# Supplementation With Vitamin $B_{12}$ Decreases Homocysteine and Methylmalonic Acid But Also Serum Folate in Patients With End-Stage Renal Disease

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**Hyperhomocysteinemia is frequently found in patients with end-stage renal disease (ESRD). Plasma total homocysteine (tHcy) concentrations may be reduced by supplementation with folic acid or combinations of folic acid, vitamin $B_{12}$, and vitamin $B_6$. Supplementation studies with vitamin $B_{12}$ alone in patients with ESRD have not yet been published. In this study, we investigated the effects of intravenous injection of cyanocobalamin (1 mg/wk for 4 weeks) in ESRD patients (N = 14) with low serum cobalamin concentrations (<180 pmol/L). All patients had elevated levels of plasma tHcy, methylmalonic acid (MMA), and cystathionine before supplementation. After supplementation, plasma tHcy and MMA decreased 35% and 48%, respectively; however, cystathionine levels were unchanged. The extent of the plasma tHcy reduction tended to be influenced by the C677T polymorphism of methylenetetrahydrofolate reductase (MTHFR). Serum cobalamin increased significantly upon supplementation, whereas serum folate levels were substantially reduced by 47%. In contrast, red blood cell (RBC) folate was unchanged. This study shows that vitamin $B_{12}$ supplementation effectively decreases both MMA and plasma tHcy in ESRD patients with low $B_{12}$ levels. Furthermore, it illustrates the close interrelation between vitamin $B_{12}$ and folate metabolism.**


TOTAL HOMOCYSTEINE (tHcy) is a metabolite of the essential amino acid methionine. Two known major pathways for the transformation of tHcy are remethylation to methionine or transsulfuration to cysteine. The remethylation requires methylcobalamin (one of the coenzyme forms of vitamin $B_{12}$) as a coenzyme and methyltetrahydrofolate (one of the metabolic active forms of folic acid) as a substrate, whereas the transsulfuration is dependent on pyridoxal-5-phosphate (the coenzyme form of vitamin $B_6$).

An elevated plasma concentration of tHcy is an independent risk factor for cardiovascular disease in patients with normal renal function. This association has been shown in both retrospective and prospective case-control studies.[1-3] Most studies demonstrated that the cardiovascular risk is increased already at the upper quartile of the reference range (12 to 16 μmol/L). Patients with end-stage renal disease (ESRD) have mildly to moderately elevated plasma tHcy concentrations, typically in the range of 20 to 80 μmol/L.[4,5]

Mild to moderate hyperhomocysteinemia in subjects with normal renal function can be normalized in most cases by supplementation with either folic acid alone or combinations of folic acid with vitamin $B_{12}$ and/or vitamin $B_6$.[6-8] However, there is only limited knowledge about the tHcy-lowering effect of vitamin $B_{12}$ alone. Ubbink et al[7] studied healthy middle-aged men who received oral vitamin $B_{12}$ supplementation (400 μg daily for 6 weeks), and a 14% reduction of the elevated plasma tHcy was observed. Patients with non–insulin-dependent diabetes mellitus were investigated by Araki et al.[9] The patients received 1 mg vitamin $B_{12}$ intramuscularly daily for 3 weeks, and a 30% decrease of plasma tHcy was reported.

Elevated plasma tHcy concentrations are typically found in folate and/or cobalamin deficiency[10-12] but also in vitamin $B_6$ deficiency.[13] Furthermore, the effects of these vitamin deficiencies may be modulated by the C677T genotype of methylenetetrahydrofolate reductase (MTHFR). A TT genotype is associated with decreased enzyme activity and increased tHcy in humans with low or low-normal serum folate concentrations.[14] This has been observed both in humans with normal renal function and in patients with ESRD.[15]

Patients with ESRD have an increased risk to develop vitamin deficiencies. In particular, vitamin $B_{12}$ metabolism may be affected by the disease itself,[16] and low serum cobalamin concentrations are frequently observed in these patients. However, studies attempting to decrease tHcy using vitamin $B_{12}$ supplementation alone have not been performed in ESRD patients. Therefore, we investigated the effect of high-dose vitamin $B_{12}$ supplementation in patients with ESRD and low baseline serum cobalamin concentrations in relation to the MTHFR genotype.

## SUBJECTS AND METHODS

Patients with ESRD from the Department of Nephrology at University Hospital Magdeburg who were clinically stable at the time of the study were eligible to participate. All underwent hemodialysis three times per week. Of 85 patients with ESRD, 14 had low serum cobalamin concentrations (<180 pmol/L) and were included in the study after provision of written informed consent. Characteristics of the patients are listed in Table 1. High-dose vitamin $B_{12}$ supplements (cyanocobalamin) were administered intravenously (1.000 μg once per week) for 4 weeks, each after dialysis. Blood samples were taken before dialysis at baseline and before dialysis after 4 weeks for measurement of plasma tHcy, serum and red blood cell (RBC) folate, and serum cobalamin, methylmalonic acid (MMA), and cystathionine. Serum and EDTA test tubes were cooled immediately, and the plasma was separated from the blood cells within 30 minutes and frozen at −20°C until analysis. Lysates of EDTA blood in ascorbic acid solution lysis reagent (Abbott Lab) were prepared for analysis of RBC folate according to the manufacturer's instructions and frozen at −20°C until analysis.

Plasma tHcy was determined in EDTA plasma with a high-performance liquid chromatographic method with fluorescence detection after derivatization with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate.[17] The method has a between-day coefficient of variation of 5%; the reference range for plasma tHcy is 5 to 15 μmol/L.

MMA and cystathionine levels were measured in EDTA plasma using

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**Table 1. Characteristics of the Hemodialysis Patients (N = 14)**

| Characteristic | Median | Range |
|---|---|---|
| Age (yr) | 63.0 | 42-79 |
| BMI (kg/m²) | 25.2 | 20.2-33.6 |
| Duration of dialysis (mo) | 33 | 5-78 |
| Creatinine (μmol/L) | 800 | 499-1,329 |
| Male/female ratio | 7/7 | |
| C677T MTHFR genotype | CC = 5; CT = 7; TT = 2 | |

Abbreviations: BMI, body mass index; CC, wild-type; CT, heterozygous; TT, mutant.

a newly developed gas chromatographic method combined with mass spectrometry as described by Guttormsen et al.[18] The method has a between-day coefficient of variation of 3% to 15% for both metabolites. The reference range for MMA is 0.05 to 0.26 μmol/L, but a reference range has not been established for cystathionine. However, the reference range for cystathionine in serum reported in the literature, based on a different method, is 0.065 to 0.301 μmol/L.[19]

Serum cobalamin and folate were analyzed using commercial test kits (Abbott IMx; Abbott Laboratories, Wiesbaden, Germany). The test kit measures total cobalamin in serum. Folate was analyzed both in serum and in RBCs. The reference range was more than 135 pmol/L for serum cobalamin, more than 6.4 nmol/L for serum folate, and more than 425 nmol/L for RBC folate.

For analysis of the C677T genotype of MTHFR, DNA was extracted from EDTA blood and the C677T genotype was assessed using polymerase chain reaction with subsequent enzymatic restriction analysis (*Hin*fI) as described by Kluijtmans et al.[20]

Statistical analysis was performed using the Statistical Package for the Social Sciences Version 6.13 (SPSS, Chicago, IL). Nonparametric tests were used for comparison of baseline and postsupplementation measurements. Spearman rank correlation coefficients are presented. A $P$ level less than .05 was regarded as significant.

## RESULTS

At baseline, none of the 14 patients were folate-deficient with RBC folate as the sole criterion to define folate deficiency; however, four patients had serum folate less than 6.4 nmol/L (cutoff point for folate deficiency). Serum cobalamin correlated weakly with RBC folate ($r = .43$, nonsignificant [NS]) but not serum folate ($r = .17$, NS). RBC folate was significantly correlated with serum folate ($r = .58$, $P = .03$). Plasma tHcy was elevated in all patients. It was correlated significantly with serum folate ($r = -.75$, $P = .001$) but only weakly with RBC folate ($r = -.49$, $P = .08$) and not with serum cobalamin ($r = .08$). MMA concentrations were not correlated with plasma tHcy ($r = .21$), serum folate ($r = -.07$), or RBC folate ($r = -.12$), but correlated weakly with serum cobalamin ($r = -.36$, $P = .2$).

**Table 3. tHcy and Effect of Vitamin $B_{12}$ Supplementation in Relation to C677T Genotype of MTHFR**

| | No T Allele, CC (n = 5) | | T Allele, CT or TT (n = 9) | | |
|---|---|---|---|---|---|
| Parameter | Median | Range | Median | Range | $P$ |
| tHcy (μmol/L) | | | | | |
| Baseline | 34.6 | 26.7-84.3 | 45.7 | 31.2-71.2 | NS |
| Postsupplementation | 21.4 | 17.3-48.1 | 33.5 | 22.8-55.7 | .04 |
| tHcy ratio (%)* | 62.7 | 54.3-65.2 | 72.8 | 45.2-110.1 | .09 |
| Serum folate ratio (%)* | 58 | 43.7-63.1 | 50.5 | 28.5-96.7 | NS |

*Calculated as value after supplementation/value at baseline × 100.

The effects of $B_{12}$ supplementation on the plasma concentration of the investigated vitamins and metabolites are listed in Table 2. Plasma tHcy was significantly reduced after supplementation (−35%). The extent of this reduction was not correlated with baseline plasma tHcy ($r = -.25$), serum or RBC folate, or serum cobalamin ($r = .06$). Patients without a T allele in the MTHFR genotype had significantly lower plasma tHcy concentrations after supplementation ($P < .05$; Table 3 and Fig 1). MMA decreased, on average, by 48% after supplementation. The decrease was weakly related to the baseline serum cobalamin concentration ($r = -.49$, $P = .07$) but was independent of the MTHFR status. The reduction in plasma tHcy was strongly related to the reduction in MMA ($r = .64$, $P = .001$), and the correlation was influenced by the presence of a T allele in the MTHFR gene (Fig 2).

After supplementation, the median serum cobalamin increased significantly, whereas the mean corpuscular volume was unaltered. Also, the RBC folate concentration did not change significantly, whereas serum folate decreased 47% (Table 2). Of 14 patients, 13 had serum folate levels less than 6.4 nmol/L after supplementation. This decrease was positively related to the baseline folate level ($r = .49$, $P = .06$) and independent of the MTHFR genotype.

## DISCUSSION

Thus far, recommendations for the treatment of hyperhomocysteinemia in ESRD patients focus on supplementation with folic acid either alone or in conjunction with other vitamins.[21] In our study, we describe for the first time the efficacy of intravenous supplementation with vitamin $B_{12}$ alone in ESRD patients with low serum levels of cobalamin. High-dose vitamin $B_{12}$ substantially reduced the elevated plasma levels of both tHcy and MMA in this subgroup of ESRD patients.

The elevated levels of plasma tHcy and MMA and the concurrent reduction of these metabolites upon vitamin $B_{12}$

**Table 2. Effect of Vitamin Supplementation (N = 14)**

| | Baseline (n = 14) | | After Supplementation (n = 14) | | |
|---|---|---|---|---|---|
| Parameter | Median | Range | Median | Range | $P$ |
| tHcy (μmol/L) | 40.8 | 26.7-84.3 | 29.4 | 17.3-50.1 | .01 |
| MMA (μmol/L) | 1.12 | 0.37-2.87 | 0.54 | 0.21-0.84 | .001 |
| Cystathionine (μmol/L) | 1.40 | 0.50-5.10 | 1.68 | 0.61-2.46 | NS |
| Vitamin $B_{12}$ (pmol/L) | 146 | 109-179 | 1,166 | 443-2,900 | .001 |
| RBC folate (nmol/L) | 875 | 431-1,428 | 1,013 | 591-1,543 | NS |
| Serum folate (nmol/L) | 9.4 | 5.0-19.6 | 4.5 | 2.4-8.8 | .001 |
| Mean corpuscular volume (fL) | 93.8 | 83.3-104.0 | 95.1 | 82.6-106.0 | NS |

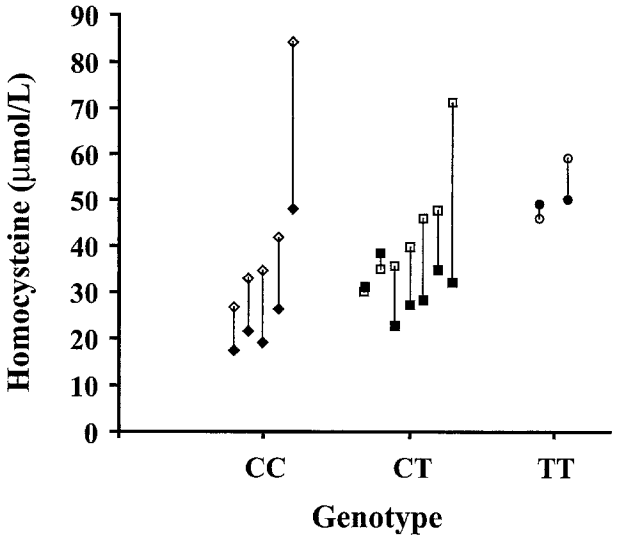


**Fig 1. Effect of vitamin $B_{12}$ supplementation on tHcy in relation to the C677T MTHFR genotype. Open symbols are the baseline tHcy concentration, and solid symbols are the tHcy concentration after supplementation. Blood samples were taken before dialysis.**

supplementation are regarded as indicative of true vitamin $B_{12}$ deficiency.[22,23] However, the diagnostic usefulness of elevated MMA levels in patients with renal disease has been questioned.[24] We were able to show a substantial reduction of MMA after vitamin $B_{12}$ supplementation. Yet despite an average 35% reduction of tHcy and 48% reduction of MMA, a complete normalization of both metabolites was not achieved in any of the patients before dialysis. This tHcy reduction seemed to be influenced by the MTHFR genotype. Although the number of patients is too small to make firm conclusions, it is worth noting that of nine patients with a T allele, four showed a reduction of homocysteine of less than 20% after vitamin $B_{12}$ supplementation (Fig 2).

We also found a pronounced decrease of serum folate but not RBC folate after supplementation with vitamin $B_{12}$. This was unexpected, as both folate parameters were within the normal range in all patients before $B_{12}$ supplementation. The reduction was strongly related to the baseline serum folate: the higher the

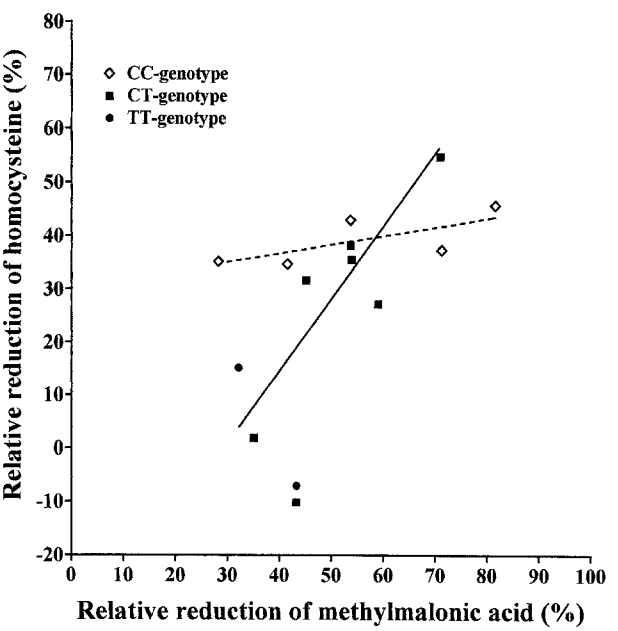


**Fig 2. Relative reduction of MMA and tHcy in relation to the C677T MTHFR genotype in dialysis patients after vitamin $B_{12}$ supplementation. (---) Regression line for patients with the CC genotype (slope not significantly different from zero); (—) regression line for patients with the CT (n = 7) and TT (n = 2) genotype.**

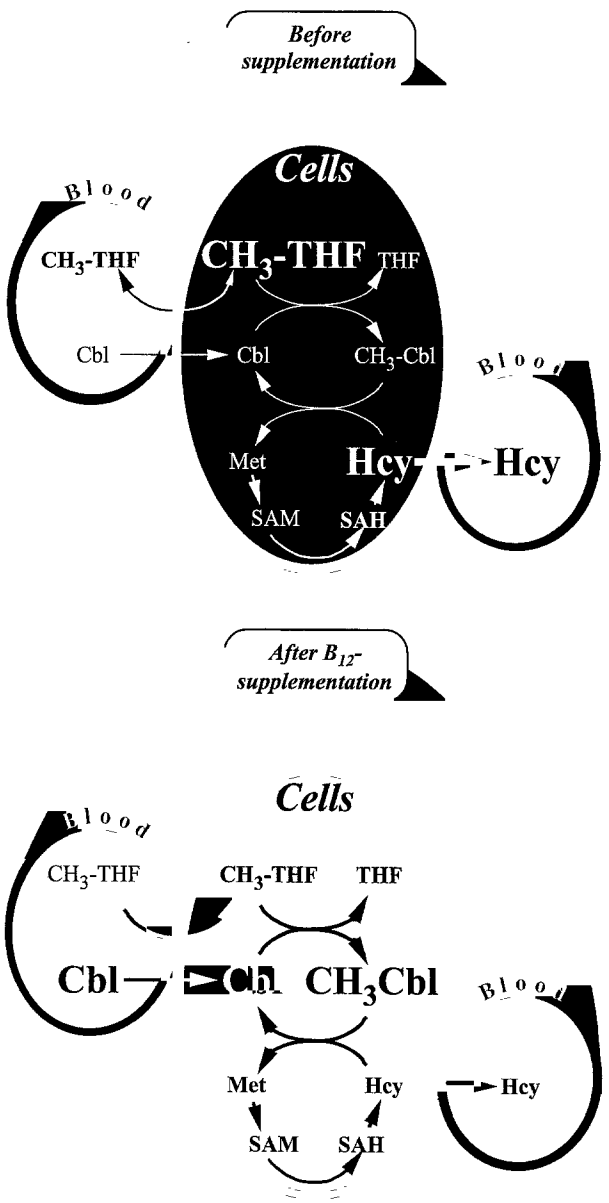


**Fig 3. Schematic representation of potential changes in the extracellular concentration of tHcy and 5-methyltetrahydrofolate ($CH_3$-THF) in cobalamin deficiency and in response to supplementation with vitamin $B_{12}$. Shown are physiological (small print), subphysiological (small boldface), and supraphysiological (large boldface) concentrations. SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; Met, methionine; THF, tetrahydrofolate; $CH_3$-THF, 5-methyltetrahydrofolate; Cbl, cobalamin; $CH_3$-Cbl, methylcobalamin.**

folate concentration at baseline, the stronger the decrease of folate. After supplementation, 13 of 14 patients had serum folate concentrations below the reference limits (<6.4 nmol/L). Whether the pronounced decrease of serum folate is indicative of an impaired folate status or just a temporary phenomenon remains unclear, as RBC levels did not show a significant change. The last cobalamin injection was given 7 days before blood sampling for the postsupplementation status. 5-Methyltetrahydrofolate is necessary for the methylation of Hcy in the vitamin $B_{12}$–dependent methionine synthase reaction. Supplementation with vitamin $B_{12}$ results in an increased activity of methionine synthase and consequently increases the intracellular demand for 5-methyltetrahydrofolate (Fig 3). An increased cellular uptake of 5-methyltetrahydrofolate, the predominating folate form in serum, might explain the substantial reduction of serum folate. Since the serum folate concentration is typically only 1% to 2% of the intracellular concentration, changes in the distribution of folate between the intracellular and extracellular compartments are likely to have a stronger influence on the serum level. A similar phenomenon is also described in connection with the methionine loading test. Methionine loading, which indirectly stresses the Hcy remethylation pathway, may also result in a temporary decrease of serum folate levels.[25,26]

Steady-state concentrations of tHcy in plasma are influenced by the amount of cellular export into the extracellular matrix and by the total plasma clearance. The kidney plays an important role in the plasma clearance of Hcy,[27,28] although this has been questioned by a recent study.[29] However, since vitamin $B_{12}$ supplementation is unlikely to influence the renal clearance of Hcy,[30] the observed decrease of plasma tHcy is most likely due to reduced cellular export. We found that cystathionine levels were well above the normal range[19] and remained virtually unchanged upon $B_{12}$ supplementation. Cystathionine concentrations are markedly elevated in renal failure,[31,32] but may also be increased in patients with $B_{12}$, $B_6$, and folate deficiency.[19] Since cystathionine levels did not respond to $B_{12}$ supplementation in our patients, the elevation is most likely caused by renal insufficiency.

In conclusion, vitamin $B_{12}$ supplementation has a substantial tHcy-lowering effect in ESRD patients with low serum cobalamin. The decrease of plasma tHcy is most likely the result of reduced cellular efflux of Hcy rather than changes in the renal clearance, and is influenced by the MTHFR genotype. Further studies will be necessary to elucidate whether concomitant administration of folate and vitamin $B_{12}$ and $B_6$—and possibly betaine—could more effectively decrease plasma tHcy levels in ESRD patients. In addition, our findings indicate that supplementation with a single vitamin may negatively influence the status of other vitamins. For this reason alone, coadministration of vitamins should be considered in ESRD patients with hyperhomocysteinemia.

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