# Maternal Nutrition and Fetal Development[1,2]

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**ABSTRACT Nutrition is the major intrauterine environmental factor that alters expression of the fetal genome and may have lifelong consequences. This phenomenon, termed "fetal programming," has led to the recent theory of "fetal origins of adult disease." Namely, alterations in fetal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology, and metabolism of the offspring, thereby predisposing individuals to metabolic, endocrine, and cardiovascular diseases in adult life. Animal studies show that both maternal undernutrition and overnutrition reduce placental-fetal blood flows and stunt fetal growth. Impaired placental syntheses of nitric oxide (a major vasodilator and angiogenesis factor) and polyamines (key regulators of DNA and protein synthesis) may provide a unified explanation for intrauterine growth retardation in response to the 2 extremes of nutritional problems with the same pregnancy outcome. There is growing evidence that maternal nutritional status can alter the epigenetic state (stable alterations of gene expression through DNA methylation and histone modifications) of the fetal genome. This may provide a molecular mechanism for the impact of maternal nutrition on both fetal programming and genomic imprinting. Promoting optimal nutrition will not only ensure optimal fetal development, but will also reduce the risk of chronic diseases in adults. J. Nutr. 134: 2169–2172, 2004.**

KEY WORDS: • epigenetics • fetus • growth • pregnancy

Maternal nutrition plays a critical role in fetal growth and development. Although considerable effort has been directed towards defining nutrient requirements of animals over the past 30 y, suboptimal nutrition during gestation remains a significant problem for many animal species (e.g., cattle, pigs, and sheep) worldwide (1). Despite advanced prenatal care for mothers and fetuses, ~5% of human infants born in the U.S. suffer from intrauterine growth retardation (IUGR)[4] (2). Over the past decade, compelling epidemiological studies have linked IUGR with the etiology of many chronic diseases in adult humans and animals (**Table 1**) (3). These intriguing findings have prompted extensive animal studies to identify the biochemical basis for nutritional programming of fetal development and its long-term health consequences [e.g., (4–8)]. This article reviews the recent advances in this emerging area of research.

***The Intrauterine Environment as a Major Factor Contributing to IUGR.*** Multiple genetic and environmental factors contribute to IUGR (1). Although the fetal genome plays an important role in growth potential in utero, increasing evidence suggests that the intrauterine environment is a major determinant of fetal growth. For example, embryo-transfer studies show that it is the recipient mother rather than the donor mother that more strongly influences fetal growth (9). There is also evidence that the intrauterine environment of the individual fetus may be of greater importance in the etiology of chronic diseases in adults than the genetics of the fetus. For instance, in twin pregnancies, a baby with fetal growth retardation is more likely to develop noninsulin dependent (type-II) diabetes mellitus than a sibling with normal fetal growth (10). Among intrauterine environmental factors, nutrition plays the most critical role in influencing placental and fetal growth (3).

***Undernutrition and IUGR.*** Maternal undernutrition during gestation reduces placental and fetal growth of both domestic animals and humans (1,3). Available evidence suggests that fetal growth is most vulnerable to maternal dietary deficiencies of nutrients (e.g., protein and micronutrients) during the peri-implantation period and the period of rapid placental development (4–6). In animal agriculture, fetal undernutrition frequently occurs worldwide. For example, the nutrient uptake of grazing ewes in the western United States is often <50% of the National Research Council (NRC) requirement (11). Unsupplemented grazing ewes lose a significant amount of body weight during pregnancy, and their health, fetal growth, and lactation performance are seriously compromised (11). In pigs, a disproportionate supply of nutrients along the uterine horn results in 15–20% low-birth-weight piglets (<1.1 kg), whose postnatal survival and growth performance are severely reduced. Therefore, the poor performance of certain livestock during the postnatal growth and finishing phases may be a consequence of growth restriction in utero.

Undernutrition in pregnant women may result from low intake of dietary nutrients owing to either a limited supply of food or severe nausea and vomiting known as hyperemesis gravidarum (12). This life-threatening disorder occurs in 1–2% of pregnancies and generally extends beyond the 16th week of gestation (12). Pregnant women may also be at increased risk of undernutrition because of early or closely-spaced pregnancies (13). Since pregnant teenage mothers are themselves growing, they compete with their own fetuses for nutrients, whereas short interpregnancy intervals result in maternal nutritional depletion at the outset of pregnancy. Low

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[3] To whom correspondence should be addressed. E-mail: g-wu@tamu.edu.
[4] Abbreviations used: $BH_4$, tetrahydrobiopterin; IUGR, intrauterine growth retardation; NO, nitric oxide; NOS, nitric oxide synthase; NRC, National Research Council; ODC, ornithine decarboxylase; SAM, S-adenosylmethionine.

**TABLE 1**

*Hormonal imbalance, metabolic disorders, and diseases in adult animals and humans with prior experience of intrauterine growth restriction*

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| Hormonal imbalance |
| Increased plasma levels of glucocorticoids and renin; decreased plasma levels of insulin, growth hormone, insulin-like growth factor-I, and thyroid hormones |
| Metabolic disorders |
| Insulin resistance, β-cell dysfunction, dyslipidemia, glucose intolerance, impaired energy homeostasis, obesity, type-II diabetes, oxidative stress, mitochondrial dysfunction, and aging |
| Organ dysfunction and abnormal development |
| Testes, ovaries, brain, heart, skeletal muscle, liver, thymus, small intestine, wool follicles, and mammary gland |
| Cardiovascular disorders |
| Coronary heart disease, hypertension, stroke, atherosclerosis |

birth weights and preterm deliveries in adolescent pregnancies are more than twice as common as in adult pregnancies, and neonatal mortality in adolescent pregnancies is almost three times higher than for adult pregnancies (13). Further, placental insufficiency results in reduced transfer of nutrients from mother to fetus, thereby leading to fetal undernutrition and IUGR (1). Finally, due to competition for nutrients, multiple fetuses resulting from assisted reproductive technologies are often at risk of undernutrition and therefore fetal growth restriction (2). Thus, various nutritional and pathological conditions can result in IUGR.

***Overnutrition and IUGR.*** Significant health problems for animals (particularly companion animals) and women of reproductive age also result from being overweight or obese due to overeating. Overnutrition can result from increased intake of energy and/or protein. Extensive studies have shown that maternal overnutrition retards placental and fetal growth, and increases fetal and neonatal mortality in rats, pigs, and sheep (14). Results of recent epidemiological studies indicate that almost 65% of the adult population in the U.S. is overweight [defined as a body mass index (BMI) $> 25$ kg/m$^2$], while 31% of the adult population is obese (defined as BMI $> 30$ kg/m$^2$) (15). Many overweight and obese women unknowingly enter pregnancy and continue overeating during gestation (16). These women usually gain more weight during the first pregnancy and accumulate more fat during subsequent pregnancies. Maternal obesity or overnutrition before or during pregnancy may result in fetal growth restriction and increased risk of neonatal mortality and morbidity in humans (16).

***Health Problems Associated with IUGR.*** IUGR causes both perinatal and neonatal medical complications. For example, IUGR is responsible for about 50% of nonmalformed stillbirths in humans (2). Infants who weigh <2.5 kg at birth have perinatal mortality rates that are 5 to 30 times greater than those of infants who have average birth weights, while those <1.5 kg have rates 70 to 100 times greater (2). Surviving infants with IUGR are often at increased risk for neurological, respiratory, intestinal, and circulatory disorders during the neonatal period. Both epidemiological and experimental evidence suggest that IUGR contributes to a wide array of metabolic disorders and chronic diseases in adults (Table 1). For example, individuals exposed to the Dutch winter famine of 1944–1945 in utero had higher rates of insulin resistance, vascular disease, morbidity, and mortality in adulthood (17). A cohort study of 15,000 Swedish men and women born between 1915 and 1929 provides by far the most convincing evidence for the close association between reduced fetal growth rate and increased risk of death from ischemic heart disease (18). Thus, the intrauterine environment of the conceptus may alter expression of the fetal genome and have lifelong consequences. This phenomenon is termed "fetal programming," which has led to the recent theory of "fetal origins of adult disease" (3). Namely, alterations in fetal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology and metabolism of the offspring, thereby predisposing individuals to metabolic, endocrine, and cardiovascular diseases in adult life.

***Biochemical Mechanisms of IUGR.*** The lack of knowledge about the mechanisms of IUGR has prevented the development of effective therapeutic options, such that the current management of growth-restricted infants is empirical and is primarily aimed at selecting a safe time for delivery (2). Because nutritional and developmental research often involves invasive tissue collections and surgical procedures, it is neither ethical nor practical to conduct these experiments with the human placenta and fetus. Thus, animal models (e.g., mice, rats, pigs, and sheep) are instrumental for defining the mechanisms of IUGR and developing therapeutic means. Available evidence, which is discussed in the following sections, suggests that arginine [a nutritionally essential amino acid for the fetus (19)] plays a key role in development of the conceptus (embryo/fetus, associated placental membranes, and fetal fluids).

***Crucial Roles of NO and Polyamines in Placental and Fetal Growth.*** Arginine is a common substrate for nitric oxide (NO) and polyamine syntheses via NO synthase (NOS) and ornithine decarboxylase (ODC) (19). NO is a major endothelium-derived relaxing factor, and plays an important role in regulating placental-fetal blood flows and, thus, the transfer of nutrients and $O_2$ from mother to fetus (20). Likewise, polyamines regulate DNA and protein synthesis, and therefore, cell proliferation and differentiation (19,21). Thus, NO and polyamines are key regulators of angiogenesis (the formation of new blood vessels from preexisting vessels) and embryogenesis (22), as well as placental and fetal growth (**Fig. 1**). These crucial roles of NO and polyamines are graphically illustrated by the following findings. First, inhibition of NO synthesis by NOS inhibitors in rats or the absence of NO synthesis in eNOS-knockout mice results in IUGR (23). Second, inhibition of polyamine synthesis prevents mouse embryogenesis, and inhibition of placental polyamine synthesis reduces placental size and impairs fetal growth (21). Third, IUGR in humans is associated with impaired whole body NO synthesis (24) and with decreases in arginine transport, eNOS activity, and NO synthesis in umbilical vein endothelial cells (25). Finally, maternal arginine deficiency causes IUGR, increases fetal resorption and death, and increases perinatal mortality in rats, whereas dietary arginine supplementation reverses fetal growth restriction in rat models of IUGR induced by hypoxia or inhibitors of NOS (26).

***Unusual Abundance of the Arginine-Family Amino Acids in the Conceptus.*** We recently discovered that arginine is particularly abundant in porcine allantoic fluid (4–5 mmol/L) at d 40 of gestation (term = 114 d), when compared with its maternal plasma level (0.13–0.14 mmol/L) (27). Remarkably, concentrations of arginine and its precursor ornithine in porcine allantoic fluid increase by 23- and 18-fold, respectively, between Days 30 and 40 of gestation, with their nitrogen accounting for ~50% of the total free α-amino acid

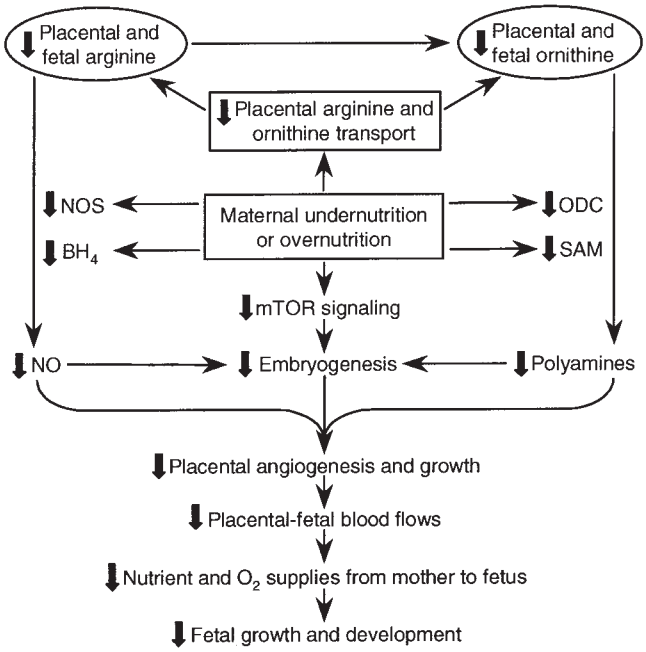


**FIGURE 1** Proposed mechanisms for fetal growth restriction in underfed and overfed dams. Both maternal undernutrition and overnutrition may impair placental syntheses of NO and polyamines, and therefore placental development and utero-placental blood flows. This may result in reduced transfer of nutrients and $O_2$ from mother to fetus, and thus fetal growth restriction. mTOR, mammalian target of rapamycin. The symbol "⬇" denotes reduction.

nitrogen in allantoic fluid (27). Most recently, we found that citrulline (an immediate precursor of arginine) is very rich (10 mmol/L) in ovine allantoic fluid at Day 60 of gestation (term = 147 d) (28). Concentrations of citrulline and its precursor glutamine in ovine allantoic fluid increase by 34- and 18-fold, respectively, between Days 30 and 60 of gestation, with their nitrogen representing ~60% of total α-amino acid nitrogen in ovine allantoic fluid (28). The unusual abundance of the arginine-family amino acids in fetal fluids is associated with the highest rates of NO and polyamine syntheses in ovine placentae in the first half of pregnancy (29,30), when their growth is most rapid (1). These novel findings support the proposed crucial roles of the arginine-dependent metabolic pathways in conceptus development (Fig. 1).

***IUGR and Impaired Syntheses of NO and Polyamines in the Conceptus.*** Maternal undernutrition and hypercholesterolemia during pregnancy (frequently occurring in obese subjects) have profound effects on the synthesis of NO and polyamines. For example, feeding a low-protein diet to pregnant pigs decreases arginine concentration by 21–25% in fetal plasma, allantoic fluid, and placenta, at Day 60 of gestation (31). In addition, allantoic fluid concentrations of arginine and ornithine decrease by ~45% in hypercholesterolemic pigs, compared with normocholesterolemic pigs, at Day 40 of gestation (31). Further, placental NOS and ODC activities are 40–45% lower in protein-deficient pigs than in protein-adequate pigs (4). Similarly, placental NOS activity is reduced by 26% in hypercholesterolemic pigs compared to normocholesterolemic pigs (4). The decreases in substrate availability and enzyme activity contribute to impaired placental syntheses of NO and polyamines in both protein-deficient and hypercholesterolemic pigs (4,31).

Maternal undernutrition in sheep (50% of NRC requirements) between Days 28 and 78 of gestation decreases ($P < 0.05$) concentrations of arginine, citrulline, and polyamines in maternal plasma, fetal plasma, and allantoic fluid by 23–30% at Day 78 of gestation (32). Notably, concentrations of biopterin [an indicator of de novo synthesis of $BH_4$ (an essential cofactor for NOS)] in fetal plasma, amniotic and allantoic fluids are reduced by 32–36% in underfed ewes, compared with control ewes (G. Wu, Texas A&M University, College Station, TX, unpublished results), indicating reduced availability of $BH_4$ for NO production in the conceptus. These changes would impair placental and fetal NO synthesis, thereby resulting in reduced placental-fetal blood flows in underfed ewes (1). Consistent with these findings, maternal undernutrition impairs NO-dependent vasodilation and increases arterial blood pressure in the ovine fetus (33). Similarly, uterine and umbilical blood flows are reduced in overnourished adolescent sheep (14), suggesting a reduction in NO generation by vascular endothelial cells of the uterus and placentae. In obese subjects, high levels of low-density lipoprotein and/or hypercholesterolemia are expected to impair endothelial NO synthesis through mechanisms involving: *1*) reduced availability of $BH_4$ likely due to oxidative stress; *2*) reduced expression of NOS; and *3*) inactivation of NOS due to its close association with caveolin-1 (34). These results have led to our hypothesis that impaired placental syntheses of NO and polyamines may provide a unified explanation for IUGR in response to the two extremes of nutritional problems with the same pregnancy outcome (Fig. 1).

***Molecular Mechanisms of Fetal Programming.*** Nutritional insult during a critical period of gestation may leave a permanent "memory" throughout life, and some of the effects (e.g., insulin secretion and action) may be gender-specific (5). There is growing evidence that maternal nutritional status can alter the epigenetic state of the fetal genome and imprint gene expression. Epigenetic alterations (stable alterations of gene expression through covalent modifications of DNA and core histones) in early embryos may be carried forward to subsequent developmental stages (6). Two mechanisms mediating epigenetic effects are DNA methylation (occurring in 5′-positions of cytosine residues within CpG dinucleotides throughout the mammalian genome) and histone modification (acetylation and methylation) (35). CpG methylation can regulate gene expression by modulating the binding of methyl-sensitive DNA-binding proteins, thereby affecting regional chromatin conformation. Histone acetylation or methylation can alter the positioning of histone-DNA interactions and the affinity of histone binding to DNA, thereby affecting gene expression (35).

DNA methylation is catalyzed by DNA methyltransferases, with S-adenosylmethionine (SAM) as a methyl donor (35). SAM is synthesized from methionine and ATP by methionine adenosyltransferase. One-carbon unit metabolism, which depends on serine, glycine, and B vitamins (including folate, vitamin B-12, and vitamin B-6), plays an important role in regulating the availability of SAM (6). Thus, DNA methylation and histone modifications may be altered by the overall availability of amino acids and micronutrients. This notion is supported by several lines of evidence. First, a deficiency of amino acids results in marked reduction in genomic DNA methylation and aberrant expression of the normally silent paternal H19 allele (an imprinted gene) in cultured mouse embryos (36). Second, uteroplacental insufficiency causes hypomethylation of p53 gene in postnatal rat kidney (7), as well as global DNA hypomethylation and increased histone acetyla-

tion in postnatal rat liver (8). Third, maternal supplementation of methyl donors and cofactors (folic acid, vitamin B-12, choline, and betaine) increases CpG methylation at the $A^{vy}$ locus of agouti mice, and the methylation patterns are retained into adulthood (6). It remains to be determined whether maternal nutrition affects CpG methylation of the genes for NOS, GTP cyclohydrolase I (the rate-limiting enzyme for $BH_4$ synthesis) and ODC, or alters histone modifications, in the uterus, placenta, as well as fetal and postnatal tissues (e.g., the vascular bed, adipose tissue, liver, kidney, skeletal muscle, or pancreas). Nevertheless, epigenetics may provide a molecular mechanism for the impact of maternal nutrition on fetal programming of postnatal disease susceptibility and on genomic imprinting (the parent-of-origin-dependent expression of a single allele of a gene) (6–8).

***Concluding Remarks and Perspectives.*** Placental and fetal growth is most vulnerable to maternal nutrition status during the peri-implantation period and the period of rapid placental development (the first trimester of gestation). Maternal undernutrition or overnutrition during pregnancy can impair fetal growth. Although full enteral feeding may be potentially effective in reversing IUGR in underfed mothers, this approach is not applicable under such conditions as severe nausea and vomiting. Conversely, reducing food intake may help prevent IUGR in overfed dams, but this intervention may not be as simple as one would think due to the powerful biological mechanisms that control food intake in mammals (including humans) (15). Thus, new knowledge of the mechanisms regulating fetal growth and development will be beneficial for designing new therapeutic strategies to prevent and treat IUGR. Understanding the multiple roles of nutrients in DNA methylation (which can influence genome stability, viability, expression, and imprinting) will have a broad impact on reproductive health and disease prevention. We expect that studies utilizing animal models of IUGR will provide the much-needed scientific basis for the development of patient management practices that will improve pregnancy outcome in humans. In view of the crucial roles of the arginine-dependent metabolic pathways, intravenous or oral administration of arginine may provide a potentially novel solution to enhancing placental-fetal blood flows (and therefore transfer of nutrients and $O_2$ from mother to fetus), thereby improving fetal growth. Promoting an optimal intrauterine environment will not only ensure optimal fetal development, but will also reduce the risk of chronic diseases in adults.

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