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Fetal nutrition

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Fetal nutrients are derived largely from the mother, and fetal nutrition is thus closely related to maternal nutrition. However, it is important to appreciate that maternal nutrition is not the same as fetal nutrition. Firstly, the mother has her own nutrient demands which may be in conflict with those of the fetus. For example, pregnant adolescent sheep deliver smaller fetuses, especially when the ewes are very well nourished and therefore growing well, and the growth restriction appears to be predominantly secondary to reduced placental growth.[1–3] Human adolescents also tend to give birth to lighter infants, and birth weight has been reported to be less in offspring of adolescents with a higher dietary sugar intake.[4,5] Secondly, the fetus lies at the end of a long supply line which can be impaired at many points. Nutrients are used by the fetus predominantly for growth and metabolism, with little energy expenditure on other processes such as thermoregulation, movement and digestion. Fetal nutrients are in fact the main drivers of fetal growth, with genetic factors playing a much smaller role. Indeed, the genetic regulation of fetal growth itself appears to be under nutritional regulation, with levels of all the major hormones involved in fetal growth being regulated by circulating nutrient levels. The placenta is also a very metabolically active organ with its own nutrient demands and metabolic pathways. The demands of the fetus and placenta must be in close harmony, particularly in situations where the nutrient supply is precarious, as if the placenta is starved of nutrients and fails the fetus will also not survive. Therefore, in extreme cases the placenta may even consume substrates provided by the fetus. This chapter will attempt to describe the physiology of fetal nutrient supply as we currently understand it, and to relate some aspects of fetal nutrition to clinical data.

The influence of maternal nutrition on fetal nutrient supply

The associations between maternal prepregnancy weight, pregnancy weight gain and birth weight are well known. Birth weight increases with increasing maternal prepregnancy size,[6] and has also been associated with maternal weight gain in pregnancy, particularly with increases in maternal fat mass.[7] Similarly, poor maternal weight gain in all trimesters of pregnancy has been associated with lower birth weight,[8–10] although there is some disagreement about which period of pregnancy is most crucial. Customized growth charts have been developed which take into account maternal height, weight, parity and ethnic group,[11] which may assist in the detection of babies that are not growing appropriately.[12,13] However, these factors account for, at best, 15% of the variability in fetal growth,[14] with the best predictor of birth weight being the mother's own growth in utero. This is true in both developed and developing nations.[14,15]

The fact that less than 15% of the variability in birth weight is accounted for by markers of maternal nutrition before and during pregnancy may explain why maternal nutrient supplementation during pregnancy has little effect on birth weight. A meta-analysis of studies of maternal balanced protein/energy supplementation does demonstrate a reduction in the incidence of small-for-gestation-age (SGA) babies, but not a significant effect on birth weight.[16] However there is increasing evidence that some aspects of maternal diet may have stronger effects on birth weight. High protein supplements during pregnancy actually had a negative effect on birth weight.[17] A study from Southampton found that placental and fetal weights

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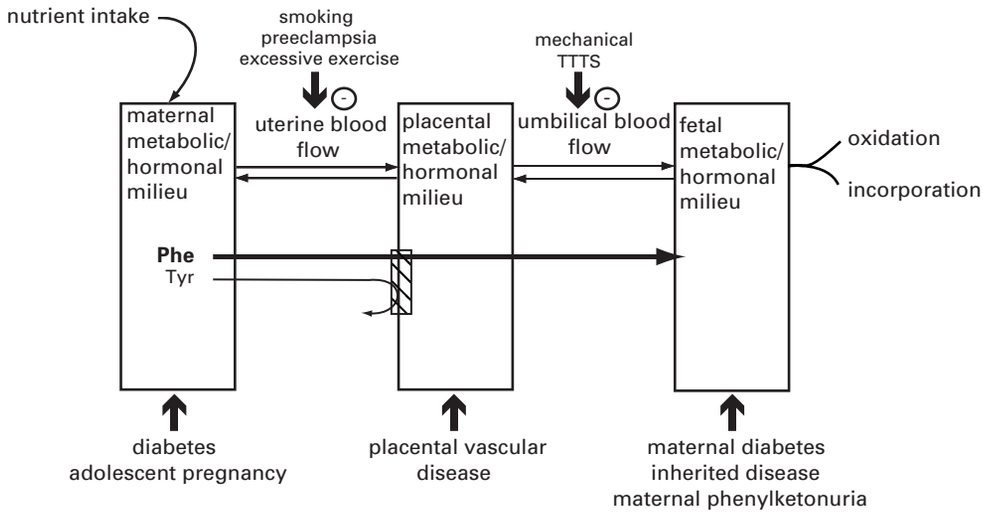

Figure 1.1. The fetal supply line. Nutrients ingested by the mother must pass along a supply line before being utilized by the fetus. The amount of any given nutrient finally utilized by the fetus for growth or metabolism may be affected at any point along this supply line. A few examples are given. Phe = phenylalanine, Tyr = tyrosine, TTTS = twin-twin transfusion syndrome.

were related to the balance of energy obtained from protein and carbohydrate at different times in pregnancy, with high carbohydrate intake in early pregnancy being related to smaller placentae and lower birth weights, and higher protein intake in late pregnancy being related to increased birth weights.[18] This study, and others, have also reported that as many as 40% of pregnant women fail to reach the recommended daily intake for many nutrients.[18–20] Another recent study from the UK found no association between the intake of any macronutrient and birth weight,[21] but a significant, although small, increase in birth weight with increasing vitamin C intake. The potential role of micronutrients in fetal growth is also supported by a recent study in rural Nepal which demonstrated an increase in birth weight with folic acid and iron supplementation and with supplementation with multiple micronutrients.[22] Rural Indian women who consumed more green vegetables and milk, rich sources of several micronutrients including folate, also gave birth to bigger babies.[22] Thus although total maternal energy consumption may have little effect on size at birth, the balance of macronutrients and the micronutrient content of the mother's diet may have an important influence on the nutrition of her baby.

One explanation for the lack of effect of maternal dietary supplementation may be the fact that the fetus lies at the end of a long "supply line" involving maternal metabolic and hormonal status, uterine and umbilical blood flows, placental size and transport capacity and the fetal metabolic and hormonal status. Maternal nutrient supply to the fetus may be affected at any point along this supply line, for example by placental disease, variations in uterine blood flow (e.g. smoking) etc., thus influencing fetal nutrition (Figure 1.1). An alternative, or additional, explanation may be that certain micronutrients are deficient, or borderline deficient. If one particular micronutrient is supplemented, this may simply lead to the next most marginal nutrient becoming limiting, so that there is little overall effect on fetal growth. Analogous situations may occur in conditions of excess of certain nutrients, e.g. phenylalanine in phenylketonuria, when the high amounts of phenylalanine may saturate placental amino acid transporters and prevent transport of other essential amino acids such as tyrosine and tryptophan (Figure 1.1).[23,24] Another example may be diets high in methionine, which requires glycine for detoxification via transulphuration. Excess methionine in a diet already marginal in glycine may lead to glycine availability to the fetus becoming limiting.[25]

The potential role of folate has been mentioned above. Folate cannot be synthesized by humans, yet is an essential vitamin for many cellular processes including the recycling of methionine and homocysteine, steps in the formation of purines and pyrimidines, and for the formation of glycine from serine. Intake of green leafy vegetables, a rich source of folate, has been found to be strongly associated with birth weight in a rural Indian community.[26] Erythrocyte folate concentrations were also independently associated with birth weight and with intake of green leafy vegetables in this study. It has also been proposed that

the intrauterine growth restriction (IUGR) seen in millions of low birth weight babies born to mothers infected with malaria may be, in part, due to disturbances within the folate pathway.[27] Malaria increases folate demand secondary to hemolysis and to a functional folate deficiency caused in part by hyperhomocysteinaemia and also the coexisting deficiency of other vitamins such as B_{12}. A secondary effect of folate deficiency, or of functional disruption of the folate cycle, is glycine deficiency. Glycine is considered to be a conditionally essential amino acid for the fetus and neonate.[28] During growth demands for glycine are high, and it is used in many metabolic processes essential for growth such as purine and porphyrin synthesis, interconversion with serine and also for the production of the free radical scavenger glutathione from α-glutamylcysteine. Glycine is also necessary for the detoxification of excess methionine. Up to 90% of fetal glycine is produced from serine by the placenta, and folate is essential for this interconversion.[29–31] Urinary excretion of 5-L-oxoproline has been used as a measure of glycine insufficiency.[32] 5-L-oxoprolinuria increases throughout pregnancy, and has been found to be higher in women with a poorer diet compared with better-nourished women,[33] suggesting that glycine may be relatively deficient in pregnant women.

Another amino acid that has received attention recently is taurine, the most abundant free amino acid in the body. Taurine is involved in cholesterol degradation, is a neurotransmitter, an osmoregulator and an antioxidant. Reduced activity of placental taurine transporters has been reported following maternal undernutrition and in IUGR in rats.[34,35] Recent interest, however, has focussed upon the role of taurine in pancreatic beta cell development. Rats fed a low protein diet have reduced circulating taurine levels, as do their fetuses.[36,37] Pups from mothers fed a low protein diet have reduced β cell mass and reduced islet area, and this persists into adult life. Supplementation of the mothers' drinking water with 2.5% taurine reversed the IUGR, restored a normal balance of proliferation and apoptosis in pancreatic islets[38] and restored insulin secretion *in vitro* to normal.[36] Furthermore, fetal plasma insulin levels were significantly correlated with fetal taurine concentrations.[37]

Fetal nutrition before placentation is established

Nutrient transfer to the fetus via the placenta in the second and third trimesters is relatively well understood in comparison to fetal nutrient supply in the first trimester. The human blastocyst implants at a relatively early stage and the developing conceptus is enveloped by the superficial layer of the endometrium by day 10 after fertilization.[39]

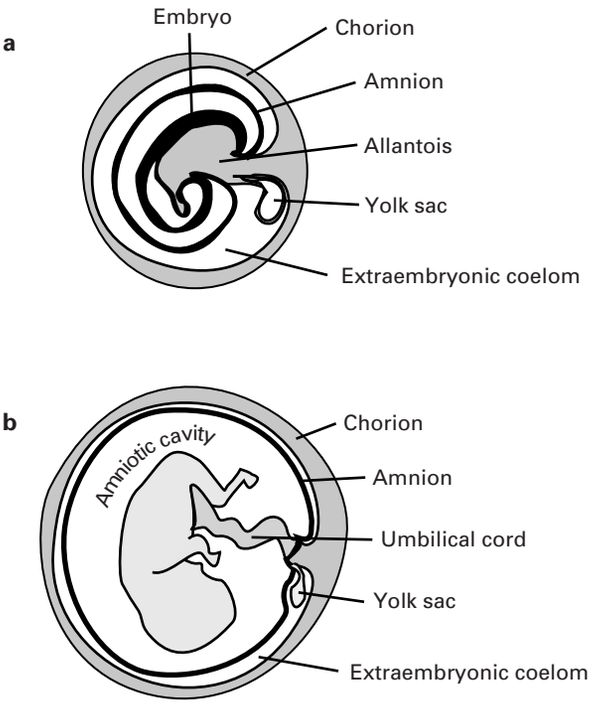

Figure 1.2. Demonstration of the changes in formation of the extraembryonic membranes and fluid cavities over the first 3 months of gestation.

Trophoblast then rapidly invades the vascular network of the endometrium. It has been proposed that maternal blood flows through the developing placenta during the third week, thus establishing the beginnings of hemotrophic nutrition to the embryo.[39,40] However, recent *in vivo* data suggest that significant maternal blood flow through the intervillous spaces may not occur much before the end of the first trimester. The finding of an increase in oxygen tension in the placenta between 10 and 12 weeks of gestation[41–43] may support this hypothesis, although it is still contentious.[44]

Until the placenta does develop sufficiently for hemotrophic nutrition to be established, the developing embryo must be supplied by histiotrophic nutrition. Human trophoblast is highly phagocytic, and has been shown to endocytose maternal erythrocytes and proteins.[45] It has been proposed that this nutrition is supplied via the uterine glands via the fetal fluid compartments.[46] These glandular secretions contain glycogen, glycoproteins and lipids.[45,47] During the first trimester the embryo is surrounded by two fluid-filled cavities, the amniotic sac and the extraembryonic coelom (Figure 1.2a). The allantoic cavity is very small in the human, in contrast to other species such as the cow, pig and sheep, and the secondary yolk

sac is devoid of any yolk. As the amniotic sac increases in size the extraembryonic coelom is progressively obliterated and the fluid reabsorbed (Figure 1.2b). Amino acid concentrations have been measured in the extraembryonic coelom, and are significantly higher than amniotic concentrations, suggesting passive diffusion from extraembryonic coelomic fluid to amniotic fluid. Jauniaux *et al.* propose that the fetal fluid compartments, including the secondary yolk sac, may provide a means for nutrient supply to the developing fetus from the uterine glands.[48] Data from studies in the cow and pig also suggest that amino acids may be concentrated in the allantoic fluid of those species, thereby acting both as a potential source of nutrients and as a possible storage place for waste.[49,50]

During this early phase of development, the nutrient requirements of the developing conceptus are very small and are unlikely to ever place a demand upon maternal nutrition that cannot be met. Yet recent intriguing data suggest that nutritional factors during this period of development determine the rate of fetal growth for the rest of gestation, and may also determine the length of gestation. *In vitro* culture of animal embryos for the first few days of pregnancy in media supplemented with human serum results in fetal overgrowth in sheep, goats and cows.[51] In humans, birth weight in donor egg pregnancies is more closely related to the body weight of the recipient rather than the donor.[52] Further evidence of the setting of the fetal growth trajectory early in pregnancy comes from the observation that the growth of twins is different from that of singletons from very early in gestation. Postmortem[53] and ultrasound studies[54] show that growth of twins diverges from that of singletons as early as 8 weeks gestation. Reduction of fetal number in early gestation in higher-order pregnancies does not alter the fetal growth trajectory, nor abolish the risk of prematurity and IUGR.[55–59] Furthermore, there is a significant relationship between birth weight, gestation length and the original number of fetuses.[56,58] Bovine twins are also smaller than singletons from early in gestation.[60] These data strongly suggest that the growth of twins is fundamentally different from that of singletons, and is not merely restricted by fetal space or nutrient supply in late gestation. The mechanism by which growth in twins is regulated differently from so early in gestation is not known.

We have recently demonstrated that the growth trajectory of singleton fetuses can also be set very early in gestation. In sheep subjected to a modest nutrient restriction around the time of conception, fetal growth rate measured in late gestation was significantly less than that in fetuses of ewes that were well nourished throughout.[61] Furthermore, this brief and relatively minor period of undernutrition resulted in preterm birth in half of the undernourished ewes.[62] Whether these changes are the result of hormonal or nutritional signals from the mother to the developing embryo is not clear, but alterations in both individual amino acids and maternal hormonal profiles can be demonstrated.[63–65]

In summary, the nutritional environment of the developing embryo is obviously of great importance, yet there are few data on the route of nutrient supply or on the nutrient requirements during the first trimester.

Fetal nutrition after placentation is established

The main fetal substrates for oxidative metabolism are glucose, lactate and amino acids, with free fatty acids also crossing the placenta in variable amounts (Table 1.1) (Figure 1.3).[66] The amounts of these substrates taken up by the fetus can be calculated in experimental paradigms, such as the sheep, using the Fick principle. Blood flow is measured using the diffusion of an inert substance (such as ethanol, deuterium, tritiated water or antipyrine) across the placenta during steady state.[67–69] As these substances are essentially inert, their loss due to metabolism, accumulation etc. is minimal (usually $< 5\%$) and can usually be ignored. Once blood flow is determined, substrate uptakes can be calculated from arteriovenous differences across the maternal and fetal sides of the placenta. The technique can be further refined using radioactively labeled tracers to determine substrate utilization.[70] The potential contribution of each metabolite to total fetal oxidation can be calculated by comparing the amounts of the metabolite consumed with the amount of oxygen consumed, and taking into account the moles of oxygen required for the complete oxidation of one mole of substrate (the constant, k). Thus, the substrate:oxygen quotient $= (k \times \Delta\text{substrate}) / \Delta\text{oxygen}$, where Δ represents the arterio-venous concentration difference across the organ/fetus/uteroplacental unit. However, it is important to remember that this quotient gives the *maximum* possible contribution of the substrate in question to oxidative metabolism, as it does not allow for carbon incorporation into tissue.

Oxygen consumption

Fetal oxygen consumption varies little between mammalian species and is about 300 μmol $Kg^{-1} \cdot min^{-1}$.[71] The fetal carcass (skeleton, muscle and skin) accounts for approximately 50% of fetal oxygen consumption, although the heart utilizes the most oxygen per unit weight.[71,72]

Table 1.1. Nutrient transfer by the placenta

Substrate	Mechanism	Transporters	Regulation of fetal uptake
Glucose	Facilitated diffusion	GLUT-1 and 3	Concentration gradient Uterine and umbilical blood flow Insulin, IGF-I Placental metabolism
Lactate	Active transport	Proton-dependent, Na^+ independent lactate transporter	Bi-directional Placental metabolism IGF-I
Amino acids	Active transport	Many different amino acid transporters	Concentration gradient Uterine and umbilical blood flow Insulin, IGF-I and -II Placental metabolism
Fatty acids	Facilitated diffusion	Fatty acid binding protein	Concentration gradient Uterine and umbilical blood flow Hormones? Placental metabolism
Oxygen	Simple diffusion	-	Blood flow Extraction fraction by tissues Redistribution of blood flow Prior exposure to episodes of reduced oxygen tension

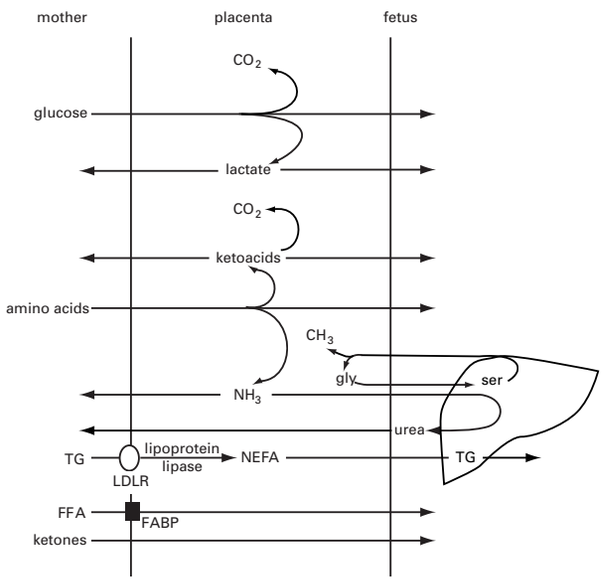

Figure 1.3. Placental transfer and metabolism of nutrients. CO_2 = carbon dioxide, NH_3 = ammonia, ser = serine, gly = glycine, CH_3 = methyl group, TG = triglyceride, LDLR = low density lipoprotein receptor, NEFA = non-esterified fatty acid, FFA = free fatty acid, FABP = fatty acid binding protein.

Oxygen consumption remains fairly constant with changes in nutritional state and with hyperoxia,[73] although consumption can be increased by provision of excess nutrients such as glucose or amino acids,[74] or by increased levels of metabolic hormones such as thyroxine.[75] Hypothyroidism reduces fetal oxygen consumption,[76,77] but in sheep there is little reduction in oxygen consumption if glucose supply is restricted by fasting the ewe.[74] Fetal oxygen supply is determined by maternal oxygenation, and thence by uterine and umbilical blood flows. The fetus operates at the upper end of the cardiac function curve and thus has limited capacity for increasing tissue oxygen supply by increasing cardiac output. However, the fetus can adapt to a limitation in oxygen supply by extracting more oxygen from hemoglobin,[78,79] increasing oxygen-carrying capacity by increasing hemoglobin and by making cardiovascular adaptations. Studies in sheep have demonstrated that when fetal oxygen supply is reduced below a critical level, there is redistribution of cardiac output away from "non-essential" organs, such as the carcass, to essential organs, such as the brain.[80,81] Ultrasound studies in humans suggest that similar changes occur.[82,83] If oxygen deprivation is severe or prolonged, fetal oxygen consumption falls and becomes proportional to oxygen delivery.[69,84] Interestingly, it appears that the fetal response to an acute episode of hypoxemia in late gestation can be altered by the presence of an earlier

insult. Fetal sheep exposed to reversible umbilical cord compression which reduced umbilical blood flow by 30% for 3 days then failed to increase oxygen and glucose extraction and blood lactate levels in response to a later acute hypoxemic insult.[85] Gardner *et al.* propose that this adaptation may be a protective mechanism against elevated lactate levels during hypoxic stress.

Glucose metabolism

Glucose is the major fetal oxidative substrate in utero. Glucose crosses the placenta by facilitated diffusion down a concentration gradient from mother to fetus. Thus fetal glucose concentration is always directly related to but lower than that of the mother, although the ratio of maternal:fetal glucose concentrations varies between species. In humans, fetal glucose concentrations are 60–70% of maternal levels, and the glucose:oxygen quotient is about 0.8. In sheep, fetal levels are only 25–30% of maternal levels, and the glucose:oxygen quotient is about 0.55.

In the ovine fetus glucose utilization is between 20–40 $\mu mol\,Kg^{-1} \cdot min^{-1}$, but this can double when extra glucose is provided experimentally, demonstrating that utilization is probably limited by supply rather than by the capacity of the fetus to metabolize glucose. Although the glucose:oxygen quotient in mammals varies between 0.5 and 0.8, not all the glucose entering the fetal circulation is oxidized.[86] The amount that is oxidized increases with increasing glucose concentration, suggesting that when glucose is in plentiful supply, other substrates are spared from oxidation. Non-oxidized glucose is used in other metabolic pathways. Thus glucose oxidation only accounts for about 30% of oxygen consumption in the sheep fetus.[86] In late gestation the fetal liver is capable of gluconeogenesis from substrates such as lactate and alanine,[87,88] but it appears that the contribution of endogenous gluconeogenesis to fetal glucose supply is normally negligible.[25,88,89]

The supply of glucose across the placenta appears to be limited by its diffusion characteristics rather than by blood flow. The main factors affecting these diffusion characteristics are the transplacental concentration gradient, placental utilization of glucose and capacity of the glucose transporters (GLUT) to transport the substrate. In the sheep, placental glucose transfer capacity increases 10-fold over the second half of pregnancy, maintaining glucose supply to the fetus as the fetus grows. This increase arises in part due to an increase in placental transfer capacity, presumably due to the increase in numbers of glucose transporters,[90–92] and in part to a fall in fetal glucose concentration, thus increasing the maternal–fetal glucose concentration gradient.[93] A similar fall in fetal glucose concentrations in late gestation, with an increase in the maternal–fetal glucose concentration gradient has been reported in human pregnancies.[94] The placenta has a high metabolic rate of its own, and extracts 60–75% of the glucose taken up from the uterine artery for its own metabolism. Thus placental glucose uptake has an important influence on fetal glucose supply (see below). If uterine glucose supply from the mother is reduced by decreasing uterine blood flow, the placenta may even take up glucose from the fetus to maintain its own metabolic requirements.[95] Some of the glucose taken up by the placenta is recycled to the fetus as lactate or fructose, but as placental glucose uptake increases with further reductions in uterine blood flow there is a net loss of glucose from the fetus to the placenta.

The transport of glucose across the placenta is mediated by glucose transporters. At least six different glucose transporters are now known, and several members of the GLUT family have been described in the human placenta, although only GLUT-1 is found in the syncytium.[96] In the rat and the sheep, both GLUT-1 and GLUT-3 are present in the placenta,[90–92,97,98] and the levels of both increase with increasing gestation.[90–92] However, in the sheep GLUT-1 expression peaks at around 120 days (term = 145 days) whereas GLUT-3 expression continues to increase until term.[90] In the rat placenta GLUT-3 expression is polarized to the maternal microvillous membrane (MVM), whereas GLUT-1 is expressed on both the MVM and the fetal-facing basal membrane (BM).[97] In the human, the distribution of GLUT-1 in syncytium is also asymmetric, with higher concentrations on the MVM than the BM. When combined with the greater surface area of the MVM (the maternal facing membrane) compared with the BM (the fetal facing membrane), it is likely that GLUT-1 density on the BM is the determinant for the rate of placental glucose transport.[99] GLUT-1 concentrations in the MVM of the human placenta do not appear to increase with increasing gestation.[100,101] However, GLUT-1 expression and activity in the BM increase significantly in later gestation.[101] Placental glucose transport also increases in late gestation.

The regulation of glucose transporter levels has been studied in several tissues, although there is little work specifically looking at regulation in the placenta. In the sheep, hypoglycemia down-regulates placental GLUT-1 levels. Hyperglycemia initially up-regulates placental GLUT-1 levels, although with chronic hyperglycemia there is subsequently a decline in levels.[102] No changes in placental GLUT 1 levels were seen in streptozotocin-induced diabetic rats, although hypoglycemia together with hypoxia following uterine artery ligation resulted in a 50% fall in GLUT-1 levels.[102] In the human, placental

GLUT-1 levels appear to be inversely related to high glucose concentrations,[103,104] although other data suggest that variations in glucose concentration within the physiological range do not affect GLUT-1 levels.[105] In both human and animal IUGR no change in placental GLUT-1 levels have been seen,[101,106] although down-regulation of GLUT-3 has been reported in the placentae from undernourished rats.[107] In diabetic pregnancies a substantial increase in GLUT-1 levels on the BM has been reported with no change in MVM GLUT-1 levels.[108,109] Placental glucose transport was increased by between 40 and 60%. It has been proposed that this up-regulation in BM GLUT-1 levels and consequent glucose transport may be involved in macrosomic growth of the fetus in diabetic pregnancies.[96]

In other tissues, such as brain and muscle, glucose transporters are up-regulated by IGF-I.[110–113] Insulin also up-regulates membrane translocation of both GLUT-1 and GLUT-3 in brain and myotubules.[111,112] The regulation of the glucose transporters by insulin and IGF-I has not been well studied in placental tissue.

Lactate metabolism

Lactate is also an important fuel for the fetus. In ruminants, such as the sheep and cow, the fetal lactate:oxygen quotient is between 0.25 and 0.4, compared with only 0.1 for the human.[71] Endogenous production of lactate by the fetus is high even in unstressed fetuses, and most of this is derived from glucose, although some is derived from other carbon sources. The major site of lactate production in the fetus is the carcass, and lactate release from here may provide fuel for other fetal organs in times of substrate deprivation.[114]

The placenta is also a major source of fetal lactate. A significant proportion of placental glucose utilization in sheep is directed towards lactate production which, in late gestation, is released into both the uterine and umbilical circulations.[115,116] Most of the lactate taken up by the sheep fetus is oxidized to CO_2, and this CO_2 contributes substantially to total fetal CO_2 production.[117] However some lactate is incorporated into fetal tissue, including hepatic glycogen, non-essential amino acids and lipids.[118] Thus, the lactate:oxygen quotient underestimates the proportion of fetal oxygen consumption that is accounted for by lactate oxidation.[71] Lactate utilization by the fetus may increase substantially in the face of undernutrition.[119]

Lactate concentrations in the fetus are higher than in the mother, and fetal pH is lower. Both the MVM and the BM of the placenta express proton-dependent, sodium-independent lactate transporters.[1,5,120–122] These transporters appear to be reversible, allowing transport of lactate in either direction.[120] Thus, lactate can be provided to the fetus as a fuel, or removed should lactate accumulate in the fetus posing a risk to tissues.

Amino acid metabolism

Amino acids are utilized by the fetus for protein synthesis and for oxidation, and certain amino acids are also essential components of pathways such as purine and pyrimidine synthesis. Essential amino acids must be derived from maternal circulating amino acids, whereas non-essential amino acids could be derived either from de novo synthesis by the fetus, from transplacental transfer or via placental synthesis. As well as the traditionally accepted essential amino acids, arginine is regarded as conditionally essential in the fetus. Total amino-nitrogen concentrations in the fetus are higher than in the mother, and concentrations in the placenta for some amino acids are higher than in either the maternal or fetal circulations.

Calculations of uterine and umbilical uptakes of amino acids in the sheep using the Fick principle[123] have demonstrated large uptakes of most basic and neutral amino acids by both the placenta[124] and fetus.[125] Uteroplacental amino acid uptake provides nitrogen in excess of amounts required by the fetus for protein accretion, and the difference is accounted for by ammonia production by the placenta.[124,126] The ammonia produced is released into both the maternal and fetal circulations, where it is converted into urea by the fetal liver.[127] Most of the ammonia is produced by placental metabolism of the branched chain amino acids leucine, isoleucine and valine.[128] Both ovine[129,130] and human placentae[131] have high branched chain amino acids (BCAA) amino transaminase activities, and significant amounts of the products of BCAA deamination, the branched-chain alpha-keto acids, are released from the ovine placenta into maternal and fetal circulations.[132,133] The role of placental metabolism of BCAA may include oxidation as an energy source, conversion to glutamate by transamination and to make nitrogen available for purine synthesis. The proportion of amino acids utilized by the placenta for oxidation increases as glucose availability falls, and in severe conditions BCAA and glutamate may be extracted from the fetal circulation for consumption by the placenta.[130,134]

Amino acids are transported across the placenta by active transport. Many different classes of amino acid transporter have now been described in the placenta[135] and are present on both the MVM and the BM.[136] Experiments on isolated human placental cotyledons have demonstrated that the placenta can take up amino acids from both the maternal

and fetal circulations against a concentration gradient.[137] Thus the amino acid transporters on the MVM probably have a more important effect on net transfer of amino acids from mother to fetus.

Placental levels of amino acid transporters are related to fetal amino acid concentrations and thus to fetal nutrition. For example, levels of the system A amino acid transporter have been related to anthropometric measurements at birth,[138] but it is not clear if down-regulation of amino acid transporters follows a reduced growth trajectory, or if the reduced growth is secondary to lower levels of amino acid transporters.[139] MVM levels and activity of both system A transporters[140–144] and β-amino acid transporters[35] are reduced in IUGR, and the activity of the system A transporter is associated with the severity of the IUGR.[140] Oxygenation of the uteroplacental unit has also been correlated with levels of system A and cationic amino acid transporter activity.[145,146] Transplacental transfer of the BCAA leucine has been studied in sheep with IUGR induced by heat stress using tracer techniques.[147] Net uterine uptake, uteroplacental utilization, flux from placenta to fetus and direct maternal–fetal flux were all reduced in IUGR animals.

Further good evidence for a direct role of the amino acid transporters in fetal nutrition and thus growth comes from studies in mice with deletion of a placental-specific transcript (P0) of the *Igf2* gene.[148] Placental growth was restricted from embryonic day 12 (E12), but the transfer of ^{14}C-methylaminoisobutyric acid (MeAIB) per gram of placenta was significantly increased compared with wild type until E16, resulting in transfer of identical amounts of ^{14}C-MeAIB across the placenta. At this time (E16) fetal weight was also not different between mutant and wild type mice. By E19 the increase in transfer of ^{14}C-MeAIB per gram placenta was reduced and therefore, when combined with the reduced placental size, total transfer was also reduced by 26%. By E19 fetal weight in mutants was reduced by 22% compared with wild type.[148]

In addition to amino acid transfer, the placenta is also involved in metabolism of amino acids. The most notable examples of placental amino acid metabolism are the glycine-serine and glutamine-glutamate placenta-hepatic shuttles. These appear to be mechanisms by which nitrogen and carbon can be shuttled between the placenta and fetal liver.[29] In sheep, there is very little umbilical uptake of serine, with almost all fetal serine arising from hepatic production from placental glycine.[149] Serine derived from the fetal pool is used within the placenta for glycine production, some of which is then returned to the fetal circulation. The net effect of such cycling is the transfer of methyl groups derived from glycine oxidation within the liver to the placenta.[149,150]

The ovine placenta takes up glutamate from the fetal circulation,[126] and also forms glutamate by oxidizing branch chain amino acids taken up from the maternal circulation.[151] Amidation of glutamate produces glutamine, which is released into the fetal circulation.[152] Some of the glutamine delivered to the fetus from the placenta is converted back to glutamate by the fetal liver, which is the main source of glutamate consumed by the placenta.[152] Thus a glutamate-glutamine shuttle is established, promoting glutamate amidation by the placenta and allowing hepatic utilization of the amide group of glutamine. This amide group, together with glycine and methylene tetrahydrofolate (derived from the conversion of serine to glycine) can be used for purine synthesis.

Fatty acid metabolism

The human baby is born with a large proportion of fat, and fat deposition increases exponentially with gestational age. Near term the accretion rate is ~7 g day^{-1}.[153] Early in gestation, the fetus derives most of the fatty acids from the mother, but as gestation progresses there is increased de novo synthesis.[74,154,155] Fatty acids are required by the fetus for membrane formation, as precursors of compounds such as prostaglandins, and as a source of energy. All fatty acids can be used as an energy source, but the structural functions are largely performed by the polyunsaturated fatty acids (PUFA). Humans cannot synthesize the ω3 and ω6 fatty acids, and these essential fatty acids must therefore be provided by the mother. Intrauterine requirements for ω3 and ω6 fatty acids in late gestation have been calculated to be approximately 400 and 50 mgKg^{-1} · day^{-1} respectively.[154] In tissues such as the brain, almost half of the lipid content is comprised of long chain polyunsaturated fatty acids (LCPUFA) such as arachidonic acid (AA) and docosahexanoic acid (DHA). The percentage of fatty acids in fetal circulation composed of LCPUFA is higher than in the mother,[156] despite the fact that the human placenta lacks Δ5- and Δ6-desaturase activity and is therefore unable to convert γ-linolenic acid (18:3, ω6) into AA (20:4, ω6).[157] The placenta must therefore be able to extract LCPUFA from the maternal circulation and deliver them to the fetus.

Free fatty acids (FFA) can directly cross the placenta, probably via facilitated membrane translocation involving a plasma membrane fatty acid-binding protein (FABP). There appears to be a specific placental FABP that has higher affinities and binding capacities for AA and DHA compared with FABPs in other tissues.[120,158,159] Placental FABP are found on both the MVM and the BM. Transport of FFA across the placenta via FABP is ATP dependent at

the MVM and ATP and Na^+ dependent at the BM,[160] but appears to occur predominantly as facilitated transport down a concentration gradient. However, FFA represents a very small amount of PUFA in the maternal circulation, as most are esterified and associated with lipoproteins (VLDL and LDL). Unlike FFAs, triglycerides (TG) and glycerol are not able to cross the placenta in any significant amount.[161] Transfer of LCPUFA from mother to fetus therefore involves placental uptake and metabolism of maternal lipoproteins and TGs. The MVM of trophoblast expresses receptors for both VLDL and LDL,[162–164] enabling uptake of circulating maternal lipoproteins into the placenta. The TG are then hydrolyzed by lipoprotein lipase and the FFA diffuse into the fetal circulation, from where they are taken up by the fetal liver and re-esterified into TG before being released back into the circulation.

In other species TG also do not cross the placenta in any appreciable quantities, and most of the fetal FFA are derived from hydrolysis and re-esterification.[154,157,165–167] The ovine placenta does express increasing levels of Δ6-desaturase during late gestation,[156,168,169] suggesting that there may be some placental synthesis of AA by the ovine placenta, although there is no direct transfer of TG from mother to fetus.[167]

Fetal growth factors and fetal nutrition

Hormones involved in fetal growth are nutritionally regulated in the fetus and also regulate substrate uptake and metabolism. The major fetal growth factors are the insulin-like growth factors (IGF)-I and II, with insulin having a passive role in fetal growth. The roles of other hormones such as growth hormone (GH), placental lactogen, leptin and ghrelin are as yet less clear.

Insulin levels in the fetus are clearly regulated by fetal nutrient supply. Fetal glucose infusion stimulates insulin secretion by the fetus,[170,171] and insulin and glucose concentrations were closely correlated in studies of different nutritional regimens in the sheep.[172] Amino acids appear to potentiate glucose-induced insulin release.[173,174] In turn, insulin stimulates glucose and amino acid uptake into fetal tissues. Fetal pancreatectomy, to prevent fetal insulin secretion, results in impaired fetal growth.[175] However, infusion of high doses of insulin restores growth only to the rate of that in controls, demonstrating that insulin itself cannot stimulate fetal growth in the absence of additional nutrient supply. In diabetic pregnancies the fetus is exposed to increased concentrations of maternal glucose. As placental transfer of glucose occurs down the maternal – fetal concentration gradient, fetal glucose concentrations are also increased. Fetal insulin release and circulating insulin concentrations are increased in response to the elevated glucose concentrations, and the combination of increased insulin and substrate results in increased fetal growth.

In prenatal life the IGFs are critical in the regulation of fetal growth, acting in both paracrine and endocrine fashion. Direct evidence for the role of IGF-I in fetal growth comes from experiments in mice using homologous recombination of defective IGF-I or IGF type 1 receptor genes to produce animals homozygous for these defects.[176] Less direct evidence is provided by the finding in all species studied that birth-weight correlates with cord blood IGF-I concentrations.[177–179] In babies, levels of IGF-I in umbilical cord blood and blood obtained in utero by fetal blood sampling are reduced in IUGR.[180–183] A case report of partial deletion of the IGF-I gene in a boy with severe IUGR is definitive evidence in the human for the role of IGF-I in fetal growth.[184] Deletion of the IGF type 1 receptor gene has also been reported to result in IUGR, with a Silver–Russell phenotype.[185]

The IGFs are anabolic hormones, and in fetal life circulating levels of these important growth-regulating hormones are regulated by fetal nutrient supply. In fetal sheep IGF-I, IGF-II and IGFBP-3 levels fall with severe maternal undernutrition while IGFBP-1 and -2 levels rise.[186,187] Replacement of glucose or insulin restores fetal IGF-I levels within 24 hours.[188,189] Circulating maternal IGF-I levels in pregnancy are also partly regulated by nutritional status, and maternal IGF-I levels also influence birth weight.[190] In pregnant rats IGF-I concentrations were correlated with changes in nitrogen balance.[191]

In turn, IGF-I regulates fetal nutrient uptake. In fetal sheep IGF-I infusion reduces fetal protein breakdown, increases fetal glucose uptake and appears to alter nutrient distribution between fetus and placenta to enhance fetal nutrient availability.[192,193] IGF-I infusion is also anabolic, increasing the fractional protein synthetic rate,[194] and increasing the conversion of serine to glycine, which would increase the availability of one-carbon groups for biosynthesis.[195] Chronic IGF-I infusion alters placental glucose transfer and placental clearance of MeAIB.[196] Recent evidence from sheep studies suggests that growth-restricted fetuses may be resistant to some of the anabolic effects of IGF-I.[159,193,197]

IGF-II is thought to be most important in embryonic and early gestational growth,[198] acting as an embryonic growth factor by activating cell cycle entry/progression.[199] IGF-II is first expressed in the placenta by 18 days gestation in the human,[200] and is highly expressed in proliferative cytotrophoblasts of the first trimester placenta,

acting as a placental growth factor.[201] Recently Constância *et al.* have demonstrated in mice that deletion from the *Igf2* gene of a transcript (P0) that is specifically expressed in the labyrinthine trophoblast of the placenta results in reduced growth of the placenta that occurs before fetal growth restriction.[148] Interestingly, placental transport of methylaminoisobutyric acid (MeAIB, a non-metabolizable analog of amino acids utilizing the system A amino acid transporter) is initially up-regulated in these mice. When this up-regulation fails, fetal growth restriction ensues. Passive permeability of the mutant placenta is also decreased. The temporal separation of placental from fetal growth restriction in this P0 knockout is distinct from the contemporaneous growth restriction of both that occurs in the *Igf-II(p-)* knockout,[198] leading the authors to propose that fetal IGF-II may signal to the placenta to up-regulate amino acid transport.[202] There are many other imprinted genes, some of which are also expressed in the placenta. In general, paternally expressed imprinted genes, such as *Igf2, Peg1, Peg3*, and insulin enhance fetal growth, whereas maternally expressed genes, such as *Igf2r* and *H19* suppress fetal growth.[203–205] This has led Reik *et al.* to propose that imprinted genes may be involved in the regulation of the balance of fetal nutrient demand and maternal nutrient supply.[202]

Growth hormone levels are also nutritionally regulated, with maternal and fetal GH levels rising in response to undernutrition.[206] It is increasingly becoming apparent that GH does have a role in fetal growth and anabolic metabolism, although the extent of this role is still not clear. GH receptors (GHR) are present in a large number of fetal tissues[207] and GHR/BP mRNA has been shown to co-localize with IGF-I mRNA in the rat fetus.[208] Congenital GH deficiency is associated with a reduction in length at birth,[209] and hypophysectomized fetal lambs supplemented with thyroxine (to abolish the effects of hypothyroidism) have shorter limbs and long bones[210] and reduced IGF-I levels.[211] IGF-II levels were unaffected. Recent data from Bauer *et al.* provide the first evidence that GH supplementation in fetuses can influence IGF-I levels in utero. A 10-day pulsatile infusion of GH to growth-restricted ovine fetuses resulted in an increase in IGF-I levels and an increase in liver and thymus weight.[212] Placental lactate production and fetal lactate uptake were also increased in this study.

Thyroid hormones are also involved in regulating fetal metabolism and thus growth. The metabolic action of thyroxine is mainly via stimulation of oxygen utilization by fetal tissues.[213] This appears to be a general increase in oxidative metabolism, rather than in glucose oxidation alone.[76] Thyroid hormones are reduced, and thyroid stimulating hormone increased (TSH), in fetuses with impaired substrate supply, suggesting that these hormones are also nutritionally regulated in utero. [214]

The role of other hormones that are involved in postnatal nutrition and growth, such as leptin and ghrelin, are beginning to receive more attention in the fetus.[215] Both leptin and ghrelin are expressed in the placenta[216–218] and both can be nutritionally regulated.[218–221] The precise role of these hormones, and other nutritionally regulated hormones such as placental lactogen, remains to be elucidated, but a recurring theme for hormones that are also expressed in the placenta is the possibility that they may play a role in nutrient partitioning between mother and fetus.

Thus there is a close and reciprocal relationship between many of the fetal hormones involved in fetal growth (and thus the utilization of fetal nutrients) and the fetal nutrient supply. Furthermore, there appears to be input from the maternal hormonal milieu on nutrient supply, and the placenta, which is exposed to both maternal and fetal hormonal and nutrient influences, itself produces many of the hormones involved in fetal growth.

The placenta and fetal nutrition

Once the placental circulation is established the fetus receives nutrients via the placenta. The placenta grows throughout gestation, but not in constant proportion to the fetus, comprising 85% of the combined fetal/placental weight at 8 weeks and only 12% at 38 weeks in human pregnancy. However, placental function continues to develop in the second half of gestation with increases in surface area for exchange, transfer capability and diffusion permeability. In mid-gestation in the sheep, when the placenta weighs twice as much as the fetus, the placenta consumes more than 80% of the glucose and oxygen taken up by the uterus.[222] Even in late gestation, when the fetus weighs five times more than the placenta, the placenta still consumes more than half of the glucose and oxygen delivered via the uterine circulation.[95,115,134] Thus, under normal conditions the placenta has a greater oxygen and glucose consumption per unit weight than the fetus.

Response of the fetus to a reduction in substrate supply

Most of the information on fetal metabolic responses to alterations in substrate supply is derived from studies in the sheep.