Homocysteine clearance and methylation flux rates in health and end-stage renal disease: association with S-adenosylhomocysteine

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Stam, Frank, Coen van Gulden, Piet M. ter Wee, Willem Kulik, Desirée E. C. Smith, Cornelis Jakobs, Coen D. A. Stehouwer, and Kees de Meer. Homocysteine clearance and methylation flux rates in health and end-stage renal disease: association with S-adenosylhomocysteine. *Am J Physiol Renal Physiol* 287: F215–F223, 2004; 10.1152/ajprenal.00376.2003.—Hyperhomocysteinemia is an independent cardiovascular risk factor for cardiovascular disease and occurs frequently in end-stage renal disease (ESRD), but its pathogenesis is poorly understood. We aimed to evaluate one-carbon flux rates of methionine and homocysteine (Hcy) in ESRD patients and healthy controls. Transmethylation (TM), remethylation (RM), and transsulfuration (TS), as well as Hcy clearance by TS (i.e., TS/plasma total Hcy concentration) and by RM (i.e., RM/plasma total Hcy concentration) were evaluated in relation to body composition, vitamins, and S-adenosylhomocysteine (AdoHcy) and S-adenosylmethionine (AdoMet) levels. After a fixed protein diet for 3 days, primed-continuous infusion of [3H]methyl-1-13C]methionine was performed in the postabsorptive state in 12 hemodialysis patients and 16 healthy volunteers. Hcy clearance by TS (−80%, P < 0.001) and by RM (−77%, P < 0.001) in ESRD patients was decreased compared with healthy controls. The absolute flux rates of TM (−27%, P < 0.01) and RM (−28%, P = 0.02) were lower in the ESRD patients. After adjustment for age, TS was not significantly reduced. Whole blood AdoHcy was significantly elevated in ESRD and was a significant determinant of TM (standardized β = 1.24, P = 0.01) and RM (standardized β = 1.43, P = 0.03). In conclusion, patients with ESRD have impaired Hcy clearance by TS and RM. Elevated whole blood AdoHcy levels are associated with impaired RM and TM flux rates in these patients, and AdoHcy may be a key regulatory compound in one-carbon flux.

Stable isotopes: S-adenosylmethionine; transsulfuration; remethylation; transmethylation.

**Hyperhomocysteinemia is an independent cardiovascular risk factor in patients with end-stage renal disease (ESRD), with a prevalence reported as high as 85–100% (25). Treatment regimens containing folic acid decrease plasma homocysteine (Hcy) concentration in ESRD patients (3, 23, 34), but only a small proportion of treated patients attain circulating Hcy levels in the normal range. The persistence of hyperhomocysteinemia in ESRD patients with supraphysiological folate levels is poorly understood. Elucidation of the regulation of the methionine-Hcy metabolism is necessary to understand the pathophysiology of hyperhomocysteinemia in patients with chronic renal failure and to develop more effective therapies.

In the one-carbon cycle (Fig. 1), Hcy is the transmethylation (TM) product of the essential sulfur-containing amino acid methionine, with S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy) as intermediates. Hcy can be either remethylated to methionine or degraded by transsulfuration (TS). There are two remethylation (RM) reactions in which Hcy is involved. In one reaction, methionine synthase converts Hcy into methionine with 5-methyltetrahydrofolate as a methyl donor and reduced vitamin B12 as a cofactor; 5-methyltetrahydrofolate is generated by a reaction catalyzed by the enzyme 5,10-methylenetetrahydrofolate reductase. In the other RM reaction, betaine is used as a methyl donor. In the irreversible catabolic (TS) pathway, the rate-limiting reaction is catalyzed by cystathionine β-synthase, requiring the active form of vitamin B6 as a cofactor.

Patients with chronic renal failure have a substantially decreased plasma disappearance rate of Hcy after Hcy loading (9). Diminished renal function (i.e., glomerular filtration rate) is strongly correlated with elevated plasma Hcy levels over a wide range of glomerular filtration capacity (28), suggesting a central role for the kidney in Hcy metabolism. As urinary Hcy excretion is negligible (24, 27), loss of renal TS capacity has been hypothesized as a cause of hyperhomocysteinemia in renal failure (2, 13). However, no net renal extraction of Hcy occurs (33), which does not exclude Hcy metabolism in the kidney with a zero balance. Alternatively, whole body Hcy metabolism may be impaired in ESRD. In a previous pilot study, we have reported that the in vivo flux rates of RM and TM were diminished in patients with ESRD compared with healthy controls on the same protein intake, whereas TS was not significantly different (35). The latter finding could have been due to the small number of individuals studied. It was argued by Hoffer (12) that the TS rate is not an adequate index of the whole body Hcy disposal capacity, i.e., the metabolic Hcy clearance by TS. Therefore, in addition to TS, we calculated Hcy clearance as TS divided by the plasma total Hcy (tHcy) concentration.

Impaired regulation of one-carbon cycling, possibly through accumulation of uremic toxins, may be an explanation for abnormalities in RM and TM rates in ESRD patients. In patients with chronic renal failure, plasma AdoHcy and AdoMet levels are elevated (20), but methionine levels are not increased (22). Whether and how these metabolite changes are related to tissue one-carbon fluxes have hitherto not been studied. From single-enzyme kinetics, the following interactions can be deduced (7, 18). In TS, increased levels of
AdoMet and AdoHcy are activators of cystathionine β-synthase. 2) In RM, AdoMet and AdoHcy are inhibitors of betaine-Hcy methyltransferase, and AdoHcy inhibits MS, whereas AdoMet inhibits 5,10-methylenetetrahydrofolate reductase, and both methyltransferases are inhibited by their metabolic product, methionine. 3) In TM, AdoHcy inhibits the activity of most methyltransferases. From these data, elevated intracellular levels of AdoMet and AdoHcy are expected to be associated with diminished RM (and TM) flux rates, whereas TS flux is expected to be stimulated by methionine ingestion and the subsequent elevations of AdoMet and AdoHcy by adenosyltransferase activity. However, little is known about the in vivo regulation of one-carbon metabolism by AdoMet and AdoHcy in healthy humans and in patients with chronic renal failure.

In view of these considerations, the aims of the present study were to test the hypothesis that in vivo one-carbon fluxes (TM, RM, and TS) and the Hcy clearance by RM and TS are decreased in ESRD patients compared with healthy individuals and to explore the relationships of these fluxes with plasma Hcy and blood AdoMet and AdoHcy levels.

MATERIALS AND METHODS

Subjects

For the present study, eight ESRD patients on maintenance hemodialysis treatment and 10 healthy controls, with an age >18 yr, were included. The patients were on chronic standard bicarbonate hemodialysis (thrice weekly for at least 6 mo) and received a multivitamin tablet (once daily) containing 2 mg vitamin B6, 2 mg thiamine, 2 mg riboflavin, 15 mg nicotinamide, and 70 mg ascorbic acid. Five ESRD patients received a tablet (once weekly) containing 1 mg folic acid. The healthy controls did not use any medication or vitamin supplements. To enhance the power of the study to measure a clinically relevant difference in methionine-Hcy metabolism, data from a previous study in four ESRD patients and six healthy controls were combined with those of the present study group. The results from these 10 individuals have been published previously (35). The total study group thus consisted of 12 ESRD patients and 16 healthy individuals. The study protocol was approved by the ethics committee of the VU University Medical Center, and written informed consent was obtained from all individuals.

Experimental Protocol

The experimental protocol has been described earlier (35). Fat-free mass. Body weight (BW) was measured on a balance scale (accuracy 50 g), and four skinfolds were measured using a caliper (Holtain, accuracy 0.1 mm). Fat-free mass (FFM) was calculated from skinfold measurements according to Durnin and Womersky (6). This method has been shown to be a reliable method of quantifying body composition in healthy individuals as well as ESRD patients (15, 16).

Stable isotopes. All subjects were kept on a fixed diet containing 1.0 g of protein·kg BW·day$^{-1}$ for 3 days before the study. The experiments were conducted after an overnight fast. Fasting was continued throughout the infusion period. Only drinking of small amounts of tap water was allowed. The hemodialysis patients were studied 1 day before a regular midweek dialysis session. All subjects were kept in bed during the study period. A priming bolus of NaHCO$_3$ (99% 13C; ARC Laboratories, Apeldoorn, The Netherlands) was administered, followed by a primed constant infusion of L-[3H$_2$methyl-1-13C]methionine (95% dilabeled; 99% 1-13C; 99% 2H$_1$, Isotec, Miamisburg, OH), according to the infusion protocol described previously (35).
Sample analysis. Plasma total (free plus protein bound) Hcy was measured with the use of a microparticle enzyme immunoassay method based on fluorescence polarization (IMX analyzer; Abbott, Chicago, IL). AdoMet and AdoHcy were measured using stable-isotope dilution tandem mass spectrometry (30). AdoHcy and AdoMet levels were not determined in the 10 individuals described in the previous study (35). Serum folate and vitamin B12 concentrations were measured in plasma and whole blood with radioassay (ICN, Costa Mesa, CA) and serum vitamin B6 with the use of fluorescence HPLC (32). The methionine concentration in the infusion was measured with the use of an amino acid analyzer equipped with a high-pressure analytic column packed with Utrapac 8 resin (Pharmaacia Biotech, Cambridge, UK). Isotopic enrichments of methionine in plasma were measured in the acetyl-3,5-bis(trifluoromethyl)benzyl derivative with the use of gas chromatography-mass spectrometry (GCMS), as previously described (17). Enrichments [in mole percent excess (MPE)] were calculated on the basis of the relative abundance of the (m + 0), (m + 1), and (m + 4) methionine species (29), and calibration curves obtained from weighed amounts of tracer (m + 1 and m + 4) and trace methionine were used to correct for minor instrument variation (17). The 13C enrichment of CO2 in breath samples was measured on a dual-inlet isotope ratio mass spectrometer (VG Optima, Fisons Instruments, Middlewich, Cheshire, UK) and expressed in atom percent excess (APE, %), as previously described (35).

Gene polymorphisms. The polymorphisms of 5,10-methylenetetrahydrofolate reductase (C677T transition), cystathionine β-synthase (844ins68 variant), and methionine synthase (A2756G transition) were measured in plasma and whole blood with radioassay (ICN, Costa Mesa, CA) and serum vitamin B6 with the use of high-pressure analytic column packed with Utrapac 8 resin (Pharmaacia Biotech, Cambridge, UK). Isotopic enrichments of methionine in plasma were measured in the acetyl-3,5-bis(trifluoromethyl)benzyl derivative with the use of gas chromatography-mass spectrometry (GCMS), as previously described (17). Enrichments [in mole percent excess (MPE)] were calculated on the basis of the relative abundance of the (m + 0), (m + 1), and (m + 4) methionine species (29), and calibration curves obtained from weighed amounts of tracer (m + 1 and m + 4) and trace methionine were used to correct for minor instrument variation (17). The 13C enrichment of CO2 in breath samples was measured on a dual-inlet isotope ratio mass spectrometer (VG Optima, Fisons Instruments, Middlewich, Cheshire, UK) and expressed in atom percent excess (APE, %), as previously described (35).

Calculations

The features of the 1,3H3-methyl-1,13C]methionine plateau model have been previously summarized (5, 29). In the isotopic steady state, whole body fluxes of TM, TS, and RM can be estimated from enrichments at plateau from the sum of the enrichments of methionine (m + 4) and (m + 1), and methionine-COOH enrichment in plasma and treatment infusion rate as follows

\[ \text{RM} = \frac{Q_m - Q_i}{Q_i} \]

From the infusion rate of the tracer and with correction for isotopic enrichment of the tracer, \( Q_m \), can be calculated from the enrichments of methionine (m + 4) and (m + 1), and methionine-COOH enrichment in plasma and treatment infusion rate as follows

\[ \text{TS} = \frac{V_{CO2} \times (1/13C]methionine enrichment in plasma - 1/[13C]methionine enrichment in tracer infusion)}{1} \]

in which \( V_{CO2} \) equals whole body CO2 production (in µmol/h) * breath air 13CO2 enrichment * 0.72, where 0.72 is the bicarbonate retention factor (11). As methionine is the only precursor of Hcy, the disappearance of Hcy (RM + TS) equals Hcy appearance, thus

\[ \text{TM} = \text{RM} + \text{TS} \]

CO2 production was measured for 30 min with a ventilated hood and using an indirect calorimeter (2900 Metabolic Measurement Cart, SensorMedics, Yorba Linda, CA). Internal calibration for CO2 and volume was conducted before each infusion. Gas volumes were automatically corrected for temperature and air pressure. The flux rates for RM, TS, and TM were expressed as micromoles per hour per kilogram BW and per kilogram FFM. Metabolic Hcy clearance by TS and by RM was calculated by dividing the flux rates (TS and RM, respectively) by plasma tHcy concentration and is expressed in liters per kilogram per hour.

Statistical Analysis

Values are expressed as means ± SD or as median (range) if data were skewed. Differences between patients and controls were tested with unpaired Student’s t-tests, Mann-Whitney tests, and Fisher’s exact tests as appropriate. Pearson’s correlation coefficients were calculated, with log-transformed data in case of a skewed distribution of a variable. The distribution of genetic polymorphisms (wild-type or heterozygote mutant = 0; homozygote mutant = 1) was analyzed with the \( \chi^2 \) test. Multivariate regression analysis was performed to analyze ESRD (absent = 0; present = 1), sex, age, and vitamin status (plasma concentrations) as determinants of FFM-adjusted flux rates and Hcy clearance. All regression coefficients (β) are reported as standardized β. Differences were considered significant if \( P < 0.05 \) (2-tailed).

RESULTS

The median (range) time on dialysis of the 12 ESRD patients was 17 (10–140) mo. Baseline characteristics are presented in Table 1. Patients with ESRD had significantly higher plasma levels of tHcy and folate, and a significantly higher mean age compared with the 16 healthy controls.

Whole Body Flux Rates of RM, TM, TS, and Hcy Clearance in ESRD Patients and Controls

CO2 production was similar in patients (2.79 ± 0.21 ml·kg\(^{-1}\)·min\(^{-1}\)) and controls (2.72 ± 0.15 ml·kg\(^{-1}\)·min\(^{-1}\)). Patients with ESRD had a RM rate that was 28% lower than the controls (95% confidence interval (CI) −50 to −5%, \( P = 0.02 \)), whereas TM was 27% lower (95% CI −43 to −11%, \( P < 0.01 \)) and TS was 26% lower (95% CI −45 to −7%, \( P < 0.01 \)). Hcy clearance by RM was 77% lower (95% CI −100 to −55%, \( P < 0.001 \)) and Hcy clearance by TS was 80% lower (95% CI −100 to −52%, \( P < 0.001 \)) in ESRD patients compared with controls (Table 2).

RM and TM rates were positively correlated, both within the patient group (\( r = 0.84, P < 0.001 \)) and within the control group (\( r = 0.89, P < 0.001 \)). Correlations between RM and TS and between TM and TS were not significant in either group (data not shown).

After adjustment for sex and age, RM (−18%; 95% CI −45 to +9%, \( P = 0.19 \)), TM (−15%; 95% CI −33 to +2%, \( P = 0.09 \)), and TS (−10%; 95% CI −30 to +9%, \( P = 0.29 \)) were no longer significantly decreased in the ESRD group, but Hcy clearance by RM (−65%; 95% CI −97 to −32%, \( P < 0.001 \)) and Hcy clearance by TS (−65%; 95% CI −97 to −33%, \( P < 0.001 \)) were still significantly decreased. Age itself was a significant determinant of TS (β = −0.51, \( P = 0.01 \)) and TM (β = −0.45, \( P = 0.03 \)), but not of RM (β = −0.29, \( P = 0.21 \)). Hcy clearance by RM (β = −0.25, \( P = 0.14 \)), and Hcy clearance by TS (β = −0.29, \( P = 0.08 \)). Plasma tHcy, the other component of the equation for Hcy clearance by TS and RM, was not significantly determined by age (β = −0.04, \( P = 0.80 \)).
Because ESRD status and age were strongly associated, analyses adjusted for age may to some extent result in overadjustment. We therefore repeated the multivariate analysis in two age-matched subgroups, which consisted of 8 ESRD patients (patients 2, 3, 4, 5, 6, 7, 10, and 11; mean age 44.9 ± 15.7 yr) and 8 healthy controls (patients 1, 3, 4, 6, 9, 14, and 15; mean age 43.3 ± 14.4 yr). After adjustment for sex and age, the ESRD group had a 23% lower RM (95% CI -44 to -3%, P = 0.03), a 19% lower TM (95% CI -38 to -1%, P = 0.048), a 72% lower Hcy clearance by RM (95% CI -96 to -49%, P < 0.001), and a 72% lower Hcy clearance by TS (95% CI -100 to -41%, P < 0.001) compared with the healthy controls, but TS was not significantly different (−8%; 95% CI −33 to +17%, P = 0.51).

Separate addition of serum levels of vitamins to the statistical models did not change the results (data not shown).

**Determinants of Plasma tHcy**

Plasma tHcy was about fourfold higher in the ESRD group than in the healthy controls.

- **Multivariate analysis**, with forced entry of the covariates sex and age, showed that patients with ESRD had a 322% higher plasma tHcy (95% CI 209 to 436%, P < 0.001). To test the possibility that this relationship was mediated by one-carbon flux rates, plasma and whole blood levels of AdoMet and AdoHcy (and their ratio), and vitamins, these individual variables were sequentially added to the multivariate model. This did not materially change the relationship between ESRD status and plasma tHcy. After adjustment for age and sex, RM (β = −0.37, P = 0.07) and TM (β = −0.42, P = 0.08) were associated with plasma tHcy with borderline significance, and TS (β = −0.08, P = 0.75) was not. Plasma levels of AdoMet (β = 0.78, P < 0.001), AdoHcy (β = 0.74, P < 0.001), and their ratio (β = −0.69, P = 0.001) were related to plasma tHcy, as were whole blood levels of AdoHcy (β = 0.63, P < 0.01) and the AdoMet/AdoHcy ratio (β = −0.55, P = 0.01), but whole blood levels of AdoMet were not significantly related to plasma tHcy (β = −0.15, P = 0.46). Of the vitamins, serum folate was significantly related to plasma tHcy (β = −0.38, P = 0.01), but vitamin B6 (β = −0.12, P = 0.63) and vitamin B12 (β = −0.16, P = 0.24) were not.
Blood AdoMet and AdoHcy and Their Relationship to Flux Rates and Hcy Clearance

Table 3 shows the levels of AdoMet, AdoHcy, and their ratio in whole blood and plasma for patients with ESRD and healthy controls. After adjustment for sex and age, plasma levels of AdoMet ($\beta = 0.87, P < 0.001$) and AdoHcy ($\beta = 0.97, P < 0.001$) were significantly higher in the ESRD patients compared with the healthy controls, and the AdoMet/AdoHcy ratio was lower ($\beta = -1.00, P < 0.001$). In whole blood, the same results were found for AdoHcy ($\beta = 0.95, P < 0.001$) and the AdoMet/AdoHcy ratio ($\beta = -1.01, P < 0.001$), but AdoMet levels in the ESRD patients were not significantly different ($\beta = -0.59, P = 0.08$) from the healthy controls.

After adjustment for sex and age, whole blood levels of AdoHcy were a significant determinant of RM ($\beta = -1.43, P = 0.03$), TM ($\beta = -1.24, P = 0.01$) (Fig. 2), but not of TS ($\beta = -0.37, P = 0.48$), Hcy clearance by RM ($\beta = -0.55, P = 0.23$), and Hcy clearance by TS ($\beta = 0.02, P = 0.97$) in the whole group. The whole blood levels of AdoMet were significantly associated with TS ($\beta = 0.40, P = 0.049$), but not with RM ($\beta = -0.06, P = 0.85$), TM ($\beta = 0.14, P = 0.57$), Hcy clearance by RM ($\beta = 0.02, P = 0.92$), or Hcy clearance by TS ($\beta = 0.25, P = 0.20$). The whole blood AdoMet/AdoHcy ratio was significantly associated with TM ($\beta = 0.51, P = 0.02$), and less with RM ($\beta = 1.27, P = 0.07$) and TS ($\beta = 0.68, P = 0.15$). The whole blood AdoMet/AdoHcy ratio was not a significant determinant of Hcy clearance by RM ($\beta = 0.49, P = 0.27$) and Hcy clearance by TS ($\beta = 0.22, P = 0.63$). Plasma levels of AdoMet, AdoHcy, or the AdoMet/AdoHcy ratio were not significantly associated with one-carbon fluxes or Hcy clearance (data not shown).

Data were insufficient to test whether the relationship between ESRD status on one hand and one-carbon fluxes and Hcy clearances on the other were mediated by AdoMet, AdoHcy, or their ratio.

DISCUSSION

Our study shows that, in ESRD patients, metabolic Hcy clearance by TS and RM is severely reduced and indicates that absolute RM and TM rates are reduced to a much lesser extent, whereas the TS rate is unchanged. We also found evidence for an inverse relationship between whole blood AdoMet/AdoHcy concentration and TM and RM rates.

In steady-state conditions in weight-maintaining adults, the TS rate reflects oxidation of methionine from dietary intake, because methionine is the only precursor of Hcy, and TS is the
only method of Hcy disposal (methionine catabolism by transamination is negligible in humans) (1). In the design of our study, protein intake was the same in ESRD patients and healthy controls. It is thus not surprising that in the present study the TS rate (after adjustment for age and gender) was similar for ESRD patients and controls. This is in agreement with observations in our previous study (35).

As an explanation of the elevated plasma tHcy concentrations in ESRD patients, the concept of decreased metabolic Hcy clearance has been put forward (12). In this concept, any block in Hcy TS has to be followed by an increase in plasma tHcy to maintain a constant (diet-dependent) methionine oxidation. The initial rise in Hcy is followed by an accumulation of its precursors AdoHcy and AdoMet, which are activators of cystathionine β-synthase (7, 19), the rate-limiting enzyme in the TS pathway. The rise in the concentration of these compounds in ESRD may stimulate TS to such an extent that it equilibrates with methionine intake. We and others (20) found elevated AdoMet and AdoHcy levels in ESRD patients, and we also found that whole blood levels of AdoMet were positively correlated with observations in our previous study (35).

In ESRD patients, the metabolic consequence of diminished whole body RM in Hcy clearance by RM in the majority of the ESRD population. Treatment regimens containing folic acid decrease plasma tHcy concentration in ESRD patients (3, 23, 34), which indicates that this block is modifiable.

TM was diminished in ESRD patients, despite accumulation of the methyl group donor AdoMet. However, in accordance with Loehrer and co-workers (20), we found that the ratio of AdoMet to AdoHcy in plasma was eight times lower in ESRD patients than in healthy individuals. From in vitro studies, the AdoMet/AdoHcy ratio is known to have a regulatory function in the enzymatic TM reactions (7). The metabolic consequence of diminished whole body TM flux in ESRD patients is impaired capacity to provide methyl groups in tissues. This may explain the impaired DNA methylation in hyperhomocysteinemic ESRD patients as recently reported (14). Less requirement for creatine biosynthesis, and therefore methyl donation, due to diminished muscle mass in ESRD patients has been suggested to result in a smaller need for TM (and RM) by Hoffer (12). However, in our study we expressed TM per kilogram FFM, which was similar in healthy controls and ESRD patients. Whether reducing tissue AdoHcy accumulation can increase methylation flux rates in patients with ESRD and will improve DNA methylation merits further study.

### Table 3. Plasma and whole blood levels of S-adenosylmethionine and S-adenosylhomocysteine in the postabsorptive state

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<th>AdoMet, nmol/l</th>
<th>AdoHcy, nmol/l</th>
<th>AdoMet/AdoHcy</th>
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<td>Plasma Whole blood</td>
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<td>ESRD patients</td>
<td>268 (190–390)</td>
<td>1.260 (1.120–1.610)</td>
<td>410 (139–562)</td>
<td>294 (124–407)</td>
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<td>Healthy controls</td>
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|          | 85 (61–102) | 1,580 (1,095–1,760) | 12 (9–13) | 63 (53–88) | 6.7 (5.7–10.4) | 24.7 (13.9–26.1) | 0.38 ± 0.03 |
|          | <0.001 | 0.06 | <0.001 | <0.001 | <0.001 | <0.001 | 0.20 |
This is the first study in humans that shows that the whole body flux rates of RM and TM are negatively related to the whole blood AdoHcy level. The inverse relationship between AdoHcy levels and RM rate may be explained by inhibitory effects of AdoHcy on methionine synthase activity in the tissues and most other acceptor methyltransferases, as suggested by Finkelstein (7). Also, in our study design, protein intake was fixed and TS unchanged, implying that RM and TM are linearly related with each other (see Eq. 3). Therefore, RM and TM showed the same trend in their relationships with AdoHcy. The whole blood AdoMet/AdoHcy ratio showed only a weak relationship with TM and none with RM, and plasma AdoHcy was not related to RM and TM flux. This suggests that the intracellular AdoHcy concentration, reflected by its whole blood levels, is a possible regulator of methyl group flux from methionine demethylation and folate-dependent RM in humans. Plasma tHcy was not significantly associated with RM or TM in our ESRD and healthy control individuals. Taken together, these findings indicate that in humans, and particularly when renal function is impaired, AdoHcy may be a key intracellular regulator of one-carbon flux.

The limitations of the study need to be addressed. First, in retrospect, the present study had a power of 80% to detect a difference of 27% in TS and TM and 38% in RM, but, despite inclusion of data from our previous stable-isotope study (35), the number of subjects was too small to quantify the impact of AdoHcy and AdoMet on the relationship between ESRD and flux rates. The conclusions regarding relationships have to be interpreted with caution, as we cannot exclude that other factors associated with ESRD (e.g., uremic compounds) interact with enzyme function in methionine and Hcy metabolism.

Second, the validity of the stable-isotope model as a reflection of intracellular methyl group metabolism merits discussion. Isotope retention in the body’s bicarbonate pool is a recognized cause of underestimation of catabolism when {\textsuperscript{13}C}{\textsubscript{2}}O enrichment in breath air is used, and therefore TS flux was corrected with the bicarbonate retention factor (10). Also, RM, TM, and TS fluxes are underestimated to some extent when plasma enrichments are used in the calculations, due to tracer dilution in the intracellular compartment. Young and co-workers (29) assumed, from measurements of dilution of other indispensable amino acids, that use of plasma values underestimates RM and TM in postabsorptive adults by ~20%. In labeled methionine studies in humans, enrichment in plasma Hcy, a surrogate measure of intracellular methionine enrichment, was 90% of tracer methionine enrichment in plasma after 9 h (4). Lower values for plasma enrichment of Hcy (the precursor for RM) compared with methionine have been found in studies using priming of the methionine pool (21, 26), because, in the absence of priming of the Hcy pool, plasma enrichment rises much more slowly than when priming is used. There are no known pathways for Hcy production apart from TM, rendering additional dilution of intracellular Hcy unlikely and underpinning the validity of the use of {\textsuperscript{13}C}methionine enrichment for methionine and Hcy flux estimation. With the use of our study protocol, plasma methionine enrichment reaches a plateau in primed steady-state protocols within 6 h even in ESRD patients (17), in whom hyperhomocysteinemia coexists with normal methionine levels (22). With these considerations, we do not expect the underestimation of TM and RM in ESRD patients and healthy controls to be much different from 10–20% under the conditions of our study.

Third, with respect to the role of selection bias on interpretation of the study data, the effect of age and vitamin supplementation must be addressed. There was a difference of ~22 yr in mean age between the ESRD patients and healthy controls in our study, and multiple regression analysis showed a significant negative relationship between their age and TS and TM rates. With the same stable-isotope infusion technique, others have reported similar flux rates in whole body methionine and Hcy metabolism in healthy elderly (n = 12; age 74 ± 2 yr) and young individuals (n = 8; age 27 ± 6 yr) on the same protein intake (8, 10). In post hoc analyses, ESRD patients had a significantly lower TM and RM than age-matched controls, but TS was similar. The patient-control difference in TS rate between the post hoc analysis (−8%) and the overall analysis (−27%) cannot be

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**Fig. 2.** Relationship between whole blood S-AdoHcy levels and 1-carbon flux rates in 7 patients with end-stage renal disease (■) and 9 healthy controls (○). Whole blood S-AdoHcy is expressed as the natural logarithm (ln) of the concentration (in nmol/l), and RM and TM fluxes are expressed per kg fat-free mass. The correlation coefficient (r) in A (RM) for the whole group is −0.61 (P < 0.01); −0.68 (P = 0.09) for end-stage renal disease; and −0.65 (P = 0.11) for controls. In B, r for whole group TM is −0.75 (P < 0.001); −0.71 (P = 0.03) for end-stage renal disease; and −0.66 (P = 0.06) for controls. Dotted lines depict logarithmic regression lines for all individuals and are added for illustrative purposes.
easily explained entirely by age alone. A difference in vitamin intake is another potential bias. Five ESRD patients received low-dose folic acid supplementation (1 mg once weekly). Compared with the other seven ESRD patients, these supplemented patients had significantly lower plasma tHcy levels (21.2 ± 6.0 vs. 42.2 ± 16.3 μmol/l, P = 0.02), but similar rates of RM (3.8 ± 0.9 vs. 3.8 ± 1.3 μmol·kg FFMM⁻¹·h⁻¹, P = 0.92) and the other fluxes (data not shown). When plasma folate levels were used in multivariate analysis, to correct for confounding by folic acid substitution, no significant changes in outcome were found. No conclusions on whether or to which extent folic acid supplementation may increase the rate of the folate-dependent RM pathway in ESRD can be drawn from the data of the present study.

In summary, this study shows that one-carbon metabolism in patients with ESRD is characterized by impaired in vivo metabolic Hcy clearance by TS and RM. Elevated levels of AdoHcy, AdoMet, or both stimulate TS until the absolute rates of RM and TM are diminished and are negatively related to whole blood AdoHcy levels. We hypothesize that the elevated intracellular concentration of AdoHcy in ESRD mediates the impairment in RM and TM flux rates. Given the persistence of hyperhomocysteinemia in ESRD patients supplemented with high doses of folic acid, the development of methods to normalize TM by reducing AdoHcy levels is of major interest.

GRANTS

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REFERENCES

18. Stamm F, van Gulden C, Schalkwijk CG, te Wee PM, Donker AJM, and Stehouwer CDA. Determination of S-adenosylhomocysteine and S-adenosylhomocysteine in plasma and cere-