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Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations

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Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations.

Background. Hyperhomocysteinemia is a risk factor for atherosclerosis that is common in chronic renal failure (CRF), but its cause is unknown. Homocysteine metabolism is linked to betaine-homocysteine methyl transferase (BHMT), a zinc metalloenzyme that converts glycine betaine (GB) to *N*,*N* dimethylglycine (DMG). DMG is a known feedback inhibitor of BHMT. We postulated that DMG might accumulate in CRF and contribute to hyperhomocysteinemia by inhibiting BHMT activity.

Methods. Plasma and urine concentrations of GB and DMG were measured in 33 dialysis patients (15 continuous ambulatory peritoneal dialysis and 18 hemodialysis), 33 patients with CRF, and 33 age-matched controls. Concentrations of fasting plasma total homocysteine (tHcy), red cell and serum folate, vitamins B_6 and B_{12}, serum zinc, and routine biochemistry were also measured. Groups were compared, and determinants of plasma tHcy were identified by correlations and stepwise linear regression.

Results. Plasma DMG increased as renal function declined and was twofold to threefold elevated in dialysis patients. Plasma GB did not differ between groups. The fractional excretion of GB (FE_{GB}) was increased tenfold, and FE_{DMG} was doubled in CRF patients compared with controls. Plasma tHcy correlated positively with plasma DMG, the plasma DMG:GB ratio, plasma creatinine, and FE_{GB} and negatively with serum folate, zinc, and plasma GB. In the multiple regression model, only plasma creatinine, plasma DMG, or the DMG:GB ratio was independent predictors of tHcy.

Conclusions. DMG accumulates in CRF and independently predicts plasma tHcy concentrations. These findings suggest that reduced BHMT activity is important in the pathogenesis of hyperhomocysteinemia in CRF.

An elevated plasma total homocysteine (tHcy) concentration is an independent risk factor for atherosclerotic disease [1–3]. Homocysteine is a sulfur amino acid product of methionine metabolism that undergoes autoxidation in plasma, producing free radicals that damage endothelial cells and promote low-density lipoprotein oxidation [4–6]. Homocysteine has also been shown to enhance platelet aggregation, blood coagulation, and vascular smooth muscle growth in vitro [6–8]. Hyperhomocysteinemia is very common in uremia [9] and is considered to be a factor contributing to the high prevalence of atherosclerotic complications in people with end-stage renal disease (ESRD) [2].

The cause of hyperhomocysteinemia in ESRD has not been established. Although homocysteine is measurable in urine, renal excretion is a trivial part of its total clearance [10, 11], and altered metabolism is likely to be much more important. The most studied pathways of homocysteine metabolism are transulfuration, involving cystathionine β synthetase, and remethylation by N^5,N^{10}-methylenetetrahydrofolate reductase. An alternate remethylation pathway regulated by betaine-homocysteine methyl transferase (BHMT) has received less attention. This zinc-dependent enzyme, which is abundant in liver and renal cortex [12, 13], catalyzes the folate-independent transfer of a methyl group from *N*,*N*,*N*-trimethylglycine [glycine betaine (GB)] to homocysteine, producing methionine and *N*,*N*-dimethylglycine (DMG; Fig. 1). DMG is a feedback inhibitor of BHMT [14] and is normally excreted in urine or metabolized to sarcosine (Fig. 1) [15]. Whether DMG metabolism is disturbed in chronic renal failure (CRF) is unknown.

The objectives of this study were to determine whether disturbances in the BHMT pathway are present in CRF and whether these are associated with hyperhomocysteinemia. Our hypothesis was that plasma DMG concentrations might increase in CRF. This would contribute to hyperhomocysteinemia by inhibiting BHMT activity, and this inhibition might be exacerbated by zinc deficiency.

Key words: kidney failure, hemodialysis, peritoneal dialysis, glycine betaine, atherosclerosis, hyperhomocysteinemia.

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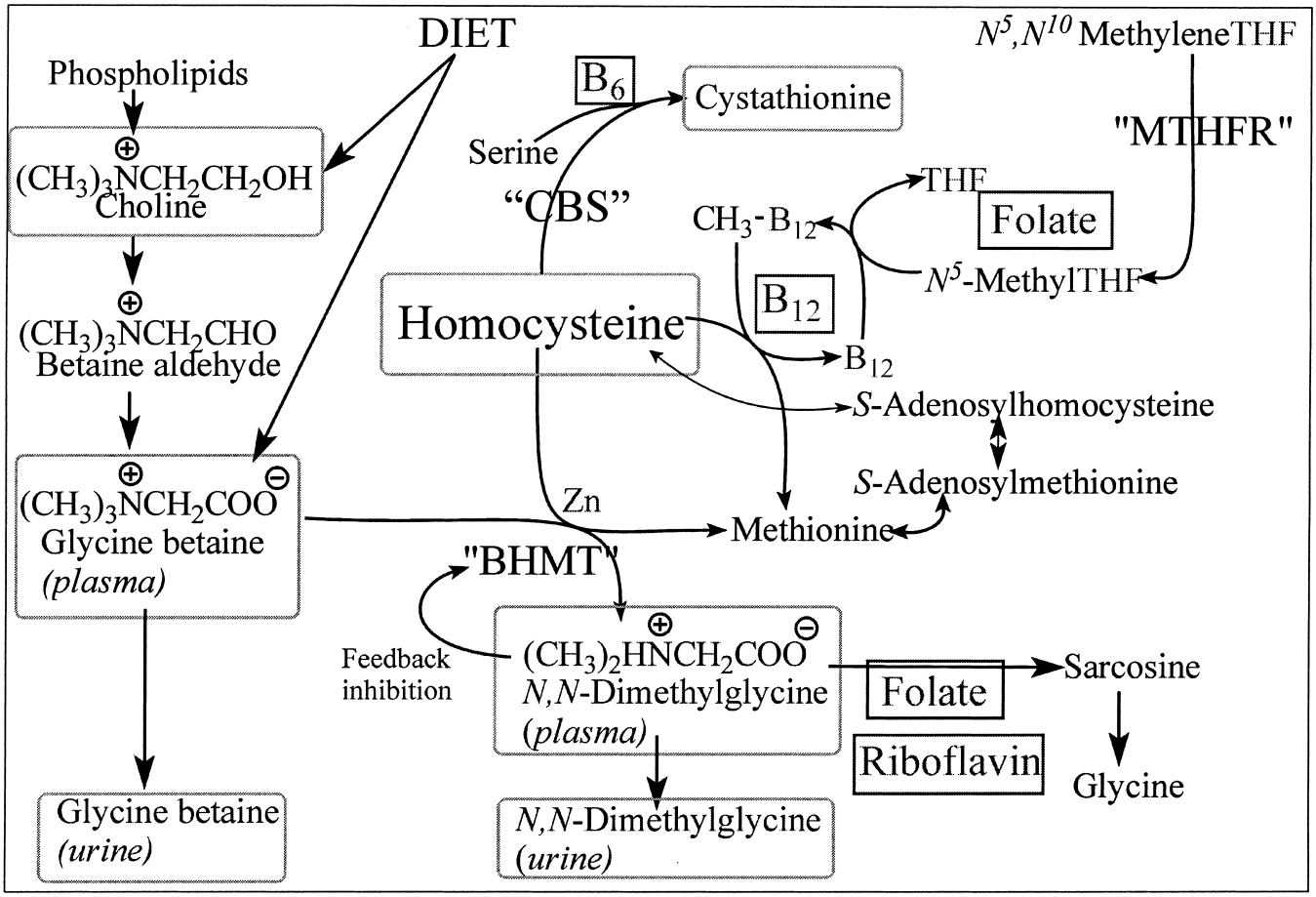

Fig. 1. Betaine-homocysteine methyl transferase (BHMT) metabolic pathway: Glycine betaine (GB) is obtained from the diet or by conversion of dietary choline. BHMT transfers a methyl group from GB to homocysteine, producing methionine and *N,N*-dimethylglycine betaine (DMG) that is excreted in urine or metabolized to sarcosine by dimethylglycine dehydrogenase. Abbreviations are: CBS, cystathionine beta-synthetase; MTHFR, N^5,N^{10}-methylenetetrahydrofolate reductase.

METHODS

Patients

The Canterbury Ethics Committee granted ethical approval for this study. Ninety-nine subjects (54 males, 95 Caucasian) were enrolled. They were aged 59.5 ± 15.9 (SD) years (range 23 to 86 years) and were divided into four groups (Table 1). Group 1 (N = 33) consisted of patients with CRF who were not receiving dialysis. Group 2 (N = 33) comprised normal volunteers who were age and sex matched to group 1. Group 3 (N = 18) consisted of patients who were on chronic ambulatory peritoneal dialysis (CAPD), and group 4 (N = 15) consisted of patients on long-term hemodialysis (HD).

Patients were excluded if they had suffered any acute illness within two weeks.

The diagnoses of the renal patients were chronic glomerulonephritis (29%), hypertension (15%), reflux nephropathy (6%), polycystic disease (6%), vasculitis (6%), renovascular disease (5%), interstitial nephritis (5%), diabetic nephropathy (3%), other diagnoses (14%), or unknown (11%). Group 1 had plasma creatinine concentrations of 0.14 to 0.68 mmol/L [calculated creatinine clearance (C_{Cr}) = 8.4 to 55.2 mL/min]. No patients were taking folate supplements. All HD patients took a vitamin supplement containing thiamine 5 mg, riboflavin 2 mg, pyridoxine 2 mg, and nicotinamide 20 mg. All dialysis patients had been on dialysis for more than six months. Those on HD were receiving five to eight hours of [mean 7.2 ± 1.1 hours (SD)] dialysis at home three days each week with an acetate buffer, using a 1.1 m^2 low-flux polysulfone dialyzer.

All patients were seen in a morning clinic after a 14-hour overnight fast. In HD patients, this was always before the first dialysis of the week. A history and examination were performed to establish whether they had clinical evidence of vascular disease, and their height and weight were measured for calculation of body mass index (BMI). All patients in groups 1 and 2 gave a midstream urine sample. Venous blood was drawn from the nonfistula arm for routine biochemistry (Aeroset; Abbott,

Table 1. Characteristics and blood measurements in all patients ($N = 99$)

	Controls ($N = 33$)	CRF ($N = 33$)	CAPD ($N = 15$)	HD ($N = 18$)
Age *years*	69 (53–75)	68 (54–74)	61 (48–67)	50 (36–62)[a]
Sex *male:female*	19:14	19:14	8:7	11:7
BMI kg/m^2	25.5 ± 4.8	25.8 ± 4.4	25.9 ± 3.3	26.2 ± 5.9
Homocysteine *μmol/L*	9.8 (7.6–14.9)	21.0 (18.5–25.7)[c]	25.5 (16.9–32.6)[c]	29.7 (25.7–38.2)[c]
Total protein *g/L*	76.0 ± 5.2	74.4 ± 7.1	70.7 ± 4.0	75.4 ± 6.6
RBC folate *nmol/L*	662 (571–782)	759 (570–837)	987 (734–1107)[a]	878 (616–1256)
Serum folate *nmol/L*	17.9 (13.7–24.4)	16.5 (13–21.9)	13.7 (10.7–17.3)	15.3 (12.3–17.6)
Plasma creatinine *mmol/L*	0.07 (0.06–0.08)	0.25 (0.22–0.38)[c]	0.89 (0.76–1.02)[c]	0.83 (0.72–1.17)[c]
Plasma DMG *μmol/L*	2.6 (1.8–3.7)	4.8 (3.6–6.2)[c]	5.4 (3.1–7.2)[b]	5.6 (4.8–10.1)[c]
Plasma GB *μmol/L*	33.6 (23.9–42.1)	32.5 (23.9–42.5)	25.6 (23.4–36.6)	31.9 (28.3–36.2)
Plasma DMG:GB ratio	0.09 (0.06–0.13)	0.13 (0.11–0.20)[c]	0.15 (0.11–0.24)[c]	0.17 (0.14–0.30)[c]
Serum zinc *μmol/L*	13.5 ± 2.2	12.0 ± 1.5[b]	12.4 ± 2.7	11.0 ± 2.3[c]

Data are median (interquartile range) or mean ± SD. Abbreviations are: GB, glycine betaine; DMG, *N*,*N*-dimethylglycine; BMI, body mass index; RBC, red blood cell; CRF, chronic renal failure; CAPD, continuous ambulatory peritoneal dialysis; HD, hemodialysis.
[a] $P < 0.05$, [b] $P < 0.005$, [c] $P < 0.001$ compared to the control group

North Chicago, IL, USA) and hematology (Coulter counter) profiles and to measure the other compounds of interest.

Plasma homocysteine

Venous blood was collected into chilled ethylenediaminetetraacetic acid (EDTA) tubes and was centrifuged immediately, and the plasma was frozen at −20°C until analysis. The plasma tHcy concentration was measured within two weeks of collection using a commercially available fluorescent polarizing immunoassay method (IMX; Abbott). The assay was subject to regular quality control had an interassay variability (coefficient of variation) of 4%.

Glycine betaine and dimethylglycine

Blood was collected into EDTA tubes and separated immediately. The separated plasma was stored at −20°C until analysis. Concentrations for DMG and GB were measured by high-performance liquid chromatography (HPLC) using a method adapted from Lever et al [16]. The compounds were extracted into 10% methanol in acetonitrile and were then derivatized with naphthacyl-triflate [17]. Separation of the derivatives was performed using HPLC on a 250-4 (5 μm) alumina column (Aluspher®; Merck, Darmstadt, Germany) and with ultraviolet detection (wavelength 249 nm). The mobile phase contained 10 mmol/L succinic acid and 3.7 mmol/L triethylamine in acetonitrile. Data were integrated using Delta for Windows version 5.0 (DataworX Ltd., Brisbane, Australia). Standards were used to quantitate HPLC peaks, and spiked plasma samples were used to determine plasma recoveries, which were always over 90%. The interassay and intra-assay coefficients of variation were less than 8%. A second HPLC system was used to verify the DMG results. This system used the same extraction procedure; however, 2-fluorenacyl triflate was used as the derivatizing agent, and a Phenosphere® SCX ion-exchange column was used for the HPLC with a mobile phase containing 10.5 mmol/L glycolic acid and 5.2 mmol/L trimethyl ammonium hydroxide in acetonitrile.

Folate and vitamin B_{12}

Serum folate and vitamin B_{12} concentrations were measured by a commercial chemiluminescence assay (ACS: 180; Chiron Diagnostics, Medfield, MA, USA). Red blood cell folate was calculated from the folate concentration of a hemolysate of 25 μL whole blood in 0.5 mL ascorbic acid, using the formula: Folate (rbc) = [Folate (hemolysate) × 21] ÷ [hematocrit].

Vitamin B_6

Pyridoxal-5-phosphate was extracted from heparinized blood with hydrochloric acid and derivatized with semicarbazide. The semicarbazide derivative was separated by HPLC with postcolumn adjustment of the eluate to pH 10 and fluorescence monitoring at 365 nm/460 nm (Instruchemie, Hilversum, The Netherlands).

Serum zinc

A protein-free filtrate was prepared by mixing the plasma sample with an equal volume of 10% trichloroacetic acid, standing for 10 minutes, and then centrifuged. The supernatant was analyzed for zinc by flame atomic absorption spectroscopy at 213.9 nm on a Varian AA10 (Varian Australia Pty Ltd., Springvale, Victoria, Australia) using an air acetylene flame.

Urine measurements

Urine creatinine concentrations were measured by an autoanalyzer (Aeroset; Abbott), and the urinary concentrations of DMG and GB were determined by the same HPLC method used for the plasma samples. The ratio of urine:plasma concentrations was used to estimate the fractional excretion using this formula:

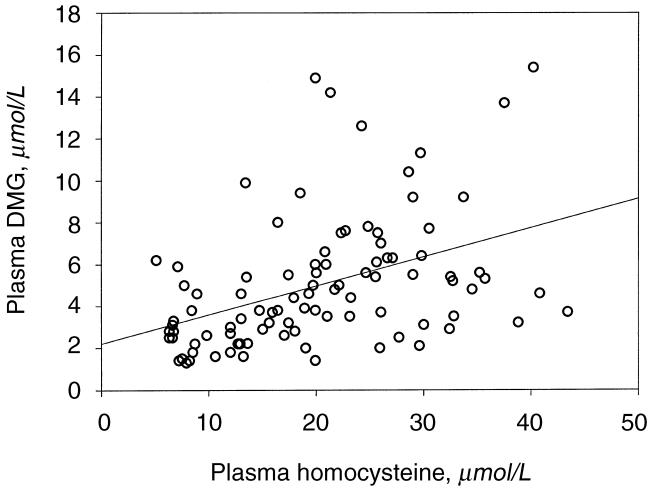

Fig. 2. Plasma DMG and homocysteine concentrations ($N = 99$, $r = 0.42$, $P < 0.001$).

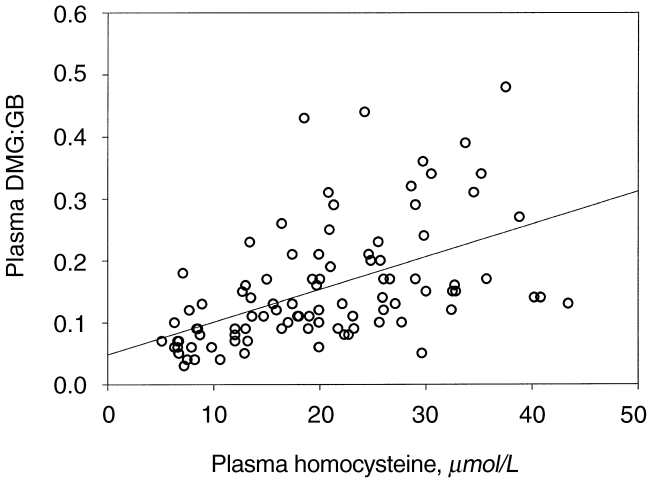

Fig. 3. Plasma DMG:GB ratio and plasma homocysteine ($N = 99$, $r = 0.54$, $P < 0.001$).

$$FE_{GB}\ (\%) = (U_{GB}/P_{GB})/(U_{Cr}/P_{Cr}) \times 100$$

where U and P denote urine and plasma concentrations of GB, DMG or creatinine, respectively. Creatinine clearance (C_{Cr}) was calculated for groups 1 and 2 from plasma creatinine using the Cockroft and Gault formula [18].

Statistics

Measurements in normal controls were compared with those made in the other groups by independent Student-*t* or Mann–Whitney tests, as appropriate. Pearson's test was used to determine correlations between plasma tHcy and the other measured variables. To find independent predictors of tHcy, all variables that correlated significantly with plasma tHcy were entered into a stepwise multiple linear regression model. Results with P values <0.05 were considered statistically significant.

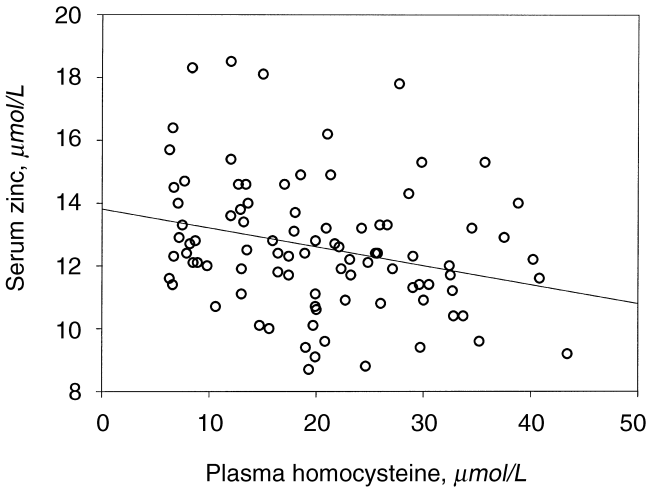

Fig. 4. Serum zinc and plasma homocysteine concentrations ($N = 99$, $r = -0.31$, $P = 0.001$).

RESULTS

Complete data were obtained for the four patient groups (Table 1). Compared with controls, patients with renal disease had significantly higher plasma tHcy and DMG concentrations. Plasma GB concentrations did not differ significantly between groups. Red blood cell folate was higher in CAPD patients, but serum folate did not differ between groups. Serum zinc was lower in HD patients than in controls. There were no significant differences between those treated with HD and those receiving CAPD.

Plasma tHcy correlated positively with plasma creatinine ($r = 0.73$, $P < 0.001$), DMG ($r = 0.42$, $P < 0.001$; Fig. 2), and the ratio of plasma DMG:GB ($r = 0.54$, $P < 0.001$; Fig. 3). Plasma tHcy correlated negatively with serum folate ($r = -0.35$, $P = 0.001$), zinc ($r = -0.31$, $P = 0.002$; Fig. 4), and plasma GB ($r = -0.25$, $P = 0.015$).

Statistical analysis was repeated for the nondialysis patients only (groups 1 and 2). In these 66 patients, similar correlations with plasma tHcy were present, including DMG ($r = 0.45$, $P < 0.001$), the plasma DMG:GB ratio ($r = 0.64$, $P < 0.001$), plasma GB ($r = -0.28$, $P = 0.03$), and serum zinc ($r = -0.3$, $P = 0.012$). Plasma tHcy correlated negatively with vitamin B_6 ($r = -0.31$, $P = 0.01$), but correlations with red cell ($r = -0.1$) and serum folate ($r = -0.22$) were nonsignificant. As C_{Cr} decreased, plasma tHcy ($r = -0.71$, $P < 0.001$), plasma DMG ($r = -0.43$, $P < 0.001$), and the plasma

DMG:GB ratio ($r = -0.429$, $P < 0.001$) all tended to increase.

The relationships between plasma DMG, the DMG:GB ratio, and tHcy were consistent in HD and CAPD groups and across all 33 dialysis patients ($r = 0.36$, $P < 0.05$ for DMG:GB). Other correlations, including serum zinc ($r = -0.29$, $P = 0.15$) and serum folate ($r = -0.24$, $P = 0.2$), did not reach statistical significance in these 33 patients.

Variables that independently predicted tHcy in the stepwise linear regression model were the plasma creatinine concentration ($R^2 = 0.41$, partial correlation = 0.55, $P < 0.001$) and the plasma DMG concentration ($R^2 = 0.446$, partial correlation = 0.249, $P < 0.05$). When the plasma DMG:GB ratio was used instead of DMG (since it is derived from DMG), it was a stronger independent predictor of tHcy ($R^2 = 0.528$, partial correlation = 0.447, $P < 0.001$).

Urine DMG was similar in CRF patients and controls [median = 6.4 (CRF) vs. 8.2 μmol/mmol creatinine (controls), P = NS], but urine GB was elevated in the CRF group (28.4 vs. 9.0 μmol/mmol creatinine, $P < 0.001$). The FE_{DMG} increased as C_{Cr} declined ($r = -0.35$, $P = 0.005$) and was nearly two times higher in CRF patients compared with controls (34.1 vs. 20.2%, $P < 0.001$). The FE_{GB} increased more markedly with declining C_{Cr} ($r = -0.65$, $P < 0.001$) and was tenfold higher in CRF patients (28.5 vs. 2.6%, $P < 0.001$). The FE_{GB} correlated positively with plasma tHcy in the univariate analysis ($r = 0.47$, $P < 0.001$).

DISCUSSION

We have demonstrated, to our knowledge for the first time, that plasma DMG is elevated in CRF and that it correlates with homocysteine. Dimethylglycine increased as renal function declined, but it also predicted tHcy independent of renal function. The ratio of plasma DMG:GB, which is likely to best reflect the flux through BHMT, was the strongest predictor of plasma tHcy concentrations. Serum zinc also correlated inversely with tHcy in the univariate analysis, which lends further support to the hypothesis that BHMT is an important determinant of plasma tHcy concentrations in CRF.

Homocysteine concentrations increase with progressive renal failure so that by the time ESRD is reached, more than 80% of patients have elevated tHcy levels [9]. Folate-based treatment regimens reduce fasting tHcy by 30 to 50%, but even with doses of 60 mg folate daily, tHcy concentrations are seldom normalized [19]. Although evidence of benefit from randomized controlled trials is lacking, it is widely believed that lowering tHcy should be a goal in ESRD patients. However, more effective therapies that can normalize tHcy may be required before any clinical benefits from homocysteine reduction will be seen.

Theoretically, dietary supplementation with betaine might overcome competitive inhibition of dimethylglycine on BHMT and reduce tHcy levels. However, two studies of betaine supplementation in dialysis patients have failed to show any benefit from betaine administration [20, 21]. The reasons for this are not clear. Plasma GB concentrations were not measured in either study, and it is possible that the doses used (4 to 6 g daily) were inadequate in the face of continued removal of GB by dialysis. Alternatively, there may have been insufficient clearance of DMG to prevent it from inhibiting BHMT activity. Optimal tHcy lowering therapy may require new approaches to overcome DMG feedback inhibition of BHMT. Possible methods include increasing DMG clearance by longer or more efficient dialysis or the addition of GB to dialysate, which might shift the equilibrium allowing BHMT to continue transmethylation of homocysteine.

Studies in experimental animals have shown that BHMT activity can be increased by dietary manipulations. Park and Garrow reported that rats fed a diet low in methionine had a fourfold increase in BHMT activity, and further increases were observed when supplemental betaine or choline was added [22]. Conversely, dimethylglycine is available as a dietary supplement in health food stores, and there are currently several Internet Web sites marketing it for human consumption. Most claim that dimethylglycine supplements improve aerobic muscle activity, immunologic responses, and a variety of neurological disorders. From our results, however, we would question whether they would be ideal for the cardiovascular health of people with renal impairment.

This study is limited by its cross-sectional design, and we cannot prove that the associations are causal. The FE_{GB} was markedly increased in those with CRF, and it correlated positively with plasma tHcy. This could simply mean that both are sensitive markers of renal injury. Alternatively, it might suggest reduced tubular GB uptake is a cause or result of decreased renal BHMT activity. The FE_{DMG} was increased to a much lesser extent, but it is unclear whether the increased plasma levels of DMG result primarily from reduced renal clearance or from impaired metabolism. Further research is needed to elucidate the cause of increased plasma DMG levels, and the relationship between disturbed renal GB handling and BHMT activity. To understand better BHMT's role in hyperhomocysteinemia, further studies using interventions that alter its activity are also needed so that effects on plasma tHcy can be determined.

In conclusion, we found that plasma DMG concentrations were increased in patients with CRF, whereas GB levels were unchanged. Previous in vitro studies indicate that such a disturbance would inhibit BHMT activity,

and we have shown that the ratio of DMG:GB predicts plasma tHcy concentrations independent of renal function. These data suggest that decreased BHMT activity may be an important contributor to hyperhomocysteinemia in CRF.

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APPENDIX

Abbreviations used in this article are: BHMT, betaine-homocysteine methyl transferase; CAPD continuous ambulatory peritoneal dialysis; C_{Cr}, creatinine clearance; CRF, chronic renal failure; DMG, *N,N*-dimethylglycine; EDTA, ethylediaminetetraacetic acid; ESRD, end-stage renal disease; FE, fractional excretion; GB, glycine betaine; HD, hemodialysis; HPLC, high-pressure liquid chromatography; tHcy, total homocysteine.

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