REVIEW

Dietary methyl-consuming compounds and metabolic syndrome

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The metabolic syndrome, a major risk factor for type 2 diabetes and cardiovascular disease, is a cluster of metabolic abnormalities including obesity, insulin resistance, hypertension and dyslipidemia. Although systemic oxidative stress and aberrant methylation status are known to have important roles in the development of metabolic syndrome, how they occur remains unclear. The metabolism of methyl-consuming compounds generates reactive oxygen species and consumes labile methyl groups; therefore, a chronic increase in the levels of methyl-consuming compounds in the body can induce not only oxidative stress and subsequent tissue injury, but also methyl-group pool depletion and subsequent aberrant methylation status. In the past few decades, the intake amount of methyl-consuming compounds has substantially increased primarily due to pollution, food additives, niacin fortification and high meat consumption. Thus, increased methyl consumers might have a causal role in the development and prevalence of metabolic syndrome and its related diseases. Moreover, factors that decrease the elimination/metabolism of methyl-consuming compounds and other xenobiotics (for example, sweat gland inactivity and decreased liver function) or increase the generation of endogenous methyl-consuming compounds (for example, mental stress-induced increase in catecholamine release) may accelerate the progression of metabolic syndrome. Based on current nutrition knowledge and the available evidence from epidemiological, ecological, clinical and laboratory studies on metabolic syndrome and its related diseases, this review outlines the relationship between methyl supply-consumption imbalance and metabolic syndrome, and proposes a novel mechanism for the pathogenesis and prevalence of metabolic syndrome and its related diseases.

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INTRODUCTION

The metabolic syndrome, a major risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD), is a cluster of metabolic abnormalities including obesity, insulin resistance, hypertension and dyslipidemia.^{1,2} In the past few decades, there has been an exponential increase in the prevalence of metabolic syndrome worldwide not only in adults but also in children and adolescents. For example, in the United States, the prevalence of obesity, a major component of metabolic syndrome, has doubled in the adult population³ and tripled in the adolescent population only in two decades (1980s and 1990s),⁴ while the prevalence of diabetes, the primary clinical outcome of metabolic syndrome, has also doubled during the past two decades (1988–2008) and still shows an increasing trend.⁵ To date, although the exact cause of metabolic syndrome remains unknown, it is known that diet may have a major role in the pathogenesis and prevalence of metabolic syndrome.⁶

Increasing evidence indicates that the pathogenic mechanism underlying metabolic syndrome and its clinical outcomes may involve oxidative stress, characterized by an increased production of reactive

oxygen species (ROS),¹ and aberrant methylation status, characterized by changes in the levels of plasma methyl donors⁷ and homocysteine^{8–12} and altered DNA methylation (an epigenetic mechanism that alters gene expression).² Recently, emerging evidence suggests that DNA methylation may be involved in the regulation of insulin gene expression in T2D and the pathogenesis of insulin resistance.^{13–15} Therefore, understanding the origin of oxidative stress and aberrant methylation status is of both fundamental and practical importance in identifying the cause of metabolic syndrome.

Methyl consumers include a variety of compounds that are metabolized by methylation and are known to be involved in both the generation of ROS and aberrant methylation status of the body. Methyl consumers from dietary sources are usually toxic xenobiotics, such as pesticides, heavy metals, food additives and even clinical drugs. In the past few decades, there has been a significant increase in methyl consumers in dietary sources that is primarily due to pollution, food additives and food fortification. Therefore, the focus of this review was on the possible role of methyl consumers in the development of metabolic syndrome.

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METHYL CONSUMERS AND THE METHIONINE-HOMOCYSTEINE CYCLE

Biological methylation is ubiquitous and important in many cellular mechanisms including inactivation/elimination of small molecules and xenobiotics, stabilization of DNA and proteins, small molecule biosynthesis and cellular signaling.¹⁶ Methylation status reflects a balance between methyl supply from dietary methyl donors (primarily betaine, choline and methionine) and methyl consumption by a variety of endogenous and exogenous methyl consumers.

All biological methylation reactions in the body use *S*-adenosylmethionine (SAM), that is derived from the methionine-homocysteine cycle, as methyl donor.¹⁶ As shown in Figure 1, a substrate is converted to a methylated metabolite by its own methyltransferase, in which SAM, after donating its methyl group to the acceptor molecule, is converted to *S*-adenosylhomocysteine (SAH). The resulting SAH is rapidly hydrolyzed by adenosylhomocysteinase into homocysteine and adenosine. The methionine-homocysteine cycle is completed by the remethylation of homocysteine via either a folate-dependent pathway catalyzed by vitamin B₁₂-dependent methionine synthase, or a betaine-dependent pathway catalyzed by betaine-homocysteine *S*-methyltransferase. Betaine is derived from either diet or the conversion of choline by choline oxidase.¹⁷ It has an important role in maintaining the methionine-homocysteine cycle, not only for its direct participation in the methylation of homocysteine to form methionine, but also for its metabolites that contribute to the formation of 5,10-methylenetetrahydrofolate, the precursor of 5-methyltetrahydrofolate.^{18,19}

Because all of the methyl consumers from either endogenous or exogenous sources share the same pool of labile methyl donors, it is conceivable that an increase in any methyl consumer(s) may influence not only the methylation of other methyl consumers due to its increasing demand for labile methyl groups. Therefore, there seems to be no doubt that the level of methyl consumers is an important

factor in determining the methylation profiles of the body, which has been largely ignored in the etiological study of metabolic syndrome and its related diseases.

METHYL CONSUMERS AND HYPERHOMOCYSTEINEMIA

Increased plasma concentration of homocysteine (that is, hyperhomocysteinemia) is frequently associated with T2D and CVD.^{8–12} Since both diseases are the primary clinical outcomes of metabolic syndrome, understanding of the cause of hyperhomocysteinemia may shed light on the etiology of metabolic syndrome. Plasma homocysteine levels are determined by three main factors: (1) the remethylation rate of homocysteine to methionine; (2) the conversion rate of homocysteine to cystathionine; and most importantly, (3) the amount of methylation of substrates.

One possible cause for the increasing prevalence of hyperhomocysteinemia and CVD may be folate deficiency, as evidence suggests that folate deficiency and methylenetetrahydrofolate reductase gene C667T polymorphism, a common genetic variation, may be involved in elevated fasting homocysteine.^{20,21} However, increasing evidence from clinical trials indicates that although folic acid and vitamin B₁₂ supplementation could reduce plasma homocysteine levels, they have no effect on T2D and cardiovascular events.^{22–25} This implies that the increased prevalence of CVD may not be a simple matter of folate deficiency. Moreover, excessive folic acid may lead to a waste of labile methyl-group donors. The underlying mechanism will be discussed in the later part of the review.

Another possible cause for the increasing prevalence of homocysteine-related diseases may be related to cystathionine β-synthase deficiency. However, cystathionine β-synthase deficiency in homozygous form, which can cause extreme elevations in homocysteine levels, is rare with a worldwide birth prevalence of 1:300 000. Moreover, although heterozygosity for cystathionine β-synthase deficiency, a mutation at a low prevalence (<1%), may cause an elevated

Figure 1 The methionine-homocysteine cycle and methylation of exogenous/endogenous substrates. The role of methionine-homocysteine cycle is to generate universal methyl donor *S*-adenosylmethionine for methylating a variety of exogenous/endogenous substrates. Homocysteine, produced in the methylation reaction, can be remethylated to methionine by a folate-dependent pathway or a betaine-dependent pathway. Adequate supply of methyl donors, especially betaine and choline, is needed to maintain the methionine-homocysteine cycle. Unlike natural folate, synthetic folic acid has to be converted to 5-methyltetrahydrofolate before it can function, which is a methyl-consuming reaction. (1) Various *S*-adenosylmethionine-dependent methyltransferases; (2) glycine *N*-methyltransferase; (3) adenosylhomocysteinase; (4) betaine-homocysteine *S*-methyltransferase; (5) methionine synthase; (6) methionine adenosyltransferase; (7) cystathionine β-synthase; (8) dihydrofolate reductase; (9) glycine hydroxymethyltransferase; (10) dimethylglycine dehydrogenase; (11) sarcosine dehydrogenase; (12) glycine cleavage system; (13) methylenetetrahydrofolate reductase; (14) choline oxidase. THF, tetrahydrofolate. A full color version of this figure is available at the *Hypertension Research* journal online.

postmethionine loading plasma homocysteine, this mutation has little effect on the fasting homocysteine levels.²⁶ Supplementation of vitamin B₆, an essential cofactor for cystathionine β -synthase, also has no effect on cardiovascular events.²⁵ Therefore, the frequency of carriers of cystathionine β -synthase deficiency alone cannot explain the high incidence of hyperhomocysteinemia, neither in healthy nor in vascular populations.²⁶

One unexplored cause for the increasing prevalence of metabolic syndrome, T2D and CVD are changes in dietary content of methyl consumers. It is known that almost all of the homocysteine is derived as an intermediate in the methionine-homocysteine cycle, because the amount of homocysteine from dietary sources is very limited.²⁷ This means that the methylation of both endogenous and exogenous methyl consumers provides the dominant source of plasma homocysteine. Although guanidinoacetate and phosphatidylethanolamine are known to be two major endogenous methyl consumers used to synthesize creatine and phosphatidylcholine, respectively,²⁸ there is no evidence of increase in the level of guanidinoacetate or phosphatidylethanolamine in metabolic syndrome or its related diseases. In contrast, increased exogenous methyl consumers, such as niacin,^{26,29,30} levodopa³¹ and arsenic,³² may contribute to elevated plasma homocysteine levels. Given that diet is a major risk factor for hyperhomocysteinemia³³ and that there has been a significant increase in dietary methyl consumers in the past few decades due to pollution, food additives, food fortification and high meat intake,^{34,35} it seems very likely that increased dietary methyl consumers may be a significant factor in the prevalence of hyperhomocysteinemia and its related diseases.

Some studies indicated that hyperhomocysteinemia is rarely seen in metabolic syndrome,^{36,37} but frequently seen in its primary clinical outcomes, T2D^{9–11} and CVD.^{8,12} This phenomenon may reflect a difference in the compensatory capacity in the conversion of homocysteine to methionine or cystathionine, since plasma homocysteine levels are also found to go up with age.^{9,38,39} Moreover, because the production of homocysteine is mainly determined by diet,³³ metabolic syndrome patients, even those with CVD, may have normal plasma homocysteine if they have shifted to a low methyl consumer diet and/or have a higher capability to convert homocysteine to methionine (for example, due to folate supplementation) or to cystathionine. From this point of view, it seems that plasma homocysteine may not be a reliable indicator of the imbalance between methyl supply and consumption, especially in the early stage of metabolic syndrome.

METHYL CONSUMERS AND THE METABOLIC SYNDROME

To date, although few studies have attempted to explain the etiology and prevalence of metabolic syndrome from the standpoint of increased methyl consumers, numerous studies have provided strong evidence that methyl consumers, both endogenous and exogenous, may be involved in the pathogenesis of metabolic syndrome and its related diseases. To outline the relationship between increased methyl consumers and the increased prevalence of metabolic syndrome and its related diseases, common examples are given below.

Arsenic

Arsenic is a ubiquitous environmental contaminant and is also one of the most common methyl consumers. In the human body, inorganic pentavalent arsenic is converted to trivalent arsenic, with subsequent methylation to monomethylarsonic acid, then to dimethylarsinic acid, and finally to trimethylarsine oxide,⁴⁰ which indicates that one inorganic arsenic may consume three methyl groups derived from SAM. Therefore, arsenic exposure may lead to a depletion of the SAM pool.

The human health effects of arsenic have been extensively studied. Chronic exposure to arsenic has been recognized to cause various types of human diseases, including metabolic syndrome, T2D, CVD and various cancers,^{40–44} which may contribute to the prevalence of metabolic syndrome in arsenic-polluted areas. The mechanism underlying arsenic toxicity is believed to be due to methyl depletion-induced aberrant DNA methylation, and excessive ROS generation and subsequent oxidative cell injury.^{40–42,45}

Niacin

Niacin (nicotinic acid and nicotinamide) is a water-soluble vitamin and a common methyl consumer.⁴⁶ Dietary nicotinic acid is derived from plant foods, while nicotinamide is derived from animal foods and typically used for food fortification. Fortified grains have provided an additional niacin source. Excessive niacin is converted to N¹-methylnicotinamide via an SAM-dependent methylation, and then to pyridinones.⁴⁶ This metabolic pathway depends on the supply of SAM and methyl donors. However, there is some difference between the degradation of the two active forms of niacin. Nicotinic acid can be either converted to nicotinamide and then degraded via the nicotinamide catabolic pathway (methyl-consuming catabolic pathway), or excreted in the urine unchanged and as nicotinuric acid (non-methyl-consuming catabolic pathway).⁴⁷ In contrast, nicotinamide is rarely excreted into the urine in its original form due to a high rate of tubular reabsorption.⁴⁸ Thus, equivalent doses of nicotinamide may consume more methyl groups than nicotinic acid.

The well-known toxicities of niacin are glucose intolerance, insulin resistance, liver enzyme elevations, fatty liver and steatosis.^{46,49–55} Our recent studies have shown that there is a strong lag-correlation between the prevalence of obesity and diabetes in the US population and the increased per capita consumption of niacin, which was attributed primarily to the consumption of fortified grains.^{34,35}

The toxic effects of niacin may involve a variety of mechanisms, including methyl depletion, changes in NAD-dependent reactions and excessive ROS generation. Methyl depletion as a consequence of niacin toxicity has been confirmed by the observations that hepatic toxicity can be prevented by choline and betaine supplements.^{30,55} It is known that methyl deficiency may limit the formation of very low density lipoprotein and low density lipoprotein in the liver due to decreased phosphatidylcholine synthesis, which may result in a decrease in the levels of plasma very low density lipoprotein and low density lipoprotein, as demonstrated in rats by Mookerjee *et al.*⁵⁶ On the other hand, the decreased transport of fat from the liver may lead to an accumulation of fat in the liver, and as a result, fatty liver and steatosis may occur.⁵⁷ Moreover, adequate phosphatidylcholine is also required for the formation and clearance of chylomicrons from the intestinal mucosa.⁵⁸ Therefore, methyl deficiency-induced decrease in phosphatidylcholine synthesis is also expected to reduce the plasma chylomicron levels. Similarly, choline deficiency has been demonstrated to lower the level of plasma chylomicrons.⁵⁶ Interestingly, niacin has been found to induce both lipid-lowering effect⁵² and adverse hepatic outcomes.^{46,52,54,55} Thus, it seems that the lipid-lowering effect of niacin may be mediated by methyl depletion, which, although improving cardiovascular outcomes, may increase the risk of adverse hepatic outcomes.

Niacin is the precursor of the important coenzymes NAD and NADP. NAD levels are dramatically altered by dietary levels of niacin.⁵⁹ Since NAD and NADP are crucial in redox reactions,⁵⁹ excessive niacin may lead to significant impacts on the redox status of the body by affecting NAD contents. Our recent study demonstrated that excessive nicotinamide might increase the generation of

hydrogen peroxide, a highly ROS, and have a role in the development of insulin resistance.^{35,60}

It is interesting to know whether niacin fortification may have adverse effects. Although niacin fortification has been implemented to prevent niacin deficiency (that is, pellagra) for more than half a century, there are virtually no studies that document and track the long-term changes of the levels of plasma niacin and labile methyl donors, or the changes in the redox status. The fact that there is no prevalence of niacin deficiency (that is, pellagra) occurring in non-niacin-fortified developed countries in the past half century suggests that the risk for developing niacin deficiency may be overestimated and that niacin fortification may be redundant. In the absence of niacin deficiency, fortification-induced long-term high-level niacin exposure may lead to persistent niacin overload. It has shown that nicotinamide at physiological concentrations is degraded mainly via the methyl-consuming mechanism,⁶¹ and that the appearance of non-methylated metabolites of nicotinamide in urine indicates nicotinamide overload and toxicity.^{47,62} Most importantly, long-term high-level niacin exposure has been observed to induce a methyl-group deficiency state and fatty liver due to an increased need for methylation of niacin in animal studies.^{55,63}

Based on current evidence, it seems likely that increases in niacin consumption may be correlated to the following phenomena: (1) the prevalence of T2D in the United States has increased in parallel with the increase in per capita consumption of niacin-fortified grains since the implementation of niacin fortification, whereas traditional high intake of non-fortified grain is associated with very low rates of obesity and diabetes;³⁴ (2) the prevalence of metabolic syndrome-related diseases in niacin-fortified countries is much higher than that of non-fortified developed countries;³⁴ (3) high consumption of meat, a nicotinamide-rich food, may increase the risk for metabolic syndrome;⁶⁴ and (4) consumption of processed meat, in which niacin is used in meat coloring in some non-European countries, can significantly increase the risk of T2D.⁶⁵

Catecholamines

Catecholamines, primarily norepinephrine and epinephrine that mediate the cardiovascular effect of the sympathetic system,⁶⁶ are important endogenous methyl consumers. Catecholamines are known to be a major risk factor for hypertension and CVD, because they can increase heart rate, blood pressure and ROS production.^{67–70}

Elevated catecholamines may be caused by two factors: (1) an increase in catecholamines release from the sympathetic nervous system and (2) a decrease in their degradation (that is, inactivation). Methylation is a crucial step in the degradation of catecholamines, which involves two methyltransferases: phenylethanolamine *N*-methyltransferase and catechol *O*-methyltransferase, where the former catalyzes norepinephrine to epinephrine and the latter converts catecholamines to methylated derivatives.⁶⁶ A lack of methyl groups may decrease the degradation of catecholamines and thus contribute to elevated circulating catecholamines. Hence, the increased level of unmethylated intermediates of catecholamines, such as dihydroxyphenylglycol and dihydroxymandelic acid^{71–73} observed in patients with hypertension and CVD may be related to methyl depletion/deficiency.

Since the methylation reactions of both catecholamines and exogenous methyl consumers utilize SAM as a methyl donor, it is expected that the decrease in the levels of SAM and methyl donors as induced by excessive exogenous methyl consumers may limit the methylation-mediated degradation of catecholamines. In this regard, we have recently observed that in healthy subjects, an excess of niacin can significantly increase the levels of plasma norepinephrine and

homocysteine and was associated with a significant decrease in the levels of plasma betaine and normetanephrine as the methylated derivative (unpublished data), which suggests that chronic high niacin intake may contribute to elevated circulating catecholamines.

Folic acid

Folic acid, the synthetic oxidized form of folate used for supplementation and fortification, is an unusual methyl consumer. Unlike 5-methyltetrahydrofolate which is the predominant natural form of folate in plants,⁷⁴ folic acid has to be reduced to tetrahydrofolate, and then further converted to the active form 5-methyltetrahydrofolate in the body (Figure 1). As shown in Figure 1, the formation of 5,10-methylenetetrahydrofolate from tetrahydrofolate requires a one-carbon unit from serine, dimethylglycine, sarcosine or glycine. The latter three compounds may be derived from betaine or its precursor choline. Also, the one-carbon unit for the formation may be derived from SAM through the glycine-sarcosine cycle (Figure 1), which is an important factor in determining the level of SAM.²⁰ Evidently, the formation of 5-methyltetrahydrofolate from folic acid is a process also dependent on SAM and other methyl donors.

Most importantly, excessive folic acid is eliminated through the urine primarily in its metabolic forms,²⁰ especially 5-methyltetrahydrofolate,⁷⁵ and thus results in a waste of SAM and the methyl donors. Folic acid supplementation and fortification have been found to significantly increase the urinary excretion of 5-methyltetrahydrofolate.⁷⁵ Theoretically, excessive folic acid may worsen rather than alleviate methyl-group deficiency. In fact, studies have demonstrated that folic acid supplementation neither lowers SAH, nor increases SAM, nor alters SAM/SAH ratio.⁷⁶ This may explain why folic acid supplementation has generally failed to reduce vascular events in clinical trials,^{22–25} or even has potential harmful effect on individuals with high homocysteine at baseline.²⁴ It should be pointed out that in most case, the reverse association between low vegetable intake and metabolic syndrome-related diseases is attributed to folate deficiency.²⁰ Vegetables generally contain much more betaine than folate. For example, 100 g of spinach, one of the richest vegetable sources of folate, contains only ~50 µg of folate,⁷⁴ but has ~600 mg of betaine and 22 mg of choline.⁷⁷ Therefore, diet-induced folate deficiency may be accompanied by a more severe deficiency of methyl-group donors. Thus, it seems that the importance of folate in the increasing prevalence of metabolic syndrome and its related diseases is overestimated.

Taken together, the dramatic worldwide increase in the intake of methyl-consuming compounds due to pollution and fortification might be responsible for the increasing prevalence of metabolic syndrome and its related diseases. The amount of methyl consumers from dietary sources may vary depending on the degree of pollution, implementation of food fortification and fortification standards. All of these differences may account for the difference in the prevalence of metabolic syndrome among countries or regions.

PROPOSED MECHANISM FOR THE EFFECTS OF METHYL CONSUMERS

Figure 2 shows the mechanism we proposed to explain the relationship between increased methyl consumers and metabolic syndrome. Methyl consumers may cause metabolic syndrome and its related diseases by a combination of oxidative stress and methyl-group depletion.

The metabolism of methyl consumers generates ROS that may lead to systemic oxidative stress, which is a key pathogenic factor in the development of T2D and CVD. Thus, chronic exposure to excessive

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outcomes. The changes in skin functions may, at least in part, underlie the following phenomena: (1) sweating exercise can reduce the incidence of diabetes,⁸² whereas sedentary lifestyles may increase the risk for metabolic syndrome;⁸³ (2) there is a high incidence of hyperhomocysteinemia in athletes of winter sports,⁸⁴ and the symptoms of T2D and CVD are worse in winter;^{85,86} (3) severe burn is frequently associated with persistent insulin resistance;⁸⁷ and (4) extensive skin diseases such as psoriasis are frequently associated with metabolic diseases including metabolic syndrome, diabetes and non-alcoholic fatty liver disease.⁸⁸

Figure 2 shows that the toxicity of methyl consumers is not merely a matter of methyl depletion. Supplementation of methyl donors, although alleviating methyl consumer-induced methyl depletion, cannot prevent methyl consumer-induced ROS generation and the resultant oxidative cell damage. This implies that without reducing the level of methyl consumers, supplementation of methyl donors alone may not achieve the desired therapeutic effect.

CONCLUSIONS

The pathogenesis of metabolic syndrome may involve an imbalance of methyl supply consumption. In the past few decades, in addition to a significant increase in exogenous methyl-consuming compounds, especially from dietary sources due to pollution, food fortification and high meat consumption, there is also a significant decrease in the elimination/metabolism of xenobiotics, especially due to the decrease in skin function as influenced by lifestyle changes. These may be major causes of the increased prevalence of metabolic syndrome and its related diseases worldwide. Therefore, it seems that decreasing dietary methyl-consuming compounds (for example, using natural foods rather than fortified or additive-containing foods) may be a key factor in the prevention and treatment of metabolic syndrome, T2D and CVD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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