Oxygenation In Utero: Placental Determinants and Fetal Requirements

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Oxygen is an essential substrate for life before birth; it is required to produce energy, maintain tissues already laid down, and support accretion of new tissues. Fetal oxygen requirements are therefore ultimately determined by the rate of fetal growth and the factors that regulate this process. These needs clearly vary with body size, composition, and metabolic activity, and therefore with stage of development (1). In addition, fetal oxygen requirements can vary in the short term, at least during the second half of gestation, when substantial changes in fetal oxygen consumption occur with sleep state and physical movement; at the same time, changes in oxygen delivery can occur with uterine activity (2). Wide variations in oxygenation can exist in utero, and in the human fetus hypoxemia is associated with altered growth and with increased morbidity and mortality, both during the perinatal period and in later postnatal life (2).

In mammalian species, the placenta is the organ responsible for the transfer of oxygen from the mother to the fetus (3). The placenta is therefore a major—although not the sole—influence on fetal oxygenation (4). The aim of this brief review is to outline current understanding of placental influences on oxygen supply to the fetus, the nature of fetal oxygen requirements, and the consequences for fetal development of limiting oxygen delivery to the conceptus. In particular, the mechanisms by which oxygen supply influences fetal growth are discussed. The focus of this review is primarily on experimental studies in the sheep, with the relevance to human physiology and pathophysiology indicated when possible.

OXYGENATION IN UTERO: PLACENTAL DETERMINANTS

Following implantation, a sequence of events, in part common to all species and in part species-specific, results in the formation of the placenta, an organ in which
fetal tissues are closely associated with maternal blood and tissues, to enable controlled transfer of essential substrates from maternal to fetal blood. In addition, as described elsewhere in this volume, the placenta has other important functions; it acts to modulate immune interactions between the mother and fetus and, through the production of steroid and polypeptide hormones, to regulate and coordinate maternal adaptation to pregnancy and fetal growth and development.

The external environment and maternal factors—such as respiratory and cardiovascular adaptations to pregnancy, hemoglobin concentration, and affinity for oxygen—influence the availability of oxygen in maternal blood, the rate of uterine blood flow, and hence the rate of oxygen delivery to the placenta for subsequent transfer to the fetus (3,4) (Table 1). The characteristics of the placenta that influence the rate at which oxygen is transferred from mother to fetus are physiologic and structural in nature (Table 1). The rates of placental perfusion or of maternal and fetal blood flows and their matching, together with the high rate of placental oxygen consumption, are key determinants of placental oxygen transfer (3,4). Within the fetal villi, the close proximity of the arterioles and venules of the umbilical circulation allows direct exchange of highly diffusible substances to occur. This diffusional "shunting" bypasses the area of maternal-fetal exchange and reduces the efficiency of the process. Placental diffusing capacity for oxygen is an important influence on the rate of oxygen transfer and is determined by structural characteristics of the placenta, as well as by physicochemical processes such as oxygen reaction rates with hemoglobin and diffusion rates through plasma and tissues (5). The structural characteristics of the placenta that determine placental diffusing capacity for oxygen

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<td>Surface area of exchange epithelia and fetal capillaries (and of maternal capillaries in some species)</td>
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<td>Volume of fetal villi and fetal capillaries (and of maternal capillaries in some species)</td>
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<td>Spatial arrangement of vasculature</td>
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<td>Hemoglobin type and concentration</td>
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<td>Cardiac output and distribution</td>
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OXYGENATION IN UTERO

include the thickness of the placental barrier (the diffusion distance between maternal and fetal blood) and the surface areas of exchange epithelia and of fetal capillaries. In general terms, these physiologic and structural characteristics are common to the placentae of different mammalian species, although great diversity exists between species in the quantitative and qualitative nature of these placental characteristics and in their relative importance in influencing oxygen transfer by the placenta (3). The fetus also influences placental oxygen transfer through characteristics such as hemoglobin concentration and affinity for oxygen and cardiac output and its distribution.

The importance of these various factors for oxygen supply to the fetus under different circumstances in several species has been reviewed recently (3,4). In sheep, the effects on oxygen transfer by the placenta of experimental variations in maternal oxygenation, of maternal and fetal hemoglobin concentration and affinity for oxygen, and of rates of fetal and maternal placental blood flows have been extensively studied (3,4). In this species, placental oxygen transfer to the fetus is determined by both placental oxygen diffusing capacity and placental perfusion, and the former is small relative to fetal and placental oxygen demand (3). Placental oxygen transport in the human is less well understood, with conflicting views on the pattern of exchange and on the main determinants of transfer. The human placental diffusing capacity for oxygen has been estimated morphometrically from quantitative analysis of placental structure and mathematical modeling of the various physicochemical processes involved (5). Morphometric estimates of diffusing capacity are higher than the available physiologic estimates for the human placenta, probably because of a variety of factors that include "shunting," placental oxygen consumption, and the technical and biologic difficulties in obtaining accurate physiologic estimates. The morphometric modeling of diffusion across the human placenta suggests that the major contributor to placental diffusional resistance is the thickness of the villous membrane, followed by the surface areas of the fetal villi and capillaries (6). The relative importance of placental diffusing capacity for oxygen compared with the rates of placental blood flows—or indeed with other factors—in influencing oxygen transfer across the human placenta remains to be determined.

Ontogenic Changes in Placental Oxygen Transfer and Characteristics

Placental growth in terms of mass occurs largely in the first half of gestation (7). In the latter part of pregnancy, fetal size increases exponentially and rapidly exceeds that of the placenta. To meet the ever-increasing substrate demands of the fetus, the functional characteristics of the placenta undergo substantial changes throughout pregnancy, resulting in increases in placental transfer capacity—including that for oxygen—approximately in parallel with fetal growth (1,8). Thus, ontogenic increases in maternal and fetal placental blood flows, villous surface area, vascularity, and placental diffusing capacity for carbon monoxide, and reductions in the thickness of the placenta barrier or the diffusion distance between maternal and fetal blood,
have been variously demonstrated in several species, including the human, sheep, cow, and guinea pig (1,8). These physiologic and structural changes increase the placental transfer capacity for oxygen. We currently understand little of the factors that regulate or modulate these processes, although, as discussed later, they can undergo modification in response to various perturbations, including hypoxemia in utero.

Relation Between Placental Growth and Oxygen Delivery to the Fetus

In late gestation and at term, much of the variation in fetal or birth weight can be accounted for by variations in placental weight in the human, sheep, and other mammalian species (2,7). Experimental restriction of placental growth has shown that this is a causative relation and that restricting growth reduces placental function in terms of substrate transfer capacity and so limits fetal growth (9–13). In sheep, surgical reduction of the number of implantation sites in the uterus before pregnancy results in a placenta with reduced numbers of placentomes and reduced total weight by late gestation. As the extent of restriction of placental growth increases, the rates of uterine and umbilical blood flow and of flow-determined antipyrine clearance decrease, as does the surface area of the exchange epithelia (10–14) (Fig. 1). Consequently, the rates of delivery of oxygen to the pregnant uterus and to the fetus

![Graph showing the relation between placental growth and function in the sheep in late gestation.](image)
OXYGENATION IN UTERO

RATES OF OXYGEN DELIVERY OR CONSUMPTION (mmol/min)

FIG. 2. Relation between placental growth and oxygen delivery to (empty circles) and consumption by (filled circles) the gravid uterus and fetus in sheep in late gestation. Placental growth was restricted in some sheep as described in Fig. 1.

decrease with decreasing placental weight (Fig. 2). This occurs despite concomitant compensatory changes in the placenta, which should help to maintain the oxygen supply to the fetus. Restriction of placental growth in sheep reduces the connective tissue content of fetal villi, which form part of the barrier to diffusion between maternal and fetal blood, and increases the surface density of the epithelial cell layers responsible for exchange, which should also help to maintain transfer (14). In addition, the rate of oxygen uptake by uteroplacental tissues decreases (Fig. 3), which would further help to maintain oxygen delivery to the fetus by reducing placental competition for this substrate.

In sheep, the consequences of restricted placental growth for the fetus are hypoxemia, hypoglycemia, and disproportionate growth restriction, with relative maintenance of brain growth, a disproportionate reduction in growth of the liver and gut, and a reduction in the ponderal index (9–13). The growth-restricted human fetus is characterized by similar metabolic and phenotypic stigmata (2,15). Studies in sheep and other animals show that restricting placental growth decreases the rate of delivery of glucose as well as of oxygen to the fetus (10–13). In late gestation, the rates of uptake of oxygen and glucose by the placentally restricted fetus are reduced in absolute terms, but they occur at normal rates relative to body weight (Figs. 2 and 3). This suggests that fetal growth is slowed to an extent commensurate with the rate at which the fetus can obtain these essential substrates. The mechanisms by which reduced substrate supply restricts fetal growth are increasingly defined and appear to involve both direct and indirect actions, mediated in part by endocrine pathways. What is less clear are the respective roles and importance of deficits in particular substrates, such as oxygen vs. glucose, in mediating placental control of fetal development.
FIG. 3. Partitioning of substrates between placenta and fetus: effect of restricting placental and fetal growth. Rates of flux between mother, placenta, and fetus in control sheep and in sheep with restricted placental and fetal growth in late gestation. Placental growth was restricted as described in Fig. 1. Mean placental and fetal weights are shown in italics.

OXYGENATION IN UTERO: FETAL REQUIREMENTS

Acute variations in the rate of oxygen delivery to the fetal sheep generally do not alter fetal oxygen uptake until delivery falls below 0.6 mmol/min/kg, or 60% of the normal rate (4). This is a consequence of the substantial capacity of the fetus to maintain oxygen uptake by increasing extraction. Both the conceptus as a whole and the fetus are capable of increasing oxygen extraction, and maintaining this increased extraction chronically, in response to reduced oxygen availability (12). When placental growth is restricted in sheep, both the gravid uterus and the fetus increase oxygen extraction to help maintain oxygen uptake (Fig. 2). This occurs at the cost of reducing the margin of safety between oxygen delivery and consumption for the placenta and fetus (7,12). Acute variations in fetal oxygen requirements occur with fetal body movements or changes in sleep state, and increased demand may transiently exceed oxygen supply in the growth-restricted fetus, with a reduced margin between delivery and consumption (2). In growth-restricted fetal sheep, acute or chronic episodes of hypoxemia or asphyxia have been observed with onset of uterine contractions. To elucidate the influence of oxygen alone on fetal growth and development and the mechanisms involved, various methods have been adopted
specifically to reduce oxygen delivery to the conceptus and fetus, and the consequences have then been determined.

**Reduced Oxygen Supply to the Conceptus: Consequences for Growth**

In human populations, high altitude is associated with a reduction in birth weight (16). This occurs despite compensatory changes in the human placenta, such as attenuation of the villous membrane and increased vascularization of the fetal villi, which should help to increase the placental diffusing capacity for oxygen (16). Experimental reduction of the supply of oxygen to the fetus has been shown to restrict growth in several species, including the sheep (17–26) (Table 2; Fig. 4). Various direct methods have been used to alter oxygen supply to the fetus, including reduction in the partial pressure of oxygen ($P_{O_2}$) in maternally inspired air by means of nitrogen gas, increased altitude, or hypobaric conditions, and low-level maternal exposure to carbon monoxide (17–25). Other approaches have been to reduce the rates of uterine or umbilical blood flows by ligation, partial mechanical occlusion, or embolization of the fetal or maternal placental circulations with microspheres. These latter methods have mostly been used in sheep, and they do reduce the delivery of oxygen to the fetus. However, many of these procedures also reduce the rate of delivery or supply of other substrates to the fetus, including glucose, and are therefore not considered here. Although chronic reduction of uterine blood flow reduces the rates of delivery of both oxygen and glucose to the gravid uterus, it appears to reduce the delivery of oxygen to the fetus specifically, and not that of other major substrates,

![Graph showing the relation between fetal growth and prolonged alterations in maternal oxygenation in different species. Data from studies in rat (circles), guinea pig (diamonds), and sheep (squares) as summarized in Table 2. References are indicated by italicized numbers.](image-url)
<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Timing</th>
<th>Fraction of gestation</th>
<th>Fetal pO₂</th>
<th>Fetal O₂ content</th>
<th>Change in weight and other parameters, %</th>
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<td>na</td>
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<td>0.14–0.9</td>
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<td>0.45–1.0</td>
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<td>0.27–1.0</td>
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<td>−38</td>
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<td>(13 days)³³</td>
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<td></td>
<td>Normobaric maternal hypoxia³⁴</td>
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<td>−30</td>
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<td></td>
<td></td>
<td>0.73–0.92</td>
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<td>−34</td>
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<td>(4 days)³³</td>
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<tr>
<td></td>
<td>Reduced uterine blood flow³⁶</td>
<td>0.86–0.91</td>
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<td>−50</td>
<td>−1</td>
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<td></td>
<td></td>
<td>(7 days)³³</td>
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CRL, crown rump length; na, data not available.
* Superscripted numbers refer to references.
* Duration of reduction in O₂ content of fetal blood.
OXYGENATION IN UTERO

The consequences for fetal growth of specifically restricting the supply of oxygen to the fetus are summarized in Table 2, in which studies have been ranked in order of increasing degree and duration of treatment.

From these studies, it is evident that restricting oxygen supply to the fetus reduces the fetal growth rate. Moreover, increasing the degree of maternal hypoxia and to a lesser extent the duration of exposure to hypoxia, is broadly associated with a greater reduction in fetal weight in the rat and guinea pig (17–21) (Table 2; Fig. 4). In the sheep, fetal growth is not clearly related to the degree of fetal hypoxia achieved, in terms of fetal PO$_2$. Rather, fetal growth appears more to reflect the duration of the reduction in fetal oxygen content (22–26) (Table 2). In these studies, oxygen content in fetal blood usually returned to normal before the end of the period of imposed hypoxia, because of a compensatory increase in fetal hematocrit and hemoglobin concentration in the blood (Table 2). When hypobaric hypoxia is imposed on pregnant sheep during late gestation alone (121 to 140 days) or during a longer period of gestation (35 to 135 days), quantitatively similar reductions in fetal growth are observed (22). This suggests either adequate compensation by the fetus during the longer period of hypoxia or developmental changes in sensitivity of fetal growth to limitations of oxygen supply.

In general, restricted oxygen supply is associated with disproportionate fetal growth, with length reduced to a lesser extent than weight and a relative sparing of brain size (17–21) (Table 2). In contrast, growth of the liver is reduced to a similar or lesser extent than fetal body weight. Other changes are disproportionate reductions in the weights of lymphoid tissues and increases in adrenal weight. This suggests that some but not all of the changes in fetal growth following restricted placental growth can be ascribed to hypoxemia (2,7). Chronic fetal hypoxemia caused by hypobaric conditions or reduced uterine blood flow also reduces the weight of skeletal muscle tissues (22), and in the most severely chronically hypoxic fetal sheep, the rate of oxygen consumption by the hindlimb (26). Thus, reduced growth of skeletal muscle tissues as well as of visceral tissues occurs in chronic fetal hypoxemia. Overall, reduced fetal uptake of oxygen could not be demonstrated up to 7 days after the onset of reduction of uterine blood flow and fetal hypoxia, possibly because oxygen consumption was still being largely determined by the size of tissues already laid down, and because there was at least partial maintenance of their oxygen consumption by increased extraction of oxygen (26).

The outcome of chronic restriction of oxygen supply to the conceptus for the placenta varies, with a maintenance of placental weight relative to fetal weight in the rat and guinea pig, which is more marked with earlier onset of restriction of oxygen supply (17–21) (Table 2). This may represent differential sensitivity of the fetus and placenta to oxygen restriction, or a compensatory response by the placenta aimed at maintenance of placental size and function. This does not occur in sheep, particularly when fetal hypoxemia is produced by reducing uterine blood flow (22–26) (Table 2). However, the latter reduces the rate of delivery of glucose as well as of oxygen to the uteroplacental tissues and is accompanied by reductions in uteroplacental uptake of glucose (26). The mechanism by which oxygen deficiency
restricts fetal growth may be direct in nature, through inhibition of oxidative metabolism and a reduction in the metabolic rate of sensitive tissues, which in the long term reduces growth. Consistent with this is the observation of reduced hindlimb oxygen uptake in the most severely hypoxic fetal sheep when uterine blood flow is chronically reduced (26). However, reductions in total fetal oxygen uptake are not evident immediately or subsequently when measured in vivo during chronic hypoxia (24,26), suggesting that any changes are modest and not detectable by these methods.

Reduced Oxygen Supply to the Conceptus: Consequences for Supply of Other Substrates

Does placental and fetal hypoxia influence growth indirectly by altering the supply of other substrates in utero? Chronic maternal hypobaric hypoxia does not alter maternal and fetal arterial concentrations of glucose, confirming that hypoxemia, but not hypoglycemia, is present (23). The concentration of another important fetal nutrient, lactate, increases substantially after 1 day of hypobaric hypoxia, then falls subsequently to a level approximately twice that of the control sheep (23). Similarly, when the rate of uterine blood flow is chronically reduced in sheep, glucose availability is unchanged, and that of lactate increased in the fetus (26). Prolonged reduction in the rate of uterine blood flow reduces oxygen delivery to the uterus and fetus and reduces fetal oxygenation, linear fetal growth, and fetal weight without changes in fetal arterial glucose (26). The rates of fetal uptake of oxygen, glucose, and lactate are not altered by reduced uterine blood flow and consequent fetal hypoxemia for up to 7 days. These findings suggest that reduced oxygen delivery to the fetus and fetal hypoxia do not slow fetal growth by reducing carbohydrate availability (23,26).

The other major class of nutrient used by the fetus for oxidative metabolism and growth is the amino acids. Placental transfer of amino acids from mother to fetus occurs by active transport against a concentration gradient and requires energy (1). In addition, as described elsewhere in this volume, some amino acid requirements of the fetus and placenta may be met by placental and fetal modification and exchange or cycling of certain amino acids (1). To determine if hypoxia in utero alters the availability of this class of nutrients, we have examined the consequences of chronic maternal hypobaric hypoxemia on the quantity of 17 individual amino acids in fetal blood in sheep (27). Prolonged hypoxemia does not alter circulating concentrations of individual amino acids in maternal plasma, but it results in substantial reductions in the concentrations of the branched-chain amino acids and phenylalanine, tyrosine, and serine in fetal plasma. Thus, chronic maternal hypoxemia reduces the fetomaternal amino acid gradients for the branched-chain amino acids—and alters the availability of many amino acids in the fetal circulation—by mechanisms not involving altered concentrations in the mother (27). To determine whether the changes in amino acid concentration result from impairment of placental amino acid transfer and exchange with the fetus, the precise consequences of chronic hypoxemia in regard to these processes need to be examined. Equally, it is not known whether
Reducing the concentrations of particular amino acids in fetal blood alters their uptake and utilization by the fetus for energy production and growth. This question must be addressed and the consequences of chronic hypoxemia for fetal uptake and utilization of amino acids known, to determine whether fetal hypoaminoacidemia contributes to the concomitant reduction in fetal growth. This is particularly important, because similar changes in circulating amino acids and fetomaternal amino acid concentration gradients are frequently observed in the growth-restricted human fetus (15,28), raising the possibility that placental and fetal hypoxemia may impair amino acid supply to the fetus and potentially perturb growth in the human.

Reduced Oxygen Supply to the Conceptus: Endocrine Consequences

Reduced delivery of essential substrates to the fetus may also restrict fetal growth indirectly by endocrine pathways. Experimental and human fetal growth restriction accompanied by fetal hypoxemia and hypoglycemia is typically characterized by a decreased quantity of anabolic hormones and an increased quantity of catabolic hormones in late gestation (2,7). Some of these changes may be specific consequences of fetal hypoxemia and constitute endocrine mechanisms by which oxygen deficit slows and perturbs the pattern of fetal growth. The altered patterns of organ growth in fetal growth restriction resulting from chronic fetal hypoxemia parallel the redistribution of fetal cardiac output to various tissues that occurs in response to acute and chronic fetal hypoxemia (2,29). Normobaric maternal hypoxia increases the concentrations of epinephrine, and transiently those of norepinephrine, in the fetal sheep (25). Therefore, the redistribution of cardiac output appears to be mediated by the sympathoadrenal medullary responses of the fetus to chronic hypoxia, suggesting one mechanism by which the pattern of fetal growth may be perturbed. Hypobaric hypoxia in pregnant sheep does not alter circulating concentrations of insulin or thyroxine in the fetus, but it does delay the rise in concentrations of triiodothyronine in fetal blood that occurs in late gestation (27). The latter normally parallels the ontogenic increase in plasma cortisol near term. The emergence of a reduced quantity of triiodothyronine after prolonged hypoxemia may contribute to fetal growth restriction, as fetal growth is thyroid hormone-dependent (30). With the onset of hypoxia, fetal plasma cortisol increased and underwent a further substantial and accelerated rise late in gestation (27). This is also consistent with the increase in adrenal growth concomitantly observed in response to chronic hypoxemia. Exogenous cortisol inhibits the growth of the fetal sheep in late gestation (30). Therefore, the increased quantity of cortisol in the hypoxic fetal sheep may also contribute to the inhibition of fetal growth by chronic hypoxemia.

Each of these hormonal factors—catecholamines, thyroid hormone, and cortisol—may themselves act in part through another endocrine/paracrine/autocrine axis, that of the insulin-like growth factors, IGF-1 and IGF-2. The IGFs are small, growth-promoting polypeptides that are important mediators of the influence of essential
substrates and hormones on fetal and placental growth (7,31). The IGFs have metabolic, mitogenic, and differentiating actions that they exert through cell surface receptors, principally the type 1 IGF receptor (31). Gene deletion studies in mice show that IGF-1 and IGF-2 are both required for normal fetal growth, whereas we have shown that an increased quantity of IGF-1 can promote growth in fetal sheep (32). Recent studies suggest that insulin deficiency and thyroid hormone deficiency are associated with reduced concentrations of IGF-1 in fetal blood in the sheep, whereas cortisol inhibits hepatic IGF-2 gene expression (2). Thus, fetal hypoxia, by inducing thyroid hormone deficiency and increasing cortisol levels, may reduce the amount of IGF-1 and IGF-2 within the conceptus and slow fetal growth. Certainly, acute hypoxia has been shown to reduce the concentrations of IGF-1 rapidly in the blood of fetal sheep (33). In addition, the biologic activities of the IGFs are modulated by up to six IGF binding proteins (IGF-BPs) (31); these sequester the IGFs in blood by slowing their clearance from the vascular space, and usually inhibit the bioactivity of the IGFs by controlling their availability to IGF receptors (31). Postnatally, the levels of the various IGF-BPs are regulated by a range of factors, including various hormones (31). IGF-BP-1 is postulated to have an important acute glucoregulatory role and in its usual highly phosphorylated form is a marked inhibitor of IGF actions (31). The levels of IGF-BP-1 increase rapidly in fetal blood in response to both acute hypoxia and more prolonged hypoxia resulting from either induction of maternal hypoxia or a reduction of uterine blood flow in sheep (33,34). This effect of hypoxia has been shown to be partly caused by an increase in circulating catecholamines, which rapidly induce hepatic IGF-BP-1 synthesis (35). Consistent with this, chronic normobaric hypoxia in the pregnant rat restricts fetal growth and increases the amounts of hepatic IGF-BP-1 and IGF-BP-2 messenger RNA and of circulating IGF-BP-1, -2, and -4 in the fetus (17). Thus, an increase in IGF-BP-1 and other IGF-BPs may reduce the bioactivity of IGFs within the chronically hypoxic fetus and further slow growth.

Reduced Oxygen Supply to the Conceptus: Therapeutic Approaches

As noted earlier, the development of percutaneous sampling of fetal blood has made it possible to show that a significant proportion of human growth-restricted fetuses are hypoxemic (36–38). Can such intrauterine hypoxemia be ameliorated? Experimental studies in rats showed improved survival of fetuses in which growth was restricted by ligation of a single umbilical artery when the mothers were exposed to hyperoxic conditions (39). Similarly, we found that placental restriction and reduced fetal oxygenation in sheep could be overcome by maternal hyperoxia, which increased fetal PO2 and the margin of safety between delivery of oxygen and its consumption by the growth-restricted fetus. This suggests that placental limitation of oxygen delivery to the conceptus can indeed be ameliorated by increasing maternal oxygenation.

There has recently been controversy about the effectiveness of exposing women
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135
to hyperoxic conditions to improve fetal oxygenation. In a randomized trial, maternal hyperoxia in the second stage of labor resulted in a deterioration of cord blood gas values at the time of birth (40). However, during the first stage of labor in women with normal pregnancies, maternal hyperoxia has been shown to increase the concentration of fetal cerebral oxyhemoglobin, with a concomitant reduction in deoxyhemoglobin (41). These changes reversed when the hyperoxia ceased.

Maternal hyperoxia in women significantly increases the umbilical arterial PO\textsubscript{2} in pregnancies complicated by growth restriction (42). A small, randomized trial suggested a benefit from maternal hyperoxia (43). Continuous hyperoxia may be better than bed rest for fetuses of less than 26 weeks’ gestation with absent end-diastolic flow in the umbilical artery (44). These are all small studies without sufficient sample size to determine the benefits and hazards of hyperoxia, and larger studies are required before the induction of maternal hyperoxia can be considered as a treatment for growth restriction into clinical practice.

In addition, there are potentially adverse effects of maternal hyperoxia for the fetus that will have to be considered when future trials of maternal hyperoxia are designed. Our studies in sheep show that although maternal hyperoxia increased PO\textsubscript{2} in normal and placentally-restricted fetal sheep, there was no increase in fetal oxygen consumption; however, these were acute studies. After cessation of the hyperoxia, oxygenation of the growth-restricted fetal sheep fell to less than that in the prehyperoxic period. Similar consequences in the human may be inferred by the increase in the number of fetal heart rate decelerations in preterm growth-restricted fetuses after cessation of maternal hyperoxia (45). Furthermore, as maternal hyperoxia improves fetal oxygenation and PO\textsubscript{2}, it may result in a redistribution of cardiac output and blood flow to less vital organs in the growth-restricted fetus. Hyperoxia causes an increase in cerebral vascular resistance and a decrease in resistance in the descending aorta of the fetus (46). This may cause a redistribution of essential but scarce nutrients away from vital organs such as the brain and heart, as the supply of several amino acids is marginal in human pregnancy (47). All these considerations have led to a note of caution about adopting apparently simple and effective treatments for fetal growth restriction (48).

SUMMARY

Oxygen is an essential substrate for life before birth, required for the production of energy, the maintenance of tissues already laid down, and the accretion of new tissues. Studies in animals show that that chronic oxygen deficiency restricts and alters the pattern of fetal growth. Reduced oxygen delivery to the fetus does not alter fetal growth by reducing the availability of the carbohydrate substrates glucose and lactate. However, chronic fetal hypoxemia is associated with reduced concentrations of the branched-chain amino acids and of phenylalanine, tyrosine, and serine in fetal plasma and a reduced fetomaternal concentration gradient for the branched-chain amino acids. It remains to be determined whether these changes are a result
of hypoxemia inhibiting placental amino acid transport, and if the consequent fetal hypoaminoacidemia limits amino acid utilization for energy production and protein accretion in the fetus and thus restricts growth. In addition, chronic fetal hypoxemia is characterized by reductions in the levels of anabolic hormones, with lowered concentrations of triiodothyronine and possibly lowered concentrations of IGF-1 and IGF-2 in fetal plasma. Concomitantly, the levels of catabolic hormones are increased, with increased concentrations of catecholamines and cortisol, which may limit and alter fetal growth. Increased concentrations of IGF binding protein, particularly IGF-BP-1, in fetal plasma occur in response to fetal hypoxemia, which inhibit the anabolic activities of IGFs within the conceptus. Thus, reduced oxygen delivery to the fetus and placental and fetal hypoxemia may restrict fetal growth directly as well as indirectly—by altering amino acid availability within the fetus and through endocrine mechanisms involving an increase of factors inhibitory to fetal growth and a decrease of other hormonal factors required for normal fetal growth and development. Both experimental animal studies and limited human trials show that maternal hyperoxia can improve oxygenation of the growth-restricted fetus, but that cessation of maternal hyperoxia may be associated with adverse changes in the fetus. Further studies are required to characterize more fully the consequences of maternal oxygen supplementation for the hypoxemic fetus before the adoption of such treatment in clinical practice.

ACKNOWLEDGMENTS

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**DISCUSSION**

*Dr. Rennie:* Have you attempted to look at the stoichiometry between the rise of alanine and the fall in the branched-chain amino acids? Because it seems to me that in a situation in which there is an oxygen deficit that is limiting the ability to oxidize fuels, and because protein synthesis is an energy-requiring process, it is unlikely that you are getting an increased disappearance of leucine, valine, and isoleucine into protein, especially as you see an opposite change for lysine, which you would have expected to have gone in the same direction. Is there a connection between the amount of a-amino nitrogen that is disappearing onto alanine and the amount that is coming from the branched chains? If so, have you looked at the ketoacid concentrations, because one would predict that there would be some export of ketoacids back.

*Dr. Owens:* No, we haven’t looked at the relation between, for example, the increase in alanine and the reduction in the branched-chain amino acids, which are the two major quantitative changes, although that would be interesting to do. Like you, intuitively we would suspect it is unlikely that the reductions in concentrations of amino acids that we are seeing are caused by increased utilization. But I think it is very clear that there are many processes that may be determining plasma concentrations, and although we can go a bit farther in doing the sorts
of calculations that you have suggested to clarify what may be happening, we really need to set up suitable experiments with catheterized umbilical and uterine circulations, blood flow measurements, and tracers to be able to say conclusively what is happening. I think it is interesting that we see reductions in the branched-chain amino acids, which are transferred from the mother to the fetus intact and in which there is substantial placental deamination. We also see reductions in glycine and serine, and these arrive in fetal blood by such a different route, maternal serine being converted to glycine by the placenta, which then supplies it to the fetal liver, which in turn produces serine. But my view is that there would be quite different pathways and mechanisms involved there.

**Dr. Soothill:** In your studies of maternal oxygen supplementation, did you find any improvements—increases in glucose or amino acids?

**Dr. Owens:** We found no change in fetal glucose concentrations or in fetal glucose uptake. It was only 4 hours, so it is quite a short-term experiment. We haven't looked at amino acid concentrations or amino acid exchange in those animals, although we have the samples available.

**Dr. Soothill:** Why was the experiment only 4 hours? In terms of what is known about induction of some of the transport systems, it would be quite nice to have data for many days, if it is possible.

**Dr. Owens:** This was really just a first step. What we want to know is whether, when we produce a placenta that is not just small in size but has reduced rates of perfusion, reduced rates of uterine umbilical blood flow, and, as we know now, very much reduced surface exchange area, can we in fact increase oxygenation within the fetus at all—the fetus that is very growth-restricted and with a very small placenta? I agree with you, I think it would be fascinating to go on for longer and perhaps to look at the impact on amino acids.

**Dr. Pardi:** You reported on the fetal-to-placental weight ratio going from 8 in controls to 12 in severe growth retardation. Could you comment on these data?

**Dr. Owens:** Yes, when we restrict implantation in the sheep we variably restrict placental growth, and what we consistently find in that perturbation is that there is an increase in the ratio of fetal weight to placental weight in late gestation. This probably reflects compensatory changes on the part of the placenta and the fetus that may help to maintain fetal growth to some extent. The nature of these compensations varies, but within the placenta, for example, we see that although there are reductions in the absolute rates of uterine and umbilical blood flows, umbilical blood flow per gram of placenta seems to increase in the small placenta.

**Dr. Pardi:** In other words, very roughly and clinically, the placenta loses weight first and then the fetus.

**Dr. Owens:** We have ontogenic data now in this particular model, and certainly up to early in late gestation the placenta is growing more slowly; in contrast, the fetus up to about day 90, which is late in midgestation in the sheep, is of normal weight, but some tissues such as the gut are starting to show decrements in weight and changes in structure, suggesting that limitation has set in. Thereafter, with cross-sectional data fetal growth rate slows. We don't know whether we actually see placental wasting per se, because in the sheep the placental weight normally decreases late in gestation. But there is clearly placental restriction first, and fetal restriction follows subsequent to that.

**Dr. Battaglia:** I think people are getting misled because you have presented oxygen data in milliliters per minute, and there are good reasons in physiology to express them in milliliters per minute per kilogram. The mouse consumes a lot less oxygen than the elephant, but the oxygen consumption of the mouse is much higher than that of the elephant, and there is certainly no reduction in ATP supply in the mouse. So if you have a small fetus, in which
you are studying growth retardation, it seems to me absolutely essential to present the data on a weight-specific basis, because otherwise people are misled. I'm assuming that if you express it per unit weight you will find as we do in growth retardation that you are not getting a reduction in oxygen consumption. So ATP supply is not the problem. I wonder why you are presenting it in absolute terms. The other comment I have is about amino acids. I know of no relation between changes in concentration and uptake. When we are talking about growth and nutrition, we are talking about uptake. So again, I have a problem with your interpretation of concentrations alone.

Dr. Owens: In terms of looking at the impact of these perturbations on fetal oxygen consumption, what I did today was simply to present the data in absolute terms. You can see in our papers that indeed, at least up to 130 days' gestation, the placentally restricted fetal sheep is consuming oxygen at a normal rate on a per kilogram basis. We now have some data showing that eventually oxygen consumption may start to decrease, but it is only very late that you start to get a decrement in oxygen flux when corrections for weight are made. Interestingly, when we look at several of our studies combined, we find that the very small placenta in late gestation is consuming oxygen at a reduced rate per gram. Although there are changes in structure and composition of such placentae, nevertheless it seems that the restricted implantation placenta may be suffering from reduced energy production. On your other point about trying to relate circulating concentrations of amino acids to their utilization, particularly for growth, I think you are absolutely right. All we can say here, for example, where we have a chronically restricted oxygen supply using hypobaric hypoxemia and induced changes in concentrations of amino acids in fetal plasma, is that there is evidence that some perturbation is occurring. We have no idea, however, what is happening to flux, what is happening to transfer, or what is happening to utilization. I think that all these data are telling us is that some of the changes that you see in plasma concentrations in the growth-restricted human fetus can be produced in the sheep by restricting oxygen supply alone, but how that relates to growth and whether it has any relevance to it is another question.

Dr. Doris Campbell: I was interested in the idea that the placenta is restricted from the time of implantation, because in terms of the human work there is a great deal of interest in what is controlling implantation and trophoblast invasion, particularly in the field of hypertension, and what the signal is for trophoblast development. I wonder if you have any information about what the decidual fetal signals might be that control that.

Dr. Owens: We don't have that information from our studies directly. What we see early on in terms of the placentomes that can form at the few sites of implantation that are left, and what we see subsequently up to midgestation at least, is that at those sites there is if anything an overgrowth, or compensatory growth, so in that sense I don't think it is analogous to what you are looking at in the human, where you have impaired or imperfect implantation and impaired subsequent placentation. The signals, of course, would be fascinating to investigate, because treatment such as maternal oxygen supplementation may prove to be valuable when you have identified that you have a problem on your hands, and I would really like to know in addition whether we can intervene very early on in women who we know are likely to go on and have problems with placentation and pregnancy.

Dr. Sibley: There are very interesting data coming from Susan Fischer's lab at the University of California in San Francisco that oxygen might play a key role in the implantation process in inducing various integrins and similar molecules. I would like to know what happens to blood flow in the hypoxic situation, because it strikes me that all your data might be explained by reduced blood flow and a change in vascular resistance. A third point: Everything you said in your model in terms of amino acid concentrations is quite similar to
the human studies, except for alanine. As I understand it, in the Milan study and I think in the London study also, alanine, if anything, goes down despite the fact that, as in your study, lactate goes up. So I really have two questions: What happens to blood flow, and is there any explanation for the discrepancy between alanine in the human and sheep model?

Dr. Owens: Taking the first question, in our study in which we imposed hypobaric hypoxemia in the sheep, we don't know what happened to blood flow. There is only one study that I know of, again in sheep but with normobaric hypoxemia, in which uterine blood flow was looked at, and they did not find any chronic changes in uteroplacental flow (1). But these sorts of measurements are extremely difficult to do, so I think it remains an open question. Intuitively, you would expect no change or perhaps even an increase in umbilical blood flow in response to maternal hypoxia, but I don't think we know whether that could be sustained. On your second question in relation to alanine, I should say that in our placentally restricted animals, we don't find increases in lactate until quite late in gestation, so there may be an element of timing in that once metabolic acidosis sets in, that may be when you see a change in alanine.

Dr. Battaglia: Dr. Meschia, would you like to comment? You did high-altitude studies at about 14,000 feet. What was the uterine flow? Was there a consistent change in flow?

Dr. Meschia: At that time we could not establish any increase in blood flow, but that was many years ago.

Dr. Battaglia: But you were looking for an increase. At least there was no reduction in flow.

Dr. Meschia: No. In acute hypoxia there can be some reduction, but it is very modest. What amazes me is how modest all the changes are with changes in oxygenation; even in growth retardation the effects are relatively small compared with what you get with heat stress.

Dr. Battaglia: Just on the alanine and lactate, I don't think it is going to end up being a species difference. In sheep with acute hypoxia, alanine and lactate concentrations are directly correlated because they are freely exchangeable. The transaminase reaction is readily reversible, so alanine, pyruvate, and lactate are really interchangeable. With chronic hypoxia that relationship breaks down, so something else is going on, but over 4 to 6 hours you would expect a tight correlation. If lactate is increased threefold or fourfold, it is almost impossible to visualize why you wouldn't have an increase in alanine.

Dr. Milliez: Is there any evidence in humans that hyperoxygenation improves fetal growth?

Dr. Owens: There is no evidence that it improves fetal growth. However, Battaglia et al. carried out a randomized controlled trial of maternal hyperoxia in human fetal growth restriction in a small, but I think sufficient, cohort and halved the mortality rate (2). They did not find a restoration of fetal growth rate, but they did improve fetal survival. I certainly agree with Professor Meschia about the limited effect of chronic hypoxemia on fetal growth. It is surprising, particularly when you look at the human growth-restricted fetuses, among which there certainly seems to be a large category that are hypoxic but not hyperglycemic, yet very growth-restricted. So there may be species differences there, and there may be questions of timing of onset and of magnitude as well.

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