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Maternal-Placental-Fetal Interactions in the Endocrine Regulation of Fetal Growth

Role of Somatotrophic Axes

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Inadequate growth *in utero* is associated not only with adverse fetal, perinatal, and neonatal outcomes, but also with an altered propensity for disease later in life. Conversely, fetal overgrowth is also associated with increased medical risks for both mother and fetus. The interaction between the fetal genome and the intrauterine environment determines in great part how fetal growth will progress. The placental, maternal, and fetal somatotrophic axes (growth hormone/insulin-like growth factor-1) play key roles in modulating this interaction. Experimental undernutrition in animal models has numerous effects over these axes and provides insight into understanding fetal growth and its abnormalities. This review addresses the contributions made by the placental, maternal, and fetal somatotrophic axes to the regulation of fetal growth.

Key Words: Fetal growth; growth hormone; insulin-like growth factor-1; placenta; nutrition; intrauterine growth retardation.

Introduction

"Birth size" of the developing conceptus—reflected in birth weight, length, body composition, and organ size—is influenced by the interaction between the fetal genome and its *in utero* environment, an interaction that is ongoing from conception to delivery. There are now numerous reports in the literature that the fetus subjected to a less-than-optimal *in utero* environment has multiple, subtle abnormalities of physiology, some of which may manifest postnatally as metabolic and cardiovascular disease in adult life. Because weight remains the most common measure of fetal growth and development, and because fetal weight varies more in late gestation, when placental function is more likely to be limiting, the bulk of the available data relate to endocrine function in late gestation and its relationship to fetal weight. However, it is now becoming clear that influences earlier in development, while poorly understood, may be much more important in growth regulation. This review examines the role of the somatotrophic axes of mother, fetus, and placenta in the broader context.

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Factors That Regulate Fetal Growth

Endocrine Regulation

Several hormonal axes are involved in the regulation of fetal growth in late gestation, such as the hypothalamic-pituitary-adrenal axis, thyroid hormones, and the sex steroids *(1)*. However, the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis and insulin secretion play dominant roles and are the focus of this review.

It is by now well understood that GH, which is synthesized in the pituitary, triggers postnatal, somatic growth by stimulating IGF-1 release from the liver, and that GH mediates its actions by binding to the GH receptor *(2)*. However, GH has functions outside of the traditional view of growth; it is present in reproductive tissues and in the developing embryo, where it may influence processes such as steroidogenesis, gametogenesis, and implantation *(3)*. GH may also have mitogenic effects in some fetal tissues, such as the liver and pancreas *(4,5)*.

IGF-1 and IGF-2 are known to stimulate cell division, differentiation, and migration, and to inhibit apoptosis (for a review, see ref. *6*). Their biologic effects are mediated primarily by binding to the type 1 IGF receptor (IGF-1R), although IGF-2 also binds to the IGF-2/mannose-6-phosphate receptor (IGF-2R), and to an uncertain extent by binding to the insulin receptor. The IGFs are present in low concentration in the circulation, complexed with one of six specific binding proteins that are either inhibitory or stimulatory to the biologic action of IGF *(7,8)*.

The relative importance of IGF-1, IGF-2, and the IGF receptors in the regulation of embryonic growth has been demonstrated in a series of elegant studies using null mutant mice *(9,10)*. A targeted double deletion of IGF-2 and IGF-1R results in a more drastic growth restriction phenotype than a double deletion of IGF-1 and IGF-1R, implicating IGF-2 as the dominant embryonic growth factor.

In mice and humans, the IGF-2 gene is imprinted and expressed from the paternal allele *(11)*, whereas IGF-2R is imprinted only in mice and expressed from the maternal allele *(12)*. Haig and Graham *(13)* have hypothesized that imprinting arose as a result of an evolutionary "conflict" that exists between maternal and paternal alleles. Growth enhancers acting early in development will be maternally inactivated in an effort by the mother to conserve resources, whereas growth inhibitors will be paternally inactivated because it is in the father's best interest for his genes to be expressed (in evolutionary terms, most evident when considering multiple paternity) *(14)*.

While IGF-1 and IGF-2 exert their growth regulatory function by binding to IGF-1R, IGF-2R is equally important as a "clearance receptor" in removing excess IGF-2 from the circulation. With respect to Haig and Graham's *(13)* hypothesis, the role of IGF-2R is to "capture and degrade IGF-2 produced by the paternal genome" *(13)*, effectively reducing IGF-2 concentrations to keep growth in check. Null mutants of IGF-1R demonstrate drastically diminished fetal growth, but null mutants of IGF-2R exhibit fetal overgrowth *(10, 15)*, as would double expression of IGF-2, as seen in cases of isopaternal inheritance evident in Beckwith-Weidemann syndrome *(16)*.

These gene deletion studies have shown that placental growth is decreased in mice with a deletion in IGF-2, but there is no effect on placental growth with deletion of IGF-1. The possibility that IGF-2 may regulate placental growth through another receptor has been suggested. Deletion of the insulin receptor alone resulted in only mild growth effects, but when combined with deletion of IGF-1R, the growth deficiency was as severe as observed with the double deletion of IGF-2 and IGF-1R. These results suggest that the growth-promoting function of IGF-2 may be mediated in part by binding to the insulin receptor *(17)*. More recently, IGF-2 has been shown to bind to isoform A of the insulin receptor *(18)*, extending the possibilities of the mode of IGF-2 action in the placenta.

Maternal Constraint

Classic crossbreeding experiments performed by Walton and Hammond *(19)* showed that birth size is primarily determined by the uterine environment rather than by genetic factors, suggesting that there are limitations in the capacity of the uterus to support fetal growth. This constraint is evident in polytocous species such as the rodent, in which there is an inverse relationship between litter size and mean birth weight, and in humans, in which the mean birth weight in multiple pregnancies is less than that for singletons.

Limitations based on the capacity of the uterus suggest that the fetus generally does not grow to its maximum genetic potential, a phenomenon termed *maternal constraint (1, 20)*. Such limitations on fetal growth have the advantage of reducing the risk of fetal overgrowth and dystocia, the latter a powerful negative selection factor. Other factors, such as the number of previous births by the mother, ethnicity, and infant gender, may also influence fetal growth *(21)*.

The connection between maternal size and birth weight is likely to reflect genetic determinants of body size and other indirect genetic linkages rather than direct genomic regulation of birth size. Some confirmation of this has been provided by Robson *(22)*, who reported that there is significant birth weight correlation between half-siblings who have the same mother but different fathers, but no correlation between half-siblings of different mothers, but same father *(22)*.

An alternative hypothesis has recently been suggested in which mitochondrial inheritance from the mother may have some connection to low birth weight, through mechanisms involving specific mitochondrial gene(s) and/or via the role of the mitochondria as an energy-converting organelle *(23)*. We have shown in embryo transfer experiments that the maternal phenotype, not genotype, is critical to growth, suggesting that this *(23)* hypothesis may be unlikely.

Nutritional Influence During Early Embryogenesis

While our understanding of early trophoblast function is relatively poor, recent evidence suggests that the early embryo develops under hypoxic conditions for a much longer period than previously considered *(24)*. Moreover, while trophoblast function is still immature, the delivery of nutrients to the conceptus may not be under tight regulation. However, it is becoming clear that nutrient and possibly hormonal signals influence the very early embryo prior to implantation, in some instances with long-term consequences. Kwong et al. *(25)* reported on the effects of maternal undernutrition at the preimplantation stage. They showed that when rats were fed a low-protein diet in the first 4 d following mating, there was an increased potential for blastocyst abnormalities in resulting embryos. Offspring exposed to such an insult have reduced birth weight, higher blood pressure, as well as disproportionate growth of specific organs *(25)*, phenotypic outcomes commonly observed following "programming" *(26–28)*.

Wolff et al. *(29)* have provided evidence that nutritional signals in the periconceptual period may alter imprinting. Methyl-supplemented diets fed to pregnant mice altered in their offspring the expression of an imprinted gene specific to coat color. The influence of nutritional status is interesting considering that the GH/IGF-1 axis is subject to nutritional regulation (which is discussed in more depth further in this review), and that IGF-1 promotes the conversion of the amino acid serine to glycine, giving rise to methylenetetrahydrofolate, a source of one-carbon units that may be utilized for DNA methylation or imprinting *(30)*. Therefore, not only would nutrition-mediated alterations in the IGF axis affect growth, but there is the added dimension of potentially altered genetic imprinting as well.

More recently, the broader implications of periconceptual undernutrition have started to become apparent. In sheep fetuses exposed to periconceptual undernutrition, our labo-

ratory and others have observed slower growth in late gestation *(31)* associated with insulin resistance altered placental lactogen (PL) and placental metabolism production, as reflected in lactate *(32)* and urea responses to acute undernutrition; persistent changes to maternal metabolism, even after a return to normal nutritional intake *(33)*; premature activation of the fetal hypothalamic-pituitary function in late gestation (unpublished observation); and an increased propensity for premature birth (unpublished observation). Taken together, these observations suggest that altered nutritional signals in the periconceptual period shape placental and embryonic development in a manner that either allows for fetal adaptation to such an environment (i.e., by altering fetal growth) or promotes fetal survival by more extreme mechanisms (i.e., premature delivery).

Hence, fetal nutrition is not simply the passive consequence of maternal intake but, rather, the culmination of hormonal and other influences on maternal nutrient compartmentalization and placental metabolism.

Placental Function

Once trophoblast function is fully established early in the second trimester *(34)*, fetal growth becomes dependent on the functionality of the uteroplacental unit, and its regulation by maternal, fetal, and placental factors. The placenta is a metabolically active tissue consuming between 40 and 60% of glucose and oxygen extracted from the uterine circulation (for reviews, see refs. *35* and *36*). Thus, the regulation of placental metabolism can determine, in significant part, the net nutrient supply to the fetus. Placental dysfunction, impaired uterine blood flow, and/or impaired fetal nutrient uptake will compromise nutrient exchange across the maternal-fetal boundary, often leading to the development of intrauterine growth retardation (IUGR) and its resulting complications.

The presumptive human placenta forms soon after fertilization, at the formation of the blastocyst stage, with the outside cell layer composed of a trophoblast layer (the placental precursor) and within this layer a cluster of cells forming the inner cell mass or embryo. In humans, the trophoblast layer comprises two distinct cell types: cytotrophoblasts and syncytiotrophoblasts. Cytotrophoblasts differentiate into syncytiotrophoblasts that attach to and invade the uterus in order to come into direct contact with the maternal circulation; they also form the boundary between the maternal and fetal compartments to mediate nutrient and gas exchange *(37)*. The syncytiotrophoblast is the site of synthesis for estrogens, progesterone, human chorionic gonadotropin (hCG), PL, and placental GH (GH-V), thus providing hormones critical to maintaining pregnancy.

Human and rodent placentae are similar in structure and typically discoid in shape, with a villous pattern of interdigitation that functions to maximize the maternal-fetal exchange area. As their respective trophoblast layers come into direct contact with the maternal blood supply, a hemochorial barrier is formed *(38)*. By contrast, sheep and other ruminants have cotyledonary placentae that form multiple sites of attachment to maternal tissues. Cotyledons (a specialized area of trophoblast) face the fetal side of the placenta in nonrandom arrangements that are complementary in position to protrusions of maternal tissue termed *caruncles*. Together, a cotyledon and a caruncle form a functional unit called a *placentome*, and anywhere between 75 and 125 placentomes comprise the ruminant placenta *(39)*. In contrast to humans or rodents, the ruminant trophoblast layer attaches to, but does not invade, the endometrium. A unique feature of the ruminant placenta is that the cotyledons invade and fuse with the uterine epithelium, resulting in the formation of fetal chorionic binucleate cells (BNC), the source of PL in these species. Because this fusion forms a hybrid fetomaternal syncytium, the sheep placental barrier is often classified as epitheliochorial or synepitheliochorial to denote this hybrid layer (for a review, see ref. *40*). Despite these differences, enough functional similarities exist that allow one to apply information obtained from animal models to the human situation.

The placenta utilizes a variety of mechanisms to transport nutrients across the maternal-fetal boundaries. As pregnancy progresses, the metabolic needs of the fetus and placenta become greater, and placental transport capacity and the expression of specific transporters are modified to meet these demands *(41–43)*. Oxygen is transported by simple diffusion, glucose via the GLUT1 and GLUT3 transporters *(44)*, and amino acids by a variety of specific uptake systems (for a review, see ref. *45*). The somatotrophic axis may influence placental transport capacity. For example, treatment of pregnant sheep with GH for a period of 10 d has been shown to increase the capacity for simple diffusion *(46)*.

In the case of glucose and amino acids, much of these substances are in fact allowed to transfer from maternal to fetal circulation, where they are then reextracted by the placenta *(47)*. Roberts et al. *(48)* observed in guinea pig that a period of maternal undernutrition reduces placental exchange surface area and increases the thickness of the syncytiotrophoblast area, as well as introduces alterations in the placental transfer capacity for glucose and urea. Placental glucose transporters may be affected by metabolic and endocrine status. Maternal diabetes reduces uteroplacental blood flow *(49)* and causes overexpression of GLUT3 mRNA *(50)* and downregulation of GLUT1 expression, the latter proposed as an alternative means of protecting the fetus from high circulating levels of glucose *(51)*. Maternal undernutrition also results in elevated glucocorticoid levels, and GLUT transporters are downregulated by glucocorticoids *(52)*. Maternal IGF-1 treatment, which may promote fetal growth, is associated with an increase in mRNAs for GLUT1 and GLUT3 *(44)*, indicating that GLUT1 may have a role consistent with glucose transport between the mother and placenta and that GLUT3 maybe responsible for transport between the fetus and placenta *(53)*. Hence, there is a role for exogenous or

endogenous levels of IGF-1 to positively mediate nutrient exchange.

Lactate is an important metabolic fuel for the fetus and is largely derived from placental metabolism of glucose and amino acids. Lactate is released from the placenta into the fetal circulation, where, in some species, such as sheep, it may function as a substrate reservoir because it does not cross the placenta readily. Lactate production by the placenta may not be autonomous. Fetal IGF-1 infusion decreases placental lactate production, whereas maternal IGF-1 infusion increases placental lactate production *(54,55)*.

In IUGR, the fetus may become catabolic to try to sustain the placenta, and under drastic circumstances, both fetus and placenta may become compromised to sustain the mother. The placenta can also metabolize key substrates for utilization by the fetus. For instance, in sheep, all fetal glycine requirements are met by the placenta *(56)*.

At least 15 transporter systems mediate amino acid transport in the human placenta *(57)*. In humans, rodents, and sheep, there is evidence that IUGR results in reduced amino acid transport *(58,59)*. Some of these alterations may arise owing to the downregulation of specific amino acid transport systems, by reduction in surface area exchange and/or reduction in specific transporter number and activity *(45)*. In rats exposed to experimental IUGR, there is a reduction in system A (neutral amino acids), $X_{\overline{AG}}$, (acidic amino acids), and y^+ transport (cationic amino acids) *(60,61)*, and in clinical IUGR, a reduction in taurine transport *(62)*.

Placental, Maternal, and Fetal Somatotrophic Axes

Placental Somatotrophic Axis

During human pregnancy from 24 wk of gestation until term, the level of pituitary-derived GH diminishes to undetectable levels and is gradually replaced by the synthesis of GH-V *(63)*. GH-V is synthesized by syncytiotrophoblasts and is secreted in a continuous fashion in contrast to pulsatile GH secretion from the pituitary *(64)*. For the remainder of gestation, GH-V is the primary form of GH in the maternal circulation until birth, when its levels drop dramatically *(63)*.

The function of human GH-V is not completely clear. Because it is secreted solely into the maternal circulation in humans, its role is likely to be similar to that proposed for hPL—to induce relative insulin resistance that allows for preferential transfer of glucose to the fetus, and to encourage maternal dependence on lipolysis as part of metabolic homeostasis. In addition, its altered secretory pattern may contribute to maintaining maternal IGF-1 levels during pregnancy *(65,66)*. GH-V is not regulated by GH-releasing factors but appears to be suppressed acutely by elevated maternal glucose *(67,68)*.

It has been observed that in cases of clinical IUGR, GH-V levels are reduced, as are maternal IGF-1 levels *(65)*, though whether these observations are simply parallels or are causally related as a result of inadequate placental function in these pregnancies has not yet been determined.

Human GH-V has been extensively characterized at the level of the gene, transcript and protein (for a review, see refs. *69* and *70*). In humans, the GH-V gene is part of a gene cluster on chromosome 17 that includes pituitary GH (GH-N) and the three genes encoding variants of hPL or chorionic somatomammotropin: PL/CS-A, PL/CS-B, and PL/CS-L. These five genes share sequence similarity within their coding sequences, intron-exon boundaries, and flanking DNA, suggesting that they evolved by gene duplication *(70)*.

Structurally, GH-V differs from GH-N in 13 amino acids dispersed throughout the molecule. GH-V, a 22-kDa protein, can be N-linked glycosylated at position 140–142, giving rise to a 25-kDa form of this hormone *(71)*. The placental GH-V transcript is alternatively spliced to generate at least four transcript variants (for a review, see ref. *72*): the 22-kDa form just described; a 20-kDa form (also referred to as GH-V) in which amino acids 32–46 are deleted (similar to GH-N); a 26-kDa form (GH-V2) in which the fourth intron is retained, resulting in a new, divergent carboxy-terminal sequence (this variant may be membrane bound); and, finally, a 24-kDa form (GH-V3) that utilizes an alternative splice site at the end of exon 4 such that the first 124 amino acids are identical but there is sequence divergence at the carboxy-terminal end.

Localization studies confirm the placental-specific expression of GH-V variants; hGH-V is localized to syncytiotrophoblasts and hGH-V2 to placental villi *(73,74)*. However, more recently, alternatively spliced forms of GH-V have been localized to extraplacental sites, such as in the testis, where a physiologic basis for its localization is unclear *(75)*.

There is evidence to suggest that GH may be important during early embryogenesis, implantation, and blastocyst development *(76,77)*. Pantaleon et al. *(76)* reported on the expression of GH mRNA and its receptor in the preimplantation mouse embryo. They propose that this GH may be of embryonic origin and that it functions in an autocrine/paracrine fashion to influence development *(76)*. Following placentation, however, GH-V is the primary version of GH reported in several species (human, monkey, sheep, rodents), although it does not enter the fetal circulation.

Early developmental expression of GH is conserved across species. Lacroix et al. *(78)* observed GH mRNA in sheep placenta from embryonic d 27, with peak expression between embryonic d 40 and 45. They could not detect GH mRNA in the fetal pituitary gland at embryonic d 40. In addition, there are reports of GH mRNA in 12-d-old rat embryos and placentae *(79)*.

Placental Lactogen

PL is a member of the GH/prolactin(PRL) gene family and is capable of binding to their respective receptors since it does not appear to have a distinct PL receptor *(80)*. However, oPL is likely to act by binding to a heterodimer of a GH and PRL receptor (for a review, see ref. *81*). Whether an analogous heterodimer can form in humans is unknown.

PL is likely to play a role in mammogenesis, as well as possibly in protecting fetal carbohydrate supply through its lipolytic actions *(82–84)*. PL is detectable in both the maternal and fetal circulation, but its role within the fetal circulation is largely unknown *(85,86)*. In sheep, PL levels rise in response to maternal starvation, leading some to suggest that PL stimulates repartitioning of maternal nutrients to the fetus, as well as stimulates the fetus to use its own substrates *(1,87, 88)*. Infusion of recombinant oPL to the fetus has no notable effect on the level of circulating plasma IGFs, though it does cause a decrease in the level of IGF-binding protein-3 (IGFBP-3) *(86)*. Interestingly, the administration of exogenous oPL to lambs increases voluntary food intake *(89)* and therefore may also have a role in increasing maternal appetite during gestation.

GH Receptor

Pantaleon et al. *(76)* reported that GH receptor transcripts can be detected in the preimplantation mouse embryo as early as the two-cell stage. In fetal sheep, Lacroix et al. *(78)* detected GH receptor mRNA from embryonic d 20 to 120, with peak expression between embryonic d 25 and 43. There are also reports of immunoreactive GH receptor in the human fetus from the second trimester *(90,91)*. Assuming that the GH receptor is functional at these earliest stages of development, it lends support for a role for GH action in implantation and placentation.

The GH receptor can be alternatively spliced to give rise to transcript variants. One variant that has garnered the most attention is detected in human placenta, where it was observed that the GH receptor had a deletion of exon 3 that was not observed in maternal tissues *(92)*. This deletion is not conserved across species because it is absent in the mouse, and it has no effect on the ability to bind GH, GH-V, PRL, or PL, suggesting that exon 3 is not required for basic functional activity *(93,94)*. More recent studies now indicate that the exon 3 deletion in the placental variant is individual specific and derived from a polymorphism in the hGH receptor gene *(95)*.

A GH receptor/GH-binding protein knockout mouse (GH-R-KO) has been generated. These mice fail to synthesize the GH receptor or GH-binding protein and have severe postnatal growth retardation and diminished serum IGF-1 *(96)*. When normal or KO females are crossed to normal or KO males, the resulting progeny have reduced fetal weight but also demonstrate an unexpected increase in placental weight *(97)*.

Placental IGF

In the human placenta, transcripts encoding IGF-1 and IGF-2 have similar distribution patterns, yet IGF-2 expression levels exceed that of IGF-1 until the end of pregnancy *(98)*. IGF-2 is detected in developing placental villi and invasive trophoblasts as early as 6 wk of gestation, which suggests that IGF-2 is involved in trophoblast proliferation, differentiation, and implantation. By contrast, IGFBP expression is primarily localized in the maternal decidua, where IGFBP-1 expression is more robust than that of the other IGFBPs.

Based on these data, Han et al. *(98)* suggest that the IGF-1GFBP distribution pattern may facilitate cell-cell communication across the fetomaternal interface. There is support for this hypothesis from a related study by Gibson et al. *(99)*. IGFBP-1 normally exists in a phosphorylated state, but its phosphorylation pattern is altered during pregnancy to reveal phosphorylated (p) and nonphosphorylated (np) variants *(100)*. pIGFBP-1 has higher affinity for IGF-1 than npIGFBP-1, whereas affinity for IGF-2 is unaffected by phosphorylation status *(101)*. Gibson et al. *(99)* show that the maternal decidua is stimulated by trophoblast-derived IGF-2 to produce primarily npIGFBP-1, leaving this binding protein vulnerable to protease digestion. Because IGFBP-1 is inhibitory to IGF-1 and IGF-2 activity, they propose that this dephosphorylation step increases the bioavailable IGF *(99)*.

More recently, Constancia et al. *(102)* analyzed in mouse a transcript variant of IGF-2, termed P0, that exerts a strong influence over placental and fetal growth. P0 is an alternate promoter region in the IGF-2 gene that is responsible for the labyrinth-specific expression of IGF-2 variant; it is also paternally imprinted, resulting in its strictly placental localization pattern *(103)*. The P0 transcript accounts for <10% of the population of placental IGF-2 mRNAs, but its absence in targeted deletion mutants results in global restriction of placental size, as well as reduced fetal size *(102)*. Constancia et al. *(102)* also demonstrate that these mutants have a decrease in passive permeability for nutrients, but an increase in active transport that at first compensates, but then fails, suggesting that the purpose of IGF-2 in the placenta is to divert nutrients from the mother to the fetus.

The placenta also serves in some capacity as a clearance site for fetal IGF-1 and thereby regulates the circulating level of IGF-1: it can remove IGF-1 from fetal circulation when levels are high and secrete IGF-1 to the fetus when levels are low *(104,105)*.

Crosslinking studies in diabetic rats have demonstrated that there is a two- to threefold increase in IGF-1R autophosphorylation and kinase activity, suggesting that maternal diabetes, which induces fetal overgrowth, may be associated with functional alterations in IGF-1R *(106)*. Interestingly, however, approx 70% of the IGF-1 binding in the placenta is mediated by insulin-IGF-1R hybrid receptors. Valensise et al. *(107)* reported that women with gestational hypertension and associated insulin resistance have increased placental expression of insulin/IGF-1 hybrid receptors. It is suggested that ligand specificity varies depending on whether isoform A or B of the insulin receptor is incorporated into the hybrid complex: hybrid receptors containing isoform A bind to and are activated by IGF-1, IGF-2, and insulin, whereas hybrid receptors containing isoform B are bound with high affinity by IGF-1 and with low affinity by IGF-2 *(108)*.

Maternal Somatotrophic Axis

Prior to and during pregnancy, the mother must undergo a variety of metabolic adaptations to meet the needs of the placenta and growing fetus. These include increases in blood volume, alterations in blood flow, and metabolic changes that induce insulin resistance. All of these changes, particularly the latter, have been proposed as a means of ensuring adequate nutrient stores for the developing fetus and placenta.

There are mixed reports in the literature as to whether the administration of exogenous GH to the mother can influence fetal growth. Some data in rodents have demonstrated that GH can enhance fetal growth if administered late in gestation *(109)*, whereas other data have demonstrated an inhibition of growth *(110)*. More recently, in sheep we have shown that maternal administration of GH increases placental capacity for simple diffusion; increases maternal IGF-1 and insulin levels *(46)*; and increases fetal growth in late, but not early, gestation *(111)*. Others have reported that administration of GH-releasing factor to ruminants *(112,113)* and porcine GH to the pregnant sow can enhance fetal growth *(114)*. The mechanism behind such effects may relate to increased glucose utilization and free fatty acids in the maternal compartment, which affect placental transfer, or it may relate to effects on the placenta.

We have suggested that IGF-1 is key to the partitioning of substrates between mother and fetus. Short-term (3 to 4 h) IGF-1 infusion to the maternal circulation increases substrate uptake from the uterine circulation and increases placental lactate production, whereas IGF-1 infusion to the sheep fetus increases fetal uptake of glucose and amino acids from the placenta and decreases placental lactate production *(54,55)*. Interestingly, IGF-1 treatment throughout pregnancy abolishes maternal constraint, possibly by augmenting the ability to supply nutrients to the fetus and support growth *(115)*.

Fetal Somatotrophic Axis

GH concentrations are high in fetal life *(83)*, and GH receptors are present in fetal tissues. However, owing to relative receptor immaturity, it was generally believed that GH had little role in the regulation of fetal growth. There are data showing that human infants with congenital GH deficiency have somewhat reduced linear growth at birth *(116)*. We have detected GH receptor mRNA in fetal hepatic tissue as early as embryonic day 51 in sheep (term is 147 d) and have demonstrated binding using radiolabeled GH, indicating the presence of functional receptor *(117)*. While the data suggest the presence of functional receptor in the fetus, the concentration of GH receptor is approx 30% of what can be detected postnatally. We had previously suggested that GH expression at the early stages of development may be more a reflection of the immaturity of the somatotrope and somatotropic axis in integrating the responses to both stimulatory and inhibitory control *(83,118–120)*. However, the data now strongly suggest a paracrine/autocrine influence of embryonic-derived GH action at the earliest stages of development.

A role for the IGFs in fetal growth has been demonstrated in many species. Fetal plasma IGF-1 levels correlate well with birth weight and placental weight, and reduced fetal IGF-1 levels are associated with an increased risk of IUGR *(121–126)*. Short-term infusion of IGF-1 to late-gestation fetal sheep promotes an increase in fetal glucose uptake and a reduction in fetal protein oxidation *(54)*, whereas long-term IGF-1 infusion primarily promotes the maturation of certain organs *(127)*. In addition, asphyxia, a common risk factor in IUGR, leads to an acute transient reduction in fetal IGF-1 and an elevation in IGFBP-1 levels *(128)*.

The GH/IGF-1 axis is perturbed by a period of maternal undernutrition. After 72 h of maternal starvation, maternal and fetal concentrations of GH rise, but the level of fetal IGF-1, insulin, and glucose decreases *(129,130)*. With respect to IGF-2, some report that undernutrition paradigms induce a decrease in fetal IGF-2 levels, whereas others report no change *(129,130)*. Also affected by nutritional status are the IGFBPs. During periods of adequate nutrition, IGFBP-3 is the major IGFBP in adult sheep, but its levels decrease following 72 h of starvation. In the sheep fetus, IGF-binding capacity is somewhat equally distributed among IGFBP-2, IGFBP-3, and IGF-2R. Following starvation, levels of IGFBP-1 and IGFBP-2 increase, raising the IGF-binding capacity *(131)*.

The level of circulating IGF-1 during late gestation is regulated by fetal glucose and insulin levels *(132,133)*. Fetal pancreatectomy results in dramatically reduced IGF-1 levels and reduced fetal growth despite the resulting hyperglycemia *(134)*. Following a period of maternal undernutrition during late gestation, IGF-1 levels can be restored in fetal sheep by either maternal refeeding or infusion to the fetus of glucose or insulin *(132,133)*. Fetal IGF-2 levels, on the other hand, respond only to glucose infusion, but not to insulin, suggesting differential regulation of IGF-1 and IGF-2 in response to nutrition. There is also evidence that a period of maternal undernutrition in early gestation followed by maternal refeeding until near term is not without effects on the IGF axis *(135,136)*. Hence, glucose taken up by the fetus stimulates the release of insulin, which, in turn, regulates IGF-1 release, suggesting that placental glucose transfer is a first step in the path toward regulating fetal growth. If glucose and insulin levels in the mother or fetus are altered, this is likely to affect circulating levels of IGF-1 and, hence, the distribution of substrates.

IGF-1 infusion to the growth-retarded sheep fetus does not have the same effects on fetoplacental metabolism as it does in the normal fetus *(137)*. The cause, duration, and severity of growth retardation may influence the response to IGF-1 treatment. We have also observed in both rodent and sheep models of growth retardation that there is altered IGF-1 sensitivity, implying a degree of IGF-1 resistance *(138, 139)*. A similar resistance to IGF-1 is observed in small-for-gestational-age children *(140)*.

The IUGR infant is often born with severe gut anomalies and is at risk for gut-acquired infections. We have shown

in growth-restricted fetal sheep that administration of IGF-1, either by direct fetal infusion *(141)* or via a single daily injection directly into the amniotic fluid *(142)*, significantly improves gut growth and development. These findings indicate promising first steps as a means of treating IUGR *in utero*.

Conclusion

Birth size is the product of the interaction between the genome and environment, an interaction that is ongoing from conception to delivery. It is clear that nutritional availability to the fetus—which must be distinguished from dietary intake—is a dominant environmental influence. The placental, maternal, and fetal somatotrophic axes appear to modulate this interaction and may offer therapeutic possibilities.

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