PART I

Fetal Origins of Adult Disease/Programming
CHAPTER 1
Maternal Undernutrition and Fetal Programming: Role of the Placenta

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Introduction

What is developmental origins of health and disease (DOHaD)?

DOHaD is an area of research that emerged following retrospective cohort studies of David Barker and colleagues during the late 1980s. These investigators studied the association of geographical distribution of heart disease in the United Kingdom to a person’s birthplace, irrespective of the place where individuals develop disease [1]. Their data suggested that environment in early life causes permanent changes in fetal physiology that predispose the adult to disease later in life. Association of early undernutrition with low birth weight is a major component of fetal programming of the Barker hypothesis. The key contention of the Barker hypothesis is that the undernourished fetus is programmed to exhibit a “thrifty phenotype,” and this predisposes to a lifetime of increased food intake and fat deposition. Such individuals develop obesity, diabetes, and hypertension as adults, due to alterations in homeostatic regulatory mechanisms as a fetus.

The placenta is a multifunctional organ that synthesizes, metabolizes, and transports nutrients required by the fetus. The placenta is also a source of hormones that influence fetal, placental, and maternal metabolism and the course of fetal development. By virtue of these roles, the placenta plays a pivotal role in fetal programming.

Scope of problem

Four hundred thousand children in the United States alone are born annually with low birth weight resulting from intrauterine growth restriction (IUGR). IUGR is variably defined, but a common definition is a fetal weight below the 10th percentile for gestational age as determined by antenatal ultrasound (hence the phrase IUGR) or by newborn birth weight percentiles (hence the phrase small for gestational age). IUGR babies exhibit aberrant development and require higher neonatal intensive care. In addition to the short-term risks, the long-term risk of developmental programming includes metabolic disorders later in life. Up to 63% of adult diabetes, hypertension, and heart disease may be attributed to low-birth-weight conditions in conjunction with an accelerated newborn-to-adolescent weight gain and obesity. Therefore, the DOHaD field is increasingly recognized as an important contributor to the epidemic of obesity and metabolic syndrome in Western populations.
### Why should we care?

**We as scientists**

The DOHaD field is now unequivocally established, yet still in its infancy. There is still a lack of specific mechanisms to explain the effects for most of the epidemiological observations described for adult human disease. Fetal growth is directly related to placental growth and placental phenotype, which are regulated by the genetic background. We as scientists have great potential to study the signals for fetal nutrient demand that control placental transfer capacity. Importantly, there are great opportunities to dissect molecular mechanisms that regulate placental nutrient transfer in early pregnancy and that program nutrient transfer closer to term.

**We as clinicians**

Clinicians should aim to identify IUGR placentas and fetuses early enough to institute appropriate monitoring, and ideally, interventions that can limit adverse outcomes for the offspring. Examination of the maternal diet prior to and during pregnancy together with early detection of "placental disease" may help improve outcomes in IUGR.

**We as patients with a "placental disease"**

Patients should improve lifestyle, including a healthy diet, physical exercise, and prenatal care to optimize fetal and neonatal outcomes.

### Importance of maternal nutrients for fetal development

During normal pregnancy, the primary determinant of fetal growth is the concentration of nutrients in the maternal circulation and the blood supply to the placenta. Glucose, amino acids (AA), and fatty acids (FA) are among the nutrients vital for fetal growth and development. Collectively, the data show that deficiency of nutrients in the mother causes alterations in placental nutrient transport and reduced body weight in the offspring.

#### Glucose

The majority of fetal glucose derives from maternal metabolism of carbohydrate in the diet. Glucose supply to the fetus is a facilitated process mediated by members of the glucose transporter (GLUT) family. Four isoforms GLUT1, GLUT3, GLUT4, and GLUT8 have been identified in human and rodent placentas. Glucose deprivation leads to hypoglycemia. This suggests that less glucose availability affects fetal growth. In rats, severe maternal glucose deprivation reduces placental transfer and fetal uptake of glucose, which results in fetal growth restriction.

#### AA

Fetal AA come from maternal AA pools derived from the diet. Fetal concentrations of nearly all AA are greater than maternal concentrations, suggesting that the placenta actively transfers AA from the maternal compartment to the developing fetus. Essential AA must be supplied in food, while nonessential AA are synthesized by the fetus from essential AA. Several AA transporter systems have been identified in human and rat placentas. Abnormalities in AA transport may be the reason that total AA concentrations are lower than controls in IUGR babies. AA transport by the placenta is downregulated following maternal protein deprivation and may contribute to fetal growth restriction.

#### Fatty acids

FA content and character in fetal plasma directly correlates with the FA composition of maternal plasma and with the maternal diet. Essential FA cannot be synthesized and are dietary essentials (e.g., linoleic and linolenic acid). Essential FA in human pregnancy are transported from maternal to fetal circulations as triglyceride-rich lipoproteins, which are hydrolyzed by placental lipases. This results in free FA (FF) release, which are transported by saturable plasma membrane FA-binding proteins, FA translocase, and a family of FA transport proteins. Low birth weight in both human and rat pregnancy correlates with low intake of essential FA.

### Clinical Pearl

Balanced diets containing complex carbohydrates, essential AA, and essential FA optimize the substrates needed for normal fetal growth and development.

### Factors affecting placental capacity for nutrient transfer

Multiple factors interact to influence the placental delivery of nutrients to the fetus. Size, histopathology, blood flow, transporter abundance, and organ consumption are factors responsive to environmental changes. Key studies...
address placental size, morphology, and transport abundance.

Size
Placental size affects the capacity for nutrient transport through changes in surface area, and placental weight correlates with fetal weight at term in many species. Timing, duration, and etiology of nutritional restriction yield variable phenotypes for placental mass. The Dutch Famine of 1944–45 reflects a highly cited example of this premise. Exposure to famine only during the first trimester of pregnancy enhanced placental weight at delivery without any impact on newborn weights when compared to control women, resulting in an increased placental-to-birth-weight ratio. In contrast, women subjected to starvation in their third trimester of pregnancy had reduced weight placentas and low-birth-weight newborns but an unaltered ratio of placental-to-birth-weight as compared with nonstarved women [1]. These results suggest that human placental adaptations in early pregnancy can overcome some environmental stressors such that fetal nutrition is maintained in late gestation. Collectively, these data suggest the placenta may compensate for insults to minimize fetal growth restriction. The histomorphology of the placenta ultimately determines placental function.

Histomorphology
Small placentas exhibit altered histopathology and ultrastructure compared to normal size placentas. Notably, the maternal undernutrition that yields IUGR in human pregnancy generates placentas with a reduced surface area for nutrient exchange, a lower volume density of trophoblasts, and increased placental apoptosis at term. In IUGR placentas, absent or reversed end-diastolic flow in the umbilical artery, as assessed by Doppler velocity waveform analysis, is indicative of poorly branched and capillarized villi, and thickened exchange barrier. In these placentas, vascular resistance occurs as a result of inadequate trophoblast invasion of the spiral arteries. In contrast, in less severe IUGR, positive end-diastolic umbilical artery flow is associated with a normal stem artery development, increased capillary angiogenesis, and adequate terminal villous development. Thus, the thicker placental exchange barrier and the increased placental vascular resistance in severe IUGR may correspond to alterations in placental structure directly involved in fetal programming of cardiovascular disease. These structural alterations in the human placenta are mirrored in the guinea pig exposed to global maternal undernutrition compared to control diets. The nutrient deprived gestations exhibit a labyrinthine placenta with a 70% lower surface area and a barrier thickness 40% higher in late gestation. A reduction in the length of the labyrinthine vessels and decreased expression of vascular endothelial growth molecules in the murine placenta in response to maternal protein malnutrition are compatible with the possibility that alterations in maternal nutrition change placental vascular function [2]. These histopathological changes predispose to lower nutrient transfer to the fetus. Our work in the rat exposed to maternal undernutrition showed enhanced apoptosis in junctional and labyrinthine zones of the placenta [3], suggesting that both hormone production and maternal-fetal exchange are impacted. Taken together, these data indicate that restriction of nutrients impairs the functional capacity of the placenta disproportionately compared to the reduction in placental weight alone.

Clinical Pearl
Doppler velocimetry techniques may be used to detect increased placental vascular resistance and predict adverse pregnancy outcome.

Transport abundance
Reductions in maternal-fetal nutrient transfer may derive from an inadequate maternal supply, inadequate placental blood flow, impaired placental transport, or a combination of these processes. Maternal nutritional status affects transporters in the placenta, which is time-dependent. For example, rats fed 50% less food during the last week of gestation have lower than control glucose levels in maternal plasma, a lower maternal-to-fetal glucose concentration gradient, and downregulation of GLUT3 expression, suggesting a mechanism for placental glucose transport dysfunction. These changes suggest that transport-mediated mechanisms may effectively reduce fetal levels of glucose. Placentall transport of AA is affected by the activity and location of AA transporter systems. In humans, circulating essential AA concentrations are decreased in growth-restricted human fetuses, likely from reduced AA transport activity. In rats, maternal protein restriction downregulates placental nutrient transport prior to the onset of fetal growth restriction, suggesting that a
reduced placental supply of AA is a causal factor for IUGR, not simply a consequence of this malady. Undernourished women exhibit placental and offspring deficiency in essential FA, leading to altered placental FA metabolism and IUGR. These placentas not only have decreased levels of arachidonic acid and docosahexaenoic acid, but also show an altered ratio of both these FA relatives to their essential FA precursors, linoleic and \( \alpha \)-linolenic, consistent with abnormal metabolism [4].

Taken together, these studies show the pivotal role played by the placenta in assuring that multiple nutrients are available to sustain normal fetal growth.

**Placental nutrient synthesis and metabolism**

Uteroplacental tissues in humans, ruminants, and equids metabolize glucose derived from the maternal circulation. Placental glucose consumption is reduced during short periods of maternal undernutrition, but this reduction has no effect on the partitioning of glucose between the uteroplacental and fetal tissues in humans [5]. Conversely, prolonged maternal hypoglycemia induces uteroplacental tissues to use less of the more limited supply of glucose available, thereby sparing glucose for the fetus. These adaptations correlate with reduced GLUT1 expression, offering a mechanism for the effect. The placenta metabolizes glucose to lactate during normal pregnancy [5], and this event increases the maternal-to-fetal concentration gradient for glucose. Placental lactate production decreases in response to maternal undernutrition in sheep, making glucose less readily available for fetal consumption [5].

The placenta synthesizes some of the AA required for fetal growth. For example, fetal glycine in sheep and human placentas are from endogenous synthesis. Serine derived from the fetus is converted in the placenta to glycine, and this AA is released back to the fetus. Interestingly, explant cultures from IUGR human placentas accumulate less serine in vitro than normal term villous explants. Besides placental synthesis of AA, uteroplacental tissues metabolize AA, supplying the fetus with essential AA [5].

The placenta synthesizes significant concentrations of FA in humans, sheep, and pigs. FA synthesis in term human placenta is lower than its oxidation. IUGR placentas commonly show a deficiency in oxidative enzymes, resulting in excess lipid peroxidation and free radical formation, both of which are harmful to maternal endothelial cells when released.

Collectively, these data show that placental nutrient synthesis and metabolism influence fetal growth and development.

**Placental hormone synthesis and metabolism**

The placenta releases hormones into both the maternal and fetal circulations, and synthesis and secretion of these hormones are responsive to environmental changes. Human placental lactogen, progesterone, insulin-like growth factors (IGF), and glucocorticoids play critical regulatory roles in fetal homeostasis.

Human placental lactogen and progesterone influence maternal metabolism to favor glucose delivery to the fetus (6). Concentrations of both hormones are lower in undernourished mothers, and this may contribute to limited delivery of glucose to the fetus. This suggests that changes in placental endocrine dysfunction may be a cause and not a consequence of altered fetal growth.

### Clinical Pearl

Maternal plasma concentration levels of lactogen and progesterone may be used to predict adverse outcomes for the offspring.

The IGF family of hormones modulates growth, cell division, and differentiation. The action of the IGFs is regulated by IGF-binding proteins (IGFBPs), and together may modulate fetal growth. IGF-I is mitogenic for placental stromal fibroblasts and has insulin-like effects to increase AA transport in human placental cells. The ovine placenta clears IGF-I from the umbilical circulation when fetal IGF-I concentrations are high, but secretes IGF-I when fetal concentrations are low. Fetal IGF-I concentrations positively correlate with fetal body weight to suggest that hormone production, metabolism, or both adjust to conditions prevailing in utero to yield optimal fetal growth. IGF-II modulates trophoblast development at the feto–maternal interface. Disturbances in IGF-II expression and activity associate with IUGR in human pregnancy [7].
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Research Spotlight

There are fetal sex differences in the IGF axis. IGF-II concentrations in umbilical cord serum from male neonates are significantly higher than those in female neonates, and cord plasma IGF-I and IGFBP-3 are higher in female neonates than in males.

Glucocorticoids are key regulators of organ development and maturation. The placenta is not a site for synthesis of glucocorticoids, but the placental 11β-HSD2 converts active glucocorticoids to inactive metabolites. This enzyme is affected by exogenous exposure to glucocorticoids and by fetal and maternal glucocorticoid concentrations. The human 11β-HSD2 enzyme is localized to the syncytiotrophoblast and is thus positioned to limit glucocorticoids transfer to the fetus. Extended periods of maternal undernutrition downregulates placental 11β-HSD2 activity, increasing placental exposure to glucocorticoids. This leads to feto-placental growth restriction and abnormalities in cardiovascular and metabolic function in the adult offspring. Therefore, changes induced by elevated glucocorticoids may have beneficial effects on offspring viability, but they also impact negatively on fetal growth and development. Thus, 11β-HSD2 enzyme plays a vital role to protect the fetus from exposure to excess maternal glucocorticoids.

Collectively, these studies show that hormone synthesis and metabolism by the placenta are affected by maternal nutritional status and that the biological effects of the hormones influence fetal growth and development.

Mechanisms of placental programming

The placenta regulates fetal development by regulating nutrient transfer to the fetus and by controlling the bioavailability of specific hormones important to fetal growth and development. The placenta therefore plays a pivotal role in mediating the programming effects of suboptimal conditions during development. Mechanisms likely involved in programming the effects of maternal undernutrition include modulation of placental vascular resistance, regulation of the nutrient supply, epigenetic, gene imprinting, and metabolism of glucocorticoids.

Maternal undernutrition increases placental vascular resistance, and this subjects the fetal heart to an excess workload. This observation provides a direct link between altered placental structure and programming the risk for cardiovascular diseases in IUGR fetuses. The placenta functions as a nutrient sensor and directly regulates the nutrient supply available for fetal growth. Genomic imprinting is an epigenetic phenomenon whereby the expression of a gene depends on the parent of origin. For example, IGF-I is an imprinted gene and is crucial to fetal development as described above. IGF-I is downregulated in placentas exposed to nutrient restriction. Moreover, a placenta-specific transcript (P0) for the IGF-II gene is expressed exclusively in the labyrinthine trophoblast of the mouse, and deletion of this transcript yields diminished placental growth, reduced placental nutrient transfer, and fetal growth restriction. Methylation of DNA restricts the genes available for transcription in cells. Maternal undernutrition affects the methylation status of the placental IGF-II gene and, in so doing, may control placental supply of maternal nutrients to the fetus. Imprinted genes in the placenta may be modified by perturbations of the maternal environment and altered fetal programming results. Moreover, the placenta strongly influences fetal endocrinology and metabolism. A well-documented example is rise in fetal glucocorticoid levels that follows decreased activity in placental 11β-HSD2. The adverse effects of excess fetal glucocorticoids on fetal development of the hypothalamic pituitary axis may program the fetus to be at higher risk for metabolic diseases as an adult.

Intervention strategies targeting the placenta to prevent altered fetal growth, fetal programming, or both should dissect in more detail how placental growth, nutrient transport function, and placental oxidative stress are modulated by maternal administration of IGFs or pharmacological levels of methyl donors. Targeted upregulation of the activity of placental 11β-HSD2 may also beneficially modulate feto-placental health.
Summary

Maternal nutrition during pregnancy is an important determinant of optimal fetal development, pregnancy outcome, and ultimately, lifelong health. Barker’s epidemiological studies have stimulated new ideas about both intrauterine development and risks for adult diseases. Animal models of programming have shown that most fetal organs are vulnerable to the effects of maternal undernutrition during critical periods of development. Importantly, these studies show that programming the placenta, as illustrated in Figure 1.1, may mediate effects on the fetus. Maternal undernutrition reduces fetal growth in part by impairing placental development and function. Placental alterations include decreases in placental weight, altered vascular development, reductions in glucose, AA, and FA transport, and hormone synthesis and metabolism. The plasticity of the placenta allows this pivotal tissue to respond to exogenous insults and to compensate for many environmental influences. Moreover, maternal diet may alter the placental genome through gene imprinting, an effect that may affect future generations. When the placental response is not sufficient to maintain fetal growth, IUGR results and suboptimal outcomes result (Table 1.1). The elucidation of further roles for the placenta in fetal programming will increase our understanding of DOHaD and hopefully will provide new strategies to prevent and treat suboptimal fetal development in the future.

Teaching Points

1. Fetal programming may occur following natural or experimental environmental changes in both humans and animals.
2. Maternal undernutrition-mediated fetal programming results in different outcomes depending on species, sex, and type of diet. It is dependent on time and length of insult.
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Table 1.1 Consequences of maternal nutrient restriction on adult offspring.

<table>
<thead>
<tr>
<th>Natural or Controlled Diet</th>
<th>Species</th>
<th>Adult Offspring Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor living conditions: Low-birth-weight baby</td>
<td>Human</td>
<td>Coronary heart disease, hypertension, obesity</td>
</tr>
<tr>
<td>Twin pregnancies: The growth restricted baby</td>
<td>Human</td>
<td>Non-insulin-dependent type II diabetes mellitus</td>
</tr>
<tr>
<td>Food restriction due to increased litter size</td>
<td>Pig</td>
<td>Hypertension, glucose intolerance</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>Guinea-pig</td>
<td>Glucose intolerance, insulin deficiency</td>
</tr>
<tr>
<td>Global nutrient restriction</td>
<td>Ovine</td>
<td>Hypertension, smaller livers, females have reduced progesterone secretion during the luteal phase of their estrous cycles and markedly reduced fertility</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>Hypertension, glucose intolerance, insulin deficiency</td>
</tr>
<tr>
<td>Protein deprivation</td>
<td>Rat</td>
<td>Glucose intolerance, relative insulin resistance, hyperinsulinemia, hypertension</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Delay in physical and neurodevelopment</td>
</tr>
<tr>
<td>Global mineral (sodium, copper, iron, magnesium, zinc) or vitamin restriction</td>
<td>Mice</td>
<td>Longevity affected</td>
</tr>
<tr>
<td>Chromium restriction</td>
<td>Rat</td>
<td>Glucose intolerance, insulin resistance, obesity</td>
</tr>
<tr>
<td>Low-sodium diet</td>
<td>Rat</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>Rat</td>
<td>Hypertension</td>
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</tbody>
</table>

3. Placental development during pregnancy has a major impact on pregnancy outcome. Thus, small-for-gestational-age placentas are more likely to result in offspring with metabolic diseases later in life.

4. Genetic imprinting has a major role in placental development. Maternal undernutrition during pregnancy significantly decreases placental IGF2, which negatively affects placental growth.

5. Glucocorticoid treatment changes placental handling and fetal delivery of lactate and selected AA. Glucocorticoids also impact placental expression of GLUT1 and GLUT3 in a dose- and time-dependent manner in both human and rat placentas.

References


