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.

Nutrient partitioning: Pregnancy: Adolescent: Placenta: Fetus

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 O_2 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 undernutrition 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,

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.

Corresponding author: Dr Jacqueline M. Wallace, fax +44 (0)1224 716622, email jwra@rri.sari.ac.uk

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

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 ($P < 0{\cdot}05$) 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 ($P < 0{\cdot}05$) and multiparous ($P < 0{\cdot}01$) 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

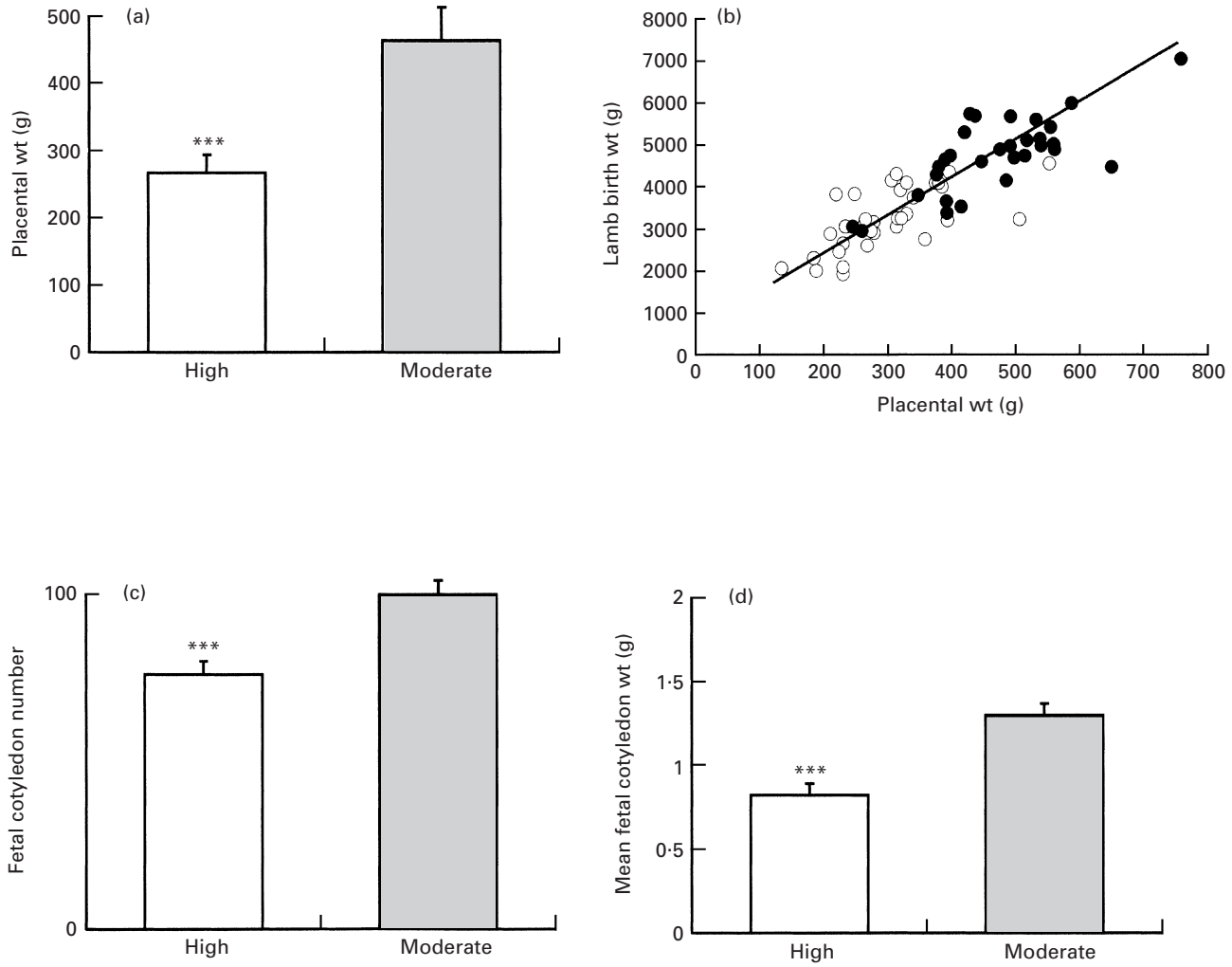

Fig. 1. (a) Total fetal placental mass at term, (b) relationship between placental mass and lamb birth weight (from Wallace *et al.* 1999*a*), (c) fetal cotyledon no. and (d) mean fetal cotyledon weight in adolescent dams overnourished (□; *n* 37; ○) or moderately-fed (■; *n* 34; ●) throughout their 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: ***$P < 0{\cdot}001$. (Data from Wallace *et al.* 1999*a*.)

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

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, 1997*b*, 1999*a*,*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*. 1997*a*). 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 Frisancho *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 variously 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, O_2 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 homeorhesis (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, placentally-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*. 1997*b*). 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*. 1999*b*). 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.

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

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; Oddy *et al*. 1985). Furthermore, in both well-fed polytocous and undernourished monotocous 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*. 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*. 1997*b*). 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*. 1997*a*). 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, placentally-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 O_2 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 somatotrophic 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 somatotrophic 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, $P < 0{\cdot}001$; Wallace *et al*. 1999*a*). 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

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

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 mammary 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*. 1997*b*). 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 (Ehrhardt & 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*. 1999*a*). 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 ($P < 0{\cdot}001$; Fig. 1). As these studies are the first to consistently demonstrate that maternal dietary intake can influence the number of maternal caruncles 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, 1997*a*) and, thus, it seemed probable that suboptimal progesterone in overnourished adolescent dams could compromise growth of the differentiating conceptus, resulting in fewer uterine caruncles 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-1 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

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

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 O_2 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, 1997*b*, 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*. 1999*b*). 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 ($P < 0{\cdot}009$), total placental weight ($P < 0{\cdot}001$), total fetal cotyledon weight ($P < 0{\cdot}001$) and mean fetal cotyledon weight per placenta ($P < 0{\cdot}001$). Fetal cotyledon number was dependent on maternal dietary intake during the first trimester only and was significantly lower ($P < 0{\cdot}007$) 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 ($P < 0{\cdot}001$) 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*. 1999*b*).

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 100 d of gestation. Thereafter, and for the rest of pregnancy, in 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 ($P < 0{\cdot}05$) in lamb birth weight at term, which was associated with an increase ($P < 0{\cdot}01$) 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

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

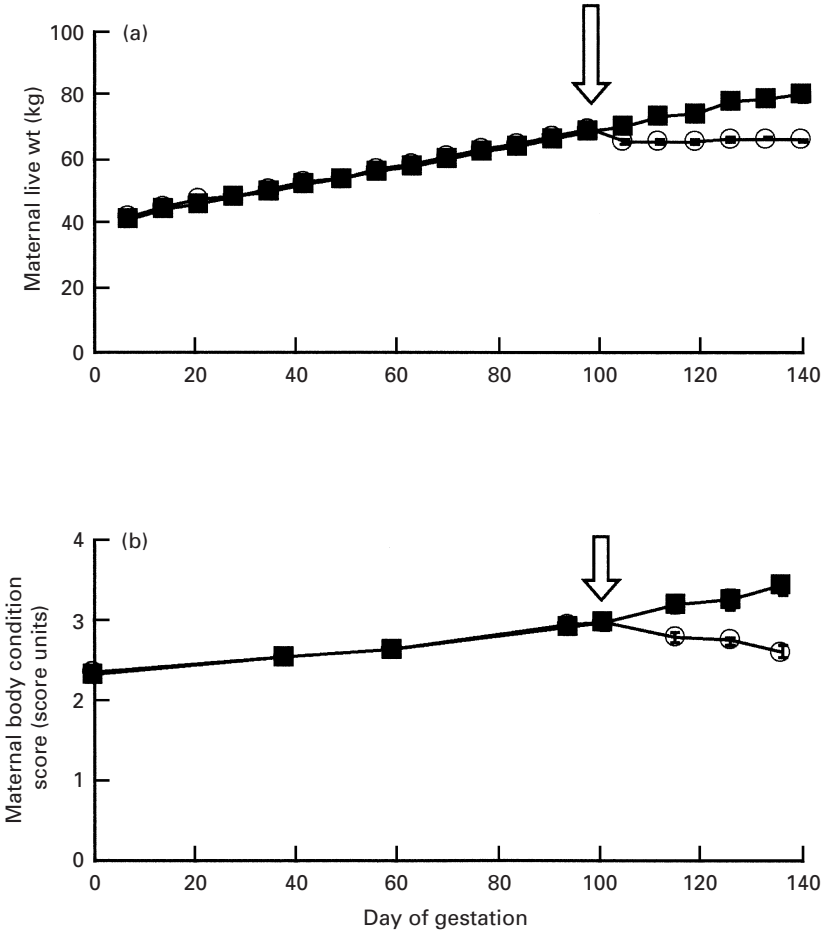

Fig. 2. Changes in (a) maternal live weight and (b) body condition score throughout gestation. Ewes were offered a high level of a complete diet to promote rapid maternal growth throughout their entire pregnancy (■) or a high level until day 100 (↓) of gestation and a low level thereafter (○). Values are means with their standard errors represented by vertical bars for ten ewes.

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

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

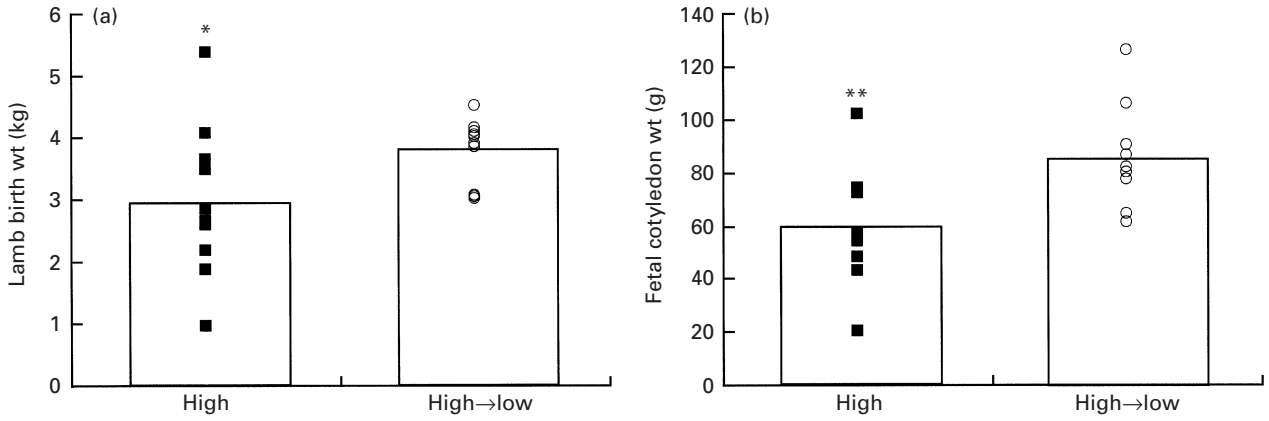

Fig. 3. (a) Lamb birth weight and (b) total fetal cotyledon weight in ewes offered a high level of a complete diet throughout their entire pregnancy (■) or a high level until day 100 of gestation and a low level thereafter (○). (□), Mean values for the individual data points. Mean values were significantly different from those for high → low dams: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.

from H dams and were highly correlated (minimum $P < 0{\cdot}01$) 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, 1948*a*,*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, $P < 0{\cdot}05$) 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 somatotrophic 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 β 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 β mRNA expression was enhanced, while the

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press

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).

Acknowledgements

This work was funded by the Scottish Office Agriculture Environment and Fisheries Department. I wish to gratefully acknowledge the support and many contributions made to these studies by my colleagues: Deirdre Bourke, Patricia Da Silva, Raymond Aitken and Robert Palmer.

References

Alexander G & Williams D (1971) Heat stress and development of the conceptus in domestic sheep. *Journal of Agricultural Science, Cambridge* **76**, 53–72.

Anthony RV, Pratt SL, Liang R & Holland MD (1995) Placental–fetal hormonal interactions: impact on fetal growth. *Journal of Animal Science* **73**, 1861–1871.

Barcroft J (1946) *Researches on Prenatal Life*. Oxford: Blackwell Scientific Publications.

Barcroft J & Kennedy JA (1939) The distribution of blood flow between the foetus and placenta in sheep. *Journal of Physiology* **95**, 173–186.

Barker DJP (1995) Fetal origins of coronary heart disease. *British Medical Journal* **311**, 171–174.

Barker DJP, Bull AR, Osmond C & Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. *British Medical Journal* **301**, 259–262.

Bauer MK, Harding JE, Bassett NS, Breier BH, Oliver MH, Gallaher BH, Evans PC, Woodall SM & Gluckman PD (1998) Fetal growth and placental function. *Molecular and Cellular Endocrinology* **140**, 115–120.

Bauman DE & Currie WB (1980) Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science* **63**, 1514–1529.

Bell AW (1984) Factors controlling placental and foetal growth and their effects on future production. In *Reproduction in Sheep*, pp. 144–152 [DR Lindsay and PT Pearce, editors]. Canberra: Australian Academy of Science.

Bell AW (1992) Foetal growth and its influence on postnatal growth and development. In *The Control of Fat and Lean Deposition*, pp. 111–127 [KN Boormann, PJ Buttery and DB Lindsay, editors]. Oxford: Butterworth-Heinemann Ltd.

Bell AW (1993) Pregnancy and fetal metabolism. In *Quantitative Aspects of Ruminant Digestion and Metabolism*, pp. 405–431 [JM Forbes and J France, editors]. Wallingford, Oxon: CAB International.

Bell AW & Bauman DE (1994) Animal models for the study of adipose regulation in pregnancy and lactation. In *Nutrient Regulation during Pregnancy, Lactation, and Infant Growth*, pp. 1–84 [L Allen, J King and B Lonnerdal, editors]. New York: Plenum Press.

Bell AW & Bauman DE (1997) Adaptations of glucose metabolism during pregnancy and lactation. *Journal of Mammary Gland Biology and Neoplasia* **2**, 265–278.

Bell AW, Hay WW Jr & Ehrhardt RA (1999) Placental transport of nutrients and its implications for fetal growth. *Journal of Reproduction and Fertility*, Suppl. 54, 401–410.

Brown CA & Thorburn GD (1989) Endocrine control of fetal growth. *Biology of the Neonate* **55**, 331–346.

Carter AM & Myatt L (1995) Control of placental blood flow: Workshop report. *Reproduction, Fertility and Development* **7**, 1401–1406.

Da Silva P, Aitken RP, Brooks AN, Rhind SM & Wallace JM (1998) Perturbed pituitary gonadotrophin gene expression and gonadal development in growth restricted fetal lambs at day 128 of gestation. *Journal of Reproduction and Fertility Abstract Series* **22**, 10.

Da Silva P, Aitken RP, Rhind SM & Wallace JM (1999) Placentally-mediated fetal growth restriction influences the onset of puberty in male but not in female lambs. *Journal of Reproduction and Fertility Abstract Series* **23**, 24.

Dodic M, May CN, Wintour EM & Coghlan JP (1998) An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. *Clinical Science* **94**, 149–155.

Ehrhardt RA & Bell AW (1995) Growth and metabolism of the ovine placenta during mid-gestation. *Placenta* **16**, 727–741.

Flint DJ, Sinnett-Smith PA, Clegg RA & Vernon RG (1979) Role of insulin receptors in the changing metabolism of adipose tissue during pregnancy and lactation in the rat. *Biochemistry Journal* **182**, 421–427.

Fraser AM, Brockert JE & Ward RH (1995) Association of young maternal age with adverse reproductive outcomes. *New England Journal of Medicine* **332**, 1113–1117.

Frisancho AR, Matos J, Leonard WR & Yarouch LA (1985) Developmental and nutritional determinants of pregnancy outcome among teenagers. *American Journal of Physical Anthropology* **66**, 247–261.

Giudice LC (1994) Growth factors and growth modulators in human endometrium: Their potential relevance to reproductive medicine. *Fertility and Sterility* **61**, 1–17.

Grizard J, Dardevet D, Papet I, Mosoni L, Mirand PP, Attaix D, Tauveron I, Bonin D & Arnal M (1995) Nutrient regulation of skeletal muscle protein metabolism in animals. The involvement of hormones and substrates. *Nutrition Research Reviews* **8**, 67–91.

Guesnet M, Massoud MJ & Demarne Y (1991) Regulation of adipose tissue metabolism during pregnancy and lactation in the ewe: the role of insulin. *Journal of Animal Science* **69**, 2057–2065.

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

Harding JE, Liu L, Evans PC & Gluckman PD (1994) Insulin-like growth factor 1 alters feto-placental protein and carbohydrate metabolism in fetal sheep. *Endocrinology* **134**, 1509–1514.

Hartwich KM, Walker SK, Owens JA & Seamark RF (1995) Progesterone supplementation in the ewe alters cell allocation to the inner cell mass. *Proceedings of the Australian Society of Medical Research* **26**, 128 Abstr.

Heasman L, Clarke L, Firth K, Stephenson T & Symonds ME (1998) Influence of restricted maternal nutrition in early to mid gestation on placental and fetal development at term in sheep. *Paediatric Research* **44**, 546–551.

Hoggard N, Hunter L, Duncan JS, Williams LM, Trayhurn P & Mercer JG (1997) Leptin and leptin receptor mRNA and protein

expression in the murine fetus and placenta. *Proceedings of the National Academy of Sciences USA* **94**, 11073–11078.
Holemans K, Aerts L & Van Assche FA (1998) Fetal growth and long-term consequences in animal models of growth retardation. *European Journal of Obstetrics and Gynecology and Reproductive Biology* **81**, 149–156.
Hough GM, McDowell GH, Annison EF & Williams AJ (1985) Glucose metabolism in hindlimb muscle of pregnant and lactating ewes. *Proceedings of the Nutrition Society of Australia* **10**, 97.
Houseknecht KL, Bauman DE, Vernon RG, Byatt JC & Collier RJ (1996) Insulin-like growth factors-I and II, somatotropin, prolactin and placental lactogen are not acute effectors of lipolysis in ruminants. *Domestic Animal Endocrinology* **13**, 239–249.
Jenkinson CMC, Min SH, Mackenzie DDS, McCutcheon SN, Breier BH & Gluckman PD (1999) Placental development and fetal growth in growth hormone-treated ewes. *Growth Hormone and IGF Research* **9**, 11–17.
Johnsson ID & Hart IC (1985) Pre-pubertal mammogenesis in the sheep 1. The effects of level of nutrition on growth and mammary development in female lambs. *Animal Production* **41**, 323–332.
Johnsson ID & Obst JM (1984) The effects of level of nutrition before and after 8 months of age on subsequent milk and calf production of beef heifers over three lactations. *Animal Production* **38**, 57–68.
Kelly RW (1992) Nutrition and placental development. *Proceedings of the Nutrition Society of Australia* **17**, 203–211.
Kleemann DO, Walker SK & Seamark RF (1994) Enhanced fetal growth in sheep administered progesterone during the first three days of pregnancy. *Journal of Reproduction and Fertility* **102**, 411–417.
Lacroix MC, Servely JL & Kann G (1995) IGF-I and IGF-II receptors in the sheep placenta: evolution during the course of pregnancy. *Journal of Endocrinology* **144**, 179–191.
Langley-Evans SC, Gardner DS & Welham SJM (1998) Intrauterine programming of cardiovascular disease by maternal nutritional status. *Nutrition* **14**, 39–47.
Lea RG, Hannah L, Blades J, Howe D & Hoggard N (1998) Placental leptin in normal and abnormal pregnancies. *Journal of Reproduction and Fertility Abstract Series* **21**, 21.
Lenders CM, Hediger ML, Scholl TO, Khoo CS, Slap GB & Stallings VA (1997) Gestational age and infant size at birth are associated with dietary sugar intake among pregnant adolescents *Journal of Nutrition* **127**, 1113–1117.
Lok F, Owens JA, Mundy L, Robinson JS & Owens PC (1996) Insulin-like growth factor 1 promotes growth selectively in fetal sheep in late gestation. *American Journal of Physiology* **270**, R1148–R1155.
McAnarney ER (1987) Young maternal age and adverse neonatal outcome. *American Journal of Diseases of Children* **141**, 1053–1059.
McMillen IC, Phillips ID, Ross JT, Robinson JS & Owens JA (1995) Chronic stress – the key to parturition. *Reproduction, Fertility and Development* **7**, 499–507.
Mellor DJ (1983) Nutritional and placental determinants of foetal growth rate in sheep and consequences for the newborn lamb *British Veterinary Journal* **139**, 307–324.
Mellor DJ (1987) Nutritional effects on the fetus and mammary gland during pregnancy. *Proceedings of the Nutrition Society* **46**, 249–257.
Molina RD, Meschia G & Wilkening RB (1990) Uterine blood flow, oxygen and glucose uptakes at mid-gestation in the sheep. *Proceedings of the Society for Experimental Biology and Medicine* **195**, 379–385.
Oddy VH, Gooden JM, Hough GM, Teleni E & Annison EF (1985) Partitioning of nutrients in Merino ewes II. Glucose utilisation by skeletal muscle, the pregnant uterus and the lactating mammary gland in relation to whole body glucose utilisation. *Australian Journal of Biological Science* **38**, 95–108.
Oddy VH & Holst PJ (1991) Maternal-foetal adaptation to mid pregnancy feed restriction in single-bearing ewes. *Australian Journal of Agricultural Research* **42**, 969–978.
Olausson PO, Cnattingius S & Haglund B (1999) Teenage pregnancies and risk of late fetal death and infant mortality. *British Journal of Obstetrics and Gynaecology* **106**, 116–121.
Oliver MH, Bloomfield FH, Harding JE, Breier BH, Bassett NS & Gluckman PD (1999) The maternal, fetal and postnatal somatotrophic axes in intrauterine growth retardation. *Biochemical Society Transactions* **27**, 69–73.
Oliver MH, Harding JE, Breier BH & Gluckman PD (1996) Fetal insulin-like growth factor (IGF)-I and IGF-II are regulated differently by glucose or insulin in the sheep fetus. *Reproduction, Fertility and Development* **8**, 167–172.
Owens JA (1991) Endocrine and substrate control of fetal growth: placental and maternal influences and insulin-like growth factors. *Reproduction, Fertility and Development* **3**, 501–517.
Owens JA, Kind KL, Carbone F, Robinson JS & Owens PC (1994) Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *Journal of Endocrinology* **140**, 5–13.
Owens JA, Owens PC & Robinson JS (1995) Experimental restriction of fetal growth. In *Fetus and Neonate, Physiology and Clinical Applications*, pp. 139–175 [MA Hanson, JAD Spencer and CH Rodeck, editors]. Cambridge: Cambridge University Press.
Palmer RM, Thompson MG, Millet C, Thom A, Aitken RP & Wallace JM (1998) Growth and metabolism of fetal and maternal muscles of adolescent sheep on adequate or high feed intakes: possible role of protein kinase C-α in fetal muscle growth. *British Journal of Nutrition* **79**, 351–357.
Parr RA, Williams AH, Campbell IP, Witcome GF & Roberts AM (1986) Low nutrition of ewes in early pregnancy and the residual effect on the offspring. *Journal of Agricultural Science, Cambridge* **106**, 81–87.
Rattray PV, Garrett WN, East NE & Hinman N (1974) Growth development and composition of the ovine mammary gland during pregnancy. *Journal of Animal Science* **38**, 613–626.
Reynolds LP & Redmer DA (1995) Utero-placental vascular development and placental function. *Journal of Animal Science* **73**, 1839–1851.
Reynolds TS, Stevenson KR & Wathes DC (1997) Pregnancy-specific alterations in the expression of the insulin-like growth factor system during early placental development in the ewe. *Endocrinology* **138**, 886–897.
Reynolds TS, Wathes DC, Aitken RP & Wallace JM (2000) Effect of maternal nutrition on components of the insulin-like growth factor (IGF) system and placental growth. *Journal of Reproduction and Fertility* (In the Press).
Robinson JJ (1983) Nutrition of the pregnant ewe. In *Sheep Production*, pp. 111–131 [W Haresign, editor]. London: Butterworths.
Robinson JJ, McDonald I, McHattie I & Pennie K (1978) Studies on reproduction in prolific ewes 4. Sequential changes in the maternal body during pregnancy. *Journal of Agricultural Science, Cambridge* **91**, 291–304.
Robinson JJ, Sinclair KD & McEvoy TG (1999) Nutritional effects on foetal growth. *Animal Science* **68**, 315–331.
Rush D (1986) Nutrition in the preparation for pregnancy. In *Prepregnancy Care: A Manual for Practice*, pp. 113–139 [G Chamberlain and J Lumley, editors]. Chichester, West Sussex: John Wiley and Sons Ltd.

Samad AR & Ford EJH (1981) The effects of progesterone on glucose and lactate metabolism in ovariectomised sheep. *Quarterly Journal of Experimental Physiology* **66**, 73–80.
Schneider H (1996) Ontogenic changes in the nutritive function of the placenta. *Placenta* **17**, 15–26.
Scholl TO & Hediger ML (1993) A review of the epidemiology of nutrition and adolescent pregnancy: maternal growth during pregnancy and its effect on the fetus. *Journal of the American College of Nutrition* **12**, 101–107.
Scottish Needs Assessment Programme (1994) Teenage pregnancy in Scotland- report. *Scottish Forum for Public Health Medicine*, pp. 3–4. Glasgow: Scottish Forum for Public Health Medicine.
Steyn C, Koser F, Hawkins P, Saito T, Ozaki T, Noakes D & Hanson M (2000) Effect of mild undernutrition in early gestation on fetal villous density in sheep. *Placenta* (In the Press).
Thissen JP, Ketelslegers JM & Underwood LE (1994) Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews* **15**, 80–101.
Torry DS & Torry RJ (1997) Angiogenesis and the expression of vascular endothelial growth factor in endometrium and placenta. *American Journal of Reproductive Immunology* **37**, 21–29.
Umberger SH, Goode L, Caruolo EV, Harvey RW, Britt JH & Linnerud AC (1985) Effects of accelerated growth during rearing on reproduction and lactation in ewes lambing at 13 to 15 months of age. *Theriogenology* **23**, 555–564.
Vatnick I, Schoknecht PA, Darrigrand R & Bell AW (1991) Growth and metabolism of the placenta after unilateral fetectomy in twin pregnant ewes. *Journal of Developmental Physiology* **15**, 351–356.
Vernon RG, Clegg RA & Flint DJ (1981) Metabolism of sheep adipose tissue during pregnancy and lactation. *Biochemistry Journal* **200**, 307–314.
Vernon RG & Finley E (1986) Endocrine control of lipogenesis on adipose tissue from lactating sheep. *Biochemical Society Transactions* **14**, 635–636.
Vincent IC, Williams HLI & Hill R (1985) The influence of a low-nutrient intake after mating on gestation and perinatal survival of lambs. *British Veterinary Journal* **141**, 611–617.
Wallace JM, Aitken RP & Cheyne MA (1994) Effect of post-ovulation nutritional status in ewes on early conceptus survival and growth in vivo and luteotrophic protein secretion in vitro. *Reproduction, Fertility and Development* **6**, 253–259.
Wallace JM, Aitken RP & Cheyne MA (1996) Nutrient partitioning and fetal growth in rapidly growing adolescent ewes. *Journal of Reproduction and Fertility* **107**, 183–190.
Wallace JM, Aitken RP, Cheyne MA & Humblot P (1997*a*) Pregnancy-specific protein B and progesterone concentrations in relation to nutritional regimen, placental mass and pregnancy outcome in growing adolescent ewes carrying singleton fetuses. *Journal of Reproduction and Fertility* **109**, 53–58.
Wallace JM, Bourke DA & Aitken RP (1999*a*) Nutrition and fetal growth: paradoxical effects in the overnourished adolescent sheep. *Journal of Reproduction and Fertility*, Suppl. 54, 385–399.
Wallace JM, Bourke DA, Aitken RP & Cruickshank MA (1999*b*) Switching maternal nutrient intake at the end of the first trimester has profound effects on placental development and fetal growth in adolescent sheep carrying singleton fetuses. *Biology of Reproduction* **61**, 101–110.
Wallace JM, Bourke DA & Aitken RP, Da Silva P & Cruickshank MA (1998) Influence of progesterone supplementation during the first trimester on pregnancy outcome in overnourished adolescent ewes. *Journal of Reproduction and Fertility Abstract Series* **22**, 23.
Wallace JM, Bourke DA, Aitken RP, Palmer RM, Da Silva P & Cruickshank MA (2000) Relationship between nutritionally-mediated placental growth restriction and fetal growth, body composition and endocrine status during late gestation in adolescent sheep. *Placenta* **21**, (In the Press).
Wallace JM, Da Silva P, Aitken RP & Cruickshank MA (1997*b*) Maternal endocrine status in relation to pregnancy outcome in rapidly growing adolescent sheep. *Journal of Endocrinology* **155**, 359–368.
Wallace LR (1948*a*) The growth of lambs before and after birth in relation to the level of nutrition. *Journal of Agricultural Science, Cambridge* **38**, 243–300.
Wallace LR (1948*b*) The growth of lambs before and after birth in relation to the level of nutrition. *Journal of Agricultural Science, Cambridge* **38**, 367–398.
Wathes DC, Reynolds TS, Robinson RS & Stevenson KR (1998) Role of insulin-like growth factor system in uterine function and placental development in ruminants. *Journal of Dairy Science* **81**, 1778–1789.
Wilson V, Dandrea J, Stephenson T, Webb R & Symonds ME (2000) The influence of maternal nutrient restriction between early to mid gestation on placental weight and leptin abundance. *Proceedings of the Nutrition Society* **59**, OCA

https://doi.org/10.1017/S0029665100000136 Published online by Cambridge University Press