SEMINARS IN
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Causes of Hyperhomocysteinemia in Patients With Chronic Kidney Diseases

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Plasma homocysteine (Hcy) levels are increased significantly in patients with moderate renal failure and increase markedly in patients with end-stage renal disease. An increase in plasma Hcy level theoretically could be caused by an increased production rate (ie, transmethylation), a decreased rate of removal by transsulfuration or remethylation, or a decrease in the excretion of Hcy. Current evidence indicates that the major mechanism for hyperhomocysteinemia in renal failure is a decrease in Hcy removal from the body. However, it is debated whether this effect is the result of a decrease in the renal metabolic clearance or a result of extrarenal metabolic changes. The human kidney plays a major role in the removal of several aminothiols or Hcy-related compounds from the circulation (eg, cysteine-glycine, glutathione, AdoMet, and AdoHcy). However, the glomerular filtration of Hcy seems to be restricted because of protein binding. Besides glomerular filtration, the normal kidney can remove Hcy by plasma flow and peritubular uptake. Although in the low normal range in absolute terms, the flow through the transsulfuration pathway is reduced if related to Hcy levels in uremia; in addition, the remethylation pathway also is impaired. Besides the potential effect of the reduced renal mass on Hcy removal, available evidence suggests the occurrence of a generalized down-regulation of the methionine cycle and catabolism in uremia. AdoHcy, sulfate, and dimethylglycine currently are being investigated as retained solutes that can inhibit 1 or more pathways of Hcy metabolism. In addition, the high Hcy levels decrease in malnourished end-stage renal disease patients and change according to nutrient intake and several other nutritional parameters, indicating that circulating Hcy levels become an expression of nutritional status.

KEYWORDS homocysteine, aminothiols, uremia, methionine, transsulfuration, transmethylation

Homocysteine (Hcy) arises from methionine (Met) degradation at the cross-way of 2 different intracellular sequences: the Met cycle and the transsulfuration pathway.[1] The Met cycle includes the conversion of Met to S-adenosylmethionine (AdoMet), the use of AdoMet in diverse transmethylation reactions with a production of a methylated product plus S-adenosylHcy (AdoHcy), and the cleavage of AdoHcy into Hcy and adenosine. These 3 reactions take place in all cells in mammals, even if at different rates. The Met synthase reaction, which requires methylcobalamin as a cofactor and uses methyltetrahydrofolate (serine is the major methyl donor), also is present in all mammalian cells. In this reaction Hcy is remethylated to Met. Another remethylation reaction is catalyzed by betaine-Hcy S-methyltransferase and is restricted to liver and kidney. In some tissues (mainly liver, kidney, pancreas, and small intestine), Hcy is metabolized further in the transsulfuration pathway. Hcy is coupled with serine to form cysthathionine in the reaction catalyzed by cystathionine-β-synthase. Hydrolysis of cystathionine to cysteine (Cys) completes the transsulfuration sequence. In addition to the complete transsulfuration pathway, Hcy can be removed by the synthesis of glutathione (GSH), the major intracellular thiol.

Hcy mainly is found intracellularly. It has been noted that normal plasma Hcy represents the amino acid in transit from the site of production to a site of catabolism.[2] Tissues in which transsulfuration or remethylation are restricted must

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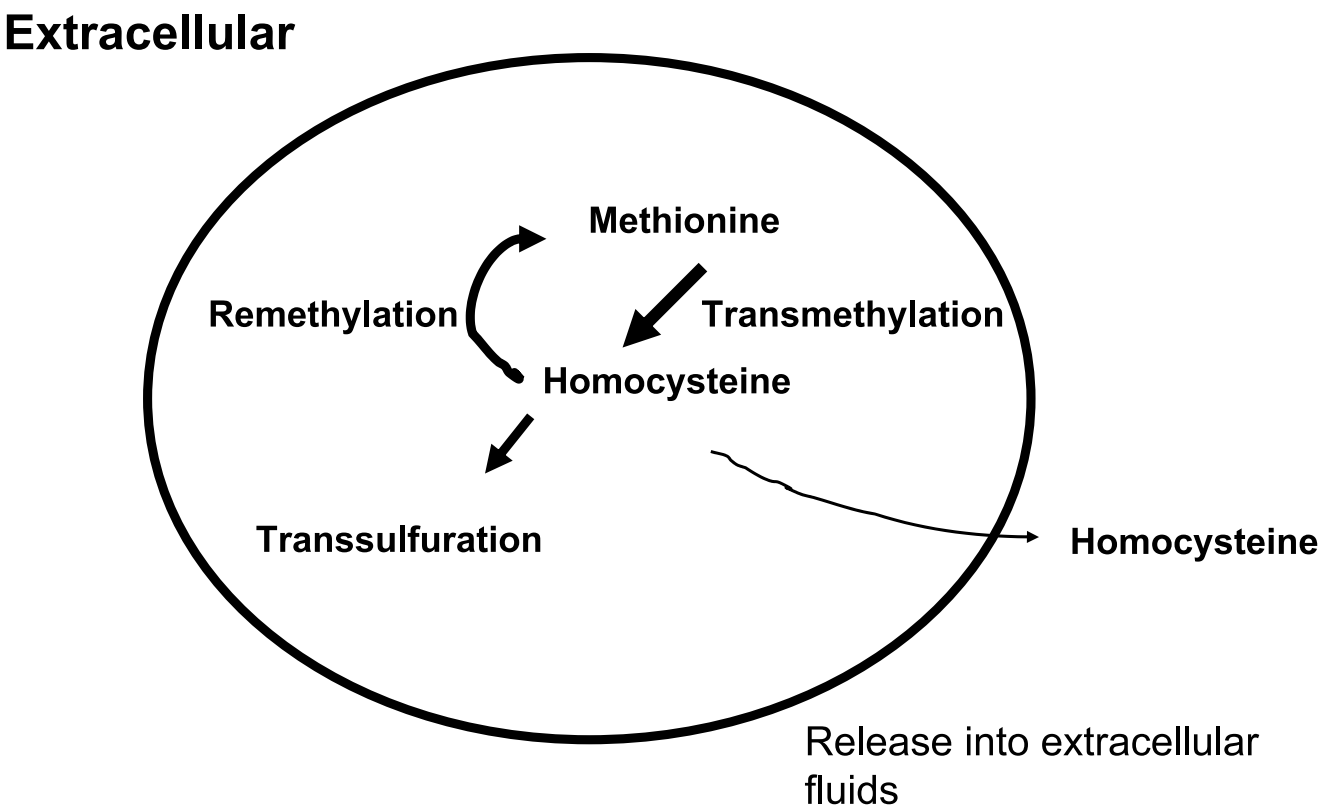

Figure 1 Pathway of methionine degradation. Decreased transsulfuration, remethylation, or reduced Hcy excretion from body fluids could increase plasma Hcy levels.

export Hcy for further metabolism by other tissues. Therefore, an increase in plasma Hcy level theoretically could be caused by an increased production rate (ie, transmethylation), by a decreased rate of removal by transsulfuration or remethylation, or by decreased elimination from body fluids (Fig 1).

The kidney plays a major role in maintaining the homeostasis of several plasma aminothiols.[1] However, the mechanisms by which renal function is linked to Hcy levels are understood poorly. Hypohomocysteinemia occurs during pregnancy[3] and in diabetes with hyperfiltration,[4] whereas hyperhomocysteinemia is related mainly to creatinine levels and glomerular filtration rate (GFR).[5] The odds of an increased Hcy level, adjusted for age, race, and vitamin levels, are increased approximately 2-fold for people with an estimated GFR of 60 to 90 mL/min/1.73 month^2, but are increased 10-fold in individuals with an estimated GFR of less than 60 mL/min/1.73 month^2.[6] Population-based studies also have shown that, besides renal function, increased plasma Hcy concentrations are associated with age, male sex, body mass index, serum creatinine level, folate level, vitamin B_{12} level, vitamin B_6 level, riboflavin level, caffeine and alcohol intakes, smoking status, and hypertension.[7,8]

It is of note that whole-body Hcy clearance is estimated to be about 80 to 100 mL/min (very close to creatinine clearance) in the healthy subjects.[9] However, whole-body Hcy clearance decreases progressively along with the decrease in renal function in patients with chronic kidney diseases (CKDs).[9] Metabolic removal of Hcy from tissues, including the kidney, have to account for the majority of body clearance because urinary Hcy clearance is very low (1-6 mL/min).[5,9] Calculations based on steady-state kinetics in healthy adult human beings estimate that 1.2 mmol of Hcy, which is a small fraction (~5%-10%) of the total daily cellular production, is delivered daily to the plasma compartment.[9] This figure increases markedly in folate-depleted patients.[9,12] The relative constancy of the plasma levels of Hcy suggests the occurrence of an active interorgan homeostatic regulation. However, scanty data are available on the ability of individual tissues and organs to remove or to add this amino acid to the plasma compartment in human beings.

Hcy Metabolism and the Kidney

Given the availability of metabolizing enzymes and transport systems in kidney cells one could expect that the loss of kidney tissue normally involved in Hcy handling would decrease Hcy removal from blood and increase its plasma level. Indeed, the rat kidney plays a major role in the maintenance of Hcy plasma homeostasis.[2]However, the concept of a major role played by the kidney in Hcy metabolism has been challenged by studies performed in human beings that have not shown the occurrence of any significant arteriovenous gradient of Hcy across this organ.[10,11] However, it has been noted that given the coefficient of variation for the measurement of Hcy in plasma (~3%-5%), the arteriovenous-difference technique cannot detect kidney removal of a significant part of the daily Hcy production. In addition, sizable amounts of

Hcy theoretically could be removed by the human kidney in the fed state.[2,5]

Glomerular filtration and tubular reabsorption are probably the mechanisms by which Hcy is removed by the rat kidney. However, besides glomerular filtration, which is restricted in human beings because of protein binding, Hcy may be taken up by the peritubular basolateral surface.[2,5] In a previous study performed in hypertensive patients we observed that the fractional extraction of Hcy across the kidney was related positively to renal plasma flow but not to GFR.[11] By examining the slope between renal Hcy clearance and renal plasma flow in that study, it appears that the Hcy renal clearance decreases rapidly from approximately 70 mL/min to values close to 0 when plasma flow decreases from normal (ie, 600-650 mL/min) to subnormal values (~400 mL/min). This finding is in agreement with data indicating that whole-body Hcy clearance decreases from approximately 100 to approximately 30 mL/min in patients with advanced CKD.[9,12] These data, although limited by the small number of patients studied, suggest the dependency of Hcy uptake by the human kidney on blood supply. It is noteworthy that in keeping with this renal transport modality, an increase in Hcy levels has been observed during conditions that preferentially can decrease blood flow across the kidney such as aging, hypertension, and heart failure.

Does an Interorgan Exchange of Hcy Take Place in Human Beings?

The tissues and organs that are a source of Hcy in plasma until now were a matter of speculation. Splanchnic organs (both liver and intestines) possess the entire transsulfuration pathway, and are major candidates to play a role in Hcy homeostasis. Under certain conditions, liver cells are able to release Hcy into the medium.[1,2] Such a possibility might occur in vivo during a Met load (or after a meal) when a sizable amount of the Met administered can account for the increase in Hcy levels. In addition, splanchnic organs might remove Hcy from plasma in the basal postabsorptive state because this occurs for several amino acids. Although we were not able to document a significant gradient for Hcy between the artery and the liver veins in human beings,[11] we observed that the exchange of Cys and Hcy across splanchnic organs were related closely and directly ($r^2 = 0.62$), with an uptake of Hcy taking place when Cys uptake was in the high range. This suggests that there may be similar dependency of Cys and Hcy handling by splanchnic organs on ongoing intracellular events. Because Cys uptake in splanchnic organs is related to the need for GSH synthesis,[1,2] is likely that a high GSH turnover rate increases the demand for Cys and Hcy, therefore depleting their intracellular pools. In agreement with this hypothesis we observed that the exchange of Hcy and Cys across splanchnic organs was related inversely to the estimated breakdown of GSH.[11]

If the splanchnic organs potentially both may remove or add Hcy to the systemic circulation, other tissues seem to be able only to add Hcy to the plasma compartment. Possible candidates for Hcy release are the peripheral tissues and lung. The sustained rates of protein degradation occurring in the postabsorptive state provide continuous Met availability within muscle cells as a precursor of thiol synthesis. The enzymes that are needed for the methylation process and Met synthase are available in mammal skeletal muscle.[1] In addition, the estimate of muscle Met remethylation pathway in vivo in dogs accounts for 8% to 10% of protein synthesis,[13] a figure that, although low in relative terms, may be considerable if one takes into account the entire muscle mass of the body. The transsulfuration route is not adaptable to changes in Met supply and plays a minor role in Met catabolism[1] in skeletal muscle. In a previous study in which we measured the arteriovenous difference of Hcy across leg tissues (which are composed mainly of muscle but also contain bone marrow and skin) in human beings, we observed that this amino acid was released into the circulation.[11] Considering the contribution of the leg to whole-body muscle mass the estimate of the amount of Hcy released into the circulation gives a figure of 0.9 mmol/d, which is similar to the estimated body release in plasma in healthy human beings.

Methionine Metabolism and Hcy Elimination From Blood in Patients With CKDs

The plasma Hcy level is increased significantly in patients with moderate renal failure and increases markedly in patients with end-stage renal disease (ESRD).[3,6] The prevalence of hyperhomocysteinemia in dialysis patients is more than 85%, and the mean plasma Hcy concentration is 3 to 4 times greater than normal. Patients with chronic renal failure show reduced rates of Hcy disappearance from plasma after Hcy loading and increased urinary Hcy excretion, suggesting the occurrence of intrarenal adaptive phenomena.[9] The major mechanism for hyperhomocysteinemia in renal failure is a decrease in Hcy clearance from the body.[9,12] However, it is unclear if these alterations are caused by diminished uptake by the kidney, an organ in which the transsulfuration pathway is developed fully, or if these are the expression of a more generalized disturbance in the remethylation and/or transsulfuration pathways, which progressively develop along with the decrease in renal function. It is of note that the progressive increase in Hcy levels in body fluids does not occur as an isolated alteration in CKD patients, but it is associated with a parallel accumulation in all downstream products of Met metabolism, with the exclusion of taurine (Fig 2). Moreover, these changes are associated with a decrease in the interorgan fluxes of several amino acids involved in methionine metabolism. The uptake of serine (an amino acid that participates as the major methyl-donor for Hcy remethylation and in the transsulfuration pathway) by both splanchnic organs and muscle is reduced in patients with chronic renal failure.[14-16] The uptake of Cys by splanchnic organs also is reduced.[16] These findings suggest the occurrence of a generalized down-regulation of Met catabolism in uremia. It is curious that a

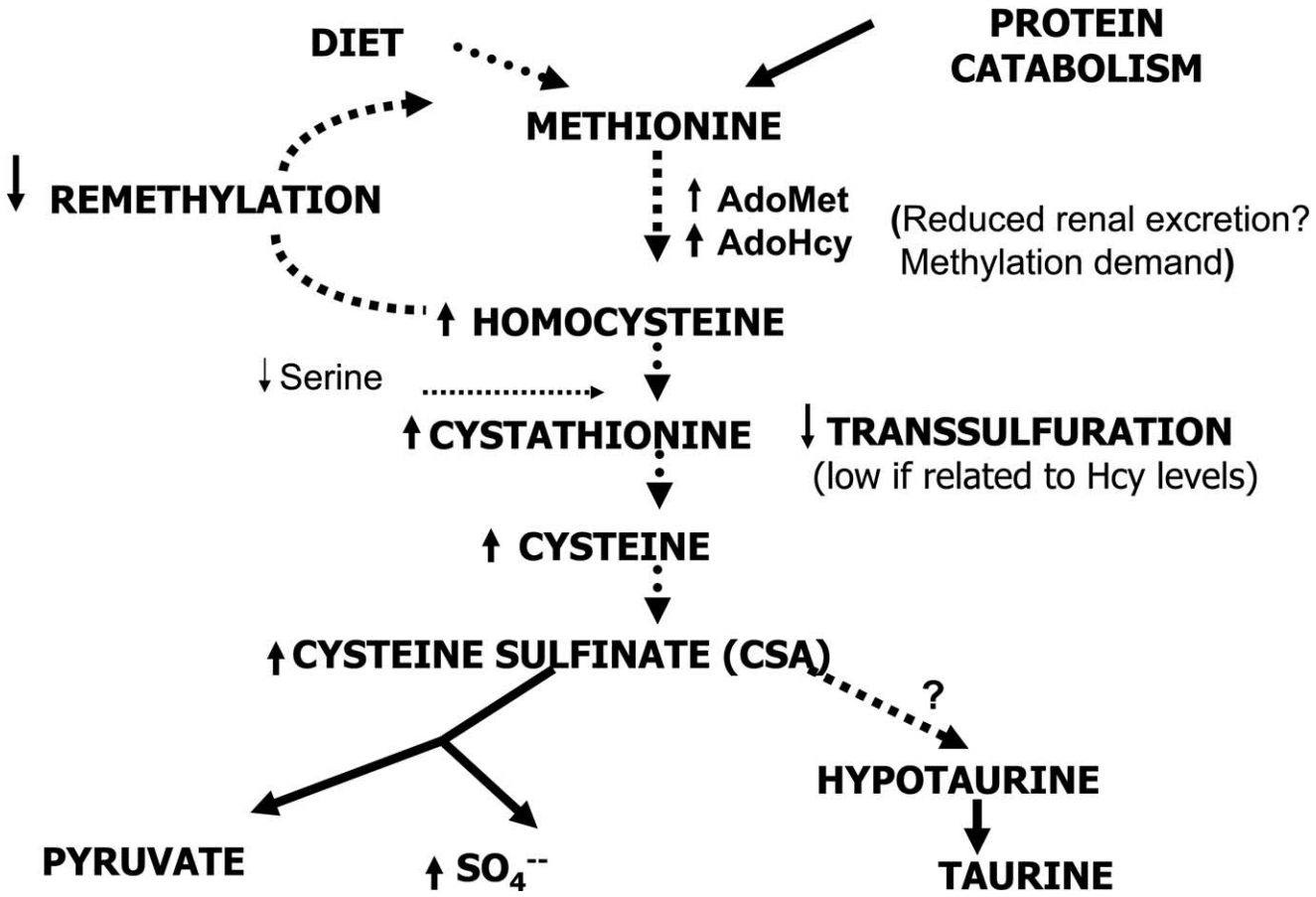

Figure 2 Summary of the results from various studies on methionine metabolism in patients with CKD and their interpretation. Downstream products of methionine metabolism, with the exclusion of taurine, accumulate in uremia. Serine levels are reduced and the uptake of serine and cysteine by splanchnic organs decreases. Flow by remethylation is reduced, and the transsulfuration is low if related to the high Hcy levels (defective clearance by transsulfuration). Several retained metabolites, for example, sulfate and AdoHcy, could cause an inhibitory action on transsulfuration and remethylation, respectively.

clear-cut decrease in the transsulfuration pathway has not been shown clearly in the few stable isotope studies on Met metabolism in ESRD patients performed to date. Van Guldener et al[16] observed that transsulfuration rates were only borderline low, whereas transmethylation and remethylation rates were decreased significantly in 4 ESRD patients. Recently, Stam et al[17] used methione isotopes to track Met metabolism in 12 ESRD patients and 16 healthy controls. Patients with ESRD had a remethylation rate that was 28% lower than the controls, whereas transmethylation and transsulfuration rates were 26 to 26% lower. These values were not statistically significant when they were corrected for age. However, when the absolute rates were related to the increased Hcy levels (as an expression of clearance), whole-body Hcy clearance by transsulfuration and remethylation were reduced markedly (by 77%-80%). Taken together, these data indicate a retention of downstream metabolites of Met metabolism in uremia and a decrease of Hcy clearance by both the transsulfuration and remethylation pathways.

A possible feedback inhibitory substrate for the transsulfuration pathway is sulfate, the end product of Met catabolism. Sulfate accumulates progressively along with the decrease in kidney function, and is responsible for part of the increase in anion gap observed in patients with ESRD. The increased plasma sulfate level has been observed to be associated with the plasma Hcy level.[18,19]

Other unidentified uremic substances could cause an inhibition of Hcy remethylation. In this regard, AdoHcy may be a key regulatory compound. A recent observation indicates that the urinary excretion of both AdoMet and AdoHcy by the normal kidney is high, and this could in part explain their accumulation in patients with CKD.[20] In addition, the increased AdoHcy level also may result from the increase in Hcy levels.[21] AdoHcy is an activator of cystathionine–β-synthase in vitro, but inhibits methyltransferases. Recently, Stam et al[17] observed that the blood AdoHcy level was correlated significantly with both transmethylation and remethylation fluxes. It also has been observed that plasma dimethyl glycine is increased in chronic renal failure and that it correlates with Hcy in ESRD patients. Previous in vitro studies indicated that such a disturbance would inhibit betaine-Hcy S-methyltransferase activity.[22]

Studies in ESRD patients have shown that, besides reduced renal function, other factors can influence plasma Hcy level.

Hyperhomocysteinemia in patients with ESRD has been shown to be associated with deficiencies of folate and vitamins B_{12} and B_6 and/or with genetic variants of several enzymes of folate and 1-carbon pool metabolism.[1] Suliman et al[13] observed that Hcy level was related strongly to serum albumin level, and that patients with malnutrition had lower levels of both Hcy and serum albumin than those with normal nutritional status. Recently, Kalantar-Zadeh et al[15] obtained similar findings in a large population of ESRD patients. In their study, plasma Hcy levels were correlated strongly with normalize Protein Nitrogen Appearance (nPNA), suggesting the dependence of Hcy on a high protein intake. Similar but even stronger correlations were found between values of Hcy and serum concentrations of other nutritional markers such as total amino acids, prealbumin level, creatinine level, and urea nitrogen level, with serum creatinine level showing the strongest correlations. Moreover, some anthropometric measures correlated with Hcy levels, with higher Hcy concentrations in patients whose triceps skinfold or arm muscle circumference were larger. According to these data protein intake, several nutritional markers and, at least in part, muscle mass appear to account for variability in Hcy levels in hemodialysis patients.

In conclusion, recent findings outline how the sensitive sequences of checks and balances by which methionine metabolism is controlled can be altered in patients with CKD. The major mechanism for hyperhomocysteinemia in patients with CKD is a decrease in Hcy removal from the body. It is debated whether this effect is the result of a decrease in the renal metabolic clearance or of extrarenal metabolic changes. The human kidney plays a substantial role in the removal from the circulation of several aminothiols or Hcy-interrelated compounds, such as cysteine-glycine, glutathione, AdoMet, and AdoHcy. Besides glomerular filtration, which seems to be restricted because of protein binding, the normal kidney can remove Hcy by plasma flow and peritubular uptake. Although in the low normal range in absolute terms, the flow through the transsulfuration pathway is reduced if related to Hcy levels in uremia; in addition, the remethylation pathway also is impaired. Besides the potential effect of the reduced renal mass on Hcy removal, available evidence suggests the occurrence of a generalized down-regulation of methionine cycle and catabolism in uremia. AdoHcy, sulfate, and dimethylglycine are investigated as retained solutes that can inhibit 1 or more pathways of Hcy metabolism. In addition, Hcy levels decrease in malnourished ESRD patients and change according to nutrient intake and several other nutritional parameters, suggesting that circulating Hcy levels become an expression of nutritional status.

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