxygen uptake used for glucose carbon oxidation by the fetus, the glucose oxidation fraction, the total uterine, uteroplacental and fetal uptake of oxygen and the distribution of the total uterine oxygen uptake between the uteroplacental and fetal tissues in intact and adrenalectomized fetuses (n = 5 in each group) in fed conditions at 136-138 days and after maternal fasting for 48h at 139-141 days in the same animals.

<table>
<thead>
<tr>
<th></th>
<th>Intact Fed</th>
<th>Intact Fasted</th>
<th>Adrenalectomized Fed</th>
<th>Adrenalectomized Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical ml/min</td>
<td>592 ± 38</td>
<td>570 ± 47</td>
<td>617 ± 82</td>
<td>541 ± 75</td>
</tr>
<tr>
<td>ml/min/kg ‡</td>
<td>174 ± 13</td>
<td>170 ± 21</td>
<td>174 ± 19</td>
<td>161 ± 11</td>
</tr>
<tr>
<td>Uterine ml/min</td>
<td>1224 ± 151</td>
<td>1206 ± 119</td>
<td>1472 ± 182</td>
<td>1310 ± 11</td>
</tr>
<tr>
<td>ml/min/kg ∑</td>
<td>796 ± 129</td>
<td>794 ± 136</td>
<td>1140 ± 251</td>
<td>1082 ± 211</td>
</tr>
<tr>
<td>Umbilical O₂ uptake Rate</td>
<td>µmol/min/kg ‡</td>
<td>266 ± 10</td>
<td>255 ± 27</td>
<td>297 ± 19</td>
</tr>
<tr>
<td>Fraction</td>
<td>0.294 ± 0.023</td>
<td>0.179 ± 0.034*</td>
<td>0.228 ± 0.023</td>
<td>0.123 ± 0.023*</td>
</tr>
<tr>
<td>Glucose oxidation fraction</td>
<td>0.469 ±0.077</td>
<td>0.398 ± 0.039</td>
<td>0.405 ± 0.031</td>
<td>0.393 ± 0.053</td>
</tr>
<tr>
<td>Total O₂ uptake ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>1344 ± 41</td>
<td>1221 ± 51</td>
<td>1611 ± 174</td>
<td>1660 ± 132</td>
</tr>
<tr>
<td>Uteroplacental tissues</td>
<td>591 ± 104</td>
<td>418 ± 100</td>
<td>560 ± 180</td>
<td>672 ± 44</td>
</tr>
<tr>
<td>Fetus</td>
<td>842 ± 56</td>
<td>803 ± 92</td>
<td>1158 ± 132</td>
<td>864 ± 114*</td>
</tr>
<tr>
<td>% of Uterine O₂ uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uteroplacental tissues</td>
<td>37.1 ± 3.7</td>
<td>33.8 ± 5.9</td>
<td>33.4 ±8.1</td>
<td>44.3 ± 2.3</td>
</tr>
<tr>
<td>Fetus</td>
<td>62.9 ± 3.4</td>
<td>66.2 ± 6.8</td>
<td>66.6 ± 8.9</td>
<td>55.6 ± 2.6</td>
</tr>
</tbody>
</table>

*Significantly different from the value in the fed state in the same group of fetuses (P<0.05, paired t-test). ‡ per kg fetal body weight. ∑ per kg total weight of uterus, placentomes and fetal membranes.
A. Glucose uptake

B. Glucose utilization

C. Glucose production

D. CO₂ production from glucose carbon

Rate of glucose metabolism

μmol/min/kg

Intact          AX

Rate of CO₂ production from glucose carbon

μmol/min/kg

Intact          AX
A. Liver glycogen

Glycogen content mg/g wet wt

Fed               Fasted

B. G6Pase

Liver                                   Kidney

Enzyme activity U/g wet wt

Fed               Fasted

C. PEPCK

Liver                                   Kidney

Enzyme activity U/g wet wt

Fed               Fasted
### Total Maternal Glucose Utilization (μmol/min)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fed</th>
<th>Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>18.0 (1.0%)</td>
<td>54.2 (6.5%)</td>
</tr>
<tr>
<td>Fasted</td>
<td>27.6 (7.0%)</td>
<td>36.5 (8.6%)</td>
</tr>
<tr>
<td></td>
<td>13.0 (3.6%)</td>
<td>17.9 (3.3%)</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>49.5 (4.2%)</td>
<td>51.9 (5.7%)</td>
</tr>
<tr>
<td>Fasted</td>
<td>31.4 (4.7%)</td>
<td>30.8 (5.9%)</td>
</tr>
<tr>
<td></td>
<td>17.9 (3.3%)</td>
<td>17.1 (4.3%)</td>
</tr>
</tbody>
</table>

*Significant differences indicated by asterisk (*)