Metabolism of Methionine in Vivo: Impact of Pregnancy, Protein Restriction, and Fatty Liver Disease

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Abstract

The coexistence of intrauterine and neonatal malnutrition and the development of obesity, type 2 diabetes and related comorbidities have been confirmed in a number of studies in humans and animal models. Data from studies in animals suggest that epigenetic changes as a result of altered methylation of the genomic DNA may be responsible for such metabolic patterning. Methionine, an essential amino acid, plays a critical role in the methyltransferases involved in the methylation by providing the one-carbon units via the methionine transmethylation cycle. Because of its interaction with a number of vitamins (B12, folate, pyridoxine), its regulation by hormones, i.e. insulin and glucagon, and by the changes in redox state, methionine metabolism is effected by nutrient and environmental influences and by altered physiological states. In the present review the impact of human pregnancy, dietary protein restriction and fatty liver disease on methionine metabolism is discussed. The role of methionine in metabolic programming in a commonly used model of intrauterine growth retardation and in propagation of fatty liver disease is briefly described.

Introduction

The rapid urbanization and improving economic wellbeing in emerging societies has been associated with high rates of obesity, insulin resistance, type 2 diabetes and coronary heart disease. At the same time, low birthweight or intrauterine growth retardation (malnutrition) remains a persistent problem, accounting for as many as 30% of the births in several countries, including India [1]. Almost 75% of low birthweight infants are born at full term. A number of epidemiological studies in humans from different parts
of the world have shown a correlation between intrauterine growth restriction (IUGR), resulting in thinness or small size at birth (low birthweight) and chronic disease in adults. Data from animal studies, particularly in the rodent, show that alterations in the intrauterine environment caused by nutrient interventions in the mother, e.g. protein or calorie restriction, iron deficiency or by hypoxemia, result in restricted growth of the fetus and long-term consequences. The latter include, amongst others, changes in hypothalamic pituitary axis, altered expression of glucose transporters and changes in glucose uptake by the fetus and neonate, chronic hypertension, changes in vascular reactivity, obesity and type 2 diabetes [2–5]. The mechanism of the ‘permanent’ effect, also termed imprinting or programming, remains a subject of investigation.

In the commonly used model of IUGR, i.e. dietary protein restriction of the mother, Rees et al. [6] have shown hypermethylation of the genomic DNA in the fetal liver. Methylation of cytosine in the DNA has been related to the activities of a number of mammalian genes, somatic inheritance and cellular differentiation [7]. The activation of some genes has been attributed to the demethylation of critical CpG loci. However, as reviewed by Jones and Takai [8], this is an oversimplification. Methylation changes the interactions between protein and DNA, leading to alterations in chromatin structure and in either a decrease or increase in the rate of transcription. Studies of transgene methylation have shown that methylation patterns can be inherited in a parent-of-origin specific manner, suggesting that DNA methylation may play a role in genomic imprinting (or programming) [9]. DNA methylation can be effected by the availability of methyl groups and by changes in one-carbon metabolism by dietary and vitamin modifications. In this context, Lillycrop et al. [10] observed that dietary protein restriction of pregnant rats resulted in lower methylation and higher expression of PPARα, acyl-CoA carboxylase and glucocorticoid receptor genes in the livers of the offspring after weaning. Of interest, supplementation with folic acid prevented these epigenetic modifications. These studies are particularly relevant when interpreting the data of nutrition–gene interactions and other epidemiological correlations, particularly from societies with nutrient deficiencies [11]. Methionine, along with homocysteine and folate, are key components of the one-carbon metabolism [12]. Perturbations in the methionine cycle and secondarily DNA methylation could cause metabolic programming in the fetus and neonate that lead to morbidity in adult life. On the other hand, in the developed organism, perturbations in the methionine metabolism due to endocrine and metabolic changes in the tissue and organism could make it more prone to injury and thus propagation of the disease.

In the present review the alterations in the metabolism of methionine induced by normal human pregnancy, dietary protein restriction and with ectopic fat accumulation in nonalcoholic fatty liver disease (NAFLD) are discussed. The changes during development are speculated to result in the
Metabolism of Methionine

Methionine, an essential amino acid required for protein synthesis, is an important source of methyl groups for a number of important methylation reactions such as transmethylation of nucleic acids (methylation of DNA in gene expression), protein, biogenic amines and phospholipids, etc. [12, 13]. Methionine is metabolized first by the ubiquitous transmethylation (or methionine) cycle wherein the methyl groups from methionine or from the folate-dependent one-carbon pool participate in various methyltransferase reactions (fig. 1). During transmethylation, methionine is converted to its active form S-adenosylmethionine (SAM), which is the major substrate for the methylation reactions. Following SAM-dependent transmethylation, the product S-adenosylhomocysteine (SAH) is metabolized to adenosine and homocysteine. Homocysteine is either converted to cysteine via transsulfuration pathway, or remethylated back to methionine. The methyl group required for remethylation is obtained from either the folate-dependent one-carbon pool (5-methyltetrahydrofolate) or from betaine (in the liver). SAM is also converted to SAH by glycine N-methyltransferase (GNMT), an enzyme abundant in the liver where it makes up a very large component (~1%) of soluble proteins in the cytosol. It transfers a methyl group from SAM to glycine, resulting in the formation of N-methylglycine (sarcosine) and SAH. The function of GNMT is believed to serve as an alternate pathway for the conversion of SAM to SAH in order to maintain a normal SAM/SAH ratio [13, 14]. SAH is a potent inhibitor of most methyltransferases, and therefore the ratio of SAM/SAH is an index of methylation potential. SAM is also an allosteric inhibitor of 5,10-methylenetetrahydrofolate reductase (MTHFR), the enzyme that catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and an allosteric activator of cystathionine β-synthase (CBS). On the other hand, 5-methyltetrahydrofolate is an inhibitor of GNMT. Finally, tetrahydrofolate also plays an important role in the catabolism of histidine in the conversion of N-formimino glutamate (Figlu) to glutamate. The synthesis of SAM by SAM synthase is regulated by hypoxia [15], glutathione [16], availability of methionine [17], and modified by oxidant injury [18] and redox state [19] of the cell.

The transmethylation cycle does not result in catabolism of methionine. While methionine provides the carbon carrier for the cycle, the majority of the methyl groups for the methyltransferase reactions are provided by glycine and serine via the folate-dependent one-carbon pool. As shown in figure 2, the methionine transmethylation cycle is interdependent upon the folate cycle as the source of methyl groups for the conversion of patterns of expression of certain genes in the fetus, while the perturbations in NAFLD are suggested to cause injury and propagation of the disease.
homocysteine to methionine catabolized by methionine synthase (tetrahydrofolate methyltransferase).

As discussed by Brosnan and Brosnan [13], methionine metabolism requires a number of vitamins as cofactors, so that nutrient deficiencies can easily impact methionine metabolism (table 1). The key vitamins required for methionine metabolism are B12 for methionine synthase, folic acid for one-carbon pool, riboflavin for MTHFR, and pyridoxine for the transsulfuration pathway – CBS and CGL. In addition, the oxidative decarboxylation of the \( \alpha \)-ketobutyrate catalyzed by pyruvate dehydrogenase requires niacin/thiamine and pentothenic acid (coenzyme A). Thus it is not surprising that deficiencies of these vitamins can impact methionine metabolism and may be associated with elevated plasma levels of homocysteine and other metabolic intermediates.

**Fig. 1.** Pathways of metabolism of methionine and its relation to the folate cycle are displayed (details in text).

Human Pregnancy

There are very few data on methionine metabolism in human pregnancy. With the increased interest in the relationship between perturbations in one-carbon metabolism and its role in epigenetics and the developmental origin
of adult health and disease, some new data have been published. However, human data are often confounded by the interactions between nutrient intake, nutrient status and other regional/societal interactions, and hence require careful interpretation. This is particularly important when evaluating data from societies with nutritional (vitamin) insufficiency. The data from studies in humans have been further confounded by the recent fortification of flour with folic acid in many countries.

The primary source of plasma methionine during fasting is that released from protein breakdown. Since the rate of breakdown of proteins has been shown to decrease during normal human pregnancy, there is a decrease in the concentrations of all amino acids in the plasma compartment [20]. Thus the plasma concentrations of all essential amino acids, including methionine, are lower during pregnancy when compared with non-pregnant women. Recent data from several studies show that the concentration of total homocysteine in the plasma is lower in normal healthy women in the 3rd trimester [21–23]. In fact, a progressive decrease in homocysteine concentration is evident starting early in pregnancy. This is associated with a progressive increase in plasma choline concentration. Of interest, the circulating levels of folate and vitamin B₁₂, respectively, increase and decrease with advancing gestation. The increase in plasma levels of choline have been interpreted as a mechanism to provide the increasing demands for choline by the fetus. In studies in the rat, the increase in plasma choline is associated with a decrease in the content of choline in the liver with advancing gestation. A decrease in

**Fig. 2.** Potential mechanism of propagation of hepatocellular injury in fatty liver disease (see text for details).
hepatic choline content would impact the remethylation of homocysteine by lowering the availability of betaine. Although a number of significant statistical correlations have been observed for these metabolites, the mechanism of these changes and their physiological significance remains to be elucidated. Examination of the maternal–fetal gradients for these metabolites show that homocysteine is transported across the placenta along a concentration gradient so that the umbilical venous concentrations of tHcy are lower than in the simultaneously obtained maternal venous blood. In addition, the umbilical arterial concentrations of tHcy are lower than that in the umbilical vein, suggesting fetal utilization of homocysteine. Finally, the umbilical venous concentrations of methionine, choline, betaine and dimethylarginine were higher when compared with those in maternal venous blood [21].

Data from studies by Sturman et al. [24] and Gaull et al. [25], and recent studies of Levonen et al. [26], have shown the absence of transsulfuration activity in the human fetal liver and thus the inability of the fetus to synthesize cysteine. This is because of the low activity of CβS and the absence of cystathionine γ-lyase (CγL; protein) in the fetal liver. The fetal utilization of homocysteine and the umbilical uptake of betaine could be interpreted to support the transmethylation of methionine and homocysteine, and methylation demands in the fetus. These data would also suggest that any perturbations in the maternal methionine metabolism as a result of nutritional/environmental perturbations could have a significant impact on the fetal one-carbon metabolism.

### Dietary Protein Restriction and IUGR

The dietary protein restriction during pregnancy model of IUGR has been used by a number of investigators to study the consequences of fetal growth retardation. These data show that protein restriction in pregnancy causes IUGR, selective changes in organ growth and permanent growth retardation, impaired insulin secretion, alterations in hepatic glucokinase expression and hepatic glucose output, and hypertension in the offspring [2–4]. These

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<th>Table 1. Vitamins and methionine metabolism</th>
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<td>Cobalamine (B₁₂)</td>
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<td>Niacin (NAD)</td>
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changes have been associated with changes in the hypothalamic-pituitary adrenal axis, changes in the reactivity of the vasculature, changes in the renin-angiotensin system and alterations in the catecholamine levels and adrenoceptors in various tissues. Data from our studies suggest a higher rate of protein synthesis in the maternal liver and a lower rate of protein synthesis in the fetal liver in response to protein restriction [27]. In relation to methionine metabolism, dietary protein restriction in non-pregnant rats resulted in a marked increase in plasma, hepatic and skeletal muscle concentrations of serine and glycine, a significant change in hepatic SAM:SAH ratio, and downregulation of the hepatic transsulfuration pathway. These changes were associated with a change in the arterial-protein vein gradient for glycine and serine, resulting in a significant release of serine and a lower uptake of glycine by the gut [28]. Gene array analysis showed a marked upregulation of genes involved in serine and taurine biosynthesis (unpublished data). The biological significance of the changes in relation to whole body metabolism and to fetal growth remain to be determined.

**Methionine and Nonalcoholic Fatty Liver Disease**

NAFLD constitutes a wide spectrum ranging from the benign accumulation of triglycerides in the hepatocytes (hepatic steatosis) to steatohepatitis with inflammation, fibrosis and cirrhosis. Insulin resistance, obesity and ectopic fat (in the liver) are the hallmarks of the disorder [29]. It has been proposed that as a result of insulin resistance, there is an increase in the rate of lipolysis, which is not suppressed by infusion of glucose and insulin (clamp), resulting in a high rate of delivery to and uptake by the liver of free fatty acids. The accumulation of triglycerides in the liver (steatosis) has been attributed to the high uptake, high de novo lipogenesis, and to a decreased export of the lipids [30]. Hepatic steatosis results in hepatic insulin resistance, altered peroxisomal and microsomal lipid metabolism, lipid peroxidation and in increased production of reactive oxygen species (ROS), which can disrupt the mitochondrial membrane potential and cause a decline in the generation of hepatic ATP and lower ATP levels.

The changes in methionine metabolism are related to the insulin resistance and to the changes in hepatic metabolism as a result of ectopic fat accumulation. The data from human studies are confounded by the associated disorders, e.g. change in renal function, in addition to the possible differences in micronutrients, especially vitamin intake. Although the effects of insulin and insulin resistance on the metabolism of methionine have not been examined in humans, certain inferences can be drawn from the published data of studies in humans and animals. Studies in the rat show that a lack of insulin induced by streptozotocin results in lower plasma homocysteine levels and an increase in the activity of hepatic CβS and CyL [31]. The activities of enzymes
involved in the transmethylation of methionine (MS, betaine:homocysteine methyltransferase, MTHFR) were not impacted by a lack of insulin [31, 32]. Insulin treatment reversed these changes. Similar changes in enzyme activity have been reported by the longitudinal studies of ZDF (fa/fa, type 2) diabetic rats [33]. Studies in patient with type 1 diabetes show that insulin deprivation results in a lower rate of transmethylation and a higher rate of transsulfuration, and that both effects could be reversed by insulin administration [34]. Somewhat variable effects have been observed by other investigators in studies in healthy subjects and in those with type 2 diabetes [35, 36]. Other data from humans in the insulin resistance state have shown that hyperhomocysteinemia is associated with hyperinsulinemia and insulin resistance [37].

In contrast to the repressive effect of insulin on transsulfuration and therefore decreased synthesis of cysteine, both insulin and hydrocortisone have been shown to increase the levels of glutathione in cultured hepatocytes. The increase in glutathione was related to the increase in the activity of γ-glutamylcysteine synthase (GCS), the key enzyme in the synthesis of glutathione [38]. A similar effect of insulin on glutathione metabolism was seen in vivo in streptozotocin-induced diabetes in the rat and in the plasma and red blood cell glutathione levels of human diabetic subjects.

In addition to insulin resistance, alterations in mitochondrial function resulting in lower mitochondrial oxidation, decreased synthesis of ATP, and increased peroxisomal production of ROS could also impair methionine metabolism in NAFLD. The high rate of ROS production may impact methionine metabolism by increasing glutathione consumption and thus change the hepatic redox state and impact the activity of methionine adenosyltransferase and the transmethylation of methionine. Finally, altered transmethylation of methionine may impact the hepatic VLDL export by altering the synthesis of phosphatidylcholine from phosphatidylethanolamine [39], and thus further contribute to the development of steatosis and steatohepatitis.

Preliminary data from our studies on subjects with biopsy-proven hepatic steatosis and nonalcoholic steatohepatitis (NASH) show that subjects with NASH, and without diabetes, are insulin-resistant, have lower plasma levels of glutathione and higher plasma concentrations of homocysteine and cysteine. A significant association of MTHFR 677C→T homozygosity and NASH was also observed. Insulin resistance (HOMA) was negatively correlated with plasma glutathione (r = 0.42, p < 0.001) and positively correlated with plasma cysteine levels (r = 0.25, p < 0.05).

Based on these data, we propose the following hypothesis for the propagation of hepatic injury in NAFLD and the role of methionine in this process (fig. 2). Hepatic insulin resistance associated with obesity, type 2 diabetes or metabolic syndrome, results in an increased flux of fatty acid to the liver and in hepatic steatosis. As a consequence of lipid accumulation and increased fatty acid oxidation, there is a high rate of production of ROS, leading to decreased activity of methionine adenosyltransferase, disruption of
Mitochondrial membrane potential and cellular ATP level. A lower activity of methionine adenosyltransferase and a decreased rate of production of ATP leads to a lower rate of production of SAM. The lower concentration and the lower production of SAM then causes changes in the methionine transmethylation and transsulfuration pathway. In addition, insulin resistance or the lack of insulin action may also cause the downregulation of glutathione synthesis. Decreased cellular glutathione levels lead to an unbalanced ROS and propagate these events. Decreased availability of SAM could impact the methylation of phosphatidylethanolamine to phosphatidylcholine, required for VLDL export, and thus interfering with fat mobilization.

In conclusion, metabolism of methionine, with all its interactions with folic acids, SAM-dependent methylation and one-carbon metabolism, is profoundly impacted by nutrient, hormone and environmental influences. During development, perturbations in methionine metabolism can result in long-term changes in the metabolism of the organism, possibly by causing changes in gene expression. In contrast, in mature organisms changes in methionine metabolism by impacting the physiological function may contribute to the propagation of injury.

Acknowledgment

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References


Metabolism of Methionine


Discussion

Dr. Bohles: I would like you to speculate on two extremes. On one hand, what is the implication of a soy diet, soy protein being basically free of methionine? And, on the other hand, the other extreme, if homocysteinuria patients are treated with betaine they get tremendously high methionine concentrations as a result of the remethylation, and this is a constant phenomenon. What is the intellectual basis of handling these two extreme situations?

Dr. Kalhan: All soy formulas for infants are supplemented with methionine; it is a regulatory requirement; so you will not see a methionine-deficient state in babies. I would imagine that you will not want to eat protein alone without added methionine. In India they always eat dhal with bread and native American Indians mix corn with another source of protein. Soy protein by itself is an incomplete protein without methionine, it requires an additional source of methionine. I am not sure where exactly you are leading with this question because I don’t think there is anybody on exclusively soy protein diet.

Dr. Bohles: My question refers to the constant effect on epigenetic phenomenon, like methylation, and possibly it is better viewed from the point of betaine therapy for homocysteinuria.

Dr. Kalhan: I will come back to homocysteinuria later. The source of methyl carbon is important for the maintenance of all these methyl reactions and there are some pilot data to suggest that if the source of methyl carbons is decreased in the diet, the methylation pattern of the genome can change. What are the implications of this in relation to the expression of physiological functions; to my knowledge this has not been studied and obviously needs to be examined in carefully done experiments. Yes, there is no question about it, we do need a constant source of methyl carbons in our diet and that is why, for example, when an animal is put on a choline betaine-deficient diet, it develops fatty liver disease and the whole phenomenon related to it. My impression is that, in vulnerable patients with homocysteinuria, when they are treated with betaine i.e. source of methyl groups, there is a tremendous transient increase in methionine which then gradually falls and returns to more reasonable levels. I have never managed a patient with homocysteinuria, so I can’t tell you more than that.

Dr. Lafeber: Since we are both neonatologists, it would be interesting for us to speculate whether it is possible to create an intervention in order to influence this methylation process. For instance, if we observe the process of intrauterine growth retardation which occurred during the last trimester of gestation in the so-called Dutch hunger winter in Holland in 1944–1945, it is a process that is very similar to that observed in preterm infants at the neonatal intensive care unit. If we try to treat these infants optimally with oral and parenteral feeding, we still observe a growth deficit at
the time of corrected term gestational age. At this time their weight is usually about 1 SD below that of normal term infants. We can then make our intervention by giving them a special diet. I just finished a study in which these preterm infants were provided with extra protein, but no extra calories. After 6 months we found that they were less fat and had lower insulin sensitivity [1]. We hope that this extra protein diet contributes to less metabolic syndrome in these preterm infants. Could you speculate on the total amount of protein and the quality of amino acids around the term age in preterm infants? I am sure you cannot say much about which specific amino acid is most important, although you have performed a trial similar to ours [2, 3] adding taurine or glutamine, for instance.

Dr. Kalhan: This is a very complex question. In spite of our best efforts, across the world when premature babies of less than 28–29 weeks gestation leave the neonatal intensive care units, their weight for the chronological gestation age is 1 or 2 SD below the fetal growth curve. The question whether this is permanent or can be reversed has always arisen. What Dr. Lafeber is referring to is that by changing the protein diet of these babies he has seen some remarkable changes at 6 and 8 months. Is this a reversible phenomenon? Time will tell. Actually we don’t even know how these premature babies do in adulthood; this is a new population, it is a new cohort. There is some evidence to suggest that low birthweight babies have metabolic syndrome as adults, but remember the low birthweight babies are not the premature babies we are talking about right now. Low birthweight babies in the Delhi cohort or Dr. Yajnik’s cohort are a very different population from the population we have in the west. The question of intervention is obviously very important. As was recently discussed at a meeting recently, the organism changes tremendously at birth, from fetus to newborn. It is probably the most dramatic event in life; the baby has to start breathing, has to maintain temperature, all sorts of things have to occur. But now we are feeding these babies for 15–16 weeks with all kinds of nutrients and we don’t know the implications of doing these things. We know quite a bit, but still we don’t know a whole lot about it. Have we destined this person to staying permanently small, we don’t know that yet, and we are just about to start studying these phenomena. As Dr. Whitelaw, I also want to give caution that collecting DNA and measuring on the lymphocytes is not going to work when we talk about epigenetics or this permanent change in methylation. These are going to be tissue-specific changes and we will have to do studies on the tissues and not collect DNA in the cord blood lymphocytes. That is the best answer I can give today it is a rapidly evolving field.

Dr. Agarwal: Some 20 years back we started doing studies on the brain and neurotransmitters. The methionine-deficient diet showed a dissociation. Normally on a protein-deficient diet, it is the 9th to the 10th generation that ends up with a reduced brain size, but with the methionine diet it happens in the first generation. Therefore methionine is very important and this dissociation is something which could just be carried on.

Dr. Kalhan: I also want to point out that protein restriction is not the only one associated with methionine metabolism. Another commonly used model which has a human counterpart is hypoxia. The Chinese people who move up to Tibet or Peru have low birthweight babies, and it will take generations before that will change because they are recent migrants to the Tibetan altitude. Some beautiful studies have been done. The first generations have small babies compared to the local inhabitant Tibetans who have what we call the normal growth curve for that, which is very comparable to our population.

Dr. Agarwal: Latent iron deficiency without hypoxia reduces the size of the baby and changes the brain and neurotransmitters irreversibly; latent iron deficiency, not hypoxia.
Dr. Kalhan: Yes, hypoxia has been demonstrated to be associated with changes in methylation patterns in the fetus, in the brain and liver. Lane et al. [4] did those studies. There is also evidence without intervention. Iron deficiency also causes growth retardation, not severe iron deficiency, just restricting iron and there is probably a lot of those which ultimately have a common denominator to programming.

References
