

Disturbed Homocysteine and Methionine Cycle Intermediates S-Adenosylhomocysteine and S-Adosylmethionine Are Related to Degree of Renal Insufficiency in Type 2 Diabetes

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Background: Diabetic nephropathy is a common complication in patients with type 2 diabetes that may increase the atherothrombotic risk. Hyperhomocysteinemia (HHcy) further increases the risk in those patients. We studied concentrations of total homocysteine (tHcy), its related metabolites S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy) in relation to B-vitamin status and renal function in patients with type 2 diabetes who developed diabetic nephropathy.

Methods: The study included 93 patients with renal failure and type 2 diabetes. Chronic kidney disease was classified into four subgroups according to the National Kidney Foundation and into stages based on glomerular filtration rate plus pathologic abnormalities or markers of kidney damage.

Results: Serum or plasma concentrations of the metabolites increased significantly with worsening of renal function, whereas serum concentrations of the B vitamins (folate, vitamins B₁₂ and B₆) did not differ appreciably between the groups. Moreover, plasma concentrations of AdoHcy and AdoMet were markedly increased in patients with kidney failure compared with those in stage 2 (median AdoHcy, 112.7 vs 10.5 nmol/L; median AdoMet, 162.0 vs 80.0 nmol/L). The AdoMet/AdoHcy ratio was more than 80% lower in patients with renal failure compared with stage 2. Vitamin B₁₂ was a

significant determinant of concentrations of AdoMet, tHcy, methylmalonic acid (MMA), and cystathionine.

Conclusions: Increased plasma concentrations of tHcy and methionine cycle intermediates (AdoMet, AdoHcy) are related to disturbed renal function in patients with type 2 diabetes. Vitamin B₁₂ and/or folate are significant predictors of tHcy, cystathionine, MMA, and AdoMet. The effect of therapeutic doses of the B vitamins on AdoMet, AdoHcy, and their ratio should be tested in renal patients.

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Impaired renal function, a major complication in patients with type 2 diabetes mellitus, increases the risk for atherothrombotic diseases (1). Increased total homocysteine (tHcy;⁵ i.e., 15–80 μmol/L) is very common in renal patients (2–4). Hyperhomocysteinemia (HHcy) is an additional factor that increases the risk of vascular diseases in general and in renal patients in particular (3, 4). Furthermore, persons with type 2 diabetes are more susceptible to the harmful effects of HHcy than are nondiabetic individuals (5). Diabetic patients with HHcy have a higher mean intima-media thickness (5) and a higher susceptibility for fatal and nonfatal coronary events (6) than do normohomocysteinemic patients.

The kidney plays an important role in Hcy metabolism (7). Variations in renal function, even within the physiologic range, are an important determinant of interindividual differences in plasma concentrations of tHcy (7). Glomerular filtration rate (GFR) is a rate-limiting factor

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⁵ Nonstandard abbreviations: tHcy, total homocysteine; HHcy, hyperhomocysteinemia; GFR, glomerular filtration rate; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; MMA, methylmalonic acid; holoTC, holo-transcobalamin; and PLP, pyridoxal-5-phosphate.

for the renal clearance of Hcy (8). The urinary excretion of Hcy has been found to be negligible in healthy persons (9). Furthermore, the fractional extraction of Hcy across the human kidney varies according to renal blood flow (10). These findings imply major alterations in the metabolism of this aminothiol in patients with end stage renal disease. Moderate HHcy in rats was associated with remarkable glomerular damage and sclerosis (11), renal tubulointerstitial injury, increased urinary albumin excretion, and decreased renal blood flow, GFR, and sodium and water excretion (12). In line with these data, treatment with folic acid and vitamin B₆ lowered not only plasma tHcy, but also urinary albumin excretion in humans (13).

Hcy is produced from the hydrolysis of *S*-adenosylhomocysteine (AdoHcy) in a reversible reaction catalyzed by AdoHcy hydrolase. This reaction proceeds in the forward direction as long as the product, Hcy, is efficiently removed via the remethylation or the transsulfuration pathways. However, the conversion of Hcy into AdoHcy is favored under conditions of HHcy, which is the case in renal insufficiency. Previous studies underlined that AdoHcy is profoundly increased in plasma and erythrocytes of renal patients (14, 15). Furthermore, many transmethylation reactions are inhibited by AdoHcy through its competition with the natural substrate, *S*-adenosylmethionine (AdoMet), for binding domains in transmethylation enzymes (15–18). The AdoMet/AdoHcy ratio is a more important determinant of cellular methylation potential than the absolute amount of AdoMet (18–21).

Serum concentrations of cystathionine and methylmalonic acid (MMA) are also increased in renal patients (22). The pathogenesis of HHcy and the disturbed methylation potential are not fully understood. Folate and vitamins B₁₂ and B₆ are important regulators in the metabolism of Hcy. Evaluating some cardiovascular risk factors, such as Hcy and related metabolites in patients with diabetes who have mild to moderate decreased GFR values may allow better management and reduce further complications. We aimed at investigating tHcy and its related metabolites, AdoMet, AdoHcy, cystathionine, cysteine, and MMA, in addition to vitamin B₁₂, holo-transcobalamin (holoTC), vitamin B₆, and folate in patients with overt diabetic nephropathy and various stages of chronic kidney disease.

Patients and Methods

PATIENTS

Patients ($n = 93$) were recruited from the Department of Medicine, Division of Nephrology of the University Hospital of Saarland, Germany. Inclusion criteria included age >17 years, established diabetic nephropathy as primary cause of renal disease, and type 2 diabetes for at least 6 years. Exclusion criteria included cancer, stroke, thrombosis or myocardial infarction within the previous 3 months, and the consumption of any vitamin supplements within the previous 4 weeks. No patient had

received antifolate or antiepilepsy medications or medications known to affect Hcy metabolism. All patients were clinically stable at the time of recruitment. Chronic kidney disease was classified according to the National Kidney Foundation into stages based on GFR plus pathologic abnormalities or markers of kidney damage (23). Stage 2 included patients with kidney damage and mildly decreased GFR (60–89 mL/min); stage 3 included patients with moderately decreased GFR (30–59 mL/min); stage 4 included patients with severely decreased GFR (15–29 mL/min); and stage 5 included patients with kidney failure (GFR <15 mL/min or dialysis). GFR was estimated from serum creatinine concentration, age, and gender by use of the well-validated MDRD equation. The study was approved by the Medical Ethical Committee of the University of Saarland, and all patients gave informed consent for the study.

BLOOD COLLECTION AND LABORATORY PROCEDURES

Blood from dialysis patients was obtained at the start of a regular dialysis treatment and in nondialysis patients during their visit in our outpatient clinics. Blood samples without anticoagulant and with EDTA were immediately chilled on ice and centrifuged within 1 h for 10 min at 2000g and 4 °C. Serum aliquots were stored at –70 °C for further analysis. EDTA-plasma samples (1 mL) were immediately deproteinized by addition of 0.625 mL of 100 g/L perchloric acid. Deproteinized plasma samples were kept at –70 °C until analysis. The concentrations of AdoHcy and AdoMet were measured in deproteinized plasma by reversed-phase chromatography with fluorescence detection according to the method described by Loehrer et al. (14). Briefly, etheno derivatives of both metabolites were separated on a Hypergrom ODS column [200 × 4 mm (i.d.); 3 μm bead size] with a guard column [20 × 4 mm (i.d.)] filled with the same packing material. Serum concentrations of tHcy, cystathionine, and MMA were measured by gas chromatography–mass spectrometry as described elsewhere (24).

The concentrations of vitamin B₁₂ and folate were determined in serum by a chemiluminescence immunoassay (ADVIA Centaur System; Bayer). Serum vitamin B₆ [pyridoxal-5-phosphate (PLP)] was measured by HPLC with fluorescence detection, using reagents from Immundiagnostik. HoloTC, the biologically active bound form of vitamin B₁₂, was measured in serum by a RIA method as described previously (Axis-Shield) (25). Other analytes were measured by routine methods at our laboratory.

Data analyses were performed with the software package SPSS (Ver. 11.0; SPSS). Continuous variables were examined for distribution by the Kolmogorov–Smirnov test. All continuous variables were skewed and were therefore log-transformed before application of tests that assume a gaussian distribution. Differences in continuous variables between the groups were examined by ANOVA and post hoc Tamhane tests. Backward stepwise multivariate regression analysis was conducted to predict fac-

Table 1. Main characteristics of the study patients.^a

	Stage 2 ^b	Stage 3	Stage 4	Stage 5 ^c
n	18	23	16	36
Female, n (%)	9 (50)	9 (39)	6 (38)	19 (53)
Age, years	61 (2.0)	63 (2.1)	64 (1.6)	67 (1.5)
Hemoglobin, g/L	138 (3)	137 (3)	119 (3) ^d	111 (2) ^d
Hematocrit, %	41 (0.9)	40 (0.8)	35 (1.0) ^d	34 (0.5) ^d
Mean corpuscular volume, fL	88 (0.9)	88 (0.9)	89 (1.0)	91 (0.9) ^d
Albumin, g/L	39 (0.8)	38 (0.9)	37 (0.9)	36 (0.5) ^d
Creatinine, μmol/L	83.6 (3.7)	131.3 (6.3) ^d	235.3 (14.6) ^d	556.7 (38.4) ^d
Urea, mg/L	360 (19)	540 (28) ^d	1140 (88) ^d	1430 (56) ^d
GFR, mL/min	75 (2.4)	44 (1.8) ^d	22 (1.1) ^d	11 (0.7) ^d
Hemoglobin A _{1c} , %	7.4 (0.3)	6.9 (0.2)	7.3 (0.3)	6.5 (0.2)
hs-CRP, ^e mg/L	2.7 (1.1)	3.7 (2.2)	3.1 (2.1)	6.1 (3.3) ^d

^a Data except for number of individuals and number (%) of females are the geometric mean (SE).

^b See *Patients and Methods* section for the definition of stages 2 through 5.

^c GFR values were available from the predialysis patients (n = 15).

^d P < 0.05 compared with stage 2 (ANOVA and Tamhane-T2 tests).

^e hs-CRP, high-sensitivity C-reactive protein.

tors that influenced serum concentrations of the metabolites. A P value < 0.05 was considered statistically significant.

Results

The main characteristics of the study population are listed in Table 1 according to the stage of renal dysfunction. As would be expected, patients in stage 5 had the worst renal function as indicated by a lower mean GFR and higher serum creatinine and urea. In addition, hematologic changes were common in patients in stage 5, such as lower hemoglobin and hematocrit and higher mean corpuscular volume (Table 1). Patients in stage 5 had significantly higher concentrations of C-reactive protein and lower albumin than those in stage 2. The concentrations of hemoglobin A_{1c} did not differ significantly among the groups.

Concentrations of tHcy and the related metabolites showed a clear increase in relation to decreasing renal function (Table 2). AdoHcy and AdoMet were markedly

increased in renal patients. The degree of increase was apparently related to the degree of HHcy and the degree of disturbance of renal function (Table 2). Differences in serum concentrations of MMA among the five groups were also significant. Increased concentrations of tHcy, cystathionine, and MMA were very common in our patients. In contrast, low concentrations of the vitamins were less prevalent: 2% had B₁₂ < 156 pmol/L, and 12% had PLP < 17.4 nmol/L. Median serum concentrations of cystathionine and cysteine were significantly lower in the patients in stage 2 than in patients in stage 5 (cystathionine, 334 vs 2399 nmol/L; cysteine, 335 vs 448 μmol/L; Table 2). The increase in AdoHcy was more pronounced than those of tHcy and AdoMet (Table 2). Furthermore, the mean AdoMet/AdoHcy ratio markedly decreased with worsening renal function (Fig. 1). Additionally, the cystathionine/cysteine ratio substantially increased from stage 2 through stage 5 (Fig. 1).

The cystathionine/cysteine ratio correlated directly with concentrations of AdoMet ($r = 0.45$; $P < 0.001$) and

Table 2. Concentrations of the metabolites and the vitamins.^a

	Stage 2	Stage 3	Stage 4	Stage 5 ^b	P ^c	P ^b
Hcy, μmol/L	10.2 (9.0–12.9)	16.2 (12.6–19.4) ^d	21.2 (17.6–23.4) ^d	27.0 (17.9–35.4) ^d	<0.001	<0.001
Cystathionine, nmol/L	334 (232–469)	532 (375–1262) ^d	921 (622–1894) ^d	2399 (1641–3526) ^d	<0.001	<0.001
Cysteine, μmol/L	335 (294–379)	396 (333–441) ^d	429 (352–492) ^d	448 (362–488) ^d	0.001	0.003
MMA, nmol/L	188 (163–282)	278 (219–394)	432 (302–667) ^d	949 (607–1253) ^d	<0.001	<0.001
AdoHcy, nmol/L	10.5 (7.3–16.4)	16.4 (12.4–22.2)	31.4 (22.4–63.3) ^d	112.7 (51.4–206.7) ^d	<0.001	<0.001
AdoMet, nmol/L	80.0 (55.3–151.3)	83.0 (71.1–105.0)	118.0 (91.1–140.1)	162.0 (128.3–185.2) ^d	<0.001	<0.001
Folate, nmol/L	22.4 (18.8–24.9)	20.6 (15.4–26.7)	23.6 (19.3–28.8)	17.2 (13.7–22.8)	0.349	0.663
Vitamin B ₁₂ , pmol/L	290 (237–415)	310 (259–415)	296 (213–632)	332 (231–422)	0.796	0.666
HoloTC, pmol/L	59 (38–94)	85 (57–96)	81 (64–103)	78 (61–112)	0.072	0.083
PLP, nmol/L	42.0 (28.1–55.2)	42.2 (21.9–75.8)	53.9 (30.0–80.9)	29.2 (15.7–67.7)	0.150	0.058

^a Data are the median (25th–75th percentiles). See *Patients and Methods* section for the definition of stages 2 through 5.

^b P values after adjusting for age, sex, and albumin.

^c P values between groups (ANOVA test).

^d P < 0.05 compared with stage 2 (ANOVA followed by Tamhane-T2 tests).

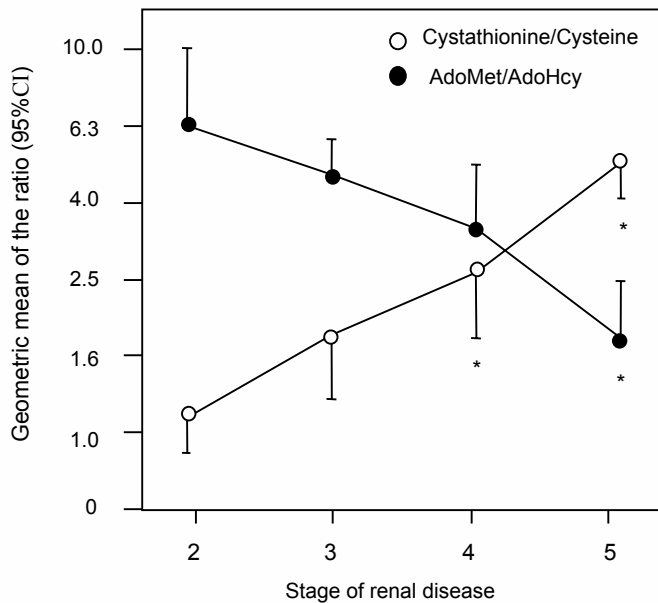


Fig. 1. Geometric mean [95% confidence interval (CI; error bars)] of AdoMet/AdoHcy ratio (●) and cystathionine/cysteine ratio (○) in diabetic patients with different degrees of chronic renal failure.

*, $P < 0.05$ compared with stage 2 (ANOVA).

tHcy ($r = 0.57$; $P < 0.001$) and inversely with PLP ($r = -0.31$; $P = 0.003$; Fig. 2). These correlations remained significant after adjustment for age, sex, and serum albumin. Other important correlations between the metabolic markers and the vitamins are reported in Table 3. Correlations between serum concentrations of tHcy and con-

Table 3. Correlations between serum concentrations of the metabolites and the vitamins.^a

	Spearman correlation coefficient					
	tHcy	Cystathionine	Cysteine	MMA	AdoMet	AdoHcy
Creatinine	0.62	0.71	0.30	0.75	0.59	0.70
AdoHcy	0.35	0.43	0.25	0.62	0.52	
Folate	-0.32	0.25	NS ^b	NS	NS	NS
Vitamin B ₁₂	-0.22	NS	NS	NS	0.27	NS
HoloTC	NS	NS	NS	NS	0.24	NS
PLP	-0.30	-0.29	NS	NS	NS	NS
AdoMet	0.26	0.43	NS	0.45		
MMA	0.65	0.59	0.24			
Cysteine	0.59	0.24				
Cystathionine	0.65					

^a $n = 93$ patients. All P values are < 0.05 .
^b NS, not significant.

centrations of MMA and cystathionine were strong, as was the correlation between AdoHcy and AdoMet ($r = 0.52$; $P < 0.001$). The last correlation remained significant after adjustment for all possible confounding variables (creatinine, age, sex, hemoglobin, and albumin; $r = 0.50$; $P < 0.001$). Moreover, we found a positive correlation between vitamin B₁₂ and AdoMet ($r = 0.27$; $P = 0.009$), but not between vitamin B₁₂ and AdoHcy or the ratio AdoMet/AdoHcy. We also found significant correlations between tHcy and both AdoHcy and AdoMet ($r = 0.35$ and 0.26 , respectively). The correlation between PLP and folate was strong in our patients ($r = 0.66$; $P < 0.001$).

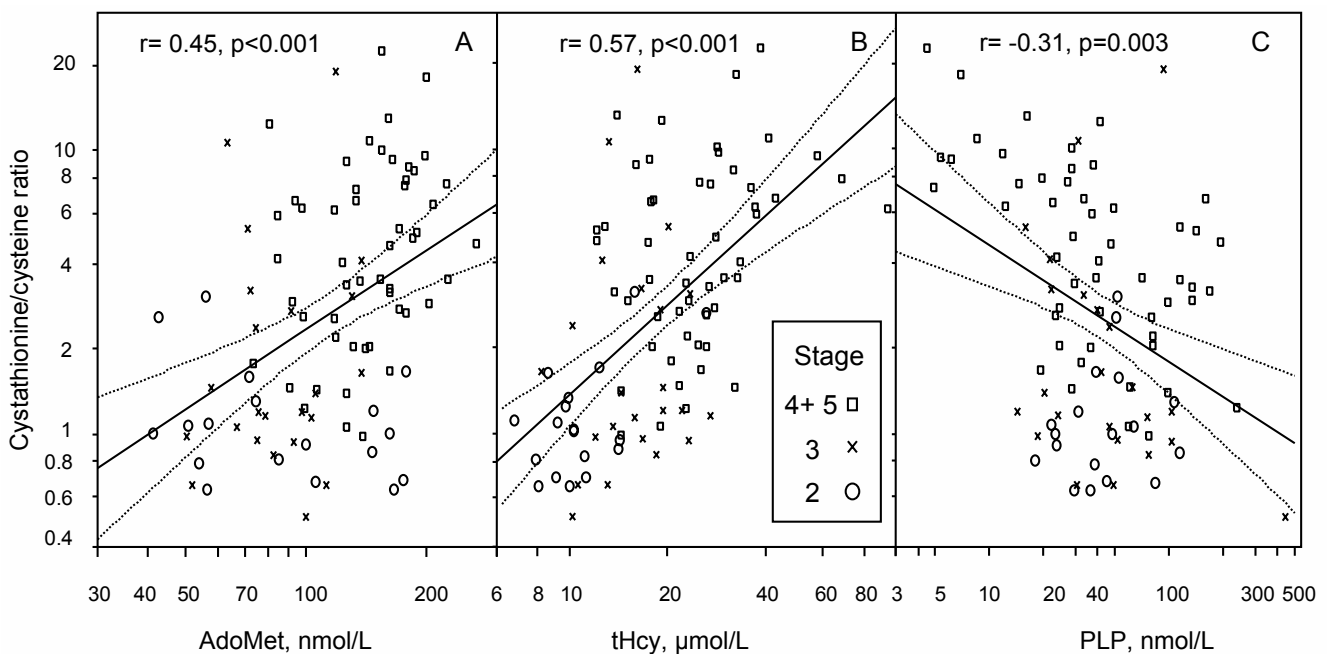


Fig. 2. Scatter plots of the cystathionine/cysteine ratio plotted vs AdoMet (A), tHcy (B), and PLP (C) concentrations.

Spearman correlation coefficients are shown. Values on the x and y axes are in logarithmic format. For simplicity, stages 4 and 5 were combined. □, stage 4 plus 5; ×, stage 3; ○, stage 2.

Hemoglobin A_{1c} did not correlate to any of the metabolites or the vitamins.

Multivariate backward regression analyses were applied to find out predictors of each of the metabolites (Table 4). Concentrations of tHcy were determined independently by serum concentrations of folate, vitamin B₁₂, cysteine, and creatinine. AdoMet was determined by creatinine, vitamin B₁₂, and AdoHcy, whereas creatinine was the only analyte that influenced AdoHcy. Furthermore, both creatinine and vitamin B₁₂ were significant factors that determined serum concentrations of MMA and cystathionine (Table 4).

Discussion

The current study represents the first comprehensive investigation of metabolic disturbances associated with HHcy in patients with diabetic nephropathy and various stages of chronic kidney disease. The study provides important information on the interrelationships between crucial metabolites of methionine and vitamin status in relation to renal function. In patients with diabetes and mildly decreased GFR (stage 2), serum concentrations of tHcy and the metabolites were comparable to those usually found in healthy individuals. This indicates that probably advanced renal injury, but not diabetes itself, is associated with disturbed Hcy metabolism. In line with this suggestion, hemoglobin A_{1c}, an indicator of a long-term diabetes control, was comparable among the groups and did not correlate to any of the markers investigated. Our results extend observations of other studies that showed normal tHcy and AdoHcy in patients with diabetes without overt diabetic nephropathy (no albuminuria and normal serum creatinine) (26).

Numerous studies, including ours, revealed a high incidence of HHcy in renal patients (2, 22, 27). These studies have also suggested that AdoHcy mediates the harmful effects of Hcy in the vascular system (28–30). Our study shows a relationship between HHcy, AdoHcy, and the degree of impaired renal function (Table 2). On the other hand, we observed no appreciable differences in

serum concentrations of the vitamin with decreasing renal function.

Several possible causes of HHcy can be considered in renal patients. The role of the kidney in Hcy metabolism is supported by the finding that Hcy concentrations increased in living kidney donors after uninephrectomy and remained high 6 months thereafter, despite folate and vitamin B₁₂ remaining unchanged (31). It is unlikely that altered Hcy metabolism is attributable to a decrease in the net filtration of Hcy from the diseased kidney because this is minimal in healthy persons (32). On the other hand, a lower rate of plasma Hcy clearance was observed in HHcy in renal patients compared with healthy persons, which suggests a role for the kidney in the elimination of this aminothiol (33). The remethylation of Hcy to methionine is lower in hemodialysis patients (34). In addition, Hcy remethylation was shown to be impaired in dialysis patients despite serum concentrations of vitamin B₁₂ and folate well above those found in the general population (4). In keeping with this, vitamin intervention studies show a significant decrease in tHcy after administration of supraphysiologic doses of the B vitamins intravenously (2, 22). We therefore propose that the requirement for B vitamins in renal patients may be much higher than that in the general population. In support of this idea, mononuclear cells isolated from renal patients showed a diminished ability to internalize vitamin B₁₂ (35).

One notable finding in our study is the marked increase in serum concentrations of cystathionine and the cystathionine/cysteine ratio. PLP is a cofactor for two enzymes in the transsulfuration pathway, cystathionine β -synthase and cystathionase, and is also a cofactor in the serine hydroxymethyltransferase reaction, which catalyzes the formation of 5,10-methylenetetrahydrofolate. The strong correlation between PLP and folate suggests that the availability of folate depends on PLP. Increased cystathionine may be partly attributed to reduced urinary excretion. The negative correlation between PLP and the cystathionine/cysteine ratio suggests that the reaction mediated by cystathionase could be impaired in renal

Table 4. Backward stepwise regression analysis.^a

	Dependent variables				
	Hcy	AdoHcy	AdoMet	Cystathionine	MMA
Intercept ^b	-1.135	-1.405	0.558	-1.712	2.313
β estimate (SE)					
Creatinine	0.283 (0.056)	0.738 (0.154)	0.252 (0.059)	0.565 (0.139)	0.559 (0.119)
Vitamin B ₁₂	-0.263 (0.101)		0.312 (0.107)	0.555 (0.245)	-0.575 (0.198)
Cysteine	0.897 (0.142)			0.735 (0.422)	
Folate	-0.160 (0.076)				
AdoHcy			0.09 (0.041)		
Total R ²	0.73	0.43	0.46	0.63	0.57

^a Only variables with significant influence are presented. All *P* values are <0.05. Other confounding variables, such as age, sex, albumin, and hemoglobin, were considered in the analyses. The regression analyses were applied on the log-transformed data (*n* = 93).

^b The units for the intercepts are μ mol/L for Hcy and nmol/L for all other variables.

patients. Additionally, an increased cystathionine/cysteine ratio was closely related to higher Hcy, which indicates increased production of cystathionine from Hcy. AdoMet has been shown to enhance the activity of cystathionine β -synthase (36). This may explain why a higher AdoMet was associated with a higher cystathionine/cysteine ratio in our patients (Fig. 2).

In line with previous studies (37), low serum concentrations of vitamin B₁₂ (<156 pmol/L) or holoTC (<35 pmol/L) were rare (2% and 3%, respectively) despite markedly increased MMA in 70% of the patients. However, the vitamin B₁₂ concentration was a stronger determinant of tHcy, cystathionine, and MMA than was folate (Table 4). Furthermore, therapeutic doses of vitamin B₁₂ have been shown to lower tHcy and MMA concentrations in dialysis patients (2, 36). The active form of vitamin B₁₂, holoTC, may be increased in patients with renal involvement (38) (Table 2). The reason for holoTC accumulation in renal patients remains unclear, but it is probably related to a generalized peripheral resistance to the vitamin. An altered conformation of the transcobalamin-B₁₂ complex that may influence the interaction of transcobalamin with its cellular receptor has also been suggested (37).

In agreement with previous reports, plasma concentrations of AdoHcy were markedly increased in our patients with chronic HHcy (14, 26, 39). In erythrocytes, however, a transient increase in plasma tHcy after methionine loading in humans did not lead to increases in AdoHcy or AdoMet (39). Plasma concentrations of AdoHcy were decreased after vitamin B₁₂ treatment (40). Moreover, treatment of dialysis patients with methyltetrahydrofolate led to an appreciable increase in AdoMet and in the AdoMet/AdoHcy ratio (41). In contrast to a previous study on persons with normal renal function (42), vitamin B₁₂ was a significant determinant of plasma AdoMet in our patients (Table 4), which suggest that higher B₁₂ status may increase AdoMet by increasing Hcy remethylation to methionine (40).

A striking finding in this study is the clear increase in the AdoHcy concentration, which exceeded that of AdoMet and was strongly related to decreasing renal function. AdoMet and AdoHcy concentrations have been reported to be ~100- and 30-fold higher, respectively, in urine than in serum (43). In addition to a lower urinary excretion of these metabolites, AdoMet is increasingly consumed in renal patients in the formation of creatine released from the muscles (a precursor of creatinine). The accumulation of AdoMet may well be explained by the suppression of transmethylation reactions by AdoHcy. AdoHcy is a potent inhibitor of intracellular methylation reactions, and the AdoHcy/AdoMet ratio represents the methylation potential in the cell (44).

In conclusion, concentrations of tHcy, cystathionine, and MMA in patients with diabetic nephropathy were related to the degree of renal insufficiency as well as to folate,

vitamin B₁₂, and PLP status. Higher concentrations of these vitamins were associated with a better metabolic profile. Functional vitamin B₁₂ deficiency causes impaired folate utilization and thus a relative shortage of AdoMet. On the other hand, HHcy induces a marked increase in plasma concentrations of AdoHcy. The magnitude of the increase of AdoHcy in plasma was severalfold higher than that of tHcy or AdoMet in patients with kidney failure. The strong decrease in the AdoHcy/AdoMet ratio points to a lower methylation potential. Further studies should investigate the effect of pharmacologic doses of folate and vitamins B₆ and B₁₂ on AdoMet, AdoHcy, and their ratio.

References

1. Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in chronic renal failure. *Lancet* 2002;356:147–52.
2. Obeid R, Kuhlmann M, Köhler H, Herrmann W. Response of homocysteine and related metabolites to vitamin treatment in hyperhomocysteinemic dialysis patients. *Clin Chem* 2005;51:196–201.
3. Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart KL, Jacobsen DW, et al. Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 1998;97:138–41.
4. Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 1996;94:2743–8.
5. Becker A, Henry RM, Kostense PJ, Jakobs C, Teerlink T, Zweegman S, et al. Plasma homocysteine and S-adenosylmethionine in erythrocytes as determinants of carotid intima-media thickness: different effects in diabetic and non-diabetic individuals. The Hoorn Study. *Atherosclerosis* 2003;169:323–30.
6. Soinio M, Marniemi J, Laakso M, Lehto S, Ronnema T. Elevated plasma homocysteine level is an independent predictor of coronary heart disease events in patients with type 2 diabetes mellitus. *Ann Intern Med* 2004;140:94–100.
7. Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. *J Am Soc Nephrol* 2001;12:2181–9.
8. Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int* 1999;55:1028–35.
9. Refsum H, Helland S, Ueland PM. Radioenzymic determination of homocysteine in plasma and urine. *Clin Chem* 1985;31:624–8.
10. Garibotto G, Sofia A, Saffioti S, Russo R, Deferrari G, Rossi D, et al. Interorgan exchange of amino thiols in humans. *Am J Physiol Endocrinol Metab* 2003;284:E757–6.
11. Li N, Chen YF, Zou AP. Implications of hyperhomocysteinemia in glomerular sclerosis in hypertension. *Hypertension* 2002;39:443–8.
12. Chen YF, Li PL, Zou AP. Effect of hyperhomocysteinemia on plasma or tissue adenosine levels and renal function. *Circulation* 2002;106:1275–81.
13. Vermeulen EG, Rauwerda JA, van den Berg M, de Jong SC, Schalkwijk C, Twisk JW, et al. Homocysteine-lowering treatment with folic acid plus vitamin B₆ lowers urinary albumin excretion but not plasma markers of endothelial function or C-reactive protein: further analysis of secondary end-points of a randomized clinical trial. *Eur J Clin Invest* 2003;33:209–15.

14. Loehrer FM, Angst CP, Brunner FP, Haefeli WE, Fowler B. Evidence for disturbed S-adenosylmethionine:S-adenosylhomocysteine ratio in patients with end-stage renal failure: a cause for disturbed methylation reactions? *Nephrol Dial Transplant* 1998;13:656–61.
15. Perna AF, Ingrosso D, Castaldo P, Galletti P, De Santo NG. Homocysteine and transmethylation in uremia. *Kidney Int Suppl* 2001;78:S230–3.
16. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, et al. S-Adenosylmethionine and methylation. *FASEB J* 1996;10:471–80.
17. Schatz RA, Wilens TE, Sellinger OZ. Decreased in vivo protein and phospholipid methylation after in vivo elevation of brain S-adenosyl-homocysteine. *Biochem Biophys Res Commun* 1981;98:1097–107.
18. Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem* 1990;1:228–37.
19. Lee ME, Wang H. Homocysteine and hypomethylation. A novel link to vascular disease. *Trends Cardiovasc Med* 1999;9:49–54.
20. McKeever M, Molloy A, Weir DG, Young PB, Kennedy DG, Kennedy S, et al. An abnormal methylation ratio induces hypomethylation in vitro in the brain of pig and man, but not in rat. *Clin Sci (Lond)* 1995;88:73–9.
21. Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML, et al. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet* 2003;361:1693–9.
22. Henning BF, Zidek W, Riezler R, Graefe U, Tepel M. Homocyst(e)ine metabolism in hemodialysis patients treated with vitamins B₆, B₁₂ and folate. *Res Exp Med (Berl)* 2001;200:155–68.
23. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. http://www.kidney.org/professionals/kdoqi/guidelines_ckd/toc.htm (accessed May 19, 2004).
24. Stabler SP, Lindenbaum J, Savage DG, Allen RH. Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. *Blood* 1993;81:3404–13.
25. Ulleland M, Eilertsen I, Quadros EV, Rothenberg SP, Fedosov SN, Sundrehagen E, et al. Direct assay for cobalamin bound to transcobalamin (holo-transcobalamin) in serum. *Clin Chem* 2002;48:526–32.
26. Poirier LA, Brown AT, Fink LM, Wise CK, Randolph CJ, De-longchamp RR, et al. Blood S-adenosylmethionine concentrations and lymphocyte methylenetetrahydrofolate reductase activity in diabetes mellitus and diabetic nephropathy. *Metabolism* 2001;50:1014–8.
27. Herrmann W, Schorr H, Geisel J, Riegel W. Homocysteine, cystathionine, methylmalonic acid and B-vitamins in patients with renal disease. *Clin Chem Lab Med* 2001;39:739–46.
28. Dudman NP, Temple SE, Guo XW, Fu W, Perry MA. Homocysteine enhances neutrophil-endothelial interactions in both cultured human cells and rats *In vivo*. *Circ Res* 1999;84:409–16.
29. Wang H, Yoshizumi M, Lai K, Tsai JC, Perrella MA, Haber E, et al. Inhibition of growth and p21ras methylation in vascular endothelial cells by homocysteine but not cysteine. *J Biol Chem* 1997;272:25380–5.
30. Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci U S A* 1994;91:6369–73.
31. Tsai MY, Aras O, Sozen H, Hanson NQ, Woll PS, Arends VL, et al. Plasma homocysteine levels in living kidney donors before and after uninephrectomy. *J Lab Clin Med* 2004;143:340–3.
32. van Guldener C, Stam F, Stehouwer CD. Homocysteine metabolism in renal failure. *Kidney Int Suppl* 2001;78:S234–7.
33. Guttormsen AB, Ueland PM, Svarstad E, Refsum H. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney Int* 1997;52:495–502.
34. van Guldener C, Kulik W, Berger R, Dijkstra DA, Jakobs C, Reijngoud DJ, et al. Homocysteine and methionine metabolism in ESRD: a stable isotope study. *Kidney Int* 1999;56:1064–71.
35. Obeid R, Kuhlmann KM, Kirsch CM, Herrmann W. Cellular uptake of vitamin B₁₂ in patients with chronic renal failure. *Nephron Clin Pract* 2005;99:c42–8.
36. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. *J Biol Chem* 1984;259:9508–13.
37. Carmel R. Measuring and interpreting holo-transcobalamin (holo-transcobalamin II). *Clin Chem* 2002;48:407–9.
38. Dierkes J, Domrose U, Ambrosch A, Schneede J, Guttormsen AB, Neumann KH, et al. Supplementation with vitamin B₁₂ decreases homocysteine and methylmalonic acid but also serum folate in patients with end-stage renal disease. *Metabolism* 1999;48:631–5.
39. Fu W, Dudman NP, Perry MA, Young K, Wang XL. Interrelations between plasma homocysteine and intracellular S-adenosylhomocysteine. *Biochem Biophys Res Commun* 2000;271:47–53.
40. Guerra-Shinohara EM, Morita OE, Pagliusi RA, Blaia-D'Avila VL, Allen RH, Stabler SP. Elevation of serum S-adenosylhomocysteine in severe cobalamin deficient megaloblastic anemia. *Blood* 2001;98:13a.
41. Perna AF, D'Aniello A, Lowenson JD, Clarke S, De Santo NG, Ingrosso D. D-Aspartate content of erythrocyte membrane proteins is decreased in uremia: implications for the repair of damaged proteins. *J Am Soc Nephrol* 1997;8:95–104.
42. Becker A, Smulders YM, Teerlink T, Struys EA, de Meer K, Kostense PJ, et al. S-Adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine are not related to folate, cobalamin and vitamin B₆ concentrations. *Eur J Clin Invest* 2003;33:17–25.
43. Stabler S, Allen RH. Quantification of serum and urinary S-adenosylmethionine and S-adenosylhomocysteine by stable-isotope-dilution liquid chromatography-mass spectrometry. *Clin Chem* 2004;50:365–72.
44. Detich N, Hamm S, Just G, Knox JD, Szyf M. The methyl donor S-adenosylmethionine inhibits active demethylation of DNA: a candidate novel mechanism for the pharmacological effects of S-adenosylmethionine. *J Biol Chem* 2003;278:20812–20.