One-Carbon Metabolism, Fetal Growth and Long-Term Consequences

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Abstract

One-carbon metabolism, or methyl transfer, is critical for metabolism in all cells, is involved in the synthesis of purines, pyrimidines, in the methylation of numerous substrates, proteins, DNA and RNA, and in the expression of a number of genes. Serine is the primary endogenous methyl donor to the one carbon pool. Perturbations in methyl transfer due to nutrient and hormonal changes can have profound effect on cell function, growth and proliferation. It is postulated that at critical stages in development, nutrient and environmental influences by their effect on methyl transfer can impair fetal growth, reprogram metabolism and cause long-term morbidity in the offspring. The potential for their effects is underscored by the unique gestation-related changes in methyl transfer in healthy women, the late expression of transsulfuration cascade in the fetus and the unique metabolism of glycine and serine in the fetus. Dietary protein restriction in animal models and protein malnutrition in humans causes remarkable changes in the methyl transfer in vivo. Although the specific consequences of perturbation in maternal and fetal methyl transfer remain to be determined, a profound influence is suggested by the demonstrated relationship between maternal folate and B12 insufficiency and metabolic programming.

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Introduction

Epidemiological and observational data from studies in human and data from experimental animal models have now established the relationship between impaired fetal growth and long-term morbidity in the offspring [1–4]. These data suggest that nutritional and environmental influences at critical times during
development can lead to programming of the metabolism of the fetus, cause alterations in growth and the development of chronic noncommunicable diseases in adult life (the Developmental Origins of Health and Disease paradigm). Although much has been learnt regarding the molecular, metabolic and physiological associations and mechanisms of the changes in the offspring, the metabolic and physiological changes in the mother that mediate the observed changes in the developing organism have not been studied in detail. Methyl transfer or one-carbon metabolism is the key component of cellular metabolism, is involved in synthesis of purines, pyrimidines, and methylation of a number of substrates, proteins, DNA, RNA and indirectly, expression of a number of genes. The non-essential amino acid serine, folate and the essential amino acid methionine constitute the key components of methyl transfer. Since the methionine and folate cycles are ubiquitously present in every cell in the body and participate in key metabolic reactions, perturbation in their metabolism by nutrient deficiency, or by nutrient, hormonal and environmental interactions can have profound impact on the cell function, metabolism, growth and proliferation. Interest in the physiological changes in maternal-fetal and neonatal one-carbon metabolism, particularly in humans, has been primarily focused on the consequence of micronutrient deficiencies. Clinical, physiological and molecular data from studies in human and those from animal models suggest that alterations in methyl transfers may be critical contributors to the impaired fetal growth and to the re-programming of the metabolism of developing embryo and the fetus, leading to morbidity in the offspring. In this review, some of the data in support of such a concept are presented. Future studies will delineate the hormonal, nutritional and environmental contributors to such perturbations and allow us to develop targeted intervention strategies.

One-Carbon Metabolism (Methyl Transfers) in vivo

One-carbon pool refers to the pool of the methyl groups that are available for the methylation of a variety of compounds, including protein, DNA, RNA, etc. This process involves the methyl form of tetrahydrofolate (THF) and is carried out by s-adenosyl methionine (SAM). As shown in figure 1, the transfer of the methyl group involves both the folate cycle and the ubiquitous methionine cycle, also called the transmethylation cycle or the ‘activated’ methionine cycle. L-serine, a nutritionally non-essential amino acid, is the primary endogenous methyl donor. Stable isotope tracer studies in young healthy volunteers show that almost 100% of the methyl groups used for whole body remethylation of homocysteine are derived from serine under the conditions of their study [5]. The
latter is important, since the tracer studies were done after an overnight fast, however in a fed state, while the subjects were given a protein-free isocaloric diet. Previous data in isolated perfused liver preparation had also shown serine to be the major contributor to the methyl groups used for the transmethylation of homocysteine. During fasting, serine is released into the circulation by the kidney and is taken up by most organs, including the skeletal muscle and the liver, the latter being quantitatively the most important consumer of serine [6, 7]. As shown in figure 1, the methyl group (β-carbon) of serine is transferred to THF, catalyzed by serine hydroxyl methyl transferase, resulting in the formation of glycine and N5-N10-methylene THF, which is then converted to 5-methyl THF, catalyzed by methylenetetrahydrofolate reductase. The methylation of homocysteine involves the transfer of the methyl group from 5-CH3THF by methio-
nine synthase. Vitamin B₁₂ is a cofactor for this reaction. The other contributor to the methyl groups for methylation of homocysteine, primarily in the liver, is betaine; however, its quantitative contribution has not been measured in vivo. SAM is the key intermediate of the activated methionine cycle, is the universal bioactive methyl donor and participates in numerous methyl transferase reactions resulting in methylated products (X-CH₃). SAM is also the primary allosteric regulator of the methionine metabolism in vivo. The catabolic pathway of methionine is the transsulfuration cascade. This pathway involves the condensation of homocysteine with serine to form cystathionine, which is then converted to cysteine, α-ketobutyrate and ammonia catalyzed by cystathionine γ-lyase. Cysteine is the precursor of taurine as well as a component of glutathione, the major intracellular antioxidant.

The metabolism of serine, folate and methionine has been studied extensively and discussed in several reviews. It is important to note that folate cobalamine (B₁₂) and pyridoxine (B₆) are directly involved in the metabolism of methionine. Insulin and glucagon exert their effect on methionine metabolism by (a) directly affecting the activity of transsulfuration cascade and methionine synthase and (b) indirectly by their effect on whole-body protein turnover and therefore affecting methionine flux. In addition, several reactions in the methionine metabolism respond to the change in the cellular redox state.

*Methionine and Serine Metabolism in Human Pregnancy*

The adaptive changes in methionine metabolism during pregnancy have been examined by us in healthy women studied early and late in gestation and compared with non-pregnant controls [8]. The data show that the fractional rate and the total rate of transsulfuration of methionine were significantly increased during early gestation with a decrease to the rate seen in non-pregnant subjects in the third trimester. In contrast, the rate of transmethylation, a measure of one-carbon transfers, was markedly higher in the third trimester of pregnancy. The high rates of transmethylation were speculated to be the consequence of higher methylation demands in the later part of pregnancy.

The rate of appearance of serine was quantified in healthy pregnant women by Kalhan et al. [9] using [2-¹⁵N¹³C]serine tracer. Plasma serine concentration and the rate of appearance of serine were lower in pregnant women in both early and in late gestation compared with healthy non-pregnant women. The rate of appearance of serine was significantly less in the 3rd trimester of pregnancy when compared with early pregnancy [serine Ra: early (n = 12) 123.7 ± 21.5, late (n = 8) 102.8 ± 18.2 μmol·kg⁻¹·h⁻¹, mean ± SD]. The isotopic tracer used in this
study would have recycled between serine and glycine and therefore would have resulted in an underestimation of serine flux. The lower estimation of serine flux would be proportional to the magnitude of tracer recycling. Assuming no significant change in actual serine turnover between early and late gestation, the higher rate of recycling is qualitatively consistent with the data in sheep fetus showing a higher rate of serine glycine flux in the fetal compartment (discussed below) and with the higher rate of transmethylation of methionine observed in healthy pregnant women [8].

**Serine and Glycine Metabolism in the Placenta and the Fetus**

The transport of serine and glycine from the mother to the fetus has been examined both in animal models and in humans. The data in humans have been obtained at term gestation. These data show a higher concentration of both serine and glycine in the fetal umbilical vein than in a simultaneously obtained maternal arterial sample [10, 11]. In addition, an infusion of amino acid mixture to the mother prior to caesarean section delivery resulted in a significant increase in all amino acids including serine and glycine in the fetus [12]. Bolus infusion studies using tracer isotopes of glycine, leucine and phenylalanine in human pregnancy show a much greater dilution or lower enrichment of glycine tracer in the fetal compartment as compared with leucine or phenylalanine [13]. The authors interpreted these data to suggest a slower transfer rate for glycine when compared with leucine or phenylalanine. However, as also suggested by them, these data could indicate a significant production of glycine by the fetus or by the placenta [13]. However, as shown by Lewis et al. [14], a low serine hydroxymethyltransferase activity in the human placenta (as compared with sheep) would suggest that placental production of glycine from serine may not be a significant source of fetal glycine. Other data in chronically catheterized sheep fetus show a uteroplacental uptake of serine from the mother, no net transport of serine from the mother to the fetus and a virtual equimolar (to serine uptake) release of glycine from the placenta into the fetal compartment [15, 16]. These data suggest that the ovine placenta converts large quantities of maternal serine into glycine and releases it into the fetus. Additionally, studies by Cetin et al. [17, 18] demonstrated production of serine from glycine by the fetal liver in a chronically catheterized sheep preparation. Thus, a unique inter-organ cycling of serine and glycine occurs in the sheep fetus and placenta, where glycine is released by the placenta into the fetal circulation and is then taken up by the fetal liver and converted to serine which in turn is taken up by the placenta in substantial quantities. Of significance were remarkably high turnover rates of serine and glycine, as compared with leu-
cine, in the fetus (glycine: \( \sim 720 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \); serine: \( \sim 2,700 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \); leucine: \( \sim 52 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)). Taken together, these studies suggest a high rate of serine and glycine interconversion, and therefore methyl (one-carbon) transfers in the placenta and the fetal liver with some quantitative differences between species. Such studies cannot be done in humans; however, these data are consistent with the higher rates of transmethylation measured in human pregnancy during the third trimester using stable isotopic tracers [8].

**One- Carbon Metabolism and the Fetus**

*Effect of Dietary Protein*

Isocaloric protein restriction in pregnancy in the rodents impairs fetal growth and causes programming of the metabolism in the offspring [reviewed in 3, 4]. Collectively, these data show that protein restriction during various periods of pregnancy results in fetal growth retardation and is associated with pancreatic dysfunction, impaired glucose homeostasis, hypertension, changes in the circadian rhythm and other metabolic dysfunctions in the offspring. The possible mechanisms of these changes in the fetus and the offspring have been reported and discussed in several excellent reviews [1–4]. However, there is paucity of data regarding changes in the maternal metabolism that are responsible for the observed responses in the fetus. Petrie et al. [19] reported high serum concentrations of homocysteine early in pregnancy (day 4) in rat placed on protein-restricted diet. Other changes in maternal metabolic and amino acid patterns have been reported. These data are difficult to interpret because of the differences in time of sampling in relation to gestation, the controls not being pair fed and significant differences in dietary regimens employed. It is also important to recognize the differences in the metabolic responses to isocaloric protein restriction as compared with simple protein restriction. Simple protein, energy restriction could be in part compensated by an increase in overall food consumption. In addition, isocaloric restriction results in suppression of adipose tissue and skeletal muscle responses seen with total energy restriction, i.e. mobilization of fatty acids and amino acids from lipolysis and proteolysis.

Parimi et al. [20] examined changes in plasma amino acids in pair-fed pregnant rats on a protein-restricted diet. They showed no change in total \( \alpha \)-amino nitrogen in the plasma in early gestation and an increase late in pregnancy. The increase in total \( \alpha \)-amino nitrogen was primarily due to increase in serine, glycine and glutamine concentration. Rees et al. [21] also reported a significant increase in the plasma glycine concentration in response to dietary protein restriction in
pregnant rat. Since serine and glycine are primary methyl donors, a change in their levels, along with higher homocysteine levels noted above, suggests change in one-carbon or methyl transfer in response to dietary protein restriction. Although such studies cannot be done in humans, an observational study in (non-pregnant) humans showed that subclinical protein malnutrition evidenced by lower transthyretin levels was associated with increase in plasma homocysteine levels, suggesting an impaired methionine-homocysteine metabolism [22]. The plasma concentration of most essential amino acids was lower in the malnourished group, while there was no significant change in the plasma methionine levels, suggesting an independent regulation of methionine levels possibly by down-regulating the transsulfuration cascade. The plasma concentrations of pyridoxal-5-phosphate (vitamin B₆) and folate of the malnourished group were not different from controls, while cobalamines (vitamin B₁₂) were lower only in the severely malnourished (state III) subjects [22]. Recently, the same investigators have reported hyperhomocysteinemia in a vegetarian, plant-eating population of Chad, who had a lower dietary intake of protein and sulfur amino acids [23]. Their data suggest a direct effect of lower protein intake on methionine-homocysteine and one-carbon metabolism. Our group has examined the effect of isocaloric protein restriction in the rat on methyl transfers and methionine metabolism [24]. The data show that dietary protein restriction, in this instance for 7–10 days, resulted in profound change in hepatic metabolism (fig. 2), was associated with differential expression of a number of genes involved in cell cycle, differentiation, transcription, transport, and other metabolic processes. Of importance, there was a marked increase in serine biosynthesis and methionine transmethylation and a decrease in the activity of transsulfuration cascade (fig. 2). All these data underscore the important effect of adequate protein intake on methyl transfers, an effect that is independent of the vitamin status. The observed fetal programming effects of maternal protein restriction could be mediated via changes in one-carbon metabolism in the mother. Such a hypothesis is supported by the data demonstrating an amelioration of the changes caused by protein restriction when the animals were supplemented with methyl donors like glycine and folate [25].

Folate and Vitamin B₁₂

The clinical evidence relating maternal folate and vitamin B₁₂ status during pregnancy and the long-term consequences for the offspring is discussed in the chapter by Deshmukh et al. [pp. 145–156]. As noted above, folate is an integral part of the transfer of methyl groups from serine or other methyl donors like betaine for the methylation of homocysteine to form methionine (fig. 1). Vi-
tamin B\textsubscript{12} is a cofactor for the enzyme methionine synthase (homocysteine methyl transferase). Insufficiency of either folate or B\textsubscript{12} will affect the methyl transfer by influencing methylation of homocysteine. Iatrogenically induced folate deficiency in healthy human subjects resulted in significant increase in plasma homocysteine levels and attenuation of the rate of methionine synthesis via remethylation of homocysteine\textsuperscript{[26]}. Such kinetic studies have not been done in B\textsubscript{12}-deficient subjects; however, B\textsubscript{12} deficiency by decreasing the activity of methionine synthase will be expected to suppress methylation of homocysteine. These data indicate that both folate and B\textsubscript{12} deficiencies can influence maternal-fetal metabolism and cause long-term consequences by effecting methyl transfers in the mother and the fetus. A direct evidence of such a cause and effect relationship is not available as yet. Folate deficiency during pregnancy in the rat has been shown to effect methyl metabolism; however, it did

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**Fig. 2.** Effect of isocaloric protein restriction on methyl transfer in the rat. Rats were placed on a casein-based 6\% protein diet for 7–10 days; controls were on a 24\% protein diet and were pair fed. Dietetic protein restriction resulted in a marked upregulation (dark grey boxes) of 3-phosphoglycerate dehydrogenase (3PGDH), phosphoserineaminotransferase (PSAT), glutamate-cysteine ligase (GCL), and cysteinesulfonic acid decarboxylase (CSAD). The activity of \textgamma S and \textgamma L was decreased (light grey boxes). Tracer dilution-measured rate of appearance of serine and the rate of transmethylation of methionine were increased (thick arrows). Figure reproduced from Kalhan et al.\textsuperscript{[24]}. 
not impact global DNA methylation in the fetus [27]. Whether there were changes in the methylation pattern of specific genes was not determined in that study.

**Homocysteine**

Alterations in one-carbon metabolism as a result of nutrient or other influences result in an increase in plasma concentrations of homocysteine, the demethylated product of methionine and an intermediate amino acid in the methionine transmethylation cycle (fig. 1). Homocysteine does not participate in protein synthesis and an increase in plasma levels of homocysteine reflects altered intracellular homocysteine and one-carbon metabolism. The relationship between elevated homocysteine levels and vascular disease, thrombosis, vascular endothelial dysfunction has been studied extensively and discussed in excellent scientific reviews [28]. A number of studies have shown a pregnancy-related decrease in plasma homocysteine concentration in healthy women. Whether homocysteine directly causes impaired fetal growth or via its effect on placental growth and function via its vascular effects has not been delineated in studies in human. An association between elevated levels of homocysteine and pregnancy-related disorders such as preeclampsia, early pregnancy loss and abruptio placentae has been reported [reviewed in 29]. A recent systematic review and meta-analysis by Hogeveen et al. [30] concluded that higher maternal total homocysteine concentrations are associated with a small increased risk of small-for-gestational-age offspring. It corresponded to a decrease in birthweight of 31 g (95% CI: –13 to –51 g) for 1-SD increase in maternal total homocysteine. The authors concluded that the small birthweight difference might be of little clinical relevance, but may be of greater importance at the population levels. However, the long-term programming consequences of such impairment of growth have not been determined. It is likely that the attenuated fetal growth, as reflected in lower birthweight is only a crude reflection of the altered metabolic and epigenomic changes in the growing fetus that may lead to programming and morbidity in later life.

**Multinutrient Deficiencies**

As noted above, folate and B₁₂ deficiencies cause a decrease in the transmethylation pathway be impacting the transfer of the methyl groups to homocysteine. In contrast, protein malnutrition in humans and in animal models causes an increase in transmethylation, in addition to suppressing the transsulfuration
cascade. On the other hand, deficiency of B₆ did not appear to have any significant effect on activated methionine cycle. Thus the net effect of these opposing responses to multiple nutrient insufficiency, commonly seen in developing societies, is not known. It is speculated that the combined effect of multiple nutrient insufficiency on one carbon metabolism will be the sum of individual affects and could result in a spectrum of responses from low rate of transmethylation (dominant vitamin B₁₂ and folate deficiency) to high rates of transmethylation (in a predominant protein deficiency state) and modified by the hormonal milieu and the physiological adaptation (as in healthy pregnancy) of the organism. Such effects cannot be discovered from single measurement of biomarkers such as measurement of plasma homocysteine, SAM/SAH ratio or by measurement of single micronutrient. These responses may also explain the lack of significant effect on for example birthweight when nutrient intervention involves a single micro- or macronutrient.

**Conclusions**

Methionine, an essential amino acid, along with folic acid, are key components of one-carbon metabolism in every cell in the body. Specific changes in the one-carbon metabolism have been identified in the mother during pregnancy, in the placenta and in the fetus during development. Methionine and one-carbon metabolism can easily be altered by nutrient and hormonal mediators and may cause specific changes in many organs in the mother, placenta and the fetus. We hypothesize that changes in one-carbon metabolism as a result of nutrient (vitamins and protein) insufficiency cause fetal growth retardation by nutrient-gene interaction and cause permanent changes leading to adult disease like diabetes, obesity and hypertension.

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The author declares that no financial or other conflict of interest exists in relation to the content of the chapter.
References