PLACENTAL METABOLISM: SUBSTRATE REQUIREMENTS AND THE RESPONSE TO STRESS.

Owen R. Vaughan and Abigail L. Fowden

Centre for Trophoblast Research,

Department of Physiology, Development and Neuroscience,

University of Cambridge, Cambridge CB2 3EG, UK

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Address for correspondence: Abigail L. Fowden
Department of Physiology, Development and Neuroscience
Downing Street
Cambridge
CB2 3EG
Tel: 44 (0)1223 333855
Fax: 44 (0)1223 333840
Email: alf1000@cam.ac.uk

ABSTRACT

The placenta is a dynamic, metabolically active organ with significant nutrient and energy requirements for growth, nutrient transfer and protein synthesis. It uses a range of substrates to meet its energy needs and has a higher rate of oxygen (O_2) consumption than many other fetal and adult tissues. Placental metabolism varies with species and alters in response to a range of nutritional and endocrine signals of adverse environmental conditions. The placenta integrates these signals and adapts its metabolic phenotype to help maintain pregnancy and to optimise offspring fitness by diversifying the sources of carbon and nitrogen available for energy production, hormone synthesis and feto-placental growth. The metabolic response of the placenta to adversity depends on the nature, severity and duration of the stressful challenge and on whether the insult is maternal, placental or fetal in origin. This review examines placental metabolism and its response to stresses common in pregnancy with particular emphasis on farm species like the sheep. It also considers the consequences of changes in placental metabolism for the supply of O_2 and nutrients to the fetus.

INTRODUCTION

As the interface between the mother and fetus, the placenta has multiple functions important to the successful outcome of pregnancy. It transports O_2, nutrients, ions and key micronutrients from mother to fetus as well as wastes, such as carbon dioxide (CO_2) and urea, in the opposite direction (Sibley et al. 1997). The placenta also converts nutrients that it receives to other forms to provide alternative substrates for feto-placental metabolism and growth (Figure 1). In addition, the placenta produces hormones and growth factors that are released into the maternal and fetal circulations (Burton and Fowden 2012). These have key roles in the maintenance of uterine quiescence and the maternal physiological adaptations to pregnancy that are essential for meeting the increasing nutrient demands of the growing fetus. Finally, the placenta acts as a barrier restricting access of maternal hormones and xenobiotics to the fetus by enzymatic inactivation or transporting them back into the maternal circulation. Consequently, the placenta is a metabolically active organ with significant nutrient and energy requirements.

The placenta uses a range of substrates to meet its energy needs and has a higher rate of oxygen (O_2) consumption than either the adult or the fetus (Hay 1991). Placental metabolism varies with species and alters in response to the fetal nutrient demands for growth with increasing gestational age (Fowden 1997; Pere 2003). It is also responsive to homeostatic challenges that evoke stress responses in both the mother and fetus (Vaughan et al. 2012; Sferruzzi-Perri et al. 2016). The mother signals adverse changes in the general environment, such as scarcity or excess of nutrients, low oxygen availability or extremes of temperature, as well as physiological changes in the individual, like the availability of fuel reserves and the degree of glycaemic control and physical activity (Fowden et al. 2010; 2015; Gaccoli et al. 2013: Sferruzzi-Perri and Camm 2016). The fetus signals mismatches between its supply and demand for nutrients in relation to its mass, genotype, and degree of maturation (Burton and Fowden 2012; Vaughan et al. 2012). In many circumstances, the signalling is via stress and other metabolic hormones, like the glucocorticoids, catecholamines, leptin and insulin (Fowden et al. 2015).

The stresses experienced during pregnancy may be chronic or acute depending on their origin. Chronic stress induced early in pregnancy often reduces feto-placental growth, whereas, more acute stresses tend to alter availability of specific nutrients and hormones transiently without major effects on intrauterine growth (Fowden et al. 2006). In farm animals and other species, there are also changes in placental morphology and nutrient transport capacity in response to stressful conditions including undernutrition, hypoxia, and manipulations of dietary composition, maternal adiposity, glucose availability and glucocorticoid concentrations (Fowden et al. 2010; 2015; Gaccoli et al. 2013). However, much less is known about the effects of these stresses on placental metabolism *per se*. This review, therefore, examines placental metabolism and its response to stresses common during pregnancy with particular emphasis on farm animals like the sheep. It also discusses the consequences of changes in placental metabolism for the supply of O_2 and nutrients to the fetus. It does not consider the effects of stressful conditions on placental growth and development as these topics have been reviewed recently (Gaccoli et al. 2013; Fowden et al. 2015; Sferruzzi-Perri and Camm 2016).

OXYGEN

Normal requirements

Like most tissues, aerobic respiration is the main source of placental ATP during normal conditions. In the farm animals studied to date, the respiratory rate of the combined uteroplacental tissues is higher per kg total tissue that seen per kg of fetus (Fowden et al., 1997). Since most of this O_2 consumption is placental rather than myometrial (Hay 1991), rates of O_2 consumption per kg placenta are at least 3-5 fold higher than those per kg of fetus as a whole (Table 1) and similar to that of the fetal brain (Hay 2006). Oxygen consumption rates calculated per kg placenta are of the same order of magnitude in different species with an epitheliochorial placenta and similar those of the haemochorial human placenta (Table 1; Hay 2006). Of the O_2 consumed by the ovine placenta about 70-75% is used to generate ATP by mitochondrial oxidative phosphorylation using a variety of substrates including carbohydrates, amino acids, probably certain volatile fatty acids (VFA) and possibly also some free fatty acids (Figure 1). The majority of this ATP is used for protein synthesis and active transport processes (Carter 2000). Oxygen is also used in placental mitochondria without generating ATP through proton leak and superoxide production along the electron transport system and for synthesis of progesterone or other steroids (Figure 1). These processes account for another 15-20% of the O_2 consumed by the ovine placenta while the remaining 10-15% is non-mitochondrial due to cellular oxidative reactions unrelated to energy production (Carter 2000).

Oxygen consumption by the combined uteroplacental tissues increases by 25-50% between mid and late gestation in sheep and horses but not in pigs when expressed per kg wet weight (Reynolds et al 1985; Bell et al. 1986; Molina et al. 1991; Fowden et al. 2000). In contrast, when values are calculated per gram dry weight of placenta alone, there is little change in placental O_2 consumption during the second half of gestation in sheep (Vatnick and Bell 1992). However, growth rates of the placenta and fetus differ over this period of gestation and also show wide species variation (Wooding and Burton 2008). Consequently, the amount of O_2 consumed by the uteroplacental tissues as a proportion of the total

uterine O_2 uptake alters with gestational age depending on the species. For instance, in sheep, the percentage of uterine O_2 uptake used by the uteroplacental tissues decreases from 80% to 40-45% between mid and late gestation, whereas, in horses and pigs, this percentage remains at 55% and 65%, respectively, throughout the second half of gestation (Reynolds et al. 1985; Bell et al. 1986; Molina et al. 1991; Fowden et al. 2000).

Response to stress

Absolute rates of uteroplacental O_2 consumption vary with placental weight and are often reduced in response to chronic stresses like hyperthermia and hypoglycaemia that compromise placental growth from early in development (Thureen et al. 1992; Carver and Hay 1995; Carter 2000). However, when all the data available for pregnant sheep in late gestation are summarised, rates of O_2 consumption calculated per kg of placenta are relatively unaffected by the range of acute and chronic stresses studied to date (Figure 2). It is only when placental growth is severely compromised by carunclectomy before pregnancy that O_2 consumption per kg placenta is reduced (Figure 2). Even when uterine O_2 delivery is reduced by 50 % by maternal anaemia, uteroplacental O_2 consumption is maintained by increasing O_2 extraction (Delpapa et al. 1992). Normal rates of uteroplacental consumption and umbilical uptake of O_2 are also sustained in a similar manner when uterine O_2 delivery is reduced for 24h by restricting uterine blood flow (Hooper et al. 1995; Carter 2000). In contrast, when O_2 availability is reduced chronically by pregnancy at high altitude, O_2 consumption per unit weight of human placenta appears to decline relative to sea level values (Illsley et al. 2010). Collectively, these findings suggest that the rate of placental respiration varies little with nutritional stresses but may adapt when the O_2 supply is restricted chronically by hypoxia or low uterine blood flow.

To date, little is known about placental energetics or mitochondrial function during stressful conditions in farm animals. In the human and rodent placenta, both nutritional and hypoxic stresses alter mitochondrial function. More specifically, there are changes in mitochondrial biogenesis, morphology, apoptosis and abundance of electron transport complexes and uncoupling proteins during common pregnancy stresses including maternal diabetes,

obesity, pre-eclampsia, calorie restriction, protein deprivation and high altitude hypoxia (Belkacemi et al 2011; Mayeur et al. 2013; Colleoni et al 2013; Hercules et al. 2013; Hastie and Lappas 2014; Mando et al. 2014; Chiaratti et al 2015). Increased abundance of uncoupling protein-2 has also been observed in the ovine placenta at mid and late gestation of ewes undernourished during early pregnancy (Gnanalingham et al. 2007). These mitochondrial changes are likely to affect the efficiency of ATP production and superoxide generation with wider implications for placental function (Figure 1). Certainly in humans and mice, nutritional and hypoxic stimuli alter placental ATP content (Tissot et al. 2010; Chiaratti et al. 2015). Consequently, even though placental O_2 consumption is maintained during many stressful conditions (Figure2), there may be changes in placental energetics and consumption of oxidative substrates that affect fetal delivery of nutrients and O_2. Indeed, rates of umbilical O_2 uptake per kg sheep fetus vary little in response to acute and chronic stresses, which indicates that the fetus grows primarily in relation to its overall O_2 availability (Figure 2).

CARBOHYDRATES

Normal requirements

In all farm animals studied to date, the main carbohydrate used by the uteroplacental tissues is glucose (Figure 1). Its primary source in normal conditions is the mother. Glucose crosses the placenta by facilitated diffusion down a materno-fetal glucose concentration gradient using glucose transporters (GLUTs). However, when this gradient is abolished experimentally in sheep, uteroplacental glucose consumption remains at 80% of normal values by deriving glucose from the fetal circulation (Simmons et al. 1979). Two GLUT isoforms, GLUT1 and GLUT3, have been detected in ruminant and equine placenta and are used sequentially in transplacental glucose transfer (Wooding and Burton 2008). GLUT 8 has also been identified in the ovine placenta and may be involved in transporting glucose across the fetal facing membranes (Limesand et al. 2005).

In late gestation, fetal and placental rates of glucose consumption calculated by kg tissue vary between species but are consistently 5-10 fold higher in the placenta than fetus (Table 1). Consistent with the lower rates of fetal glucose uptake in sheep and cows in late gestation (Table 1), the cotyledonary epitheliochorial placenta of these ruminants appears to use a greater proportion of the glucose taken up from the uterine circulation (55-85%) than the diffuse epitheliochorial placenta of horses and pigs (25-50%, Fowden 1997). Glucose consumption per kg of combined uteroplacental tissues increases between mid and late gestation in sheep but decrease over the last third of gestation in the horse, although, in both species, the percentage of total uterine glucose uptake used by the uteroplacental tissues is less near term than earlier in gestation (Bell et al. 1986; Molina et al. 1991; Fowden et al. 2000).

In sheep, the glucose consumed by the uteroplacental tissues is known to be used for oxidative phosphorylation and synthesis of polyols, other sugars and carbohydrates (Figure 1). Measurements made with tracer glucose indicate that, of the glucose used by the uteroplacental tissues, 15-20% is oxidised to CO_2, about 30% is converted to lactate via glycolysis and 5-10% is metabolised to fructose via sorbitol (Aldoretta and Hay 1999). The remaining 40-50% of the glucose carbon is unaccounted for but may contribute to the short term turnover of amino acids, glycerol and keto acids and/or to the synthesis of substances with longer turnover times such as glycoaminoglycans, proteins and lipids (Aldoretta and Hay 1999; Kim et al., 2012). Some of the lactate and fructose produced by the ovine placenta may also be used oxidatively for ATP generation, which, together with glucose, could account for up to 50% of the normal rate of uteroplacental O_2 consumption (Sparks et al. 1982; Meznarich et al. 1987). However, the majority of the lactate and fructose produced by the ovine placenta in late gestation appears to be transported into either the umbilical and/or uterine circulations (Figure 1). GLUT8 may be responsible for fructose transport but little is known about placental expression of the monocarboxylate transporters (MCTs) that transport lactate in any farm animal (Limesand et al. 2005). Two MCT isoforms, MCT1 and MCT4, have been identified in human and mouse placenta with species specific polarised expression on maternal and fetal facing membranes indicative of different transport kinetics at the two surfaces (Settle et al. 2004; Nagai et al. 2010).

Fructose is also detected in high concentrations in fetal pigs, cows and horses but whether the placenta produces fructose and releases it into the fetal circulation in late gestation in these species remains unclear (Silver 1984). Porcine trophectoderm cells can use fructose *in vitro* to synthesise glycoaminoglycans such as hyaluronic acid and the ovine placenta uses small amounts of fructose oxidatively and to produce lactate *in vivo*, although little is known about these metabolic processes in other species (Meznarich et al. 1987; Kim et al. 2012). In contrast, lactate production by the uteroplacental tissues has also been observed in pigs, horses and cows (Table 1). In the ovine and bovine placenta, net production of lactate appears to be derived solely from glucose and varies directly with the rate of uteroplacental glucose consumption in normal conditions (Comline and Silver, 1976; Aldoretta et al. 1994; Aldoretta and Hay, 1999). Uteroplacental lactate production per unit weight of total tissue increases between mid and late gestation in sheep and horses in association with changes in its relative distribution between the uterine and umbilical circulations (Sparks et al. 1982; Bell et al. 1986; Fowden et al. 2000). In sheep at mid gestation, uteroplacental lactate production is low and distributed almost entirely into the uterine circulation, whereas, by late gestation, production is 3-4 fold higher per unit weight and distributed equally into the fetal and maternal circulations (Bell et al. 1986; Figure 1). In horses, uteroplacental lactate production is undetectable at mid gestation while near term it occurs at a significant rate and is distributed solely to the fetus (Fowden et al. 2000). Similarly, in cows near term, the majority of lactate produced by the uteroplacental tissues is released into the umbilical circulation, although absolute rates of production vary with breed (Comline and Silver 1976; Ferrell 1991). Like cows, uteroplacental lactate production in pigs appears to be delivered primarily to the fetus near term and makes a greater contribution to the daily fetal carbon requirement in pigs than other farm animals (Fowden et al. 1997). The mechanisms involved in these ontogenic changes in uteroplacental production and distribution of lactate remain unknown but may involve alterations in cell types or cellular O_2 availability within the placental tissues and, possibly, a switch from oxidative to more glycolytic metabolism of glucose towards term. However, since lactate and fructose can both be utilised by feto-placental tissues (Sparks et al. 1982; Meznarich et al. 1987), their placental production

provides alternative sources of carbon for fetal metabolism and growth, which may be beneficial in stressful conditions.

Response to stress

During nutritional stresses, uteroplacental consumption and umbilical uptake of glucose alter largely in line with the changes in maternal glycaemia and the transplacental glucose concentration gradient (Hay 2006). In sheep, stresses which produce maternal hypoglycaemia, therefore, tend to reduce glucose consumption calculated per kg placenta while, conversely, maternal hyperglycaemia increases these rates (Figure 2). During maternal hypoglycaemia lasting 2-7 days, percentage distribution of uterine glucose uptake between ovine uteroplacental and fetal tissues does not alter and both share equally in the reduced glucose availability (Hay et al. 1983; 1990; Fowden and Forhead 2011). However, as maternal hypoglycaemia becomes prolonged, the uteroplacental tissues appear to use proportionally more of the uterine glucose uptake than in normoglycaemic conditions (Hay et al., 1983; Carver and Hay 1995). The relationship between uteroplacental glucose consumption and maternal glucose levels is, therefore, more complex during stressful than normal conditions. Particularly in late gestation, fetal sheep can activate glucogenesis when hypoglycaemic or hypercortisolaemic, which raises their glucose levels independently of the maternal concentrations (DiGiacomo and Hay 1989; Ward et al. 2004; Houin et al. 2015). This has consequences for the transplacental glucose concentration gradient and carbon fluxes from the placenta to fetus and vice versa (DiGiacomo and Hay 1989). Indeed, net uteroplacental glucose consumption varies directly with the fetal glucose concentration when fetal glucose levels are manipulated experimentally independently of the mother (Hay et al. 1990; Thureen et al. 1992; Ward et al. 2004).

Chronic stresses that reduce placental growth like hyperthermia and hypoglycaemia alter the glucose transport capacity of the ovine placenta at any given transplacental gradient, which suggests that other morphological and/or functional factors are influencing placental fluxes and consumption of glucose in these circumstances (Fowden et al. 2010). Certainly, placental GLUT expression is affected by longer term variations in maternal glycaemia with decreases in GLUT1 abundance in hypoglycaemia and hyperthermic conditions, and in both

GLUT1 and GLUT 3 abundance in response to maternal hyperglycaemia in ewes (Das et al. 1998; 2000; Zhu et al. 2010; Ma et al. 2011). Similar changes in the glucose transport capacity associated with altered GLUT expression are observed in the small placenta of carunclectomised ewes and in the mouse placenta after maternal undernutrition and other dietary manipulations (Owen et al. 1987b; Vaughan et al. 2012).

During uterine artery constriction, ovine uteroplacental tissues use less glucose, which sustains umbilical glucose uptake in the face of the reduced uterine glucose delivery (Hooper et al. 1995). Since placental O_2 consumption is maintained in these and other stressful conditions in which placental glucose consumption is reduced (Figure 2), the ovine placenta must switch from glucose to other oxidative substrates to maintain its respiratory rate (Figure 1). In contrast, when O_2 availability is reduced at high altitude, the human placenta uses 60% more glucose and 20% less O_2 than at sea level (Illsley et al. 2010). The hypoxic human placenta, therefore, appears to switch from oxidative phosphorylation of glucose to a greater dependence on glycolysis to meet its ATP requirements, thereby sparing O_2 but reducing glucose availability for fetal delivery.

In sheep, placental production and umbilical uptake of lactate appear to parallel placental glucose consumption during most stressful conditions (Figure 2). However, in the small placenta of carunclectomised ewes, uteroplacental lactate production exceeds the rate of uteroplacental glucose consumption so there must be other carbon sources for lactate synthesis and/or oxidative phosphorylation in these animals (Owens et al. 1987b). Similarly, when either maternal or fetal cortisol levels are raised, placental lactate production appears to vary independently of placental glucose consumption (Figure 2). With cortisol overexposure from the fetus, uteroplacental lactate production is unaffected despite increased uteroplacental glucose consumption, whereas, when cortisol is infused maternally, uteroplacental lactate production increases without a significant rise in uteroplacental glucose consumption (Ward et al. 2004; Vaughan et al. 2016). Thus, lactate production by ovine uteroplacental tissues is regulated dynamically and is responsive to fetal and maternal environmental cues. Indeed, the ovine placenta can switch rapidly from net production to net consumption of lactate during exercise and from releasing lactate into

the fetus to clearing it from the fetal circulation within 4h of uterine artery restriction (Chandler et al. 1985; Hooper et al. 1995).

AMINO ACIDS

Normal requirements

The placenta transports, utilises, produces and interconverts amino acids. The ovine placenta has a high rate of protein synthesis and, given the changes that occur in placental morphology over the second half of gestation, its rate of protein turnover is also likely to be high (Vatnick and Bell 1992; Bell and Ehrhardt, 2002; Wooding and Burton, 2008). In sheep, all 9 essential amino acids that cannot be synthesised *de novo* and most of the other amino acids needed for protein synthesis are taken up from the uterine circulation against their concentration gradients using energy dependent active transport (Bell and Ehrhardt, 2002). In late gestation, fetal concentrations of most amino acids are also higher than those of the mother in cows and pigs although not consistently in the horse (Silver et al. 1994; Ashworth et al. 2013; Zicker et al. 1994). There are also breed differences in fetal and maternal amino acid profiles and in fetal to maternal concentration ratios for specific amino acids in sheep, pigs and horses, which may relate, in some instances, to differences in nutrition (Zicker et al. 1994; Silver et al. 1994; Wu et al. 1998; Kwon et al. 2004; Jobgen et al. 2008; Ashworth et al. 2011; 2013). In addition, fetal to maternal concentration amino acid ratios may change with increasing gestational age in pigs and horses (Silver et al. 194; Zicker et al. 1994; Wu et al. 1998; Ashworth et al. 2013).

Although net amino acid transport is from mother to fetus for most amino acids, significant bidirectional fluxes have been observed across ovine placental membranes using labelled amino acid tracers (Battaglia 2002). For three amino acids (glutamate, aspartate and serine), there is no net uteroplacental uptake from the ovine uterine circulation (Regnault et al. 2002). Instead, the uteroplacental tissues derive these amino acids from the fetal circulation. Multiple amino acid transporter systems have been identified in the ovine placenta, which differ in their amino acid specificity, sodium-dependence and localisation

within the placental barrier (Regnault et al. 2002; Wooding and Burton 2008). Amino acid specificity of the transporter systems overlaps for some amino acids so there is competition between these amino acids for uteroplacental uptake and transplacental transport which depends on their concentrations in the maternal circulation (Regnault et al. 20002).

The ovine placenta is a net consumer of glutamate, serine and three branched chain amino acids (BCAA), valine, leucine and isoleucine, and also releases glutamine, methionine and glycine into the fetus in excess of the uterine uptakes (Chung et al, 1998). Thus, significant catabolism and/or transamination of amino acids occurs within the ovine placenta, which leads to the production of ammonia and α-keto acids (Figure 1). The ammonia is released primarily into the uterine circulation but can also be used to synthesis other amino acids such as glutamate (Leichty et al. 1991). The α-keto derivatives may be oxidised to produce ATP, released into the fetal circulation or metabolised into amino acids and other substances, such as fatty acids, proteins and peptides that are, in turn, metabolised or secreted by the placenta (Figure 1). Placental mitochondria have been shown to use several amino acids for oxidative phosphorylation *in vitro* and glutamate is oxidised at high rates by the ovine placenta *in vivo* in late gestation (Moores et al. 1994: Battaglia 2002). Given its large placental uptake and synthesis *in utero* from BCAA (Battaglia and Regnault 2001), glutamate is likely to be quantitatively the most important fuel amongst the amino acids. If complete, its oxidation would account for 10% of the uteroplacental O_2 consumption and provide NADPH for placental steroidogenesis, lipogenesis and nucleoside production.

In addition to oxidation, 6% of the glutamate taken up by the ovine placenta is converted to glutamine, which is then released into the fetal circulation in amounts exceeding its uterine uptake (Moores et al. 1994). Glutamine is also synthesised from BCAA and glutamate by the porcine and equine placenta (Self et al 2004; Manso Filho et al. 2009). It is used for feto-placental synthesis of protein and glycosaminoglycans and is re-converted back to glutamate by fetal ovine liver (Battaglia 2000; Kim et al. 2012). There is also significant metabolic interconversion of alanine, pyruvate and lactate in the ovine placenta without net uteroplacental alanine consumption (Timmerman et al. 1998). Alanine derived from the

maternal circulation is, therefore, exchanged for endogenously produced alanine with the result that net umbilical uptake of alanine is derived from placental transamination and protein turnover with only a small fraction coming from direct transplacental flux (Timmerman et al. 1998). Similarly, serine taken up from both circulations is metabolised to glycine in the ovine placenta, which results in significant umbilical glycine uptake without net uterine uptake (Geddie et al. 1996; Regnault et al. 2002). In addition, the methylenetetrahydrofolate produced by conversion of serine to glycine can be used in purine synthesis or for remethylation of homocysteine to methionine. If homocysteine is taken up from the uterine circulation, this metabolic pathway may also account for the umbilical uptake of methionine in the sheep fetus. Placental amino acid metabolism is, therefore, complex and involves metabolic cycling between the maternal, placental and fetal compartments with important consequences for the amounts and composition of the amino acids delivered to the fetus.

Response to stress

In farm animals, fetal and maternal amino acid concentrations are affected by a wide range of stressful conditions including heat stress, undernutrition, hypoglycaemia, protein deprivation and glucocorticoid administration (Schaefer et al. 1984; Silver et al. 1994; Wu et al. 1998; Timmerman et al. 2000; Kwon et al. 2004; Ashworth et al. 2011; 2013; Regnault et al. 2013). For instance, maternal undernutrition influences maternal and fetal amino acid profiles, reduces specific amino acid concentrations and alters the fetal to maternal concentration ratios for specific amino acids in sheep, pigs and horses, which suggests that placental amino acid transport or competition amongst the amino acids for the transporters and/or feto-placental amino acid metabolism are altered in these circumstances (Schaefer et al. 1984; Silver et al. 1994; Kwon et al. 2004; Ashworth et al. 2011). In sheep, these changes persist after restoration of normal nutrition which indicates that feto-placental amino acid metabolism may be permanently altered by nutritional stress earlier in gestation (Kwon et al. 2004). Certainly, undernutrition of pregnant ewes for 7 days leads to increased placental BCAA utilisation and ammonia production, indicative of increased placental amino acid deamination (Leichty et al. 1991). There are also reductions in the umbilical uptake, transplacental flux and feto-placental back flux of leucine and threonine after heat stress,

even when the lower placental weight is taken into account (Ross et al. 1996; Anderson et al. 1997). Similarly, umbilical leucine uptake per kg fetus is less during prolonged maternal hypoglycaemia and coupled with a trend for greater percentage utilization of the uterine uptake by the uteroplacental tissues (Carver et al. 1997). In addition, both maternal undernutrition and fetal dexamethasone administration reduce fetal glutamate concentrations and placental glutamate uptake from the fetal circulation, which indicates that ovine placental-fetal amino acid cycling is also responsive to environmental conditions during late gestation (Schaefer et al. 1984; Leichty et al. 1991; Timmerman et al. 2000; Houin et al. 2015). Reduced placental uptake and metabolism of glutamate may also lower NADPH availability consistent with the decrease in progesterone synthesis seen when fetal glucocorticoids rise in late gestation (Silver 1984; Timmerman et al. 2000). Similar changes in amino acid cycling between the fetal and placental compartments are also seen in response to manipulation of other fetal hormone concentrations (Teng et al. 2001).

When availability of single amino acids is increased experimentally in pregnant ewes, their umbilical uptake and placental utilization is increased significantly, probably at the expense of other amino acids using the same transporters (Timmerman et al. 1998; Battaglia 2002; Thureen et al. 2002). Similarly, maternal BCAA infusion increases their umbilical uptake and uteroplacental utilization by deamination as indicated by the increased uteroplacental production of ammonia (Jozwik et al. 1999; 2001). However, when mixtures of amino acids are infused, umbilical uptake may increase, decrease or be unaffected depending on the specific amino acid due to competitive inhibition by the other amino acids for the different transporter systems in the ovine placenta (Battaglia 2002). Collectively, these findings suggest that placental amino acid metabolism and transport adapts to environmental stresses in farm animals with implications for fetal growth as seen in humans and rodents (Vaughan et al. 2012; Gaccoli et al. 2013; Lewis et al. 2013; Day et al. 2015)

LIPID, FATTY ACID AND VOLATILE FATTY ACIDS

Normal requirments

Although lipids and free fatty acids (FFA) are required for growth and development of feto-placental tissues, the epitheliochorial placenta of ruminants, pigs and horses appears to relatively impermeable to these substances compared to the human and rodent haemochorial placenta (Herrera and Ortega-Senovilla 2014). Both the uterine arterio-venous and the umbilical venous-arterial concentration differences in fatty acids are negligible in sheep, cows and horses during late gestation (James et al. 1971; Elphick et al. 1979; Stammers et al. 1988). There is also little evidence for transfer of labelled short or long chain fatty acids across the ovine placenta, despite the presence of fatty acid transporters in the placentomes at mid and late gestation (Elphick et al. 1979; Leat and Harrison 1980; Zhu et al. 2010; Ma et al. 2011). However, in sheep and horses, the placenta does appear to hydrolyse esterified lipids and to desaturate and elongate fatty acids, including the essential C18 fatty acids, which, together with placental synthesis of lipids from glucose and keto acids, may provide an adequate supply of essential lipids and fatty acids to the feto-placental tissues (Stammers et al. 1988; Bell and Ehrhardt 2002).

In sheep and cows, rumen fermentation leads to significant amounts of acetate and other volatile fatty acids (VFA), such β-hydroxybutyrate and acetoacetate, in the maternal circulation. Although these substances are taken up by the uterus in relatively small amounts compared to other nutrients, they are utilized by the uteroplacental tissues and transported to the fetus (Figure 1). In both sheep and cows, rates of VFA consumption are higher per kg of placental than fetal tissues (Comline and Silver 1976; Carver and Hay 1995). In sheep, the β-hydroxybutyrate taken up from the uterine circulation is utilised almost entirely within the uteroplacental tissues with no significant onward transfer to fetus whereas uterine acetoacetate uptake is distributed equally between the uteroplacental and fetal tissues, although the uterus takes up significantly less acetoacetate than β-hydroxybutyrate (Smeaton et al. 1989; Carver and Hay 1995). In cows near term, there is significant uteroplacental consumption of acetate at rates 8-10 fold higher than those of the fetus when expressed per unit weight (Comline and Silver 1976). The fate of the VFA used *in utero* remains unknown but may involve oxidative phosphorylation to generate ATP and/or synthesis into steroids and fatty acids (Dhand et al. 1970; Miodovnik et al. 1982; Christie and Noble 1982)

Response to stress

Compared to carbohydrates and amino acids, much less is known about the effects of adverse conditions on the placental metabolism and transport of lipids, FFA and VFA in farm animals. In sheep and horses, there are changes in the lipid and FFA profiles of fetal and maternal plasma in response to maternal undernutrition, which may be related, in part, to altered placental lipid metabolism (Stammers et al. 1988; 1995). Certainly, in both these species, maternal hypoglycaemia induced by short term fasting or insulin infusion is associated with increased uteroplacental synthesis and release of prostaglandins, which are hormones derived from arachnidonic acid through phospholipid metabolism (Silver & Fowden 1982; Fowden and Silver 1983). This has led to the suggestion that the placenta may switch from glucose to a greater use of lipids as metabolic fuels when glucose availability is limited, thereby increasing the supply of precursors for prostaglandin synthesis (Fowden et al. 1994). This is consistent with the increase in fatty acid transporters seen in the ovine placenta during undernutrition (Ma et al. 2011). Similar increases in placental lipid metabolism are believed to occur in the human and rodent placenta during intrauterine growth restriction (Cetin and Alvino, 2009; Herrera and Ortega-Senovilla, 2014)

Fetal VFA concentrations have been shown to vary naturally with maternal concentrations in sheep and cows but little is known about the factors regulating placental VFA metabolism and transport in adverse conditions (Comline and Silver 1976). Infusion of β-hydroxybutyrate into pregnant ewes increases its fetal concentration and causes fetal lactacideamia and hypoxaemia (Miodovnik et al. 1982). Prolonged maternal undernutrition also increases maternal concentrations and uterine uptake of β-hydroxybutyrate through increased maternal fat utilisation, which may provide the placenta with alterative oxidative substrates to glucose (Chandler et al. 1985). In contrast, prolonged insulin-induced maternal hypoglycaemia leads to decreased uterine uptake and uteroplacental utilization of both β-hydroxybutyrate and acetoacetate in the absence of changes in the maternal or fetal concentrations (Carver and Hay 1995). Taken together, these findings suggest that VFA

metabolism by the ruminant placenta is responsive to environmental stresses but is determined largely by maternal nutrient availability.

CONCLUSIONS

The placenta is a metabolically labile organ that is responsive to a range of interdependent nutritional and endocrine signals of adversity (Figure 3). It integrates these multiple signals and adapts its metabolic phenotype accordingly to maintain pregnancy and maximise the chances of fetal survival *in utero*. The metabolic response of the placenta depends on the nature, severity and duration of the stressful challenge and also on whether signals of stress are maternal, placental or fetal in origin (Figure 3). By diversifying the sources of carbon and nitrogen available to the fetus, the metabolic responsiveness of the placenta also helps to optimise offspring fitness for the prevailing environmental conditions and, thus, improves the likelihood of the offspring reaching reproductive age.

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FIGURE LEGENDS

Figure 1: Schematic diagram showing the rates of transport of oxygen and nutrients from the uterine circulation and into the umbilical circulation in pregnant sheep with a fetus of an average weight of 3kg with a 300g placenta (complete placentomes) at about 80-90% of gestation. Solid lines = Major routes of metabolism. Dashed lines = Minor routes of metabolism Trace = ≤ 5 μmol/min. VFA = Volatile fatty acids. FFA = Free fatty acids. NH_3 = Ammonia. Data from Sparks et al. 1982; Meznarich et al. 1987; Smeaton et al. 1989; Hay et al. 1990; Carver and Hay, 1990; Chung et al. 1998; Teng et al. 2002; Regnault et al., 2007; 2010; Fowden and Forhead, 2011; Vaughan et al 2016.

Figure 2: Rates of placental consumption or production (calculated from uteroplacental measurements and expressed per kg total whole placentomes) and of fetal umbilical uptake (per kg fetus) of oxygen, glucose and lactate in sheep in late gestation in response to a range of acute and chronic stresses presented as a percentage of the normal control values.
* significantly different from control animals as identified in the individual studies.
Data derived from Chandler et al. 1985; Owens et al. 1987a,b; DiGiacomo and Hay 1989; Carver and Hay 1995; Thureen et al. 1992; Aldoretta et al 1994; Aldoretta and Hay, 1998; Carver et al. 1997; Wallace et al., 2001; Ward et al. 2004; Regnault et al. 2007; Limesand et al. 2009; Fowden and Forhead 2011; Vaughan et al. 2016.

Figure 3: Schematic diagram of the stressful and other environmental factors in the mother and fetus influencing placental metabolism during late gestation showing the known placental processes affected by environmental changes.

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Table 1: Average weight specific rates of consumption (or production †) of oxygen, glucose and lactate by the placenta (calculated using uteroplacental values expressed per unit of total weight of whole placentomes) and fetus (using body weight) of different species during late gestation (≥ 80% gestation).

	Placental consumption or production μmol/min/kg placenta			Fetal umbilical uptake μmol/min/kg fetus		
	Oxygen	Glucose	Lactate†	Oxygen	Glucose	Lactate
Sheep	1700	350	250	310	30	30
Cow	1200	270	160	300	30	30
Pig	1070	200	250	340	40	40
Horse	1900	400	50	290	40	10

837 Data derived from Comline and Silver 1976; Reynolds et al., 1985; Ferrell, 1991; Fowden and Silver
838 1995; DiGiacomo and Hay 1989; Fowden et al. 1997; 2000; Aldoretta and Hay 1999.

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Figure 1

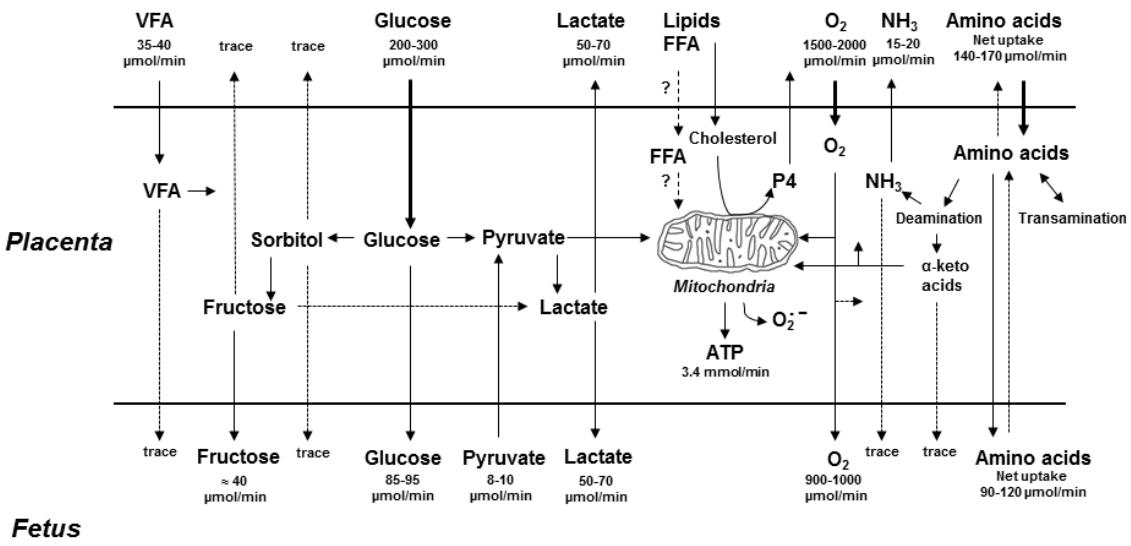
Mother
VFA
35-40 µmol/min
trace
trace
Glucose
200-300 µmol/min
Lactate
50-70 µmol/min
Lipids
FFA
?
O2
1500-2000 µmol/min
NH3
15-20 µmol/min
Amino acids
Net uptake
140-170 µmol/min
Cholesterol
FFA
?
P4
O2
NH3
Amino acids
Deamination
Transamination
α-keto acids
Placenta
VFA
Sorbitol
Glucose
Pyruvate
Fructose
Lactate
Mitochondria
O2·-
ATP
3.4 mmol/min
trace
Fructose
≈ 40 µmol/min
trace
Glucose
85-95 µmol/min
Pyruvate
8-10 µmol/min
Lactate
50-70 µmol/min
O2
900-1000 µmol/min
trace
trace
Amino acids
Net uptake
90-120 µmol/min
Fetus

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Figure 2

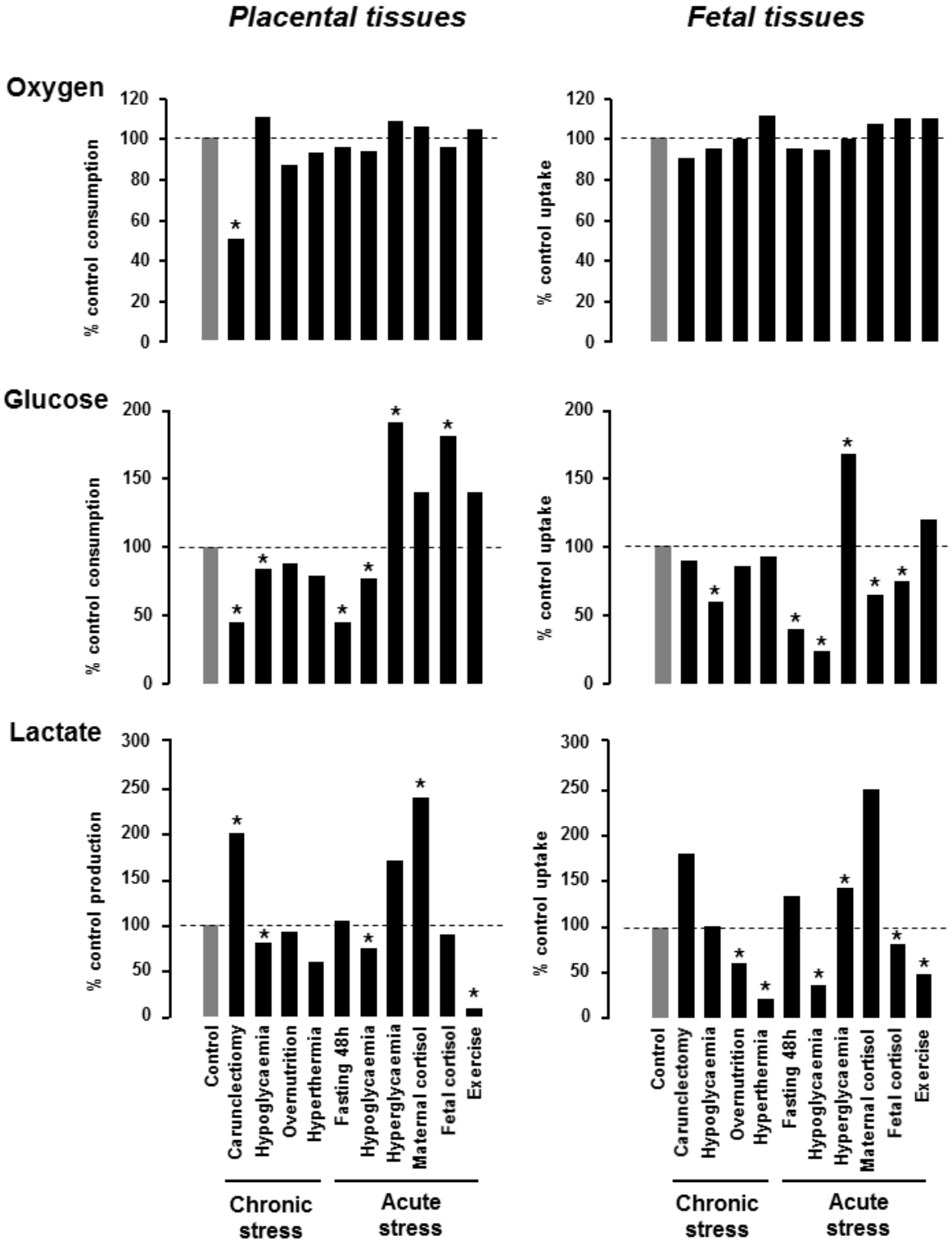

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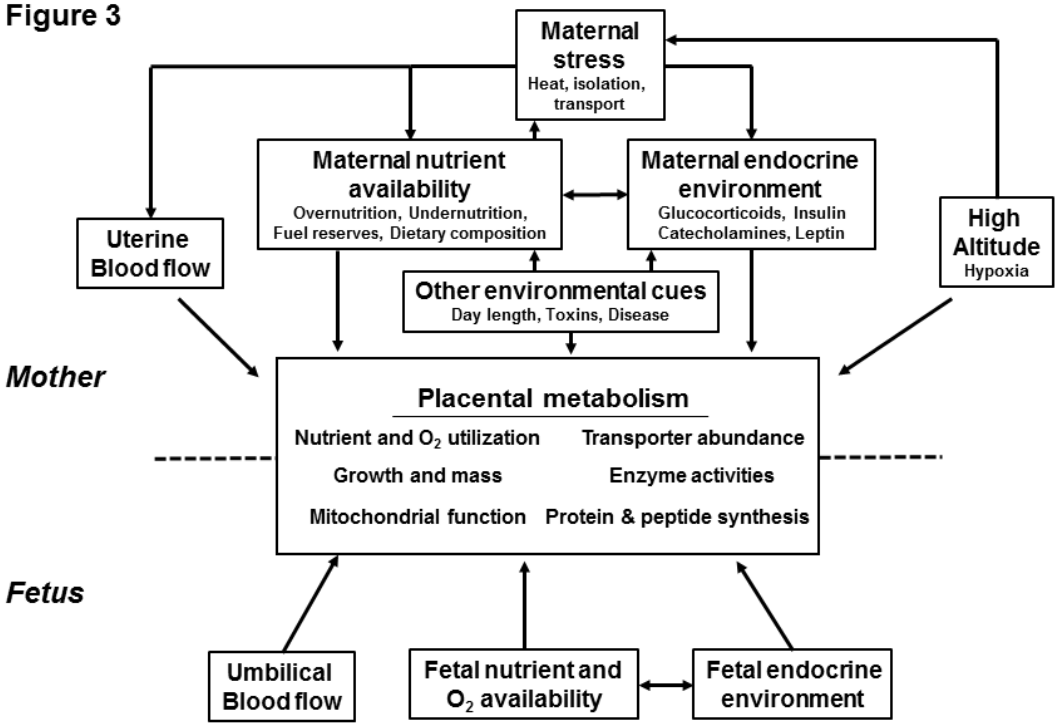
Figure 3



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