Placental origins of chronic disease

[1]Graham J Burton, [1]Abigail L Fowden and [2]Kent L Thornburg

[1]Centre for Trophoblast Research and Department of Physiology, Development and Neuroscience, University of Cambridge, UK

[2] Department of Medicine, Knight Cardiovascular Institute, and Moore Institute for Nutrition and Wellness, Oregon Health and Science University, Portland, USA

Address for correspondence:

Professor GJ Burton,

Physiological Laboratory,

Downing Street,

Cambridge CB2 3EG, UK

Tel: + 44 1223 333856

Email: gjb2@cam.ac.uk

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ABSTRACT

Epidemiological evidence links an individual's susceptibility to chronic disease in adult life to events during their intrauterine phase of development. Biologically this should not be unexpected, for organ systems are at their most plastic when progenitor cells are proliferating and differentiating. Influences operating at this time can permanently affect their structure and functional capacity, and the activity of enzyme systems and endocrine axes. It is now appreciated that such effects lay the foundations for a diverse array of diseases that become manifest many years later, often in response to secondary environmental stressors. Fetal development is underpinned by the placenta, the organ that forms the interface between the fetus and its mother. All nutrients and oxygen reaching the fetus must pass through this organ. The placenta also has major endocrine functions, orchestrating maternal adaptations to pregnancy and mobilising resources for fetal use. In addition, it acts as a selective barrier, creating a protective milieu by minimizing exposure of the fetus to maternal hormones, such as glucocorticoids, xenobiotics, pathogens and parasites. The placenta shows a remarkable capacity to adapt to adverse environmental cues and lessen their impact on the fetus. However, if placental function is impaired, or its capacity to adapt is exceeded, then fetal development may be compromised. Here, we explore the complex relationships between the placental phenotype and developmental programming of chronic disease in the offspring. Ensuring optimal placentation offers a new approach to the prevention of disorders such as cardiovascular disease, diabetes, and obesity, which are reaching epidemic proportions.

I. INTRODUCTION

The intrauterine phase of development is key to life-long health, for the foundations of the body plan and the major organ systems are laid down during this period. Perturbation of gene expression or cell proliferation and differentiation during vulnerable periods by nutritional and other environmental influences can alter the structure and functional capacity of major organ systems for life, a process known as developmental programming. These changes predispose the offspring to a variety of disorders that may become manifest in later life, often following exposure to a second precipitating challenge. This concept has profound implications for public health and our approach to the management of chronic diseases, some of which are now reaching epidemic proportions.

The programmed outcomes and the mechanisms by which they occur in the developing fetus, together with their significance for future health have been reviewed previously (37, 56, 215, 237, 374, 426, 528, 565). Here, we focus on the impact of the placenta, the organ that forms the interface between the mother and her offspring while *in utero*, on the causation of chronic disease. The placenta evolved to transfer nutrients to the fetus, and also to create a stable milieu in which the fetus can develop, isolated as far as possible from maternal and environmental stressors. To achieve these functions, it performs a remarkably diverse range of activities, including active and passive transport, endocrine secretion, immunological protection and xenobiotic detoxification. As well as being multifunctional, the placenta is also a remarkably plastic organ, capable of considerable structural and functional adaptations that help to mitigate adverse maternal insults, such as nutrient deprivation, and exposure to drugs, toxins or hypoxia.

However, if normal placental function is impaired, or the organ's capacity for adaptation exceeded, then the fetal milieu may be perturbed with major consequences for the life-long health of the offspring (Figure 1). Ensuring women of childbearing age have access to sufficient and appropriate nutrition is essential, but so too is an understanding of maternal physiological adaptations during pregnancy, in particular the mechanisms by which resources are allocated such that her own needs, and those of her offspring, are suitably met. There is now compelling evidence that the placenta plays a central role in orchestrating this process.

In order to achieve our aim we will consider: 1) the various functions of the mammalian placenta, 2) how placental structure and development facilitate those functions in the human and in the two main experimental models, the mouse and the sheep, 3) the epidemiological evidence linking changes in human placental phenotype to adult disease, 4) the mechanisms by which placental cells may sense oxygen and nutrient availability, 5) how maternal nutrient supply and fetal demand may be integrated at the placental interface, 6) the mechanisms by which the placenta can impact on developmental programming of the offspring, and finally, 7) areas for future research.

We start by briefly describing the general concept of developmental programming of chronic disease.

II. DEVELOPMENTAL PROGRAMMING OF CHRONIC DISEASE

It has long been known that the intrauterine environment has a major impact on development of the adult phenotype (558), but the significance of this phenomenon for

adult health was first highlighted by David Barker and colleagues. In the late 1980s, they reported on ~15,000 records from men and women in Hertfordshire, UK, and showed that rates of death from ischemic heart disease were ordered across the birth weight scale (40). Babies born at the lower end of the scale (5 lb or 2.3 kg) had the highest mortality rates as adults, while those at the opposite end (9 lb or 4.0 kg) were two-thirds lower. At the time, Barker and his colleagues had just concluded a study examining cardiac-related death rates across England (31). The finding that people in the industrial areas of the north of the country died more often of cardiovascular disease than those in the rural south was not surprising, since the impact of an adverse social environment on mortality was already known. The new insight gained was that their findings showed a similar geographic distribution as for the death rates of neonates some sixty years earlier.

The Barker team reasoned that both the neonates and adults died for the same reason, namely that their development had been compromised before birth. Thus, they suggested that an adverse intrauterine environment rendered them vulnerable to death as neonates, and more likely to acquire heart disease later if they survived childhood (33). This relationship between poor growth in the womb and the risk of adult disease has since been confirmed in many other countries, including Finland (26), Sweden (332), China (183), India (517), and the USA (464).

The conclusion that growth rates before birth predict later disease was initially received with skepticism, principally because a mechanistic explanation was not immediately apparent. Eventually, however, experimental evidence accumulated showing clear biological links between stresses that occurred during the first 1000 days after

conception and elevated risks for chronic conditions. These links revolve around permanent structural changes in organ systems, premature aging of tissues and epigenetic changes. For example, a growth-restricted fetus has smaller coronary arteries (288), fewer but more immature cardiomyocytes (55, 348, 394), less elastin in the arteries (152, 362, 526), and fewer nephrons in the kidney (22, 352). In addition, the pancreas has fewer insulin-producing beta cells and reduced vascularization (159, 340, 473), and the structure and maturation of the brain (142), lungs (358, 359, 424, 460) and liver (209, 474) are compromised. All these outcomes have been linked experimentally to impaired placental function. These links go beyond an abnormal maternal nutrient supply, and include, for example, intrauterine hypoxia (213), maternal social stress of the severity that leads to hypercortisolemia (128) and, increasingly, environmental toxins. Thus, diverse stressors acting alone or in combination can lead to alterations in fetal development. Developmental plasticity is a well-described process in nature (43), but little research has addressed the phenomenon within the placenta. This is an area ripe for study.

The placenta does not function in isolation, however, and the mother's nutritional status has a powerful modifying influence on allocation of resources. Accumulating data show that maternal size, a marker of the mother's own growth history, and body composition, a marker of her current nutritional state, combine with placental size and shape to predict chronic disease outcomes. This is perhaps not surprising given that a proportion of the nutrients that support fetal growth, particularly in late pregnancy, come from turnover of maternal fat reserves that are built up in early pregnancy (414). More research is needed to understand how mothers and their offspring communicate

through the placenta to regulate nutrient flow so that the needs of both parties are adequately met.

III. FUNCTIONS OF THE PLACENTA

When considering the potential impact that perturbation of placental function may have on developmental programming, it is essential to bear in mind the variety of activities that the organ performs. Different stressors, for example undernutrition or hypoxia, may affect different placental functions, either in isolation or across the range. Here we consider those functions that have the greatest impact on the embryonic/fetal milieu, namely the transport of nutrients and respiratory gases, the secretion of hormones, and its action as a selective barrier.

A. Transport of nutrients and mechanisms

Although a wide diversity of morphological types exists amongst mammals, a common feature is that the placenta provides for an extensive and intimate apposition of the maternal and fetal circulations. The tissue separating the two circulations is best referred to generically as the interhemal membrane, and it may vary in the number and nature of its cell layers (583). Transport across the membrane has recently been extensively reviewed (19, 61, 95), and so this account is restricted to those aspects most pertinent to placental adaptations to environmental cues.

There are three main mechanisms by which exchange across the interhemal membrane can take place; diffusion, transporter-mediated mechanisms and endocytosis/exocytosis (Figure 2).

253

254 *1. Diffusion*

255 Simple diffusion is the passage of molecules through the lipid bilayers of the cell
256 membranes and the intervening cytoplasm, and is a passive process that does not
257 involve the expenditure of ATP. For small uncharged molecules the rate of diffusion is
258 governed by Fick's Law of diffusion, being proportional to the surface area for exchange
259 and inversely proportional to the thickness of the interhemal membrane:

260

$$Rate = \frac{surface\ area \times concentration\ gradient \times Krogh's\ constant}{thickness\ of\ interhaemal\ membrane}$$

261 where Krogh's constant is a measure of the diffusivity of the molecule

262

263 Small hydrophobic molecules cross cell membranes easily, and so their transplacental
264 flux depends principally on the concentration gradient driving exchange. The main
265 factor maintaining that gradient is the rate of circulation of blood on either side of the
266 membrane, refreshing and depleting the reservoir and recipient pools respectively.
267 Hence, exchange of molecules such as the respiratory gases and lipophilic drugs is
268 considered to be 'flow-limited', and changes in maternal or fetal blood flow have a
269 profound impact on the net flux (573). However, under limiting conditions, such as
270 pregnancy at high altitude, changes in surface area or membrane thickness may be
271 considered adaptive responses to facilitate exchange (275, 369, 458). By contrast, more
272 hydrophilic molecules traverse lipid bilayers slowly, and so the transmembrane
273 concentration gradient is generally more stable. Exchange of these molecules is said to
274 be 'membrane- or 'diffusion'-limited, and structural parameters such as surface area
275 and membrane thickness will play a more major role in determining the flux.

Another mechanism influencing diffusion across the interhemal membrane is the presence of water-filled channels or pores. These are most relevant in species where the trophoblastic layer of the membrane is syncytial in nature and there are no paracellular pathways available, such as the human and mouse. The presence of such pores is evidenced by data showing that the human placenta is permeable to solutes of up to 5,200 daltons (529, 575). Changing the number or diameter of these pores represents a potential mechanism by which the diffusion characteristics of the membrane could be altered in response to environmental cues. Identifying the morphological correlates of these pores has proved problematic in the human due to the complexity of the syncytiotrophoblast. Occasional membrane-lined clefts resembling intercellular spaces have been reported, but may represent areas of repair (339). However, the apical portions of such clefts are sealed by tight junctions and are impenetrable to the extracellular marker ruthenium red instilled at the time of post-fixation. Another approach has been to perfuse the fetal vasculature of the placenta at elevated pressures. Pressures of 100 mmHg and above cause dilation of basal invaginations of the syncytiotrophoblast, and enlargement of vacuoles within the syncytioplasm (304), but connections with the apical surface are not found. Hence, the physiological significance of these morphological observations remains uncertain. An alternative explanation for the apparent existence of pores is that localized areas of damage to the syncytiotrophoblast represent paracellular routes of transport (68). These are discussed in more detail in Section III.C.*1*.

2. Transporter-mediated mechanisms

Transporter-mediated processes are dependent on carrier proteins being inserted into cell membranes to facilitate the passage of highly hydrophilic molecules (Figure 2). Although contrasting in their functional characteristics, they are characterized by common features such as substrate specificity, saturation kinetics and the ability to be competitively inhibited (19). Some transporter proteins are also capable of pumping against a concentration gradient, utilizing ATP. As will be discussed later, there is considerable evidence indicating that expression of the transporter proteins, and their insertion into the appropriate membrane are responsive to nutritional and hormonal cues. This flexibility allows the placenta to adapt functionally, independent of structural changes. Transporter-mediated processes are responsible for the exchange of key nutrients such as glucose, amino acids and fatty acids as outlined below. In addition, there are a variety of other transporter proteins localized to the apical surface of the syncytiotrophoblast in the human, including ones specific for micronutrients, such as copper, iron and folate (49, 371).

i. Glucose

Transport of glucose and related hexoses is dependent on the GLUT family of transporters that enable the sugar to pass down a concentration gradient at rates up to 10,000 times faster than possible by simple diffusion (169). Hence, it is commonly referred to as facilitated diffusion. The density of glucose transporter proteins is considerably greater on the apical surface of the syncytiotrophoblast of the human placenta than on the basal surface, which is thought to reflect the fact that much of the glucose taken up from the maternal circulation is used to meet the placenta's own considerable metabolic needs (141, 270). It is likely, therefore, that the density of the transporters on the basal surface represents the rate-limiting step for exchange. In the

human, GLUT1 is the principal isoform involved in transport across the trophoblast, and protein levels in the apical membrane of the syncytiotrophoblast remain constant from 16 weeks until term. By contrast, levels in the basal membrane double during the late second trimester (280), and this change may explain the increase in glucose transport seen towards term. GLUT1 in the placenta is insensitive to insulin.

GLUT3 is also present on the apical, but not the basal, membrane of the syncytiotrophoblast (67), and is the principal isoform on fetal capillary endothelial cells (245, 270). It has a higher affinity for glucose than GLUT1, and may be more important for transport during early pregnancy (67). In the murine placenta, GLUT1 has been immunolocalized at the ultrastructural level to the apical surface of layer II of the syncytiotrophoblast and the basal surface of layer III (409), suggesting these layers may operate in terms of glucose transport as one functional unit. Both GLUT1 and GLUT3 are expressed in the sheep placenta, but in different layers of the interhemal membrane (95, 582). GLUT1 is localized to the basal surfaces of the maternal-fetal synepithelium and the fetal trophoblast, while GLUT3 is present on the apical surface of the trophoblast. Therefore, a glucose molecule must interact with the two isoforms sequentially to transit between the circulations. Expression of GLUT1 and GLUT3 increases across gestation in the sheep, but the ratio alters with GLUT3 becoming more predominant towards term (165). The implications for glucose transport are not obvious, but clearly caution needs to be exercised when extrapolating data across species (270).

ii. Amino acids

Amino acid transport across the placenta is a key determinant of fetal growth as it provides the essentials for protein synthesis. Single amino acids diffuse slowly across

cell membranes, and most uptake is mediated by a large family of transporter proteins. Amino acid transporters can be classified according to their properties, for example whether they are sodium-coupled or not, and whether they convey neutral, cationic or aromatic amino acids (19, 113). Alternatively, on a more functional basis they fall into three broad categories, accumulative, exchange and facilitative that interact to modulate net transfer across the placenta against a concentration gradient (335). Accumulative transporters are present on both the apical and basal cell membranes of the trophoblast, and mediate the uptake of amino acids driven by the intra-extracellular electrochemical gradient previously described. These transporters generate a pool of amino acids within the trophoblast that drives the activity of other transporters. Efflux from the basal membrane is performed by facilitative transporters, and the rate is determined principally by the concentration gradient across the membrane. The gradient for specific amino acids is modulated by the action of exchange transporters, which, as their name suggests, exchange an amino acid of one type in the intracellular pool generated by the accumulative transporters for an amino acid of another type. Hence, interaction between the three groups of transporters is required to effect transfer, and the net flux per unit area will be dependent upon the, density of the transporter proteins in the apical and basal membranes, the metabolic and anabolic demands of the intervening trophoblastic cytoplasm, and the rate of blood flow in the two circulations.

iii. Lipids

Lipids are essential for the formation of cell membranes, and may be an important fuel for fetal growth, especially among Asian Indians (319). Triglycerides cannot cross the placenta, but may be conveyed in lipoproteins. A number of binding sites for lipoproteins have been identified on the apical and basal membranes of the

syncytiotrophoblast, including those for very low density (VLDL-R) (580), low density (LDL-R) (179), and high density lipoproteins (HDL-R) (9). The scavenger receptors SR-B1 and CLA-1 that bind LDL and HDL respectively are also present (179, 322). Expression of the mRNAs encoding the VLDL-R and LDL-R increases across gestation (402, 580), but is suppressed at term in pregnancies complicated by pre-eclampsia and severe growth restriction (402, 552). The impact of these changes is uncertain, as is, indeed, the contribution of lipoprotein uptake to overall lipid transport. However, placental lipoprotein uptake represents an important step in the maternal-fetal transfer of cholesterol (584).

Alternatively, triglycerides can be converted into free fatty acids (FFAs) by the actions of lipases. Endothelial lipase and lipoprotein lipase have been immunolocalized to the apical membrane of the syncytiotrophoblast during the first trimester, although only the former is seen at term (208). The mRNA encoding endothelial lipase is notably lower in growth-restricted placentas compared to normal counterparts (208), but the significance of this finding for transfer of FFAs is not known. The mechanisms underlying transport of FFAs across the placenta are not fully understood, but at least three membrane systems have been implicated that may act in concert, in addition to simple diffusion. A family of fatty acid transport proteins, (FATP 1-6), have been identified in plasma membranes of the human placenta (162). These are particularly important for transfer of medium to long chain fatty acids. Targeted deletion of FAT-4 results in embryonic lethality, but little is known regarding the specificity of the different transporters. There is also a fatty acid binding protein (FABPpm) located in the apical membrane of the syncytiotrophoblast that appears to preferentially bind and transport long chain polyunsaturated fatty acids. Finally, fatty acid translocase

(FAT/CD36) is present in both the apical and basal membranes of the syncytiotrophoblast. Expression of these transporters is responsive to nutrient availability through fatty acid activated transcription factors (PPARs, LXR, PXR and SREBP-1) (162), and is also influenced by maternal obesity (156).

Computational modeling has suggested that transport of fatty acids across the placenta is modulated by the presence of an intracellular metabolic pool (438), which had been assumed to be within the syncytiotrophoblast. However, recent data derived from placental explants demonstrate that esterification of long-chain fatty acids and their incorporation into lipid droplets occurs within the cytotrophoblast cells, and not the syncytium (314). Further research into the role of the cytotrophoblast cells in lipid transfer to the fetus is therefore clearly required.

3. Endocytosis/exocytosis

Endocytosis/exocytosis is the final mechanism for transplacental transport (Figure 2). Immunoglubulin G (IgG), other large proteins, and cholesterol are considered to be transported by this route. Early studies suggested IgG binds to the apical membrane of the syncytiotrophoblast surface and then concentrates in clathrin-coated pits. However, further work has indicated that IgG is internalized initially through non-specific endocytosis, and delivered, along with other proteins, to early endosomes (486). In the acidic microenvironment, IgG binds to the neonatal Fc receptor, FcRn, which routes it for transcytosis and exocytosis at the basal membrane. There, the more neutral pH of the interstitial fluid favors release of the IgG, promoting transport into the fetal circulation. In this way, a proportion of the IgG internalized is protected from lysosomal degradation, and specificity of transport of Ig subclasses is conferred.

Endocytosis of macro- and micro-nutrients is particularly important in the yolk sac of rodents during the period of early organogenesis (18, 45, 617). The multifunctional endocytic receptors megalin and cubilin have been immunolocalized to the visceral endoderm layer of the rodent yolk sac (13, 186), and potential ligands include folic acid, retinoic acid, vitamins B12 and D, cholesterol, insulin and aminoglycosides (110). Targeted disruption of these receptors leads to failure of somite formation, indicating their key role in supporting early embryogenesis (506). Endocytic uptake of maternal proteins has been described in the human syncytiotrophoblast (325, 571), and is particularly prominent during the first trimester when maternal glycoproteins secreted by the endometrial glands, such as MUC-1 and glycodelin, are engulfed (83). A large proportion of the endosomes co-localize immunohistochemically with lysosomes (83), but some maternal glycodelin crosses the placenta intact and accumulates in the amniotic fluid (296). Megalin and cubilin are expressed in the syncytiotrophoblast, and are also present in the yolk sac, raising the possibility that it too may play a role in nutrient exchange during the earliest stages of human pregnancy (72).

B. Endocrine functions

The importance of the placenta's endocrine role is reflected in the fact that many of the large-placenta-specific gene families arising during evolution through gene duplication encode hormones (450). A wide array of hormones is secreted from the placenta with major impacts on maternal physiology, ranging from suppression of reproductive cycles to mobilization of nutrient resources. The evolution and function of the principal placental hormones was reviewed by Carter (95).

In the earliest stages of pregnancy, the most important function is to signal the presence of the conceptus to the mother, and prevent onset of the next ovarian cycle. In the human chorionic gonadotropin (hCG) secreted by the syncytiotrophoblast acts via luteinizing hormone receptors to maintain progesterone output from the corpus luteum. In the sheep, secretion of interferon τ by the conceptus blocks endometrial production of the luteolytic prostaglandin $F_{2\alpha}$, and so establishes pregnancy. Continuing high levels of progesterone keep the myometrium in a quiescent state, and in the human prevent menstruation.

There is strong evidence in the sheep and other domestic species that interferon τ performs additional functions, combining with placental lactogens secreted by the trophoblast to upregulate the expression of genes encoding uterine milk proteins and growth factors in the endometrial glands (514). This signaling loop represents a mechanism by which the trophectoderm is able to enhance the nutrient supply to the conceptus, and stimulate early development of the placenta. Circumstantial evidence suggests that an equivalent mechanism may operate in the human based on hCG and placental lactogen from the trophoblast (76), but details of the pathways involved are not available as yet.

Progesterone also stimulates maternal appetite during early pregnancy, as does human placental lactogen (hPL), enabling the deposition of maternal adipose energy reserves that can be utilized later in pregnancy and during lactation (414). This build-up is facilitated by the development of leptin-resistance (321), which prevents the negative feedback on appetite centers in the hypothalamus that would normally occur as leptin levels rise with fat accumulation. Evidence from rodent models suggests this central

resistance may be mediated by placental lactogens. Deposition of fat reserves is also facilitated by increased levels of insulin secretion following stimulation of pancreatic ß cell proliferation by placental lactogens in early pregnancy.

Later in pregnancy, a state of insulin resistance develops in the peripheral maternal tissues, mediated, in part, through the actions of placental growth hormone (23). There is also an accompanying rise in circulating triglycerides and free fatty acids. This may serve to enhance nutrient transfer to the fetus by elevating the concentration gradients across the villous membrane, particularly after meals. The placenta may further stimulate its own development by the action of placental growth hormone on the secretion of insulin-like growth factor 1 (IGF-1) by the maternal liver. IGF-1 is a powerful mitogen that increases placental cell proliferation, and increases maternal blood flow to the organ (196, 494).

Finally, it is important to note that the placenta secretes a number of hormones that are traditionally associated with the hypoxic kidney, including erythropoietin, angiotensin II and adrenomedullin (123, 357). Erythropoietin, in particular, is synthesized at rates far higher than the fetal kidney, and may mediate both classical hematopoietic responses to hypoxia and non-classical changes, including increased placental vascularity and defense against oxidative stress (534).

C. Protective functions of the placenta

As well as facilitating the transport of nutrients to the fetus, the placenta plays an equally important role in minimizing xenobiotics, inorganic toxins, pathogens and also maternal hormones from reaching the fetus. It therefore acts as a selective barrier to

create an internal milieu in which the fetus, and in particular its endocrine systems, can develop independently. Nonetheless, perturbations of this function due to mechanical damage, polymorphisms (267), or environmental factors (287), may lead to increased fetal exposure. A range of drugs and toxins are well known to disrupt normal development and mediate teratogenesis, and one might speculate that lower doses, insufficient to cause malformations, may play a role in programming.

1. A Physical barrier

The syncytiotrophoblast is often cited as a physical barrier, impeding the entry of pathogens and maternal immune cells into the fetal compartment. Whilst this is true, defects in the surface are seen in all pregnancies and represent potential portals of entry. These defects, usually 10-20 μm in diameter, can arise through physical interactions between neighboring villi, or the rupture of syncytial bridges that form between terminal villi (73). Abnormal hemodynamics within the intervillous space as a result of deficient conversion of the spiral arteries may also cause damage to the syncytium (265). Defects in the villous surface stimulate activation of maternal platelets and deposition of fibrin (82). These deposits, which are seen in all pregnancies (367), have been demonstrated to be permeable to creatinine and so may represent sites of paracellular transport through the syncytiotrophoblast (68). They are also potential portals for infectious agents; indeed, incubation of placental villi with *listeria in vitro* revealed that the bacteria are only able to penetrate at sites where the syncytiotrophoblast is damaged or absent (465). Despite these defects, the majority of pathogens and parasites do not cross the placenta, most likely due to the large number of marcophages within the villous stroma. These are actively phagocytic, and generally only those pathogens that can survive within the macrophages are associated with

vertical transmission *in utero* (345, 346). Infection of the fetus can lead to growth restriction (3), and hence developmental programming.

2. Efflux transporters

Efflux transporters, such as members of the multidrug resistance protein family, the breast cancer resistance protein, P-glycoprotein, organic anion (OAT and OATP) and cation (OCTN) transporters, and the noradrenalin and serotonin transporters are present on the apical and basal surfaces of the syncytiotrophoblast and the fetal endothelial cells in the human placenta (20, 407, 500, 540). These transporters aid the efflux of a broad range of anionic and cationic organic compounds, and are thought to provide protection to the fetus from maternally-administered drugs and exposure to environmental chemicals. The mRNA and protein levels of P-glycoprotein reduce across gestation, suggesting the fetus may be more exposed to toxic insults later in pregnancy (519).

Assessing the efficacy of these mechanisms in preventing placental transfer is difficult in the human, and in the clinical setting is limited to correlative studies. Thus, during the first trimester the teratogenic effects of drugs that are targets of P-glycoprotein is greater if they are administered in combination with other P-glycoproteins substrates than by themselves, suggesting competitive interactions at the level of the transporter (140). Other studies have compared maternal and fetal blood levels at the time of delivery; for example, levels of dioxins in the fetal circulation were found to be approximately half those in the mother (536). Experimentation is obviously possible in animal models, but species differences in the expression of efflux transporters raises questions as to the applicability of the resultant data to the human (406).

Dual-perfusion of the delivered placenta provides an experimental system, albeit technically challenging, in which to explore transfer of drugs and toxins (266, 408). Comparison of the data with maternal-fetal *in vivo* measurements has validated transfer for approximately 50 drugs (266). In addition, the system is manipulable; for example, inhibition of G-glycoprotein increases transfer of the antiretroviral drugs lopinavir and retinavir to the fetal perfusate, confirming its role as an efflux transporter (100).

3. Enzymatic defenses

A range of defensive enzymes capable of detoxifying xenobiotics and drugs is present within the syncytioplasm. This includes cytochrome P450 enzymes, alcohol dehydrogenase, glutathione transferase and (407). These enzymes provide a measure of defense against agents such alcohol and components of cigarette smoke, but can be overwhelmed, as evidenced by the occurrence of fetal alcohol syndrome. Also present is the enzyme 11-ß-hydroxysteroid dehydrogenase 2 (11-ßHSD2) that catalyzes the conversion of maternal cortisol to its inactive metabolite cortisone. Glucocorticoids are powerful inhibitors of cell proliferation for most fetal organs, except the heart and kidney, and the importance of this enzyme for normal development is demonstrated by the fact that there is a significant correlation between its activity in the human placenta and birth weight (497, 518). In addition, deletion of the *11-ßHSD2* gene in mice is associated with fetal growth restriction (261). The amount of cortisol reaching the fetus will be dependent both on maternal circulating levels and the activity of placental 11-ßHSD2. Maternal concentrations are elevated in response to stress, which may be emotional (300), induced by undernutrition (495), or the result of thermal (491) and other adverse stimuli. Equally, the expression and activity of placental 11-ßHSD2 are

influenced by a number of factors, including intrauterine growth restriction (221), the sex of the fetus (132), hypoxia (6), heavy metals such as cadmium that are present in tobacco smoke (591), and MAPK stress response pathways (498). Resultant exposure to elevated levels of cortisol may contribute to developmental programming of the fetal hypothalamic-pituitary-adrenal axis and other organ systems (127, 129, 306).

D. Sexual dimorphism

The placenta is of the same genotype of the fetus, and there is increasing evidence that sexual dimorphism in terms of its gene expression may modulate its responses to environmental stimuli, and so influence the likelihood of fetal developmental programming. Placentas associated with female fetuses tend to have higher expression of genes involved in immune regulation, endocrine functions and placental growth (71, 512), whilst those from males have more inflammatory profiles (136). These observations have lead to the suggestion that females invest more resources in building the placenta, while males invest more in fetal growth and consequently may have less placental reserve capacity under adverse conditions (71). The situation is made more complex by the finding that sex-dependent expression patterns vary amongst the tissue types comprising the placenta, with differences being observed among purified isolates of syncytiotrophoblast, cytotrophoblast cells, and arterial and venous endothelial cells (136).

Nonetheless, sex-dependent differences in gene expression are likely to underlie the contrasting placental responses observed following exposure to high-fat/low-fat diets (202, 356), glucocorticoids (132), or hypoxia (133, 363) in mice. Similarly, lower levels of mRNAs encoding key enzymes regulating glucocorticoid transfer, including *11ß-HSD2*,

were found in female placentas from women suffering anxiety or depression compared to male counterparts (381). Hence, female fetuses may be exposed to higher levels of maternal stress hormones in these cases, but as yet no data on protein levels or enzyme activity are available to confirm this suggestion.

At present there are few details of the molecular mechanisms involved, but clearly the genetic sex plays an important role in determining the placenta's responses to environmental insults, and hence how it transduces these to the fetus. The impact of the sex of the placenta on its various functions is an important area for future research, and may explain some of the sex-specific aspects of fetal developmental programming (63, 203, 472, 522).

IV. PLACENTAL STRUCTURE AND DEVELOPMENT

While the functions of the placenta are common across all species, its structure is the most varied of any organ. Although major differences exist among species in terms of the gross shape of the placenta, the most striking difference is in the degree of invasion by derivatives of the fetal chorion into the maternal tissues. This varies from no invasion in the epitheliochorial placenta of ruminants, equids and suids, in which the trophoblast simply abuts the uterine epithelium, through the partially invasive endotheliochorial placenta of carnivores, to the fully invasive hemochorial placenta of the human and rodents where the trophoblast is bathed by maternal blood (583). The reduction in the number of tissue layers constituting the interhemal membrane as a result of increased invasion was considered for many years to represent an evolutionary progression. Molecular phylogenetic data have, however, overturned this view. It is now appreciated

625 that the non-invasive epitheliochorial placenta is a derived form that arose by
626 convergent evolution in different orders, and that the ancestral mammal was most likely
627 a shrew-like creature with an invasive hemochorial placenta (96, 98, 171, 572).
628 Epitheliochorial placentation avoids many of the immunological and hemodynamic
629 problems associated with the invasive forms that underlie complications of human
630 pregnancy, such as pre-eclampsia, and these may have operated as selective pressures
631 over the millennia (131, 170, 231).
632
633 Placentas also vary in the degree of interdigitation at the maternal-fetal interface, which
634 impacts on the surface area for exchange. Patterns vary from the folded type,
635 characteristic of pigs where there are poorly branched ridge-like folds, through the
636 more complex villous type, seen in the human and ruminants, to the labyrinthine type of
637 rodents where intricate networks of maternal and fetal vascular channels permeate a
638 block of trophoblast tissue (583). Comparative studies have demonstrated that species
639 with a labyrinthine placenta have gestation lengths less than half those associated with a
640 villous or folded placenta, although there are no relationships with birth weight or brain
641 size (91, 92). Hence, the labyrinthine placenta is capable of delivering nutrients at a
642 faster rate, which may be traded-off against gestational length in order to prevent
643 maternal depletion. Short gestations are presumed to have a selective advantage in
644 environments with marked seasonal changes in food availability.
645
646 Hence, the form of placentation needs to be considered in the context of the
647 reproductive strategy of the species concerned and the environment and habitat that it
648 lives within, for all forms are equally successful in supporting the development of live
649 offspring. Nonetheless, extreme care needs to be taken when extrapolating data from

one species to another. Extensive descriptions of different placental types are available elsewhere (395, 449, 583), and here we restrict our consideration to the human placenta and that of the two main animal species used in research into developmental programming, the mouse and the sheep. The mouse is favored because of the ease of genetic manipulations, which enable, for example, imbalances to be created among maternal supply, placental size and fetal demands (482). Furthermore, placental transport capacity can be assayed *in vivo* (502), and assessed in relation to the maternal and fetal blood flows monitored using high-resolution ultrasound (399). While ultrasound permits longitudinal assessment of placental and fetal development, the small size of the mouse prohibits repeated blood sampling, which represents a significant limitation for metabolic studies. By contrast, the sheep offers opportunities for extended experimentation in conscious, ambulant animals through chronic catheterization of the maternal and fetal circulations. The neonate is also of approximately the same size as that of the human, and born at a similar degree of maturation.

Although research has been performed on other species, including the rat, rabbit, guinea-pig, pig, horse and non-human primates (94, 520), the data are limited in comparison. There is no perfect model of human placentation, except for the great apes in which experimentation is ethically unacceptable. Hence, one has to select the species most suitable for the question being addressed, giving consideration to factors such as the number of offspring, the histology of the interhemal membrane, the length of gestation and the relative mass of the conceptus to that of the mother at term.

A. The human placenta

675

676 *1. The mature placenta*

677 The mature human placenta is usually a circular or oval disc approximately 22 cm in
678 diameter (435, 478). The disc is bounded on the fetal surface by the chorionic plate to
679 which the umbilical cord is attached, and over which the branches and tributaries of the
680 umbilical vessels radiate. The branching pattern of the chorionic arteries may be
681 monopodial or dichotomous, and varies depending on the site of insertion of the
682 umbilical cord. The first two-three generations are always dichotomous, and thereafter
683 are mostly monopodial if the cord insertion is marginal and dichotomous if it is central
684 (223). Computational models indicate that energy losses are small in monopodial
685 branching, and this may be beneficial when perfusing placental territory over a long
686 distance (224). Conversely, dichotomous branching is more efficient in distributing
687 blood over large areas near the bifurcation. On the maternal surface is the basal plate
688 that abuts the decidua, and this is divided into a number of lobes by septa that are
689 directed towards, but do not reach, the chorionic plate. Hence, the placenta is divided
690 into a variable number of compartments, and this arrangement may assist in directing
691 the flow of maternal blood (49). Lobes are alternatively known as cotyledons, but we
692 prefer to use the former term to avoid confusion with the ovine placenta.

693

694 Internally, it comprises a series of highly branched villus trees that in total contribute a
695 surface area for exchange of 12-14 m^2 (75). Each tree arises via a stem villus from the
696 chorionic plate and forms a lobule that is centered over the opening of a maternal spiral
697 artery through the basal plate, so constituting an individual maternal-fetal exchange unit
698 (Figure 3A). There may be one or more lobule per lobe. While some villi, the anchoring
699 villi, extend between the two plates, the majority are free-floating within the cavity of

the placenta, the intervillous space. The finest branches of the villus tree, the terminal villi, are highly vascularized with fetal capillaries. Dilations of the capillaries, referred to as sinusoids, bring the endothelium into close apposition with the overlying syncytiotrophoblast, which is often locally thinned to form a vasculosyncytial membrane. Consequently, the diffusion distance between the two circulations may be reduced to 1-2 μm at these sites, aiding diffusional exchange (Figure 3B).

The syncytiotrophoblast forms the epithelial covering of the villus tree, and during the second and third trimesters and is bathed directly with the maternal blood circulating in the intervillous space (Figure 3B). Hence the human placenta is described as being of the hemochorial type (49). The syncytiotrophoblast is a terminally differentiated, multinucleated syncytium. The apical surface bears numerous microvilli, amplifying the surface area for receptor-mediated transport by a factor of ~ x7 (302). A wide variety of receptors have been localized to the microvillous surface, and their activity is responsive to maternal nutrition (204). Coated pits are observed at the base of the microvilli for endocytic transport (291, 420).

The syncytioplasm is dense with organelles, including rough endoplasmic reticulum and mitochondria, reflecting its high synthetic and metabolic activity. Hence the tissue is vulnerable to oxidative and endoplasmic reticulum stress, which if not resolved leads to activation of the unfolded protein response. These stresses may impact severely on its endocrine and transport functions, and are associated with growth restriction and other complications (85, 405, 596). In this respect, comparisons can be drawn between the syncytiotrophoblast and other endocrine-active cells, for example pancreatic ß cells (21).

The basal surface of the syncytiotrophoblast contacts either the progenitor unicellular cytotrophoblast cells, or the trophoblastic basement membrane. In early pregnancy the cytotrophoblast cells form a complete layer, and so nutrients must either pass through the cells or through the narrow intercellular clefts. Towards term these cells become more dispersed, with studies finding that they occupy 44% (290) or up to 90% (392) of the basement membrane, creating larger gaps for potential paracellular transport.

The fetal capillaries lie within the stromal core, often closely approximated to the trophoblastic basement membrane. They form the third layer of the interhemal membrane, and potentially play an important in regulating maternal-fetal transport (168, 185). The endothelial cells are of the non-fenestrated type, and are connected by both tight and adherens junctions (339). The composition of these complexes differs with gestational age, and with their location in the villus tree. During the first trimester the tight junctions lack occludin and claudin-1 and -2, suggesting that they are still plastic and highly permeable (329). This arrangement persists in later pregnancy within the terminal villi (330), pointing again to the importance of these villi for exchange. Numerous caveolae are present within the cytoplasm of the endothelial cells, which may play a role in transcellular endocytic transport. The GLUT3 transporter protein has also been immunolocalized to the endothelial cells (245), as has the multidrug resistance protein (540), tocopherol transfer protein (400), and phospholipid transfer protein (487). However, no data are yet available describing the importance of the different transcellular and paracellular pathways for the transfer of specific types of nutrients. In a co-culture model of the interhemal membrane, the endothelial layer was found to offer greater resistance to the transport of glucose than the trophoblast layer (333). Whilst a

step forward, these results cannot necessarily be extrapolated to the *in vivo* condition as the unicellular HTR8 trophoblast cell line was used, which may not reflect the same transport properties as the syncytiotrophoblast. Insulin receptors are detectable on the cell surface from the start of the second trimester onwards (143), and may regulate villus angiogenesis in metabolic disorders (327).

2. Development

The human placenta undergoes major transformations in its structure during pregnancy, and it is important to be aware of these changes when considering the impact of environmental insults on fetal-placental development.

Placental development begins with differentiation of the trophoblast lineage at the morula stage, and there is evidence of plasticity at this early stage. For example, embryos derived from oocytes retrieved from women with a body mass index greater than 25 kg/m^2 develop faster than those from lean women and have fewer trophectoderm cells (331). The embryos also display differences in metabolism, with reduced glucose consumption and altered amino acid usage. Mammalian zygotes do not form functional gap junctions until around the 8-cell stage, and so the individual cells behave metabolically in an autonomous fashion (62). It has been speculated that this lack of cell-cell communication heightens sensitivity to stressors. Thus, the zygote may be affected by environmental cues transduced through the oviductal secretions during its passage into the uterus. Equally, it may be influenced by the culture conditions during assisted reproduction techniques (ART), which can have significant effects on birth weight (160). The impact of ART on pregnancy outcomes (508), and cardiovascular health (427), has recently been reviewed, but few data relating to

placental development are available. A large study of over 500,000 births showed that placentas arising from ART are heavier than those from natural conceptions (233). The placental-fetal weight ratio is also increased in ART pregnancies, and this relationship is independent of the technique employed, the method of delivery and other potential confounders. However, the mechanism underpinning the effect, and the timing at which it operates, are still unknown.

The first morphological evidence of placental development is seen at implantation, which occurs around day 7 post-fertilization. On attachment to the uterine epithelium the trophectodermal cells differentiate and fuse to form the syncytiotrophoblast. Projections from the latter penetrate between the epithelial cells and into the underlying stroma, so that the zygote is completely embedded within the superficial endometrium by day 11. The syncytiotrophoblast expands through the proliferation and fusion of underlying cytotrophoblast cells, and surrounds the entire surface of the original blastocyst. As it expands, the syncytiotrophoblast erodes into dilated capillaries within the endometrium, and also the apical parts of the endometrial glands. As a result, maternal erythrocytes and gland secretions enter into spaces that form within the syncytiotrophoblastic mantle, the forerunners of the intervillous space (49). Development of the placenta is precocious, but the factors stimulating and regulating this rapid development are poorly understood, principally through the difficulty of obtaining suitable specimens. However, it is now accepted that during the first trimester the conceptus is supported by histotrophic secretions from the endometrial glands, the 'uterine milk' (80, 83).

799 The full composition of the endometrial secretions during pregnancy is not yet known,
800 but evidence from proteomic analysis during the secretory phase of the non-pregnant
801 cycle indicate that they likely contain large glycoproteins, including MUC-1, glycodelin-A
802 and uteroglobin, carbohydrates and lipids (46, 50, 238). These secretions are
803 phagocytosed by the syncytiotrophoblast (83, 254), and maternal proteins and amino
804 acids accumulate in the coelomic fluid inside the placental sac (282). From there, they
805 may be transported to the embryo via the secondary yolk sac, which floats within the
806 coelom. The yolk sac is the first of the extraembryonic membranes to be vascularized,
807 and abnormalities in its development are associated with early pregnancy loss (416).
808 Recent immunohistochemical studies have located transporter proteins, such as GLUT1,
809 folate receptor-α, and tocopherol transfer protein, to the outer mesothelial surface (49,
810 281, 285), but no data are available regarding the yolk sac's functional capacity for
811 uptake *in vivo*. However, deficiency in the transport of retinol and other key signaling
812 molecules, potentially involving the yolk sac, has been implicated in the causation of
813 major embryonic defects seen in chromosomally normal spontaneous miscarriages
814 (440).
815
816 The glandular epithelial cells are also immunopositive during early pregnancy for an
817 array of powerful mitogens, such as epithelial growth factor (EGF) and vascular
818 endothelial growth factor (VEGF) (254), and so the secretions may play an important
819 role in stimulating early development of the placenta. Indeed, evidence from animal
820 species indicates that the conceptus promotes its own development by signaling to the
821 glands through placental lactogens and upregulating expression of uterine milk proteins
822 and growth factors (514). It is suspected, but not yet proven, that the same happens in
823 the human, possibly augmented by prolactin secreted by the decidual cells (76, 80). The

824 fact that the morphology of the glandular epithelial cells changes to a characteristic
825 hypersecretory type, the Arias-Stella reaction, suggests this may be the case (17).
826
827 Taken together, these data indicate that the endometrium plays a greater role in
828 stimulating and supporting placental development during early pregnancy than
829 previously anticipated. The first trimester is a critical period for placental development,
830 for expression of markers of trophoblast stemness decline rapidly after 12 weeks of
831 gestation (252), suggesting loss of proliferative potential. Perturbation of endometrial
832 and, in particular, gland function may therefore have a profound effect on the ultimate
833 growth of the villus trees and the surface area for exchange. Whether the secretome is
834 altered in response to maternal nutritional status, obesity or other conditions during
835 early pregnancy is not known. Further research is necessary to test this hypothesis, and
836 also to determine whether the endometrial glands may themselves be subjected to
837 developmental programming. Ultrasound data indicate that the size of the uterus is
838 reduced in girls born with low birth weight (268), but whether the density or activity of
839 the glands are also compromised is not known. If so, this could represent a mechanism
840 mediating intergenerational effects on birth weight.
841
842 Placental metabolism is heavily glycolytic in early pregnancy due to the prevailing low
843 oxygen concentration (286). The phylogenetically old polyol pathways are highly active,
844 avoiding excessive fermentation of glucose to lactate (284). Whether these pathways are
845 more robust to environmental stressors than oxidative phosphorylation is not known,
846 but it is notable that the placental ATP/ADP ratio is the same as in later pregnancy, and
847 that there is no evidence of hypoxic stress in early placental tissues (112).
848

849 The maternal arterial circulation to the placenta is established towards the end of the
850 first trimester, and is associated with transformation of the early placenta to its
851 definitive form. Establishing the circulation requires invasion into, and remodeling of,
852 the endometrial spiral arteries. This is performed by a sub-population of migratory
853 trophoblast cells, the extravillous trophoblast, which in normal pregnancies penetrate
854 the underlying decidua and reach as far as the inner third of the myometrium.
855 Remodeling of the spiral arteries involves the loss of smooth muscle cells and elastic
856 tissue from their walls, and their replacement by fibrinoid material (441, 570). As a
857 result, the vessels loose their vasoreactivity, and their terminal portions dilate as they
858 approach the basal plate of the placenta. Together, these changes ensure a constant flow
859 of maternal blood into the placenta at a low velocity and pressure (84). Failure of
860 trophoblast invasion and arterial remodeling is associated with the 'Great Obstetrical
861 Syndromes', including growth restriction, pre-eclampsia and late spontaneous abortion,
862 due to impaired maternal perfusion (64). Early in pregnancy the invading trophoblast
863 cells plug the maternal spiral arteries, preventing flow of maternal blood into the
864 placenta (264). Towards the end of the first trimester these plugs dislocate, leading to
865 onset of the maternal arterial placental circulation and the switch from predominantly
866 histotrophic to hemotrophic nutrition.
867
868 Events at this stage appear to play a major role in determining the final size and shape of
869 the organ. Villi initially form over the whole of the chorionic sac, but at around 7-8
870 weeks of gestation those over the superficial pole begin to regress, leaving the smooth
871 membranes or chorion laeve. This regression is linked with locally high levels of
872 oxidative stress and apoptosis, for blood flow starts in the periphery of the early
873 placenta and gradually extends to the central region (283). This pattern reflects the

874 extent of extravillous trophoblast invasion and plugging of the maternal spiral arteries
875 across the placental bed (65). In normal pregnancies, this centripetal regression results
876 in an approximately discoid placenta with the umbilical cord near the center. However,
877 we have speculated that if onset of the circulation is more erratic, possibly due to
878 uneven trophoblast invasion, excessive villous regression may lead to small, abnormally
879 shaped placentas with eccentrically inserted umbilical cords (77). Unfortunately, this
880 hypothesis cannot be tested experimentally. However, the site of cord insertion
881 identified by ultrasound at the end of the first trimester correlates closely with that
882 observed at delivery, confirming the location is determined early in pregnancy (479,
883 489). Equally, placentas that are growth restricted at term are smaller than normal at
884 the end of the first trimester (122, 235), whereas the converse is the case for
885 macrosomic placentas (490).
886
887 An important question that has not been fully addressed is whether compensatory
888 lateral growth of the placenta is possible in later pregnancy. There are three aspects of
889 human placentation that are critical when considering this possibility. First, the
890 conceptus is completely embedded in the uterine wall, and so it is not just a question of
891 the placenta expanding over the uterine surface. Any enlargement with respect to the
892 uterus must be associated with erosion into the maternal tissues. Second, there must be
893 recruitment of additional spiral arteries to supply any significant increase in territory.
894 Recruitment is possible during the first trimester when there is an alternative source of
895 nutrients from the endometrial glands, and a prolific supply of extravillous trophoblast
896 cells from the cytotrophoblast columns to initially plug the arteries while remodeling
897 takes place. However, that supply wanes during the second trimester as the columns
898 become short and sparse, and the villi at the margin of the disc regress. Thus, it is

899 probable that the final complement of arteries is essentially fixed at the end of the first
900 trimester. Third, the uterus obviously expands and remodels as pregnancy advances,
901 and consequently the relative position of the placental attachment within the uterus
902 changes with gestational age. This is not achieved through migration or trophotropism
903 as suggested by early investigators (594), but is principally due to the drawing-out of
904 the lower uterine segment (257, 404). Hence, whilst in early pregnancy the placenta
905 grows faster than the uterus and the syncytiotrophoblast mantle expands within the
906 superficial endometrium (130), it is likely that the placental footprint is established
907 around the end of the first trimester when formation of the chorion laeve is complete.
908 Thereafter, it has been suggested that the placenta and uterine wall expand together
909 (229). Rough estimates based on the density of the spiral arteries in the non-pregnant
910 uterus and their final disposition in the placental bed at term indicates that this may be
911 the case. The arteries are initially 2-3 mm apart (41), but at term must be 10-20 mm
912 apart based on the diameter in the lobules that each supplies (253). Thus, the placental
913 bed has expanded ~5 fold whereas the diameter of the placenta increases similarly from
914 5 cm at 11 weeks to 22 cm at term (49). Whether all areas of the uterus expand equally
915 or whether some areas, such as the fundus, expand preferentially is not known.
916 However, differential expansion could explain why some placentas are circular and
917 others elliptical dependent on the implantation site. Equally, it is not known whether
918 the density of the spiral arteries is uniform in the uterine wall. If not, then the site of
919 implantation may affect the ultimate blood supply to the placenta. This linkage provides
920 a potential mechanism by which the shape of the placenta may be associated with its
921 functional capacity and the ensuing phenotype of the offspring.
922

If the first trimester sees the establishment of the framework of the placenta, the second and third trimesters see an increase in its functional capacity, principally owing to the exponential increase in villous surface area created by the formation of terminal villi and a reduction in the maternal-fetal diffusion distance (274). It is notable that the theoretical diffusing capacity for oxygen expressed per kg of fetal weight remains constant across gestational age (364, 368), suggesting placental development determines the rate of fetal growth or that the two are closely co-regulated. Formation of terminal villi is believed to be driven through angiogenesis causing capillary loops to obtrude from the side of the containing villus (303). Hence, it is likely to be heavily influenced by the prevailing oxygen tension (312). The vascular network appears to be particularly plastic during the first trimester due to its low coverage with stabilizing pericytes at that time (607). Pericyte coverage is also reduced in placentas from pregnancies at high altitude, which may facilitate vascular adaptations to increase gaseous exchange, as will be discussed later.

B. The murine placenta

1. The mature placenta

The mouse has a single, discoid hemochorial placenta that in terms of its gross morphology is similar to that of the human. Internally, however, there are significant differences (210), the most major being that the placenta is divided into two morphologically and functionally distinct zones; the labyrinth zone that is responsible primarily for exchange and the junctional zone that serves an endocrine function (Figure 3C). The proportion of these two zones displays considerable plasticity, varying within a

947 normal litter depending on the overall placental size and also following dietary and
948 other manipulations (114, 118, 119).
949
950 The labyrinth zone is closest to the chorionic plate and consists of a dense meshwork of
951 interconnecting lamellae of trophoblast. Within the lamellae are the fetal capillaries,
952 whereas between them lie the maternal blood spaces (Figure 3D). The labyrinthine
953 trophoblast comprises three layers. The outer layer is formed of uninucleate cells that in
954 the past were referred to as cytotrophoblast cells. However, it is now recognized that
955 they do not equate in progenitor terms to the cells of the same name in the human
956 placenta, and their expression of genes encoding placental lactogen suggests they have
957 an endocrine function (504). They display a large nucleus with evidence of limited
958 endoreduplication (117), and so are now classified as sinusoidal giant cells (503).
959 Beneath these cells are two layers of syncytiotrophoblast that are closely approximated
960 to each other and linked by extensive gap junctions (378, 409). This arrangement is
961 often referred to as hemotrichorial, although as gestation advances the sinusoidal giant
962 cells become perforated, allowing maternal blood access to the outer layer of
963 syncytiotrophoblast (117). The extent to which the two syncytiotrophoblast layers
964 function as one is also debatable, for the presence of the gap junctions will allow small
965 molecules to pass easily between them. This is evidenced by the fact that GLUT1 glucose
966 transporter proteins are only immunolocalized to the apical surface of layer II and the
967 basal surface of layer III, with none being located at the interface between the two layers
968 (409). They are also not present on the layer I, the sinusoidal giant cells. Hence, the
969 arrangement in the mouse may be more analogous to the single layer of trophoblast in
970 the human than previously anticipated. These proteins, and a variety of amino acid
971 transporters, appear responsive to maternal nutrition and genetic manipulations of the

placental to fetal size ratio (14, 204, 566). The trophoblast layers rest on a basement membrane to which the fetal capillaries are closely apposed on the other side, with no intervening stromal cells. Unlike the human, the murine syncytiotrophoblast has no endocrine function (355).

The junctional zone, in contrast, does not contain fetal blood vessels, and is only traversed by the maternal spiral artery delivering blood to the labyrinth and venous channels conveying maternal blood back to the uterine veins. It is composed of two principal cell types, spongiotrophoblast cells and glycogen cells, and the proportion of these changes with gestational age. Glycogen cells are sparse before E14.5, but numbers then expand before declining around E18.5 as they migrate into the decidua (115). As their name suggests, these cells accumulate large quantities of glycogen that may act as an energy reserve to be released when growth of the fetus is maximal. Spongiotrophoblast cells display large quantities of rough endoplasmic reticulum, suggesting a high secretory output. Many members of the placental lactogen family have been localized to these cells (355, 507), but the full range of their output is still unknown. These cells are more vulnerable to stress than the syncytiotrophoblast of the labyrinth, which may reflect a higher metabolic rate (599). The venous channels are lined by other types of polyploid trophoblast giant cells (4, 446, 503). These too have a potential endocrine function through the release of placental lactogens (504), raising the possibility that they may relay information to the mother concerning the composition of her blood following exchange with the fetus. Integration of signals from the sinusoidal giant cells and the giant cells lining the venous channels could thus provide an indicator of fetal demand.

In addition to the discoid chorioallantoic placenta, an inverted yolk sac placenta is functional in the mouse from early in pregnancy until term (583) (Figure 3C). The yolk sac is highly vascularized, and the visceral endodermal layer is exposed to the uterine lumen and any nutrients secreted by the endometrial glands. The apical surface of the cells resembles in many respects that of the syncytiotrophoblast in the human placenta. There is an abundance of microvilli and coated pits, and numerous absorptive droplets and vacuoles within the underlying cytoplasm (232). The absorptive function is reinforced by the presence of the multifunctional endocytic receptors megalin and cubilin that potentially transport a wide variety of vitamins and micronutrients (617). The large number of mitochondria and cisternae of rough endoplasmic reticulum suggest that the endodermal cells have a high metabolic rate. Experiments in the rat have revealed that more than 95% of amino acids transported during the period of organogenesis are derived from the uptake and subsequent breakdown of maternal proteins by the yolk sac (59, 344). Perturbation of yolk sac function can thus have profound effects on embryo development (455), and so impact on yolk sac function is often targeted in screening of potential teratogens.

2. Development

Development of the placenta starts with differentiation of the trophectoderm lineage at around E3.5. This process shows considerable plasticity in response to environmental cues, such as maternal diet, that influence the ratio and number of trophectoderm and inner cell mass cells. A low protein diet during the perimplantation period induces an increase in the total number of trophectoderm cells in the blastocyst, suggesting an early compensatory reaction (163). However, maintenance on such a diet throughout

pregnancy results in reduced placental and pup weights, indicating that growth of the conceptus is ultimately constrained by the impoverished nutrient supply (120). Implantation commences at E4.5. At this time, the polar trophectoderm cells overlying the inner cell mass differentiate into two cell types, extraembryonic ectoderm and the ectoplacental cone. The remaining mural trophectoderm cells undergo limited proliferation before exiting the cell cycle and transforming into polyploid primary trophoblast giant cells (210, 503, 566). These mediate the initial invasion of the conceptus at the implantation site, and hence lie at the boundary between the mature placental disc and the decidua. They have an endocrine role, expressing several members of the prolactin/ placental lactogen gene family, and are also thought to secrete angiogenic and vasodilatory factors (503).

It is likely that in rodents embryogenesis and early placental development take place in a low oxygen environment, as in the human, for at E6 the antimesometrial decidual cells form an avascular zone around the conceptus, separating it from the maternal blood (430). Nutrition at this time is histotrophic, absorbed principally through the visceral yolk sac. The yolk sac grows at a prolific rate and soon encapsulates the conceptus except for the region of the ectoplacental cone. Initially, the yolk sac comprises an outer avascular parietal layer formed from the primary trophoblast giant cells and endoderm, and an inner vascularized visceral layer. Nutrients must diffuse through the parietal layer to be absorbed by the visceral layer. Later in pregnancy the parietal layer breaks down with migration of the giant cells, exposing the visceral layer directly to the uterine epithelium and forming an 'inverted' yolk sac (583).

The chorioallantoic placenta develops from both the ectoplacental cone and the extraembryonic ectoderm. The former gives rise to the spongiotrophoblast and glycogen cells of the junctional zone, and a second wave of giant cells (503). These secondary trophoblast giant cells invade along the lumens of the spiral arteries, and are therefore analogous to the endovascular extravillous trophoblast of the human placenta. The extraembryonic ectoderm gives rise to the trophoblast forming the labyrinth. At E8.5 the allantois attaches to the expanding extraembryonic ectoderm, bringing in mesoderm from which the fetal vasculature differentiates. Allantoic attachment stimulates folding within the ectoderm layer, initiating the formation of the trabecular network of trophoblast and maternal blood spaces. The genes and transcriptional networks regulating placental development in the mouse have recently been extensively reviewed (511, 566).

The fetus becomes dependent on the chorioallantoic placenta from E10.5, and hence gene mutations that severely compromise placental function cause embryonic lethality at this time. The placenta undergoes rapid growth, with weight reaching a maximum around E16.5 and plateauing, or even declining, thereafter (116). By contrast, peak fetal growth is seen around E18.5. As in the human, it appears that trophoblast proliferative potential is limited to early pregnancy, for progenitor cells positive for EpCAM, a marker of stemness, are not detectable within the labyrinth after E14.5 (538). However, stereological analyses reveal that the labyrinth continues to expand in volume until E16.5 and more slowly thereafter (116). This enlargement is associated principally with an increase in the volume of the maternal blood spaces and fetal capillaries. While the surface area of the maternal blood spaces reaches a maximum at E16.5, that of the fetal capillaries continues to increase until term, allowing for the possibility of adaptations

during late pregnancy. Continuing fetal placental angiogenesis is reflected in a progressive reduction in the thickness of the interhemal membrane, and consequently the theoretical diffusing capacity of the placenta rises until term (116). By the end of pregnancy, the conductance for oxygen in the murine placenta is approximately the same as in the mature human placenta (364).

By contrast, the volume of the junctional zone peaks at ~E16.5 due to an increase in both the number and mean cell volume of the spongiotrophoblast and glycogen cells, and then declines (115). The decline in volume towards term reflects the migration of the glycogen cells into the decidua, but this cannot account for the whole change and there may be additional cell loss through apoptosis.

C. The ovine placenta

1. The mature placenta

Morphologically, the placenta of the sheep is very different from those of the human and the mouse, although there are many functional similarities. The ovine placenta is of the cotyledonary type, comprising approximately 70 placentomes of 0.5 – 4.0 cm diameter in a singleton pregnancy (516). A placentome is formed when villous outgrowth creates a fetal cotyledon opposite a pre-existing non-glandular specialization, a caruncle, in the wall of the uterus (Figure 3E). Thus, placentomes are only formed at predetermined sites, and there is no villus regression as in the human. The fetal villi interdigitate with crypts in the maternal caruncle, and the complexity of branching increases with gestational age. Each cotyledon functions as an independent maternal-fetal exchange unit, and is therefore analogous to a single lobule of the human placenta.

Histologically, the maternal-fetal interface is also different. The trophoblast covering the fetal villi remains unicellular, and the cells are linked at their apices by tight junctions to form a columnar epithelium. There is no invasion by the fetal tissues comparable to that seen in the human and murine placentas, and the interface is formed by a microvillar interdigitation with the maternal tissues (Figure 3F). The exception is the migration of binucleate cells that arise in the trophoblast layer just prior to implantation, and form 15-20% of the layer throughout gestation (583). These cells migrate across the interface and fuse with the uterine epithelial cells to form localized plaques of maternal-fetal syncytium that are interspersed amongst the otherwise unicellular uterine epithelium (583). The placental interface in the sheep is therefore referred to as synepitheliochorial. The binucleate cells contain large numbers of dense granules that are immunoreactive for ovine placental lactogen (581). Their migration appears to be a way of delivering this hormone, and possibly other effectors, into the maternal circulation, where it plays an important role in early pregnancy by stimulating activity of the endometrial glands and the secretion of uterine milk (415).

Dense capillary plexuses are present within both the fetal villi and the maternal crypts. The fetal capillaries display sinusoidal dilations, as in the human, which may serve to reduce the vascular resistance (234). Nutrients and respiratory gases thus have to pass through six tissue layers; the maternal endothelium, maternal stromal tissue, the maternal-fetal syncytium, the trophectoderm, fetal stromal tissue and the fetal endothelium (Figure 3F). Diffusional exchange is facilitated by the invagination of the fetal capillaries into the trophectoderm, which along with the apposing syncytium is locally thinned, forming the equivalents of vasculo-syncytial membranes in the human

placenta. Exchange of glucose is aided by the presence of GLUT1 and GLUT3 that are expressed on different membranes (95, 582). Amino acid transporters have been characterized functionally *in vivo*, although not localized to individual cell layers (44).

In addition, there are two specialized accessory structures that contribute to nutrient transfer. Firstly, in the center of each placentome is a hemophagous zone where maternal blood is released by limited degradation of the uterine tissues, sequestered and then phagocytosed by the trophoblast cells (79). This is considered to be the principal pathway for the maternal-fetal transfer of iron. Since there is no evidence of circulation of maternal blood through these regions, they cannot be considered analogous to a hemochorial placenta. Secondly, openings of uterine glands are found clustered in the uterine wall between the placentomes. The trophoblast cells opposite are transformed from cuboidal to columnar, and form small elevations known as areolae (Figure 3E). Histotrophic secretions from the glands are endocytosed by the trophoblast, representing a pathway for the transfer of large proteins. The areolae reach their maximum diameter of ~3 mm in the last third of pregnancy although this decreases considerably towards term, most likely due to diminishing activity of the glands (578).

2. Development

As in the human and mouse, maternal diet during the periconceptional period can influence both the number of cells in the blastocyst and also the ratio between the cell lineages (299). This sensitivity is further evidenced by the impact that embryo transfer or assisted reproductive technologies has on subsequent ovine placental development, particularly its vascularization and expression of sex steroid receptors (462). Implantation is relatively later in the sheep than in the other species, and there is no

invasion of the maternal tissues. Consequently, the conceptus remains within the uterine lumen throughout the whole of pregnancy. After entering the uterus the blastocyst elongates rapidly, a response driven principally by the endometrial secretions. Their importance has been demonstrated by endocrine ablation of gland development in newborn lambs (227, 514, 515). Complete ablation results in a cessation of growth of the conceptus and loss of the pregnancy in the adult animal, whereas partial ablation leads to a small, non-expanded conceptus that fails to attach. Following expansion, trophoblastic papillae project into the mouths of the endometrial glands and immobilize the conceptus. Villous development starts opposite caruncles around days 24-26 post-fertilization, which corresponds to the timing of allantoic attachment with the chorion, as in the mouse (583). The number of cotyledons is fixed by about 5-6 weeks of pregnancy and does not increase thereafter. Each cotyledon does expand, however, reaching maximum weight at 80-90 days after which there is a decline due to loss of water and a reduction in the mesenchymal component of the fetal villi (516). Together, the caruncular and cotyledonary portions at each implantation site form 70-100 placentomes, which have been be classified into 4 different types on the basis of their gross morphology (545). The frequency distribution of the different placentome types changes with gestational age and sub-optimal environmental conditions, although functional significance of the different types and the mechanisms governing the shape changes remain unclear (198).

The surface area of the maternal-fetal interface increases in line with placental weight during gestation due to the increasing length and branching of the fetal villi. In addition, towards term the distal parts of the villi are thrown into folds that presumably generate further surface area for exchange, despite the decreasing placental weight (234).

Vascularization of the villi appears to increase continuously throughout pregnancy, although it occurs at twice the rate on the fetal as on the maternal side (54). On the maternal side the changes are predominantly in capillary diameter rather than number, whereas on the fetal side there is more branching angiogenesis. Consequently, total fetal capillary surface area increases, favoring exchange, although mean diameter is reduced, as in the mouse (54, 116). These contrasting patterns reflect different expression of angiogenic factors in the maternal and fetal tissues (54). The increase in vascularization is matched by exponential rises in uterine and umbilical blood flows throughout pregnancy, which are of critical importance for fetal growth (461).

V. EPIDEMIOLOGICAL ASSOCIATIONS BETWEEN PLACENTAL PHENOTYPE AND ADULT DISEASE

Low birth weight usually implies inadequate placental function, either as a primary or secondary cause. In recent years, an increasing number of relationships have been discovered between placental phenotypic features, such as its weight, length and width, and diseases in later life (Table 1). While correlation does not necessarily equate with causation, one possibility is that these gross morphological features are linked biologically to the functional capacity of the placenta. Alternatively, it may be that these features impose mechanical or other constraints on the developing embryo/fetus. Thus, there is accumulating evidence that the vascular arrangement of the early embryo plays an important mechanical role in regulating gene expression in the developing heart. Cells in the common ventricle and the outflow tract of the embryonic heart are sensitive to wall and shear forces (342, 442), and increases in these forces can lead to heart defects (135, 260, 379). The heart of the human embryo begins beating around day 21

post-conception. From then on, it is subject to the pulsatile pressures and flows generated by its own pumping action. At 6-8 weeks the heart has become 4 chambered, and the vitelline and allantoic circulations are increasingly perfused (Figure 4). These vascular beds offer mechanical resistance to blood flow, and there is increasing evidence that their inadequate growth promotes mechanical signals in the heart that result in structural defects (218, 260, 341, 379). We suspect that changes in the yolk sac and/or early placental vascular architecture underlie a broad spectrum of cardiovascular disorders, including a propensity for heart failure, but confirmatory data are not yet available.

A. **Placental Efficiency**

While fetal weight generally correlates with placental weight, the efficiency of the placenta, defined as the amount of fetal body mass accumulated per gram of placenta, is a key indicator of the offspring's resilience and susceptibility to chronic disease in later life (576). This index is a simple one to calculate in epidemiological studies, and encapsulates many different factors, such as placental exchange surface area, transporter density and activity, and blood flow rates, which would require more detailed individual stereological, molecular or physiological analyses. However, while it provides an overview of placental function, changes in the index provide no insight into whether specific activities have been stimulated or compromised, and, if so, the mechanisms involved. The physiological and endocrine regulation of placental efficiency has recently been reviewed (196). Efficiency, as estimated by this index, varies dramatically among species. The index is not related to the histological type of the placenta, but to the relative geometries of the maternal and fetal blood flows (137), emphasizing the importance of flow-limited transport. The human placenta is notably

1221 one of the least efficient on this basis, suggesting that other evolutionary selective
1222 pressures have been more important.
1223
1224 In the human, large placentas tend to less efficient than smaller ones (384, 480),
1225 suggesting adaptations, such as changes in transporter expression, have been successful
1226 in the latter. The risk of cardiovascular disease in adult men in relation to the birth
1227 weight to placental weight ratio does not follow a linear fashion as one might expect, but
1228 rather a "U" shaped pattern with heart-related death rates being lowest when placental
1229 weight is ~19% of birth weight (Figure 5) (25, 217, 361). The explanation for this
1230 relationship is not intuitively obvious, for it might be assumed that the more weight
1231 achieved per gram of placenta, the better the fetal outcome. However, it is possible that
1232 very small placentas are symptomatic of a severely compromised and constraining
1233 maternal-fetal supply line, and have a limited functional capacity (477). Large placentas
1234 on the other hand may imply that compensatory growth was stimulated by inadequate
1235 access to nutrients at key phases of development. This phenomenon is well known to
1236 farmers, who graze ewes on poor pasture after mating to stimulate placental growth,
1237 before placing them on good pasture during mid- to late-pregnancy. In doing so, they
1238 obtain larger lambs than ewes grazing on good pasture throughout (373). In the non-
1239 human primate, a reduction in placental mass can be compensated by expansion of the
1240 remaining placenta. However, this plasticity is lost as term approaches (466). When,
1241 and how, the human placenta is able to adapt to different nutritional conditions is poorly
1242 understood.
1243
1244 There is considerable variation in placental efficiency across the birth weight range, as
1245 demonstrated by the relationship between 17,000 birth weight-placental weight pairs

from deliveries in Saudi Arabia (11) (Figure 6). If the data are divided into quadrants based on the approximate median birth weight and placental weight then in the upper left quadrant, heavy babies were nourished by relatively light placentas, whereas in the lower right quadrant babies were light even though their placentas were at the heavier end of the scale. On the basis of the "U" shaped pattern described above, one might speculate that people born in these quadrants may have higher than average risks for chronic conditions in later life (217). It would be interesting to know whether these extreme quadrants have different sex ratios. On average, boys have heavier birth weights per gram of placenta (173), and thus would be expected to be more numerous in the upper left quadrant. By contrast, girls tend to make larger placentas for any given birth weight, and so may be more likely to populate the lower right quadrant. This remains to be tested.

There is increasing evidence that placental efficiency changes over time in any given population, and is likely to be influenced by the nutritional environment. The data from Saudi Arabia mentioned above showed that placental efficiency decreased significantly over a decade (11). This change was due solely to an increase in placental weight, which rose by more than 100g without a concomitant increase in birth weight. Studies in Mysore, India, suggest that maternal head circumference in conjunction with maternal fat mass predict placental efficiency (579). The former is related to the mother's early life nutrition, whereas the latter reflects levels of nutrition during adulthood. This finding suggests that the nutritional conditions across the life of a woman are highly influential in the establishment and growth of the placenta, and thus impact the lifelong health risks of her offspring. However, it has not been determined whether the optimal

efficiency associated with low cardiovascular death rates originally found in the UK population (Figure 5) applies to populations in Saudi Arabia and elsewhere.

B. **Placental shape and its influence on fetal development**.

The human placenta is generally described as discoid, but in large populations it has often been found on average to be slightly elliptical (435). Among ~6,000 placentas in the Helsinki Birth Cohort, the placentas were some 2.6 cm longer in one direction than in the other (172). Because the Helsinki data allow so many comparisons with a variety of diseases, it has become clear that the degree to which a placenta deviates from being perfectly round has a predictive value for specific diseases. Here, we define "length" of the delivered placenta as the longest dimension, and "width" as the longest distance measured perpendicular to the first. Assuming an elliptical surface, the average thickness can be estimated by the weight of the delivered placenta divided by the estimated surface area, the length × width × $\pi/4$ (172). A frequent finding is that although length and width correlate in any sample of placentas, the two measurements often have independent associations with fetal growth parameters as well as with postnatal disease. For example, simultaneous regression revealed that increasing ponderal index and the circumferences of the head, chest, abdomen and thigh among newborns are all highly associated with placental width; however, none are related to placental length (12). For each cm increase in placental width, birth weight increased by 125 g (95% confidence interval 88 to 162, $p < 0.001$) but only by 20 g for each cm increase in placental length (-13 to 53, $p= 0.2$). Mothers below the median height (157cm) had the strongest associations between placental width and neonatal body size.

The biological links among placental shape, size and function have not been defined. It has been proposed that different regions of the placenta have specific roles in nutrient transport, and that the placenta has a polarity related to the rostral-caudal axis of the early embryo (12, 298). However, there are no experimental data to substantiate this speculation. A more likely explanation is that placental shape is a powerful proxy indicator of processes in placentation that are related to its transport and physiological functions. As described in section IV.A.2, placental shape may reflect the site of implantation, or events taking place during the transition of the early placenta to its definitive form. For the former, implantation at different sites within the uterus, such as on the anterior or lateral walls, close to the cervix or in the fundus, may lead to variable shapes depending on whether the uterus expands symmetrically during later pregnancy or preferentially in certain dimensions. If uterine vascularity is different at these implantations sites, in terms of the density of the arcuate and spiral arteries, then placental blood flows, and hence functional capacity, will correlate with placental shape. For the latter, excessive villous regression at the time of onset of the maternal circulation may be indicative of deficient extravillous trophoblast invasion (77), which may mean that the remaining spiral arteries are not fully remodeled, compromising placental blood flow.

The lack of certainty over the role of placental shape in driving biological function is based on a paucity of basic data detailing how the placenta actually grows, and how shape is determined. Placental growth patterns measured over intervals across gestation are needed, and this may be possible with the advent of high-resolution ultrasound from which volumetric and shape data can be obtained. Once the mechanisms are known, it should be possible to determine how maternal diet, body type

and lifestyle alter the placenta over its lifespan. We also need more detailed information on the regional distribution of spiral arteries, and how the uterus and placenta co-expand during pregnancy. The visualization of placental oxygenation, metabolism and nutrient transport in real-time would further illuminate the relationships between shape and placental function, and may become possible through magnetic resonance imaging techniques. At present, data show that elliptical placentas tend to be less efficient than circular ones (590), but more research is needed to understand the underlying biology.

C. Placental inflammation

A number of maternal conditions, both infectious and non-infectious, have been linked to placental inflammation (102, 453). Elevated levels of placental inflammation have been associated independently with slower post-natal growth in pre-term infants, and so may have long-lasting effects for adult health (377).

It is becoming increasing clear that standard definitions of inflammation do not fit the findings in the placenta in non-infectious cases, such as obesity and diabetes, for it is possible for inflammatory pathways to be activated without the classic infiltration of granulocytes (418). Recently, new definitions have been applied to chronic inflammatory states in adult tissues (87). The basis for this reappraisal is the recognition that the same signaling cascades that are activated in classical inflammation in response to pathogens, including activator protein 1 (AP1), NF-κ B and interferon regulatory factors (IRFs), can be stimulated by cytokines arising from a variety of metabolically active tissues, such as adipose tissue, muscle and liver, and their resident immune cells in response to excess nutrients (228, 567). The outcome has been termed

metaflammation, or 'cold, smoldering inflammation' as it is characterized by its chronicity. Since the tissues remain in an anabolic state, there is the capacity for tissue remodeling and gradual metabolic deterioration over time (87).

It is likely that the stressors known to lead to fetal programming, including insufficient or excess nutrition, social stress and hypoxia, can lead to metaflammation in the placenta. Indeed, the same inflammatory pathways can be activated through the signaling cascades of the unfolded protein response (UPR) (228, 609), as will be discussed later. These cascades are activated in placentas from cases of growth restriction and early-onset pre-eclampsia (595, 596), but no data are yet available for pregnancies complicated by maternal obesity or other metabolic disorders. A sterile inflammatory state can also arise through senescence, when cells adopt the senescent-associated secretory phenotype (SASP) and release pro-inflammatory cytokines and proteases (425). Senescence has only recently been considered a potential feature of the syncytiotrophoblast in human placenta (220). While it may be part of the normal aging process, the fact that it can be induced by chronic stress, including oxidative and endoplasmic reticulum stress, suggests it may be more prevalent in complicated pregnancies.

Although the intermediate molecular mechanisms remain uncertain, we speculate that metaflammation is present in the human placenta in pregnancies complicated by malperfusion or maternal metabolic disorders (Figure 7). It may mediate programming of the fetus by either adversely affecting placental function, or by causing the release of pro-inflammatory cytokines into the fetal circulation.

D. Specific examples linking placental phenotype to chronic disease

Here, we explore associations between placental phenotypes and adult chronic diseases resulting from epidemiological studies. In each case the findings have been corrected for known confounders. Epidemiological studies are unable to separate cause from effect, but these associations provide the opportunity to investigate the underlying biology.

1. Hypertension

Most of the epidemiological data related to hypertension have arisen from studies of large cohorts followed from birth to the present day. One such is the Helsinki Birth Cohort that comprises 13,345 men and women born during 1934-1944. Among the 644 hypertensive subjects, treatment for hypertension was associated with low placental weight and surface area (39). Birth weight is linked to maternal body size and composition, as well as to growth of the placenta. When taking maternal characteristics into consideration, the associations were strongest among mothers whose stature was below average height (160cm), or who were of low socioeconomic status (Figure 8). This suggests an interaction between the role played by the placenta and the nutritional state of the mother during her early development. Among these shorter women, the prevalence of hypertension fell from 38% if the placental area was 200 cm^2 or less, to 21% if the area exceeded 320 cm^2 ($p=0.0007$). Poor maternal nutrition may exaggerate the adverse effects of small placental size on fetal development, possibly by restricting compensatory mechanisms.

Among men who were exposed *in utero* to the starvation effects of the post-war famine in Holland, a reduced width of the placenta was associated with hypertension (471). The surface area of the placenta also predicted hypertension with an odds ratio of 1.34

(95% CI 0.99 to 1.80) for an increase in surface area of 40 cm^2. However, hypertension was predicted by a short placental width and a more oval shape in men who were born after the war (471, 541).

There are also effects of placental development on blood pressure in children. A study of placentas of children in the longitudinal Alspac study of 13,971 births in Bristol UK, showed the number of lobes on the maternal surface was related to the blood pressure of the children at age 9 years (24). Increasing lobe number (range 4 to 40) was associated with higher blood pressures in both boys and girls. The larger the surface area of the placenta, the more lobes it contained and the heavier the birth weight of the offspring. However, among boys, the number of lobes was directly associated with higher systolic and diastolic pressure, but not with an increased pulse pressure. In that group, diastolic pressure rose by 2.2 mmHg (95% CI 0.6 to 3.7, p =0.007) for every 10 additional lobes. A greater number of lobes was associated with higher systolic pressure and pulse pressure, but not with higher diastolic pressure, in girls. Pulse pressure rose by 2.7 mmHg (1.1 to 4.3, p<0.001) for every 10 additional lobes. Adjustment for placental surface area, a powerful determinant of hypertension in adult men, did not change the relationships. Although we do not understand how lobation of the human placenta arises developmentally (49), these data are fascinating for two reasons. Firstly, since septae are first observed projecting from the basal plate as early as six weeks of gestation, they may represent an informative proxy marker of early events in placental development that are of physiological consequence. Secondly, they complicate the role of placental area as a predictor of adult hypertension, for the number of lobes is the more powerful index.

2. Heart Failure

Returning to the Helsinki Birth Cohort, 187 patients were taking medications for chronic heart failure. Their disease was associated with a small surface area of the delivered placenta, and the odds ratio for chronic heart failure was 1.7 (1.1–2.5) in men and women born with a placental area <225 cm^2 compared those with an area >295 cm^2 (27). There was no relationship with placental weight. As with hypertension, the relationships were strongest among women of below median stature (Table 2), suggesting a link with the mother's own nutritional state during her fetal and childhood development.

In a separate study of adult men, concentric enlargement of the left ventricle, a known predictor of coronary heart disease, was found to be associated with low weight at 1 year (548). It was speculated that this was due to altered hemodynamics during the fetal period, or a persisting elevation of growth factors. Taken together, these data suggest that chronic heart failure in adult life may be initiated by impaired placental growth, which subsequently adversely affects cardiac development. In addition, people born with a vulnerable heart are more likely to develop chronic heart failure if they become insulin resistant (27).

3. Coronary Heart Disease

Numerous studies have shown a relationship between cardiovascular disease and restricted growth before birth (5, 182, 428). However, the finding that the placenta is a more powerful driver of coronary heart disease in men (182) than in women (175) is a recent discovery. A study of 6975 men in the Helsinki Birth Cohort found that three different placental phenotypes were associated with disease of the coronary arteries

(175). In the first, an increasing difference between the length and width of the placenta predicted the disease in the offspring of primiparous mothers who were of below median height. The hazard ratio for each cm in difference between length and width was 1.14 (95% C.I. 1.08–1.21, P = 0.0001). The second placental phenotype was a small surface area of the delivered placenta. In tall mothers whose body mass index was above the median, a 40 cm^2 decrease in surface area was associated with a hazard ratio of 1.25 (1.10–1.42, P =0.0007). The third phenotype was found also in tall mothers, but only those whose body mass index was below the median. In these mothers, the hazard ratio was 1.07 (1.02–1.13, P = 0.01) per 1% increase in the placental weight/birth weight ratio. These data suggest an interaction between a mother's body size and composition and placentation that leads to a particular capacity for nutrient exchange and efficiency. Coronary heart disease was associated with a low ponderal index (birth weight/$length^3$) in all groups, suggesting that the fetuses were all undernourished because of poor placental growth.

Whereas placental size usually correlates with maternal body size, coronary heart disease in men was associated with a small placenta in tall mothers. This suggests poor placentation and poor placental growth throughout pregnancy. It was placental inefficiency that predicted the disease in the third group. In this group, thin tall women had large placentas in proportion to the size of the baby at term. In these women, placenta growth may have been stimulated by poor maternal nutrition at mid-gestation.

4. Sudden Cardiac Death

Because it is not possible to study the living hearts of people who have died suddenly from cardiac causes, it has been very difficult to pinpoint the electrical properties of the

myocardium in hearts whose contractility suddenly becomes inadequate to sustain life. Nonetheless, aberrant functioning of the autonomic nervous system is considered the most common explanation for sudden cardiac death (201). While it is known that sympathetic tone may be exaggerated in people who had low birth weight, the links among maternal, placental, and fetal growth have only recently been explored through the Helsinki Birth Cohort. Sudden unexplained cardiac death outside hospital was associated with a thin placenta, and for each gram/cm^2 decrease in thickness the hazard ratio was 1.47 (95% C.I. 1.11–1.93, P=0.006) (30). A high placental/birth weight ratio also predicted sudden death among women, but not men. The determinants of placental thickness are not fully understood, but if the theory that abnormal autonomic function underlies sudden death holds then one might suspect a relationship between the nutritional function of a thin placenta and development of the autonomic nervous system during fetal life.

5. Lung and Colorectal Cancers

Susceptibility to developing cancer on exposure to carcinogens, such as those in tobacco smoke, differs among individuals. Through a combination of the Helsinki Birth Cohort with an older cohort born in 1924–1933, the smoking history was known for 6,822 men and women, of which 385 developed lung cancer by 2010. The cases were characterized by having a short mother and a high ponderal index (weight/length^3) at birth, and the delivered placenta had either a small or a large surface area in three separate phenotypes (38). It was suggested that in each phenotype, low amino acid transport but normal glucose transfer was reflected in a newborn that was short in relation to its weight. These data indicate that both large and small placentas can limit the flow of

nutrients, and that poor placentation occurs more often in women who are short in stature.

In the combined Helsinki cohorts, 275 had colorectal cancer (36). The risk for acquiring the disease increased as the placental surface became longer and more oval. Among people in whom the difference between the length and breadth of the surface exceeded 6 cm, the hazard ratio was 2.3 (95% CI 1.2–4.7) compared with those in whom there was no difference. Colorectal cancer was unrelated to other placental measurements or to body size at birth. Thus, colorectal cancer had a graded association with placental elipticity.

VI. OXYGEN SENSING BY PLACENTAL CELLS

Placental cells, and in particular the trophoblast, are metabolically highly active due to their multiple functions. Thus, it has been estimated that the placenta accounts for ~40% of the oxygen consumption of the fetal-placental unit, and of that ~33% is utilized in active transport and ~33% in protein synthesis (97). It is therefore to be expected that placental cells are sensitive to oxygen availability. There are a number of potential pathways by which cells may sense the prevailing oxygen concentration (Figure 9), and there is evidence of considerable interplay between them (180, 324, 562). Given that oxygen is central to cell metabolism some of these pathways also overlap with those sensing energy levels and nutrient supply. Here, the respective pathways will be considered according to their principal function. Equally, activation of the two sets of pathways causes, to a large extent, a common outcome, for under conditions of hypoxia or nutrient deprivation there is a need to conserve energy reserves by stimulating glycolysis and suppressing non-essential protein synthesis. Responses to hypoxia

generally begin when the oxygen concentration falls below a cell's critical threshold that switches aerobic oxygen-regulated metabolism to anaerobic oxygen-conforming metabolism (225). That threshold is likely to be different for different cell types within the placenta dependent on their metabolic activity and other factors, but no data are available as to what the precise values might be. For most primary or transformed mammalian cells it is within the range of ~0.15 - 1.5% oxygen (225). Equally, although it is commonly asserted that the placenta is hypoxic in pathological states, such as pre-eclampsia, no measurements have been made *in vivo* to confirm whether or not this is the case. These claims must therefore be treated with caution.

There are a variety of signaling mechanisms that are activated in response to hypoxia, as follows.

A. Transcription factors

Central to oxygen sensing in any cell is the family of hypoxia-inducible basic Helix-Loop-Helix transcription factors, the HIFs, of which there are three members, HIF-1-3 (297). All members consist of an alpha and beta subunit, and it is the former that is oxygen dependent. In well-oxygenated conditions this sub-unit turns over rapidly and does not accumulate, whereas under hypoxia it is stabilized and the two subunits are able to combine to form an effective transcription factor. This binds to hypoxia response elements on a wide range of genes, the most important of which in the current context include those encoding VEGF, glucose transporters and glycolytic enzymes. It can also inhibit mTOR signaling, and hence regulate protein synthesis. The actual oxygen sensors are the prolyl-4-hydroxylase (PHDs) enzymes that have an absolute requirement for molecular oxygen and hydroxylate conserved proline residues on the alpha subunit.

This enables binding of the von Hippel-Lindau protein (pVHL), which targets the subunit for ubiquitination and subsequent degradation (493). HIF-1 thus provides a very rapid mechanism for responding within seconds or minutes to acute changes in oxygenation, whereas HIF-2 is thought to mediate longer-term adaptations to relatively modest changes (444).

Animal studies have confirmed that HIFs are expressed at the blastocyst stage of development (243), and that they are essential for normal placental development. Knockout in mice results in failure of allantoic attachment with the chorion, impaired vascularization in the labyrinth, and abnormal differentiation of the trophoblast sub-populations (161). Consistent with these effects, HIF-1 and HIF-2 have been immunolocalized in the human placenta to the trophoblast and endothelial cells throughout gestation (447). There has been considerable interest in the importance of HIF signaling during the first trimester when the intraplacental oxygen concentration is relatively low. Ontogenetic studies have reported contrasting results, with peaks of HIF-1α at 7-10 weeks and then again at 14-18 weeks (269), or a steady decline in HIF-1α from 5-8 weeks to 18-21 weeks and a rapid decline of HIF-2α between 5-8 weeks and 9-11 weeks (447). Interpretation of these data is difficult given that it is now realized that HIFs can be stabilized by factors other than hypoxia, including reactive oxygen and nitrogen species, angiotensin, growth factors and cytokines (433, 444), many of which are changing during early pregnancy. Another potential confounder is stress induced on the tissues during collection, especially when this is performed by curettage when they are inevitably mixed with maternal blood (112). It is notable that HIF-1 and HIF-2 are undetectable in first trimester samples collected by a chorionic villus sampling (CVS) technique and processed immediately (112). Thus, it is unlikely that HIF signaling plays

a significant role under the steady state, low oxygen conditions that prevail during the first trimester. This is supported by the observation that the ATP/ADP ratio is the same during the first and early second trimesters and at term (112). The tissues are therefore not energetically compromised, most likely due the high activity of glycolytic metabolism (284). In addition, the intraplacental oxygen concentration of ~2.5% during the first trimester exceeds the evolutionary conserved range of maximal HIF activity of 0.5 - 2.0% oxygen (225, 286).

There is no doubt, however, that the HIF signaling machinery is competent in the human early placenta and can respond to acute changes in oxygenation. Experiments on first trimester placental explants cultured at 3% oxygen compared with 21% indicate that HIF regulates trophoblast proliferation, migration and invasion through its actions on TGFß$_3$ (89). Equally, HIF-1 and HIF-2 can be stabilized in CVS samples by stress and activate downstream targets, including VEGF (112).

In later pregnancy, natural selection acting on HIF-targeted or –regulatory genes has been implicated in mediating placental adaptations to the chronic hypobaric hypoxia experienced at high altitude (388). Increased levels of HIF-1α mRNA and protein have been reported in healthy placentas from pregnancies at 3,1000 m, in association with elevated TGFß$_3$, suggestive of stimulation of HIF-mediated pathways (606). Another study of placentas from the same region found enhanced vascularization, consistent with a hypoxic response, but paradoxically HIF-DNA binding was less than in the low-altitude controls (533). Analysis of the placenta after delivery only provides a single snapshot, however, and this finding may reflect successful adaptations earlier in pregnancy, for these placentas showed no evidence of oxidative or glycolytic stress. An

excessive hypoxic response may account for the increased incidence of chorangiomas in placentas from altitudes greater than 4,000 m (459, 510), but no data are available as to whether this is HIF-mediated..

Aberrant HIF signaling has been implicated in the pathophysiology of pre-eclampsia, in particular of the early-onset form when the PHDs do not appear to sense oxygen (90, 448, 467), and may be responsible for the abnormal placental secretion of angiogenic regulatory factors that is thought to precipitate the clinical syndrome (413).

A number of other transcription factors and co-activators have been identified that respond to changes in the redox potential of a cell rather than to oxygen directly. These include AP-1, CREB, Mash2, NFκB, p53, PCC-1α, SP-1 and STAT3 (16, 106, 155, 277). Often, activation involves conformational changes secondary to formation of disulfide bonds, and so responses can be rapid. While some of these pathways have been implicated in stress responses, others are involved in trophoblast proliferation and differentiation, and secretion of extracellular matrix.

B. Epigenetics

1. Non-coding RNAs

A large number of miRNAs have been identified from the field of cancer biology as being regulated by hypoxia, and mediate events such as cell proliferation, differentiation, invasion and metastasis that are relevant to placental biology (109, 499). Many of these are miRNAS are regulated by HIF, but others are HIF-independent. The human placenta expresses a wide variety of non-coding RNAs (398), but few data are available regarding their responsiveness to hypoxia. Most attention has focused on mir-210, which is HIF-

dependent. It is increased in normal placentas from pregnancies at high altitude (121), and in placentas from pregnancies complicated by pre-eclampsia (272, 351, 401, 611). MiR-210 has a number of targets that are relevant to the placenta and developmental programming. Within mitochondria it targets regulatory proteins that assist in the assembly of the complexes of the electron transport chain, and suppresses respiration (103, 108). Consistent with this action, the complexes are reduced in these high-altitude placentas (121), as is the ATP/ADP ratio (534), suggesting energetic compromise that could adversely affect transport and synthetic activities of the organ. Furthermore, transfection of trophoblast cells with miR-210 reduces respiration and oxygen consumption (401). Besides mitochondria, other targets identified for miR-210 include the steroidogenic enzyme hydroxysteroid (17-ß) dehydrogenase 1 (272), ephrin-A3 and homeobox-A9 which are involved in trophoblast cell migration and vascular remodeling (611), and thrombospondin type 1 domain containing 7A in the placental vasculature (351).

Other micro-RNAs identified as being differentially expressed under hypoxia include miR-93, miR-205, miR-224, MiR-335, MiR-424, miR-451 and miR-491 (396, 397), and mIR-34a (154), but little is known regarding their functional significance at present.

2. mRNA stability

Transcript levels are determined by both the rate of transcription and the rate of mRNA degradation. Stability of mRNAs is regulated by association with specific binding proteins or with micro-RNAs, and can be influenced by hypoxia. A notable example is the mRNA encoding Angiopoietin-1, which becomes less stable under low oxygen conditions, shifting the balance of angiopoietin-1:angiopoietin-2 in favor of

angiopoietin-2 and vessel growth (608). By contrast, the half-life of the mRNA encoding VEGF is more than doubled under hypoxic conditions (334), again favoring angiogenesis. Such effects could contribute to the increased placental angiogenesis observed under hypoxic conditions.

3. DNA methylation and histone modifications

Hypoxia can potentially impact on DNA and histone methylation since the demethylase enzymes are dioxygenases, and so require oxygen and 2-oxoglutarate for their activity (501). Hence, there is the possibility of changes in chromatin structure in response to hypoxia. The significance for the placenta is still unknown, although it is well recognized that nuclei within the syncytiotrophoblast exhibit contrasting patterns of chromatin and different epigenetic states (187). Nuclei that display particularly condensed chromatin aggregate in syncytial knots and are transcriptionally inactive (188). Whether more subtle changes regulate gene expression under different environmental conditions awaits investigation.

Methylation of the DNA represents another level of control, affecting promoter availability. Methylation changes in response to intermittent hypoxia in experimental animals have been reported (66), and the realization that 5-hydroxymethylcytosine, an oxidation product of 5-methylcytosine, plays an important role in regulating transcription raises further possibilities for gene-environment interactions. The heavily condensed nuclei within syncytial knots stain particularly strongly for 5-hydroxymethylcytosine (187).

Epigenetics is a rapidly expanding field, and there are now many reports of changes in placental DNA methylation and histone proteins in response to environmental cues that have been associated with developmental programming of the offspring (203, 417, 522). Interpreting the significance of these findings is difficult at present, since they are based on analysis of placental homogenates. It is therefore impossible to determine in which tissue the changes have occurred, be it trophoblast, immune cell or vascular endothelium. It is also impossible to assess whether the changes have functional significance for placental transport or hormone synthesis, or whether they are just epiphenomena. The situation may be resolved as the technology advances, enabling methylation studies to be performed on single cells or laser-capture microdissected tissues, allowing more specific analyses to be undertaken.

C. Mitochondrial pathways

As the principal site of oxygen consumption within cells, mitochondria likely play an important role in oxygen sensing. There are close interactions between mitochondria and HIF signaling that operate on a number of levels, not least because mitochondria and the PHDs compete for molecular oxygen (523). Therefore, one or the other may be favored depending on the precise concentration. In addition, the metabolic intermediate 2-oxoglutarate generated in the tricarboxylic acid cycle is a co-factor regulating PHD activity (48). Another means by which the pathways may interact is through reactive oxygen species (ROS). Mitochondria are the principal source of ROS, and production is stimulated under both hypoxic and hyperoxic conditions. Leakage of electrons from the complexes of the electron transport chain, in particular complexes I and III, generates the superoxide ion, which is then converted to hydrogen peroxide. Being non-polar hydrogen peroxide diffuses out of the organelle and can stabilize HIFs (48). In addition,

it will influence the redox potential within the cytoplasm, and contribute to activation of other redox-sensitive transcription factors. It is notable that complex I and III are downregulated at the protein level in the placenta at high altitude, which may be interpreted as an adaptation to reduce production of ROS and so limit HIF signaling (121). This may explain the reduced HIF-binding reported in these placentas (533).

D. Unfolded protein response

The unfolded protein response (UPR) is a set of three evolutionary conserved pathways whose primary function is to maintain homeostasis within the endoplasmic reticulum. However, they are now recognized as being a point of convergence of cell responses to a variety of stimuli, including hypoxia. Activation occurs generally at oxygen levels below those regulating HIF (225), and although the precise sensing mechanisms are not known there are two main possibilities. Firstly the protein disulfide isomerase enzymes that facilitate formation of disulfide bonds during folding of nascent proteins have a requirement for molecular oxygen as an electron acceptor. Second, the protein folding machinery is dependent on a high concentration of Ca^{2+} ions within the lumen of the endoplasmic reticulum that is maintained by SERCA pumps in the membrane. Folding capacity may therefore be compromised if oxygen or ATP concentrations are limiting, but this is likely to be a secondary mechanism (315). The accumulation of misfolded proteins will activate the three pathways; PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring protein 1 (IRE1). PERK selectively suppresses formation of non-essential new proteins by phosphorylating eIF2α (eukaryotic initiation factor 2 sub-unit alpha) and blocking cap-dependent RNA translation. It also upregulates the transcription factor ATF4, which along with the ATF6 and IRE1 pathways selectively regulates gene expression to assist the cell to tolerate

hypoxia (180, 343, 585). These adaptations include increased amino acid transporter activity and enzyme expression to boost the concentration of glutathione, the principal intracellular antioxidant, stimulation of hematopoiesis, and upregulation of the angiogenic growth factor VEGF. However, it is now appreciated that these transcription factors have broader targets (2), and, for example, the UPR has been implicated in regulating processes as diverse as the inflammatory response (609) and stemness within the intestinal epithelium (251).

Manipulations in mice have confirmed that the IRE1 pathway is essential for normal placental development, for knockout leads to downregulation of VEGF and abnormal angiogenesis within the labyrinth (273). Mild activation of the UPR, with phosphorylation of eIF2α alone, has been reported in the high-altitude human placenta, where it may mediate homeostatic adaptations to the hypobaric hypoxia experienced (598). More severe activation is seen in human placentas from growth-restricted pregnancies (596), and particularly in cases of early-onset pre-eclampsia (595). These findings can be replicated in trophoblast cell lines by exposure to hypoxia-reoxygenation, with activation of the three pathways being dependent on the severity of the stress (596).

The regulator eIF2α can be phosphorylated by at least three other kinases besides PERK. One of these is GCN2, which is activated by the presence of uncharged tRNAs (153). Hence, if either oxygen or amino acids are in short supply protein synthesis is suppressed by a common mechanism.

E. Ion channels

Since ionic pumping is energy dependent, a number of ion channels are sensitive to the prevailing oxygen concentration. Most data have been derived from the carotid body, but may also be applicable to the placenta. The proximal sensor is still uncertain, but two main theories have been proposed, the mitochondrial and membrane models (324). In the former, it is proposed that under hypoxia the accumulation of ROS leads to opening of the mitochondrial permeability transition pore and the efflux of Ca^{2+} from the mitochondrial endoplasmic reticulum complex. In the latter, there are various K^+ channels that are influenced by hypoxia, including voltage-gated K^+ channels, Ca^{2+}-activated K^+ channels, and ATP-sensitive K^+ channels (308, 347). Suppression of these channels under hypoxia is thought to lead to membrane depolarization and influx of extracellular calcium through voltage-gated calcium channels. In addition, transient receptor potential (TRP) channels that are responsive to ROS have been identified in a number of cell types, and provide another route of entry for calcium (589). The end result of all these pathways is a rise in intracellular calcium, which at physiological levels can regulate transcription of a number of genes that assist in adaptations to hypoxia, including those encoding the ion channels themselves (347). Excessive calcium influx can lead to activation of apoptotic and necrotic cell death, dependent on the severity.

A variety of K^+ channel subtypes, many of them oxygen sensitive, are present in the vasculature of the human placenta (307, 563, 564). Hypoxia-induced fetoplacental vasoconstriction, equivalent to that seen in the pulmonary circulation, has often been proposed but never proven (563), but could assist in matching flow in the two placental circulations. Voltage-dependent K^+ channels have also been immunolocalized to the syncytiotrophoblast, cytotrophoblast and some stromal cells in the first trimester and

term placenta (382, 383), and so they may play a broader role in placental biology, including regulating the secretion of hCG (574). In addition, Ca^{2+}-activated K^+ channels have recently been demonstrated to be important for trophoblast syncytialization and for syncytial volume homeostasis (145). The significance of these findings for the expansion and functional well-being of the syncytiotrophoblast under different environmental conditions awaits confirmation.

F. Gasotransmitters

Allied to the functions of these ion channels are the gasotransmitters, and in particular hydrogen sulfide. Increasing evidence suggests that this evolutionary ancient gas can act as an oxygen sensor (423, 436). Hydrogen sulfide is generated from cysteine by two enzymes cystathionine-γ-lyase (CSE) and cystathionine-ß-synthase (CBS). It is notable that expression of *CSE* is induced by the UPR through the PERK pathway and ATF4 (146), illustrating crosstalk between these pathways. Hydrogen sulfide is metabolized principally in the mitochondria by oxidation to thiosulfate, but if oxygen availability is limited then the concentration in the cytosol will increase (423). There, it can activate ATP-sensitive K+ channels with all the downstream consequences of hyperpolarization. In addition, it can scavenge ROS and so act as a defense against ischemia-reperfusion injury (310), affect the redox balance, and also inhibit a number of related enzymes, including NADPH oxidase and nitric oxide synthase.

CBS and CSE have been immunolocalized to the syncytiotrophoblast and also to the smooth muscle cells surrounding the stem villus arteries in the human placenta (111, 262). Perfusion experiments *ex vivo* have demonstrated that hydrogen sulfide is a powerful vasodilator of the fetal placental vasculature, and that its effects are mediated

through actions on both ATP-sensitive K^+ channels and nitric oxide (111). The functional significance of the gas is reflected in the observation that downregulation of CSE is associated with evidence of increased resistance within the umbilical circulation as assessed by Doppler waveforms. Its role in trophoblast biology has not yet been explored, although it is likely to impact on the redox potential within the cytosol. Inhibition of CSE activity also inhibits trophoblast invasion from first trimester explants *in vitro*, and increases the release on anti-angiogenic factors from umbilical vein endothelial cells (559). It has been proposed that dysregulation of placental CSE activity may contribute to the syndrome of pre-eclampsia (559).

VII. NUTRIENT AND ENERGY SENSING BY PLACENTAL CELLS

In the same way that placental cells are sensitive to oxygen due to their high proliferative and metabolic rates, they are also sensitive to variations in the nutrient supply. The available evidence suggests that they utilize pathways common to most mammalian cells, in particular the mTOR/AKT and the AMPK pathways (Figure 10). These networks combine with the UPR to regulate protein synthesis over the short and long term periods to promote cell survival.

D. mTOR/AKT pathway

The mTOR (mechanistic/mammalian target of rapamycin) complex integrates signals from a diverse array of pathways, including oxygen- and nutrient-sensitive sensors. As it is central to the control of cell proliferation, the complex also has strong input from the AKT (protein kinase B) pathway that transduces growth factor stimulation. These pathways will therefore be considered together (Figure 10).

mTOR comprises two major complexes, mTORC1 and mTORC2, which share the same core kinase but have the different adaptor proteins, raptor and rictor respectively. mTORC1 has the most direct effect on cell proliferation due to its actions on cap-dependent RNA translation mediated through phosphorylation of the binding protein 4E-BP1, and the ribosomal protein S6. In its non-phosphorylated state, 4E-BP1 modulates formation of the ribosomal complex (246), while activation of S6K1, (p70 ribosomal protein S6 kinase 1) has multiple actions. These include phosphorylation of S6, a component of the 40S sub-unit, of regulators of translation elongation (560, 561), and of the insulin receptor substrate (IRS) 1, creating a negative feedback loop that limits signaling through the insulin/PI3K/AKT pathway (Figure 10). Conversely, mTOR inhibits autophagy and other catabolic processes by promoting the ubiquitination of ULK1 (410). Hence, stimulation of mTORC1 promotes protein synthesis, increases cell mass and leads to cell proliferation and growth.

mTORC1 is regulated by amino acid availability, although for many years the mechanism has been uncertain. Early experiments revealed that withdrawal of amino acids, in particular leucine, led to inhibition of mTOR, growth restriction and the stimulation of autophagy. It was also clear that the amino acids acted independently of insulin, and so were not sensed through the insulin/PI3K pathway. More recent research has identified a Ragulator-RAG GTPases multiprotein complex associated with the lysosomal surface that regulates mTOR activity by controlling its sub-cellular location, recruiting it to the lysosome (164). The membrane-resident amino acid transporter SLC38A9 is a key component of this sensing machinery (452).

Glucose availability also regulates mTORC1, with accumulation of ADP and AMP under conditions of energy shortage stimulating AMPK (AMP-activated protein kinase). AMPK has a direct inhibitory action on raptor, the adaptor protein for mTORC1, and can also stimulate the tuberous sclerosis complex TSC1/2 (164). TSC1/2 is a major upstream regulator of mTORC1, suppressing activity under stress conditions. This complex plays a major role in integrating insulin and growth factor signaling through the PI3K and AKT pathway (Figure 10), although recent data suggest growth factors may also stimulate mTORC1 by enhancing the delivery of amino acid-laden macropinosomes to the Ragulator complex (593). TSC1/2 is also responsive to hypoxia through the actions of REDD (70). The latter involves phosphorylation of HIF-1α (88), illustrating again the overlap between these pathways. TSC1/2 also acts on mTORC2, which in turn regulates the activity and substrate specificity of AKT through phosphorylation (597).

AKT is a serine/threonine protein kinase that has a wide range of targets, but the most important for cell growth are TSC1/2 and glycogen synthase kinase 3β (GSK-3β). The latter plays a major role in glucose homeostasis, and is likely to be key to the deposition of glycogen in the human extravillous trophoblast cell and the murine glycogen cells. It is also a major regulator of protein synthesis through its actions on eIF2B (568).

The involvement of mTOR/AKT signaling in the regulation of placental growth has only recently been addressed, but there is evidence that it is of key importance from the earliest stages. mTOR/AKT signaling is essential for maintenance of embryonic and hematopoietic stem cells (419), and the same is likely to be true for trophoblast. Indeed, treatment of mouse blastocysts with rapamycin or knockout of *mTOR* causes lethality at E5.5 associated with a failure of trophoblast outgrowth and maintenance of stem cells in

the inner cell mass (206, 360). Disruption of just *Akt1* causes placental and fetal growth restriction in the mouse, with a particularly severe effect on the development of the glycogen cells (592). Equally, a reduction of AKT and mTOR at the protein level, and reduced phosphorylation of mTOR, TSC1/2, 4E-BP1 and GSK-3β have been reported in growth restricted human placentas associated with maternal vascular compromise, but there was no effect on S6Ks and eEF2K (596). Reduced p-S6K1, but no change in p-4E-BP1, was observed in placentas with growth restriction of unknown origin (468). By contrast, increased placental mTOR signaling is associated with large for gestational age babies delivered by obese women (279). *mTOR* expression is also inversely correlated with levels of maternal exercise, and total sugar content in her diet (60).

The effects of diet on placental mTOR signaling have been more fully explored in animal models. Evidence from downstream signaling indicates that nutrient restriction leads to reduced mTOR activity, along with reduced insulin and AKT signaling, in rats fed a low protein diet (470), and mice fed 80% of the control *ad libitum* diet (495), as might be expected. However, no effect was observed at mid- to late-gestation in sheep fed 50% of the control diet (353). Data arising from over-nutrition models have been more conflicting. Thus, whilst an obesogenic diet has been shown to cause activation of mTOR in rat placentas (205), the opposite was found following over-nutrition in sheep (150% of control diet) and mice fed an obesogenic diet (323, 615).

Nonetheless, it seems reasonable to conclude that the mTOR/AKT pathway plays an important role in matching placental growth to the available nutrient supply, an idea originally proposed a decade ago (569). Indeed, a linear relationship between placental mTOR activity and birth weight has been found across a wide range of maternal body

mass index (279). In addition, the mTOR pathway regulates the activity of system A, system L and taurine amino acid transporters in the placenta at the post-translational level, either through modifications or by influencing translocation to the apical membrane (469). Thus, activity of system A, but not system L, transporters in the apical membrane of the syncytiotrophoblast correlates positively with birth weight, and may contribute to fetal overgrowth in cases of maternal obesity (279).

The mTOR/AKT pathway thus plays a central role in modulating anabolic and catabolic pathways in response to fluctuations in the nutrient and oxygen supply reaching the placenta. Such fluctuations may arise from either variations in maternal diet or compromise of utero-placental blood flow secondary to deficient trophoblast invasion. By regulating the activity of amino acid transporters, the pathway will also be pivotal in integrating the maternal supply and fetal demand signals that underpin resource allocation between the mother and her fetus. Genes encoding components of the mTOR and protein translation pathways are amongst the most sexually dimorphically expressed genes in the placenta (71). This could account for the different growth rates displayed by male and female placentas, and the variations in their adaptations observed in response to stress. Other functions, such as the regulation of extravillous trophoblast invasion (86), have also been proposed.

E. AMP-activated protein kinase

AMPK is an evolutionarily conserved and ubiquitously expressed regulator of cell metabolism that is activated by depletion of ATP. Hence, it acts as a key metabolic sensor to match energy demand with supply (240). It comprises three sub-units, each of which have multiple isoforms and confer tissue specificity (549). Classically, it acts to

promote glucose uptake and mitochondrial biogenesis, while reducing energy demands by inhibiting mTORC1 (239). It may also regulate more physiological functions as it has been implicated in stimulating endothelial nitric oxide production and regulating vascular smooth muscle tone (219).

Within the placental field there are limited data concerning the involvement of AMPK in placental development and function. It has recently been implicated in controlling uterine blood flow, and in adaptations to pregnancy at high altitude (505). Activation has been demonstrated to be a key step in the differentiation of mouse trophoblast stem cells under stress conditions (613), confirming that the pathway is functional in this cell type. Knockdown of the isoforms AMPKα1 and AMPKα2 in murine SM10 trophoblast progenitor cells has been shown to affect cell nutrient transport, inhibiting expression of *Glut3* and blocking translocation of the protein to the cell surface, but increasing the activity of system A transporters (93). In addition, knockdown inhibits cell proliferation and cytokine-induced differentiation. Dietary restriction (50% of controls) during early to mid-gestation in ewes resulted in increased activation of AMPK in the fetal cotyledonary tissues at d78 but not later on d135 (353), whereas the reverse was the case with over-nutrition (150% of controls) (615). In the latter situation there was reduced vascularity within the placentomes, suggesting perturbation of VEGF signaling. Similar inhibition of AMPK signaling has been reported in the placenta of rats fed an obesogenic diet of high-saturated fats (205).

F. Protein synthesis inhibition

A common, immediate response of cells to oxygen or nutrient deprivation is to suppress non-essential energy-demanding processes in order to harbor what resources are

remaining and maximize the chance of survival. Protein synthesis represents one of these processes, for incorporation of a single amino acid into a polypeptide chain involves four high-energy bonds, two ATP and two GTP. Most of the data relating to regulation of protein synthesis in response to hypoxia come from the cancer field (542, 585, 586), but it appears that the same pathways are operative in the placenta under physiological and pathological conditions (596, 598). There are two principal mechanisms that operate, a rapid response involving phosphorylation of eIF2α through PERK and the UPR, and a longer term response dependent on suppression of the mTOR pathway (181, 585). Both of these mechanisms regulate cap-dependent mRNA translation.

It should be appreciated that these blocks lead to a selective rather than a global inhibition of protein synthesis. Indeed, one of the actions of the UPR is to upregulate cellular antioxidant defenses and ER chaperone proteins, and to stimulate ER biogenesis to increase the folding capacity and promote cell survival. Selected mRNAs must therefore be able to bypass this translational arrest, and it has been suggested that those containing small upstream open reading frames (uORFs) within their 5'-UTR regions or internal ribosome entry site (IRES) sequences are able to do so (278, 349). More recently, it has been proposed that HIF-2α is able to combine with RBM4 at hypoxia response elements within the 3'UTR of a selective sub-set of mRNAs to initiate translation under hypoxic conditions (539). It is to expected, therefore, that the secretory output of the trophoblast might change both quantitatively and qualitatively under conditions of stress.

Protein synthesis is essential for normal development, and blocking dephosphorylation of eIF2α in mice results in severe growth restriction and early embryonic lethality (241). Equally, evidence of translational arrest has been reported in human placentas from normal healthy pregnancies at high altitude (3,100 m) where growth of the villus tree is impaired (598). These placentas displayed increased phosphorylation of eIF2α, reduced phosphorylation of AKT and 4E-BP1, and an increase in total 4E-BP1 that will favor sequestration of eIF4E. These changes can be recapitulated by exposing trophoblast cell lines to hypoxia (1% O_2), when they are associated with reduced cell proliferation. Such mechanisms may provide the homeostatic means for matching placental and fetal growth to the reduced ambient oxygen concentration, as previously described. The same placentas also show a decrease in the complexes of the mitochondrial electron transport chain at the protein, but not mRNA, level, consistent with translational arrest (121). Indeed, treating trophoblast cell lines with salubrinal, an eIF2α-phosphatase inhibitor, is sufficient to lower the complexes under normoxic conditions (121). There is thus a danger of a feed-forward vicious circle developing if glycolysis is insufficient to maintain energy levels under stress conditions.

Evidence of more severe translational arrest has been reported in placentas from growth restricted pregnancies of maternal vascular origin when placental weight is significantly reduced (596). Marked increased phosphorylation of eIF2α and decreased phosphorylation of 4E-BP1 were observed, and more significantly, all three isoforms of AKT were reduced at the protein, but not at the mRNA, level. Activation of the UPR has also been reported in placentas from cases of early-onset, but not late-onset, pre-eclampsia (595), when there is often accompanying growth restriction. It is notable that certain placental proteins, such as leptin, VEGF and its receptor soluble fms-like tyrosine

kinase (sflt), are markedly increased in early-onset pre-eclampsia. Genomic sequence analysis revealed that the encoding genes contain either uORFs or IRES sequences or both. These factors may be responsible for the maternal endothelial cell activation that typifies pre-eclampsia.

VIII. INTEGRATION OF SUPPLY AND DEMAND AT THE PLACENTAL INTERFACE

The placenta is not just a passive conduit for nutrient transfer. It has a dynamic role in optimising resource allocation between the fetus and mother during pregnancy (74). This is particularly apparent in late gestation when the fetus is growing rapidly in absolute terms or when resources are scarce due to poor maternal nutrition or nutrient reserves. The importance of this balance to the successful outcome of pregnancy also depends on the total uterine mass relative to maternal body size both within and between species, and on the particular mix of nutrients required by the fetus(es) relative to the metabolic ability of the mother to supply these nutrients in the correct proportions and absolute amounts (193).

The fetus demands nutrients from the mother via the placenta to improve its fitness since a large neonate with significant fuel reserves is more likely to survive at birth and onto reproductive age (372). This drive is mediated partly through the expression of imprinted genes that are expressed from paternal alleles and promote growth of the placental tissues (42). In evolutionary terms the role of imprinting has been explained as a means by which the male optimises the spread of his genome through the population (391). While the mother also benefits from this resource investment as her genes too are transmitted to the next generation, a balance must be struck as maternal investment

in the current fetus leaves less reserves for future pregnancies (193). There is, therefore, both co-operation and conflict between the mother and her fetus, and between siblings in litter-bearing species, in resource allocation at the placental interface (193). This leads to adaptations in placental phenotype designed to optimise maternal-fetal fitness with respect to the conditions prevailing during the current pregnancy. Changes in placental phenotype in response to environmental cues can, therefore, be seen as a co-adaptive response mutually beneficial to the mother and fetus in the successful outcome of pregnancy (305). However, in the polyandrous mating systems used by many mammals, competition between mother and fetus at the placental level is potentially more intense because of the differing contributions to the fetal genomes of half-siblings in demanding resources from the mother to the detriment of other half-siblings in future pregnancies (390). This leads to differential selection pressures on maternally and paternally inherited alleles in the conceptus with respect to resource allocation and is manifested as genomic imprinting, a mechanism for monoallelically regulating gene dosage in a parent-of-origin fashion (305, 391). Imprinted genes, therefore, have an important role in the developmental plasticity of the placenta, particularly with respect to resource allocation (189, 445).

Inter- and intra-species crosses between breeds of several species have shown that the mother can constrain the fetal genetic drive for growth while, conversely, the fetal genome can influence the mother to provide more resources with consequences for fetal growth and pregnancy outcome (8, 457, 551). Similarly, direct manipulation of the fetal genome by gene deletion or disruption is known to alter resource allocation to fetal growth via alterations in placental phenotype (198). Thus, the placenta acts as an environmental sensor, integrating signals of the current availability of oxygen and

nutrients, maternal stores of nutrient reserves and of the fetal nutrient demands for growth driven by the genes and actual mass of the fetus (74, 204). The adaptive responses of the placenta to these signals depends on the species, stage of gestation, total uterine mass, evolutionary history and on the specific nature of the environmental cues (546). However, the integration of nutrient-response systems in the placenta has not been fully determined.

A. Hypoxia

Hypoxia is one of the most common complications of human pregnancy, occurring in 9-10% of pregnancies at sea level and all pregnancies at high altitude (484, 600). It affects both the placenta and fetus and is associated with impaired trophoblast invasion, poor villous development, altered vascularity, reduced spiral artery remodelling and low blood flow on both sides of the placenta (387, 543, 600). The placental effects of hypoxia have been studied in a number of species using both *in vivo* and *in vitro* approaches. In humans, the studies have concentrated on the chronic hypoxia of high altitude and pathologies such as pre-eclampsia, whereas, in experimental animals like sheep and mice, they have focussed more on short term, acute hypoxia at different stages of pregnancy (255, 271, 363, 543, 600). At the placental and fetal levels, maternal hypoxia, the diminished availability of oxygen, manifests most frequently as hypoxemia, a low level of oxygen in the blood.

1. Placental size, morphology and blood flow

Development of the human placenta at high altitude has been studied in populations in Saudia Arabia, Kirghizstan, the Himalayas and in both South and North America (7, 276, 458, 532, 607, 612). These show no consistent effect of chronic hypoxia on placental

2066 weight or size with increases, decreases and no change depending on the study and/or
2067 population. Amongst studies at high altitude, the most consistent findings are
2068 alterations in the uterine vasculature and blood flow, and an increase in fetal capillary
2069 density in the placenta in association with fetal growth restriction. On average, there is a
2070 100g decrease in birth weight per 1000m elevation in altitude, although the exact figure
2071 varies with the ethnicity of the population (387). Lower birth weight at higher altitudes
2072 is seen with both long and short residency at high altitude regardless of nutrition or
2073 socioeconomic class (214, 386, 389). However, the high-altitude decline in birth weight
2074 is less in populations with longer residency at high altitude, such as the Tibetans and
2075 Andeans, than in populations like the Han and Europeans who have settled at altitude
2076 more recently (214, 387, 443). These ethnic differences in birth weight have been
2077 related to better placental adaptation to low pO_2 in populations with a longer
2078 evolutionary history at high altitude.
2079
2080 Although alterations in the uterine vasculature and blood flow are a common feature of
2081 pregnancy at high altitude, both increases and decreases in these parameters have been
2082 reported during late gestation relative to lowland populations (387). This is likely to
2083 relate to differences in the ethnicity, altitude and obstetric history of the populations
2084 studied and in the methods used to measure and calculate blood flow in the different
2085 studies (69). However, in the majority of studies, the normal pregnancy-induced rise in
2086 uterine blood flow is blunted at high altitude in association with a reduced diameter of
2087 the uterine arteries and/or lower nitric oxide synthesis, regardless of ancestry (294,
2088 295, 577, 603, 604). This suggests that remodelling of the uterine arteries during human
2089 pregnancy is impaired at high altitude (532), in line with the greater incidence of pre-
2090 eclampsia in these populations (429). An increase in placental weight was recently

reported in mice exposed to 13% oxygen throughout gestation, but only in those associated with male fetuses (363) (Table 3). Male placentas also showed more resistance to oxidative stress, and fetal growth restriction was notably significantly less than in their female littermates, suggesting the placentas had been able to compensate. In pregnant sheep at high altitude, the luminal cross sectional area, but not the number, of maternal vessels in the placentomes is increased, which results in a greater percentage area of maternal blood vessels and normal fetal growth (317, 437). Sheep evolved at higher altitudes than human populations and, hence, may be better adapted to pregnancy in hypoxic conditions (600).

In human populations, the adverse consequences of high altitude on uterine artery diameter and uterine blood flow are less pronounced with long than short ancestry at high altitude (295, 387). Pregnant Tibetan women have higher uterine artery diameters and blood velocity than Han women at high altitude (107, 389), while Andean women have twice the increment in uterine artery diameter during pregnancy than European women at high altitude (295, 577, 604). This results in differences in uterine blood flow between Andean and European women, which are detectable at 20 weeks of gestation before fetal growth slows (295). In some studies, the protective effect of Andean ancestry on uterine artery haemodynamics is only seen at high altitude while in others, the ethnic differences are also evident at sea level (295, 604). Indeed, in Andean women, the increase in uterine blood flow during pregnancy at high altitude can exceed that seen at low altitude (69, 295). Thus, in some studies of Andeans, absolute oxygen delivery to the gravid uterus at high altitude is maintained or even increased above lowland values, despite the low maternal pO_2, while in others the uterine oxygen supply is less at high than low altitude irrespective of ancestry (69, 295, 443, 604). However,

for any given altitude or ancestry group, fetal size is related to the absolute rate of uterine oxygen delivery, so weight specific rates of uterine oxygen delivery vary less with altitude and ancestry than the absolute values (295, 604). The fetus is, therefore, growing in relation to its overall oxygen availability.

At high altitude, there are also changes in the vasculature and blood flow on the fetal side of the placenta in both humans and sheep (317, 431, 443). In human infants near term, the diameters of the umbilical vein and artery are both smaller at high than low altitude, which results in a lower absolute blood flow in the umbilical circulation, irrespective of ethnicity (295, 443). However, babies of European ancestry are more adversely affected than those of Andean descent (443). In all high-altitude populations studied to date, there is increased villous vascularisation as a result of increased vasculogenesis and angiogenesis (7, 78, 176, 509, 532). Depending on the study, the increase in fetal capillary density in the human placenta may be due to an increased capillary number, diameter or length. At high altitude, there is increased branching and reduced coiling of the fetal capillaries in the placenta with more densely packed capillary loops in the terminal villi responsible for gas exchange (7, 532). The fetal capillaries are also longer and thinner in Andean than European/Mestizo placentas (275). This increase in villous vascularisation at high altitude appears to occur without any consistent increase in villous surface area or volume (176, 365, 370, 387, 458). In addition, there is thinning of the interhemal membrane in the human placenta at high altitude (276, 366, 458). This is achieved through selective dilation of the capillary sinusoids at the vasculosyncytial membranes (78), a mechanism that increases placental diffusing capacity but has minimal effects on extracorporeal blood volume and the load on the fetal heart.

Similar increases in fetal vascularity are seen in ovine placentomes at high altitude, but, in contrast to the findings in the human placenta, these are accompanied by an increase in the total surface area of fetal-maternal contact for gas exchange (317, 431). In the mouse placenta, vascularity has been shown to be increased in late gestation by 48h of hypoxia, but decreased by longer exposures (133, 212) (Table 3). Earlier in gestation, there appears to be little, if any, change in placental morphology during severe hypoxia of the mouse dam (483). In neither of these species is there evidence for thinning of the interhemal membrane in response to hypoxic conditions (133, 317). Overall amongst species, the morphological adaptations of the placenta will increase its oxygen diffusion capacity and aid oxygen delivery to the fetus at low maternal pO_2 (370, 387, 431). Indeed, per kg of fetus, placental oxygen delivery to the human fetus and its rate of oxygen consumption near term are normal at high altitude, although the fetuses are smaller (443, 605). Similarly, in sheep, fetal oxygen consumption is maintained when fetal-placental hypoxia is induced by restricting uterine blood flow and, hence, uterine oxygen delivery for 24h (53, 263).

2. Placental metabolism and nutrient transport

In addition to the morphological adaptations in the high-altitude placenta, there are changes in placental metabolism that may spare oxygen for onward passage to the fetus (271). In Bolivian women, oxygen consumption by the utero-placental tissues near term appears to be about 20% less at high than low altitude in the absence of any change in placental weight (605). This is greater than the 13-15% reduction in absolute uterine oxygen delivery observed between these high- and low-altitude populations of pregnant women (604, 605). Measurements of absolute rates of uterine and umbilical glucose

uptake suggest that the placenta may be using up to 60% more glucose at high altitude (605). The placental content of glucose and lactate also tend to be lower and higher, respectively, at high relative to low altitude (534). In addition, in the high-altitude human placenta, there is reduced abundance of all four complexes of the mitochondrial electron transport system (ETS) responsible for oxidative phosphorylation (121). The lower ATP/ADP ratio and the trend towards higher levels of the energy store, phosphocreatine, in these high-altitude placentas also suggests that there is a greater coupling of ATP demand to production and alternative sources of energy other than oxidative phosphorylation at high altitude. Similarly, during *in vitro* studies of cultured mouse trophoblast cells, hypoxia decreases abundance of cytochrome oxidase c, a component of the ETS involved in generating the proton gradient used for mitochondrial ATP synthesis (588). Collectively, these observations suggest that the chronically hypoxic placenta at high altitude may switch from oxidative phosphorylation to a greater dependence on anaerobic glycolysis to meet its ATP requirements, which may increase fetal oxygen availability albeit at the expense of the fetal glucose supply (271, 403). The finding that placental mitochondrial oxygen consumption under state III conditions (ADP stimulated) are higher in Tibetan than Han women also suggests that these metabolic adaptions may be dependent on ethnicity and/or duration of high altitude residency (612).

The proposed metabolic switch in the high altitude placenta will have consequences for fetal metabolism because, although fetal oxygen consumption is maintained on a weight specific basis, the fetus has lower than normal glucose concentrations and uses less glucose per kg body weight measured as umbilical glucose uptake (605). Fetal oxygen consumption must, therefore, be maintained by oxidation of substrates other than

glucose, such as amino acids and/or fats, which will then reduce their availability for other purposes (403). Since the percentage decrease in fetal glucose delivery appears to be greater than the percentage reduction in net fetal glucose consumption measured as umbilical glucose uptake at high altitude (605), there may also be activation of gluconeogenesis from lactate and amino acids by the high-altitude fetus near term. There is an increase in the fetal arterial concentration of lactate in these fetuses, but little is known about their rate of lactate consumption or about the rates of placental production and delivery of lactate at high altitude (534, 605). The reduced availability of both glucose and amino acids for tissue accretion will, therefore, decrease fetal growth in line with the oxygen supply.

With shorter episodes of severe hypoxia (<48h) in pregnant sheep, there is evidence for activation of fetal glucogenesis and increased delivery of glucose and lactate to the placenta from the fetal circulation (230, 263, 292). This short-term type of hypoxic challenge also induces changes in umbilical blood flow, with increases or decreases in flow immediately after the onset of maternal hypoxia depending on its severity (380, 524). Similarly, umbilical flow increases transiently 1-4 h after inducing placental-fetal hypoxemia by uterine artery constriction in ewes, but then normalises as the period of restricted uterine flow is extended to 24h or more (52, 230, 263, 380, 524). In line with the alterations in umbilical flow, there are changes in the placental delivery and fetal consumption of oxygen. Initially, fetal oxygen consumption decreases in line with placental delivery but then recovers to normal values despite the sustained low delivery by increasing oxygen extraction (230, 263, 380). Placental oxygen consumption is maintained at normal values for up to 48 h of hypoxemia (230, 263). Consequently, there is little evidence for placental oxygen sparing in response to acute normobaric

hypoxia in the sheep (230, 263, 380), as may occur in the human placenta in response to the chronic hypoxia of high altitude (271).

In contrast to oxygen, the ovine placenta appears to spare glucose for onward passage to the fetus during acute hypoxemia as fetal glucose delivery and consumption are maintained at the expense of utero-placental glucose consumption for periods of up to 24h (53, 230, 263, 380, 525). In these circumstances, the normal rate of placental oxygen consumption may be maintained by oxidation of lactate derived from the lactacidemic, hypoxemic fetus (230, 263). Certainly, lactate production by ovine utero-placental tissues is reduced by 24 h of placental-fetal hypoxemia induced by restricting uterine blood flow or placental growth by hyperthermia (263, 456). Little is known about the changes in abundance of the glucose or lactate transporters in the ovine placenta during hypoxic conditions (610). In the human placenta *in vitro,* acute hypoxia leads to upregulated expression of the GLUT1 and GLUT3 (178, 249). By contrast, in the high-altitude human placenta, GLUT1 is reduced at the basal but not the microvillous membranes, consistent with the decreased placental transfer of glucose to the fetus in conditions of chronic maternal hypoxia (601, 605). There is also a sex-linked decrease in placental *Slc2a1* (GLUT1) gene expression in the mouse placenta after 4 days of maternal hypoxia in late gestation (Table 3).

Much less is known about fetal-placental amino acid metabolism during either acute or chronic hypoxemia. In the human placenta, concentrations of several key essential amino acids were unaffected by altitude, with the exception of glutamine and the antioxidant, taurine, which were higher in concentration at high altitude than sea level (534). However, there is evidence for a decrease in protein synthesis in the placenta of

non-native women at high altitude (598). In human placentas *in vitro*, exposure to acute episodes of hypoxia reduces expression and/or activity of accumulative System A amino acid transporters but increases activity of System L amino acid transport (313, 412, 547). In pregnant sheep, acute hypoxia for 4 h decreases the placental supply and fetal use of leucine in association with a general increase in amino-nitrogen availability in the fetal circulation and reduced rates of fetal protein synthesis and proteolysis (380). The net effect of these changes is a reduction in protein accretion by the fetus. In the mouse placenta, maternal hypoxia for 2-4 days in late pregnancy leads to reduced expression of an isoform of the y+ system of cationic amino acid transporters and increased expression of the *Slc38a1* isoform of the System A amino acid transporters (Table 3).

Collectively, the studies show that the placenta tolerates hypoxemia well, but adapts its metabolism and transport characteristics to cope with the reduced oxygen availability (485). However, its strategy appears to differ with species, duration, severity and timing of the hypoxic insult, and/or the presence of fetal hypoxemia (393). At high altitude with chronic maternal hypoxemia but mild fetal hypoxemia, the human placenta reduces consumption of oxygen but increases use of glucose (271). At low altitude in response to acute hypoxia and fetal hypoxemia, the sheep placenta maintains its rate of oxygen consumption but reduces its use of glucose, while increasing uptake of lactate from the fetal circulation. In both scenarios, fetal metabolism is altered. In the high-altitude human fetus there is reduced glucose consumption but normal oxygen consumption, while in fetal sheep exposed to 4 h or more of acute hypoxia, normal rates of glucose and oxygen consumption are maintained coupled with fetal glucogenesis and altered amino acid turnover. In both species, the changes in placental and fetal metabolism will have adverse consequences for fetal growth.

2266

2267 *3. Placental endocrine function*

2268 Both *in vivo* and *in vitro* studies have shown that hypoxia affects placental production of
2269 a wide variety of hormones including protein, glycoprotein, eicosanoid and steroid
2270 hormones (Table 4). Altered placental endocrine function is seen in response to both
2271 chronic and acute hypoxia and reflects changes in gene expression, protein synthesis,
2272 and in metabolism and secretion of hormones (Table 4). These endocrine changes do
2273 not appear to be a strategy to reduce placental energy expenditure, as there are both
2274 increases and decreases in placental hormone production (Table 4). In addition to the
2275 endocrine outcomes of poor oxygen availability, there are also paracrine changes within
2276 the placenta itself, which will contribute to the adaptations in its morphological and
2277 transport phenotype (191). Furthermore, hypoxia induces changes in the placental
2278 barrier to transfer of maternal hormones to the fetal circulation (Table 4). For instance,
2279 in human and mouse placentas hypoxia reduces expression of 11βHSD2, potentially
2280 compromising the inactivation of maternal cortisol and exposing the fetus to
2281 hypercortisolemia (133, 242). Conversely, hypoxia increases expression of the thyroid
2282 hormone binding protein involved in transferring maternal thyroid hormones to the
2283 human fetus (434).

2284

2285 The changes in placental hormone synthesis and metabolism in response to hypoxia are
2286 likely to have consequences for both the mother and her fetus. They may contribute to
2287 the observed changes in uterine and umbilical blood flows, and influence the maternal
2288 metabolic adaptation to pregnancy during hypoxic conditions. Certainly, the normal
2289 pregnancy-induced increase in maternal insulin resistance associated with increased
2290 placental production of somatotrophic and steroid hormones is absent in women

chronically hypoxic at high altitude (316). Studies in women and sheep at high altitude have also shown that placental steroid production depends on the length of residency at altitude (105, 432). In turn, this may explain some of the ethnic differences in birth weight seen between populations with long and short ancestry at altitude (69).

B. Nutrition

The ability of the mother to provide nutrients to the fetus is determined, in part, by her nutritional state. This involves her diet, body composition and fuel reserves both before and during pregnancy, as well as her metabolic and physiological adaptations to the pregnancy *per se*. In addition, maternal nutrition is a major determinant of placental development and alters the morphological, transport and endocrine characteristics of the placenta with consequences for the fetal nutrient supply in a wide range of species including laboratory and farm species as well as human and non-human primates (47, 144, 204, 522, 550). Both under- and over-nutrition during pregnancy are effective at altering placental development in human and other species. There are also interactions between the current nutritional environment of the mother and her past nutritional history, as indicated by her body mass index (BMI) and body composition, in determining placental phenotype (29, 521, 527, 556). In experimental animals, the role of nutrition in regulating placental phenotype has been studied by varying dietary composition and maternal intake of calories, macro- and micro-nutrients and of other substances with metabolic actions such as alcohol, antioxidants and hormones (546). These dietary manipulations have been applied to induce obesity, for example, prior to conception and/or after establishment of pregnancy, or to investigate the effects of more acute nutritional changes later in gestation. In addition, changes in nutritional state and food intake during pregnancy often accompany, and are confounding factors in

studying, other environmental challenges such as hypoxia, heat stress, exercise and alterations in housing, lighting and noise levels (482).

1. Placental size, morphology and blood flow

Relative to the hypoxia of high altitude, much less is known about the effects of nutrition on development of the human placenta. The size of the human placenta at term is known to be affected by the calorie intake and dietary composition during the pregnancy (216, 350, 471). It is also positively related to maternal BMI across the normal spectrum, from the underweight to the morbidly obese (556). Variations in the balance between protein and carbohydrate intake at different stages of human pregnancy are related to placental and infant size at birth, with reduced protein intake in late gestation associated with a smaller placenta (216). Women who gain weight between pregnancies are more likely to have a large placenta subsequently whereas those with significant inter-pregnancy weight loss are more prone to placental growth restriction in their second pregnancy (553). Formal fasting in late pregnancy during Ramadan is also associated with reduced placental weight at term in Saudi Arabian and Tunisian women (10, 12). In the Dutch hunger winter populations, placental weight at term was increased when the famine occurred in the 1st trimester but was reduced, along with infant birth weight, in women who were in their 3rd trimester at the time of the famine (350, 471). However, despite this, placental efficiency measured as the fetal to placental weight ratio was greater in response to the undernutrition in late pregnancy than seen in control pregnancies before the famine (350, 471). Similar increases in placental efficiency are seen in the human populations fasting for Ramadan and in underweight women delivering small infants with a small placenta (10, 556). Taken together, these observations suggest that the human placenta either has a significant

reserve capacity or can adapt its nutrient transport capacity during nutritional compromise to support fetal growth. There is also some evidence of changes in angiogenesis in the placenta of obese women (157). In non-human primates, there are increases in placental infarction and reductions in utero-placental blood flow in response to feeding a high fat diet, which are more pronounced in mothers that become obese than in those who remained lean on the diet (200).

Similar changes in placental growth and efficiency are seen in murine and ovine placentas in response to maternal undernutrition (198, 546, 550). In pregnant mice, decreases in placental vascularity are seen in response to maternal obesity, calorie restriction and feeding diets high in fat or low in protein, particularly in the labyrinthine zone (Table 5). There are also changes in the relative proportions of the placental zones and reductions in surface area of the labyrinth and thickness of the interhemal membrane in response to nutritional manipulations before, and during, mouse pregnancy, irrespective of the level of calorie intake or maternal adiposity (Table 5). Even relative modest changes in dietary composition within the range recommended for rodent pregnancy can alter placental weight and zonal proportions near term (119, 301).

In sheep, undernutrition during the early stages of pregnancy when the placenta is growing most rapidly leads to an increase in term placental weight, whereas nutrient restriction later in pregnancy once the placenta is fully formed leads to reduced placental weight at term (198). These changes in weight are accompanied by alterations in the gross morphology of the ovine placentomes and in their vascularity (454, 463). Similar changes in placental morphology are also seen in response to overnutrition in juvenile and adult sheep (250, 354, 454). In particular, there are decreases in capillary

vascular density and/or volume in the caruncular part of the ovine placentomes, irrespective of whether the mothers were over- or under-nourished during pregnancy (454, 463). In contrast, vascularity of the fetal cotyledonary part of the placentomes is increased in response to undernutrition during mid-gestation (614). When over-nutrition begins before ovine pregnancy, there are increases in placentome capillary diameter at mid-gestation that are not sustained until term (354). In some, but not all, studies these changes in placental vascularity are accompanied by decreases in cell proliferation, angiogenesis and uterine and/or umbilical blood flow (166, 550, 557). At present, the mechanisms that regulate the responses to under- or over-nutrition are unknown, but likely involve the mTOR pathway and/or the generation of ROS.

2. Placental metabolism and nutrient transport

There have been relatively few studies of placental metabolism and nutrient transport with respect to nutritional state in women. The majority have concentrated on maternal obesity rather than on undernutrition or dietary composition. A recent study has shown that placental expression of GLUT1 is positively related to sugar intake in normal pregnant women (60). System A amino acid transport activity is lower in the placenta of women with smaller upper arm muscle areas, which suggests that reduced protein accretion in the mother, a proxy measure of nutritional state, may limit fetal amino acid availability (336). Reduced taurine and System A transport activity, and lower SNAT4 expression, are also seen in placental villous fragments from obese women delivering infants of normal birth weight (151, 184). By contrast, in obese women delivering larger infants, placental GLUT1 expression and System A activity are higher in the basal and microvillous membranes, respectively, than seen in women with a lean BMI (1, 279). There are also changes in placental fatty acid transport and transporter expression in

obese relative to lean women irrespective of the weight of their infants (101, 156). Furthermore, mitochondrial density and expression of the electron transport chain ETS complexes decrease in the human placenta as maternal BMI increases, with the result that placental respiration is lower in obese than lean women (244, 376). Taken together, these observations suggest that nutritional state and obesity, in particular, alter the energetics and nutrient transport capacity of the human placenta, which will have consequences for fetal development. However, the heterogeneity of the placental responses to maternal obesity in relation to infant birth weight suggests that there may be additional metabolic or other factors involved in regulating placental transport phenotype in these circumstances. Certainly, the specific changes in placental ETS function in obese women appear to depend, in part, on the degree of maternal glucose intolerance (244).

More is known about the effects of maternal nutrition on placental transport and consumption of nutrients in experimental animals. In pregnant mice, both under- and over-nutrition influence the transport phenotype of the placenta in late gestation (Table 5). Even relatively minor changes in dietary composition are known to alter placental clearance of glucose and amino acids with consequences for growth of the mouse pups near term (119). In part, the responses of the mouse placenta depend on the severity and duration of the altered dietary regime, and on the degree of placental and/or fetal growth restriction (Table 5). For example, a 50% reduction in food intake for the second half of mouse pregnancy leads to reduced placental glucose and amino acid delivery and severe feto-placental growth restriction, whereas a 20% reduction in food intake for most of pregnancy up-regulates amino acid transport per gram of placenta in association with relatively small reductions in fetal-placental weight close to term (120,

207). With several of the dietary manipulations including those inducing maternal obesity, the compromised growth and morphology of the mouse placenta is associated with up-regulation of nutrient transport or transporter expression (Table 5). Collectively, these studies suggest that a smaller, morphologically compromised placenta adapts its transport characteristics to help maintain fetal growth in late gestation (Table 5). For instance, feeding a diet high in fat and sugar reduces fetal-placental growth at day 16 of mouse pregnancy yet upregulates placental glucose and amino acid clearance with the result that fetal weight is restored to normal by D19, despite persisting placental growth restriction (496). However, the extent to which this strategy is successful in altering maternal-fetal resource allocation in favour of the mouse fetus depends on the actual nutritional and endocrine environment of the mother and on the mass, gestational age and genetic background of her litter (74, 193, 546).

In sheep, changes in maternal glucose levels induced by fasting, over-nutrition or direct experimental manipulation by maternal glucose or insulin infusion alter uterine glucose uptake and, hence, placental consumption and transfer of glucose in relation to the transplacental glucose concentration gradient driving glucose flux (247, 557). Even when hyperglycaemia or hypoglycaemia is prolonged in the ewe, placental glucose consumption still varies directly with the maternal glucose concentration (99, 147). Similarly, in overnourished obese adolescent ewes, there is no evidence for a change in the placental glucose transfer capacity, despite feto-placental growth restriction, as placental glucose consumption and transfer vary normally with maternal glucose concentrations on a weight-specific basis (554). However, in late gestation once the sheep fetus has developed the capacity for glucogenesis, the placenta can consume glucose from the fetal circulation if the maternal supply is limited or concentration

gradients are manipulated experimentally (190, 194, 248). With prolonged maternal hypoglycaemia, distribution of uterine glucose uptake between the ovine utero-placental and fetal tissues shifts to favour the utero-placental tissues, although absolute rates of glucose consumption remain lower than normal (99). This too, may reflect the ability of the sheep fetus to supplement its own glucose supply by activating gluconeogenesis, thereby reducing its demand for maternal glucose and sparing glucose for placental functions essential to maintaining pregnancy (148, 194). Ovine placental GLUT expression is altered in response to longer term variations in maternal glycaemia with decreases in GLUT1 abundance in hypoglycaemia conditions, and in both GLUT1 and GLUT 3 abundance in response to hyperglycaemia (138, 139, 247, 353). However, these changes may not have significant effects on glucose transport as the maximal capacity for placental glucose transport is much greater than the actual transport rate in the ewe (247).

Variations in maternal nutritional state have little effect on oxygen consumption by the ovine utero-placental tissues (99, 148, 190, 554). Consequently, when glucose consumption is reduced, for example by fasting, the utero-placental tissues must be oxidising other substrates. There are increases in the uterine uptake and utero-placental utilisation of some branched chain amino acids in response to fasting ewes, which may provide an alternative source of energy (338). In addition, undernutrition of ewes from early to mid-gestation increases expression of several molecules involved in transplacental fatty acid transport at mid-gestation, although few of these changes persist until late gestation (353). Certainly, there is no measureable net uptake of fatty acids by ovine utero-placental tissues in late gestation after prolonged hypoglycaemia

(99). In contrast, diet-induced obesity of ewes increases expression of several fatty acid transporters in the placenta at both mid and late gestation (616).

3. Placental endocrine function

Changes in placental size due to variations in maternal nutrition or obesity are likely to affect placental hormone synthesis, and hence circulating hormone concentrations. There are reductions in maternal progesterone and estrogen concentrations in obese women and overnourished adolescent ewes (326, 328, 555). In the latter animals, the decrease in maternal progesterone levels was associated with reductions in both placental weight and expression of *CYP11A1* mRNA at term compared to moderately fed adolescent ewes (328). In adult ewes, there are changes in the uteroplacental synthesis and metabolism of prostaglandins in response to acute nutritional manipulations during late gestation (195). In particular, there is increased utero-placental production of prostaglandins E and $F_{2\alpha}$ when maternal glucose levels fall due to fasting or insulin infusion in the ewe (192, 197). This may reflect the switch of hypoglycaemic placental tissues from glucose to fat metabolism, and the concomitant increase in availability of arachidonic acid, the prostaglandin precursor (195). Certainly, infusion of glucose into the fasted ewes to restore normoglycemia also normalises prostaglandin output by the uteroplacental tissues (192). In addition, production of ovine placental lactogen (oPL) is nutritionally responsive and is increased in late gestation by short-term fasting and periconceptional undernutrition (421, 422). It is also increased by short-term glucose infusion (421). In contrast, maternal oPL concentrations are low for most of gestation in overnourished adolescent ewes in association with placental growth restriction (328). In the mouse, manipulation of dietary fat content during pregnancy also alters expression of both the growth hormone gene (*Gh1*) and the extensive family of prolactin

and prolactin-like genes in the placenta during late gestation (356). However, the extent to which any of these alterations in hormone synthesis and metabolism are due directly to the changes in nutrient availability remains unclear, as other factors known to affect production of these hormones, such as glucocorticoid bioavailability, are also influenced by nutritional state (128, 191).

C. Genetic manipulation of nutrient supply and demand

The responses of the placenta to environmental cues, such as hypoxia and nutrition, indicate that there is significant interaction between maternal nutrient availability and fetal demands for these resources in determining maternal-fetal nutrient allocation at the level of the placenta (74, 144). The specific nature of these interactions is difficult to establish *in vivo* when the maternal, fetal and placental contributions to the dynamics of resource allocation all change simultaneously, for example, in response to undernutrition. Consequently, gene manipulations in mice have been used to induce more discrete changes in fetal demand relative to the placental supply of nutrients. These studies have tended to concentrate on the imprinted genes, which are known to have a disproportionately important role in fetal-placental development and are involved in resource allocation more widely (126, 189, 385, 544). However, even with the imprinted genes, relatively few studies have examined the functional consequences for the mouse placenta of altering its growth and morphological development in relation to the fetal genetic demands for growth (537, 544).

Measurements of placental nutrient transfer or transporters have been made in a number of the genetic mutants with deletions in imprinted and other genes involved in resource allocation (Table 6). There is often an increased fetal to placental weight ratio

that is accompanied by up-regulation of glucose and amino acid transfer per gram of placenta, particularly when placental growth is restricted early in mouse development (Table 6). This helps to support fetal growth despite the reduced passive permeability of the small, morphologically compromised mutant placenta (Table 6). Indeed, for nutrients actively transported to the fetus, the reduced placental permeability may enhance net transfer by preventing back-flux of nutrients into the placenta (150). Comparison of the placental-specific *Igf2P0* with the complete *Igf2* null mutant demonstrates clearly that the small placenta can become more efficient by increasing its nutrient transfer and transporter abundance when there is a maintained drive for growth by feto-placental tissues still expressing *Igf2* (124, 125). Even in the large *H19*$^{-/+}$ mutant placenta, restriction of its transport capacity by simultaneous deletion of the *Igf2P0* transcript leads to upregulation of MeAIB transport and *Slc38a4* expression to meet the larger demand of the overgrown mutant fetus, with increased *Igf2* expression in all its other tissues (14). Since changes in placental *Igf2P0* expression occur in response to maternal undernutrition and feeding an obesogenic diet (120, 496), this gene transcript may have an important role in adapting placental phenotype to environmental cues. Certainly, the changes in the placental capacity for nutrient transfer induced by maternal undernutrition do not occur in the *Igf2P0* null mutant (495).

When the fetal demand and supply are mismatched in the naturally small placenta, there are changes in expression of several imprinted genes, including *Igf2* in association with sparing of the labyrinth zone, increased MeAIB and glucose transport and upregulated expression of the *Slc38a2* amino acid transporters (114). As a result, the naturally small placenta also supports more fetal growth per gram and maintains a normal fetal growth

rate in late gestation (114). Similarly, when the placental supply of glucose to the fetus is disrupted by deletion of the *Slc2a3* gene, the placenta compensates by upregulating placental amino acid transport and transporter expression sufficiently to maintain normal fetal growth and metabolism until term (207). Furthermore, when placental growth is restricted by glucocorticoid overexposure in the *11βHsd2*-null mutant, there is upregulation of placental amino acid transfer and transporter expression in association with maintained fetal growth for the first 15 days of gestation (587). However, this upregulation is not maintained into late gestation, probably due to the decreased fetal demand associated with the direct growth inhibitory effects of excess glucocorticoids on fetal tissues late in gestation (191, 587). Thus, a mismatch between nutrient supply and demand appears to drive an upregulated capacity for transplacental nutrient transport, particularly when the placenta is small in relation to the fetal genetic drive for growth and the mother has the ability to provide the additional nutrients.

Conflict between the fetal demands for nutrients and the maternal capacity to supply them may also underlie the altered transport characteristics of those mouse mutants with placentomegaly (Table 6). The reduced passive permeability, nutrient transport and/or transporter expression of these mutant placentas may reflect the dominance of maternal signals that constrain maternal-fetal resource allocation in the face of the increased drain posed by the overgrown conceptuses in late gestation (14, 15). There is also evidence for inter-sibling competition for nutrients between mutant and wild type fetuses within mixed litters, which affects the size of the wild type pups (14, 104, 124). Taken together, these observations suggest that maternal constraint is an important factor in regulating placental phenotype, not only when maternal nutritional resources are limited but also when fetal demands increase rapidly in late gestation due either to

large litter sizes or genetically induced conceptus overgrowth. Certainly, upregulation of placental amino acid transport is maintained until D19 in mice with complete *Igf2P0*-null litters but not in *Igf2P0* mutants of dams with mixed litters of mutants and wild types, which have a greater total conceptus mass and, thus, demand for nutrients in late gestation (124, 495). In addition, variations in fetal-placental growth induced genetically are known to alter the metabolic and endocrine environment of the dam (439, 495). However, whether these maternal changes are a consequence of altered placental endocrine function or alterations in fetal-placental nutrient demand remain unknown. The placenta is, therefore, integrating maternal and fetal signals of resource needs along with its own growth and metabolic requirements in controlling maternal nutrient allocation to the gravid uterus. This dynamic adaptation in placental phenotype optimises fetal fitness in the prevailing conditions while maintaining sufficient maternal resource for lactation and subsequent pregnancies (144, 193).

IX. POSSIBLE MECHANISMS LINKING THE PLACENTA AND DEVELOPMENTAL PROGRAMING

The placenta clearly plays a critical role in the maternal-fetal supply line, but it's potential influence on programming must be set in the context of other non-placental candidates, including gametogenesis in both parents, fertilization, transport of the conceptus in the oviduct, lactation and post-natal nutrition. Attributing causation, or even a proportion of it, to placental changes is therefore problematic, as the same environmental insult may affect several different systems. In addition, the placental changes may be secondary to programming within the embryo/fetus, or simply a parallel response to the same insult independent of that of the offspring (58).

Nonetheless, the placenta is in a key position to modulate signals coming from the mother before they are transduced to the embryo/fetus, and can influence programming in at least four ways (Figure 11).

Firstly, its capacity to deliver sufficient oxygen and macro- and micro-nutrients to sustain normal fetal growth may be impaired. This may be due to a number of causes, but establishment of an adequate maternal blood flow to the placenta represents the final common pathway for many in the human. The maternal circulation to the placenta is dependent on remodeling of the spiral arteries, which in turn is reliant on invasion of the endometrium by extravillous trophoblast cells during the first and early second trimesters. The remodeling process is still far from understood, but brings together genetic, endocrinological and local endometrial factors. Deficient remodeling leads to malperfusion of the placenta, causing loss of function through oxidative and ER stress, diminished surface area through reduced growth and increased infarction, mechanical damage to the syncytiotrophoblast, and hypoxemia. The end result will be altered development of the fetal organs, as seen in cases of severe maternal undernutrition (215, 374, 565).

Secondly, the same stresses may compromise the protective, barrier functions of the placenta, allowing exposure of the embryo/fetus to abnormally high levels of maternal glucocorticoids, drugs (both therapeutic and recreational), xenobiotics and pathogens (129).

Thirdly, the placenta secretes a variety of factors into both the maternal and fetal circulations. Perturbation of that secretion may impact on fetal development, either

directly or indirectly via alterations in maternal metabolism. For example, placental prostaglandins or the release of pro-inflammatory cytokines, such as TNFα in response to oxidative or ER stress, may influence fetal cardiovascular development through their effects on the ductus venosus and endothelial cells respectively. Equally, placental hormones, such as the IGFs, stimulate fetal organ growth, and impact on maternal nutrient supply through their actions on appetite, the endometrial glands, pancreatic ß cells and peripheral insulin resistance as discussed in section III.B. The impact of placental release of microRNAs in exosomes is only just beginning to be explored, but this represents another potentially powerful signaling mechanism relaying information bidirectionally from the organ.

Fourthly, there may be mechanical influences imposed by the placental and vitelline vascular beds on the developing cardiovascular system (Figure 4). The resistance offered by the extracorporeal circulations exerts a powerful influence on the development of the entire fetal arterial tree, in addition to its effects on the heart (530). The heart appears to be particularly vulnerable during the early embryonic and late fetal periods of development (528), and so both the vitelline and placental must be considered. With regards to the latter, placental surface area was found to be inversely related to ultrasound measurements of umbilical arterial resistance in a prospective cohort of nulliparous pregnancies (481). The effect on the developing heart will be exaggerated in pathological pregnancies, where poor placental development is associated with absent- or reversed-end diastolic umbilical arterial flow (513).

It is also possible that the placenta may modify fetal growth and development by mechanisms as yet unknown. For example, it may provide stem cells to the mother and

fetus, or may alter the maternal vascular tree so that nutrient flow is reduced. Equally, it may provide molecules that influence epigenetic alterations in the offspring, to suggest but a few possibilities.

All these factors will interact with the fetal drive for growth determined by its genotype, and the outcome will depend on the timing and severity of the insult (Figure 11). Their impact will also be influenced by the placenta's ability to adapt and compensate, for example by increasing vascularity, enzyme or transporter expression. There is also the question as to whether the placenta has a functional reserve capacity, or whether it is operating to its maximum potential in normal pregnancies. They are clearly differences in placental efficiency within healthy pregnancies that deliver babies within the normal birth weight range, but placental weight is an uninformative proxy measure for placental function. At the crudest levels it does not distinguish between maternal blood and placental tissue, and is heavily influenced by the mode of delivery and processing of the organ (57). Quantifying physical parameters that determine the theoretical diffusing capacity of the placenta provides a more objective assessment of placental function. The fact that the value expressed per kg of fetus remains constant across gestation suggests that development of the placenta and fetus are closely interlinked (368). But such analyses cannot take into account changes in, for example, placental blood flows, maternal-fetal concentration gradients, hemoglobin binding affinities, transporter activity and placental metabolism. The speed with which these and many other physiological adaptations on both the maternal and fetal sides can occur makes it difficult to determine if any reserve capacity exists. There might also be detrimental effects for the fetus of building too large a placenta for its immediate needs, as this will

consume extra resources and place an additional burden on the extracorporeal circulation.

X. FUTURE RESEARCH

The placenta remains the most poorly understood and under-researched organ. One of the biggest gaps in our knowledge is how the human trophoblast interacts with the endometrium to establish the placenta during the first few weeks of pregnancy. The events taking place then are of critical importance to generating the framework of the placenta, and remodeling of the maternal circulation to perfuse it. We now know this phase of development is stimulated and supported by the endometrial glands, but what are the contents of those secretions, how are they regulated, and are they affected by maternal diet? Emerging evidence suggests that the yolk sac plays a key role in the transport of nutrients from the glands to the embryo, but what is its functional capacity, and how does its vascularization impact on the developing heart?

The maternal circulation becomes fully established at the start of the send trimester, and the extent of villus regression at this time appears to be major determinant of final placental size and shape. But how does unplugging of the spiral arteries occur? Is it purely a mechanical event, or is it related in some way to decline of gland function, co-ordinating the switch from histotrophic to hemotrophic nutrition? Is onset of the circulation abnormal in pregnancies complicated by early-onset pre-eclampsia or growth restriction? Equally, little is known about the spiral arteries and growth of the uterus during pregnancy. Are the arteries evenly distributed within the non-pregnant endometrium, or are there regional differences that might affect placental efficiency? Does the uterus expand symmetrically during gestation, and if so does the shape of the

delivered placenta reflect the implantation site and its possible arterial supply? What determines placental thickness? How do genetic interactions between the invading extravillous trophoblast cells and the uterine natural killer cells influence birth weight and obstetric outcome mechanistically?

The placenta clearly receives signals from the mother regarding her nutritional resources and reserves, and from the fetus relating to its demands, but what is the nature of those signals and how are they integrated? Imprinted genes are important, but what is the role of epigenetic factors and in what tissues are these mediated? The placenta is also sending signals in the form of growth factors, hormones and potentially exosomes, into the maternal and fetal circulations to modify the maternal ability to support the pregnancy and fetal growth. The precise nature of these signals, their regulation in response to environmental cues, and their effects remain to be determined.

Answers to these, and other questions, will come in part through advances in imaging technologies, and the ability to monitor placental development, oxygenation and metabolism in real-time. Magnetic resonance imaging and associated techniques, such as BOLD, promise much, but they must be capable of being applied during early pregnancy to capture the most fundamental events in placentation. In part, the answers will come through better phenotyping of the neonates and of the delivered placenta, at both the clinical and molecular levels. Longitudinal assessments of fetal growth trajectories *in utero* are needed to identify which neonates are potentially subject to programming. For the placenta, we need more information that just weight and shape at delivery. Greater attention needs to be paid to the mode of delivery, and the collection of samples to avoid possible artifacts (81), as well as to the sex of the placenta. Ultimately, we require more

comprehensive phenotyping of the placenta, including quantification of structural parameters such as surface area and interhemal distance, characterization of maternal and fetal circulations, expression and activity levels of the different types of transporters, measurement of endocrine function and enzyme activities, assessment of placental metabolism and regulatory signaling pathways, and, ideally, single cell transcriptomics and epigenetics. Such a comprehensive approach will require multidisciplinary research groups and/or collaborations over samples, but only then might we be able to tease apart the multiple interactions occurring during pregnancy, and attribute causation with some degree of certainty.

XI. Conclusion

Over the last decade, a mass of epidemiological evidence associating the gross placental phenotype with predisposition to chronic disease has been accumulated. The statistical associations are so strong, and have been confirmed in so many different cohorts across the globe, that they are incontrovertible. However, the associations are complex, for they integrate the long-term nutritional status of the mother, environmental cues and stressors, the demands of the fetus, and development of the placenta. The challenge now is to elucidate the developmental mechanisms that link the placental phenotype to chronic disease in the offspring. These may operate at different levels for different diseases, and at different times during gestation.

The pioneering epidemiological studies of David Barker inspired a new approach to our understanding of chronic disease, highlighting the importance of pre-natal growth as the foundation for a healthy body. As life expectancy continues to increase, we need to ensure that the next generations are built optimally from the outset, so that their organ

systems endure. The placenta plays a pivotal role in this process as it represents the platform on which the individual is constructed. Its transience should not belittle its importance.

XII Dedication

This review is dedicated to memory of David Barker, FRS, FMedSci, a friend and colleague.

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Figure legends

Figure 1. Diagrammatic illustration showing how the placenta may modulate and transduce environmental cues that lead to developmental programming of the fetus. The functional capacity of the placenta will depend on its development and its ability to adapt, as well as any reserve that exists.

Figure 2. Diagrammatic representation of the three main processes by which materials can cross the interhemal placental membrane; diffusion, transporter-mediated and endocytosis. The nature of the mechanism involved will determine how readily the placenta can adapt to facilitate transport under adverse conditions.

Figure 3. Diagrammatic representation of the gross morphology of the placenta and of the histology the interhemal membrane in the human, mouse and sheep. In each case the lower panel represents detail of the area outlined by the square in the upper panel. *A*) In the human, the fetal villi arise as a series of lobules (L) from the chorionic plate (CP). The basal plate abutting the maternal decidua (D), is thrown into a series of folds forming septae (S) that partially compartmentalize the placenta into lobes. Each lobe may contain one or more lobules. Maternal blood enters the intervillous space (IVS) from the spiral arteries (SA), passes between the villi and drains into the openings of the uterine veins on the septae. *B*) A single layer of syncytiotrophoblast (Stb) covers each villus and is generated from underlying cytotrophoblast (Ctb) cells. It is bathed by maternal blood in IVS from the start of the 2nd trimester onwards. Fetal capillaries (FC) within the stromal core (Str) invaginate to reduce the length of the diffusion pathway (arrowed). *C*) The mouse placenta is divided into an exchange labyrinth zone (LZ) and an endocrine junctional zone (JZ). The visceral endoderm layer of the inverted yolk sac

(YS) is exposed to the decidua (D) after the outer parietal layer breaks down (dotted line). This represents an important route of nutrient exchange during early pregnancy, and may continue until term. *D*) In the labyrinth the syncytiotrophoblast (Stb) is two-layered, and an additional layer of sinusoidal giant cells (SGC) lines the maternal blood spaces (MBS). Little stromal tissue (Str) is interposed between the fetal capillaries (FC) and the trophoblast. *E*) In sheep, fetal villi (FV) interdigitate with maternal crypts within specialized areas of the endometrium (E), the caruncles, to form placentomes. In between placentomes, the trophoblast forms areolae (Ar) opposite the openings of the endometrial glands (EG). Histotroph from the glands is taken up by the trophoblast, representing another route for maternal-fetal transfer. *F*) Within a placentome there are six tissue layers interposed between the maternal (MC) and fetal (FC) capillaries; the maternal endothelium, maternal stromal tissue (MStr), the uterine epithelium which is converted into a synepithelium by the migration and fusion of fetal binucleate cells, the trophoblast (Tr), the fetal stroma (FStr) and the fetal endothelial cells. Differences in the nature of the interhemal interface mean that extrapolation of transport data from one species to another may not always be justified.

Figure 4. The relationship between the vascular plexuses of the secondary yolk sac and the chorioallantoic placenta, and the developing heart. Because these two beds account for a substantial portion of the total vascular impedance to flow sensed by the embryonic heart, poor vascularity in these organs would offer an increased load to the heart, altering gene expression patterns and leading to congenital defects or a myocardium that is vulnerable for later disease. (Reproduced from Netter with permission).

Figure 5. Coronary heart disease mortality in 2571 men born in Sheffield, U.K., during 1907-1930 as a function of the placental to birth weight ratio expressed as a percentage. The lowest rates of death from heart disease were found among men where the placental weight was approximately 19% of the newborn body weight. *P*=0.03. (Adapted from (217) with permission).

Figure 6. Birth and placental weights of 17,000 live births in Unizah, Saudi Arabia. The points in the upper left box represent relatively low placental weights associated with relatively large babies, which have been defined as efficient placentas. The lower right box shows low efficiency placentas where large placentas nourished low birth weight babies. These two extremes of efficiency may represent different kinds of programming. (Adapted from (11) with permission).

Figure 7. Schematic representation of how multiple environments may give rise to placental metaflammation or 'cold, smoldering inflammation', and how this may predispose the fetus to chronic disease.

Figure 8. In the Helsinki Birth Cohort, hypertension is related to the surface area of the delivered placenta, in mothers of below median height (160 cm) (p=0.002) but not for tall mothers (p=0.72). (From (531) with permission, using data from (39)).

Figure 9. A summary of the principal mechanisms for oxygen sensing in cells, and of the effects of modulating oxygen concentration on cell behavior that have been reported for the placenta.

Figure 10. Diagrammatic summary of the principal ways by which the Unfolded Protein Response pathway may interact with the mTOR/AKT pathway to modulate protein synthesis within the placenta. Both pathways receive input at various levels regarding oxygen and nutrient availability, and will influence cell proliferation and growth. See text for details.

Figure 11. Schematic summary showing how various environmental influences may interact with, and be modulated by, the placenta, and the consequences for developmental programming of the fetus.