Nutritional Influences on Implantation and Placental Development

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The placenta is critical for nourishing the fetus throughout pregnancy, and also produces hormones that alter the metabolic functions of the mother. While the effects of nutrition on fetal development and long-term outcome have been very well documented, there are only a few reports based on studies in rat, sheep, and guinea pigs on how specific nutrients or general nutritional status affect the development of the blastocyst, its implantation, and the subsequent placenta. The data suggest that placental development is highly adaptable and that many types of compensation are possible for suboptimal nutrition.

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INTRODUCTION

As mammals, the placenta is something that we can’t do without during our embryonic and fetal lives, but which we soon forget about after birth. Even for clinicians and researchers who are interested in understanding complications in fetal growth, the placenta is often either not included in the analysis or is given cursory examination—such as by simply weighing it. This treatment is unjustified, as the placenta is not simply a selective filter for nutrient transport to the growing fetus. Rather, it plays several key roles in most mammalian species in regulating the nutritional status of both the fetus and the mother by: a) forming a highly branched villous structure that forms the surface area for nutrient and gas exchange and establishes its own circulation to connect to the umbilical cord; b) promoting blood flow to the implantation site and intervillous space by producing angiogenic factors and promoting vasodilation; 3) producing metabolic hormones such as placental lactogens and placental growth hormone, which alter insulin production and promote insulin resistance in maternal tissues to increase glucose availability to the fetus, as well as leptin and ghrelin; 4) accumulating glycogen in times of glucose surplus (Figure 1).

Each of these placental functions is highly regulated and undertaken by a specific compartment of the placenta. The barrier covering the chorionic villi in the human and rodent placenta is composed of several cell layers, including a multinucleated syncytiotrophoblast (syncytiotrophoblast) cells. Nutrient transporters are highly expressed to facilitate and regulate the transfer between the maternal and fetal circulatory systems. A specialized subtype of trophoblast cell invades outside of the confines of the villi (these cells are called extravillous cytotrophoblasts in humans and trophoblast giant cells in rodents) to encounter uterine spiral arteries. They replace the endothelial cell lining of the arteries and therefore lead to the transition into the trophoblast-lined blood vessels (hemochorial). The different placental hormones are produced by the invasive trophoblast and the syncytiotrophoblast cells. Finally, glycogen accumulates in both villous and extravillous trophoblast cells, but is most dramatic in the rodent placenta in cells designated as glycogen trophoblast cells. They first start to appear in the latter part of gestation within the middle layer of placenta called the spongiotrophoblast (often also called the junctional zone), which is analogous to the cytotrophoblast columns in the human placenta. After appearing, they subsequently invade diffusely into the interstitium of the decidual tissue of the uterus.

The purpose of this review is to discuss the role of nutrition in regulating these developmental events. Although there is only limited direct experimental evidence, it is clear that some specific nutrients and general nutritional status can play key roles in altering the developmental trajectory of the placenta, effects that have
direct consequences for the survival of the fetus and the well-being of the newborn.

MOLECULAR MECHANISMS OF PLACENTAL DEVELOPMENT

The cellular and molecular mechanisms underlying the development of the placenta are best understood in the mouse as a result of studies of experimental embryology, the ability to culture trophoblast stem cells, and analysis of several mouse mutants that have defects in placental development usually resulting in embryonic mortality or intrauterine growth restriction. Whereas we knew of only a few genes essential for placental development a decade ago,9 we now know of approximately 100. These subjects have been recently reviewed in detail elsewhere,2,8 but some of the key molecular pathways regulating placental development are summarized in Figure 2.

There are several important themes that emerge from the work to date. First, the placenta normally achieves the delicate balance of having the correct proportions of the different cell types to achieve its functions. Second, differentiation of the trophoblast compartments of the placenta is regulated by largely independent molecular mechanisms (Figure 2). Third, primary defects in one developmental process can lead to secondary changes in other parts. For example, as is observed in retinoblastoma (Rb) mutant placentas, failure to properly form villi can lead to secondary attempts to hypervascularize the villi that do form.10 This suggests that development is adaptable to environmental circumstances.

Figure 1. Structures and essential functions of the human placenta.

Figure 2. Developmental origins and molecular mechanisms underlying the differentiation of trophoblast cell types in the mouse placenta.
Indeed, the differentiation potential of trophoblast cells is highly regulated by oxygen levels.\textsuperscript{11-13} Given this, it stands to reason that nutrients and nutritional status may also have similar effects.

**EFFECT OF GLUCOSE, AMINO ACIDS, AND FASTING ON BLASTOCYST DEVELOPMENT**

The progenitors of the placenta are established in the pre-implantation embryo. Many years of culturing mammalian embryos have led to a significant understanding of their metabolism and of the optimal medium for embryos that allows them to reach the blastocyst stage and to successfully implant if transferred into a recipient mother.\textsuperscript{14} Blastocyst development and subsequent implantation potential are reduced in diabetic mothers,\textsuperscript{15} and this effect can be mimicked by culturing embryos in high concentrations of D-glucose.\textsuperscript{16,17} Amino acid supplementation also affects development. Essential amino acid supplementation during in vitro culture of pre-implantation embryos enhances post-implantation fetal development.\textsuperscript{14} In addition, a strong beneficial effect of nonessential amino acid supplementation is observed in mouse embryos, leading to enhanced blastocyst formation\textsuperscript{19} and increased implantation rates.\textsuperscript{14,18}

The beneficial effect of the nonessential amino acids can be observed in an in vitro model of implantation called blastocyst attachment and outgrowth. In the absence of amino acids, blastocysts do not attach but enter a state of quiescence analogous to delayed implantation.\textsuperscript{19} With the addition of amino acids, the trophoblast cells change their adhesive characteristics and form outgrowths. The effect is mediated by the mTOR signaling system and can therefore be inhibited with rapamycin.\textsuperscript{19} Interestingly, blastocysts can be exposed to the attachment-promoting amino acid levels for as little as a critical 8-hour period. This narrow window implies that amino acid sensing by blastocysts is a checkpoint mechanism for determining whether to continue to develop. If the blastocyst does not initiate attachment to the uterus, the maternal-side window of implantation will close and pregnancy will not be established.

Another potential mechanism by which undernutrition may affect blastocyst development has recently been suggested. Ghrelin is a hormone first described for its ability to increase fat deposition by stimulating appetite and decreasing fat utilization.\textsuperscript{20} Its expression is stimulated by fasting and insulin-induced hypoglycemia.\textsuperscript{20} The ghrelin receptor is also expressed by pre-implantation embryos, and ghrelin treatment significantly reduces the number of inner cell mass and trophectoderm cells in blastocysts,\textsuperscript{21} similar to the effect of a low-protein diet during the period of blastocyst formation.\textsuperscript{22}

**EFFECT OF MATERNAL NUTRITIONAL STATUS ON VILLOUS PLACENTAL DEVELOPMENT**

While specific nutrients have not been evaluated for effects on post-implantation placental development, the effect of feed restriction and overfeeding have been evaluated in rat, sheep, and guinea pig models. Curiously, at a general level, while the responses in rats and sheep are similar, the guinea pig appears to show a different effect. In rats, protein restriction from the time of mating leads to intrauterine growth restriction (IUGR) of approximately 10%, but the placentas show enhanced villous development.\textsuperscript{23} While the overall volume of the villous placenta (called the labyrinth in rodents) is not changed, the villous surface area is enhanced by about 15%, implying that branching had become more elaborate. The underlying molecular mechanism is unclear. Interestingly, though, the underlying vasculature does not undergo a proportional expansion. This implies that the trophoblast cells alter their development, perhaps in an attempt to compensate for the protein restriction. IUGR may have occurred because the response was inadequate or because, in the absence of a proportional increase in vascularization, the uptake efficiency of the placenta may not have been sufficient.

The effect of overall energy restriction on placental development and structure in rats has not been documented, and this is significant since it is obviously unclear if the ability of the placenta to compensate (or what type of compensation occurs) is dependent on the specific type of nutrient deprivation. Maternal iron restriction before and during pregnancy also leads to an increase in villous surface area but, again, with a failure in vascular adaptation.\textsuperscript{24} This model leads to anemia, and the response indicates that trophoblast-branching morphogenesis and differentiation can be regulated by tissue oxygenation. This is consistent with previous findings with knockout mice lacking hypoxia-inducible factor (HIF).\textsuperscript{11} Most other animal models of IUGR use feed restriction rather than protein restriction.

In sheep, overall caloric intake has been tested as a model and, interestingly, both feed restriction and overfeeding can lead to IUGR.\textsuperscript{25-28} The placenta in sheep is distributed over about 100 separate cotyledons, and nutrient status can affect both the number of cotyledons that form and their subsequent size and vascularity. Trophoblast proliferation\textsuperscript{25} and the expression of angiogenic factors\textsuperscript{27,28} are reduced by overfeeding during the first and second trimesters, leading to smaller cotyledons that are relatively poorly vascularized. By focusing on shorter intervals, Wallace et al.\textsuperscript{26} have been able to show that cotyledon number is most affected by overnourishment during the first trimester, whereas final cotyledon size is
most affected by nutritional status in the second and third trimesters. This coincides with the fact that cotyledons are formed during the first trimester, and implies that once the number is established, the placenta has only a limited ability to compensate through increasing the size of the remaining cotyledons.

In guinea pigs, a feed restriction model has been widely used as a model of IUGR. In this model, pregnant dams are fed only 50% to 70% of the normal ad libidum intake and birth weights are reduced by about 30%. By term, the labyrinth layer of placenta is reduced in overall size, and the villous surface area is also dramatically reduced. The response of the placental structure is significantly different than that in the rat models discussed above. It is not clear whether this reflects a species difference, differences in response to protein only versus total diet restriction, or is due to the fact that the guinea pig model involves a more significant challenge to the pregnancy. Insulin-like growth factor (IGF) I and II expression, and the ratio of the IGFs to IGF-binding proteins is reduced in the guinea pig model. Moreover, IGF levels appear to be correlated with the extent of villous branching in the labyrinth and are inversely correlated with the trophoblast barrier thickness. These observations are interesting, because IGF-II mutant mice have defects in the extent of villous branching in the labyrinth and the barrier thickness is increased.

**EFFECT OF GLUCOCORTICOIDs ON PLACENTAL DEVELOPMENT AND FUNCTION**

In rats, glucocorticoid treatment of pregnant dams during the second half of gestation results in IUGR. The expression of several hormone and nutrient transporter genes has been reported to be altered in this model. Notably, though, placental weight is also dramatically smaller in this model, and the decrease in placental size is associated with an increase in apoptotic cell death, that occurs in both the labyrinth and junctional zone, though the specific cell type(s) have not been defined. Therefore, the data on gene expression should be interpreted with caution, since it is not clear if the changes simply reflect a change in cell composition or a physiological change in expression. Ain et al. have addressed the point by conducting a microarray experiment focused on the family of prolactin-related hormone genes, and then performing in situ hybridization to determine the relative number and spatial patterns of expressing cells. It is evident from this work that at least some of the gene expression changes induced by glucocorticoids, including the placental lactogens and IGF-II, are physiological and due to cellular expression levels rather than to the pathological loss of cell types. This type of work clearly needs to be expanded to leptin expression and particularly to the glucose transporters GLUT1 and GLUT3, since both increases and decreases in their expression have been reported. However, in a food restriction model of IUGR in rats, GLUT expression levels did not correlate with endogenous glucocorticoid levels, perhaps suggesting that glucocorticoids may not directly regulate GLUT expression.

**MODULATION OF PLACENTAL HORMONE PRODUCTION BY MATERNAL NUTRIENT STATUS**

The placenta produces a number of metabolically important hormones. The placenta in many mammalian species has been reported to produce placental-specific members of the prolactin/growth hormone superfamily. In humans, both placental lactogen and placent growth hormone are produced. In rodents, a placental growth hormone is not made but a huge cluster of over 25 prolactin-related genes that have been amplified during evolution are expressed in the placenta, largely by trophoblast giant cells. The functions of most of the prolactin-related genes are unknown. Four placental lactogen-related proteins and a single PL-II all work through the prolactin receptor, and as such can potentially mediate several important maternal adaptations to pregnancy that are attributed to prolactin. These include mammary development and lactogenesis, pancreatic islet hyperplasia and the associated increase in insulin production, as well as insulin resistance. There is very little information about what regulates the synthesis and release of the PLs. PL-I expression is suppressed by progesterone. PL-II expression is reduced by IL-6, and has been found to be expressed in a circadian rhythm. PL-II is suppressed by melatonin, which is also expressed in the placenta.

The placenta also produces leptin and ghrelin, hormones that suppress and stimulate appetite, respectively, and regulate other metabolic processes as well. While they no doubt contribute to metabolic control during pregnancy, there are few insights into the specific function of the placently derived hormones. Leptin is expressed by syncytiotrophoblast cells under the control of the Gcm1 transcription factor. Leptin receptors are also expressed in the placenta, suggesting an autocrine or negative feedback function. Ghrelin expression in the placenta has only recently been described, but the site of the expression has not been identified. Interestingly, while fasting leads to increased levels of ghrelin in circulation during pregnancy, placent expression is not altered by fasting in pregnant rats.
PLACENTAL DEPOSITS OF GLYCOGEN

In addition to transporting glucose from the maternal to the fetal circulation, the placenta can also store it in the form of glycogen. Glycogen deposits are a common pathological finding in different tissues, but placental accumulation occurs in all pregnancies and appears to be highly regulated, which suggests that it may be physiological. In mice, glycogen accumulation is limited to the latter half of gestation, and specifically occurs in a subset of cells in the spongiotrophoblast layer after embryonic day 12.5. Thereafter, the cells appear to massively invade the wall of the uterus. The extent of glycogen accumulation is increased in diabetic pregnancies, but is stimulated by insulin. By contrast, glycogen content is reduced in IGF-II mutant placentas, suggesting that IGF-II may act in a paracrine manner within the placenta to regulate glucose uptake and synthesis of glycogen, perhaps through the insulin receptor. Glucagon has also been reported to diminish glycogen content in the placenta in one study, but in another study had no effect. While the ability of the placenta to store and mobilize glycogen is interesting, it is unclear to what extent it contributes to the energy balance of the fetus and/or mother.

CONCLUSION

The effects of specific nutrients and general nutritional status on placental development, structure, and function have been largely ignored by most researchers. However, from the limited data that do exist, it is clear that placental development is highly regulated and also highly adaptable. In particular, villous morphogenesis can alter to change the maternal-fetal surface area and barrier thickness across which nutrients pass. In addition, the extent of vascularization within the villi is adaptable and may be orchestrated by trophoblast cell expression of angiogenic factors. In addition to the development of the basic structure, placental functions (endocrine, active nutrient transporters, and glycogen storage) are also highly adaptable and can change either in response to the maternal environment or to defects within the placenta itself.

REFERENCES


