Impact of maternal undernutrition on the hypothalamic–pituitary–adrenal axis responsiveness in sheep at different ages postnatal

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Abstract

Epidemiological and experimental data support the hypothesis of 'fetal programming', which proposes that alterations in fetal nutrition and endocrine status lead to permanent adaptations in fetal homeostatic mechanisms, producing long-term changes in physiology and determine susceptibility to later disease. Altered hypothalamic–pituitary–adrenal (HPA) axis function has been proposed to play an important role in programming of disease risk. The aim of the present study was to examine the effects of maternal nutrient restriction imposed during different periods of gestation on the HPA axis function in sheep, at different ages postnatal. Pregnant ewes were fed a 50% nutrient-restricted diet from days 0–30 (group R1, $n=7$), or from days 31–100 of gestation (group R2, $n=7$) or a control 100% diet throughout pregnancy, (Control, $n=8$). Blood samples were collected at 10-day intervals from day 40 of gestation to term. Lambs were born naturally and fed to appetite throughout the study period. At 2, 5·5, and 10 months of age lambs were given an i.v. injection of corticotrophin-releasing hormone (CRH) and blood samples were collected at −15, 0, 15, 30, 60, 120, and 180 min postinjection. Maternal cortisol levels were significantly higher ($P<0·05$) in group R1 compared with the other two groups, whereas maternal insulin levels were lower ($P<0·05$) in group R2 compared with control. Birth weight of lambs was not affected by the maternal nutritional manipulation. The area under the curve for ACTH and cortisol response to CRH challenge was greater ($P<0·05$) in lambs of group R1 at two months of age, whereas no difference was detected at the ages of 5·5 and 10 months. However, significantly higher ($P<0·01$) basal cortisol levels were observed in lambs of R1 group at 5·5 months of age. There was no interaction between treatment and sex for both pituitary and adrenal responses to the challenge. A significant sex effect was evident with females responding with higher ACTH and cortisol levels at the age of 5·5 months ($P<0·01$, $P<0·001$ respectively) and with higher cortisol levels ($P<0·01$) at 10 months of age than males. It is concluded that the HPA axis is programmable by altered nutrition *in utero*. The sensitivity of the axis to exogenous stimulation is enhanced during early postnatal life and attenuated with age, suggesting a role for the postnatal influences in resetting of the HPA axis and emphasizing the importance of identifying the impact of maternal undernutrition at several time points after birth.

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Introduction

A series of experimental and epidemiological studies had led to the fetal programming hypothesis, which implies that adverse environmental factors, acting *in utero* program the development of fetal tissues, producing dysfunctions and diseases in adults (Barker *et al.* 1993). Such programming reflects the action of a factor during a sensitive period or window of development to exert organizational effects that persist throughout life (Seckl 2001). The triggers operative during fetal life that have been studied most extensively are undernutrition and glucocorticoid exposure (Seckl 2001, Symonds *et al.* 2001). The glucocorticoids are proposed to act as intermediary factors that transcribe the development programming sequelae of maternal nutrient restriction (Langley-Evans *et al.* 1996). In sheep, maternal cortisol changes are related to the timing when feed restriction is imposed. Late-gestation undernutrition resulted in elevated maternal cortisol concentrations (Edwards & McMillen 2001), while early- to mid-gestation or periconceptional undernutrition resulted in reduced or unchanged cortisol levels respectively (Bispham *et al.* 2003, Gardner *et al.* 2006).

It has been shown that maternal nutrient restriction, which has no effect on birth weight, can alter the function of the hypothalamic–pituitary–adrenal (HPA) axis (Hawkins *et al.* 1999, 2000), which has been suggested to play a role in programming of the later disease (Seckl 1997). Studies examining the impact of maternal nutrient restriction on

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HPA axis have been mostly limited to the late-gestation fetus or young lambs (Hawkins *et al.* 2000, Edwards *et al.* 2001, Edwards & McMillen 2002, Bloomfield *et al.* 2004) and only a few have been undertaken to establish the long-term effects of maternal undernutrition on HPA function in the adult sheep. Brief undernutrition for 10 but not 20 days during late gestation altered the HPA function in adult offspring, further emphasizing the fact that the timing, type, and duration of fetal nutrient restriction are each important in determining the nature of the fetal adaptive responses and their pathophysiological sequelae in later life (Bloomfield *et al.* 2003). More recently periconceptional undernutrition was shown to exert a minor influence on HPA axis function in young adult sheep (Gardner *et al.* 2006).

It has been suggested that HPA axis function may undergo developmental changes, since prenatal glucocorticoid exposure in sheep resulted in different HPA axis responsiveness between 6 months and 1 year postnatal age, which highlights the importance of studying outcome at several different times postnatal (Sloboda *et al.* 2002).

To our knowledge, no study has investigated the age-related changes on stimulated HPA axis function in sheep offspring as a result of maternal undernutrition at different discrete times during pregnancy.

Therefore, the present study was undertaken in order to characterize the effects of 50% maternal nutrient restriction during two (0–30 and 31–100 days) periods of gestation on the HPA axis responsiveness to exogenously administered corticotropin-releasing hormone at three different ages in postnatal life in sheep.

Materials and Methods

Animal management and nutritional treatments

Twenty-two ewes (*Ovies aries*) of the Chios breed, of similar age (2·0±0·3 years) and body weight (63·0±0·6 kg), were used. Housing and care of animals conformed to Faculty of Animal Science guidelines. Ewes were housed indoors and were mated following estrus synchronization with intravaginal progestagen sponges (Chronogest, Intervet, Holland). At the time of mating, they were randomly allocated to one of the following treatments. Control group (C, $n=8$) fed 100% of the recommended metabolizable energy (ME) and crude protein (CP) requirements (9 MJ ME/day and 104 g CP/day for the maintenance of a 60 kg non pregnant ewe, National Research Council (NRC 1985) for the whole of gestational period, nutrient restricted group 1 (R1, $n=7$) offered 50% of the control nutrient allowance during the first 30 days of pregnancy, and nutrient restricted group 2 (R2, $n=7$) fed to 50% of the control nutrient allowance from day 31 to day 100 of pregnancy. Feed was provided at an individual basis in two equal portions at 0800 and 1600 h daily and animals had free access to water. Feed residues were recorded every day. The diet consisted of lucerne chaff, wheat straw, and a commercial concentrate mixture (in pellets) containing barley, corn, wheat bran, cottonseed meal, limestone, bicalcium phosphate, sodium chloride, and mineral–vitamin premix. The lucerne chaff, wheat straw and pelleted concentrate provided 7·2, 5·5, 10 MJ metabolizable energy/kg and contained 135, 32 and 115 g crude protein/kg respectively.

Pregnancy and fetal number were confirmed by ultrasound at day 70 of gestation. Only twin-bearing ewes were used, while two singleton-bearing ewes from group R2 were excluded from the study. All nutritional regimens were adjusted for gestational age and fetal number, while maintaining the treatment difference and taking into account the increasing fetal burden.

Ewes were weighed weekly before morning feeding throughout gestation. Blood samples were collected at 10-day intervals before feeding for the measurement of cortisol and insulin concentrations, from day 40 until parturition. All blood samples were centrifuged at 2800 r.p.m. for 15 min and plasma was separated into aliquots and stored at −20 °C for subsequent analysis.

Lambs were delivered naturally. After delivery they were dried, ear tagged, and weighed and the date of birth and sex were recorded. Two days after birth, newborns were separated from their mothers and reared on artificial milk containing coconut oil, whey powder, wheat starch, soya protein, and vitamin–antioxidants premix (Capragno, Serval S.A., France). Milk was offered *ad libitum*, in order to ensure that lambs met their daily requirements, until the age of 45 days. After this age, lambs' diet consisted of lucerne hay and a commercial pellet mixture containing barley, corn, soya meal, sunflower meal, wheat bran, powder milk, limestone, bicalcium phosphate, and mineral–vitamin premix. Lambs were weighed biweekly from birth to the end of experiment. Three lambs from the control and one from the R2 group were weighed until weaning and therefore treatment groups were: C (male (m)=7/female (f)=6), R1 (m=7/f=7), R2: (m=5/f=4).

Corticotropin releasing hormone (CRH) challenges

At three different ages (2, 5·5, and 10 months) lambs were injected with 0·5 mg ovine CRH/kg body weight (Sigma Chemical). Blood samples were collected at 15 and 0 min before and at 15, 30, 60, and 180 min after the CRH challenge, in heparinized tubes kept on ice, centrifuged at 2800 r.p.m. for 15 min and stored at −20 °C until hormone analysis. All challenges were administered between 0800 and 0900 h in order to minimize the impact of circadian variability on measurements of plasma adrenocorticotropic hormone (ACTH) and cortisol. In addition, before the last CRH challenge at 10 months female lambs were subjected to estrus synchronization using intravaginal progesterone sponges to ensure that changes in circulating steroid hormones due to variations in the estrous cycle did not confound interpretation of the results.

Measurement of plasma insulin, cortisol and ACTH levels

Plasma insulin concentrations were measured using a commercial RIA kit (Linco Research Inc, St.Charles, MO, USA). The sensitivity of the method was 0·1 ng/ml and the intra- and interassay coefficients of variation were 4·6 and 9·4% respectively.

Cortisol concentrations in both maternal and lamb plasma samples were measured by a coated-tube RIA kit (Coat-a-Count cortisol, DPC, Biermann GmbH, Germany) as described previously (Bispham *et al.* 2003). The sensitivity of the method was 1 μg/dl. The intra- and interassay coefficients of variation were 4·4 and 6·4% respectively.

Plasma ACTH levels were measured by a commercial RIA kit that detects $ACTH_{1–24}$ (DiaSorin Inc., Stillwater, MN, USA) and that has previously been validated for use in the sheep (Jeffray *et al.* 1998). The sensitivity of the assay was 15 pg/ml. The coefficients of variation were 6·3 and 6·7% respectively.

Statistical analysis

All data are presented as least square means ± S.E.M. and were analyzed using the SAS Statistical Program (Statistical Analysis System 2005). Maternal body weight and hormone levels throughout gestation were analyzed using a General Linear Model (GLM) for repeated measures with treatment and time (gestational age) as fixed effects and their interactions. Offspring body weight data were analyzed using the same procedure, which included treatment, sex, and time (lamb age) as fixed effects and their interactions.

Data for the area under the response curve (AUC), calculated by a baseline-corrected trapezoidal integration method for cortisol and ACTH after CRH challenge were initially analyzed using a GLM appropriate for repeated measures per subject with fixed effects for treatment, sex, lamb age and their interactions. In addition, data for cortisol and ACTH values (response curve) at each age were also analyzed by a GLM, which included the effects of treatment, sex, and sampling time and their interactions. Birth and current body weight were used as covariates in the initial statistical models but were excluded since no significant effect was revealed. *Post hoc* analyses were performed, when appropriate, using Duncan's method for pairwise comparisons and statistical significance was set at $P<0·05$.

Results

Maternal body weight and hormone levels during gestation

Feed restriction during the first 30 days (group R1) had no effect on body weight throughout pregnancy. In contrast, body weight of the R2 group decreased from day 70, becoming significantly lower than control and R1 groups from day 92 to 99 ($P<0·05$). After feed restoration body weight increased, but remained lower ($P<0·05$), relative to the other two groups until parturition (Fig. 1).

Ewes of R1 group exhibited significantly higher cortisol levels ($P<0·05$) from day 40 up to day 90 of gestation, compared with the other two groups (Fig. 2). On the other hand, insulin levels of ewes undernourished from days 31–100 of gestation (R2 group) were significantly lower compared with control from 40 to 120 days of gestation ($P<0·05$) (Fig. 3).

Lamb body weight

Birth weight of both male and female lambs was not affected by the maternal nutritional manipulation. At 2, 5·5 and 10 months of age there was no difference in body weight between the three groups of animals. In addition, no sex effect was detected (Table 1).

Responses to CRH challenge

ACTH response Three-way (age × treatment × sex) repeated measures ANOVA for ACTH response (AUC) revealed significant effect of age ($P<0·001$) and a significant age × sex interaction ($P<0·001$). The bases for all of these effects were the decrease in ACTH response of both sexes at 5·5 months compared with the response at 2 months and significantly higher ACTH response of female than male lambs at 5·5 months of age ($P<0·01$), (Fig. 4). However, no significant treatment × sex interaction was revealed. A significantly higher ($P<0·05$) ACTH response (AUC) to CRH challenge was observed in animals of R1

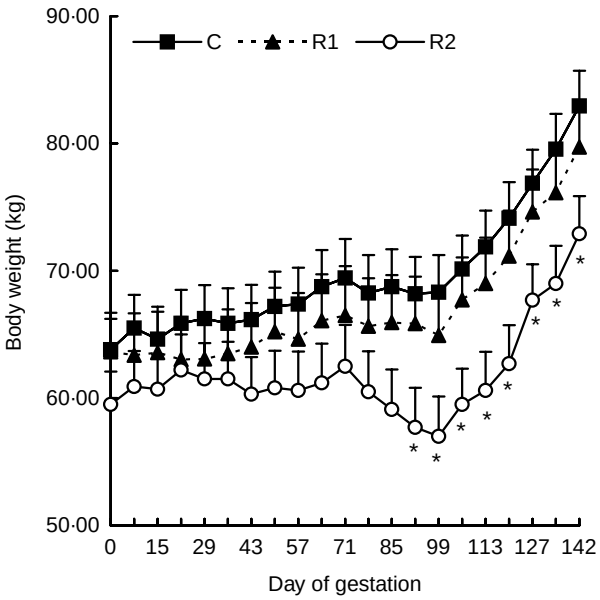

Figure 1 Maternal body weight during gestation. * denotes significant ($P<0·05$) differences between group R2 and both control and R1 groups.

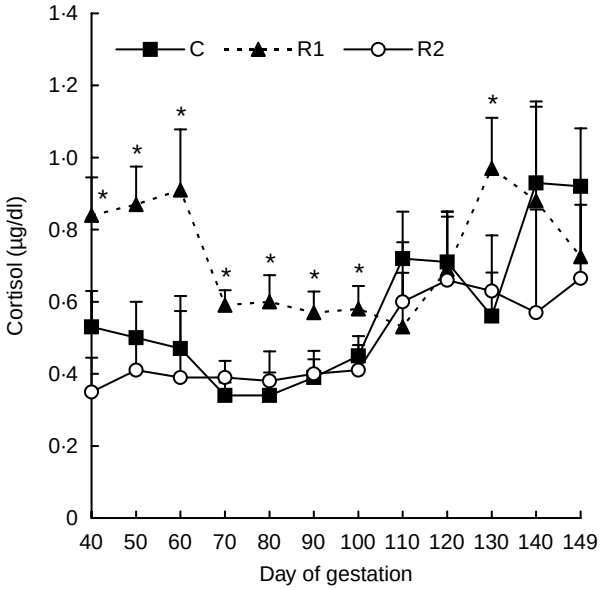

Figure 2 Maternal cortisol concentration during gestation. * Denotes significant differences ($P<0·05$) between group R1 and both control and R2 groups.

group at the age of 2 months compared with the other two groups (Table 2), but no sex effect was detected. In addition, at 120 and 180 min after the challenge ACTH concentration was higher ($P<0·05$) in R1 compared with control and R2 groups (Fig. 5). At the age of 5·5 and 10 months, no treatment effect was revealed for the ACTH response (Table 2, Figs 6 and 7).

Table 1 Body weight (kg) of female and male lambs at birth and at the age of corticotropin-releasing hormone responses

	Group[a]			
	C	R1	R2	P^b
Females				
Birth	4·2±0·29	4·0±0·26	3·8±0·35	NS
2 months	19·2±1·29	18·8±1·20	18·0±1·58	NS
5·5 months	41·4±2·09	37·7±1·92	36·4±2·53	NS
10 months	59·5±2·62	54·7±2·42	54·3±3·21	NS
Males				
Birth	4·8±0·26	4·8±0·26	4·5±0·31	NS
2 months	21·3±1·20	20·0±1·20	18·9±1·42	NS
5·5 months	48·1±1·92	49·3±1·92	43·4±2·27	NS
10 months	69·9±2·42	69·7±2·42	63·2±2·87	NS

[a]C, control; R1, undernutrition from days 0 to 30 of gestation; R2, undernutrition from days 31 to 100 of gestation.
[b]NS, non-significant.

Cortisol response Three-way (age×treatment×sex) repeated measures ANOVA revealed significant effects of sex ($P<0·001$) and age ($P<0·01$) and a significant sex×age ($P<0·001$) interaction on cortisol response (AUC), attributed to the higher cortisol response of female animals at 5·5 ($P<0·001$) and 10 months of age ($P<0·01$), than males (Fig. 8). Similar to the ACTH response, no treatment×sex interaction was revealed.

Cortisol response (AUC) to the CRH challenge followed the same pattern as ACTH, with values being significantly ($P<0·05$) higher at the age of 2 months in lambs of R1 compared with the other two groups (Table 2). In the same group cortisol levels were significantly higher at 60, 120, and

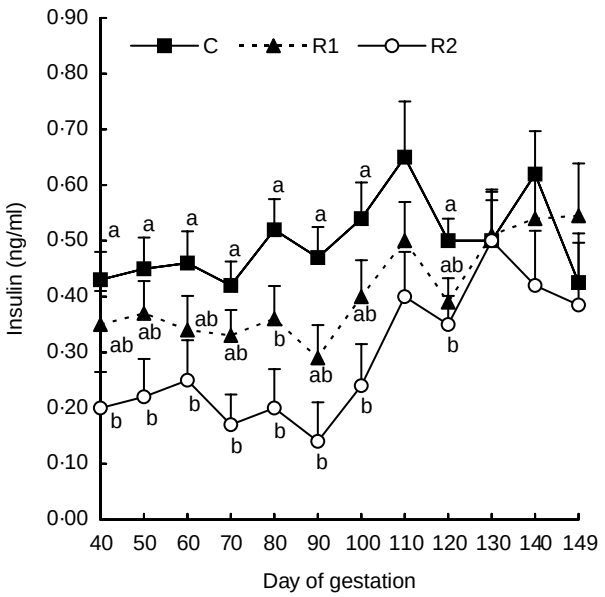

Figure 3 Maternal insulin concentration during gestation. Different letters denote significant ($P<0·05$) differences between groups.

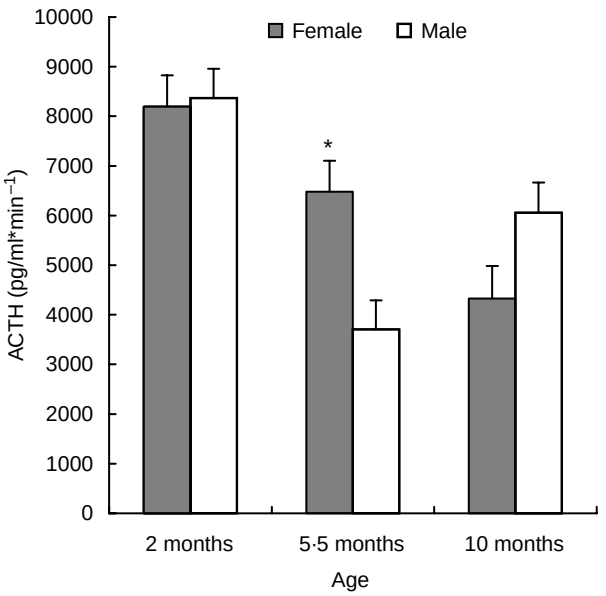

Figure 4 Comparison of ACTH response with CRH challenge between female and male lambs at different ages. *$P<0·01$.

Table 2 Area under the response curve (AUC) for cortisol and adrenocorticotrophic hormone (ACTH) of the lambs at the three corticotrophin-releasing hormone (CRH) challenges

	Group[a]			
	C	R1	R2	*P*[b]
Cortisol AUC (μg/dl per min)				
2 months	195[c]±27·7	265[d]±26·7	155[c]±32·2	*
5·5 months	268±22·6	258±21·8	288±26·3	NS
10 months	249±23·9	223±23·1	221±27·9	NS
ACTH AUC (pg/ml per min)				
2 months	7638[c]±710·4	10 031[d]±682·5	7164[c]±856·6	*
5·5 months	4700±698·1	5450±670·7	5131±841·7	NS
10 months	5523±730·9	4471±702·2	5579±881·3	NS

*$P<0·05$.
[a]C, control; R1, undernutrition from days 0 to 30 of gestation; R2, undernutrition from days 31 to 100 of gestation.
[b]NS, non-significant.
Different letters in the same row indicate significant ($P<0·05$) differences.

180 min after the challenge ($P<0·05$, $P<0·01$ and $P<0·05$ respectively), (Fig. 5).

At the age of 5·5 months cortisol response to CRH challenge (AUC) did not differ between treatment groups (Table 2), but the basal cortisol levels were higher ($P<0·01$) in R1 group, followed by elevated, although not significant concentrations at each sampling time (Fig. 6). This latter effect was not more apparent at the age of 10 months, when no differences between treatments were observed (Fig. 7).

Discussion

Feed restriction (50% of requirements) from 31 to 100 days of pregnancy resulted in a significant reduction in maternal body weight 60 days after the start of underfeeding, which lasted until parturition. In contrast to these results, Hawkins *et al.* (1999) and Bispham *et al.* (2003) reported that ewes underfed from early to mid gestation exhibited a significant decrease in their body weight during undernutrition, an effect no longer apparent when diet was restored. It seems that maternal body weight restoration depends upon the refeeding manipulation, as well as upon the duration and the intensity of previously imposed nutritional restriction.

Maternal cortisol levels exhibited a significant increase between 40 and 90 days of gestation in ewes undernourished from conception to day 30 of gestation (group R1). Previous data in sheep have shown a suppression of maternal HPA axis during periconceptional undernutrition with decreased ACTH and cortisol levels, which returned to normal or even elevated levels after cessation of undernutrition (Bloomfield *et al.* 2004). In the present study the significantly elevated cortisol levels detected after refeeding in ewes of R1 group support the hypothesis of an increased HPA axis activity after cessation of undernutrition. In contrast, undernutrition from 31 to 100 days of gestation had no effect on maternal cortisol levels. However, Bispham *et al.* (2003) reported that ewes undernourished at 60% of their requirements from day 28 to day 80 of gestation exhibited lower cortisol levels throughout the period of undernutrition, compared with well-fed ewes. The differences in maternal cortisol response to nutrient restriction between the present and other studies may be due to variations in the time, duration and intensity of the feed restriction. Maternal insulin concentrations were significantly lower in R2 group during undernutrition and for the next 20 days after cessation, which is in good agreement with the data reported by McMullen *et al.* (2005).

Lambs' birth weights were not affected by either nutritional manipulation. No effect on birth body weight has been reported in a number of previous studies in which ewes were fed at 50 or 85% of their requirements at different periods up

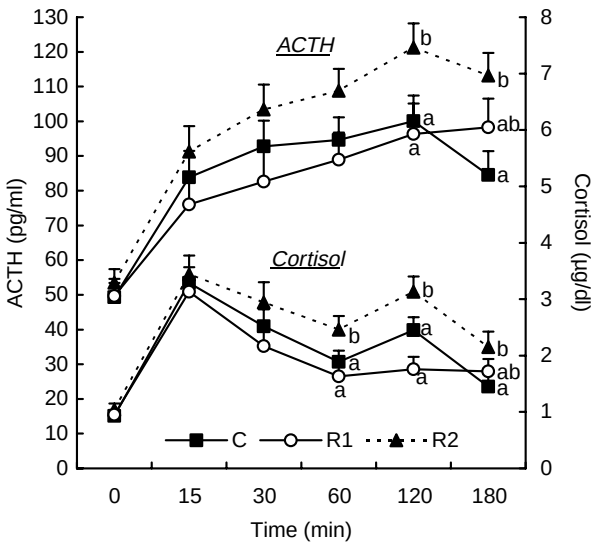

Figure 5 ACTH and cortisol response to CRH challenge in 2-month-old lambs. Different letters denote significant ($P<0·05$) differences between groups.

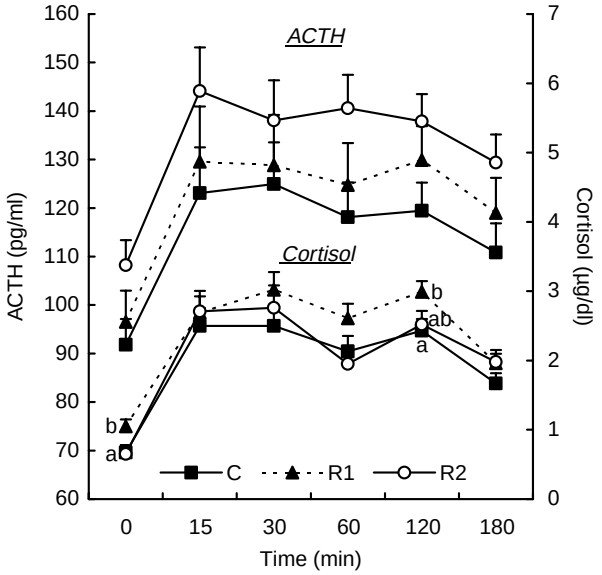

Figure 6 ACTH and cortisol response to CRH challenge in 5·5-month-old lambs. Different letters denote significant ($P<0·05$) differences between groups.

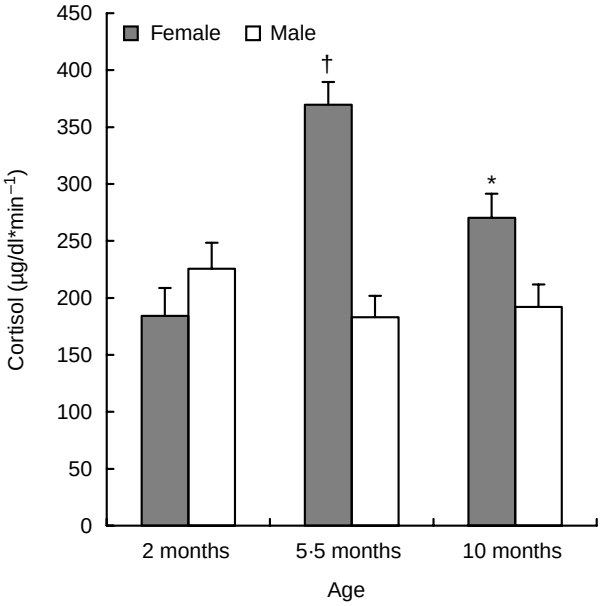

Figure 8 Comparison of cortisol response to CRH challenge between female and male lambs at different ages. $^{*}P<0·05$, $^{\dagger}P<0·001$.

to 90 days of gestation (Hawkins *et al.* 1999, 2000, Whoorwood *et al.* 2001, Gopalakrishman *et al.* 2004). On the contrary a reduction in the birth body weight was evident in newborn lambs from mothers undernourished from 105 to 115 days of gestation (Bloomfield *et al.* 2003). It is clear that the body weight at birth is affected only when undernutrition is imposed on mothers after day 100 of gestation, when rapid growth of the fetus occurs.

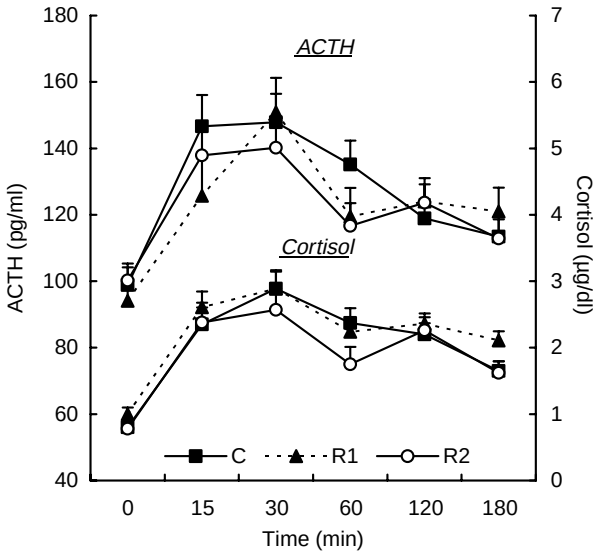

Figure 7 ACTH and cortisol response to CRH challenge in 10-month-old lambs.

It has been shown that maternal nutritional insults that have no effect on birth weight can have pronounced effects on fetal metabolism (Harding & Johnston 1995) and hormonal responses (Oliver *et al.* 2001), including alterations in fetal hypothalamic adrenal axis function (Hawkins *et al.* 1999, 2000). To date, the majority of studies have focused on the effects of maternal undernutrition in late-gestation fetal sheep, which vary from enhanced, when periconceptional undernutrition was imposed (Edwards & McMillen 2002) to reduced pituitary–adrenal response following CRH administration after early- to mid-gestation undernutrition (Hawkins *et al.* 1999, 2000). However, the timing and duration of maternal undernutrition required to produce postnatal consequences have received limited study. Nutritional restriction for a very brief (10 days), but not longer (20 days) period during late gestation resulted in increased responsiveness of HPA axis in adult (30 months old) female sheep (Bloomfield *et al.* 2003). In a more recent study Gardner *et al.* (2006) showed that periconceptional undernutrition, similar to that imposed in our study had a minor influence on HPA axis function in young adult (1 year old) offspring, with females responding differently to males and a clear influence of periconceptional nutritional environment on this response. However, the main object of the present study was to examine the age-related alterations of HPA axis responsiveness after undernutrition *in utero* imposed during different gestational periods. Feed restriction from conception to 30 days of gestation (R1 group) resulted in enhanced ACTH and cortisol response after CRH administration at the age of 2 months, a result no more apparent at the age of 5·5 and 10 months. On the contrary, undernutrition from

early- to mid-gestation (31–100 days) had no effect on HPA axis responsiveness at any age postnatal. These differences strongly indicate that both timing and duration of nutritional insults during gestation may differently affect the HPA axis function in offspring.

The above enhanced ACTH and cortisol response detected at 2 months of age are in accordance with the results of Hawkins *et al.* (2000), who reported that undernutrition during early gestation led to enhanced pituitary and adrenal responsiveness at 85 days postnatal, probably through reduced number of glucocorticoid receptors (GR) in the pituitary, indicating decreased glucocorticoid-mediated negative feedback of the axis (Hawkins *et al.* 1998).

The underlying mechanisms responsible for the altered hypothalamo–pituitary–adrenal function, as a result of maternal undernutrition are not yet clear. It has been shown that prenatal glucocorticoid exposure resulted in significant alterations in glucocorticoid receptor mRNA levels in the HPA axis, thus linking glucocorticoid exposure with alterations in negative feedback control of the axis (Levitt *et al.* 1996). Altered expression of central GR and mineralo corticoid receptors (MR) systems leading to decreased negative feedback at the paraventricular nucleus (PVN) and enhanced facilitation of the HPA response has also been reported after prenatal stress in rats and piglets (Henry *et al.* 1994, Maccari *et al.* 1995, Barbazanges *et al.* 1996, Kanitz *et al.* 2003). In the present study, given the higher cortisol concentrations detected after feeding restoration in association with data that placental 11βHSD2 activity is reduced at the day 50 of gestation after periconceptional undernutrition (Jaquiery *et al.* 2006), it is tempting to speculate that the enhanced HPA axis function seen at 2 months *post partum* was due to increased exposure to excess cortisol *in utero*. Moreover, other components of the HPA axis, such as CRH mRNA expression in hypothalamus, ACTH-R expression and/or steroidogenic enzymes expression in adrenals might have been altered by maternal undernutrition, as has also been suggested in fetal sheep (Hawkins *et al.* 2001, Edwards *et al.* 2001).

The fact that the effects on HPA axis function were detected in lambs of R1, but not R2, group further emphasizes the possibility that there must be a critical window or windows during which the fetus is susceptible to maternal stress.

The lack of an effect on cortisol and ACTH response in lambs of R1 group at the age of 5·5 and 10 months points to the fact that the altered HPA axis function seen early in postnatal life, as a result of periconceptional undernutrition, changes with age. However, at the age of 5·5 months basal cortisol levels were still significantly higher in lambs of R1 group and a tendency for higher, although not significant, cortisol levels was evident at all time points. This gradual attenuation of HPA axis responsiveness indicates that there must be some factors contributing to this enhanced control of HPA axis activity with increasing age. A possible mechanism for this shift in HPA axis response is the resetting of the axis by hypercortisolaemia observed in early postnatal life, as has also been suggested by Poore & Fowden (2003) in their studies with juvenile and adult pigs. In addition, the possibility that the sensitivity to glucocorticoid negative feedback may have been influenced by alterations in the gonadal steroid milieu as animals underwent transition from pre-to postpubertal stage cannot be ruled out, since it has been shown that sex steroids potentiate and buffer glucocorticoid feedback on the gene expression of CRH, MR and, GR in a gender-specific manner (Patchev & Osborne 1996). Interestingly, in the present study a significant sex effect was observed at 5·5 and 10 months of age, with females exhibiting higher ACTH and cortisol levels after CRH administration, confirming earlier reports of gender-related differences in hypothalamo–pituitary–adrenal axis (Viau & Meaney 1996, Owen & Mathews 2003). The absence of an interaction between treatment and gender early in postnatal life, but the significant gender effect at the age of 5·5 months further emphasizes the impact of puberty-related changes on HPA axis responsiveness, with estrogens exerting stimulatory effects on stress-induced ACTH and glucocorticoid release (Lund *et al.* 2004). A well-characterized interaction between gender and puberty on the stress-induced response has recently been reported in rats (Viau *et al.* 2005). The results of the present study do not support the hypothesis that programming of the adult HPA axis is sex specific, as was the case with guinea pigs (Lingas & Matthews 2001) and sheep (Gardner *et al.* 2006). However, the main finding of the present study is that the effects of maternal undernutrition on HPA axis function changes with age, thus highlighting the importance of studying outcome at several different times during the life course. Furthermore, the differences detected between the two experimental groups points at the significant influence that the timing of imposing insult could exert on the programming of the HPA axis. Further studies are required to identify the impact of prenatal feed restriction on development and subsequent function of the hypothalamo–pituitary–gonadal (HPG) axis, particularly with respect to the interaction between the HPG and the HPA axes in both males and females.

In conclusion the present study showed that the first month of pregnancy is very critical for the HPA axis development, as indicated by the enhanced HPA axis activity after birth. This effect may be a consequence of fetal cortisol exposure resulting in early life hypersensitivity to glucocorticoid action and altered regulation of the HPA axis. However, this heightened hypothalamic drive decreases with age, indicating the resetting of the axis to adulthood and further emphasizing the importance of identifying the impact of nutritional restriction *in utero* at several time points after birth. Whether or not this effect, although transient, is sufficient to program alterations to other systems needs to be further elucidated.

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References

Barbazanges A, Piazza PV, Le Moal M & Maccari S 1996 Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *Journal of Neuroscience* **16** 3943–3949.

Barker DJP, Gluckman PD, Godfrey KM, Harding JE, Owens JA & Robinson JS 1993 Fetal nutrition and cardiovascular disease in adult life. *Lancet* **341** 938–941.

Bispham J, Gopalakrishnan GS, Dandrea J, Wilson V, Budge H, Keisler DH, Broughton Pipkin F, Stephenson T & Symonds ME 2003 Maternal endocrine adaptation throughout pregnancy to nutritional manipulation: consequences for maternal plasma leptin and cortisol and the programming of fetal adipose tissue development. *Endocrinology* **144** 3575–3585.

Bloomfield FH, Oliver MH, Giannoulias D, Gluckman PD, Harding JE & Challis JRG 2003 Brief undernutrition in late-gestation sheep programs the hypothalamic–pituitary–adrenal axis in adult offspring. *Endocrinology* **144** 2933–2940.

Bloomfield FH, Oliver MH, Hawkins P, Holloway AC, Campbell M, Gluckman PD, Harding JE & Challis JRG 2004 Periconceptional undernutrition in sheep accelerates maturation of the fetal hypothalamic–pituitary–adrenal axis in late gestation. *Endocrinology* **145** 4278–4285.

Edwards LJ & McMillen IC 2001 Maternal undernutrition increases arterial blood pressure in the sheep fetus during late gestation. *Journal of Physiology* **533** 561–570.

Edwards LJ & McMillen IC 2002 Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo–pituitary–adrenal axis in sheep during late gestation. *Biology of Reproduction* **66** 1562–1569.

Edwards L, Bryce AE, Coulter CL & McMillen IC 2001 Maternal undernutrition throughout pregnancy increases adrenocorticotropin receptor and steroidogenic acute regulatory protein gene expression in the adrenal gland of twin fetal sheep during late gestation. *Molecular and Cellular Endocrinology* **196** 1–10.

Gardner DS, Van Bon BWM, Dandrea J, Goddard PJ, May SF, Wilson V, Stephenson T & Symonds ME 2006 Effect of periconceptional undernutrition and gender on hypothalamic–pituitary–adrenal axis function in young adult sheep. *Journal of Endocrinology* **190** 203–212.

Gopalakrishman GS, Gardner DS, Rhind SM, Rae MT, Kyle CE, Brooks AN, Walker RM, Ramsay MM, Keisler DH, Stephenson T *et al.* 2004 Programming of adult cardiovascular function after early maternal undernutrition in sheep. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **287** R12–R20.

Harding JE & Johnston BM 1995 Nutrition and fetal growth. *Reproduction, Fertility, and Development* **7** 539–547.

Hawkins P, Hanson MA, Noakes DE & Matthews SG 1998 Fetal sheep HPA axis control mechanisms following modest maternal nutrient restriction in early gestation. *Journal of Physiology* **513** 7P.

Hawkins P, Steyn C, McGarrigle HHG, Saito T, Ozaki T, Stratford LL, Noakes DE & Hanson MA 1999 Effect of maternal nutrient restriction in early gestation on development of the hypothalamic–pituitary–adrenal axis in fetal sheep at 0.8–0.9 of gestation. *Journal of Endocrinology* **163** 553–561.

Hawkins P, Steyn C, McGarrigle HHG, Calder NA, Saito T, Stratford LL, Noakes DE & Hanson MA 2000 Cardiovascular and hypothalamic–pituitary–adrenal development in late gestation fetal sheep and young lambs following modest maternal nutrient restriction in early gestation. *Reproduction, Fertility, and Development* **12** 443–456.

Hawkins P, Hanson MA & Matthews SG 2001 Maternal undernutrition in early gestation alters molecular regulation of the hypothalamic–pituitary–adrenal axis in the ovine fetus. *Journal of Neuroendocrinology* **13** 855–861.

Henry C, Kabbaj M, Simon H, Le Moal M & Maccari S 1994 Prenatal stress increases the hypothalamo–pituitary–adrenal axis response in young and adult rats. *Journal of Neuroendocrinology* **6** 341–345.

Jaquiery AL, Oliver MH, Bloomfield FH, Connor KL, Challis JRG & Harding JE 2006 Fetal exposure to excess glucocorticoid is unlikely to explain the effects of periconceptional undernutrition in sheep. *Journal of Physiology* **572** 109–118.

Jeffray TM, Matthews SG, Hammond GL & Challis JRG 1998 Divergent changes in plasma ACTH and pituitary POMC mRNA after cortisol administration to the late gestation ovine fetus. *American Journal of Physiology* **274** 417–425.

Kanitz E, Otten W, Tuchscherer M & Manteuffel G 2003 Effects of prenatal stress on corticosteroid receptors and monoamine concentrations in limbic areas of suckling piglets (*Sus scrofa*) at different ages. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine* **50** 132–139.

Langley-Evans S, Gardner D & Jackson AA 1996 Maternal protein restriction influences the programming of the rat HPA. *Journal of Nutrition* **126** 1578–1585.

Levitt NS, Lindsay RS, Holmes MC & Seckl JR 1996 Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology* **64** 412–418.

Lingas R & Matthews SG 2001 A short period of maternal nutrient restriction in late gestation modifies pituitary-adrenal function in adult guinea pig offspring. *Neuroendocrinology* **73** 302–311.

Lund TD, Munson DJ, Haldy ME & Handa RJ 2004 Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. *Journal of Neuroendocrinology* **16** 272–278.

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H & Le Moal M 1995 Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *Journal of Neuroscience* **15** 110–116.

McMullen S, Osgerby JC, Milne JS, Wallace JM & Wathes DC 2005 The effects of acute nutrient restriction in the mid-gestational ewe on maternal and fetal nutrient status, the expression of placenta growth factors and fetal growth. *Placenta* **26** 25–33.

National Research Council (NRC) 1985 *Nutrient Requirements of Sheep*, Washington, DC: National Academy Press.

Oliver MH, Hawkins P, Breier B, van Zijl PL, Sargison S & Harding JE 2001 Maternal undernutrition during the periconceptional period increases plasma taurine levels and insulin response to glucose but not arginine in the late gestational fetal sheep. *Experimental Physiology* **85** 85–96.

Owen D & Mathews SG 2003 Glucocorticoids and sex-dependent development of brain glucocorticoid and mineralocorticoid receptors. *Endocrinology* **144** 2775–2784.

Patchev VK & Osborne AFX 1996 Gonadal steroids exert facilitating and 'buffering' effects on glucocorticoid-mediated transcriptional regulation of corticotropin-releasing hormone and corticosteroid receptor genes in rat brain. *Journal of Neuroscience* **16** 7077–7084.

Poore KR & Fowden AL 2003 The effect of birth weight on hypothalamo–pituitary–adrenal axis function in juvenile and adult pigs. *Journal of Physiology* **547** 107–116.

Seckl JR 1997 Glucocorticoids, feto-placenta 11β-hydroxysteroid dehydrogenase type 2, and the early life origins of adult disease. *Steroids* **62** 89–94.

Seckl JR 2001 Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. *Molecular and Cellular Endocrinology* **185** 61–71.

Sloboda DM, Moss TJ, Gurrin LC, Newnham JP & Challis JRG 2002 The effect of prenatal betamethasone administration on postnatal ovine hypothalamic–pituitary–adrenal function. *Journal of Endocrinology* **172** 71–81.

Symonds ME, Budge H, Stephenson T & McMillen CI 2001 Fetal endocrinology and development manipulation and adaptation to long-term nutritional and enviromental challenges. *Reproduction* **121** 853–862.

Viau V & Meaney MJ 1996 The inhibitory effect of testosterone on hypothalamic–pituitary–adrenal responses to stress is mediated by the medial preoptic area. *Journal of Neuroscience* **16** 1866–1876.

Viau V, Bingham B, Davis J, Lee P & Wong M 2005 Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology* **146** 137–146.

Whoorwood CB, Firth KM, Budge H & Symonds ME 2001 Maternal undernutrition during early to midgestation programs tissue specific alterations in the expression of the glucocorticoid receptor, 11β-hydroxysteroid dehydrogenase isoforms, and type 1 angiotensin II receptor in neonatal sheep. *Endocrinology* **142** 2854–2864.

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