

The influence of maternal nutrient restriction in early to mid-pregnancy on placental and fetal development in sheep

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Placental weight is a primary factor determining size at birth in many species. In sheep, placental weight peaks at approximately mid-gestation, with structural remodelling occurring over the second half of pregnancy to meet the increasing nutritional demands of the growing fetus. Numerous factors influence placental growth and development in sheep, and many workers (see Kelly, 1992) have investigated the role of maternal nutrition as a regulator of placental and fetal size. We have studied the effects of feeding ewes approximately 50% of their recommended energy requirements during early to mid-pregnancy on fetal and placental indices measured at mid-gestation (i.e. 80 d) and close to term (i.e. 145 d). Maternal nutrient restriction is associated with a reduction in placental weight at 80 d, but an increase in placental weight at 145 d of gestation, compared with ewes fed adequately in early pregnancy. No significant effect on fetal weight was observed at either 80 or 145 d gestation, although differences in body dimensions and the insulin-like growth factor-1 axis were found in lambs from nutrient-restricted ewes delivered close to term. Maternal nutrition during pregnancy plays a pivotal role in the regulation of fetal and placental development in sheep, and therefore has the potential to influence both short- and longer-term health outcomes.

Maternal nutrition: Placenta: Fetus: Adults

The incidence of morbidity and mortality during the neonatal period is known to be high in babies of low birth weight, but a substantial body of epidemiological evidence now exists to suggest that perturbations in fetal and infant growth rates are also associated with a predisposition to certain disorders in adulthood (Barker *et al.* 1990). Subsequently, it has become apparent that subtle alterations in conformation at birth, irrespective of birth weight, may also play a role in the programming of future health outcomes (Barker, 1994). The long thin baby, for example, is proposed to be at greater risk of developing hypertension and type 2 diabetes in adulthood, whereas the short fat baby is more likely to develop CHD and thrombotic stroke. The 'fetal and infant origins of adult disease' hypothesis (Barker, 1994) has also been strengthened by data obtained following the Dutch famine of 1944–5 (e.g. Lumey, 1998). Obstetric records have shown reduced birth weights in 'second-generation' infants delivered from mothers who themselves had been exposed *in utero* to the famine in either the first or second trimester of pregnancy (Lumey, 1992). This was

found to be due, in part, to a slower fetal growth rate, in conjunction with a shorter gestation. Exposure to famine during the third trimester, however, was not associated with a reduction in birth weight in the second-generation infants, despite the mothers of these infants being themselves growth-retarded at birth. Further evidence for the 'Barker hypothesis' has been derived from numerous animal studies, with cross-breeding experiments demonstrating that the maternal environment is a more important regulator of fetal size than the genetic component (Walton & Hammond, 1938). More recently, fetal programming of adult hypertension in rats, by maternal nutrition during pregnancy, has been demonstrated by a number of studies (Woodall *et al.* 1996; Gardner *et al.* 1998).

Placental development and fetal size

In the majority of eutherian mammals studied to date, there is a positive correlation between size at birth and placental weight. Pathological alterations in placental weight in

Abbreviation: IGF-1, insulin-like growth factor-1.

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human pregnancies support this finding, with a poorly-controlled diabetic pregnancy often resulting in a heavy placenta and large infant, while intrauterine growth retardation is associated with a small placenta (Naeye, 1987). The association between placental size and birth weight has been demonstrated clearly in sheep using the carunclectomy technique, where the majority of the discrete sites of placental implantation on the maternal endometrium are surgically removed before mating (Robinson *et al.* 1979). Although there may be some compensatory growth of the remaining placentomes, the fetus is typically hypoxic and growth-retarded.

In sheep, the period of maximal placental weight gain occurs in the first half of gestation, typically between 30 and 80 d of gestation (Schneider, 1996), with term being 147 d. Peak placental weight is attained by 80 d of gestation, and placental weight may then decline by up to 30% over the final 50 d of pregnancy. Placentome number is generally established by 40 d of gestation, and is not thought to change throughout pregnancy, although individual placentomes alter in size. Fetal growth rate, however, remains relatively slow until mid-gestation, and it is only after placental weight has peaked that the exponential rise in fetal weight is seen. It is thought that alterations in the structure and morphology of the placenta allow the increasing nutrient requirements of the ovine fetus to be met during late gestation (Schneider, 1996).

Maternal nutrition and placental weight in sheep

Many studies have examined the effect of maternal nutrition at different stages of pregnancy on placental and fetal development in sheep (see Kelly, 1992). Nutrient restriction over the first 40 d or final 50 d of pregnancy generally has no marked effect on placental weight, whereas the majority of studies examining the effect of a restricted diet for periods of variable length between 30 and 107 d of pregnancy have found placental weight to be markedly altered (Table 1).

However, results obtained are inconsistent, and this may be due to a number of confounding factors, including differences in initial maternal weight, body condition and breed. Furthermore, results obtained at term from nutrient-restricted ewes are generally influenced by a compensatory increase in maternal nutrition over the remainder of pregnancy compared with controls (for example, see McCrabb *et al.* 1991). In addition, few studies have looked at placental morphology in detail, and effects on fetal conformation have been largely ignored. Other studies have looked at maternal nutrient restriction from 0 to 90 d of gestation (Everitt, 1964; Rattray & Trigg, 1979), using a mixture of singleton- and twin-bearing ewes (Mellor & Murray, 1982), triplets (Kelly & Ralph, 1988), or measured cotyledon diameter by ultrasound scanning to examine effects on placental development (Kelly *et al.* 1992; Kleemann *et al.* 1993). Data from these experiments have not been included in Table 1, as their results are not comparable with those for singleton pregnancies where placental weight data have been obtained (Heasman *et al.* 1998a).

Maternal nutrient restriction in early to mid-pregnancy

Given the variation in placental responses to maternal nutrient restriction described previously, we decided to examine, in detail, the effects of a defined alteration in maternal nutritional regimen in early to mid-gestation, over the period of maximal placental growth, on placental and fetal development. Singleton-bearing ewes of similar breed, age and weight at mating were used throughout our studies. These were fed to meet either half or twice their maintenance energy requirements (Agricultural Research Council, 1980) between 30 and 80 d of gestation, with the diet being increased at 14 d intervals to meet the higher energy requirements associated with fetal growth. For example, at maintenance levels a 40 kg ewe would receive 5.62 MJ metabolizable energy/d at 30 d of gestation, rising to 6.22 MJ/d at 74 d of gestation (Clarke *et al.* 1998). For

Table 1. Summary of the effects of maternal nutrient restriction in mid-pregnancy on placental weight in singleton-bearing ewes

Reference	Altered diet (d of pregnancy)	Outcome measured (d of pregnancy)	Maternal wt (kg)		Nutrition		Placental wt (g)		
			C	R	C	R	C	R	Difference (%)
Robinson <i>et al.</i> (1994)	30–90	90	63	63	'Unrestricted intake'	'Restricted intake'	462	561	+21
Robinson <i>et al.</i> (1994)	30–90	90	51	51	'Unrestricted intake'	'Restricted intake'	569	423	–27
McCrabb <i>et al.</i> (1991)	30–96	96	55	57	Fed to gain 100 g/d	Fed to lose 114 g/d	496	600	+21
McCrabb <i>et al.</i> (1992a)	30–96	96	40	45	Fed to gain 100 g/d	Fed to lose 114 g/d	596	473	–21
McCrabb <i>et al.</i> (1992b)	30–96	96	45	47	1.43–1.54 kg hay DM/d	0.39–0.64 kg hay DM/d	517	398	–23
McCrabb <i>et al.</i> (1992b)	50–96	96	45	46	1.43–1.54 kg hay DM/d	0.39–0.44 kg hay DM/d	517	488	–6
McCrabb <i>et al.</i> (1992b)	75–96	96	45	46	1.43–1.54 kg hay DM/d	0.44 kg hay DM/d	517	429	–17
Holst <i>et al.</i> (1992)	79–107	107	–	–	Unrestricted pasture	Pasture restricted (lost 29 g/d)	433	510	+18
McCrabb <i>et al.</i> (1992b)	30–96	140	–	–	1.43–1.54 kg hay DM/d	0.39–0.64 kg hay DM/d	442	388	–12
McCrabb <i>et al.</i> (1991)	30–96	140	53	56	Fed to gain 100 g/d	Fed to lose 114 g/d	430	560	+30
Davis <i>et al.</i> (1981)	40–95	137	49	51	'High' pasture allowance	'Low' pasture allowance	441	386	–12
Faichney & White (1987)	50–100	135	44	45	900 g hay and oats/d	500 g hay and oats/d	337	485	+44
Holst <i>et al.</i> (1992)	79–107	144	–	–	Unrestricted pasture	Pasture restricted (lost 29 g/d)	353	364	+3

C, control; R, restricted

placental and fetal sampling, ewes were humanely killed either at 80 d of gestation or at 145 d of gestation following feeding to meet their maintenance energy requirements for the remainder of pregnancy. Each placenta was classified in terms of inversion or apparent overgrowth of fetal tissue within the placenta, as described by Vatnick *et al.* (1991), with inverted placentas being classified as A and everted placentas as B, C or D depending on the extent of eversion. Placentas were then separated into the maternal and fetal components, which were weighed separately. For ewes studied until term, blood samples were taken through a jugular vein catheter at 77 and 140 d of gestation, and plasma was analysed to determine cortisol and thyroid hormone concentrations (Clarke *et al.* 1994; Bird *et al.* 1996). Furthermore, for ewes studied close to term, cord blood samples were taken at Caesarean section and later analysed for plasma insulin-like growth factor-1 (IGF-1) concentration. Each fetus was dried and weighed, and measurements were taken of crown-rump length, girth (thoracic circumference) and height (Heasman *et al.* 1998b). Lambs were humanely killed and all major organs were dissected out and weighed.

Maternal thyroid hormones and cortisol

Maternal nutrient restriction between 28 and 77 d of gestation had no significant effect on plasma cortisol concentrations at either 77 d of gestation (controls 90.7 (SE 21.0) nmol/l, n 19; nutrient-restricted 82.2 (SE 15.7) nmol/l, n 28) or 140 d of gestation (controls 26.4 (SE 10.4) nmol/l, n 19; nutrient-restricted 27.7 (SE 5.1) nmol/l, n 28). At 77 d of gestation, however, both plasma triiodothyronine and thyroxine concentrations were significantly ($P < 0.01$) reduced in the nutrient-restricted group compared with the controls (Fig. 1). Following feeding to maintenance for the remainder of pregnancy, however, plasma thyroid hormone concentrations in the nutrient-restricted group were found not to differ significantly from those of control animals.

Effects on placental morphology

We have demonstrated that feeding ewes only 50–60% of their maintenance energy requirements between 30 and 80 d of gestation results in a decrease in placental weight at 80 d of gestation, compared with adequately-fed controls (Clarke *et al.* 1998). By separating each placenta into the maternal caruncular and fetal cotyledonary tissue, we found that this difference is due to a significant ($P < 0.05$) reduction in the weight of the fetal component of the placenta, as the weight of maternal tissue was similar between groups (Fig. 2). Placenta number was found to be significantly higher ($P < 0.01$) in the nutrient-restricted group compared with controls (controls 60 (SE 13), n 5; nutrient-restricted 76 (SE 4), n 5), although the control group were found to have a greater proportion of large (i.e. > 10 g) placentas, and smaller proportion of placentas weighing < 8 g (Clarke *et al.* 1998). There was no effect of maternal nutrition on placenta type, with the majority in each group being the inverted A type (controls 85.6 (SE 4.5) %, n 5; nutrient-restricted 81.0 (SE 5.5) %, n 5).

A similar level of maternal nutrient restriction (i.e. 50%) in early to mid-pregnancy, followed by refeeding to maintenance for the remainder of pregnancy, results in an increase in total placental weight at 145 d of gestation compared with the controls (Heasman *et al.* 1998b). This is again due to a difference in the weight of the fetal component of the

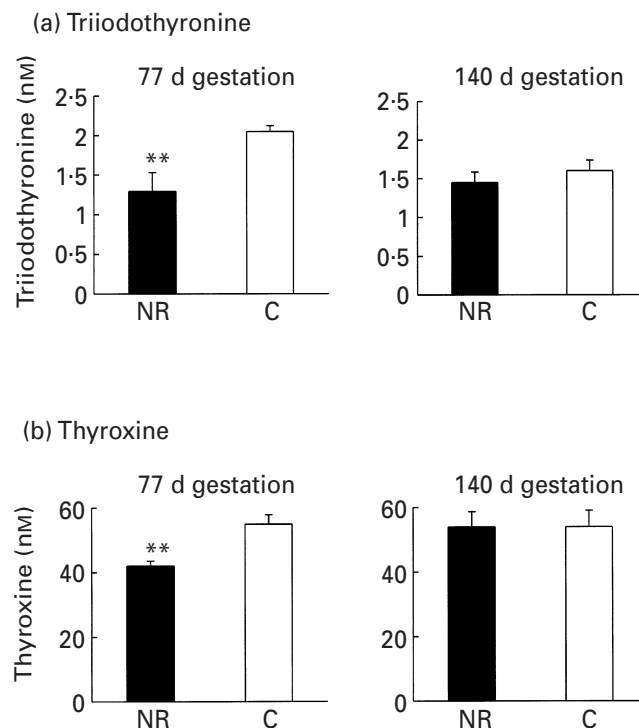
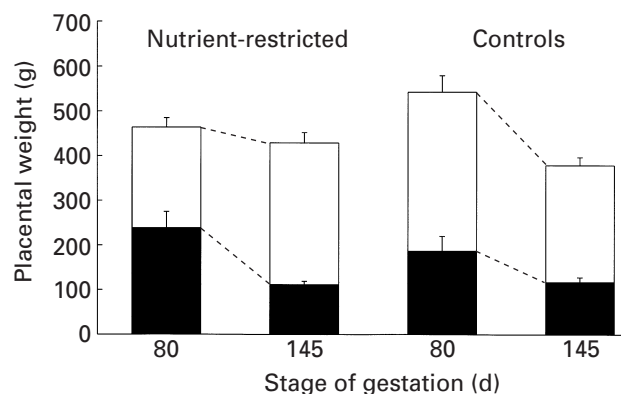


Fig. 1. Maternal plasma thyroid hormone concentrations ((a) triiodothyronine and (b) thyroxine) in nutrient-restricted (NR; ■; n 28) and control (C; □; n 19) ewes at 77 and 140 d of gestation. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those of the controls: ** $P < 0.01$. For details of procedures, see pp. 284–285.



Placental weight (g) at 80 and 145 d of gestation for nutrient-restricted (n 5 at 80 d, n 28 at 145 d) and control (n 5 at 80 d, n 19 at 145 d) ewes. Total placental weight is divided into maternal (■) and fetal (□) components. Values are means with their standard errors represented by vertical bars. For details of procedures, see pp. 284–286. (Adapted from Clarke *et al.* 1998 and Heasman *et al.* 1998b).

placenta, which is approximately 20% heavier in the nutrient-restricted group compared with the controls (Fig. 2). At 145 d of gestation, placentome number was again significantly ($P < 0.05$) higher in the nutrient-restricted group (controls 75 (SE 2), n 19; nutrient-restricted 83 (SE 2), n 28), but the distribution of placentome weights was seen to be similar between groups. Placentas from the nutrient-restricted group were found to have fewer inverted A type placentomes (controls 52 (SE 5), n 19; nutrient-restricted 40 (SE 5), n 28; NS), but more of the everted B (controls 7 (SE 2), n 19; nutrient-restricted 13 (SE 2), n 28; $P < 0.05$), C (controls 6 (SE 2), n 19; nutrient-restricted 9 (SE 1), n 28; NS) and D types (controls 9 (SE 3), n 19; nutrient-restricted 20 (SE 5), n 28 $P = 0.09$) compared with the controls.

Lamb weight and conformation

At 80 d of gestation, maternal nutrient restriction in early to mid-gestation had no significant effect on fetal weight or linear dimensions, including crown-rump length, thoracic circumference or height. All major organs were also found to be similar in weight between groups, with the exception of the brain, which was 2 g (i.e. 23%) lighter in the fetuses of the nutrient-restricted ewes (Fig. 3).

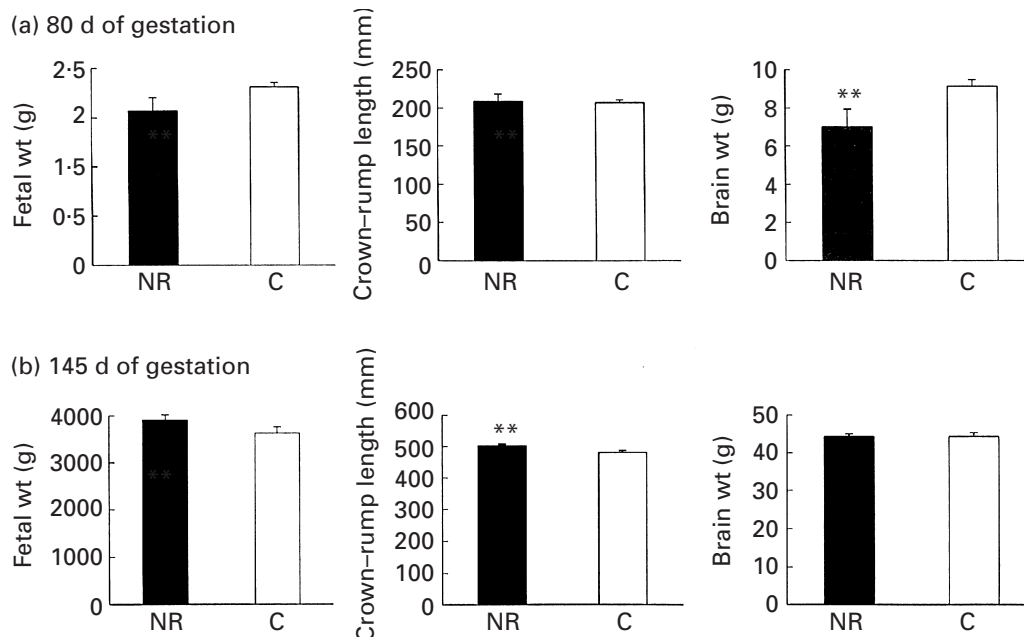
Close to term, fetal weight at Caesarean section delivery was similar between groups, but lambs delivered from ewes subjected to nutrient restriction in early to mid-gestation had significantly ($P < 0.01$) longer crown-rump lengths compared with controls. Again, the level of maternal nutrition in early to mid-gestation had no significant effect on the weight of major organs, including the brain (Fig. 3). This

finding suggests that the reduction in brain weight observed in the nutrient-restricted group at 80 d of gestation can be normalized following adequate nutrition for the second half of pregnancy, although the effects of this normalization on subsequent cerebral function remain to be determined.

IGF-1 is known to be a major determinant of fetal growth, and in particular is thought to regulate the growth of the long bones (Lok *et al.* 1996). The concentration of IGF-1 in cord blood taken at Caesarean section close to term was not found to differ significantly between groups (controls 96 (SE 10.6) $\mu\text{g/l}$, n 19; nutrient-restricted 103 (SE 7.1) $\mu\text{g/l}$, n 28), but a significant positive correlation was found to exist between all body dimensions and cord IGF-1 concentration in the control group (r^2 0.58–0.87, $P < 0.001$), a relationship not present in lambs delivered from nutrient-restricted ewes (r^2 0.02–0.25; Heasman *et al.* 1998c). We suggest this is evidence that the fetal IGF-1 growth axis is sensitive *in utero* to alterations in the maternal metabolic environment, and hypothesize that an alternative endocrine factor may regulate the growth of fetuses delivered from nutrient-restricted ewes.

Future perspectives

The level of nutrition in early to mid-pregnancy clearly influences placental development in sheep, and also has effects on fetal conformation, although fetal weight is not affected. These findings may be due directly to nutritionally-mediated alterations in maternal endocrine status, such as the reduced levels of circulating thyroid hormones observed following nutrient restriction, or indirectly by a reduction in fetal substrate supply, possibly in conjunction



Body weight, crown-rump length and brain weight at (a) 80 d of gestation and (b) 145 d of gestation of fetuses delivered from nutrient-restricted (NR; ■; n 5 at 80 d, n 28 at 145 d) and control (C; □; n 5 at 80 d, n 19 at 145 d) ewes. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those of the controls: ** $P < 0.01$. For details of procedures see pp 284–286 (Adapted from Clarke *et al.* 1998 and

with altered fetal and/or placental hormone production. Attention is now being focused on the consequences of altered placental development on both short- and long-term outcome measures. A preliminary study has found that lambs delivered by Caesarean section from ewes subjected to nutrient restriction in early to mid-pregnancy are unable to thrive when delivered into a cool ambient temperature, but that treatment with an umbilical vein injection of thyrotrophin-releasing hormone before cord clamping greatly improves survival rates (Symonds *et al.* 1998). A longer-term follow-up study by Kelly *et al.* (1996) has also shown that poor nutrition during fetal life has the potential to reduce both the quantity and quality of wool in young Merino sheep.

The concept of fetal programming is now well established in the fields of medical and biomedical research, and the role of maternal nutrition during pregnancy as a mediator of such programming is becoming increasingly recognized. Advanced molecular biology techniques have been able to show differences in gene transcription (Ozanne *et al.* 1997) and enzyme activity (Langley-Evans *et al.* 1996) in specific tissues between fetuses from mothers fed differentially during pregnancy, but the role of the placenta in these adaptations is poorly understood. Furthermore, the mechanisms whereby alterations in the metabolic environment of the fetus are able to translate into adult disease syndromes remain to be determined. It is clear, however, that only by gaining an in-depth knowledge of the interactions between maternal body reserves, nutrition during pregnancy and placental function, can important issues for both animal welfare and human disease be addressed.

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