

Placental nutrient supply and fetal growth

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ABSTRACT This review considers mechanisms by which transfer across the placenta takes place and how the capacity of the placenta to supply nutrients relates to fetal growth and *vice versa*. Blood flow through both uterine and umbilical circulations of the placenta, the structural properties of the placental exchange barrier and its related diffusional permeability, and the expression and activity of a wide range of transporter proteins in the syncytiotrophoblast, the transporting epithelium of the placenta, all need to be taken into account in considering placental supply capacity. We discuss the evidence that each of these factors affects, and is affected by, fetal growth rate and consider the regulatory mechanisms involved, with a particular focus on data that has emerged from study of the system A amino acid transporter. We consider that future work will build on the considerable foundation of knowledge regarding placental transfer mechanisms, as well as the other aspects of placental structure and function, to develop new diagnostic and therapeutic strategies for pregnancy complications, such as fetal growth restriction or overgrowth.

KEY WORDS: *placenta, fetus, syncytiotrophoblast, system A*

Introduction

The placenta is responsible for the transfer of the bulk of substances between maternal and fetal circulations including oxygen, carbon dioxide, water, and all the necessary nutrients required by the fetus. Consequently, fetal growth is intimately linked to the exchange functions of the placenta and the size of a fetus at birth directly reflects the net transfer of solutes and water across the placenta over the course of gestation. What has interested our research group over nearly three decades of placental research, is the extent to which placental transfer mechanisms regulate fetal growth and *vice versa*. This review considers some of the issues that have arisen from this theme: we compare the mechanisms of maternofetal exchange across the placenta in normal pregnancy and describe how these are altered in relation to fetal growth restriction (FGR) and fetal overgrowth.

The placental exchange barrier and exchange pathways

Any substance that passes between the maternal and the fetal circulation is faced with a number of anatomical barriers. The actual number and nature of these vary markedly between species (Atkinson *et al.*, 2006). In the human, the first step involved

in crossing the placenta requires transport across the microvillous plasma membrane (MVM) of the syncytiotrophoblast, the transporting epithelium of the placenta (Fig. 1). Once in the cytoplasm, molecules destined for the fetus exit from the syncytiotrophoblast via the fetal facing basal plasma membrane (BM). The MVM and the BM are probably the most important membrane barriers involved in maternal-fetal transfer (Sideri *et al.*, 1983). They represent the limiting step in transport of the primary fetal nutrients such as glucose and amino acids. As in other epithelial cells, the differences in the type, number, and activity of transporters in these two plasma membranes provide the basis for vectorial transport towards the fetus (Eaton and Yudilevich, 1981; Smith *et al.*, 1992). Net transport towards the fetus will result if the influx is greater than efflux across MVM and efflux is greater than influx across BM. Molecules then have to pass through the villous

Abbreviations used in this paper: BM, basal membrane; BMI, body mass index; E_m , membrane potential; FGR, fetal growth restriction; GDM, gestational diabetes mellitus; J_{fm} , fetal to maternal unidirectional transfer rate; J_{mf} , maternal to fetal unidirectional transfer rate; LGA, large for gestational age; MeAIB, α -(methylamino)isobutyric acid; MVM, microvillous membrane; NHE, Na^+/H^+ exchanger; PMCA, plasma membrane calcium ATPase; PTHrP, parathyroid hormone related peptide; SNAT, sodium coupled neutral amino acid transporter.

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stroma and cross the fetal capillary endothelium before entering the fetal circulation (Fig. 1). The fetal capillary endothelium is of the continuous type and allows unrestricted passage of small solutes within the interendothelial clefts but diffusion of small peptides and larger sized molecules through these clefts will be restricted to an extent related to their molecular radius (Brownbill *et al.*, 1995; Eaton *et al.*, 1993; Firth *et al.*, 1983; Leach and Firth, 1992; Michel and Neal, 1999; Sibley *et al.*, 1982). The issues of solute permeability will be discussed further below.

Placental transfer has been the focus of many reviews which document the various transport pathways in detail (Atkinson *et al.*, 2006; Boyd *et al.*, 1994; Morriss *et al.*, 1994; Sibley and Boyd, 2004; Smith *et al.*, 1992). These pathways include those mediated by diffusion and those utilizing transcellular mechanisms, mediated by transporter proteins in the plasma membranes, as discussed in the following sections.

Diffusion, blood flow and permeability

Diffusion

Most exchange across the placenta is driven by diffusion or modifications of this process. A maternofetal concentration difference of any molecule, or electrochemical gradient for charged molecules, will lead to diffusion of that molecule across the placenta down its concentration gradient. There are, broadly, two pathways available for diffusion; a lipophilic route (for lipid-soluble molecules), and a hydrophilic route (for lipid-insoluble molecules). The net rate of diffusional transfer (flux) (J_{net}), according to Fick's law for a particular solute, depends on the surface area available for diffusion per gram of placenta (S), the diffusion coefficient in water at 37°C (D_w) of that solute (directly proportional to molecular size), the path length over which diffusion takes place (l), or membrane thickness, and the maternofetal concentration difference of that substance ($c_m - c_f$) i.e. $J_{net} = SD_w l / (C_m - C_f)$ mol/sec/g placenta. For any mechanism of transfer within the placenta, J_{net} can be considered as having two components: $J_{net} = J_{mf} - J_{fm}$. Here, J_{mf} and J_{fm} refer to unidirectional transfer rates in the maternofetal and fetomaternal directions respectively (Sibley and Boyd, 2004). Fetal growth requires that J_{mf} for metabolic and synthetic substrates and water exceeds J_{fm} over the course of pregnancy and therefore these variables control fetal growth by influencing either unidirectional flux. J_{mf} and J_{fm} are defined above in terms of diffusional exchange but the total flux in each direction will be via diffusion plus that via all the other available transfer mechanisms as considered below.

Blood flow

Lipophilic substances such as the respiratory gases dissolve readily through the entire syncytiotrophoblast plasma membrane (Fig. 2A). Because of the large surface area available to this process, such diffusion is said to be flow limited: net transfer will depend on the maternofetal concentration difference of the gas, which itself depends on the flow rates of the uterine and umbilical circulation. It is therefore unsurprising, mechanistically, that abnormalities in blood flow through both vascular beds are associated with FGR (Cnossen *et al.*, 2008; Karsdorp *et al.*, 1994). Indeed, *in utero* cord blood sampling has demonstrated that FGR is associated with hypoxemia (Pardi *et al.*, 1993).

Failure of normal trophoblast invasion into the decidua in early

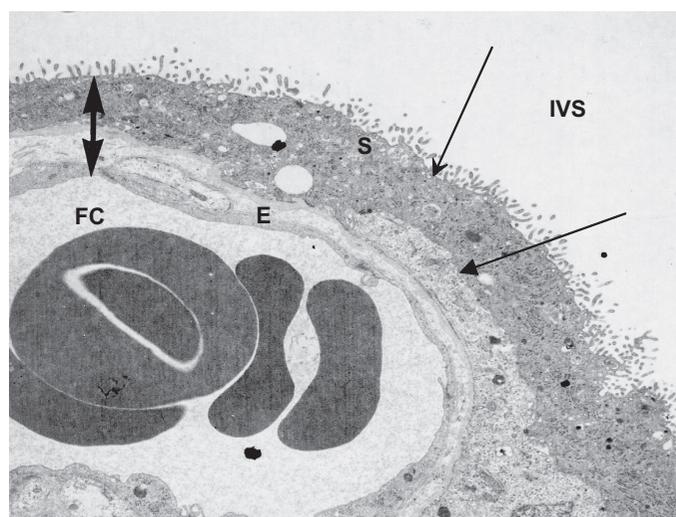


Fig. 1. Electron micrograph image (courtesy of C.J.P. Jones) of the placental exchange barrier. IVS is the intervillous space containing maternal blood; FC is the fetal capillary containing fetal blood; S is the syncytiotrophoblast; E is the fetal capillary endothelium. Single headed arrows point out the microvillous (maternal facing) and basal (fetal facing) plasma membranes of the syncytiotrophoblast. Double headed arrow demonstrates the entire barrier, at one point, across which transfer between maternal and fetal blood must take place.

pregnancy may lead to the failure of the normal transformation of the spiral arteries supplying blood directly into the intervillous space on the maternal side of the placenta (Fig. 1) (Aplin, 1991; Pijnenborg *et al.*, 1983). This will lead directly to the poor uterine artery blood flow, as detected by ultrasound Doppler velocimetry measurements, in FGR (Cnossen *et al.*, 2008; Karsdorp *et al.*, 1994). This failed invasion and transformation may also be the root cause of abnormal placental development in FGR as well as other pregnancy complications such as pre-eclampsia (Aplin, 1991; Pijnenborg *et al.*, 1983). One effect of this abnormal placental development is malformation of the fetoplacental vascular bed of the umbilical circulation (Kingdom *et al.*, 2000). Again this leads to high resistance and abnormal umbilical blood flow in FGR as assessed by ultrasound Doppler (Cnossen *et al.*, 2008; Karsdorp *et al.*, 1994).

Uterine and umbilical Doppler velocimetry is widely used in clinical practice to assess women at high risk of FGR and there is a wealth of literature on this subject (Botsis *et al.*, 2006). There is therefore a tendency for abnormal placental blood flow to be considered the sole cause of poor nutrient supply and consequently of FGR. This is not the case. Abnormal patterns of uterine artery Doppler signal show poor sensitivity and specificity for adverse outcome (Aardema *et al.*, 2001); this can be improved by combining Doppler with biochemical measurements of placental barrier function (Toal *et al.*, 2008a). Furthermore, growth restricted fetuses have lower plasma concentrations of amino acids than infants of normal size (Cetin *et al.*, 1990; Cetin *et al.*, 1988; Cetin *et al.*, 1992). The physicochemical characteristics of these hydrophilic nutrients dictate that they do not readily cross the placental plasma membrane, their rate of transfer is much slower than that of the gases, and is limited much more by the properties of the exchange barrier than by blood flow. Therefore, all aspects of the placental exchange pathways have to be considered in

understanding FGR, and other fetal growth anomalies.

In addition to the structural changes mediating altered blood flow, there are a range of endocrine, paracrine and autocrine factors which are known to alter resistance in the placental circulation (Brownbill *et al.*, 2003; Brownbill *et al.*, 2007; Carter and Myatt, 1995). These are likely to be particularly important in feedback between fetal growth and uterine and umbilical blood flow because of the absence of any innervation of the placenta. However, there is virtually no information yet available on such feedback, other than evidence that vascular reactivity in chorionic plate arteries and veins from placentas of FGR pregnancies is altered, and that the former is unrelated to changes in ultrasound Doppler indices of the umbilical circulation (Mills *et al.*, 2005; Wareing *et al.*, 2006). This is an important area for future research with the potential for therapeutic modulation of the fetoplacental vessel reactivity using drugs primarily being developed for use in other vascular beds.

Diffusional permeability of the placenta to hydrophilic molecules

Hydrophilic molecules have poor solubility in lipid bilayers and therefore do not easily cross plasma membranes (Dobson and Kell, 2008). Consequently, with reference to the Fick equation described above, the diffusional transfer of these substances is much less affected by concentration gradients (and therefore by blood flow unless this is very low) and is more affected by the surface area of the barrier available for diffusion and the thickness of this barrier. The $S \cdot Dw/l$ term in the Fick equation is conveniently described and measured as a permeability \times surface area product (P.S) in studies of diffusional permeability of the placenta to hydrophilic molecules (Atkinson *et al.*, 2006; Sibley and Boyd, 1988). P.S has been measured *in vitro* and *in vivo* in a number of different species (Atkinson *et al.*, 2006; Sibley and Boyd, 1988) and two important facts have emerged. Firstly, P.S is inversely proportional to the radius of inert hydrophilic solutes of increasing molecular size. Secondly, such P.S values are much lower in the epitheliochorial placenta of e.g. sheep, than the haemochorial placenta of humans, rats, guinea pigs and mice (Atkinson *et al.*, 2006; Sibley and Boyd, 1988) i.e. the latter species have a much higher permeability.

The fact that P.S for inert hydrophilic solutes is inversely related to molecular radius, and therefore directly related to D_w , suggests that diffusion of these molecules must take place through extracellular water filled pores, bypassing the plasma membrane (Stulc, 1989). The morphological nature of these pores – sometimes referred to as a paracellular route (Fig. 2B) – in haemochorial placentas is unclear but two possibilities appear to exist. The pores may be transtrophoblastic channels, which have been visualised in both the guinea pig and human placenta (Kaufmann *et al.*, 1982; Kertschanska *et al.*, 1994). Alternatively, a paracellular pathway may be provided during denudation of the syncytiotrophoblast (Brownbill *et al.*, 1995; Brownbill *et al.*, 2000; Edwards *et al.*, 1993). This is a normal feature of the human placenta at all stages of gestation and the denudations are either open or filled with fibrinoid deposits (Nelson *et al.*, 1990).

Whether P.S of the human is altered in relation to fetal growth has not been studied. There is stereological evidence that villous surface area of the human placenta in FGR is decreased and that mean barrier thickness is increased (Mayhew *et al.*, 2007; Mayhew

et al., 2003; Teasdale and Jean-Jacques, 1988), providing evidence that P.S might be decreased and contribute to the lower nutrient supply capacity of these placentas. Supporting evidence for this comes from mice where the placental-specific transcript of the insulin-like growth factor 2 (*Igf2*) gene was knocked out (Sibley *et al.*, 2004). Fetuses of these mice are growth restricted by about 30% at term and this is associated with reduced P.S for inert hydrophilic solutes, decreased placental surface area and increased barrier thickness. This effect of *Igf2* knockout also suggests that structural development of the placental barrier to diffusion might be hormonally modulated, perhaps enabling adjustment over the course of gestation in relation to changes in fetal growth rates.

Paracellular transfer of hydrophilic molecules across the placenta via extracellular pores may be quantitatively important for the exchange of small hydrophilic solutes such as calcium and chloride ions (Doughty *et al.*, 1996; Stulc *et al.*, 1994). The net diffusional flux of ions through the paracellular route will be determined by the electrochemical gradient across the syncytiotrophoblast. Transcellular routes of transfer across the placenta via specific transporters in the syncytiotrophoblast plasma membranes may be qualitatively of greater importance and allow fine tuning of net flux towards the fetus through cellular signaling events.

Transcellular transport and membrane transporters

Transporter proteins found in the plasma membrane responsible for the transport of hydrophilic molecules include channels and carriers. Channels are integral membrane proteins which transport ions down their electrochemical gradient (Fig. 2G). Although diffusion through channels is passive, it is also selective, gated and saturable. Carriers are integral membrane proteins which selectively combine with the solute and translocate it from one side of the plasma membrane to the other. This mechanism is saturable and may use energy from the prevailing electrochemical gradient (i.e. facilitated diffusion, Fig. 2C), or from the hydrolysis of ATP (i.e. active transport, Fig. 2F). Some carriers can transport more than one solute at a time, e.g. co-transporters which carry both solutes in the same direction (Fig. 2D), and exchangers which carry solutes in opposite directions (Fig. 2E). Carriers can harness the gradients generated by pumps by linking solute movements with, for example, Na^+ . Solute movements are moved against their concentration gradient using this process of secondary active transport (Fig. 2D). Endocytosis, another form of transcellular transport, involves invagination of the plasma membrane to form an intracellular membrane-bound vesicle which contains extracellular fluid. The contents of the vesicle are released on the other side of the cell by a process called exocytosis (Fig. 2H): reviewed by Atkinson *et al.*, 2006 (Atkinson *et al.*, 2006). In the following sections we consider some placental transporter processes key for fetal growth and nutrition.

Ion Transport

Ion transport in the placenta subserves two functions, maintenance of cellular homeostasis in terms of electrochemical gradients and volume of placental cells, particularly important for the syncytiotrophoblast with its high rates of transcellular solute fluxes, and provision of cations and anions to the fetus. Several

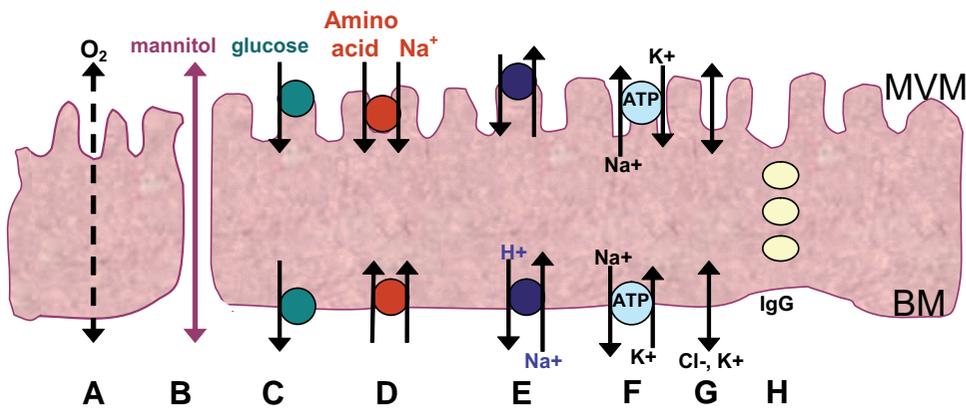


Fig. 2. Schematic diagram depicting the major mechanisms of transfer across the microvillous membrane (MVM) and basal membrane (BM) of the syncytiotrophoblast. (A) Simple diffusion of relatively lipophilic substances; transporter-mediated transport including (C) facilitated diffusion; (D) co-transport; (E) exchange; and (F) active transport. Ion channels (G) are present in the MVM and BM, and there is also evidence for endocytosis-exocytosis (H). Examples of the solutes transported by each mechanism are also given. (B) The paracellular route for the transport of hydrophilic substances is also indicated.

ion transporters and conductances have been identified in the MVM and BM of the syncytiotrophoblast. A broad review of transport mechanisms for different cations and anions can be found in Atkinson *et al.*, 2006. Here we focus on K^+ , Na^+ and Ca^{2+} .

Potassium channels

As in all epithelia, the transmembrane ion concentration difference and the electrical potential difference (the electrochemical gradient) are important determinants of the rate and direction of transport of ions and of processes which involve the net transfer of charge (e.g. Na^+ -linked co-transport). With the use of micro-electrodes, a fetal-side negative potential difference has been shown to exist across the human syncytiotrophoblast/cytotrophoblast layer (Greenwood *et al.*, 1993a; Greenwood *et al.*, 1996). The microvillous membrane potential (E_m) is significantly more negative (hyperpolarized) in first trimester villi compared to term (Birdsey *et al.*, 1997). In most cells E_m depends primarily on the K^+ conductance of the membrane with other conductances and electrogenic transporters making little or no contribution. A difference in the relative K^+ conductance of the MVM between first trimester and term is most likely to contribute to the difference in the magnitude of the E_m observed at these two gestations (Birdsey *et al.*, 1997). Some progress has been made in identifying the K^+ channels in the syncytiotrophoblast and these include Kir2.1 (Clarson *et al.*, 2002; Mylona *et al.*, 1998), TASK and TREK family members (Bai *et al.*, 2005; Bai *et al.*, 2006). There are K^+ channels present in the MVM (Fig. 2G) (Bai *et al.*, 2005; Greenwood *et al.*, 1993b) and, although an early study by Dancis *et al.*, 1983 suggests the bulk of maternofetal K^+ transfer is by paracellular diffusion (Dancis *et al.*, 1983), the regulation of K^+ channel activity is likely to be important for determining E_m , and thereby the cellular transport of charged solutes, cellular homeostasis and regulation of secretory processes. As regards cellular homeostasis, Birdsey *et al.*, (1999) showed that hypoosmotic challenge of the syncytiotrophoblast resulted in activation of both K^+ , and anion, conductances (Birdsey *et al.*, 1999) suggesting that they are involved in maintenance of cell volume. K^+ efflux is also stimulated by angiotensin and ATP, and there is evidence that the latter effects specifically the Ca^{2+} sensitive K^+ channels (Clarson *et al.*, 2002; Siman *et al.*, 2001). In relation to regulation of secretory processes, recent data show that specific K^+ channel blockers inhibit the secretion of human chorionic gonadotrophin from syncytiotrophoblast (Williams *et al.*, 2007). These data suggest the importance of K^+ channels in syncytiotrophoblast

function however there remains much still to be done in terms of identifying and characterising K^+ channels in the syncytiotrophoblast, delineating their function and determining how they are affected in fetal growth disorders.

Na^+/K^+ /ATPase

In some cells, a proportion of the E_m is generated by the activity of the Na^+/K^+ /ATPase or Na^+ pump (Fig. 2F). The Na^+ pump actively extrudes sodium from the cell in exchange for K^+ using energy in the form of ATP (Clarson *et al.*, 1996). This generates an inwardly directed Na^+ gradient which in turn facilitates the Na^+ entry mechanisms. In the placenta, this transporter was originally thought to be located predominantly on the BM (Kelley *et al.*, 1983) as in other epithelia but, a more recent, thorough study showed that both expression and activity of Na^+/K^+ /ATPase is present on the MVM at levels twice that of the BM (Johansson *et al.*, 2000). Na^+/K^+ /ATPase activity is relatively low in the placenta compared to other transporting epithelia (Birdsey *et al.*, 1997) and it does not contribute significantly to E_m in cytotrophoblast cells (Greenwood *et al.*, 1996) or intact syncytiotrophoblast (Birdsey *et al.*, 1997). It is, however, involved in maintenance of high intracellular K^+ and low intracellular Na^+ concentrations (Greenwood *et al.*, 1996) which are important driving forces for other transport systems discussed later in this chapter.

Na^+/H^+ exchanger

There are various mechanisms for Na^+ transport into the syncytiotrophoblast including sodium conductances, Na^+/H^+ exchange, and co-transport with inorganic ions and organic solutes (Shennan and Boyd, 1987). Entry of Na^+ into the cell via the conductive pathway involves movement of Na^+ down its electrochemical gradient (Chipperfield *et al.*, 1988) but the nature of this pathway has not yet been characterised in syncytiotrophoblast. The Na^+/H^+ exchanger (NHE) catalyses an electroneutral coupling of Na^+ influx into the syncytiotrophoblast with H^+ efflux (Balkovetz *et al.*, 1986; Sibley *et al.*, 2002) shown in Fig. 2E. The main function of the NHE is thought to be regulation of intracellular pH (Powell and Illsley, 1996). Co-transport with other molecules contributes significantly to the entry of Na^+ into the syncytiotrophoblast because a number of transport systems, including amino acid transporters, utilise the transmembrane Na^+ gradient as a driving force (Fig. 2D).

Na^+/K^+ /ATPase, NHE and Na^+ channels might all be involved in syncytiotrophoblast homeostasis as well as in transport of the

cation to the fetus. In the rat it has been possible, based on experimental data, to construct a model mechanism of transcellular Na^+ transport to the fetus, involving entry into the trophoblast across the maternal facing plasma membrane via NHE and Na^+ co-transport with amino acids and other solutes, followed by extrusion on the fetal side via Na^+/K^+ ATPase (Sibley, 1994; Stulc *et al.*, 1993). It is not clear how relevant this model is to the human, both because of the localisation of Na^+/K^+ ATPase described above and because it is likely that the paracellular diffusional route is more important than it is in rat (Sibley, 1994). However, whether it is involved in fetal supply or syncytiotrophoblast homeostasis, there does appear to be a strong relationship between reduced fetal growth and placental Na^+ transport. Both Na^+/K^+ ATPase and NHE activities are reduced in placentas from growth restricted fetuses (Johansson *et al.*, 2002; Johansson *et al.*, 2003; Mahendran *et al.*, 1993), as are the activities of some Na^+ -coupled amino acid transporters (see below). These effects could well be interrelated, perhaps with alterations in the electrochemical gradient for Na^+ across the plasma membranes of the syncytiotrophoblast playing a key role in the dysfunction of the epithelium in FGR.

Ca^{2+} transport

Placental transfer of Ca^{2+} to the developing fetus is essential for bone mineralization and, if this is abnormal, it may increase the risk of developing osteoporosis later in life (Tobias and Cooper, 2004). Fetal accretion of Ca^{2+} increases exponentially over the last third of pregnancy, concomitant with the development of the fetal skeleton (Comar, 1956), suggesting that there is cross talk between the two processes. Data suggest that in the mouse and rat placenta, as in other epithelia, Ca^{2+} transport is likely to involve three main steps (Atkinson *et al.*, 2006; Belkacemi *et al.*, 2005): firstly, diffusion into the trophoblast from maternal plasma down an electrochemical gradient through epithelial Ca^{2+} channels of the transient receptor potential (TRP) gene family; secondly, transfer across the trophoblast cytoplasm bound to the calcium binding protein calbindin- $\text{D}_{9\text{K}}$ (Glazier *et al.*, 1992); and, thirdly, active extrusion into the fetal compartment via plasma membrane Ca^{2+} -ATPase (PMCA) localized to the BM (Borke *et al.*, 1989; Fisher *et al.*, 1987). Over the last third of gestation, gene and protein expression for calbindin- $\text{D}_{9\text{K}}$ increases markedly in both mouse and rat placenta (An *et al.*, 2004; Glazier *et al.*, 1992; Hamilton *et al.*, 2000; Mathieu *et al.*, 1989) and correlates with the increase in unidirectional maternofetal ^{45}Ca clearance measured over the same period (Glazier *et al.*, 1992), suggesting that transcytosolic transfer on this protein and the dynamic equilibrium between bound and free Ca^{2+} in the syncytiotrophoblast might be the rate limiting step of transfer in these species. The situation may well be quite different in the human placenta where it has proved difficult to show the expression of calbindin- $\text{D}_{9\text{K}}$ (Belkacemi *et al.*, 2004), or to determine if another protein is involved instead. Interestingly, the activity, but not expression, of PMCA in the BM of human placenta increases linearly over the last third of gestation (Strid and Powell, 2000). This suggests that there is a signaling mechanism linking activity of PMCA in placental BM, and therefore Ca^{2+} transfer, to fetal bone mineralization, which is also increasing rapidly in the third trimester. Further evidence of a link

to fetal calcium demand is from data showing that PMCA activity in the BM is increased in pregnancies affected by both FGR and those with maternal insulin dependent diabetes mellitus (Strid *et al.*, 2003), where fetal overgrowth can occur. Although FGR is associated with reduced bone mineralization, the increased PMCA activity may be a response to increased fetal demand in this condition (Strid *et al.*, 2003).

There is good evidence that parathyroid hormone related peptide (PTHrP) is involved in controlling placental Ca^{2+} transfer. In fetal mice with the PTHrP gene knocked out, Ca^{2+} transfer across the placenta is altered, though there is disagreement between studies, which might have a methodological basis, as to whether knockout leads to decreased (Kovacs *et al.*, 1996) or increased (Bond *et al.*, 2008) J_{mf} for Ca^{2+} . Interestingly, Strid and colleagues have shown that PTHrP stimulates Ca^{2+} ATPase activity in human BM *in vitro* (Strid *et al.*, 2002). Furthermore, umbilical cord plasma concentrations of PTHrP are increased in FGR, perhaps providing the stimulus for the increased PMCA activity in these pregnancies (Strid *et al.*, 2003). Therefore, PTHrP might be a signal relating fetal Ca^{2+} demand to placental Ca^{2+} supply.

Glucose transport

Fetal growth and development is dependent on an adequate supply of nutrients from the mother, in particular glucose and amino acids. Glucose is a primary energy source for the fetus. Investigations using syncytiotrophoblast membrane vesicles and placental perfusion have shown that transport of glucose across the human placenta is saturable, stereo-specific, shows competition, and is independent of energy sources i.e. it shows all the characteristics of facilitated diffusion (Fig. 2C) (Bissonnette *et al.*, 1981; Johnson and Smith, 1985; Rice *et al.*, 1976). Glucose transport in mammalian tissues is mediated by the GLUT gene family of facilitated diffusion transporters of which there are at least twelve members (Joost and Thorens, 2001). The fetal plasma glucose concentration is lower than that in the maternal circulation even though one study estimated that the glucose transfer capacity of the MVM is many fold higher than would be required to supply fetal and placental needs (Johnson and Smith, 1980). It is likely the maternal-fetal glucose gradient may arise from limitation of overall transcyntial transfer by the lower transport capacity of the BM (Johnson and Smith, 1985). Interestingly, it has been shown clearly that neither MVM nor BM GLUT expression and activity is altered in pregnancies affected by FGR (Jansson *et al.*, 1993; Jansson *et al.*, 2002b). In fetal overgrowth, glucose transport across MVM is unaltered but there is some evidence of increased glucose transport across the BM (Gaither *et al.*, 1999; Jansson *et al.*, 2001; Jansson *et al.*, 1999).

Amino acid transport

The identification and kinetic properties of different amino acid transport systems in placenta has been investigated extensively using membrane vesicles prepared from MVM and BM of the human syncytiotrophoblast (Hoeltzli and Smith, 1989; Kudo *et al.*, 1987; Smith *et al.*, 1974). Analysis of the different amino acid transporters found in the placenta has been challenging due to the large number of amino acid substrates, the overlapping specificity of the transporters, and

the complicating factor of placental metabolism and utilisation of amino acids (Battaglia, 1986). Based on competitive interactions between amino acid substrates, at least 15 transport systems for neutral, basic and acidic amino acids have been identified in the placenta analogous to those described for other epithelia (Jansson, 2001; Moe, 1995). Many of the amino acid transporters have now been cloned and characterised at the molecular level (Kudo and Boyd, 2002). The genes encoding the different transporters are divided into different solute carrier (SLC) families. For example, the SLC38 family encodes the system A and system N neutral amino acid transporters (Hediger *et al.*, 2004).

In healthy fetuses, umbilical cord blood concentrations of most amino acids are significantly higher than maternal concentrations at mid gestation and term with the exception of aspartate and glutamate (Cetin *et al.*, 1990; McIntosh *et al.*, 1984; Philipps *et al.*, 1978; Soltesz *et al.*, 1985). Placental tissue concentrations of free amino acids appear to be higher than in both fetal and maternal plasma (Camelo *et al.*, 2004; Philipps *et al.*, 1978). Energy is therefore required to transport amino acids into the syncytiotrophoblast against their concentration gradient. The most generally accepted classification of amino acid transporters divides them into two systems – those that co-transport Na⁺ with the amino acid (Na⁺-dependent systems), and those that do not (Na⁺-independent systems) (Christensen, 1982). Na⁺-dependent amino acid transporters are secondary active transporters because they do not directly utilise ATP. Instead they harness the active energy of the Na⁺/K⁺ATPase which creates the Na⁺ gradient. It is the energy stored in the Na⁺ electrochemical gradient that is used to transport the amino acid substrates of the transporters against their concentration gradient (Sibley and Boyd, 1998). It is not immediately apparent what represents the driving force for the uphill transport of amino acids across MVM by Na⁺-independent transporters. System L exhibits strong trans-stimulation i.e. uptake is stimulated by high intracellular concentrations of another neutral amino acid (Christensen, 1990; Jansson *et al.*, 1998). Possible candidates for trans-stimulation of System L activity are glycine and serine, which are transported by system A. System y⁺L is a Na⁺-independent cationic amino acid transporter that carries a net positive charge. It is therefore likely that it is the electrical potential difference that provides the driving force for transport of cationic amino acids against their concentration gradient across MVM by this transporter.

Exit of amino acids from the syncytiotrophoblast across the BM towards the fetus is less well understood, although Na⁺-independent pathways are thought to play an important role. There is evidence that amino acid exchangers in the BM may function to take up amino acids required in lower quantities by the fetus in exchange for placental amino acids for which the fetus has a higher metabolic requirement (Cleal *et al.*, 2007). The non-specific permeability of BM *in vitro* to neutral amino acids is not insignificant (Hoeltzli and Smith, 1989; Jansson *et al.*, 1998; Kudo and Boyd, 1990). Therefore it is possible that non-mediated transport across BM may contribute to exit of neutral amino acids from the syncytiotrophoblast towards the fetus, down their own concentration gradient.

Because fetal amino acid requirements for both metabolic and synthetic purposes are likely to be important determinants

of fetal growth, we hypothesized that placental transport of these substrates and fetal growth are interrelated. Work in our laboratory has focused particularly on placental amino acid transport via the system A transporter and how this is altered in both FGR and fetal overgrowth.

System A amino acid transporter

Transporter mechanisms and characteristics

System A primarily transports non-essential neutral amino acids such as alanine, glutamine, glycine, methionine, proline, serine, and threonine. It is a ubiquitous transporter that actively transports small, zwitterionic, neutral amino acids with short unbranched side chains (Johnson and Smith, 1988). Substrates are co-transported with Na⁺ against their concentration gradient using the Na⁺ electrochemical potential gradient across the cell membrane as a driving force. The Na⁺:amino acid stoichiometry is 1:1 (Hatanaka *et al.*, 2000; Sugawara *et al.*, 2000a; Wang *et al.*, 2000) and Na⁺ binding has been shown to precede binding of amino acid before simultaneous transport takes place (Mackenzie *et al.*, 2003; Yao *et al.*, 2000). This transporter is pH sensitive, displaying reduced activity at lower pH (Hatanaka *et al.*, 2000; Sugawara *et al.*, 2000a; Wang *et al.*, 2000). System A is sensitive to N-methylated amino acids (Johnson and Smith, 1988) such as α -(methylamino)isobutyric acid (MeAIB). This non-metabolised amino acid analogue has a specific affinity for the System A transporter (Christensen *et al.*, 1965) and has been used extensively to study this transport system in the placenta (Champion *et al.*, 2004; Jansson *et al.*, 2003; Johnson and Smith, 1988; Mahendran *et al.*, 1993; Moe *et al.*, 1994).

Molecular characterisation and expression in human placenta

Molecular characterisation of system A has revealed there are three highly homologous protein subtypes of this Na⁺-coupled neutral amino acid transporter known as SNAT1, SNAT2, and SNAT4, which are encoded by three members of the SLC38 gene family (*SLC38A1*, *SLC38A2*, and *SLC38A4* respectively) (Hediger *et al.*, 2004; Mackenzie and Erickson, 2004). Expression of *SLC38A1* has been shown in various tissues including human placenta (Desforges *et al.*, 2006; Wang *et al.*, 2000). *SLC38A2* is expressed ubiquitously in mammalian tissues (Hatanaka *et al.*, 2000; Sugawara *et al.*, 2000a) and it is therefore assumed that this isoform represents the classical system A amino acid transporter. Functionally, SNAT1 and SNAT2 operate via similar mechanisms (Hatanaka *et al.*, 2000; Wang *et al.*, 2000; Yao *et al.*, 2000). SNAT4 has a lower substrate affinity for neutral amino acids and MeAIB than SNAT1 and SNAT2 ($K_m = 6.7\text{mM}$ versus 0.89mM and 0.39mM respectively for MeAIB) (Hatanaka *et al.*, 2001; Sugawara *et al.*, 2000b) and also has a unique ability amongst the other system A isoforms to interact with cationic amino acids in a Na⁺-independent manner such that it resembles system y⁺L (Hatanaka *et al.*, 2001). It was originally thought that *SLC38A4*/SNAT4 was a liver-specific isoform (Hatanaka *et al.*, 2001). However, we have shown this isoform is present in human placenta (Desforges *et al.*, 2006) and expression has also been confirmed in rat (Novak *et al.*, 2006a) and mouse placenta (Mizuno *et al.*, 2002; Smith *et al.*, 2003).

The high homology between the protein sequences for the

three system A isoforms has complicated development of SNAT-specific antibodies and therefore system A protein expression in placenta and its regulation is poorly understood. Using custom synthesised SNAT isoform-specific antibodies, we have used immunohistochemistry to confirm localisation of each SNAT isoform to the syncytiotrophoblast in both first trimester and term placental samples (Champion *et al.*, 2004; Desforges *et al.*, 2006, 2009). This pattern of localization allows for the possibility that each isoform of system A has the potential to transport amino acids from maternal blood in the intervillous space into the placenta.

System A transport in the placenta

System A activity in the placenta has been demonstrated in MVM and BM (Dicke *et al.*, 1993; Kudo *et al.*, 1987; Mahendran *et al.*, 1993), however maximal transport capacity per mg protein is much higher in MVM (Hoeltzli and Smith, 1989). Exit of system A substrates across the BM is poorly understood but is thought to occur predominantly via system L, a Na⁺-independent transporter with broad specificity (Jansson, 2001). It is also possible that non-mediated transport across BM may contribute to exit of neutral amino acids from the syncytiotrophoblast (Hoeltzli and Smith, 1989; Kudo and Boyd, 1990).

System A activity in placental cells, tissue, and plasma membrane vesicles has been studied by measuring Na⁺-dependent uptake of radiolabelled MeAIB, used at tracer concentrations. It is therefore likely that the majority of these studies reflect SNAT2-mediated transport as this isoform has the highest affinity for MeAIB (Hatanaka *et al.*, 2000). Localisation of SNAT1, SNAT2, and SNAT4 protein to the syncytiotrophoblast raises the question of the relative functional importance of each isoform for amino acid transport from mother to fetus. The broader substrate specificity of SNAT4 suggests it could mediate the Na⁺-independent transport of cationic amino acids such as arginine (Hatanaka *et al.*, 2001) as well as the Na⁺-dependent transport of neutral amino acids typical of system A. Our current research aims to determine the functional importance of each SNAT isoform for amino acid transport by system A across the human placenta (Desforges *et al.*, 2008; Desforges *et al.*, 2009). Our experimental approach is to knockdown the individual SNAT isoforms using siRNA technology and examine the effect on Na⁺-dependent MeAIB transport. We have recently achieved successful knockdown of *SLC38A1* mRNA in primary cytotrophoblast cells and preliminary experiments show this was associated with reduced MeAIB uptake, suggesting SNAT1 plays an important role by contributing to system A activity in the placenta (Desforges *et al.*, 2008).

Placental system A activity and fetal growth during normal pregnancy

As pregnancy advances, the fetus grows at a faster rate than the placenta (Sands and Dobbing, 1985; Thomson *et al.*, 1969). Towards the end of pregnancy, human fetal weight increases from ~1kg at 27 weeks gestation to 3.5kg at term. This is a growth rate of around 30-35g per day (Schneider, 1996) which equates to an amino acid requirement of 40-60mmol per day (Smith, 1986). It therefore seems reasonable to assume the nutrient transporting capacity of the placenta increases with gestation in order to support accelerated fetal growth. Increased transport of nutrients across the placenta over gestation is likely to be achieved

through alterations in placental perfusion, an increase in MVM and BM surface area and changes in transporter abundance or activity in the syncytiotrophoblast. There is also a progressive thinning of the placental exchange barrier towards term which further facilitates nutrient exchange (Jones and Fox, 1991).

Studies using both MVM vesicles and placental villous fragments have shown the activity of the system A amino acid transporter increases between first trimester and term (Mahendran *et al.*, 1994; Desforges *et al.*, 2009). The factors responsible for increased system A activity in the placenta during the course of normal pregnancy are currently unknown. We are exploring the possibility that there is increased expression of SNAT isoform(s) in the placenta at term compared to first trimester, accounting for the increased system A activity. A complex picture is emerging. Placental *SLC38A1* and *SLC38A2* mRNA expression is comparable between first trimester and term whereas *SLC38A4* mRNA expression is significantly higher in placentas from 6-10 weeks gestation than in term placenta (Desforges *et al.*, 2006). In contrast, protein expression for this subtype of system A is significantly higher at term compared to first trimester (Desforges *et al.*, 2006). Furthermore, our experiments also suggest SNAT4 activity is higher in the placenta during first trimester compared to term (Desforges *et al.*, 2009). These data add to the increasing body of evidence that there are complex gestational changes in placental transporter expression and activity (Atkinson *et al.*, 2006). A change in mRNA expression is not necessarily paralleled by a similar change in protein expression, perhaps attributed to transcriptional regulation, mRNA degradation, translation into protein and post-transcriptional processes. Each of these events and the rate at which they occur may differ between cell types. In the placenta, the relative proportions of syncytiotrophoblast, cytotrophoblast cells and endothelial cells changes over gestation quite markedly. For example, immunohistochemical detection of SNAT4 shows strong staining in fetal blood vessels which are widespread at term but poorly developed in first trimester. Our research into this area is ongoing and we have yet to examine levels of SNAT1 and SNAT2 protein expression in first trimester and term placenta.

Placental system A and fetal growth restriction

The availability of techniques to sample umbilical cord blood *in utero* has made it possible to measure plasma amino acid concentrations prior to delivery (Daffos *et al.*, 1985) and studies have shown that these are significantly lower in FGR compared to normal pregnancies (Cetin *et al.*, 1990; Cetin *et al.*, 1988; Cetin *et al.*, 1992). Human *in vivo* studies with stable isotopes have shown delivery of neutral amino acids to the fetus in normal pregnancy is only just sufficient to meet requirements (Cetin, 2001), and in FGR maternofetal ¹³C-leucine transfer is reduced (Marconi *et al.*, 1999). Such findings provide the rationale for studying placental amino acid transport systems in relation to FGR. Evidence of a defect in placental system A amino acid transport in fetal growth restriction was first obtained by Dicke and Henderson in 1988 (Dicke and Henderson, 1988). In a later study, Mahendran *et al.*, (1993) found that MeAIB uptake by MVM was reduced by 63% in FGR pregnancies (< 3rd percentile), and this was predominantly due to a reduction in the V_{max} of system A. The affinity of the transporters (K_m) was not altered. This would imply that either the turnover of system A transporters was reduced or the number of

active carrier proteins in MVM was reduced in FGR. The reduction in MVM system A activity has been shown to be related to the severity of FGR (Glazier *et al.*, 1997) but the molecular mechanisms responsible are unknown. mRNA expression for the three system A isoforms is comparable in placentas from pregnancies complicated by FGR versus normal (Malina *et al.*, 2005) (and M.Desforges, unpublished observations). Levels of SNAT protein expression in the placenta and localization of functional SNAT proteins has yet to be examined in cases of FGR.

The work on the system A amino acid transporter in MVM and BM from placentas of pregnancies affected by FGR has led to similar studies on other nutrient transporters. As has been reviewed recently (Atkinson *et al.*, 2006; Jansson, 2001) it is now clear that the activity of a range of transporters in MVM and BM is decreased in FGR as compared to normal. It is also apparent that not all transporters are affected (e.g. glucose) and the activities of some transporters, such as PMCA as described above, are actually increased. These data suggest changes in activity of some transporters are part of the cause of FGR, whereas others, such as PMCA, are part of a fetoplacental response to reduced fetal growth.

Evidence from three separate animal studies provides weight to the theory that alterations in the activity of system A may cause, rather than merely be associated with, altered fetal growth. Firstly, inhibition of placental system A activity in pregnant rats, achieved by injecting the mother with high concentrations of MeAIB, is associated with suboptimal fetal growth (Cramer *et al.*, 2002). Secondly, in a rat model of FGR in which dams were fed a protein restricted diet, there was a decrease in placental system A activity that preceded in gestation the decrease in fetal growth (Jansson *et al.*, 2006a). Thirdly, in a mouse model of FGR which displays alterations in placental structure and function that are very similar to those found in human FGR (Sibley *et al.*, 2004), fetal growth restriction, as in the rat model, is preceded by alterations in placental system A activity (Constancia *et al.*, 2002).

This mouse model, achieved by knocking out placental-specific *Igf2* (P0), leads to placental growth restriction from embryonic day 12 (E12) but fetal growth restriction only from E19 (Constancia *et al.*, 2002). Therefore at E16 there is a high fetal/placental weight ratio suggesting increased placental transport efficiency. Indeed, at E16, maternofetal ¹⁴C-MeAIB transport is increased in the P0 knockouts, suggesting that upregulation of system A in these placentas enables maintenance of fetal growth despite reduced placental size. Interestingly, in humans, Godfrey *et al.*, 1998) found that MVM from smaller babies (within the range of normal pregnancies) had increased system A activity (Godfrey *et al.*, 1998). The authors also found an inverse relationship between system A activity and placental size. These observations suggest there is a compensatory upregulation of system A transport in smaller placentas during normal pregnancy in an attempt to maintain sufficient transfer capacity. By E19 in the P0 knockout mice, transport of ¹⁴C-MeAIB is similar to that observed in wild type mice (Constancia *et al.*, 2002), suggesting that when upregulation of system A is lost, normal fetal growth cannot be sustained. These changes in placental system A activity in P0 knockout mice were specifically associated with changes in *Slc38a4* expression (Constancia *et al.*, 2005). Knocking-out placental *Slc38a4* in mice is associated with fetal growth restriction (Fowden *et al.*, 2006) suggesting placental SNAT4 is important for

the transport of amino acids required to maintain normal fetal growth in this species. We have been interested in the function of this isoform of system A in human placenta; however, our data suggest placental SNAT4 may not be as important in humans at later stages of pregnancy as it appears to be in mice (Desforges *et al.*, 2009).

Placental system A and fetal overgrowth

Delivery of large-for-gestational age (LGA) or macrosomic (birth weight >4000g) babies complicates 40% of pregnancies affected by diabetes (Berk *et al.*, 1989). It represents a risk factor for operative delivery, traumatic birth injury, and developing diabetes and obesity later in life (Pettitt *et al.*, 1993; Zhang *et al.*, 2008). It is thought that these LGA babies result from increased substrate availability during intrauterine development which stimulates fetal insulin secretion and fetal growth (Jansson *et al.*, 2006b). There are different forms of maternal diabetes which include gestational diabetes mellitus (GDM), Type-I and Type-II diabetes and in addition, some of these cases of diabetes will be well controlled which can complicate interpretation of data. *In vivo* studies have demonstrated that the umbilical delivery of amino acids is significantly increased even in well controlled GDM despite maternal substrate levels being comparable to normal pregnancies (Cetin *et al.*, 2005). This has prompted investigation into placental amino acid transport in diabetic mothers. One study has shown higher system A activity in the MVM of placentas from pregnancies complicated with GDM or Type-I diabetes with or without LGA births compared to normal pregnancy (Jansson *et al.*, 2002a). In contrast, our laboratory has also shown that system A activity is reduced in the MVM of placentas from macrosomic babies born to diabetic mothers (Kuruvillea *et al.*, 1994). The reasons for these disparate observations may be related to differences in study populations and the placental to fetal weight ratios of these cohorts. In well controlled diabetic women, placental morphology appears to be relatively normal (Mayhew *et al.*, 1994), however when associated with macrosomia, the placentas are often larger (Jansson *et al.*, 2002a; Kuruvillea *et al.*, 1994). It is possible that the reduced placental amino acid transporter activity we observed could be compensated for by the associated increased placental size observed.

Maternal obesity is also associated with fetal overgrowth and an increased risk of pregnancy complications (Galtier *et al.*, 2008; Guelinckx *et al.*, 2008; Robinson *et al.*, 2005). Pre-pregnancy body mass index (BMI, calculated by dividing weight by height²) correlates with fetal birthweight and placental weight in normal, uncomplicated, term singleton pregnancies without diabetes (Swanson and Bewtra, 2008). The mechanisms underlying fetal overgrowth in obese pregnant women are largely unknown. Obesity is on the increase and therefore maternal obesity and the associated pregnancy complications are becoming a significant clinical problem which is prompting research to investigate whether there is altered placental function in obese mothers (Jansson *et al.*, 2007; Jones *et al.*, 2008).

Regulation of System A by external factors

Regulation of amino acid transport across the syncytiotrophoblast by system A is likely to be complex involving multiple pathways and differential regulation of the three SNAT isoforms. By analogy with other tissues, this is likely to involve alterations in

transporter mRNA and protein expression, trafficking of pre-formed protein to the cell membrane, and changes in transporter activity/turnover. The regulatory factors responsible for altered placental system A activity in cases of FGR and fetal overgrowth are currently under investigation. There is evidence to suggest these regulatory factors could include oxygen tension, substrate levels, as well as some hormones and growth factors (Jones *et al.*, 2007).

Oxygen may regulate system A based on a study using human lung fibroblast cells which demonstrated hypoxia inhibits MeAIB uptake (Berk *et al.*, 2000). System A activity is also reduced in cytotrophoblast cells cultured in low oxygen tensions and this is associated with decreased expression of *SLC38A1* and *SLC38A2* mRNA (Nelson *et al.*, 2003). Altered blood flow to the placenta in pregnancies associated with FGR and preeclampsia, discussed earlier in this chapter, leads to reduced oxygen delivery. It is therefore possible that placental system A activity could be downregulated by reduced oxygen supply in these pregnancy complications. Oxidative stress in the placenta is often associated with FGR and preeclampsia and is thought to result from ischemia-reperfusion injury (Hung *et al.*, 2001). It is therefore interesting that reactive oxygen species have also been shown to reduce MVM system A activity (Khullar *et al.*, 2004).

System A is regulated by substrate availability. Depletion of system A substrates leads to an increase in transporter activity when substrates become available again (Smith and Depper, 1974). This response is known as adaptive regulation and is perhaps the most understood mechanism of system A regulation. In various cell types, substrate regulation of system A is associated with specific changes in the expression and localisation of *SLC38A2*/SNAT2 (Franchi-Gazzola *et al.*, 2001; Hyde *et al.*, 2001; Ling *et al.*, 2001). In studies using BeWo choriocarcinoma cells, MeAIB transport has been shown to increase following substrate deprivation and this was associated with upregulation of *SLC38A2* mRNA (Jones *et al.*, 2006; Novak *et al.*, 2006b). SNAT2 has an amino acid response element in its promoter region, possibly explaining these findings (Palli *et al.*, 2004). BeWo protein expression for SNAT2 was unaffected in substrate deprivation experiments but there was increased localisation of this isoform to the plasma membrane (Jones *et al.*, 2006). This suggests a similar mechanism to that present in other cell types is responsible for adaptive regulation of system A in placenta. Adaptive regulation of system A has also been demonstrated in term human placental villous fragments (Parrott *et al.*, 2007). System A is also trans-inhibited i.e. high intracellular concentrations of its own substrates can lead to reduced activity (Smith and Depper, 1974). The nutritional status of the mother during pregnancy could impact on substrate availability for system A, for example in teenage pregnancies where maternal diet is poor, or in overweight and obese mothers where substrate availability may be increased.

System A is also sensitive to a wide range of hormones (Shotwell *et al.*, 1983) and its activity has been reported to be upregulated in term placental villous fragments following acute incubation with insulin and leptin (Jansson *et al.*, 2003). Acute upregulation of system A by insulin has also been demonstrated in skeletal muscle cells and this was associated with increased localisation of SNAT2 to the plasma membrane (Hyde *et al.*, 2002). Chronic regulation of system A activity by hormones and

growth factors is believed to involve upregulation of transporter gene and protein expression since inhibiting these abolishes the response (Su *et al.*, 1998). A number of *in vitro* studies have reported regulation of system A activity in placenta by insulin-like growth factors (Bloxam *et al.*, 1994; Karl, 1995; Kniss *et al.*, 1994). In the liver, increased system A activity is observed following experimentally induced diabetes or glucagon treatment and this is due to a selective increase in *SLC38A2* and *SLC38A4* expression whereas *SLC38A1* is unaffected (Varoqui and Erickson, 2002). These regulatory factors are likely to be relevant in cases of maternal diabetes and obesity as well as in mothers who have a poor diet.

When considering regulation of system A activity it is important to remember the driving forces involved. As discussed earlier in this chapter, amino acid transport into the cell by system A relies upon co-transport with Na⁺ which in turn is dependent upon a relatively low intracellular Na⁺ concentration. Angiotensin II reduces system A activity in placental villous fragments however this is primarily mediated through a negative effect of angiotensin II on Na⁺/K⁺/ATPase activity (Shibata *et al.*, 2006).

Future Work

Continuing research into characterizing and understanding the cross-talk between placental nutrient supply and fetal growth is required, and also how this is related to maternal nutrition. Problems yet to be resolved include determining the relationship between all the variables which contribute to placental supply capacity. For example, does reduced uterine blood flow in FGR always occur with altered syncytiotrophoblast structure and expression of transport proteins, or can reduced activity of system A occur in isolation? These investigations will help delineate the placental phenotypes in FGR and, with the use of imaging and other non-invasive modalities, help diagnose those women at high risk of the disease, in settings such as the Placenta Clinic described by Kingdom and colleagues (Toal *et al.*, 2008b).

Our review has mainly focused on placental transport at term. However, appropriate regulation of nutrient transport earlier in gestation will be an important determinant of normal fetal development. There has been some research into placental nutrient transport earlier in gestation, which has been reviewed recently (Atkinson *et al.*, 2006), but further investigations are required into the development of placental nutrient transport across gestation and how this is regulated.

The majority of research has focused on how placental supply capacity is affected by FGR, and therefore further investigation is needed into altered placental structure and function associated with fetal overgrowth.

It will also be important to examine how the major risk factors for poor pregnancy outcome such as stress, obesity and teenage maternity affect placental function, with this information also contributing to understanding the pathophysiology of the placenta in pregnancy complications, and in finding solutions to them. The aim is to identify target molecules and strategies for early diagnosis and therapeutic intervention.

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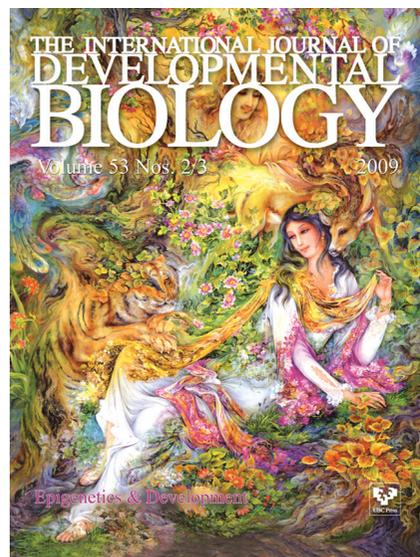
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