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 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
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 (Naege, 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 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 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 (Millor & 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 at 74 d of gestation (Clarke et al. 1998). For

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

Table 1.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Maternal wt (kg)</th>
<th>Nutrition</th>
<th>Placental wt (g)</th>
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<tr>
<td></td>
<td>Outcome measured (d of pregnancy)</td>
<td>C</td>
<td>R</td>
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<td>C</td>
<td>R</td>
<td>C</td>
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<tr>
<td>Robinson et al. (1994)</td>
<td>30–90</td>
<td>90</td>
<td>63</td>
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<tr>
<td>Robinson et al. (1994)</td>
<td>30–90</td>
<td>90</td>
<td>51</td>
</tr>
<tr>
<td>McCrabb et al. (1991)</td>
<td>30–96</td>
<td>96</td>
<td>40</td>
</tr>
<tr>
<td>McCrabb et al. (1992a)</td>
<td>30–96</td>
<td>96</td>
<td>45</td>
</tr>
<tr>
<td>McCrabb et al. (1992b)</td>
<td>50–96</td>
<td>96</td>
<td>45</td>
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<tr>
<td>McCrabb et al. (1992b)</td>
<td>75–96</td>
<td>96</td>
<td>45</td>
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<tr>
<td>McKibbin et al. (1992)</td>
<td>79–107</td>
<td>107</td>
<td>–</td>
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<tr>
<td>McCrabb et al. (1992b)</td>
<td>30–96</td>
<td>140</td>
<td>–</td>
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<tr>
<td>McCrabb et al. (1991)</td>
<td>30–96</td>
<td>140</td>
<td>53</td>
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<tr>
<td>Davis et al. (1981)</td>
<td>40–85</td>
<td>137</td>
<td>49</td>
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<tr>
<td>Falcinelli &amp; White (1987)</td>
<td>50–100</td>
<td>135</td>
<td>44</td>
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<td>Holst et al. (1992)</td>
<td>50–100</td>
<td>135</td>
<td>44</td>
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G, control; R, restricted

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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 placentomes being classified as A and everted placentomes as B, C or D depending on the extent of eversion. Placentomes 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. 1996b). 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 2·0) nmol/l, n 19; nutrient-restricted 82·2 (SE 1·5) nmol/l, n 28) or 140 d of gestation (controls 26·4 (SE 1·0) 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 placentome 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 1·3), n 5; nutrient-restricted 76 (SE 4·5), n 5), although the control group were found to have a greater proportion of large (i.e. >10 g) placentomes, and smaller proportion of placentomes weighing <8 g (Clarke et al. 1998). There was no effect of maternal nutrition on placentome 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 5·0) %, n 5).

A similar level of maternal nutrient restriction (i.e. 50 %) in early to mid-pregnancy, followed by refedding 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 placenta. Therefore, we have focused our attention on the fetal component of the placenta in the present study.
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 6 (SE 1), $n$ 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) µg/l, $n$ 19; nutrient-restricted 103 (SE 7·1) µg/l, $n$ 28), but a significant positive correlation was observed following adequate nutrition for the second half of pregnancy, although the effects of this normalization on subsequent cerebral function remain to be determined.

In our study, we found a significant positive correlation between IGF-1 concentration in the control group ($r^2$ = 0·58–0·87, $P < 0·001$) 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 with...
with altered fetal and/or placental hormone production. Attention is now being focused on the consequences of altered placentation development on both short- and long-term outcome measures. A preliminary study has found that lambs delivered by Caeasarean 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 improved 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 inter-actions 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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References


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