REVIEW ARTICLE

Homocysteine Remethylation and Trans-sulfuration

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The plasma homocysteine (Hcy) concentration represents the balance between its entry and removal from the circulation. This understanding has stimulated efforts to elucidate the causes of hyperhomocysteinemia by measuring plasma Hcy turnover. However, these studies have been performed under steady-state conditions, which do not allow for conclusions about the type and severity of the metabolic blocks that cause metabolites to accumulate. Failure to appreciate this has led to some confusion in the literature dealing with whole body Hcy metabolism.

STRONG EVIDENCE that homocysteine (Hcy) is a vascular toxin has focused interest on the factors that determine its plasma concentration in health and disease. The prevailing plasma Hcy concentration represents the balance between its production and removal from the circulation, so the usefulness of plasma Hcy kinetic measurements in normal and hyperhomocysteinemic states seems obvious. Moreover, an elegant dual-label tracer model, developed by Storch and Young, is at hand to perform such studies.[1] It needs to be appreciated, however, that the Storch-Young model is a *steady-state* model, and steady-state substrate flux does not provide information about the type and severity of metabolic blocks.[2] Failure to appreciate this will lead to misinterpretation of the results generated by this model.

HOMOCYSTEINE METABOLISM

Hcy is the sulfur amino acid created upon removal of methionine's labile methyl group. Approximately 80% of fasting whole-body methionine disappearance is due to uptake for protein synthesis; the remainder is catabolism via conversion to Hcy.[3] The first step in methionine's catabolic pathway is activation to S-adenosylmethionine (SAM), the donor in most methyl transfer reactions.[4] Methyl donation by SAM produces S-adenosylhomocysteine, which, upon hydrolysis, yields Hcy. Hcy is metabolized by 2 pathways. The first is reconversion to methionine (remethylation pathway [RM]). The second is conversion to cystathionine in a reaction catalyzed by cystathionine β-synthase (CBS). Cystathionine is subsequently cleaved to cysteine and α-ketobutyrate (trans-sulfuration pathway [TS]). Methionine catabolism by other routes is insignificant[5,6] so the TS pathway is, with rare exceptions, the only route of methionine (and Hcy) elimination from the body.

The RM and TS pathways have different metabolic functions. Organisms in metabolic balance eliminate methionine at the same rate it enters from the diet, so their TS rate matches methionine intake. RM appears to represent a mechanism by which methyl groups from diverse sources are channelled, via Hcy reconversion to methionine, into SAM-mediated transmethylation reactions.[7] Selhub and Miller[8,9] have described a plausible scheme by which the intracellular SAM concentration regulates traffic on these independent, but intersecting metabolic pathways. According to their scheme, high methionine states increase intracellular SAM, activating CBS to eliminate the surfeit methionine. High SAM simultaneously inhibits methylenetetrahydrofolate reductase (MTHFR), the supplier of methyltetrahydrofolate to the Hcy remethylating enzyme, methionine synthase, thereby limiting needless methionine synthesis via Hcy remethylation in a situation in which methionine is already abundant. Low methionine states—as would be predicted with a low methionine diet—decrease intracellular SAM. This reduces CBS activity, conserving methionine, while simultaneously stimulating MTHFR to increase Hcy remethylation and maintain sufficient methionine influx for essential transmethylation reactions. Finally, an increased transmethylation requirement is sensed as a reduction in SAM. This increases the RM rate to increase the supply of methionine to form SAM while keeping TS constant, the latter being essential to maintain methionine balance.

THE STORCH-YOUNG MODEL

Whole body TS can be measured in the steady state by means of a primed, continuous infusion of tracer [1-^{13}C]methionine. It is valid to use the steady-state $^{13}CO_2$ production rate to calculate methionine oxidation, first, because the C-1 atom of methionine becomes the C-1 of Hcy, which in turn becomes the C-1 of a molecule—α-ketobutyrate—whose only metabolic fate is irreversible C-1 decarboxylation,[1] and, second, because TS is the only quantitatively important pathway for methionine oxidation in humans.[5,6] The C-1 of methionine is not affected by demethylation to form Hcy nor by Hcy remethylation to form methionine, so the methionine disappearance rate calculated with this tracer provides no information about the RM pathway. Storch and Young realized that a label on methionine's labile methyl group will be lost in both the TS and RM pathways, so methionine turnover measured using a methyl-labeled tracer will exceed that measured using a C-1–labeled tracer by an amount equal to RM. SAM-mediated methyl transfer occurs at the rate RM + TS, which is termed the transmethylation rate (TM).[1,3]

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The interactions described here among rate of methionine ingestion, metabolite concentrations, and flux rates are pertinent to steady-state conditions; the situation is more complicated under short-term non–steady-state conditions. In the non–steady-state period immediately following methionine ingestion, serum and tissue concentrations of methionine and its metabolites such as SAM, S-adenosylhomocysteine, and Hcy may rise, creating a temporary mismatch between methionine entry and its ultimate disposal. Temporal variation could also exist in the rates of SAM-mediated transmethylation reactions. Like most tracer models of amino acid metabolism, the Storch-Young model is applicable only when metabolic and isotopic steady state prevail. The TS and RM rates it indicates apply to a steady-state situation in which metabolite pool sizes are constant in size.

The Storch-Young model assumes the TS pathway is the only quantitatively important route of methionine catabolism in the body. This is a valid assumption, since the existing data indicate that methionine transamination is quantitatively insignificant in humans.[5,6] In certain metabolic disorders, urinary excretion of methionine or its metabolites, including Hcy or cystathionine, could be quantitatively important.[6,10] In such situations the model needs to be adjusted to account for steady-state urinary excretion of these molecules.

CAUSES OF HYPERHOMOCYSTEINEMIA

The RM pathway largely depends on the cobalamin-dependent enzyme, methionine synthase, and on the availability of its substrate, methyltetrahydrofolate, to supply the methyl group necessary to reconvert Hcy to methionine. Folate and vitamin B_{12} deficiency cause hyperhomocysteinemia by inhibiting this reaction. The TS pathway depends on CBS, a pyridoxine-dependent enzyme, so vitamin B_6 deficiency or a defective CBS gene (as in classic homocystinuria) cause hyperhomocysteinemia. Since Hcy removal is the sum of RM and TS, some authors have found it appealing to ask which of these 2 processes is impaired in different hyperhomocysteinemic states. As the following paragraphs will show, the Storch-Young model cannot be used to answer this question.

A REDUCED REMETHYLATION RATE NEITHER CAUSES NOR EXPLAINS HYPERHOMOCYSTEINEMIA

As illustrated in Fig 1, the RM pathway is a closed loop that does not eliminate Hcy from the body. Slowing of the RM rate cannot, in itself, cause hyperhomocysteinemia since any reduction in Hcy's removal rate from the sampling pool by this pathway will slow its entry rate equivalently. In fact, when measured in the steady state, a disease that impairs Hcy remethylation cannot even be assumed to lower the RM rate. Rather, the onset of impaired remethylation lowers SAM concentrations. The low-SAM state feeds forward to inhibit CBS. The combination of impaired RM activity and feed-forward–reduced CBS activity causes Hcy to accumulate, increasing the substrate concentration for CBS, for methionine synthase, and for the folate- and vitamin B_{12}-independent Hcy remethylating enzyme, betaine:homocysteine methyltransferase.[11] The end result is an adaptive return of RM and TS to normal steady-state rates. The Storch-Young model indicates only these final, adaptively restored flux rates. Thus, a normal RM rate does not rule out the presence of an enzymic RM block when measured under steady-state conditions.

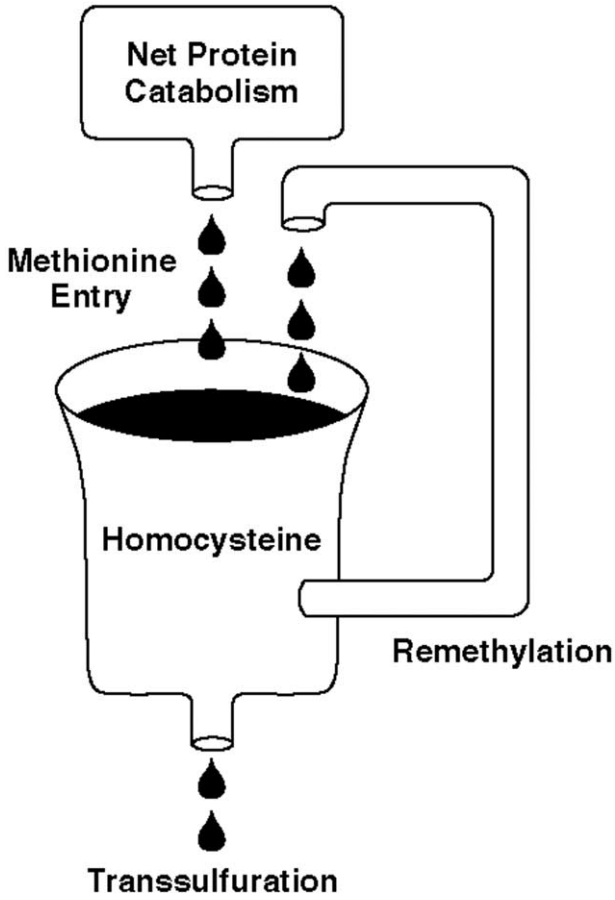

Fig 1. The Storch-Young model of homocysteine metabolism in the basal state. The remethylation pathway is a closed loop: it neither adds nor subtracts homocysteine from the body. A decreased rate of homocysteine removal by this pathway will not raise the plasma homocysteine concentration because it simultaneously decreases the entry rate.

Nor does a low-steady state RM rate prove remethylation is impaired: it might merely indicate a low requirement for transmethylation reactions. Much of SAM-mediated methyl donation is destined for creatine synthesis,[7,12,13] so it is conceivable that an individual's RM rate is influenced by his or her muscle mass.

METHIONINE CATABOLISM REQUIRES TRANSMETHYLATION

Since methionine catabolism requires SAM formation, situations arise in which SAM is formed faster than required for biologically necessary transmethylation reactions. This explains the importance of the "overflow" pathway in which SAM methylates glycine to form N-methylglycine.[7,9,14,15] This is really a methyl group disposal pathway. This pathway must be important in normal metabolism, for when it is blocked, as

in glycine N-methyltransferase deficiency, hyperSAMemia and hypermethioninemia occur.[16,17]

The basal TM rate of normal persons is approximately 6 to 8 μmol/kg-h.[3] How much of this represents biologically necessary biosynthetic reactions and how much is merely overflow? A recent study[18] casts light on this. If the RM rate is largely regulated by the need to guarantee sufficient methionine influx to allow essential transmethylation reactions to proceed, as current commentaries assume,[9] one would predict a compensatory increase in the RM rate when methionine influx from diet (and endogenous protein breakdown) falls below the minimum necessary from these sources.[9] The observation that consumption of a diet so low in methionine that it reduced the TM rate to 4 μmol/kg-h caused a marked reduction, rather than in increase, in RM rate implies that the rate of essential transmethylation reactions is not greater than 4 μmol/kg-h. If the requirement for essential transmethylation reactions was greater than 4 μmol/kg-h, the requirement could easily be met by supplying SAM at a faster rate, via the RM pathway: that is, by increasing the RM rate. Since the RM rate decreased markedly under these conditions[18] it would seem that 4 μmol/kg-h of transmethylation reactions is all the body needs, and that rates greater than this represent physiologically inconsequential "overflow." This assumes, of course, that the Selhub-Miller scheme and the Storch-Young model are substantially correct.

THE TRANS-SULFURATION RATE IS THE METHIONINE AND HOMOCYSTEINE ELIMINATION RATE

Statements to the effect that Hcy has a "choice" between RM or TS for elimination are incorrect. As shown in Fig 1, Hcy molecules have a metabolic "choice," but not for elimination. In the steady state, any reduction in the rate of Hcy removal by RM will reduce the Hcy entry rate equivalently, and hence will have no effect on Hcy's plasma concentration. Except for rare, inborn errors of metabolism, the only way the body has to dispose of Hcy is via the TS pathway. This is a key (and valid[5,6]) assumption of the Storch-Young model, in which the TS rate is measured as tracer-determined oxidation of α-ketobutyrate, a trans-sulfuration–derived molecule.[1]

Except, potentially, for severe protein catabolic states or severe dietary methionine excess, which would increase Hcy entry, all hyperhomocysteinemia must be due to reduced whole body CBS activity, since CBS is the regulatory enzyme on the TS pathway, and the TS pathway is the only pathway for Hcy elimination. This is true whether the specific cause of reduced CBS activity is direct inhibition, as in vitamin B_6 deficiency, SAM-mediated inhibition, as in folate or vitamin B_{12} deficiency, or decreased metabolic mass, as may occur in renal failure.[3] As explained earlier, under steady-state conditions the TS rate is not slowed by CBS inhibition. Rather, CBS inhibition causes Hcy to accumulate upstream of it. This accumulation increases the enzyme's catalytic rate, allowing TS flux to proceed at a normal rate despite the defect or inhibition.

The only exception to this is when CBS deficiency is so severe that even extremely high substrate Hcy concentrations do not permit TS to match normal methionine intake. Patients whose disease is this severe excrete Hcy and other metabolites in their urine, and often require a methionine-restricted, cysteine-supplemented diet for survival. In such cases TS is certainly reduced, but only because methionine intake is restricted. Under conditions of metabolic equilibrium (and when urinary losses are corrected for) methionine intake always equals TS.

Diseases that inhibit CBS activity can be revealed by the large rise and delayed fall in the plasma Hcy concentration that follows administration of a methionine load[9] or by the delayed oxidation of a single dose of [1-^{13}C]methionine[19]; these are non–steady-state responses. Under steady-state conditions, the evidence for CBS inhibition (and this may be sufficient evidence) is hyperhomocysteinemia itself. Steady-state RM and TS rates will be appropriate for the prevailing methionine intake and TM rates, and hence, unrevealing.

RENAL FAILURE-ASSOCIATED HYPERHOMOCYSTEINEMIA IS DUE TO IMPAIRED TRANS-SULFURATION

In a frequently cited study, the Storch-Young model was used in an attempt to determine whether the hyperhomocysteinemia of chronic renal failure is due to impaired RM or impaired TS.[20] Upon finding the TS rate of hemodialysis patients was normal, the authors concluded that hyperhomocysteinemia in end-stage renal disease is not due to impaired TS, and suggested that the main cause is impaired RM. This conclusion is wrong.

The TS rate is the rate at which methionine and Hcy are eliminated from the body, and in states of metabolic equilibrium it is equal to methionine intake. People with stable chronic renal failure are in metabolic equilibrium, eliminating urea nitrogen and oxidizing methionine at rates commensurate with their protein and methionine intakes. The observation that the TS rate of such persons was normal implies only that their protein intake was normal (and indeed, it was[20]). The abnormalities in renal failure are urea *clearance* and Hcy *clearance*. The difference between turnover and clearance of a metabolite can be illustrated by reference to glucose metabolism. Type 2 diabetes is characterized by impaired insulin-mediated glucose removal; that is, by reduced glucose metabolic clearance. The fact that steady-state glucose removal rates are normal in type 2 diabetes does not contradict this conclusion.

CONCLUSION

Inhibition of the regulatory enzyme on an obligatory metabolic pathway causes metabolite accumulation upstream of it. The regulatory enzyme in methionine's catabolic pathway is CBS. CBS may be inhibited directly, as in pyridoxine deficiency, or indirectly, as when vitamin B_{12} or folate deficiency cause a low-SAM state. When the block is mild or moderate, hyperhomocysteinemia increases the enzyme's catalytic rate until a new steady state is established in which Hcy conversion to cystathionine occurs at normal rate past the block. The Storch-Young model is a valuable tool for studying methionine and Hcy catabolic rates and the rate of SAM-mediated methyl transfer reactions. As it is a steady-state model, it cannot provide information about the type and severity of metabolic blocks that cause hyperhomocysteinemia.

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REFERENCES

1. Storch KJ, Wagner DA, Burke JF, et al: Quantitative study in vivo of methionine cycle in humans using [methyl-$^{2}H_{3}$] [1-^{13}C]- and [methyl-$^{2}H_{3}$] [1-^{13}C]methionine. Am J Physiol 255:E322-E331, 1988
2. Newsholme EA, Start C: Regulation in Metabolism. London, England, Wiley, 1973
3. Hoffer LJ: Methods for measuring sulfur amino acid metabolism. Curr Opin Clin Nutr Metab Care 5:511-517, 2002
4. Mudd SH, Levy HL, Kraus JP: Disorders of transsulfuration, in Scriver CR, Beaudet A, Sly WS, et al (eds): The Metabolic and Molecular Bases of Inherited Disease, vol 8. New York, NY, McGraw-Hill, 2001, pp 2007-2056
5. Blom HJ, Boers GH, van den Elzen JP, et al: Transamination of methionine in humans. Clin Sci 76:43-49, 1989
6. Tangerman A, Wilcken B, Levy HL, et al: Methionine transamination in patients with homocystinuria due to cystathionine beta-synthase deficiency. Metabolism 49:1071-1077, 2000
7. Mudd SH, Ebert MH, Scriver CR: Labile methyl group balances in the human: The role of sarcosine. Metabolism 29:707-720, 1980
8. Selhub J, Miller JW: The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 55:131-138, 1992
9. Selhub J: Homocysteine metabolism. Ann Rev Nutr 19:217-246, 1999
10. Gahl WA, Bernardini I, Finkelstein JD, et al: Transsulfuration in an adult with hepatic methionine adenosyltransferase deficiency. J Clin Invest 81:390-397, 1988
11. Finkelstein JD: Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost 26:219-225, 2000
12. Lobley GE, Connell A, Revell D: The importance of transmethylation reactions to methionine metabolism in sheep: effects of supplementation with creatine and choline. Br J Nutr 75:47-56, 1996
13. Stead LM, Au KP, Jacobs RL, et al: Methylation demand and homocysteine metabolism: Effects of dietary provision of creatine and guanidinoacetate. Am J Physiol 281:E1095-E1100, 2001
14. House JD, Jacobs RL, Stead LM, et al: Regulation of homocysteine metabolism. Adv Enzyme Regul 39:69-91, 1999
15. Luka Z, Cerone R, Phillips JA, et al: Mutations in human glycine N-methyltransferase give insights into its role in methionine metabolism. Hum Genet 110:68-74, 2002
16. Mudd SH, Cerone R, Schiaffino MC, et al: Glycine N-methyltransferase deficiency: A novel inborn error causing persistent isolated hypermethioninaemia. J Inherit Metab Dis 24:448-464, 2001
17. Augoustides-Savvopoulou P, Luka Z, Karyda S, et al: Glycine N-methyltransferase deficiency: A new patient with a novel mutation. J Inherit Metab Dis 26:745-759, 2003
18. Di Buono M, Wykes LJ, Cole DE, et al: Regulation of sulfur amino acid metabolism in men in response to changes in sulfur amino acid intakes. J Nutr 133:733-739, 2003
19. Russmann S, Junker E, Lauterburg BH: Remethylation and transsulfuration of methionine in cirrhosis: studies with L-[$^{2}H_{3}$-methyl-1-^{13}C]methionine. Hepatology 36:1190-1196, 2002
20. van Guldener C, Kulik W, Berger R, et al: Homocysteine and methionine metabolism in ESRD: A stable isotope study. Kidney Int 56:1064-1071, 1999