Nutrient partitioning during adolescent pregnancy*

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Human adolescent mothers have an increased risk of delivering low birth weight and premature infants with high mortality rates within the first year of life. Studies using a highly controlled adolescent sheep paradigm demonstrate that, in young growing females, the hierarchy of nutrient partitioning during pregnancy is altered to promote growth of the maternal body at the expense of the gradually evolving nutrient requirements of the gravid uterus and mammary gland. Thus, overnourishing adolescent dams throughout pregnancy results in a major restriction in placental mass, and leads to a significant decrease in birth weight relative to adolescent dams receiving a moderate nutrient intake. High maternal intakes are also associated with increased rates of spontaneous abortion in late gestation and, for ewes delivering live young, with a reduction in the duration of gestation and in the quality and quantity of colostrum accumulated prenatally. As the adolescent dams are of equivalent age at the time of conception, these studies indicate that nutritional status during pregnancy rather than biological immaturity predisposes the rapidly growing adolescents to adverse pregnancy outcome. Nutrient partitioning between the maternal body and gravid uterus is putatively orchestrated by a number of endocrine hormones and, in this review, the roles of both maternal and placental hormones in the regulation of placental and fetal growth in this intriguing adolescent paradigm are discussed. Impaired placental growth, particularly of the fetal component of the placenta, is the primary constraint to fetal growth during late gestation in the overnourished dams and nutritional switch-over studies indicate that high nutrient intakes during the second two-thirds of pregnancy are most detrimental to pregnancy outcome. In addition, it may be possible to alter the nutrient transport function of the growth-restricted placenta in that the imposition of a catabolic phase during the final third of pregnancy in previously rapidly growing dams results in a modest increase in lamb birth weight.

The partitioning of nutrients during pregnancy depends on a series of evolving maternal adaptations that redirect oxygen and nutrients to the gravid uterus to ensure adequate placental growth and function, and to facilitate placental delivery of these nutrients to the growing fetus (Owens, 1991; Bell, 1993). Historically, a number of experimental paradigms, primarily involving sheep, have been used to demonstrate unequivocally that appropriate growth and function of the placenta is central to the partitioning process and essential for optimum fetal growth and neonatal outcome. Thus, interference with the growth or blood supply of the placenta, by pre-mating carunclectomy (Alexander, 1964; Robinson *et al.*, 1979), uterine artery ligation (Emmanoulides *et al.*, 1968), embolism using microspheres (Charlton and Johengen, 1987) or the

imposition of chronic heat stress (Alexander and Williams, 1971; Vatnick *et al.*, 1991a; McCrabb *et al.*, 1993) have all resulted in various degrees of fetal growth restriction. These extreme and largely invasive models have been invaluable for increasing our understanding of prenatal growth and physiology. However, these models rarely result in the birth of viable offspring and hence are of limited value for studying the long-term sequelae of prenatal growth restriction. In contrast, recent studies using an adolescent sheep model provide a clinically relevant and more natural model of placental growth restriction with the added advantage that most of the resulting low birth weight lambs can survive the rigours of the neonatal period.

Pregnancy outcome in human adolescents

Human adolescent girls have a high risk of delivering low birth weight and premature babies that have high mortality rates within the first year of life (McAnarney, 1987; Adelson *et al.*, 1992; Cooper *et al.*, 1995). The risk of adverse

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Table 1. Pregnancy outcome in adolescent sheep offered either a high or moderate nutrient intake throughout pregnancy and delivering live young

	Maternal intake		Significance
	High (n = 59)	Moderate (n = 56)	
Duration of gestation (days)	142.5 ± 0.35	145.2 ± 0.37	***
Lamb birth weight (g) (range)	3481 ± 143 (1910–6940)	4890 ± 107 (2950–7050)	***
Fetal placental mass (g) (range)	312 ± 11.3 (134–553)	465 ± 13.8 (245–759)	***
Number of fetal cotyledons	78.2 ± 1.93	93.1 ± 1.99	***
Total fetal cotyledon mass (g) (range)	69.0 ± 2.73 (23–118)	128.9 ± 4.72 (61–223)	***
Fetal:placental mass	11.6 ± 0.30	10.8 ± 0.22	*

Values are means ± SEM.
*P < 0.05; ***P < 0.001.
Data summarized from Wallace *et al.* (1999a) and J. M. Wallace, D. A. Bourke and R. P. Aitken (unpublished).

pregnancy outcome has been variously attributed to poor socio-economic status, gynaecological immaturity or the growth and nutritional status of the mother at the time of conception (Fraser *et al.*, 1995). Within adolescents, the risk of spontaneous miscarriage is highest in girls aged 13–15 years (Scottish Needs Assessment Programme, 1994). Similarly, in a large population-based study involving over 300 000 pregnancies, the rates of very preterm birth (< 32 weeks) increased significantly with decreasing maternal age and almost entirely explained the increased risk of neonatal and post-neonatal mortality in babies born to girls in the 13–15 age group (Olausson *et al.*, 1999). At first glance this would indicate that gynaecological immaturity is the major factor predisposing adolescent girls to poor pregnancy outcome. However, maternal growth and nutritional status during pregnancy also appear to play a role, in that birth weight is modestly but significantly reduced in both primiparous and multiparous adolescents who are still growing during pregnancy (Scholl and Hediger, 1993). Studies assessing nutritional status in human adolescents are poorly controlled but the delivery of low birth weight babies has been associated with both the consumption of high sugar diets (Lenders *et al.*, 1997) and with protein supplementation during late gestation (Rush, 1986).

The UK has the highest adolescent pregnancy rate in Europe and currently 1 in 500 babies are born to girls who are less than 16 years of age at the time of conception, and hence in the potentially still-growing category (Scottish Needs Assessment Programme, 1994). It is against this background that we are studying nutrient partitioning during adolescent pregnancy.

The adolescent sheep paradigm

The experimental paradigm uses embryo recovery and transfer techniques (Wallace *et al.*, 1997b) to establish singleton pregnancies on day 4 of an induced oestrous cycle in peripubertal adolescent sheep (7–10 months of age). This technique 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. Immediately after embryo transfer, recipient dams are offered a high (2.0–2.5 × maintenance) or moderate (1.25 × maintenance) quantity of a complete diet to promote rapid or normal maternal growth, respectively. The diet contains 10.2 MJ metabolizable energy and 137 g crude protein kg⁻¹ dry matter. Approximately 70% of adolescents receiving embryos become pregnant, and maternal live weight gain during the first 100 days of the 145 day gestation ranges from 200 to 350 g day⁻¹ in high-intake compared with 50–85 g day⁻¹ in moderate-intake groups. Thereafter, the feed intake of the moderate-intake group is adjusted weekly to maintain body condition score and to meet the increasing nutrient demands of the pregnant uterus.

Key characteristics

Pregnancy outcome data obtained after the application of high and moderate nutritional treatments throughout gestation in studies carried out over a period of 5 years is given (Table 1). These studies were all initiated during the mid-breeding season using the same recipient genotype and a single sire. Within studies, the adolescents were also of equivalent age, live weight and body condition score at the time of embryo transfer, thus removing the confounding effect of differences in gynaecological age and pre-conception nutrition. Overnourishing adolescent dams by feeding a high intake throughout their entire pregnancy results in a major restriction in fetal placental mass (33%, *P* < 0.001), leading to a significant decrease in lamb birth weight relative to that for normally growing adolescents (29%, *P* < 0.001). Within both high- and moderate-intake groups, total placental mass and fetal mass were highly correlated (*r* = 0.730 and 0.723, respectively, *P* < 0.001). However, the fetal:placental mass ratio was higher (*P* < 0.05) in the overnourished compared with the moderate-intake dams because placental growth was more perturbed than

fetal growth in the former group. When the dataset is considered as a whole, the degree of placental and hence fetal growth restriction achieved by overnourishing is variable. It is widely accepted that when categorizing animals as growth-restricted, fetal mass must equal or be lower than the mean of the control group minus two times the standard deviation of the control group (Robinson *et al.*, 1979). Thus, for this genotype, a term fetus was considered to be growth-restricted if its mass was ≤ 3300 g. On this basis, 31 of 59 high-intake and 2 of 56 moderate-intake adolescent dams produced growth-restricted fetuses. Within the high-intake group, fetal and placental masses (mean $\pm$ SEM) for the growth-restricted ($n = 31$) compared with the non-growth-restricted pregnancies ($n = 28$) were 2653 ± 79 and 267 ± 13 versus 4397 ± 156 and 355 ± 10 g, respectively ($P < 0.001$). There was no difference in maternal live weight gain during the first 100 days of gestation in high-intake dams with growth-restricted compared with non-growth-restricted pregnancies (274 ± 8.9 and 284 ± 8.7 g day⁻¹, respectively). Mean fetal and placental masses for the non-growth-restricted pregnancies were still significantly lower ($P < 0.01$ and $P < 0.001$, respectively) than those of the moderate-intake dams ($n = 54$, 4960 ± 99 and 473 ± 13 g, respectively).

In this paradigm, high maternal dietary intakes are also associated with an increased incidence ($P < 0.01$) of non-infectious spontaneous abortion or stillbirth in late gestation (Fig. 1). Low or absent secretion of pregnancy-specific protein B by the binucleate cells of the placenta implies that this abortion or stillbirth is preceded by severe placental insufficiency during mid-gestation (Wallace *et al.*, 1997a). In ewes delivering live young, independent of the degree of placental and fetal growth restriction achieved, high maternal dietary intakes are also associated with a modest but highly significant ($P < 0.001$) reduction in duration of gestation compared with moderate-intake dams (Fig. 2, Table 1). Timing of parturition is dependent on a complex cascade of endocrine signals emanating from the fetal hypothalamic–pituitary–adrenal axis (for reviews, see Challis and Brooks, 1989; Matthews *et al.*, 1995). Fetal stress such as hypoxia and hypoglycaemia may accelerate the maturation of this endocrine cascade resulting in premature parturition (McMillen *et al.*, 1995). The precise endocrine changes underlying premature parturition in the adolescent paradigm have not been examined but may be initiated by nutritionally induced alterations in placental hormone secretion (primarily progesterone) or limitations in placental nutrient transfer capacity that cause fetal nutrient stress during late gestation.

In ewes, the mass of the mammary gland is positively correlated with total lamb birth weight, with $> 95\%$ of its growth occurring during pregnancy (Robinson, 1986). Thus, it is not surprising that in adolescent sheep, alterations in the pattern of nutrient partitioning are also evident at the mammary gland in that overfeeding is associated with a reduction in the initial yield of colostrum, which has been accumulated prenatally (Davidson *et al.*, 2000). Colostrum

Fig. 1. Proportion of spontaneous abortions (■), live births (□) and neonatal deaths (▒) in adolescent dams offered either a high or moderate nutrient intake throughout their entire pregnancy. (Summarized from Wallace *et al.*, 1999b and J. M. Wallace, D. A. Bourke and R. P. Aitken, unpublished.)

samples from high-intake dams contained a higher concentration of IgG, lower concentrations of butterfat and lactose and similar amounts of crude protein relative to moderate-intake dams (Table 2). However, when expressed relative to individual colostrum yield, the total IgG, butterfat, lactose and crude protein available to the neonate was reduced significantly in the high- compared with the moderate-intake group ($P < 0.01$ to $P < 0.001$). The metabolic requirements of growth-restricted lambs are high in that they have reduced wool cover at birth and a relatively large surface area per unit mass, making them highly susceptible to hypothermia. In the adolescent paradigm, the observed changes in the nutrient composition of the colostrum results in a significant reduction in its energy concentration (4694 ± 208 and 5393 ± 199 kJ kg⁻¹ colostrum in high-versus moderate-intake dams; $P < 0.02$). This finding, together with the observation that growth-restricted fetuses from high-intake dams have reduced body lipid and lower liver glycogen stores (Wallace *et al.*, 2000a), indicates that, in the absence of human intervention, many of the low birth weight lambs would receive inadequate energy to meet their metabolic needs after birth. Furthermore, as the absolute amount of IgG consumed, rather than its concentration, is the most important determinant of immune status in the newborn (Hunter *et al.*, 1977), many lambs born to high-intake dams would not receive adequate antibody from their mother to confer passive immunity, leaving them susceptible to both systemic and enteric infection. Indeed, at the onset of this project, few of the premature and low birth weight lambs survived (Wallace *et al.*, 1996; Fig. 1). However, with meticulous neonatal care procedures, most of these lambs are expected to survive to adulthood.

As the adolescent dams used in these studies were of equivalent age at the time of conception, the results indicate that nutritional status throughout pregnancy rather than

Fig. 2. Duration of gestation in adolescent sheep offered either a high (□) or moderate (■) nutrient intake throughout their entire pregnancy. (Summarized from Wallace *et al.*, 1999b and J. M. Wallace, D. A. Bourke and R. P. Aitken, unpublished.)

Table 2. Nutrient composition and IgG content of colostrum samples collected immediately after parturition from adolescent sheep offered either a high or moderate nutrient intake throughout pregnancy

	Maternal dietary intake		Significance
	High (n = 27)	Moderate (n = 25)	
Fetal placental mass (g)	280 ± 17.7	449 ± 24.9	***
Colostrum yield (g)	115 ± 21.4	301 ± 44.2	***
Colostrum composition			
Butterfat (g per 100 g)	7.6 ± 0.64	9.7 ± 0.61	*
Lactose (g per 100 g)	2.2 ± 0.21	2.9 ± 0.24	*
Crude protein (g per 100 g)	18.4 ± 0.86	16.6 ± 0.45	ns
IgG (mg ml ⁻¹)	163 ± 17.4	116 ± 11.9	*
Total butterfat (g)	9.8 ± 2.41	31.7 ± 3.46	***
Total lactose (g)	2.3 ± 0.39	9.3 ± 1.44	***
Total crude protein (g)	21.6 ± 5.13	53.9 ± 5.41	***
Total IgG (g)	16.3 ± 3.93	33.6 ± 3.88	**

Values are means ± SEM.
*P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.
Reproduced from Davidson *et al.* (2000).

biological immaturity predisposes the overnourished adolescents to poor pregnancy outcome. Indeed, the similarity between adolescent human and sheep pregnancy outcome data indicates that the hierarchy of nutrient partitioning in young growing females is altered to promote the growth of maternal tissues at the expense of the gradually evolving nutrient requirements of the gravid uterus and mammary gland. Furthermore, the adolescent sheep paradigm implies that impaired placental growth and hence reduced nutrient transfer capacity are central to the aetiology of adverse pregnancy outcome in both species.

Placental mass has rarely been measured in human studies but small reductions in placental mass have been measured in growing adolescent girls producing low birth weight babies (Frisancho *et al.*, 1985). Results of a preliminary study using older sheep of an identical genotype indicate that this alteration in the nutrient partitioning hierarchy in overnourished animals is a feature of very young females (J. M. Wallace, unpublished). In this study, the primiparous recipient ewes were 12 months older and approximately 10 kg heavier at the time of embryo transfer. Duration of gestation was significantly

Fig. 3. Changes in maternal concentrations of (a) insulin, (b) insulin-like growth factor 1 (IGF-I), (c) leptin and (d) placental lactogen in relation to (e) placental and (f) fetal mass at term in adolescent dams carrying a singleton fetus and offered either a high ($\square$, $n = 7$) or moderate ($\blacksquare$, $n = 6$) nutrient intake throughout their entire pregnancy. (Insulin and IGF-I profiles redrawn from Wallace *et al.*, 1999a.)

shorter in high- ($n = 12$) versus moderate-intake ($n = 9$) dams (144.8 ± 0.55 and 146.9 ± 0.65 days, respectively; $P < 0.05$) but placental mass (483 ± 25 and 508 ± 40 g, respectively) and lamb birth weight (5213 ± 282 and 4826 ± 459 g, respectively) were completely independent of maternal dietary intake. Furthermore, placental and fetal masses in these older animals were identical to those detailed previously for the moderate-intake adolescents (Table 1), adding further credence to the suggestion that gynaecological immaturity is not the major factor influencing pregnancy outcome in adolescent females.

Hormonal regulators of nutrient partitioning between the maternal and fetoplacental compartments

The partitioning of glucose, oxygen and amino acids between the dam and her gravid uterus may be orchestrated by a number of endocrine hormones of maternal, placental and fetal origin (for reviews, see Bell and Bauman, 1997; Bauer *et al.*, 1998). Maternally derived endocrine partitioning agents may operate via changes in maternal or placental metabolism, uteroplacental blood flow or placental growth

and transport functions. Similarly, placenta-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). The adolescent sheep provides an intriguing model system to study nutrient partitioning in that the dam is overnourished whereas the growth-restricted placenta limits nutrient supply to the fetus. In this paradigm, maternal insulin and insulin-like growth factor I (IGF-I) concentrations are increased from early in gestation in the high-intake dams (Wallace *et al.*, 1997a, 1999a; Fig. 3) and it is probable that these nutritionally sensitive hormones provide a sustained anabolic stimulus to maternal tissue deposition (primarily of adipose tissue) at the expense of placental growth. Indeed, the IGF system may play a role in the growth and metabolic activity of the placenta *per se* in that the various components of the IGF system have been localized in the ovine uterus and placenta, where they show spatial and temporal patterns of expression (Wathes *et al.*, 1998). The pattern of expression during early placental growth in the adolescent paradigm has not yet been examined. However, IGF binding protein 1 (IGFBP-1) mRNA expression was higher and IGFBP-3 mRNA expression was lower in the endometrial glands of high-intake compared with moderate-intake dams at the end of the second third of gestation, and these significant changes in the binding proteins are characteristic of severe undernutrition at the uteroplacental level (Gadd *et al.*, 2000).

In studies involving adult sheep and a range of maternal dietary intakes, there is a reduction in the amount of glucose available to support maternal tissues as pregnancy progresses, and this is associated with significant fat mobilization during late pregnancy (Robinson *et al.*, 1978; Hough *et al.*, 1985; Oddy *et al.*, 1985). In contrast, in the overnourished adolescent, maternal glucose concentrations remain high, non-esterified fatty acid concentrations are low and the dam continues to accumulate lipid during the final third of pregnancy (Wallace *et al.*, 1999a). Insulin and placental lactogen have been proposed to play a key role in mediating these metabolic changes (Wallace *et al.*, 1997a). In women, placental lactogen modifies maternal intermediary metabolism to the advantage of the fetus and exerts direct growth-promoting and metabolic effects in the fetus *per se* (Handwerger and Freemerk, 2000). Clear evidence for a similar role in the sheep is equivocal, but direct infusion of ovine placental lactogen into the fetus for 14 days during late gestation stimulated fetal IGF-I concentrations and was associated with increased hepatic glycogen deposition (Schoknecht *et al.*, 1996). In the adolescent sheep paradigm, maternal placental lactogen concentrations were significantly lower in the high-intake compared with the moderate-intake dams from the end of the first third of gestation (J. M. Wallace, T. R. H. Regnault and R. V. Anthony, unpublished; Fig. 3). Furthermore, irrespective of nutritional treatment, placental lactogen concentrations were positively correlated with placental cotyledon and fetal mass at term ($r = 0.764$ and 0.757 ,

respectively, $P < 0.001$) and negatively associated with maternal body condition score ($r = -0.765$, $P < 0.001$). The maternal concentrations of progesterone and pregnancy-specific protein B, which are produced by the binucleate cells of the placenta, are similarly attenuated during pregnancy in the high-intake dams (Wallace *et al.*, 1997a,b). Although progesterone has been implicated in the growth of uterine blood vessels (Caton *et al.*, 1974) and in the regulation of uterine blood flow (Caton *et al.*, 1974; Roman-Ponce *et al.*, 1983), a clear and definitive role for these placental hormones in nutrient partitioning during ovine pregnancy has not yet been established.

Growth hormone (GH) of maternal pituitary or placental origin may also play a role in nutrient partitioning during pregnancy. In the adolescent sheep paradigm, GH pulse frequency and mean concentrations during mid- and late gestation are lower in high-intake compared with moderate-intake dams and inversely related to maternal insulin concentrations. During human pregnancy, pituitary growth hormone secretion is suppressed in the mother and a placental growth hormone variant, which is postulated to play a role in ensuring adequate nutrient availability for the fetus, predominates (Handwerger and Freemerk, 2000). In cases of intrauterine growth retardation, placental GH concentrations in the maternal circulation and GH mRNA concentrations in the term placenta are reduced (Mirlesse *et al.*, 1993; Chowen *et al.*, 1996). An ovine placental growth hormone variant has also been identified (Lacroix *et al.*, 1996) and the presence of GH receptors in endometrium, placenta and fetus during early pregnancy indicates that placental GH may influence the proliferative growth of the ovine placenta *per se* (Lacroix *et al.*, 1999). GH of pituitary or placental origin may also play a role in nutrient partitioning once placental growth is complete. GH is known to inhibit the effects of insulin on lipogenesis *in vitro* (Vernon and Finley, 1986) and administration of recombinant GH twice a day between days 98 and 111 of pregnancy stimulated fetal growth (Jenkinson *et al.*, 1999), possibly by mobilizing maternal body reserves.

The recent development of a sheep-specific ELISA has allowed us to determine circulating leptin concentrations throughout pregnancy in relation to maternal body composition and pregnancy outcome (Thomas *et al.*, 2001). Maternal leptin concentrations are significantly increased in overnourished dams from the end of the first third of pregnancy and remain high throughout the remainder of gestation (Fig. 3). Irrespective of nutritional treatment, maternal leptin concentrations were positively correlated with both objective (carcass analysis) and subjective (body condition score) indices of body fat status ($r = 0.64$ and 0.85 , respectively, $P < 0.05$) and negatively correlated with fetal cotyledon mass and lamb birth weight at term ($r = -0.54$ and -0.64 , respectively, $P < 0.05$). The hormonal profiles presented (Fig. 3) indicate that nutritionally enhanced secretion of insulin in the high-intake dams may co-ordinate increased lipogenesis and leptin expression. Intriguingly, hyperleptinaemia in the high-intake dams does

not suppress appetite during the second half of pregnancy and implies that these animals may be leptin-resistant. It remains to be established whether these changes in maternal leptin concentrations play a regulatory role in the metabolic adaptations required during pregnancy or merely reflect changing maternal fat status. Although the adipose tissue is the major site of leptin secretion, leptin is also produced by the placenta in a variety of species (Hoggard *et al.*, 1997; Masuzaki *et al.*, 1997) and leptin and its receptor are present in a variety of mouse tissues (Hoggard *et al.*, 1997). This finding has led to the suggestion that placental leptin has a direct local role in nutrient partitioning and fuel handling within the gravid uterus (Holness *et al.*, 1999). However, other laboratories, including our own, have failed to detect significant leptin mRNA expression in the ovine placenta, although preliminary results indicate that leptin receptors are present (Thomas *et al.*, 2001).

Role of the placenta in the nutrient partitioning trajectory

Fetal growth during mid- to late gestation is controlled by the size, metabolism and transfer capacity of the placenta and by the prevailing maternal nutritional status (for reviews, see Mellor, 1983; Bell *et al.*, 1999; Robinson *et al.*, 1999; Wallace *et al.*, 1999b). Ewes have an epitheliochorial-cotyledonary placenta and the number and size of the individual cotyledons determine the area available for nutrient exchange between the maternal and fetal systems. The decrease in placental mass observed at term in the overnourished adolescent dams reflects a significant reduction in both the number of cotyledons per placenta and mean fetal cotyledon mass (Table 1). Initially, we proposed that sub-optimal progesterone concentrations, which are a characteristic feature of high dietary intakes in both adult and adolescent sheep (Wallace *et al.*, 1994, 1997a), compromise growth of the differentiating conceptus resulting in fewer uterine caruncles being occupied. However, when progesterone concentrations were restored in high-intake dams by exogenous supplementation from day 5 to day 55 of gestation, placental mass and number of cotyledons were equivalent in high and high plus progesterone groups (Wallace *et al.*, 1998). Progesterone supplementation enhanced lamb birth weight but this was most probably due to a direct effect of progesterone on the embryonic inner cell mass.

In three separate studies, a significant reduction in total placental cotyledon mass in high-intake versus moderate-intake dams was observed at approximately days 77, 100 and 128 of gestation (18, 20 and 51% reductions, respectively; Da Silva *et al.*, 2000; Wallace *et al.*, 2000a; J. M. Wallace, P. Da Silva, D. A. Bourke and R. P. Aitken, unpublished). The growth of the placenta precedes that of the fetus and although the relative metabolic rate of the fetus is highest during mid-pregnancy (Bell *et al.*, 1986), placental mass (and presumably transport capacity) in the adolescent paradigm does not appear to limit fetal organ or body growth until the final third

Fig. 4. Relationship between total placental cotyledon mass and fetal mass in sheep at days 77 (○, ●), 100–104 (□, ■) and 128–136 (△, ▲) of gestation. Adolescent sheep were offered either a high (open symbols) or moderate (closed symbols) nutrient intake from embryo transfer (day 4) until autopsy in three separate studies days using the same sire.

of gestation (Fig. 4). However, the subsequent pattern of fetal growth and organ development may be programmed before any measurable changes in growth of the fetal body *per se*. For example, a change in the subcellular localization of one of the protein kinase C enzymes (protein kinase C- α), which is thought to be involved in growth and differentiation, is detected in the muscles of fetuses from high-intake dams at day 100 of gestation (Palmer *et al.*, 1998). Similarly, at this same gestational time-point, a significant reduction in the number of ovarian follicles in fetuses derived from high-intake versus moderate-intake dams is detected (Da Silva *et al.*, 2000). By day 128 of gestation, when the normally growing fetus has reached 85% of its predicted birth weight, fetuses from overnourished dams were 37% smaller than those from moderate-intake dams. All measures of fetal confirmation and absolute fetal organ masses, with the exception of adrenal gland mass, were lower in the fetuses from high-intake dams and were highly correlated with total placental cotyledon mass. However, fetal organ masses expressed as g kg⁻¹ fetal body weight were independent of maternal nutritional status. In addition, fetal mass but not maternal dietary intake was predictive of individual organ mass for all organs studied, indicating that, in this paradigm, placental growth restriction mediates a symmetrical slowing of fetal growth during the final third of gestation. The postnatal sequelae of these alterations in fetal growth, organ structure and body composition largely remain to be established. However, alterations in prenatal pituitary gonadotrophin gene expression and testes development in growth-restricted male fetuses (Da Silva *et al.*, 1998) appear to have a negative impact on both the endocrine and physical onset of puberty (Da Silva *et al.*, 1999).

Table 3. Uterine and umbilical blood flows at day 130 of gestation in adolescent sheep offered either a high or moderate dietary intake throughout pregnancy

	Maternal dietary intake		Significance
	High (n = 9)	Moderate (n = 9)	
Maternal weight at autopsy (kg)	69.4 ± 1.96	50.1 ± 1.44	***
Fetal weight at study (g)	3220 ± 306	4547 ± 285	**
Placental cotyledon mass (g)	224 ± 25	426 ± 44	**
Fetal : placental cotyledon mass	14.8 ± 0.83	11.3 ± 0.90	**
Uterine blood flow (ml min ⁻¹)	1213 ± 147	1928 ± 179	**
Uterine blood flow/conceptus weight (ml min ⁻¹ kg ⁻¹)	289 ± 21	320 ± 30	ns
Umbilical blood flow (ml min ⁻¹)	489 ± 54	787 ± 42	***
Umbilical blood flow/fetus weight (ml min ⁻¹ kg ⁻¹)	153 ± 11	185 ± 11	ns

Values are means ± SEM.
**P < 0.01; ***P < 0.001; ns, not significant.
Data from J. M. Wallace, D. A. Bourke, R. P. Aitken and W. W. Hay, Jr (unpublished).

During the final third of gestation, the placenta undergoes considerable structural remodelling and placental mass decreases as a result of tissue dehydration associated with loss of hyaluronic acid and other glycosaminoglycans (Ott *et al.*, 1997). The greater response to overfeeding at late gestation may reflect the duration of exposure to high maternal intakes resulting in a higher degree of tissue dehydration in high-intake versus moderate-intake dams. The reduction in placental mass in the high-intake dams reflects a smaller number of cells rather than a change in cell size, and high nutrient intakes predominantly inhibit growth of the fetal component of the placenta (Wallace *et al.*, 1999a, 2000a). When the classification system of Vatnick *et al.* (1991b) is used, a striking difference in morphology becomes apparent. The cotyledons from the high-intake dams are largely inverted with maternal tissue surrounding fetal tissue (A type). In contrast, many of the cotyledons in the moderate-intake dams are everted with fetal tissue growing over the maternal tissue (C and D types). C and D type cotyledons also predominated when pharmacological doses of progesterone were administered to pregnant ewes ovariectomized 3 weeks after mating (Alexander and Williams, 1966). Thus, the failure of the cotyledons from high-intake adolescent dams to evert may be the result of nutritionally induced alterations in circulating progesterone concentrations, which are significantly attenuated in the high- versus moderate-intake dams (Wallace *et al.*, 1997a,b). A similar predominance of everted cotyledons in adult ewes moderately (Heasman *et al.*, 1998) or mildly (Steyn *et al.*, in press) nutrient-restricted during early to mid-gestation has been reported and, in the study by Steyn *et al.* (in press), was associated with a compensatory increase in fetal placental villous density evident by day 90 of gestation. Indeed, during the last third of pregnancy, when the absolute nutrient requirements of the placenta and fetus are at maximum values, uterine and

umbilical blood flows, placental surface area and permeability are critical regulators of nutrient partitioning among the maternal, placental and fetal compartments (Carter and Myatt, 1995). Preliminary studies using the adolescent sheep paradigm reveal that both uterine and umbilical blood flows are significantly lower in high-intake compared with moderate-intake dams at day 130 of gestation. However, when uterine and umbilical blood flows are expressed per kilogram conceptus and fetus, respectively, the differences are no longer significant (Table 3, J. M. Wallace, D. A. Bourke, R. P. Aitken and W. W. Hay, unpublished). It remains to be established whether the angiogenic growth factors, which may regulate placental vascularization from early pregnancy onwards (Reynolds and Redmer, 1995), set the trajectory for these late pregnancy changes in blood flow and hence nutrient partitioning within the gravid uterus.

Critical window of sensitivity to maternal nutrient intake

Nutritional switch-over studies have been used to define when the placenta is most sensitive to maternal nutritional status and whether the effects on placental growth and pregnancy outcome can be reversed. The number of uterine caruncles occupied by the developing trophoblast is an event of early pregnancy, which is complete by day 50 of gestation (Barcroft and Kennedy, 1939). In contrast, the growth of the placenta has variously been reported to reach an apex in placental wet mass during mid-pregnancy between days 75 and 90 of gestation (Barcroft and Kennedy, 1939; Alexander, 1964; Ehrhardt and Bell, 1995). As detailed above, the restriction in placental growth in the overnourished adolescent dams at term is associated with a reduction in both the number and size of fetal cotyledons. Thus, the relative impact of maternal nutrient intake during

the first and second third of pregnancy on placental growth and pregnancy outcome was determined by switching adolescent dams from an anabolic to a catabolic state at day 50 of pregnancy and vice versa (Wallace *et al.*, 1999a). In this study, ewes were initially offered a high (H) or moderate (M) quantity of diet to promote rapid or normal maternal growth, respectively, then at day 50 of gestation the dietary intakes of half the ewes was changed to yield HH, MM, HM and MH groups. In ewes delivering live young at term, a high plane of nutrition from day 50 of gestation (HH and MH groups) was associated with a significant decrease in duration of gestation ($P < 0.009$), total placental mass ($P < 0.001$), total fetal cotyledon mass ($P < 0.001$) and mean fetal cotyledon mass per placenta ($P < 0.001$). The number of caruncles occupied by the developing conceptus was dependent on maternal dietary intake during the first 50 days of gestation only and was significantly lower ($P < 0.007$) in HH and HM dams. The reduced 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, and highlights the importance of appropriate maternal nutrition during mid-pregnancy in setting the placental, and hence fetal, growth trajectory.

Clinically, fetal growth restriction is rarely diagnosed until the second half of pregnancy, when it is often associated with reduced placental mass, altered placental function and sub-optimal uterine blood flow (Owens *et al.*, 1995). Maternal nutrient supplementation in women has largely been ineffective at improving pregnancy outcome unless the mother is severely undernourished at the time of conception (Rush, 1989) and, indeed, in adolescent girls protein supplementation in late pregnancy appears to depress birth weight (Rush, 1986). In well-fed ewes, direct infusion of glucose into the fetus during the final month of pregnancy stimulates fetal growth by 18% (Stevens *et al.*, 1990), while intravenous infusion of a glucose and amino acid mixture prevents fetal growth restriction induced by placental embolization (Charlton and Johengen, 1987). These direct approaches are attractive therapeutically in that they bypass the placenta. However, such invasive procedures are unlikely to gain clinical acceptance as they are associated with substantial risks to both mother and baby (Harding, 1999). A preliminary study using the adolescent sheep paradigm has indicated that it may be possible to alter the nutrient transport function of the growth-restricted placenta *per se* (Wallace *et al.*, 2000b). In this study, adolescent dams were offered a high or moderate amount of feed for the first 100 days of gestation. After day 100 of gestation, the feed intake of the moderate group was adjusted weekly to maintain their body condition score during the final third of gestation, whereas the amount of feed offered to half the ewes in the high group was abruptly decreased by 64% (high–low group). The induction of a catabolic phase in the high–low group was associated with a sharp decrease in maternal insulin and glucose concentrations and an increase in non-esterified fatty acid concen-

trations relative to the dams overnourished throughout pregnancy. At term, fetal cotyledon mass (58 ± 7.2 and 147 ± 18.4 g, $P < 0.01$), number of cotyledons (77 ± 4.4 and 91 ± 4.7 , $P < 0.05$) and fetal mass (3.10 ± 0.38 and 4.98 ± 0.13 kg, $P < 0.01$) adjusted for a standard duration of gestation of 147 days, were lower in high than in moderate groups. In the high–low group, fetal cotyledon mass (86 ± 6.0 g) and fetal mass (3.90 ± 0.16 g) were intermediate between the H and M group values, whereas the number of cotyledons (77 ± 5.6) was equivalent to that in the high group. These results indicate that the structural remodelling and functional adaptation of the placenta known to occur during the final third of pregnancy (Schneider, 1996) that normally results in a decrease in placental mass during this period (Fig. 4), can be altered in favour of fetal growth after the induction of a catabolic phase in previously rapidly growing adolescent sheep.

To date, our studies have demonstrated that inadequate placental growth is the primary cause of fetal growth restriction in overnourished adolescent sheep. A future aim will be to define which nutritionally mediated endocrine hormones and paracrine growth factors play a role in placental vascularization, growth and morphology. The restriction in fetal growth occurs in spite of the ready availability of nutrients in the maternal system and studies using this paradigm are investigating whether impaired fetal growth is the result of reduced placental size *per se* or of more subtle alterations in placental nutrient uptake, metabolism and transfer to the developing fetus. Preliminary results indicate that this form of placentally mediated fetal growth restriction has a significant impact on the onset of puberty in male lambs. Future studies involving both sexes will assess the impact of prenatal growth restriction on the programming of postnatal pituitary and gonadal secretory function and on adult fertility *per se*.

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