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Comments
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Homocysteine Metabolism and its Relation to Health and Disease

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Running title: Regulation of homocysteine balance

Abstract/ Summary

Homocysteine is a metabolic intermediate in methyl group metabolism that is dependent on a number of nutritional B-vitamin cofactors. An emerging aspect of homocysteine metabolism is its relation to health and disease. Perturbations of homocysteine metabolism, particularly intracellular and subsequently circulating accumulation of homocysteine (i.e., hyperhomocysteinemia), are associated with vascular disease risk, as well as other pathologies. However, intervention with B-vitamin supplementation has been shown to successfully restore normal homocysteine concentrations, but without concomitant reductions in disease risk. Thus, the mechanistic relation between homocysteine balance and disease states, as well as the value of homocysteine management, remains an area of intense investigation.

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Introduction

Homocysteine is an amino acid generated metabolically by the S-adenosylmethionine (SAM)-dependent transmethylation pathway (1). This series of reaction occurs in most cells and tissues; however, the liver is the most prominent site for SAM-dependent transmethylation and the subsequent production of homocysteine. As shown in **Fig. 1**, the initial step is the activation of methionine to SAM by the ATP-dependent action of methionine adenosyltransferase. SAM is the universal methyl donor for numerous transmethylation reactions, including the methylation of proteins, nucleic acids, lipids, and small molecule substrates such as amino acids. A consequence of all SAM-dependent transmethylation reactions is the subsequent generation of S-adenosylhomocysteine (SAH), which is then hydrolyzed to adenosine and homocysteine by SAH hydrolase. It is important to note that the intracellular concentrations of SAM and SAH are critically important with respect to regulating methylation reactions, as SAH is an allosteric inhibitor of most methyltransferases (2). Thus, the ratio of SAM/SAH can be viewed as an index of transmethylation potential in the cell (3,4).

Following the production of homocysteine by SAM-dependent transmethylation, it can undergo remethylation back to methionine, or be irreversibly catabolized by the transsulfuration pathway. Two tissue-specific homocysteine remethylation pathways are known to exist: a folate-dependent reaction that utilizes 5-methyltetrahydrofolate (5-CH_3-THF) as a substrate and the action of the B_{12}-dependent enzyme methionine synthase (MS); and a folate-independent route catalyzed by betaine-homocysteine *S*-methyltransferase (BHMT) where betaine, an oxidation product of choline, serves as the

methyl donor. Hepatic BHMT and MS are considered to contribute equally in the process of homocysteine remethylation in the liver (5). For both reactions, the homocysteine backbone of methionine serves as an acceptor of methyl groups for the maintenance of essential SAM-dependent transmethylation reactions. Transsulfuration is an alternative and essential route for homocysteine catabolism that is initiated by the action of cystathionine β-synthase (CBS), a B_6-dependent enzyme, to form cystathionine via the condensation of homocysteine and serine. Cystathionine is further metabolized by γ-cystathionase to cysteine, a conditionally essential amino acid that, in addition to protein synthesis, is required for the synthesis of glutathione and other biologically important molecules such as taurine. Thus, homocysteine balance is dependent on the numerous SAM-dependent transmethylation reactions that result in its production, counterbalanced with the utilization of homocysteine for folate-dependent/ -independent remethylation and/ or transsulfuration. Some of the specific enzymes and regulatory proteins involved in homocysteine production and utilization are addressed further in the following sections.

Homocysteine Production: SAM-dependent Transmethylation and Regulation of Homocysteine Balance

As there are >100 SAM-dependent transmethylation reactions, all contributing to intracellular homocysteine pools, it is beyond the scope of this review to adequately address even the most critical reactions. However, it is important to recognize and discuss the transmethylation reactions that are thought to contribute the greatest extent to homocysteine production, as well as have a potential regulatory role. Collectively, the

hepatic production of phosphatidylcholine (PC) and creatine, catalyzed by the SAM-dependent enzymes phosphatidylethanolamine *N*-methyltransferase (PEMT) and guanidinoacetate *N*-methyltransferase (GAMT), respectively, represent the greatest usage of methyl groups (~85%) from SAM (6,7); thus, the action of PEMT and GAMT are major determinants of homocysteine production. The synthesis of PC is important in the maintenance of cell membranes and lipoproteins, and methylation of guanidinoacetate is the final step in the synthesis of creatine, a key molecule in energy metabolism as creatine phosphate. Traditionally, GAMT was considered the most significant consumer of methyl groups from SAM; however, this hypothesis has been recently challenged, as the reaction catalyzed by PEMT requires 3 methyl groups from SAM in the conversion of phosphatidylethanolamine (PE) to PC (8,9).

In addition to the specific reactions PEMT and GAMT function to catalyze, they have also been proposed to have a regulatory role with respect to homocysteine balance. For hepatic creatine synthesis, it has been shown that decreasing the demand for SAM-dependent methylation of guanidinoacetate by GAMT via dietary supplementation with creatine resulted in a significant decrease in homocysteine concentrations (10). Conversely, the production of homocysteine was elevated owing to the dietary provision of guanidinoacetate. Thus, the demand that creatine synthesis places on hepatic homocysteine production has a major impact on homocysteine balance, as reflected in the circulating concentrations of homocysteine.

SAM-dependent methylation of PE to generate PC is a vital component in the maintenance of normal VLD production such that when compromised, the result is hepatic

steatosis owing to an inability to export fatty acids from the liver (11). In addition to lipoprotein synthesis, Vance and coworkers have shown that PEMT is a key regulator of homocysteine balance (12,13). Using PEMT -/- knockout mice, the lack of PEMT expression significantly reduced the circulating concentrations of homocysteine, whereas its over-expression resulted in hyperhomocysteinemia. Thus, the expression and activity of PEMT appears to be directly correlated to homocysteine accumulation.

A third key enzyme in SAM-dependent methyl group metabolism is glycine N-methyltransferase (GNMT), a protein which regulates the supply and utilization of methyl groups, particularly with respect to methyl groups supplied by the folate-dependent pathway (14). Owing to its significant intracellular abundance (1-2% of soluble cytosolic protein) and regulatory role in maintaining an optimal ratio of SAM: SAH, it would be expected that regulation of GNMT expression and function would translate into having a direct impact on homocysteine balance, similar to that exhibited by PEMT. However, to date this has not been clearly demonstrated. A GNMT knockout mouse model was characterized by extremely high levels of methionine and SAM in the circulation (15); however, plasma homocysteine concentrations were not reported. Although over-expression of GNMT might be expected to result in excessive production of homocysteine, various rodent models exhibiting a marked increase in GNMT abundance and activity did not translate into similar changes in the circulating concentrations of homocysteine (16-19). In fact, homocysteine concentrations were actually diminished in these studies, owing to the increased activity of other enzymes involved in homocysteine remethylation and catabolism.

Homocysteine Utilization: Remethylation and Transsulfuration

Similar to the SAM-dependent transmethylation enzymes discussed earlier, enzymes involved in the metabolism of homocysteine, by either remethylation or transsulfuration, also have a significant impact on homocysteine balance. Consequently, these three routes for homocysteine metabolism represent an additional point for its regulation.

For folate-dependent remethylation of homocysteine by the B_{12}-dependent enzyme MS, much of the data regarding the impact of this pathway on homocysteine balance resides in studies focused on the polymorphic expression of the enzyme 5,10-methylenetetrahydrofoalte reductase (MTHFR). For humans, a reduction in the activity of MTHFR owing to the single nucleotide polymorphism C677T has been associated with hyperhomocysteinemia and vascular disease (20-22). Similarly, MTHFR-deficient mice exhibited hyperhomocysteinemia, as well as indicators of neurological and vascular problems (23). For all cases of reduced MTHFR function, the ensuing hyperhomocysteinemia can be attributed to the compromised ability of the folate/ B_{12}-dependent pathway to adequately remethylate homocysteine and is most pronounced under conditions of low folate intake. Because the MTHFR C677T genotype represents a significant determinant in circulating homocysteine concentrations, its presence is also linked to the potential disease risks associated with hyperhomocysteinemia. Moreover, a clear interaction exists between populations that exhibit the MTHFR C677T genotype in

combination with polymorphisms of other enzymes involved in homocysteine balance, including the PEMT G5465A, the GNMT C1289Y, and the MS A66G genotypes (24-26).

For folate-independent homocysteine remethylation using betaine as a methyl donor, BHMT has been shown to be a key regulator of homocysteine balance, particularly under diabetic conditions (16,17,27) and in response to dietary protein supply (28,29). To date, a rodent BHMT knockout model has not been developed as a means to further understand the relation between BHMT expression and homocysteine balance. However, the development of specific and potent inhibitors of BHMT has provided insight its role in regulating homocysteine metabolism. Mice administered *S*-(δ-carboxybutyl)-DL-homocysteine (CBHcy) as a means to knockdown BHMT function lowered BHMT activity nearly 90% and increased plasma total homocysteine concentrations 7-fold (30). This relation between BHMT and circulating homocysteine concentrations appears to be highly specific, as CBHcy treatment was without effect on the enzymatic activities of MS, MTHFR, or PEMT (Garrow, T., personal communication).

A key enzyme in the irreversible catabolism of homocysteine is CBS, the B_6-dependent enzyme that catalyzes the initial condensation of serine and homocysteine to form cystathionine. The earliest evidence linking aberrant homocysteine metabolism (i.e., hyperhomocysteinemia) to cardiovascular disease results from the observations noted in humans exhibiting a lack of sufficient CBS expression, namely homocystinuria (31). Using a transgenic mouse model, Wang et al. (32) has demonstrated that induction of hepatic and renal CBS effectively lowered serum homocysteine concentrations. In support of these observations, the inherent elevation of CBS expression in diabetic rat models was

associated with a state of hypohomocysteinemia, owing to elevated homocysteine catabolism via the transsulfuration pathway (33).

In summary, a number of key enzymes involved in homocysteine metabolism play a pivotal role in homocysteine balance. It should be noted that a variety of other factors impact the regulation and function of these enzymes, including diet, age, physiological state, and hormonal imbalance. Moreover, and in addition to the MTHFR C677T polymorphisms, the majority of these enzyme proteins exhibit polymorphic forms that certainly have the potential to impact homocysteine balance for specific individuals, as has been discussed. For humans, the most sensitive individuals for aberrant homocysteine metabolism are likely characterized by exhibiting multiple determinants.

Hyperhomocysteinemia: relation between homocysteine balance and disease

Hyperhomocysteinemia, the accumulation of homocysteine in the circulation, has received considerable attention in the last decade with respect to a number of disease states in both animal models and human studies. The predominant focus has been on hyperhomocysteinemia being recognized as an independent risk factor for cardiovascular disease (34,35). It has been estimated that a 2.5 μM rise in circulating homocysteine concentrations translates into a 10% increase in cardiovascular disease risk. This relation between homocysteine concentrations and cardiovascular disease risk has since been extended to include other vascular diseases as well (36). Moreover, hyperhomocysteinemia has been reported to be a potential risk factor in a number of other pathologies, including diabetes, birth defects, neurological disorders, and cancer

development (37-40). A key issue that remains to be resolved is whether a condition of hyperhomocysteinemia has a direct, causal impact on vascular disease, or exists as a biomarker that reflects another mechanistic basis for the adverse effects on vascular function.

Intervention studies to reduce elevations in homocysteine concentrations

Based on the preceding discussion, it is a logical approach to expect that dietary intervention to reduce circulating homocysteine concentrations represents a viable means to subsequently reduce cardiovascular disease risk. There have been numerous investigations into the impact of homocysteine-lowering treatment with B vitamins for the prevention of vascular disease. To date, most studies have demonstrated that treatment with folic acid, B_{12}, B_6 or a combination thereof results in a significant decrease in plasma homocysteine. Whether this lowering of plasma homocysteine is associated with a reduction in the incidence of disease via B-vitamin therapies remains controversial (41,42).

Near the turn of the century, the evidence from several trials suggested that B-vitamin treatment could decrease the risk of vascular diseases, but subsequent investigations were not as promising. Early studies showed improved B-vitamin status lowered plasma homocysteine levels, and decreased incidence of adverse events or improvements in indicators of vascular endothelial dysfunction in patients treated with folic acid, B_{12}, and/or B_6 vs. those treated with a placebo (43-47). However, these results have largely been refuted by the predominantly negative results of subsequent trials, including the NORVIT, WENBIT, HOPE-2, VISP, VITATOPS, and VITRO studies (48-

60). These studies generally had a mean follow-up time of several years and assessed a wide variety of vascular indicators and endpoints including carotid intima-media thickness and flow-mediated dilation (52), markers of arterial inflammation (49-58), need for revascularization procedures (54), occurrence of thromboembolism (53,55), occurrence of stroke and myocardial infarction (48,50,54,56,57,59), as well as overall or coronary/vascular-related mortality (48,50,51,54,56,59).

It is not clear as to what factors may account for the disparity in these findings. The duration of treatment, B-vitamin status, and polymorphisms of enzymes involved in homocysteine metabolism could potentially play a role. Notably, the treatment period was generally longer in duration for the later studies in comparison to those conducted earlier, i.e. several years vs. weeks or months. The apparent affect of the duration of treatment is supported by the meta-analysis by Potter et al (52) in which they found that in patients post-stroke, B-vitamin treatment had positive effects in the short term, but these effects were not sustained long term. Research in this area is ongoing and several recent short term studies have demonstrated benefits of folate supplementation alone on vascular outcomes in high-risk patients (61-63). Interestingly, the data suggests that improvements are independent of the homocysteine-lowering effect of treatment, therefore other mechanisms of action should also be considered for short-term treatment effects.. More data is also expected from additional long-term studies of high-risk populations which have been initiated, but are not yet complete (64,65). Although poor B-vitamin status and polymorphisms of MTHFR have been associated with elevations in plasma homocysteine levels and may impact the homocysteine-lowering response to treatment, there is little or

no evidence to support that these factors may account for differential results between studies.

Vascular diseases are not the only conditions in which there have been trials of B-vitamin interventions. Unfortunately, there appears to be no effect of homocysteine-lowering B-vitamin therapy on Alzheimer's disease and cognitive decline (66-68) or type 2 diabetes (69), and the results are conflicting regarding potential effects on bone mineral density and turnover, and fracture occurrence (70-72). However, not all findings have been negative; the Women's Antioxidant and Folic Acid Cardiovascular Study found that long-term daily treatment with folic acid, pyridoxine, and cobalamin in a high-risk population reduced the risk of age-related macular degeneration (73). Furthermore, studies in healthy individuals suggest that treatment with B vitamins may be an effective means of reducing the risk of stroke (74) and slowing the progression of early-stage atherosclerosis (75). Though the data is limited, the most important research area for the future use of treatments with the homocysteine-lowering vitamins is the identification of which specific populations are most expected to benefit from therapy, with particular emphasis on primary prevention.

Summary

Because of the association of hyperhomocysteinemia with a number of pathological conditions, particularly vascular disease, it is clear that homocysteine management represents a significant focus for nutrition and health. Supplementation with the various B-vitamin cofactors that are essential to maintain homocysteine balance represents the most logical and viable approach. However, much remains to be resolved to understand

the mechanistic relation between homocysteine imbalance and disease, and subsequently the most appropriate approach to maintain homocysteine balance as a means to reduce disease risk. It will also be of utmost importance to identify specific populations that are most sensitive to homocysteine imbalance, based on known polymorphisms or physiological states that have been shown to impact homocysteine metabolism.

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Figure Legends

Fig. 1. Hepatic folate, methyl group, and homocysteine metabolism.

Abbreviations of metabolites and enzymes are: betaine-homocysteine *S*-methyltransferase (BHMT); cystathionine β-synthase (CBS); cystathionine γ-lyase (CGL); dimethylglycine (DMG); methionine synthase (MS); methyltransferases (MTs); 5,10-methylene-THF reductase (MTHFR); *S*-adenosylhomocysteine (SAH); *S*-adenosylmethionine (SAM); serine hydroxymethyltransferase (SHMT); tetrahydrofolate (THF); and methyl acceptor (X). For this review, important SAM-dependent methyltransferases include: glycine *N*-methyltransferase (GNMT); guanidinoacetate *N*-methyltransferase (GAMT); and phosphatidylethanolamine *N*-methyltransferase (PEMT). These three methyltransferases respectively catalyze the conversion of glycine to sarcosine, guanidinoacetate to creatine, and phosphatidylethanolamine (PE) to phosphatidylcholine (PC). In addition to folate, note that this series of interrelated pathways are dependent on a number of other B-vitamins, including riboflavin (B_2), vitamin B_6, and vitamin B_{12}.