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REVIEW

Oxygen, the Janus gas; its effects on human placental development and function

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Abstract

The accumulation of oxygen in the earth's atmosphere enabled metabolic pathways based on high-energy electron transfers that were capable of sustaining complex multicellular organisms to evolve. This advance came at a price, however, for the high reactivity of oxygen posed a major challenge as biological molecules became susceptible to oxidative damage, resulting in potential loss of function. Many extant physiological systems are therefore adapted, and homeostatically regulated, to supply sufficient oxygen to meet energy demands whilst also protecting cells, and mitochondria in particular, from excessive concentrations that could lead to oxidative damage. The invasive form of implantation displayed by the human conceptus presents particular challenges in this respect. During the first trimester, the conceptus develops in a low oxygen environment that favours organogenesis in the embryo, and cell proliferation and angiogenesis in the placenta. Later in pregnancy, higher oxygen concentrations are required to support the rapid growth of the fetus. This transition, which appears unique to the human placenta, must be negotiated safely for a successful pregnancy. Normally, onset of the maternal placental circulation is a progressive periphery-centre phenomenon, and is associated with extensive villous regression to form the chorion laeve. In cases of miscarriage, onset of the circulation is both precocious and disorganized, and excessive placental oxidative stress and villous regression undoubtedly contribute to loss of the pregnancy. Comparison of experimental and *in vivo* data indicates that fluctuations in placental oxygen concentration are a more powerful stimulus for the generation of oxidative stress than chronic hypoxia alone. Placental oxidative and endoplasmic reticulum stress appear to play key roles in the pathophysiology of complications of pregnancy, such as intrauterine growth restriction and preeclampsia, through their adverse impacts on placental function and growth. Establishing an inviolable maternal blood supply for the second and third trimesters is therefore one of the most crucial aspects of human placentation.

Key words development; endoplasmic reticulum stress; human placenta; oxidative stress; oxygen.

Introduction

Climate change is rightly a matter of great topical concern, but by far the greatest change in the Earth's atmosphere occurred approximately 2.3 billion years ago with the accumulation of free oxygen. Life had evolved on the planet in the form of single-celled organisms about 1.4 billion years previously in an anaerobic environment heavily laden with reducing equivalents (Holland, 2006). The metabolic pathways utilized were based around low-energy electron transfers, using, for example, hydrogen sulphide or methane as potential donors (Nealson & Conrad, 1999). Geochemical analyses indicate that oxygenation of the atmosphere took place in two principal stages (Koch & Britton, 2008). It is generally acknowledged that the initiating step was the evolution of the cyanobacteria capable of using water as the source of hydrogen with which to reduce carbon dioxide ($CO_2 + H_2O \rightarrow CH_2O + O_2$) approximately 3 billion years ago. This led to the release of free oxygen as a by-product, which gradually accumulated in the atmosphere. However, levels remained relatively low at around 15 mmHg for approximately 1 billion years, due to reactions with ferrous salts in the oceans. During this period, referred to as the Great Oxidation Event, large deposits of ferric oxides were precipitated. Once this sink was exhausted, the concentration of oxygen in the atmosphere rose to the current level around 0.5 billion years ago, although there have been significant fluctuations since.

The accumulation of oxygen within the atmosphere had two great benefits for life. First, it led to the formation of an ozone layer that acted as a shield against the fierce

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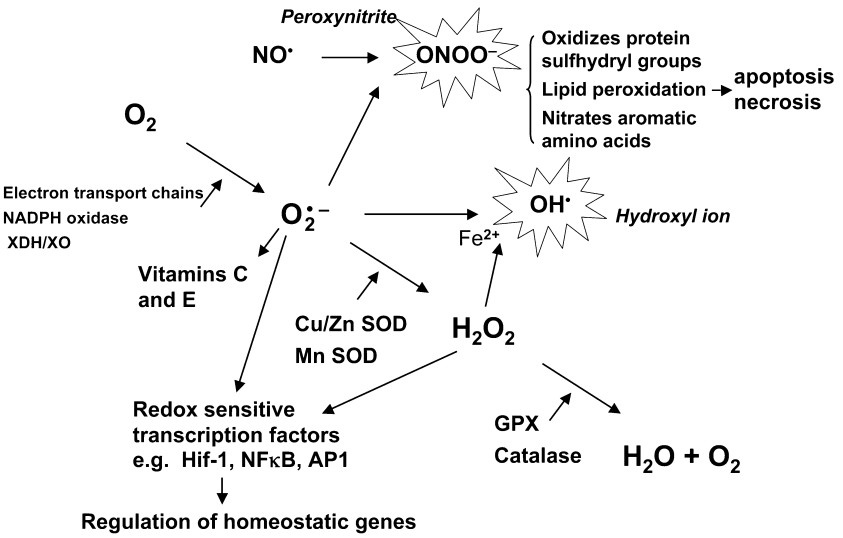

Fig. 1 Diagrammatic representation of the principal pathways for generation and detoxification of ROS. Under physiological conditions ROS regulate the activity of many homeostatic genes through redox-sensitive transcription factors. If excess ROS are produced, either through exposure to elevated oxygen concentrations or through ischaemia–reperfusion, then peroxynitrite and the highly damaging hydroxyl ion can be formed, leading to indiscriminate oxidative damage of biomolecules.

ultraviolet radiation bombarding the planet. This protection allowed life forms to emerge from the oceans and colonize the land. Secondly, with the exception of fluorine, the reduction of oxygen provides the largest release of free energy per electron transfer. These favourable thermodynamics enabled the evolution of more complex metabolic pathways and multicellular life forms (Nealson & Conrad, 1999; Catling et al. 2005; Raymond & Segre, 2006; Koch & Britton, 2008). As concentrations rose subsequent to the Great Oxidation Event, longer diffusion distances could be supported, permitting an increase in organismal size. Indeed, it has been suggested that the rapid rises during the Early Jurassic and Eocene allowed for the evolution of the large placental mammals as they provided for the necessary materno-fetal gradient to enable the fetus to survive (Falkowski et al. 2005). In support of their argument, Falkowski et al. (2005) point out that few extant mammals are capable of reproducing above 4500 m, where the prevailing oxygen concentration approximates to that at sea level in the Early Jurassic.

These benefits came at a price, however, for unless electrons are transferred to oxygen during aerobic metabolism in the correct manner, potentially highly damaging oxygen free radicals can easily be formed (Halliwell & Gutteridge, 1999). Free radicals are species characterized by the presence of an unpaired electron, and hence are highly reactive. Oxygen free radicals, such as the superoxide ($O_2^{\bullet-}$) and hydroxyl anions ($OH^{\bullet}$), and their non-radical intermediates, such as hydrogen peroxide, are collectively referred to as reactive oxygen species (ROS). Together, they are capable of attacking any biological molecule in their vicinity, whether it be a lipid, protein or nucleic acid (Fig. 1). Once initiated these reactions are often self-propagating, and so can cause widespread loss of function and damage within a cell, a situation referred to as oxidative stress. The earliest life forms were therefore faced with a stark challenge; either they were forced to retreat to anoxic environments or they had to evolve enzymatic and non-enzymatic defences that were capable of detoxifying the free radicals to water. Both strategies were employed.

Interest in ROS amongst biologists initially centred on their potential for damage to cell membranes, enzyme systems and DNA (Halliwell & Gutteridge, 1999), and dysfunctional oxygen metabolism may be at the root of diverse chronic disorders (Koch & Britton, 2007). More recently, it has been recognized that ROS play an important role in acting as second messengers in many signalling pathways. Because the rate of production of ROS is proportional to the prevailing oxygen level, ROS act as key intermediates in regulating many homeostatic systems (Fig. 1). Under normal conditions, approximately 2% of the oxygen we consume is converted to superoxide anions rather than being reduced to water. Mitochondria are one of the principal sites of ROS production through leakage of electrons from Complex III of the electron transport chain on to molecular oxygen. Physiological systems are therefore adapted to protect these organelles against excessive ROS production by restricting their exposure to oxygen. In mammals this is achieved by the progressive branching of the vascular system, with a reduction in flow and hence oxygen availability at each stage (Fig. 2A). Thus, the oxygen level in mitochondria within striated muscle is in the region of 0.5 mmHg. Equivalent restriction of oxygen availability is achieved in insect pupae by cyclical closure of the spiracles (Hetz & Bradley, 2005). A number of redox-sensitive transcription factors, such as hypoxia-inducible factor (HIF), cAMP response element-binding protein (CREB), nuclear factor-kappa B (NF-κB), activator protein 1 (AP-1), and p53 have now been identified that regulate gene expression in response to changes in the concentration of ROS (Droge, 2002; Chen et al. 2003; Fedoroff, 2006). At normal levels, reactive oxygen species therefore play an essential

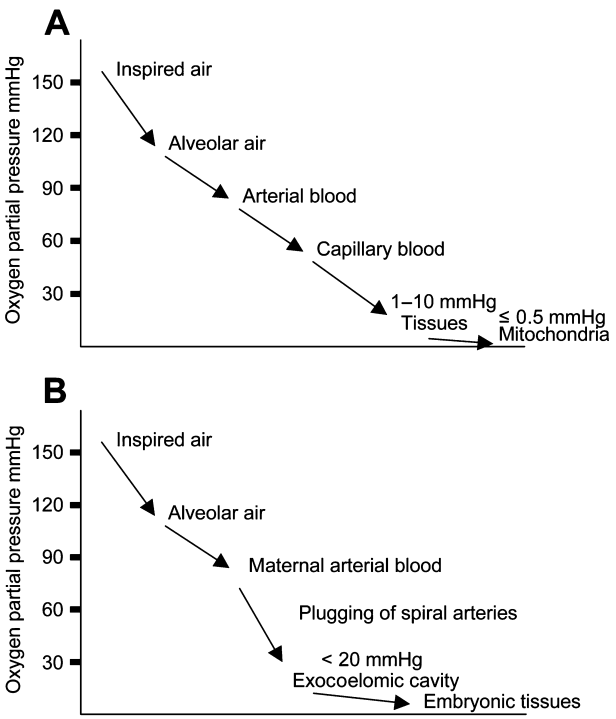

Fig. 2 Diagram showing how the delivery of oxygen to cells is regulated in (A) the adult body, and (B) the conceptus during the first trimester to avoid excessive oxidative stress. Plugging of the spiral arteries by extravillous trophoblast may serve to maintain low oxygen concentrations within the embryo during the critical phase of organogenesis. Reproduced from (Jauniaux et al. 2006) with permission.

physiological role, but if production overwhelms the capacity for scavenging, then cell damage and even cell death can ensue (Fig. 3).

Hence oxygen is often referred to as the Janus gas, for it has both beneficial and detrimental effects in biological systems. The name is taken from that of the Roman god Janus, who is depicted with two heads facing in opposite directions and was frequently used to symbolize transitions from one state to another. Janus was also the god of beginnings, and so it is appropriate to consider the role of oxygen in regulating placental development at the start of a new life.

Oxygen and early placental development

The oxygen level in the uterus at the time of implantation is low in many species; hamster 37 mmHg, rabbit 24 mmHg, rhesus monkey 11–14 mmHg (Fischer & Bavister, 1993). Considerable variation has been found between women, but the averages of 15 mmHg (Yedwab et al. 1976) and 18.9 mmHg (Ottosen et al. 2006) reported are consistent with these values. Such conditions favour optimal pre-implantation embryo development, for they keep metabolism running at a low level and thereby minimize the production of ROS (Leese, 2002). Early placental development occurs under much the same conditions, as it is now recognized that the maternal circulation to the placenta is not fully established until the end of the first trimester (Ramsey & Donner, 1980; Hustin & Schaaps, 1987; Burton et al. 1999). Aggregates of invading endovascular trophoblast plug the tips of the spiral arteries prior to this time, and instead the feto-placental unit is supported by secretions from the endometrial glands (Burton et al. 2002). Hence, although the human placenta is classified as being of the haemochorial type, this only applies from the start of the second trimester onwards. The term 'deciduo-chorial' better describes the situation during the first trimester. Measurements obtained *in vivo* have confirmed that the oxygen tension within the placental intervillous space and the exocoelomic fluid are in the region of 20 mmHg at 7–10 weeks of gestation (Rodesch et al. 1992; Jauniaux et al. 1999, 2000). By contrast, the oxygen tension in the decidua beneath the placenta during this period is approximately 60 mmHg.

The level of oxygenation in the early placenta thus approximates to that in adult muscle, and our own unpublished data indicate that the levels and ratio of ATP to ADP are the same during the first and second trimesters as at term. Interestingly, the feto-placental unit utilizes phylogenetically ancient metabolic pathways to handle

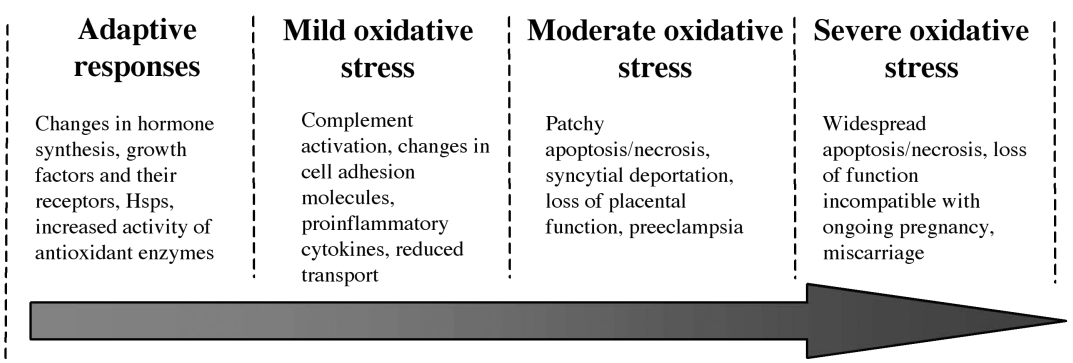

Fig. 3 Diagrammatic representation of the spectrum of changes that can be induced by ROS, ranging from homeostatic responses on the left to cell death on the right.

carbohydrates during the first trimester, producing high concentrations of polyols such as sorbitol, ribitol and erythritol (Jauniaux et al. 2005). These pathways are thought to represent some of the earliest to evolve (Horecker, 1968), and as they rely on non-phosphorylated sugars they can function in a low oxygen environment. Through their activity, NAD^+ can be regenerated from NADH, allowing glycolysis to continue without an excessive build-up of lactate. Nonetheless, the lack of an oxygen carrier in the exocoelomic fluid presumably limits the supply of oxygen to the early fetus (Fig. 2B). We speculate that this may protect against the teratogenic effects of ROS during the critical phase of organogenesis (Jauniaux et al. 2003a). Data from mice in which antioxidant enzyme activity has been genetically impaired, and from diabetic rats, have confirmed that increased levels of ROS are associated with both major and minor congenital abnormalities (Hagay et al. 1995; Cederberg et al. 2000; Nicol et al. 2000; Ornoy, 2007), and are accompanied by a rise in DNA oxidative damage (Eriksson, 1999; Nicol et al. 2000).

A low oxygen environment may also have beneficial effects on early placental development. Thus, angiogenesis is promoted under low oxygen through the transcriptional and post-transcriptional regulation of growth factors such as vascular endothelial growth factor, placental growth factor and the angiopoietins 1 and 2 (Charnock-Jones & Burton, 2000; Charnock-Jones, 2002). Increased cytotrophoblast proliferation has also been associated with hypoxia both *in vivo* (Fox, 1964; Ali, 1997), and *in vitro* (Fox, 1970; Genbacev et al. 1996). More recently, culturing human embryonic stem cells under 5% oxygen has been shown to retain a greater degree of pluripotency than culturing under ambient conditions (21% oxygen) (Ezashi et al. 2005). Daughter cytotrophoblast cells can enter either the villous or the extravillous lineages. However, low levels of oxygen suppress the expression of the fusigenic retroviral protein syncytin in trophoblast cell lines (Kudo et al. 2003). If the same occurs *in vivo* then the incorporation of cytotrophoblast into the syncytial layer may be inhibited, limiting the expansion of the latter. This may have the effect of directing the cytotrophoblast cells towards the extravillous lineage in early pregnancy.

Several groups have investigated the effects of oxygen on extravillous trophoblast invasion, but have obtained conflicting results (James et al. 2006). This most likely reflects methodological differences such as the contrasting oxygen concentrations used, the various cell lines employed and their adaptation to ambient oxygen, the different gestational ages of explants and the means of their collection, the matrix the cells/explants are grown on and the concentration of serum present, and the methods used to quantify the degree of invasion. Work by Genbacev et al. (1996) demonstrated that invasion was greater when primary cultures of cytotrophoblast cells extracted from placentas of more than 7 weeks' gestational age were cultured under 21% oxygen compared to 5%. Similar results were obtained by Caniggia et al. using a first trimester explant model in which maintenance under 3% oxygen produced more extravillous trophoblast proliferation, but not invasion, compared to 20% (Caniggia et al. 2000; Caniggia & Winter, 2002). This change was associated with increased levels of HIF-1α and TGFβ3, which has previously been shown to inhibit invasion. Furthermore, incubation under 3% oxygen in the presence of antisense to HIF-1α reduced the level of TGFβ3 and promoted invasion through increased activity of metalloproteinase 9. By contrast, Graham et al. (2000) reported increased invasiveness of the immortalized trophoblast-like cell line HTR-8/Svneo through Matrigel under 1% oxygen compared to 20%. The invading cells expressed higher levels of the urokinase-type plasminogen activator receptor on their surface, and antibodies directed against this receptor negated the effect. Incubation under 1% oxygen also stimulated an increase in plasminogen activator inhibitor-1 at both the mRNA and protein levels, and this increase again appeared to be mediated through TGFβ.

As mentioned earlier, methodological differences in the experimental approach make it difficult to resolve these conflicting results. In particular, the controls are often performed at 21% oxygen, which is unphysiological for primary cultures or comparison with the *in vivo* situation. Conversely, subjecting cell lines that are adapted to 21% oxygen to low oxygen may have a very different effect on intracellular signalling pathways than a cell adapted to low oxygen moving into a higher oxygen environment, as happens *in vivo* when the extravillous trophoblast cells migrate from the cell columns into the decidua. In an attempt to circumvent these problems Rosario et al. (2007) recently maintained pregnant rats under 11% oxygen for various periods, and compared the degree of endovascular trophoblast invasion against normoxic controls. They reported increased invasion under hypoxia, with exposure between days 8.5 and 9.5 being critical, and also an increased thickness of the junctional zone suggestive of increased trophoblast proliferation. These results indicate that the prevailing oxygen level can influence trophoblast invasion *in vivo*, but clearly further experiments conducted *in vitro* under more physiological conditions are required to elucidate the mechanism by which it acts.

The oxygen transition

If a low-oxygen environment favours feto-placental development during early pregnancy, then the placental tissues must face a major oxidative challenge when the maternal circulation to the placenta is established at the end of the first trimester. Intraplacental oxygen concentrations rise approximately three-fold at that time (Rodesch et al. 1992; Jauniaux et al. 2000). The mitochondria within the syncytiotrophoblast are particularly sensitive to changes

in the prevailing oxygen concentration during early pregnancy (Watson et al. 1998b), most likely due to the low levels of antioxidant enzymes observed in this layer (Watson et al. 1997, 1998a). Why levels should be so low compared with those in the cytotrophoblast and stromal cells is not known, but it may reflect the importance that ROS play in regulating trophoblast functions, such as fusion of cytotrophoblast cells into the syncytiotrophoblast. Thus, overexpression of the anti-oxidant enzyme copper/zinc superoxide dismutase (SOD-1) in cytotrophoblast cells *in vitro* impairs fusion to form syncytial masses (Frendo et al. 2001). This mimics the delayed fusion that is observed with primary cultures extracted from Trisomy 21 placentas where there is gene overdosage of SOD-1 (Frendo et al. 2000), and could account for the abnormally high numbers of cytotrophoblast cells observed in Trisomy 21 early placentas (Roberts et al. 2000). High levels of endogenous antioxidant defences might therefore suppress critical ROS-mediated signals, and inhibit placental development.

The fact that a delicate balance must be struck between pro- and anti-oxidants to maintain a functional homeostatic concentration of ROS means that the syncytiotrophoblast is vulnerable to changes in the oxygen concentration. Due to their charge, free radicals such as $O_2^{\cdot -}$ are lipid impermeable, and so their effects are principally restricted to the compartment in which they are generated. Exposure of first-trimester villi to elevated concentrations of oxygen causes dilation of the intracristal space in the mitochondria and loss of the membrane potential (Watson et al. 1998b). Degeneration of the syncytiotrophoblast soon follows, but the layer is able to be replaced both *in vitro* and *in vivo* by the formation of a new covering arising from differentiation and fusion of the cytotrophoblast cells (Palmer et al. 1997; Hempstock et al. 2003). It is therefore intriguing that when the maternal arterial circulation to the placenta is established it appears to be a progressive phenomenon, starting in the periphery of the placenta and gradually extending towards the more central region as gestation advances (Jauniaux et al. 2003b). Although the mechanism of unplugging of the spiral arteries is still not understood, this pattern of onset of flow correlates with the degree of trophoblast invasion across the placental bed, which is greatest in the centre and least in the periphery (Pijnenborg et al. 1981). Plugging might therefore be expected to be most extensive in the central region, rendering these arteries the last to become fully patent.

Villi sampled from the periphery of the placenta display higher levels of oxidative stress, morphological evidence of syncytiotrophoblast and endothelial cell degeneration, and molecular evidence of activation of the apoptotic cascade compared to their counterparts in the central region (Jauniaux et al. 2003b), consistent with the *in vitro* data. Furthermore, examination of archival placenta-*in-situ* sections of 8.5 weeks' gestational age reveals that the villi in the periphery of the placenta are already shorter and less dense than those under the insertion of the umbilical cord. At the microscopic level the peripheral villi are hypocellular, avascular and have a thin trophoblastic covering, appearances that are in keeping with down-regulation of VEGF and other angiogenic growth factors in a hyperoxic environment. We have referred to this phenomenon as physiological placental oxidative stress, as these changes occur in every normal pregnancy. They constitute an essential part of placental remodelling during which the villi over the superficial pole of the chorionic sac regress to create the smooth chorion, or chorion laeve, through which delivery occurs at term (Fig. 4). We have proposed that abnormalities in this process due to local variations in trophoblast invasion and arterial plugging may cause excessive villous regression, leading to the insertion of the umbilical cord becoming eccentrically placed (Burton et al. in press). Not surprisingly, such placentas are often associated with poor obstetric outcome.

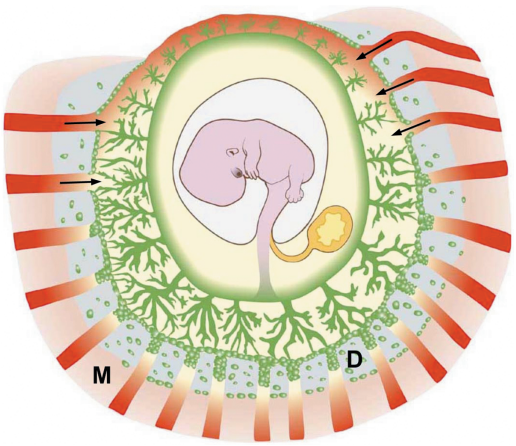

Fig. 4 Diagrammatic representation of how onset of maternal blood flow (arrowed) in the periphery of the placenta may lead to locally elevated levels of oxidative stress, which through suppression of cell proliferation and stimulation of apoptosis leads to villous regression. The deeper trophoblast invasion beneath the conceptus results in more extensive plugging in the central region of the placenta, where villous development continues. D, decidua; M, myometrium. Reproduced from (Jauniaux et al. 2004) with permission.

Qualitatively similar, yet more extensive, changes are seen when placental villi are exposed to chronic hyperoxic conditions during a missed miscarriage. In these abnormal pregnancies onset of the maternal circulation is both precocious and disorganized the whole placenta (Jauniaux et al. 2003b). As a result, levels of oxidative stress are high throughout in all the villi, and are associated with increased apoptosis and decreased cell proliferation (Hempstock et al. 2003). Although the fetus dies, the conceptus is retained *in utero* for a period of days or even weeks, during which the villi gradually regress until they form a thin shell around the chorionic sac. These pathological changes can therefore be viewed as an extension of the events that normally take place in the periphery of a healthy placenta.

Changes in placental oxygen concentrations in later pregnancy

The increased oxygen concentration following the oxygen transition is essential to support the high levels of placental active transport and protein synthesis required to support the rapid fetal weight gain that characterizes the second and third trimesters (Carter, 2000; Schneider, 2000). There are contemporaneous increases in the protein concentrations and activities of several of the principal antioxidant enzymes, suggesting that the placenta adapts its defences to suit the new conditions (Jauniaux et al. 2000). Immediately after the transition, the placenta must be considered to be relatively hyperoxic, as fetal demands are still relatively low. However, as feto-placental consumption gradually increases, the mean concentration falls progressively from approximately 60 to 40 mmHg at term (Soothill et al. 1986). These values may be influenced by different environmental and pathological conditions, and changes in placental structure have been linked to both hypoxic and hyperoxic states (Kingdom & Kaufmann, 1997).

Placental hypoxia is frequently cited as a mediator of the pathological changes observed in complicated pregnancies, although as yet no measurements of the intraplacental oxygen concentration have been presented to support this contention. One situation where the placenta is exposed to chronic hypoxia is during pregnancy at high altitude. For example, the partial pressure of oxygen in maternal arterial blood is reduced from 106 mmHg at sea level to 53 mmHg at 4300 m (Espinoza et al. 2001). Despite this dramatic reduction, the high altitude placenta appears remarkably healthy, with no increase in infarction or other lesions characteristic of preeclampsia (Reshetnikova et al. 1994; Zamudio, 2003). Indeed, recent data from placentas collected at 3100 m demonstrate a reduction in oxidative stress and the concentrations of several antioxidants compared to sea level controls (Zamudio et al. 2007). The majority of studies report increased vascularization of the terminal villi at high altitude (Mayhew et al. 2004), with one finding a strong correlation between the percentage of the villous volume occupied by fetal capillaries and the maternal pO_2 (Espinoza et al. 2001). As the greater capillary volume and surface area result in an increase in the theoretical diffusing capacity of the placenta, these may be viewed as compensatory adaptive changes (Reshetnikova et al. 1994). They most likely reflect increased concentrations of hypoxically regulated growth factors, such as vascular endothelial growth factor, and the villous vessels display greater plasticity in the high altitude placenta (Zhang et al. 2002). This combination of increased angiogenic drive and vessel instability may explain the higher incidence of placental chorangioma above 4000 m (Soma et al. 1995; Reshetnikova et al. 1996).

These findings, along with the data from the first trimester, indicate that the placenta is able to adapt well to constant low levels of oxygen. We therefore took a different approach and hypothesized that the placental pathology that characterizes cases of intrauterine growth restriction and preeclampsia is precipitated by fluctuations in oxygenation rather than hypoxia alone (Burton & Hung, 2003; Burton et al. 2007). These fluctuations may be caused by spontaneous constriction of spiral arteries that have retained smooth muscle within their walls due to deficient trophoblast invasion. Indirect support for this theory comes from several lines of evidence, but principally by comparison with the effects of labour on a normal healthy placenta. During uterine contractions the maternal arterial supply to the placenta is cut off, generating classical ischaemia–reperfusion stress (Grace & Mathie, 1999). Thus, placentas from vaginal deliveries show increased levels of lipid peroxidation compared to controls delivered by caesarean section (Diamant et al. 1980). In addition, they demonstrate increased activity of the xanthine oxidase enzyme (Many & Roberts, 1997), a hallmark of ischaemia–reperfusion, and the mothers have lower levels of vitamin C, suggesting depletion through scavenging of ROS (Woods et al. 2002). Preeclamptic placentas similarly show increased lipid peroxidation and xanthine oxidase activity (Many et al. 1996; Walsh et al. 2000) as well as elevated levels of nitrotyrosine residues (Myatt et al. 1996; Myatt & Cui, 2004), indicative of excessive $O_2^{\cdot-}$ production. Many of these changes can also be induced by exposing villous explants from non-laboured caesarean-delivered placenta to hypoxia–reoxygenation *in vitro* (Hung et al. 2001).

The pathophysiology of preeclampsia is still uncertain, but there seems general agreement that the induction of placental oxidative stress is a key intermediary step, leading to the release of pro-inflammatory cytokines and/or angiogenic factors that cause activation of the maternal endothelial cells (Roberts & Hubel, 1999; Redman & Sargent, 2005). Recent microarray studies have revealed that labour induces changes in the transcripts of many placental genes, the pattern of which closely matches that reported in preeclampsia (Cindrova-Davies et al. 2007b). Furthermore, hypoxia–reoxygenation of placental explants induces increased secretion of pro-inflammatory cytokines and activation of the apoptotic cascade (Cindrova-Davies et al. 2007a). Hence, fluctuations in intraplacental oxygen concentration *in vivo* may have highly adverse effects on placental function. These could arise through a failure of placentation as previously described, with deficient trophoblast invasion and conversion of the spiral arteries, or as a result of the unique upright posture adopted by the human. This can lead to intermittent compression of the aorta and vena cava by the pregnant uterus.

Our most recent data have identified the presence of endoplasmic reticulum stress in placentas from cases of intrauterine growth restriction associated with abnormal uterine artery blood flow (Yung et al. 2008). This is associated with molecular evidence of activation of the unfolded

protein response, leading to protein synthesis inhibition (Schroder & Kaufman, 2005). As a result, levels of AKT and other kinases involved in the AKT/mTOR pathway are greatly reduced (Yung et al. 2007, 2008). This pathway plays a central role in regulating cell proliferation (Fingar & Blenis, 2004; Hay & Sonenberg, 2007). Villous volume and surface area are severely compromised in cases of intrauterine growth restriction (Mayhew et al. 2007; Mayhew, 2008), and longitudinal ultrasound studies indicate that placental size is reduced even at 12 weeks of pregnancy compared to healthy controls (Hafner et al. 2003). These pregnancies are also associated with deficient trophoblast invasion, although to a less severe degree than in miscarriage or preeclampsia (Brosens et al. 1977; Gerretsen et al. 1981). Therefore, we speculate that plugging of the spiral arteries and onset of the maternal circulation are abnormal, resulting in excessive villous regression during the oxygen transition. This loss, coupled with chronic low-grade endoplasmic reticulum stress during the second and third trimesters induced by excessive vasoreactivity of the incompletely converted spiral arteries, could explain the smaller placental phenotype observed. Experimental support for this hypothesis is provided by the observation that JEG-3 choriocarcinoma cells proliferate at a lower rate when subjected to cycles of hypoxia–rexoygenation *in vitro* (Yung et al. 2008).

Conclusion

The accumulation of oxygen in the Earth's atmosphere provided conditions that enabled the placental mammals to evolve, but the high reactivity of oxygen ensures that it is a potentially toxic gas. Concentrations therefore need to be carefully regulated at the cellular level. This appears to be particularly important during the first trimester, for experimental and clinical evidence indicates that ROS are teratogenic. Hence, early development of the conceptus takes place in a low-oxygen environment, supported by phylogenetically ancient carbohydrate metabolic pathways. Later in pregnancy, higher concentrations of oxygen are required to maintain the rapid rate of fetal growth. The transition in intraplacental oxygenation at the end of the first trimester poses a major challenge. Failure of the tissues to adapt to the increased oxygen concentration, or fluctuations in oxygenation secondary to deficient spiral artery conversion, may cause placental endoplasmic reticulum and/or oxidative stress. These can have powerful adverse influences on placental development and function through their effects on cell proliferation and the secretion of cytokines and angiogenic factors, respectively.

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