Regulation of placental nutrient transport and implications for fetal growth

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Fetal macronutrient requirements for oxidative metabolism and growth are met by placental transport of glucose, amino acids, and, to a lesser extent that varies with species, fatty acids. It is becoming possible to relate the maternal–fetal transport kinetics of these molecules *in vivo* to the expression and distribution of specific transporters among placental cell types and subcellular membrane fractions. This is most true for glucose transport, although apparent inconsistencies among data on the roles and relative importance of the predominant placenta glucose transporters, GLUT-1 and GLUT-3, remain to be resolved. The quantity of macronutrients transferred to the fetus from the maternal bloodstream is greatly influenced by placental metabolism, which results in net consumption of large amounts of glucose and, to a lesser extent, amino acids. The pattern of fetal nutrient supply is also altered considerably by placental conversion of glucose to lactate and, in some species, fructose, and extensive transamination of amino acids. Placental capacity for transport of glucose and amino acids increases with fetal demand as gestation advances through expansion of the exchange surface area and increased expression of specific transport molecules. In late pregnancy, transport capacity is closely related to placental size and can be modified by maternal nutrition. Preliminary evidence suggests that placental expression and function of specific transport proteins are influenced by extracellular concentrations of nutrients and endocrine factors, but, in general, the humoral regulation of placental capacity for nutrient transport is poorly understood. Consequences of normal and abnormal development of placental transport functions for fetal growth, especially during late gestation, and, possibly, for fetal programming of postnatal disorders, are discussed.

Placental transport: Glucose: Amino acids: Regulatory factors: Fetal growth

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Introduction

The placenta is an organ unique to pregnancy in higher animals, and is most highly developed in eutherian mammals. It acts as the conduit for exchange of nutrients and excreta between mother and fetus, as an endocrine regulator, as a paracrine and autocrine mediator of many pregnancy-specific physiological functions in maternal and conceptus tissues, and as an immunological 'blindfold' that serves to protect the conceptus from its maternal host. The present review will address only the nutrient transport functions of the placenta, confined to the macronutrients that support energy metabolism and synthesis of soft tissues. A major theme will be extracellular regulation of placental function and fetal growth responses as studied *in vivo* in the sheep and other model species. Fundamental mechanisms elucidated in studies employing isolated systems, such as placental membrane vesicles, will be discussed in the context of organismal integration. The putative endocrine roles of the placenta in regulation of maternal nutrient partitioning and fetal growth are reviewed elsewhere (Anthony *et al*. 1995; Bell & Ehrhardt, 1998).

Mechanisms of placental nutrient transport

Glucose and its metabolites

Glucose. Glucose is a principal energy substrate for fetal and placental metabolism in all mammalian species studied. Analysis of the kinetics of placental glucose transport *in vivo* indicated that this process is achieved by facilitated diffusion (Widdas, 1952; Simmons *et al*. 1979). The predominant glucose transporter (GLUT) protein isoforms in the sheep placenta are GLUT-1 and GLUT-3 (Ehrhardt & Bell, 1997; Das *et al*. 1998), mRNA and protein abundance of which increase with gestational age, especially for GLUT-3 (Currie *et al*. 1997; Ehrhardt & Bell, 1997). The relative importance of GLUT-3 also may be inferred from its immunolocalization at the apical, maternal-facing surface of the trophoblastic cell layer that forms the fetomaternal tissue barrier in the ovine placenta (Das *et al*. 2000). In contrast, GLUT-1 was localized at the basolateral and apical membrane surfaces in fetal trophoblast cells. The authors suggest that this localization pattern allows a collaboration between GLUT-3 and GLUT-1 at the maternal–fetal tissue barrier in which apical GLUT-3, with a lower K_m, readily takes up maternal glucose while basolateral GLUT-1, with a higher K_m, facilitates its intracellular transport and exit (Das *et al*. 2000).

In distinct contrast, GLUT-1 is considered to be the primary GLUT in the human placenta (see Illsley, 2000). In the term placenta, at least, GLUT-1 protein was located on both the microvillous and basal membranes of the syncytiotrophoblast (Jansson *et al*. 1993; Barros *et al*. 1995), but its asymmetric distribution favoured the microvillous surface (Jansson *et al*. 1993; Tadokoro *et al*. 1996). GLUT-1 mRNA was localized predominantly in syncytial as opposed to vascular tissue (Jansson *et al*. 1996). Although lower abundance of GLUT-3 mRNA was distributed throughout human villous tissue (Jansson *et al*. 1995; Hauguel-de Mouzon *et al*. 1997), GLUT-3 protein was essentially absent in syncytial membranes (Jansson *et al*. 1993; Barros *et al*. 1995), and was located primarily in vascular endothelium (Hauguel-de Mouzon *et al*. 1997).

The different patterns of cellular and intracellular location, and inferred functional roles of GLUT-1 and GLUT-3 in human and sheep placentae cannot be explained simply in terms of species differences in placental structure. In the haemochorial rat placenta, gestational changes in relative mRNA abundance of the two isoforms (Zhou & Bondy, 1993), and the asymmetrical

distribution of GLUT-3 in syncytiotrophoblastic membranes (Shin *et al.* 1997), are much more similar to characteristics of the epitheliochorial sheep placenta than to those of the haemochorial human placenta.

In addition to the important influences of cellular configuration and kinetics of GLUT proteins in the placenta, placental glucose transfer is dependent on the maternal–fetal plasma glucose concentration gradient (Simmons *et al.* 1979; DiGiacomo & Hay, 1990; Hay *et al.* 1990). This gradient is influenced by numerous factors that affect maternal and/or fetal glycaemia, including maternal nutrition and endocrine status, fetal growth capacity, and the variable but always large fraction of uterine glucose uptake that is consumed by the placenta (see Hay, 1995).

Lactate and fructose. Glucose taken up from the umbilical circulation is readily converted to lactate by the ovine fetal placenta *in vivo* (Aldoretta & Hay, 1999). The placentae of other species, including man (Challier *et al.* 1986), are similarly glycolytic. Placental perfusion studies (Kastendieck *et al.* 1979), combined with analysis of the fetoplacental exchanges of radiolabelled glucose and lactate (Bassett, 1986), indicate that the fetomaternal interface of the ovine placenta is highly impermeable to lactate. Thus, little or none of the energetically significant net uptake of lactate by the ovine fetus is derived directly from the maternal circulation or glycolysis in the maternal caruncular tissues. In contrast, the placenta of the guinea-pig is much more permeable to lactate (Kastendieck & Moll, 1977). The same is presumably true for other species, including man, with haemochorial placentae.

Certain species, most notably the ungulates, also metabolize fetal glucose to fructose in trophoblastic tissues nourished by the umbilical circulation (Britton *et al.* 1967; Meznarich *et al.* 1987). Like lactate, this metabolite is mostly confined within the fetoplacental compartment by the impermeability of the maternal–fetal tissue interface, consistent with the almost complete absence of fructose in the maternal circulation of species with fructogenic placentae (Goodwin, 1952). This implies that in these species, as in man (Illsley, 2000), the placenta lacks GLUT-5 or a similar protein that is responsible for fructose transport in the jejunum and some other tissues (Mueckler, 1994).

Amino acids

Most amino acids taken up by the placenta are transported against a fetal–maternal concentration gradient, implying the use of energy-dependent, active transport processes (Young & McFadyen, 1973). Studies of isolated plasma vesicles prepared from rodent and human trophoblast have confirmed that the placenta actively transports amino acids using systems previously described for plasma membranes in other tissues (Battaglia & Regnault, 2001). These are classified as Na-dependent or Na-independent, and on their preference for neutral (zwitterionic), acidic (cationic), or basic (anionic) amino acids, leading to six fundamental groups: A, ASC, L (or *l*), y+, β, and glycine transporter systems. Not all systems are present on the same membrane and there appears to be a greater variety of transporters on the maternal-facing microvillous membrane than on the basolateral adjacent to the umbilical microcirculation. The functional significance of these systems for transporting different categories of amino acids, including membrane location, have been comprehensively reviewed and systematically organized by Battaglia & Regnault (2001).

Recent *in vivo* studies on pregnant sheep have done much to relate knowledge of amino-acid transport systems in isolated placental vesicles to the physiology of amino acid transfer

from maternal to fetal circulations. These experiments have strongly suggested that rapid pla-
cental transport of neutral amino acids requires not only Na-dependent transport at the maternal
epithelial surface, but affinity for highly reversible, Na-independent transporters located at the
fetal surface (Jozwik *et al*. 1998). Additional studies by the same group provided supporting
evidence for this hypothesis and also showed that there are major differences in placental clear-
ance among the essential amino acids (Paolini *et al*. 2001). Five of the nine essential acids,
namely, the branched-chain amino acids leucine, isoleucine, and valine, plus methionine and
phenylalanine, have similar and relatively fast clearances, in contrast to the slower clearances
of tryptophan, threonine, histidine, and lysine (Fig. 1). It is notable that the more rapidly
cleared acids are all zwitterions with hydrophobic side chains. The evidence suggests that they
have similar affinity for a rate-limiting transport system that mediates rapid flux from the
maternal to the fetal circulation, most likely the Na-independent L system located on the fetal
surface of the placenta (Jozwik *et al*. 1998). It is reassuring to note that the relative rates of *in
vivo* transport of the amino acids studied thus far in the sheep are consistent with the more lim-
ited data for the human placenta (see Battaglia & Regnault, 2001).

It can be inferred that many of the amino-acid transporter proteins cloned in other tissues
are expressed and functional in the placenta. However, relatively few of these proteins have
been cloned from placental tissue and the molecular explanation of placental amino-acid trans-
port systems in terms of the regulated, differential expression of various transporter proteins in
trophoblastic cell membranes is in its infancy (for example, Kamath *et al*. 1999; Ayuk *et al*.
2000).

Fatty acids

Placental capacity for maternal–fetal transport of short- and long-chain fatty acids and their
keto-acid derivatives varies widely among species. For example, the epitheliochorial placentae
of ruminants and swine generally appear to be much less permeable to fatty acids than are the
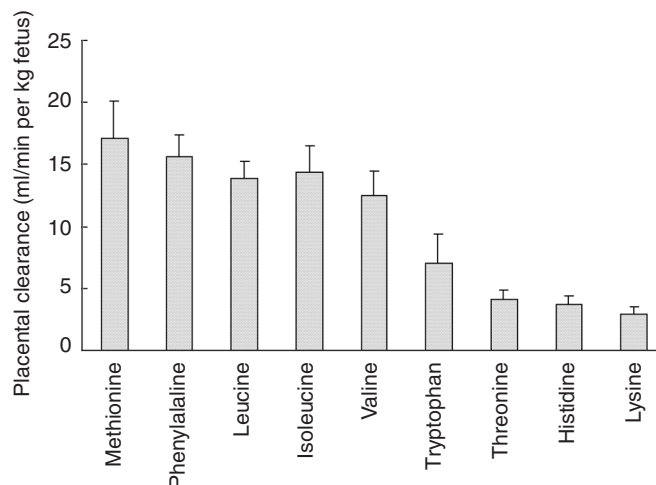
Fig. 1. Transplacental clearance of isotopically labelled essential amino acids in seven ewes during late
pregnancy. Histograms are means with their standard errors shown as vertical bars. (Data are from Paolini
et al. 2001.)

haemochorial placentae of rodents, lagomorphs, and man (Battaglia & Meschia, 1988). Thus, despite the relative abundance of acetate and other derivatives of rumen fermentation, such as 3-hydroxybutyrate, in the circulation of maternal ruminants, these substrates make only minor contributions to the energy metabolism of fetal sheep (Morriss *et al*. 1974; Char & Creasy, 1976) and cattle (Comline & Silver, 1976). Transport of long-chain, non-esterified fatty acids is similarly meagre in the sheep (Elphick *et al*. 1979; Leat & Harrison, 1980) and pig (Elphick *et al*. 1980). However, placental transfer of non-esterified fatty acids is greater and more responsive to maternal plasma non-esterified fatty acid concentration in the rat (Hummel *et al*. 1975), rabbit (Elphick & Hull, 1977), and guinea-pig (Thomas & Lowy, 1983). Transport mechanisms have yet to be defined conclusively, but a role for a placenta-specific fatty-acid binding protein identified in man (Campbell *et al*. 1995) and sheep (Campbell *et al*. 1994) has been proposed by Dutta-Roy (2000). It also has been suggested that this protein may be involved in the preferential transfer of long-chain, polyunsaturated fatty acids from maternal plasma to the fetus (Campbell *et al*. 1996*b*). As discussed later, fetal acquisition of these vital fatty acids is additionally facilitated by placental elongation and desaturation of C18 essential fatty acids.

Influence of placental metabolism on maternal–fetal nutrient transfer

Glucose metabolism

Uteroplacental tissues consume 60–70 % of uterine net uptake of glucose during late pregnancy in sheep and cattle (see Bell, 1993; Table 1). Placental glucose consumption is also substantial in other species, although apparently lower in the haemochorial placenta of man (Hauguel *et al*. 1986), rats (Leturque *et al*. 1987), and rabbits (Hauguel *et al*. 1988) than in the epitheliochorial placenta of ruminants. In sheep, uterine arterial glucose concentration determines the net uptake of glucose by the uterus from the maternal circulation, while changes in fetal arterial glucose concentration independently determine the partition of glucose between uteroplacental tissues and the fetus (Hay *et al*. 1990). However, under normal conditions, fetal arterial glucose concentration, and thus its influence on uteroplacental glucose consumption, is directly related to maternal arterial glucose concentration (Hay *et al*. 1983; Hay & Meznarich, 1988; Leury *et al*. 1990*a*).

Table 1. Effect of maternal glucose supply on uptake and metabolism of glucose by the conceptus in eight ewes during late pregnancy*
(Mean values and standard errors of the mean)

| | Glucose supply† | | | |
| | Low | | High | |
Variable	Mean	SEM	Mean	SEM
Maternal plasma glucose concentration (mM)	2·23	0·13	4·92	0·29
Uterine glucose uptake (μmol/min)	239	23	437	40
Fetal glucose uptake (μmol/min)	75	5	125	9
Uteroplacental metabolic rates (μmol/min)				
Glucose consumption	164	22	312	39
Lactate production	109	12	183	21
Fructose production from glucose	4	1	7	3
Glucose oxidation	27	4	43	8

* Data from Aldoretta & Hay (1999).
† Manipulated by fasting for 24 h (low) and intravenous infusion of dextrose (high).

Glucose consumed by the ovine placenta undergoes rapid conversion to lactate (about 35 %), fructose (about 4 %), and CO_2 (about 17 %), accounting for about 56 % of uteroplacental glucose disposal in late-pregnant ewes with low or high maternal plasma glucose concentrations (Aldoretta & Hay, 1999; Table 1). The metabolic fate of the remaining 44 % of glucose that is apparently metabolized by the placenta is not known and requires investigation. Lactate and fructose formed in trophoblastic tissues was released into the fetal circulation while lactate formed in caruncular tissues was released into the maternal circulation, at rates that were directly related to maternal glucose supply. Rapid oxidation of glucose accounted for about 23–34 % of uteroplacental oxygen consumption, depending on maternal glycaemia (Aldoretta & Hay, 1999). Oxidizable substrates that might contribute to the remaining 66–77 % of uteroplacental respiration include ketones (Carver & Hay, 1995) and acetate, at least in caruncular tissues (Bell, 1993), branched-chain and certain other amino acids (see later), and C derived from the turnover of carbohydrate and lipid stores in placental tissues.

Amino acid metabolism

Placental metabolism substantially influences the quantity and composition of amino acids delivered to the fetus. Turnover of placental constitutive proteins is very rapid (Young *et al.* 1982) but net deposition of protein is negligible during the latter half of ovine pregnancy (Ehrhardt & Bell, 1995). Thus, the appreciable net consumption of glutamate, serine, and branched-chain amino acids by the placenta (Liechty *et al.* 1991; Chung *et al.* 1998) implies significant catabolism or transamination of these acids. Secretion of placental peptides must account for an additional, small fraction of this net loss of amino acids.

The ovine placenta produces copious amounts of ammonia that is released into maternal and, to a much lesser extent, fetal circulations (Holzman *et al.* 1977; Bell *et al.* 1989). This is consistent with its lack of enzymic capacity for urea synthesis (Edwards *et al.* 1977), its extensive deamination of branched-chain amino acids to their respective keto acids, which are released into fetal and maternal circulations (Smeaton *et al.* 1989; Loy *et al.* 1990), and its rapid rate of glutamate oxidation (Moores *et al.* 1994). Transamination of branched-chain amino acids accounts for some of the net acquisition of glutamate by the placenta, the remainder of which is taken up from the umbilical circulation (Moores *et al.* 1994). That which is not quickly oxidized combines with ammonia to form glutamine, which is then released back into the fetal bloodstream (Chung *et al.* 1998). Some of this glutamine is converted back to glutamate in the fetal liver, which produces most of the glutamate consumed by the placenta (Vaughn *et al.* 1995). This glutamate–glutamine shuttle promotes placental oxidation of glutamate and fetal hepatic utilization of the amide group of glutamine.

Similarly, the placenta almost quantitatively converts serine, mostly taken up from the uterine circulation, to glycine (Chung *et al.* 1998), reconciling the discrepancy between the negligible net uptake of glycine by the uterus and the substantial net release of this amino acid into the umbilical circulation (Geddie *et al.* 1996). In addition to ensuring an adequate supply of the most abundant amino acid in fetal blood and tissues, this process is important for placental purine synthesis via donation of the side-chain β-carbon atom of serine to form methylene-tetrahydrofolate.

The complex interrelationships among placental uptake, metabolism, and transport of amino acids are further illustrated by a tracer study of alanine metabolism in the late-pregnant ewe (Timmerman *et al.* 1998). This study revealed that the trivial net placental exchange of alanine masks an appreciable rate of metabolism of maternal alanine taken up by the placenta that

exchanges with endogenously produced alanine. Thus, most of the alanine delivered to the fetus is of placental origin, derived from placental protein turnover and transamination.

Fatty acid metabolism

Fetal plasma and tissue lipids have characteristically low concentrations of the essential C18 fatty acids, linoleic and linolenic acids, and high concentrations of their respective C20 and C22 derivatives, arachidonic and docosahexaenoic acids, relative to the composition of maternal plasma lipids (see Noble, 1979). Although fetal tissues have some capacity to elongate and desaturate C18 acids, observed rates of maternal–fetal transfer of the these acids would provide too little substrate for fetal metabolism to account for more than a fraction of the abundance of C20 and C22 acids in fetal tissues, especially in ruminants (Elphick *et al*. 1979; Leat & Harrison, 1980). Active systems for desaturation and chain-elongation of linoleic and linolenic acids have been identified in sheep placenta (Noble *et al*. 1985). Also, the ovine placenta has considerable capacity for hydrolysis of esterified lipids from maternal plasma that are richer than plasma non-esterified fatty acids in linoleic and linolenic acids (Clegg, 1981). Thus, placental metabolism ensures an adequate fetal supply of the longer-chain *n*-6 and *n*-3 metabolites of the C18 polyunsaturated fatty acids, which are the forms ultimately required by tissues.

### Factors affecting placental capacity for nutrient transport

Stage of gestation

Placental capacity for glucose transport *in vivo* increases fivefold during the latter half of pregnancy in sheep (Molina *et al*. 1991). Part of this increase undoubtedly is related to placental remodelling and expansion of exchange surface area over this period (Stegeman, 1974). However, there is also a substantial gestational increase in the expression and membrane density of functional GLUT proteins as assessed by the concentration of cytochalasin B binding sites and mRNA and protein abundance of GLUT-1 and GLUT-3 (Ehrhardt & Bell, 1997). The relative importance of GLUT-3 is indicated by its greater rate of gestational increase in protein abundance, closely correlated with an increasing fraction of cytochalasin binding that cannot be accounted for by GLUT-1, the only other GLUT of quantitative significance in the ovine placenta (Fig. 2). These results are generally consistent with gestational changes in the relative abundance of GLUT-1 and GLUT-3 in the rat placenta (Zhou & Bondy, 1993).

In contrast, the relatively modest gestational increase in glucose transport capacity in the human placenta has been ascribed to increased expression of GLUT-1 (Sakata *et al*. 1995), especially in the basal membrane fraction of syncytiotrophoblastic cells (Jansson *et al*. 1993). Abundance of GLUT-3 mRNA in human villous tissue decreased with advancing gestation (Sakata *et al*. 1995).

Gestational development of placental amino-acid transport systems has not been investigated systematically in any species. However, developmental changes in expression of amino-acid transport proteins and transport kinetics have been described in rat (Novak *et al*. 1996) and human (Ayuk *et al*. 2000) placentae, and reviewed by Battaglia & Regnault (2001).
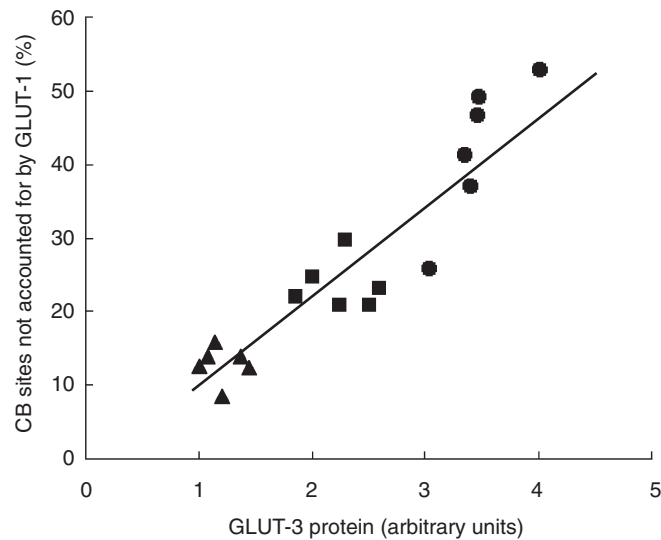
Fig. 2. Relationship (R^2 0·86) between fraction of cytochalasin B (CB) binding sites not accounted for by immunoneutralization of glucose transporter (GLUT)-1 protein and abundance of GLUT-3 protein in the placentae of ewes at 75 d (▲), 110 d (■), and 140 d (●) of pregnancy. Each point represents the placenta of a twin fetus. (Adapted from the data of Ehrhardt & Bell, 1997.)

Placental size and its functional correlates

During the latter half of pregnancy, positive correlations between fetal and placental weights become progressively stronger, such that in well-nourished, polytocous ewes close to term, variation in placental weight accounts for about 90 % of variation in fetal weight (Greenwood *et al*. 2000; Fig. 3). Various experimental manipulations have been used to demonstrate persuasively that placental weight is indeed a powerful determinant of fetal growth, especially during late gestation. These treatments include pre-mating carunclectomy, which variably reduces opportunity for establishment of cotyledonary placentation in sheep (Alexander, 1964), chronic environmental heat stress (Alexander & Williams, 1971), and, more recently, overfeeding of relatively young, primiparous ewes (Wallace *et al*. 2000). Each of these procedures causes a primary reduction in placental growth that is followed by fetal growth retardation in late pregnancy (Alexander & Williams, 1971; Vatnick *et al*. 1991; Robinson *et al*. 1995; Wallace *et al*. 2000). The similarity in patterns of association between fetal and placental weights caused by these approaches and that due to natural variation in polytocous ewes (Greenwood *et al*. 2000) suggests that they provide comparable models of placental insufficiency during late pregnancy.

Experimental and, presumably, natural reduction in placental size is highly correlated with decreases in several important determinants and indices of placental capacity for nutrient transport, and with consequent changes in fetal metabolism and growth during late gestation. For example, reduction in membrane surface area for placental exchange in carunclectomized ewes had little effect on fetal weight at 90 d but was insufficient for normal growth at 120 d of gestation (term being about 150 d) (Robinson *et al*. 1995). In both carunclectomized and heat-treated ewes there were also reductions in uterine and umbilical blood flows, consistent with reduced placental clearance of highly diffusible, flow-limited solutes such as antipyrine, ethanol, and ^3H_2O, and metabolic consequences such as reduced placental uptake and transport of oxygen,
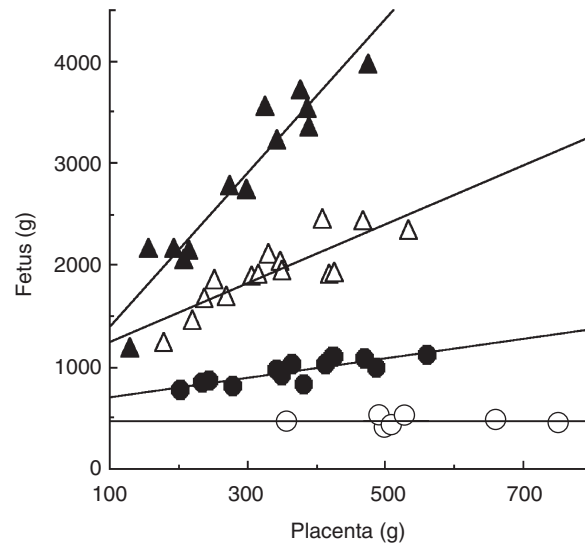
Fig. 3. Relationships between fetal weight and placental weight in ewes at 85 d ($\bigcirc$, R^2 0·00); 100 d ($\bullet$, R^2 0·70); 115 d ($\triangle$, R^2 0·73); 130 d ($\blacktriangle$, R^2 0·91) of pregnancy (Greenwood *et al*. 2000).

and development of fetal hypoxaemia (for reviews see Bell, 1987; Owens *et al*. 1989; Robinson *et al*. 1995; Bell *et al*. 1999). It is unlikely that reduced placental perfusion is a primary constraint for placental transport of glucose and amino acids because the placental clearance of these nutrients is diffusion-limited rather than flow-limited. For example, the placental delivery of glucose to the umbilical circulation is not responsive to physiological variations in uterine blood flow (Wilkening *et al*. 1985). Severe restriction of placental perfusion, as can occur during exercise or acute heat stress (Bell, 1987), may indirectly affect active transport of amino acids through negative effects on placental energetics and ion gradients (Hay, 1998).

Placental capacity for glucose transport was reduced greatly, as were uteroplacental glucose consumption and fetal glycaemia in carunclectomized (Owens *et al*. 1987*a*,*b*) and heat-treated (Bell *et al*. 1987; Thureen *et al*. 1992) ewes. Part of the absolute reduction in glucose transport capacity must be due to reduced exchange surface area of the trophoblastic membrane, implying fewer GLUT in total. In previously heat-treated (Thureen *et al*. 1992), but not in carunclectomized (Owens *et al*. 1987*b*) ewes, placental weight-specific glucose transport capacity also was reduced. Thus, chronic heat stress, which reduces average weight but not total number of placentomes, additionally reduces number and/or activity of GLUT proteins at maternal and/or fetal exchange surfaces. In contrast, carunclectomy, which reduces placentome number but may stimulate a compensatory increase in average weight of individual placentomes, caused a modest increase in placental weight-specific clearance of the non-metabolizable glucose analogue, 3-*O*-methyl glucose (Owens *et al*. 1987*b*). This implies that GLUT expression was increased somewhat in the reduced number of placentomes.

Placental insufficiency in heat-treated ewes also extends to impaired capacity for amino acid transport, including major reductions in placental uptake and maternal–fetal transfer of leucine (Ross *et al*. 1996; Table 2) and threonine (Anderson *et al*. 1997). The magnitude of

Table 2. Effect of heat-induced intra-uterine growth retardation (IUGR) on placental transfer and metabolism of leucine in thirteen ewes during late pregnancy*
(Mean values and standard errors of the mean)

| Variable	Control Mean	SEM	IUGR Mean	SEM
Fetal weight (kg)	3·34	0·14	1·44	0·11
Placental weight (kg)	0·39	0·03	0·14	0·02
Leucine fluxes (μmol/min per kg fetus)				
Uterine uptake	8·6	1·1	3·4	0·7
Umbilical uptake	3·8	0·2	3·5	0·4
Uteroplacental oxidation	1·1	0·2	0·4	0·1
Additional uteroplacental metabolism	3·0	1·2	−0·3	0·6
KIC fluxes (μmol/min per kg fetus)				
Uterine uptake	−0·2	0·2	0·2	0·1
Umbilical uptake	0·5	0·1	0·0	0·2

KIC, ketoisocaproic acid.
* Data from Ross *et al.* (1996).

these reductions implies decreased abundance and/or activity of specific transporter proteins, in addition to a reduction in exchange surface area. The normally extensive placental catabolism of leucine was also greatly reduced in heat-treated ewes (Ross *et al*. 1996; Table 2), presumably as a means of conserving fetal access to this essential amino acid. Fetal:maternal leucine enrichment decreased in human pregnancies exhibiting intrauterine growth retardation (Marconi *et al*. 1999) as in heat-treated sheep (Ross *et al*. 1996), suggesting similar mechanisms of transport and metabolism of leucine in the two species.

Maternal nutrition

Recent evidence from our laboratory indicates that the placenta is not merely a passive respondent to maternal nutrient supply, and that activity of placental transport mechanisms may be directly modulated by maternal nutrition. For example, moderate undernutrition of ditocous ewes for 2 weeks during late pregnancy caused a 50 % increase in maternal–fetal capacity for glucose transport *in vivo* (Ehrhardt *et al*. 1996*b*), determined by compartmental modelling of the bi-directional exchanges of radiolabelled 3-*O*-methyl glucose between mother and twin fetuses (Ehrhardt *et al*. 1996*a*; Fig. 4). This was at least partly explained by a 20 % increase in total GLUT abundance, assessed by binding of cytochalasin B, associated with a similar increase in GLUT-3 protein abundance (Ehrhardt *et al*. 1998). These responses help explain how placental glucose transfer remained sufficient to sustain normal fetal growth, despite chronic maternal hypoglycaemia and a 26 % decrease in the maternal–fetal gradient in arterial plasma glucose concentration (Bell *et al*. 1999).

During more severe, chronic undernutrition or starvation for several days, the development of profound fetal hypoglycaemia helps to sustain the maternal–fetal gradient in glucose concentration by restricting the reverse transfer of glucose to the placenta, and reducing placental glucose consumption (see Hay, 1995). Under these more stringent conditions, fetal gluconeogenesis is induced (Leury *et al*. 1990*b*), with amino acids being the presumed major substrate, consistent with increased fetal urea synthesis (Lemons & Schreiner, 1983; Faichney & White, 1987). The ultimate consequence is reduced fetal tissue protein synthesis (Krishnamurti & Schaefer, 1984) and slowing of fetal growth to a rate that can be sustained by the reduced placental nutrient supply.
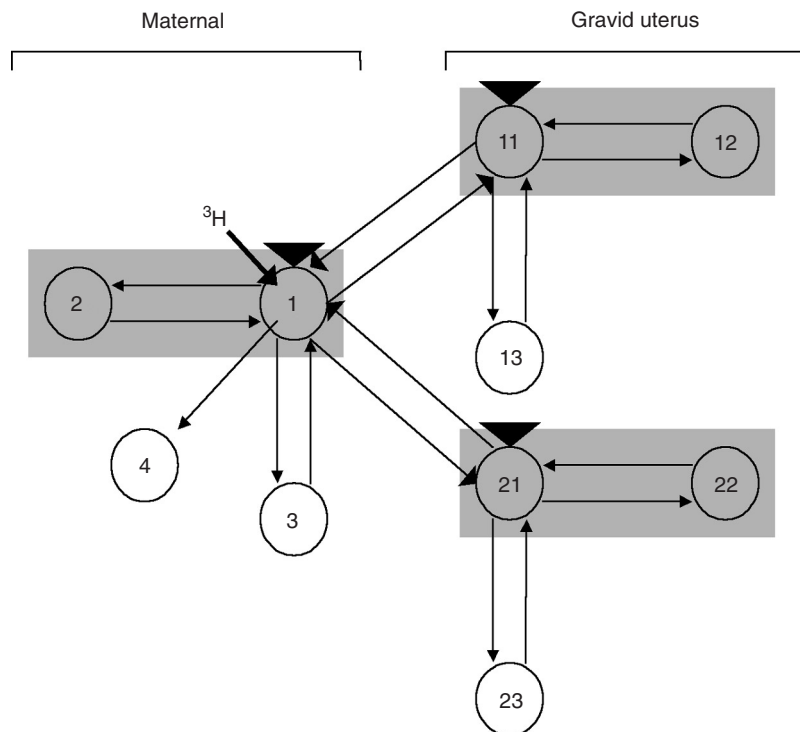
Fig. 4. Compartmental model of the kinetics of 3-*O*-methyl glucose (3MG) in ditocous ewes during late pregnancy, based on maternal injection of [³H]3MG. The fetal model was validated in monotocous ewes by simultaneous maternal injection of [³H]3MG and fetal injection of [¹⁴C]3MG (Ehrhardt *et al*. 1996*a*). (▲), Blood sampling sites; (→), fractional transfer rates; (○), compartments; (➙), [³H]3MG injection site; (●), extra-cellular distribution of 3MG. Maternal–fetal clearance of 3MG was calculated as the volume of compartment 1 cleared of tracer to compartment 11 or 21 per unit time (ml/min).

Effects of energy and/or protein supply on placental capacity for amino acid transport have been little studied. Fasting late-pregnant ewes for 5 d had an insignificant effect on umbilical net uptake of amino acids despite appreciable decreases in maternal arterial plasma concentrations of many amino acids (Lemons & Schreiner, 1983). This suggests that during short-term energy–protein deprivation, placental mechanisms for active transport of amino acids are unimpaired and may even be up regulated. Under similar fasting conditions, the uteroplacental deamination of branched-chain amino acids appeared to be increased, judging from a threefold increase in the efflux of α-ketoisocaproate, the keto-acid derivative of leucine, into uterine and umbilical circulations (Liechty *et al*. 1991). This suggests that increased amino acid catabolism may partly compensate for the likely reduction in placental glucose oxidation under these conditions.

Placental transport and metabolism of amino acids have not been studied during more prolonged restriction of energy or protein. However, in ewes fed adequate energy but insufficient protein during the last month of pregnancy, fetal growth and protein deposition over this period were decreased by 18 % (McNeill *et al*. 1997). It is also relevant that in chronically hyperglycaemic ewes with secondary hyperinsulinaemia and hypoaminoacidaemia, placental and fetal uptakes of several amino acids were reduced, and fetal total N uptake was decreased by 60 % (Thureen *et al*. 2001).

Extracellular regulation of placental capacity for nutrient transport

Maternal nutrient supply

Glucose. The experimentally determined K_m for saturable glucose transport by the ovine placenta is about 3·9 mM (Simmons *et al*. 1979), which is within the physiological range of glycaemia in well-fed, adult sheep. Thus, uterine uptake and placental consumption and transfer of glucose are very sensitive to arterial glucose concentration in pregnant ewes (see Hay, 1995). Emerging evidence suggests that chronic changes in maternal glycaemia may additionally modulate glucose transport capacity by influencing the placental expression of GLUT proteins. Maternal hyperglycaemia for up to 3 weeks during late pregnancy caused persistent decreases in placental concentrations of GLUT-1 (Das *et al*. 1998) and GLUT-3 (Das *et al*. 2000). In contrast, chronic, insulin-induced maternal hypoglycaemia decreased the concentration of GLUT-1 (Das *et al*. 1998) but had no effect on GLUT-3 (Das *et al*. 2000). The authors speculated that this 'relative' increase in GLUT-3 might help explain their repeated observation that placental glucose consumption was less severely affected by maternal hypoglycaemia than was placental glucose transfer to the fetus (Carver & Hay, 1995; Das *et al*. 2000). It is notable that, as discussed previously, chronic hypoglycaemia in underfed, late-pregnant ewes was associated with a modest but significant increase in placental expression of GLUT-3 (Ehrhardt *et al*. 1998).

The possible influence of maternal glycaemia on placental expression and activity of GLUT in the human placenta is of interest, not least because of the incidence of gestational and non-gestational diabetes in women. Illsley (2000) has summarized the conflicting evidence based on culture and incubation of human trophoblastic cells over varying periods in media containing different concentrations of glucose. He concluded that while extracellular concentration of glucose may exert short-term effects on capacity for glucose transport, placental expression of GLUT-1 is refractory to changes in glucose concentrations within the physiological range. However, it remains possible that this refractoriness of the human placenta is a consequence of the *in vitro* conditions employed, in contrast to the relative sensitivity of the sheep placenta *in vivo* to physiological variations in glycaemia.

Amino acids. Relationships between maternal plasma concentration and placental transport of amino acids are complicated by the multiplicity of active transport systems, and by differing affinities of several amino acids for the same, non-specific transporters. However, it is becoming apparent that maternal concentration is an important determinant of placental transport of neutral amino acids that have a relatively high affinity for the Na-independent L exchange system located on the fetal exchange surface (Jozwik *et al*. 1999; Paolini *et al*. 2001; Thureen *et al*. 2001). These amino acids, which are most rapidly transported from the maternal to the fetal circulation, include the branched-chain acids, plus methionine, and phenylalanine (Paolini *et al*. 2001; Fig. 5). Maternal infusion has a positive effect on umbilical uptake of the branched-chain amino acids but is a relatively ineffective means of increasing fetal supply of most other amino acids (Jozwik *et al*. 1999).

Hormones and growth factors

Extracellular regulation of placental transport functions must be effected by direct endocrine influences, as well as by nutrient supply. The following, briefly discussed examples highlight the need for systematic investigation of this area.
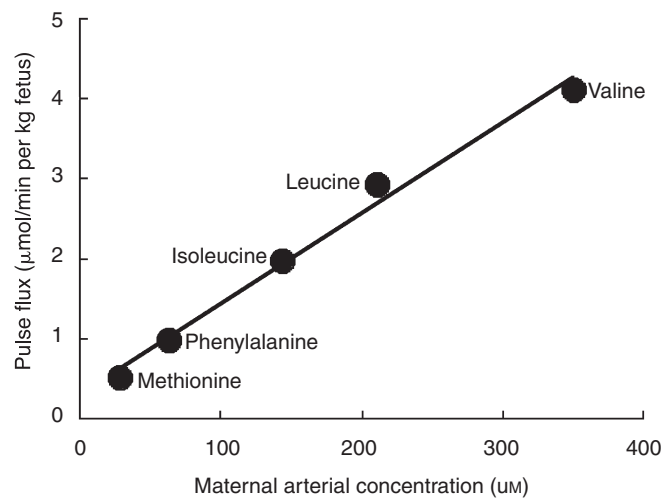
Fig. 5. Relationship (R^2 0·98) between transplacental pulse flux of isotopically labelled amino acids after single injection into the maternal femoral vein and maternal plasma arterial concentration before injection for five essential amino acids that have high and similar rates of placental clearance (see p. 222 and Fig. 1). Each value is the mean of seven observations. (Adapted from the data of Paolini *et al.* 2001.)

Insulin. Fetal plasma insulin concentration is a regulator of glucose consumption by insulin-responsive fetal tissues (Wilkening *et al*. 1987). Through consequent changes in fetal glycaemia and the maternal–fetal gradient in glucose concentration, it can also influence umbilical uptake of glucose (Simmons *et al*. 1978). However, when respective fetal or maternal plasma glucose concentrations were controlled using the euglycaemic clamp procedure, neither fetal (Jodarski *et al*. 1985) nor maternal (Rankin *et al*. 1986) insulinaemia had any effect on the placental clearance of the non-metabolizable glucose analogue, 3-*O*-methyl glucose. Similarly, maternal hyperinsulinaemia had no effect on uterine or umbilical uptake of glucose when maternal eug-lycaemia was maintained (Hay *et al*. 1984). These observations are consistent with our failure to detect significant concentrations of the insulin-responsive glucose transport protein, GLUT-4, in the ovine placenta (Ehrhardt & Bell, 1997). They also agree with the observed lack of effect of insulin on glucose uptake and transport by the human placenta (Challier *et al*. 1986; Brunette *et al*. 1990), and the failure to detect significant levels of GLUT-4 in human placental tissues (Hauguel-de Mouzon *et al*. 1994; Barros *et al*. 1995). Greater abundance of GLUT-4 has been detected in intravillous stromal cells, apparently co-localized with insulin receptors (Xing *et al*. 1998), but this probably has little relevance to the regulation of glucose transfer by syncytial cells.

It is also unlikely that fetal insulin indirectly promotes fetal growth via a direct influence on placental growth and functional capacity. Pancreatectomy of fetal lambs (Fowden *et al*. 1989) and streptozotocin treatment of fetal rabbits (Fletcher & Bassett, 1986) profoundly reduced fetal growth without overt effects on placental development.

Insulin-like growth factors. Insulin-like growth factors (IGF)-I and -II enhanced cytochalasin B-inhibitable deoxyglucose uptake in cultured human trophoblast cells (Kniss *et al*. 1994). However, infusion of IGF-1 into the maternal (Liu *et al*. 1994) or fetal (Harding *et al*. 1994; Liechty *et al*. 1996) circulation of sheep had no effect on uterine, uteroplacental, or umbilical exchanges of glucose. These were short-term experiments and it remains possible that the IGF

have a more chronic influence on expression and/or activity of placental GLUT. Possible local effects of IGF, their binding proteins, and other growth factors produced by the placenta also require systematic investigation.

Several lines of evidence indicate that IGF-II plays an important role in placental growth and functional development. It is abundantly expressed in various placental cell types throughout gestation (see Zumkeller, 2000) and the characteristic phenotype of mice with a null mutation of the IGF-II gene is profound placental growth retardation, associated with fetal stunting (Baker *et al*. 1993). This suggests an important autocrine or paracrine role for IGF-II in placental growth. In contrast, gene knockout of IGF-I had no effect on the placenta (Baker *et al*. 1993). Placental expression of IGF-II is paternally imprinted (DeChiara *et al*. 1991), offering a possible explanation for the apparently greater influence of paternal *v*. maternal genotype on prenatal growth and birth weight of cattle (Garrick *et al*. 1989).

Leptin. It is becoming apparent that leptin, the *ob* gene product that is expressed principally in white adipose tissue, may have numerous peripheral functions in addition to its role as a humoral signal of energy balance to the brain (Harris, 2000). Likely target organs include the placenta, which, in sheep, strongly expresses the leptin receptor variant Ob-Rb (Thomas *et al*. 2001; Ehrhardt *et al*. 2002) that is considered essential for intracellular signal transduction after the binding of leptin. Maternal plasma leptin concentration is elevated during early to mid pregnancy in sheep, independent of changes in body fatness (Ehrhardt *et al*. 2001), at a time when rapid placental growth may be most sensitive to extracellular influences (Ehrhardt & Bell, 1995). It is, therefore, intriguing to note that elevated levels of maternal plasma leptin tended to be associated with impaired placental growth during mid gestation in overconditioned, primiparous ewes (Thomas *et al*. 2001). The putative role of leptin in modulation of the functional development of the placenta requires direct investigation.

Implications of placental transport functions for fetal growth in late gestation

Except under the most extreme conditions, fetal growth is little affected by maternal nutrient supply or placental capacity for nutrient transport before the last third of gestation. In late pregnancy, maternal hypoglycaemia induced by fasting or severe undernutrition of ewes is associated with a perceptible slowing of fetal growth within a few days (see Mellor, 1983). The negative influence of preceding placental growth retardation and functional insufficiency is equally clear (Bell, 1987; Robinson *et al*. 1995). However, there is also strong evidence that the normal and appropriate slowing of fetal growth in well-fed animals during late pregnancy is due to placental constraint (Mellor, 1983; Bell *et al*. 1999). The degree to which this is mediated by regulation of placental transport characteristics, independently of membrane surface area for nutrient exchange, is uncertain. For example, placental GLUT normally operate well below saturation over the range of glucose concentrations normally experienced, suggesting that their membrane concentration or activity might not be limiting for glucose transport. On the other hand, even in optimally fed, monotocous ewes with normal placentae, circumvention of the placenta by direct fetal infusion with glucose during the last month of gestation increased lamb birth weight by almost 20 % (Stevens *et al*. 1990). The relative lack of fetal growth response to high levels of maternal protein intake (McNeill *et al*. 1997), consistent with the limited effect of maternal amino acid infusion on umbilical uptake of amino acids (Jozwik *et al*. 1999), also suggests placental limitation of the transfer of these vital nutrients.

Placental influences on fetal programming and its consequences in postnatal life

Beginning with the observations summarized by Barker (1994), accumulating epidemiological evidence from several human populations suggests that prenatal nutrient supply affects not only patterns of growth of conceptus tissues but also the incidence of disorders such as cardiovascular disease and type II diabetes during adulthood (see Langley-Evans, 2001). This has led to the notion of 'fetal programming', based on the postulate that nutritional experience during key phases of prenatal life can indelibly alter fetal tissue development in ways that ultimately affect predisposition to systemic pathologies in later life. Altered placental function was implicated as an influencing factor through epidemiological association of increased incidence of cardiovascular disease and type II diabetes with increased placental weight:birth weight in human infants (Phillips *et al*. 1994; Campbell *et al*. 1996*a*; Godfrey *et al*. 1996). Some of these associated effects have been mimicked experimentally in animal studies, most consistently by protein undernutrition of pregnant rats (Langley & Jackson, 1994; Langley *et al*. 1994; Langley-Evans *et al*. 1996*a,b*). Maternal undernutrition during early to mid pregnancy can also increase placental size in sheep without necessarily affecting later fetal growth (McCrabb *et al*. 1992; Heasman *et al*. 1998). It is not yet known if these effects on placental morphology are related to alterations in nutrient transport capacity. However, the relative enhancement of placental growth in protein-deprived, pregnant rats was associated with decreased placental activity of 11β-hydroxysteroid dehydrogenase type 2 (Langley-Evans *et al*. 1996*b*). Resulting impairment of the placenta's ability to prevent fetal overexposure to maternal glucocorticoids has been asserted as an important determinant of fetal programming events that lead to postnatal development of hypertension and glucose intolerance in rats (Lindsay *et al*. 1996; Nyrienda *et al*. 1998) and sheep (Dodic *et al*. 1998; Whorwood *et al*. 2001).

Conclusions

The variable constraint of fetal growth by placental capacity for nutrient transport during late gestation is well documented. The functional consequences of this phenomenon range from intra-uterine growth retardation due to placental insufficiency, commonly seen in polytocous pregnancies in domestic animals, to abnormally rapid late fetal growth that can result in maternal metabolic disease, such as pregnancy toxaemia in ruminants, or dystocia and perinatal mortality. The critically important role of the placenta in optimizing fetal growth without compromising neonatal survival or maternal health is unquestioned, but the specific mechanisms are poorly understood.

Identification and molecular characterization of the proteins responsible for placental transport of glucose, amino acids, and fatty acids is under way. This will allow detailed studies of the regulation of expression and activity of these transporters by extracellular factors of maternal and fetal origin, as well as by autocrine and paracrine factors produced in the placenta itself. The insights so provided will be necessary to determine the specific nature of placental limitation of fetal nutrient supply and growth during late pregnancy. In particular, it is unclear if the limiting effects of placental capacity for nutrient transport are primarily a function of abundance or activity of nutrient transporters, distinct from the more general constraints related to the morphometry of the maternal–fetal surface for nutrient exchange. Although not discussed in the present review, it must also be recognized that the placenta plays an active role in the endocrine modulation of fetal demand and maternal supply of nutrients, as well as being a

primary target for humoral regulatory agents. Finally, putative placental involvement in nutrition-related fetal programming of adult disease requires systematic investigation.

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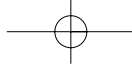
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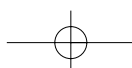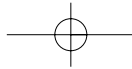
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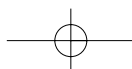
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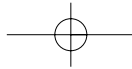
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