Nutrient partitioning during pregnancy: adverse gestational outcome in overnourished adolescent dams

Jacqueline M. Wallace
Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

Appropriate nutrient partitioning between the maternal body and gravid uterus is essential for optimum fetal growth and neonatal survival, and in adult sheep nutrient partitioning during pregnancy generally favours the conceptus at the expense of the dam. However, recent studies using an overnourished adolescent sheep model demonstrate that the hierarchy of nutrient partitioning during pregnancy can be dramatically altered in young growing females. Overnourishing the adolescent dams to promote rapid maternal growth throughout pregnancy results in a major restriction in placental mass and leads to a significant decrease in birth weight relative to moderately-fed adolescents of equivalent gynaecological age. High maternal feed intakes are also associated with an increased incidence of non-infectious spontaneous abortion, a reduction in gestation length and colostrum production, and a higher incidence of neonatal mortality. The present paper examines the putative role of a variety of endocrine regulators of nutrient partitioning in this unusual model system, where the dam is overnourished while the stunted placenta restricts nutrient supply to the fetus. The central role of nutritionally-mediated alterations in placental growth and development in setting the subsequent pattern of nutrient partitioning between the maternal body, placenta and fetus is examined, and critical periods of sensitivity to alterations in maternal nutritional status are defined. Finally, the consequences of this form of inappropriate nutrient partitioning on the growth and development of the fetus and neonate are described with particular emphasis on the reproductive axis.


Pregnancy represents the most anabolic period of the female life cycle, and appropriate nutrient partitioning between the maternal body and gravid uterus throughout gestation is essential for optimum fetal growth and neonatal survival. While low birth weight has long been associated with impaired postnatal performance in domestic livestock species (Bell, 1984, 1992), it is the recent human epidemiological studies of Professor Barker and colleagues (see Barker et al. 1990; Barker, 1995) which have rekindled a major interest in the nutritional programming of placental and fetal growth. These latter studies reveal that low birth weight or disproportionate fetal growth in relation to placental mass can have far-reaching detrimental consequences on disease aetiology which persist into adult life and even the next generation. Nutrient partitioning during pregnancy depends on a series of evolving maternal adaptations which redirect O₂ and nutrients to the gravid uterus to ensure adequate placental growth and function, and facilitate placental delivery of these nutrients to the growing fetus (Owens, 1991; Bell, 1993). Undoubtedly, maternal nutritional status is one of the major extrinsic factors programming nutrient partitioning and ultimately growth, development and function of the major fetal organ systems. Indeed, recent reviews have highlighted that the prenatal growth trajectory is sensitive to the direct and indirect effects of maternal dietary intake from the earliest stages of embryonic life when the absolute nutrient requirements for fetal growth are negligible (Robinson et al. 1999; Wallace et al. 1999a). Although in sheep severe maternal under-nutrition at all stages of pregnancy and particularly during late pregnancy largely reduces fetal growth by varying degrees (Mellor, 1983; Robinson, 1983; Vincent et al. 1985; Parr et al. 1986), the historical notion remains that during pregnancy nutrient partitioning favours the conceptus at the expense of the dam (Barcroft, 1946). Thus, in studies where moderate to severe maternal nutrient restriction were imposed between early to mid gestation (Heasman et al. 1998) and mid to late gestation respectively (Oddy & Holst, 1990; Oddy et al. 1999; Wallace et al. 1999a), it is likely that maternal under-nutrition could have an additional detrimental effect on fetal growth.

Abbreviations: GH, growth hormone; H, high intake of a complete diet to promote rapid maternal growth; IGF-I, insulin-like growth factor-I; M, moderate intake of a complete diet to promote normal maternal growth.
1991), lamb birth weight at term was unaffected. However, recent studies using an overnourished adolescent sheep model are challenging this concept, and demonstrate that the hierarchy of nutrient partitioning can be dramatically altered in young growing females. The present short review will detail these new studies with reference to human adolescent pregnancy where appropriate.

**Human adolescent pregnancy**

The UK has the highest adolescent pregnancy rate in Europe, and in the developed world the rate is second only to the USA (Scottish Needs Assessment Programme, 1994). Human adolescent mothers have an increased risk of delivering low birth weight and premature infants who exhibit high mortality rates within the first year of life (McAnarney, 1987). The underlying causes of adverse pregnancy outcome are poorly understood, but have been variously attributed to social deprivation, biological immaturity or the growth and nutritional status of the mother at the time of conception (Fraser et al., 1995). Within adolescents the risks of spontaneous miscarriage and very premature births are age-related and significantly increase ($P < 0.05$) in the 13–15-year category (Scottish Needs Assessment Programme, 1994; Olausson et al. 1999). Moreover, birth weight is modestly but significantly reduced in both primiparous ($P < 0.05$) and multiparous ($P < 0.01$) adolescents who are still growing at the time of conception compared with non-growing adolescents from equivalent socio-economic backgrounds (Scholl & Hediger, 1993). Currently in the UK one in 500 babies are born to girls who were less than 16 years of age at the time of conception, and hence in the still potentially-growing category. It was against this background that we initially developed the sheep model to investigate nutrient partitioning and the underlying causes of adverse gestational outcome during adolescent pregnancy.

**Key features of the ovine adolescent pregnancy model**

The experimental model involves using embryo recovery and transfer techniques to establish singleton pregnancies in

![Fig. 1.](https://www.cambridge.org/core/fig/...)

*Fig. 1.* (a) Total fetal placental mass at term, (b) relationship between placental mass and lamb birth weight (from Wallace et al. 1999a), (c) fetal cotyledon no. and (d) mean fetal cotyledon weight in adolescent dams overnourished ($\circ$; $n$ 37; $\sigma$) or moderately-fed (■; $n$ 34; ●) throughout their entire pregnancy. Values for a, c and d are means with their standard errors represented by vertical bars. Mean values were significantly different from those for moderately-fed dams: $***P < 0.001$. (Data from Wallace et al. 1999a.)
Maternal reserves, lactation and the young

peripubertal adolescent sheep. This procedure removes the potentially confounding influence of partial embryo loss and variation in fetal number, and by using a single sire and a small number of adult donors maximizes the homogeneity of the resulting fetuses. The adolescents are of equivalent age, live weight and body condition score at the time of embryo transfer. Following embryo transfer (day 4 after ovulation) the recipient dams are offered a high (H) or moderate (M) quantity of a complete diet to promote rapid or normal maternal growth respectively. The diet contains /kg 10·2 MJ metabolizable energy and 137 g crude protein (N×6-25), and typically the two dietary manipulations result in a live-weight gain of 200–350 g/d compared with 50–75 g/d during the first 100 d of the 145 d gestation. Thereafter, the feed intake of the normally-growing group is adjusted weekly to maintain body condition score and to meet the increasing nutrient demands of the gravid uterus. Maternal live weight and body condition score significantly diverge by day 35 of gestation.

As highlighted in Fig. 1, overnourishing adolescent dams to promote rapid maternal growth throughout their entire pregnancy results in a major restriction in total placental mass (36 %), which leads to a highly significant decrease (P<0·001) in birth weight (33 %) relative to that for normally-growing adolescents (Wallace et al. 1996, 1997b, 1999a,b). Total placental mass and lamb birth weight are highly correlated in these adolescent animals (P<0·001), and placental:fetal weight is not altered by nutritional treatment. For ewes delivering live young, high maternal dietary intakes are also associated with a reduction in gestation length (142±4 (se 0·42) and 145±1 (se 0·50) d for H v. M dams respectively, P<0·001) and, in spite of intensive care procedures, with a higher incidence of neonatal mortality within the first week of life (15 % v. 3 %; P<0·05). In this paradigm, overfeeding is also associated with an increased incidence of non-infectious spontaneous abortion and stillbirth in late gestation (18·7 % v. 4·6 %, P<0·05). Low or absent secretion of pregnancy-specific protein B by the binucleate cells of the placenta implies that this latter phenomenon is preceded by severe placental insufficiency during mid gestation (Wallace et al. 1997a). As the adolescent dams are of equivalent age, these results suggest that their nutritional status rather than gynaecological immaturity predisposes them to poor pregnancy outcome. Indeed, the similarity between the human and ovine studies in terms of low birth weight, prematurity and neonatal mortality suggests that insufficient placental growth, and hence reduced nutrient transfer, may be central to the aetiology of adverse pregnancy outcome in both species. Placental variables have rarely been measured in human studies, but Frisano et al. (1985) have reported small reductions in placental weight associated with low birth weight in still-growing adolescent mothers.

To date the ovine paradigm has involved feeding two levels of the same complete diet to achieve predetermined growth rates. Clearly, the balance between protein and energy may play a critical role in the extent of placental and fetal growth restriction observed in these pregnancies. Although studies assessing nutrient intake in human adolescents are relatively poorly controlled, the delivery of low-birth-weight infants has variably been associated with the consumption of high-sugar diets (Lenders et al. 1997) and with protein supplementation during late gestation (for review, see Rush, 1986).

Putative hormonal regulators of nutrient partitioning in the growing adolescent sheep

Glucose, O₂ and amino acids are the major substrates for growth and energy production by the gravid uterus, and the partitioning of these substrates between the maternal, placental and fetal compartments involves two types of regulation, i.e. homeostasis and homeorhetic (Bauman & Currie, 1980). These latter authors defined homeorhesis as the ‘orchestrated or coordinated changes in metabolism of body tissues necessary to support a (dominant) physiological state’. Clearly, in the overnourished and rapidly-growing adolescent, pregnancy is not, at least initially, the dominant physiological state, and results in a conflict between the maintenance and growth of maternal body tissues and the evolving nutrient requirements of the gravid uterus. A number of endocrine hormones of maternal, placental and fetal origin are postulated to play a role in both homeostatic and homeorhetic control mechanisms (for review, see Bell & Bauman, 1997; Bauer et al. 1998), and the adolescent sheep provides an unusual model system in that the dam is overnourished, while the stunted placenta restricts nutrient supply to the fetus. Maternal insulin, somatotrophic and thyroid hormones do not cross the placenta in physiologically-significant quantities (Brown & Thorburn, 1989), but may coordinate nutrient partitioning via secondary changes in maternal or placental metabolism, utero–placental blood flow or placental growth and transport functions. Similarly, placental-derived steroid and protein hormones have been implicated in the regulation of maternal and fetal amino acid, carbohydrate and lipid metabolism (Anthony et al. 1995).

Insulin and insulin-like growth factor-I (IGF-I) are highly sensitive to nutrient intake; thus, it is not surprising that in the overnourished (H) adolescent sheep maternal concentrations of these hormones are high from early in gestation (Wallace et al. 1997b). Assessment of body composition in H v. M ewes at day 104 of gestation reveals that the increase in weight in both the carcass and non-carcass components is due to a 2–3-fold increase in fat content, with a much less dramatic effect on body protein (Wallace et al. 1999b). Insulin, acting via its receptor on the adipocyte, is the major stimulator of lipogenesis in the subcutaneous and omental fat of pregnant sheep (Vernon et al. 1981; Guesnet et al. 1991), and the elevated insulin levels in the H dams are commensurate with this role. IGF-I is not an acute regulator of lipolysis in sheep (Houseknecht et al. 1996), but together with insulin can alter the protein economy of the growing sheep in favour of protein deposition (Grizard et al. 1995). Thus, as the adolescent sheep used in these studies have attained only 60 % of their adult weight at the time of embryo transfer, it is probable that the high maternal concentrations of insulin and IGF-I provide a sustained anabolic stimulus to maternal tissue deposition and a shift in the hierarchy of nutrient supply away from placental growth.
In adult animals, studies investigating the partitioning of glucose during pregnancy demonstrate that there is a reduction in the amount of glucose available to maternal tissues as pregnancy progresses over a range of maternal intakes (Hough et al. 1985; Oddly et al. 1985). Furthermore, in both well-fed polytocous and undernourished monotorous ewes, significant fat mobilization occurs during late gestation in an attempt to meet the increasing nutrient demands for fetal growth (Robinson et al. 1978). In adult sheep the decrease in maternal insulin concentrations during late gestation is thought to mediate this switch in adipose tissue metabolism in favour of lipid mobilization (Guesnet et al. 1991). Furthermore, placental lactogen has been proposed as the causative agent, as concentrations are inversely related to insulin levels at this time (Vernon et al. 1985; Oddy et al. 1985; Oddy et al. 1979), progesterone administration failed either to increase maternal or fetal insulin concentrations (Oliver et al. 1996), while indomethacin reduced in heifers receiving a high plane of nutrition (Johnsson & Obst, 1984), increased maternal plasma insulin concentrations, as measured during mid and late gestation, are reduced compared with those of M dams and inversely related to maternal insulin concentrations, as measured during mid and late gestation, are reduced compared with those of M dams and inversely related to maternal insulin concentrations (Wallace et al. 1981). While definitive evidence for a role of placental lactogen in nutrient partitioning during pregnancy is lacking (see Bell & Bauman, 1994), decreased secretion of this hormone by the growth-restricted placenta of overnourished adolescent dams may underlie the continued rise in maternal insulin concentrations observed during the final third of gestation. This factor in turn appears to result in increased glucose utilization by the maternal tissues and continued lipid accumulation during the final third of gestation.

Growth hormone (GH) may also play a role as a hormonal regulator of lipid mobilization during late pregnancy in that GH inhibits the lipogenic effect of insulin in sheep adipose tissue in vitro (Vernon & Finley, 1986). In the H adolescent dam, both GH pulse frequency and mean concentrations, as measured during mid and late gestation, are reduced compared with those of M dams and inversely related to maternal insulin concentrations (Wallace et al. 1997b). While on the face of it this finding supports a role for maternal GH in stimulating lipid mobilization in the M dams, these relationships are merely correlative and require to be supported by in vivo hormone-supplementation studies. In this respect the recent studies of Jenkinson et al. (1999) are of interest in that twice daily administration of recombinant GH to ewes between days 98 and 111 of gestation increased circulating insulin concentrations and stimulated fetal growth.

It is axiomatic that the restriction in placental mass in overnourished adolescent animals is associated with a reduction in placental hormone secretion. Indeed, we have demonstrated that maternal peripheral concentrations of progesterone and of the pregnancy-specific protein B secreted by the binucleate cells of the placenta were low throughout gestation in H dams, particularly during the second half of gestation (Wallace et al. 1997a). However, in spite of evidence that progesterone may enhance the number of insulin receptors and hence influence lipid deposition in the rat (Flint et al. 1979), progesterone administration failed to influence glucose metabolism in ovariectomized sheep (Samad & Ford, 1981).

It is highly unlikely that a single hormone will regulate the diverse metabolic adaptations required during pregnancy to ensure appropriate fetal growth. However, the recent discovery that leptin is produced by the placenta in a variety of species, including sheep, and that leptin and its receptor are present in a variety of murine fetal tissues (Hoggard et al. 1997) ensures that this hormone will be added to the list of potential nutrient-partitioning agents worthy of further study. Intriguingly, in human subjects placental leptin expression is enhanced in pregnancies of diabetic subjects and reduced in pregnancies in which there is growth restriction (Lea et al. 1998), while in sheep maternal undernutrition during the period of rapid placental growth is associated with reduced abundance of leptin within both the fetal and maternal components of the growth-restricted placenta (Wilson et al. 2000). Moreover, a reduction in fetal and/or placental leptin secretion in late gestation may serve to signal depleted energy stores or reduced nutrient availability, and hence stimulate the hypothalamic–pituitary–adrenal axis to trigger parturition (McMillen et al. 1995). This putative role for fetal and/or placental leptin is attractive in light of the observation that gestation length is reduced in overnourished adolescent dams in which the pregnancy is growth restricted.

In the overnourished adolescent, placently-mediated fetal growth restriction, as assessed during late gestation (day 128), was characterized by attenuated fetal insulin, IGF-I and glucose concentrations, while fetal urea levels were high (Wallace et al. 2000). Similarly, restriction of placental growth by carunclectomy reduces O2 and glucose supply to the fetus and is associated with a reduced abundance of anabolic factors in the fetal circulation (Owens et al. 1994). Recent reviews have highlighted the importance of the fetal somatotropic axis in the regulation of the interplay between placental and fetal nutrient uptake and utilization (Bauer et al. 1998; Oliver et al. 1999). While the precise role of the fetal somatotropic axis in the regulation of fetal growth is still controversial, acute fetal IGF-I infusions to late-gestation sheep promote an anabolic state (Harding et al. 1994), while longer-term infusions increase the weight of some of the major fetal organs but not fetal weight per se (Lok et al. 1996). In addition, the reduction in fetal IGF-I concentrations induced following maternal starvation can be quickly reversed by fetal glucose or fetal insulin infusion (Oliver et al. 1996). In the adolescent sheep model the strong positive correlations between both fetal IGF-I and insulin concentrations and fetal weight on the one hand, and between placental weight and fetal glucose, IGF-I and insulin concentrations on the other, support the hypothesis that the fetal insulin–IGF-I axis mediates the effects of decreased placental nutrient transfer, and as such plays an important role in fetal growth during late gestation.

In singleton-bearing adult ewes mammary gland weight increases 3-fold during the last third of gestation, in preparation for lactation at a time when the nutrient demands of the fetus are maximal (Rattray et al. 1974). It is perhaps not surprising, therefore, that in the overnourished adolescent sheep alterations in the hierarchy of nutrient partitioning during pregnancy are also evident at the level of the developing mammary gland, and that overfeeding throughout gestation is associated with a major reduction in colostrum yield at parturition (128 (SE 19·5) and 375 (SE 35·1) g in H v. M dams respectively, P < 0·001; Wallace et al. 1999a). Furthermore, lifetime milk production is reduced in heifers receiving a high plane of nutrition throughout the pubertal period (Johnsson & Obst, 1984), and this effect has been attributed to a decrease in the rate of
allometric growth of the mammary parenchyma (Johnsson & Hart, 1985; Umberger et al. 1985). Some of the hormones known to influence maternal–fetal nutrient partitioning also play a crucial role in the development, differentiation and function of the mammary gland. Many of these hormones, in particular steroids and protein hormones of the prolactin and growth hormone family, are secreted by the placenta in increasing amounts as pregnancy progresses, and are positively correlated with placental and maternal gland weight (Mellor, 1987). In overnourished adolescent dams the reduction in placental mass and hence lower capacity to secrete placental lactogen and progesterone, together with the nutritionally-mediated reduction in maternal GH secretion, may underlie the observed impairment in colostrum production (Wallace et al. 1997b). It is unknown whether still-growing well-nourished human adolescents experience similar competition for nutrients at the level of the mammary gland.

**Nutrient-partitioning trajectory: the role of placental growth**

The central role of placental size, metabolism and nutrient transfer capacity in the determination of birth weight has been extensively reviewed (Mellor, 1983; Bell, 1984; Schneider, 1996; Bell et al. 1999). Rapid proliferative growth of the placenta occurs primarily between days 40 and 80 of gestation (Ehrhard & Bell, 1995), and during this time maternal nutritional status has a major impact on its eventual size and transport capacity. In adult sheep the magnitude of the variable placental response to modifying maternal nutrition appears to depend on the size, body condition and age of the dam, as well as the timing of the nutritional treatment (for review, see Kelly, 1992; Robinson et al. 1999; Wallace et al. 1999a). In the highly-controlled adolescent sheep model size, body condition score and age are equivalent before the application of the nutritional treatments, and the major decrease in placental mass at term in dams overnourished throughout pregnancy reflects a significant reduction in both the number of fetal cotyledons per placenta and mean fetal cotyledon weight ($P < 0.001$; Fig. 1). As these studies the first to consistently demonstrate that maternal dietary intake can influence the number of maternal caruncules utilized by the developing trophoblast, we can now begin to investigate the putative mediators of this early pregnancy event.

Exogenous progesterone supplementation during the periovulatory period increased ovine fetal growth at day 74 of gestation by 11 % (Kleemann et al. 1994). While placental data were not reported in the latter study, progesterone administration has been shown to influence blastocyst differentiation in favour of the trophectoderm cells and stimulate early trophoblast elongation compared with control animals (Hartwich et al. 1995). In both adult and adolescent sheep maternal dietary intakes during early pregnancy are inversely related to peripheral progesterone concentrations (Wallace, 1994, 1997a) and, thus, it seemed probable that suboptimal progesterone in overnourished adolescent dams could compromise growth of the differentiating conceptus, resulting in fewer uterine caruncules being occupied. Indeed, when H dams had their peripheral progesterone concentrations restored to M levels by daily administration of progesterone during the first third of pregnancy (days 5–55), lamb birth weight was intermediate between the H and M groups (5164 (SE 151), 2893 (SE 381) and 4150 (SE 389) g for M, H and H plus progesterone groups respectively; Wallace et al. 1998). However, this increase in birth weight was not mediated by significant alterations in total placental mass, which was equivalent in H and H plus progesterone groups (294 (SE 57.2) and 318 (SE 41.5) g respectively) and significantly lower than that in the control group (498 (SE 18.9) g). While these results do not preclude the possibility of more subtle effects of progesterone on utero–placental blood flow, placental morphology or nutrient transport capacity, they do suggest a direct influence of progesterone on the embryonic inner cell mass. This hypothesis is confirmed by the results of a subsequent study in which initiation of progesterone supplementation in H ewes was delayed until day 11 of gestation, by which time blastocyst differentiation is largely complete, and it was observed that there was no longer any effect of progesterone on birth weight. Mean birth weights for the M, H and H plus progesterone groups were 4966 (SE 215), 3331 (SE 363) and 3247 (SE 476) g respectively (JM Wallace, DA Bourke and RP Aitken, unpublished results).

The IGF system may play a central role in the regulation of the placental growth trajectory. Various components of the IGF system have been localized in the uterus and placenta of a variety of species, and have been shown to exhibit spatial and temporal patterns of expression (Wathes et al. 1998). Type 1 receptors for IGF are present in the ovine placenta throughout gestation (Lacroix et al. 1995; Reynolds et al. 1997), and could be a target for locally-produced or systemic IGF from the maternal and fetal circulations. As the synthesis of many components of the IGF system are regulated by nutrition (Thissen et al. 1994), these growth factors, their receptors and binding proteins may serve to match the proliferative growth and/or metabolic activity of the placenta with current nutritional status. In the overnourished adolescent dam maternal IGF-I concentrations are high while placental growth is restricted. The pattern of expression of the components of the IGF system during early placental growth in these animals has not been examined yet, but intriguingly at the end of the second third of pregnancy, when placental growth is complete, IGF-binding protein 1 mRNA expression was higher and IGF-binding protein 3 mRNA expression lower in the endometrial glands of H dams compared with M dams. In addition IGF-I receptor expression in the lumen epithelium was reduced in the H dams (Reynolds et al. 2000). These changes, particularly with respect to the binding proteins, are characteristic of severe undernutrition and may reflect reduced nutrient availability at the utero–placental level.

Blood flow to the utero–placenta increases 3-fold between mid gestation and term (Molina et al. 1990), and during the final third of pregnancy, when the absolute nutrient requirements of the placenta and fetus are maximal, and both uterine and umbilical blood flows are critical regulators of nutrient partitioning between the maternal, placental and fetal compartments (Carter & Myatt, 1995). Indeed, experimental restriction of placental growth...
involving premating carunclectomy and heat stress are both associated with markedly reduced rates of uterine and umbilical blood flow, and with limited placental transfer of O₂ and glucose (for review, see Bell et al. 1999). It is axiomatic that factors which influence placental vascular development and function during early pregnancy will set the trajectory for these later adaptive haemodynamic changes, and hence have a major impact on fetal growth. A complex range of angiogenic growth factors are emerging as putative regulators of this process (Giudice, 1994; Reynolds & Redmer, 1995; Torry & Torry, 1997). Clearly, the impact of maternal nutritional status during early pregnancy and the associated nutritionally-mediated endocrine perturbations on the expression of these angiogenic growth factors in relation to placental growth and morphogenesis requires to be examined.

Nutrient-partitioning trajectory: reversibility of effects

In adult sheep placental and fetal growth responses to alterations in maternal nutrition are often highly variable and inconsistent, even when performed by the same group of researchers using a single genotype (Kelly, 1992). In contrast, in the highly-controlled adolescent paradigm over-nourishing the dam results in a consistent reduction in placental weight at term (40, 32:5 and 41 % decrease relative to the M group in consecutive studies; Wallace et al. 1996, 1997b, 1998). The highly-repeatable nature of this effect allows us to examine when placental growth is most sensitive to maternal nutritional status, and whether the effects on placental growth and function and hence pregnancy outcome can be reversed. The number of uterine caruncles occupied by the developing trophoblast is generally considered to be fixed by day 50 of gestation (Barcroft & Kennedy, 1939), whereas the proliferative growth of the placenta continues until the end of the second third of pregnancy. As indicated previously, the restriction in placental growth in the overnourished adolescent dam reflects a reduction in both cotyledon number and size. An initial study examined whether the placental growth trajectory could be altered by switching adolescent dams from an anabolic to a catabolic state at the end of the first third of pregnancy (day 50) and vice versa (Wallace et al. 1999b). Thus, ewes were offered the H or M diet to promote rapid or normal maternal growth as described earlier, and at day 50 of gestation half the ewes had their dietary intakes switched to yield HH, MM, HM and MH treatments. At term a high plane of nutrition from the end of the first third of gestation (HH and MH groups) compared with moderate levels (MM and HM groups) was associated with highly significant decreases in gestation length (P < 0:009), total placental weight (P < 0:001), total fetal cotyledon weight (P < 0:001) and mean fetal cotyledon weight per placenta (P < 0:001). Fetal cotyledon number was dependent on maternal dietary intake during the first trimester only and was significantly lower (P < 0:007) in HH and HM dams compared with MM and MH dams. The inhibition of placental growth during mid pregnancy (HH and MH groups) was associated with a major decrease (P < 0:001) in lamb birth weight at term relative to the MM and HM groups. Thus, in this nutritionally-sensitive paradigm, reducing maternal dietary intake from H to M at the end of the first trimester stimulates placental growth and enhances pregnancy outcome, and increasing maternal dietary intake at this time point has a deleterious effect on placental development and fetal growth.

Analysis of DNA content and protein : DNA of placentas harvested at both days 100 and 128 of gestation confirm that the reduction in placenta mass in H dams reflects a lower cell number rather than a change in placental cell size (Wallace et al. 2000). Dissection of these placentas into their component parts reveals that high nutrient intakes during mid gestation and thereafter predominantly inhibits growth of the fetal component of the placenta. Furthermore, using the classification system of Vatnick et al. (1991), the cotyledons from these H dams are largely inverted A type with maternal tissue completely surrounding fetal tissue. In contrast, the majority of cotyledons in the M dams are everted C and D type with fetal tissue growing over the maternal tissue. A similar predominance of everted cotyledons in adult ewes moderately (0:5 × maintenance; Heasman et al. 1998) or mildly (0:85 × maintenance; Steyn et al. 2000) nutrient restricted during early to mid gestation has been reported, and is thought to reflect an adaptive compensatory response by the placenta to mild maternal undernutrition during the period of rapid proliferative placental growth in order to optimize transplacental exchange efficiency. However, it is important to note that the prefeeding maternal plasma concentrations of free fatty acids and glucose in M adolescent dams are in fact characteristic of well-fed adults during mid to late gestation (Wallace et al. 1999b).

Switching maternal nutrient intake between early and mid gestation is not the only time when alterations in nutritional status can influence the placental and fetal growth trajectory. A second study investigated whether the function of a growth-restricted placenta could be altered by switching adolescent dams from an anabolic to a catabolic state, commencing at the end of the second third of gestation when the proliferative growth of the placenta had ceased but the fetus theoretically has still 75 % of its prenatal growth to complete. Ewes were on H intakes for the first 100d of gestation. Thereafter, and for the rest of pregnancy, half the ewes food offered was sharply decreased by approximately 64 % (HL group) which resulted in a decrease in maternal live weight and body condition score relative to ewes on H intakes (H group) throughout their entire pregnancy (Fig. 2). The induction of a relatively catabolic state in these previously-anabolic dams resulted in a modest increase (P < 0:05) in lamb birth weight at term, which was associated with an increase (P < 0:01) in fetal cotyledon mass (Fig. 3; JM Wallace, DA Bourke and RP Aitken, unpublished results). In contrast, fetal cotyledon number was low and similar for the two groups (78 (SE 5:6) and 77 (SE 4:6) for the H and HL groups respectively). These preliminary results imply that the structural remodelling and functional adaptation of the placenta which is known to occur during the final third of pregnancy (Schneider, 1996), and which normally results in a decrease in placental weight during this period, can be altered in favour of fetal growth following the induction of a catabolic phase in the previously-rapidly-growing adolescent. While the precise
mechanisms underlying this switch in nutrient partitioning between the maternal body and the gravid uterus are unknown, they are likely to involve one or more of the endocrine nutrient-partitioning agents outlined previously. These nutritional switch-over studies have obvious implications for agricultural practice, but more importantly they imply that in the human clinical situation, where fetal growth restriction is often accompanied by reduced placental mass (Owens et al. 1995), it may be possible to manipulate the nutrient transport function of the initially-growth-restricted placenta to the advantage of the fetus.

Consequences of inappropriate nutrient partitioning

Interfering with the growth of the placenta by over-nourishing the adolescent dam throughout pregnancy has a dramatic influence on lamb birth weight as assessed at term. However, several authors have highlighted that birth weight may be a poor summary measure of more subtle alterations in the pattern of prenatal organ growth (Mellor, 1987; Harding & Johnston, 1995; Robinson et al. 1999). Bearing this observation in mind, we have studied the consequences of a disrupted placental growth trajectory on the pattern of fetal organ growth in three separate studies corresponding to days 70, 100 and 128 of gestation. H compared with M groups were associated with a significant reduction in total placental cotyledon weight at all three developmental time points (18, 20 and 51 % at days 70, 100 and 128 respectively; Wallace et al. 2000; JM Wallace, DA Bourke and RP Aitken, unpublished results). At days 70 and 100 of gestation fetal weight, fetal conformation and individual fetal organ weights were independent of both maternal nutritional status and placental size. This finding is perhaps not surprising in view of the fact that the fetus has only reached approximately 6 and 25 % of its predicted birth weight by days 70 and 100 respectively. However, the subsequent pattern of nutrient partitioning between the maternal body, placenta and fetus may already be being set, in that a change in the subcellular localization of one of the protein kinase C enzymes (protein kinase C-α), thought to be involved in growth and differentiation, is evident in the muscle of fetuses from overnourished dams at day 100 of gestation (Palmer et al. 1998). By day 128 of gestation, when the normally-growing fetus has reached 85 % of its predicted birth weight, fetuses from H dams were 37 % smaller than those from M dams. All variables of fetal conformation and absolute fetal organ weights, with the exception of the adrenal glands, were lower in the fetuses...
from H dams and were highly correlated (minimum $P < 0.01$) with total placental cotyledon mass. However, relative fetal organ weights expressed on a g/kg fetal body weight basis were not influenced by maternal dietary intake. Furthermore, fetal weight but not maternal nutritional group was predictive of individual organ weight for all organs dissected. Together these results imply that growth restriction in the fetuses from adolescent dams overnourished throughout their entire pregnancy is largely symmetrical, and as such is in direct contrast with other forms of placentally-mediated fetal growth restriction. For example, in heat-stressed fetuses body length, brain, kidney and adrenal glands were disproportionately large while the liver, thyroid, thymus glands and biceps muscle were disproportionately small (Alexander & Williams, 1971). Similarly, early studies of fetal growth in undernourished ewes reveal that the liver and spleen are particularly sensitive to fetal nutrient restriction mediated by impaired placental growth, while the brain is relatively spared (Wallace, 1948a,b). It remains to be established whether the mid and late gestation nutritional switches detailed earlier (p. 112) can similarly alter the allometric relationship between the key fetal organs and tissues, and hence programme long-term pathological changes in the major organ systems.

While overnourishment of the adolescent dam throughout her entire pregnancy appears to result in fetuses which are proportionate miniatures, altered structure and function or immaturity of the major organs and tissues most probably underlies the high incidence of neonatal morbidity and mortality observed in the growth-restricted offspring. For example, post-mortem examination of neonates who died within 3 d of delivery has revealed that, on gross pathology, the kidneys and gut were abnormal. This finding was associated with high plasma urea concentrations and a failure to absorb colostrum and urinate before death. Indeed, high neonatal plasma urea concentrations at birth and during the first week of life are a characteristic feature of the growth-restricted offspring of the H dams, and implies that amino acids play a significant role as key energy substrates for oxidative metabolism in these lambs during the early neonatal period. Similarly, plasma IGF-I concentrations were lower in growth-restricted lambs at birth (78 (SE 6.9) v. 107 (SE 9.5) ng/ml, $P < 0.05$) and throughout the first 2 weeks of postnatal life, implying that the maturation of the hypothalamic–pituitary functions required for activation of the GH–IGF-I axis are transiently delayed in prenatally-growth-restricted lambs (JM Wallace, DA Bourke and RP Aitken, unpublished results). While all but the most severely growth-restricted lambs exhibit rapid catch-up growth (in terms of body weight) once released from the constraining uterine environment, it remains to be established whether this form of placentally-mediated growth restriction has any long-term influences on the somatotropic axis and lifetime health.

As indicated in the introduction to the present paper, recent epidemiological studies have refocused research emphasis on the prenatal programming of adult health and performance. Consequently, a range of ovine and rodent models are being utilized to examine the mechanisms underlying altered structure and function of the major organ systems during fetal and early neonatal life, and the subsequent development of a range of disease states in adulthood including hypertension, cardiovascular disease and metabolic disorders such as non-insulin-dependent diabetes and hyperlipidaemia (Dodic et al. 1998; Holemans et al. 1998; Langley-Evans et al. 1998).

In addition, and of key importance to the propagation of the gene pool in all species, is the concept that aspects of our adult reproductive potential may be programmed during prenatal life. Using the adolescent sheep model we have recently demonstrated both sex-dependent alterations in pituitary gonadotrophin gene expression and impaired gonadal development in growth-restricted fetuses derived during late gestation from overnourished dams (Da Silva et al., 1998). In male fetuses growth restriction was associated with lower follicle-stimulating hormone and luteinizing hormone $\beta$ mRNA expression in the fetal pituitary and a reduction in testicular weight and seminiferous cord number at day 128 of gestation. At this stage of development in female growth-restricted fetuses luteinizing hormone $\beta$ mRNA expression was enhanced, while the
ovaries contained fewer germ cells at a less advanced stage of development. However, perturbed ovarian development, i.e. reduced numbers of primordial and primary follicles, was also evident at day 100 of gestation, before both the subsequent alteration in gonadotrophin gene expression and restriction of fetal growth per se (P Da Silva, RP Aitken, AN Brooks, SM Rhind and JM Wallace, unpublished results), and suggests that maternal nutrition influences fetal ovarian development via gonadotrophin-independent mechanisms. While it remains to be established whether these prenatal effects on the reproductive axis are translated into impaired reproductive performance in adult life, a recent study has demonstrated that this form of placentally-mediated prenatal growth restriction influences both the onset and magnitude of pubertal activation in male lambs (Da Silva et al. 1999).

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References


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