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Homocysteine in chronic kidney disease: Effect of low protein diet and repletion with B vitamins

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Background. Data are limited on the determinants of homocysteine (tHcy) and its relationship with nutritional indices, and dietary protein intake, in the earlier stages of chronic kidney disease (CKD).

Methods. Levels of tHcy were assayed at baseline ($N = 804$) and 1 year postrandomization ($N = 678$) in the Modification of Diet in Renal Disease (MDRD) Study [study A, glomerular filtration rate (GFR) 25 to 55 mL/min/1.73 m^2 and study B GFR 13 to 24 mL/min/1.73 m^2]. Participants were randomly assigned to different blood pressure targets and protein diets and all subjects received a multivitamin supplement containing 1 mg of folic acid, 10 mg pyridoxal 5′-phosphate (PLP) and 6 μg of vitamin B_{12}. Multivariable analyses were used to evaluate determinants of tHcy at baseline and 1 year.

Results. The prevalence of hyperhomocysteinemia (tHcy >15 μmol/L) at baseline was 56% in study A and 85% in study B. Baseline tHcy was negatively correlated with measures of body fat and dietary protein intake. Folate, vitamin B_{12}, and GFR were the major determinants of tHcy levels. Of the patients with hyperhomocysteinemia at baseline, 49% and 24% reduced their tHcy levels at 1 year to ≤15 μmol/L in study A and study B, respectively. There was no association between dietary protein intake and odds of developing hyperhomocysteinemia at 1 year in study A ($P = 0.94$) or study B ($P = 0.10$).

Conclusion. Hyperhomocysteinemia is partly amenable to correction by vitamin supplementation in CKD stages 3 and 4. There is insufficient evidence to suggest that low tHcy is associated with poor nutritional status in the MDRD Study cohort. B vitamins and GFR, but not dietary protein, are the major determinants of tHcy in this patient population.

The prevalence of hyperhomocysteinemia is over 80% among dialysis patients [1]. Proposed mechanisms for hyperhomocysteinemia in kidney failure include deficiencies of folate, vitamin B_{12}, and pyridoxal 5′-phosphate (PLP), and reduced clearance of total plasma homocysteine (tHcy) secondary to defective kidney and/or extra kidney metabolism of tHcy [2, 3]. The determinants of hyperhomocysteinemia in patients in the earlier stages of chronic kidney disease (CKD) has been less well studied [4].

Hyperhomocysteinemia appears to be associated with increased cardiovascular disease risk in the general population [5, 6]. Similarly, elevated levels of tHcy may be associated with increased morbidity and mortality from cardiovascular disease in patients with kidney failure [7–9]. In contrast, low tHcy levels were associated with hospitalization and mortality in hemodialysis patients [10–12]. These studies also found a positive relationship between tHcy levels and serum albumin and protein intake. Thus, an association between low tHcy levels and malnutrition has been suggested as a possible explanation for these paradoxic findings [10, 11]. However, there is limited information concerning the relationship between tHcy levels and nutritional markers in the earlier stages of CKD.

Folate and vitamin B_{12} are the major determinants of tHcy levels in the general population and in dialysis patients [13, 14]. Several clinical trials in dialysis patients have found that folic acid and vitamin B_{12} supplementation, therapeutic measures that are highly effective in the general population, do not normalize tHcy levels in kidney failure; even when administered in supraphysiologic doses [15]. It is unknown if these strategies are more effective if initiated earlier in CKD.

Dietary protein intake may also affect tHcy levels. Although studies in the general population have noted an inverse or no association between dietary protein and methionine intake and tHcy levels [16, 17], in the dialysis population there appears to be a positive relationship between tHcy levels and both protein intake and markers of adequate or good nutritional status [18]. These

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relationships have not been examined in patients with CKD, prior to reaching kidney failure.

The Food and Drug Administration authorized the addition of folic acid to enriched grain products in March 1996, and made it mandatory in January 1998. The Modification of Diet in Renal Disease Study (MDRD Study) was a randomized controlled clinical trial of dietary protein restriction and strict blood pressure control in patients with CKD stages 3 and 4, conducted from 1989 to 1993 and thus predating the folate fortification era. The objectives of the analyses presented here were to assess the prevalence and determinants of hyperhomocysteinemia at baseline, to examine the association between tHcy and nutritional markers, and to study the effect of vitamin supplementation and dietary protein intake on tHcy levels at one year among MDRD Study participants.

METHODS

The Cleveland Clinic Foundation and Tufts-New England Medical Center Institutional Review Boards approved all data collection procedures.

MDRD Study

The MDRD Study was a randomized controlled trial of 840 patients to study the effects of dietary protein restriction and strict blood pressure control on the progression of kidney disease [19]. Eligibility criteria for enrollment into the MDRD Study included age 18 to 70 years, CKD with serum creatinine 1.4 to 7.0 mg/dL (men) and 1.2 to 7.0 mg/dL (women). Patients with insulin-requiring diabetes, class III or IV congestive heart failure, renal artery stenosis, and history of kidney transplantation or frequent hospitalizations were excluded. Study A consisted of patients with a glomerular filtration rate (GFR) of 25 to 55 mL/min/1.73 m^2 and study B of patients with a GFR of 13 to 24 mL/min/1.73 m^2. Etiologies of kidney diseases were 25% polycystic kidney disease, 25% glomerular diseases, and 50% other causes.

The interventions in the MDRD Study have been described previously [20]. To summarize briefly, patients were randomized to low (0.58 g/kg/day) protein versus usual (1.3 g/kg/day) protein diet in study A. Patients in study B were randomized to low (0.58 g/kg/day) versus very low (0.28 g/kg/day) protein diet supplemented with a mixture of ketoacids and amino acids. Patients in both studies were also randomized to usual ($\leq$107 mm Hg for age $\leq$60 years, and $\leq$113 mm Hg for age >61 years) versus low ($\leq$92 mm Hg for age $\leq$60 years, and $\leq$98 mm Hg for age >61 years) blood pressure goals. At baseline, all participants were prescribed a multivitamin supplement containing 1 mg of folic acid, 10 mg PLP and 6 μg of vitamin B_{12}.

Daily protein intake at baseline was estimated from 3-day food records [20]. Adjusted protein intake was estimated from urine urea nitrogen excretion at 1 year. Plasma levels of amino acids were analyzed at baseline at the Central Amino Acid Laboratory, Department of Pediatrics, University of Iowa, Iowa City, Iowa.

Measurement of tHCY, folic acid, PLP, and vitamin B_{12}

Serum samples from the MDRD Study cohort were available from 804 (96% of randomized participants) patients at baseline (559 in study A and 245 in study B), and 678 (81% of those randomized) patients at 1 year follow-up (481 in study A and 197 in study B). As described previously [21], after blood was drawn, samples were allowed to clot at room temperature for a maximum of 2 hours prior to sample processing for serum and placing on ice. Samples were shipped overnight to the Central Biochemistry Laboratory at The Cleveland Clinic Foundation, and stored at -70° C. Frozen samples were then thawed, split and assayed for tHcy by high-performance liquid chromatography (HPLC) with fluorometric detection [22]. Serum PLP was assessed using the tyrosine decarboxylase apoenzyme method. Serum folate was measured by a microbial (*Lactobacillus cassei*) assay, and serum vitamin B_{12} with a (Magic) radioimmunoassay from Ciba-Corning. Details of assays used for measurements of these analytes have been described previously [21].

Statistical analyses

Hyperhomocysteinemia was defined as fasting tHcy >15 μmol/L; the upper limit of the normative range in the prefortification era [23]. Descriptive statistics are reported as percentages for categoric data, and mean and standard deviation for normally distributed continuous data. Skewed continuous variables were transformed to natural logarithms and median and interquartile ranges (IQR) are reported for these variables. Differences between groups were tested using the chi-square test, Student *t* test, and the Mann-Whitney test as appropriate.

The strength of the association between tHcy and selected nutritional markers was determined using Pearson's correlations with natural log-transformed tHcy and Spearman's ρ correlation with nontransformed tHcy. The results obtained from these analyses were similar and we present results using nontransformed variables to facilitate interpretation of study findings. Multivariable regression procedures were used to evaluate the independent determinants of tHcy at baseline and at 1 year follow-up. For determinants of tHcy at baseline, a priori selected variables included age, gender, race (white versus nonwhite), cause of kidney disease (glomerular, polycystic

Table 1. Baseline characteristics of the study population stratified by study and level of homocysteine (tHcy)

	Study A		Study B	
	tHcy ≤15 μmol/L $N = 249$	tHcy >15 μmol/L $N = 310$	tHcy ≤15 μmol/L $N = 38$	tHcy >15 μmol/L $N = 207$
Age *years*[a]	52.8 ± 11.8	51.6 ± 12.3	48.4 ± 14.5	51.4 ± 12.6
Male%	51	68[c]	63	36[d]
White%	84	85	92	85
Current smoker%	8	12	21	6[d]
Etiology of kidney disease%				
Glomerular	21	26	8	26[d]
Polycystic kidney disease	30	28	55	33
Other	49	46	37	41
History of diabetes%	5	5	11	4
History of coronary artery disease%	8	10	8	11
History of hypertension%	82	85	82	86
Glomerular filtration rate *mL/min/1.73 m*^{2}[a]	41.4 ± 8.8	36.6 ± 8.4[c]	19.5 ± 3.2	18.4 ± 3.4
Creatinine *mg/dL*[a]	1.7 ± 0.4	2.1 ± 0.5[c]	3.1 ± 0.8	3.5 ± 0.9[d]
Total cholesterol *mg/dL*[a]	216.1 ± 45.5	220.5 ± 48.7	216.2 ± 39.1	212.6 ± 41.7
Proteinuria *g/day*[b]	0.2 (0.9)	0.3 (1.3)	0.6 (2.3)	0.7 (1.9)
Albumin *g/dL*[a]	4.0 ± 0.3	4.1 ± 0.4[c]	3.8 ± 0.4	4.1 ± 0.4[d]
Vitamin B_{12} *pg/mL*[b]	461.5 (233.0)	379.8 (172.4)[c]	546.5 (290.6)	433.2 (212.1)[d]
Pyridoxal 5′-phosphate *ng/mL*[b]	42.4 (38.5)	34.8 (27.1)[c]	49.9 (68.1)	37.6 (37.0)[d]
Folate *ng/mL*[b]	12.0 (17.4)	7.1 (5.6)[c]	15.1 (29.9)	7.2 (6.4)[d]
tHcy *μmol/L*[b]	12.5 (29)	18.7 (5.9)[c]	13.2 (2.2)	22.1 (8.4)[d]

[a]Mean ± standard deviation.
[b]Median (interquartile range).
[c]$P < 0.05$, patients with tHcy ≤15 μmol/L compared to those with tHcy >15 μmol/L in study A.
[d]$P < 0.05$, patients with tHcy ≤15 μmol/L compared to those with tHcy >15 μmol/L in study B.

kidney disease or other), smoking (current versus prior or never), and baseline levels of albumin, daily protein intake, GFR, folate, PLP, and vitamin B_{12}. The regression was repeated after inclusion of serum creatinine because of the known direct association between serum creatinine, independent of GFR, with tHcy [24].

For determinants of tHcy at 1 year, the model included the same set of a priori selected variables with the following differences. One-year levels were used for albumin, GFR, folate, PLP, and vitamin B_{12}, and daily protein intake was replaced with randomization to the different protein diets. The model also adjusted for randomization to different blood pressure goals. The regression was repeated using adjusted protein intake estimated from urine urea nitrogen excretion at 1 year instead of randomization assignment to protein diets. A mixed effects model was used to test differences in selected factors at baseline and 1 year.

The McNemar test was used to compare prevalence of hyperhomocysteinemia at baseline and 1 year, stratified by study. An analysis of variance (ANOVA) was performed to relate percent change in the geometric mean of tHcy from baseline to 1 year controlling for protein diet and blood pressure assignments. The effect of different protein diets on hyperhomocysteinemia was examined using a logistic regression procedure with protein diet and blood pressure assignment, and baseline tHcy as independent variables and hyperhomocysteinemia at 1 year as the dependent variable.

RESULTS

Baseline characteristics of study sample

Mean ± standard deviation and median (IQR) of tHcy was 18.8 ± 9.7 μmol/L and 16.8 (8.0) μmol/L in the entire cohort. Baseline characteristics, stratified by study and level of tHcy, for all subjects with baseline values of tHcy are presented in Table 1 ($N = 804$). The prevalence of hyperhomocysteinemia at baseline was 56% in study A and 85% in study B. More males in study A and females in study B had hyperhomocysteinemia. Patients with hyperhomocysteinemia had higher levels of creatinine and lower levels of vitamin B_{12}, PLP, and folic acid in both studies A and B, compared to patients with tHcy levels in the normal range. There were no differences in age, race, history of cardiovascular disease, diabetes, hypertension, total cholesterol, and proteinuria between the high and low tHcy groups in either study A or B. The high tHcy group in study A had significantly lower levels of GFR. In study B, there was a higher prevalence of glomerular disease and a lower prevalence of polycystic kidney disease and smoking, among patients with hyperhomocysteinemia.

Univariate correlations of nutritional indices with baseline tHcy levels

The associations between baseline tHcy and nutritional markers were examined in univariate analyses in the whole cohort (study A and study B combined).

Table 2. Correlations between baseline homocysteine (tHcy) levels and nutritional indices

	Spearman correlation coefficient
Folate *ng/mL*	−0.47[a]
Vitamin B_{12} *pg/mL*	−0.31[a]
Pyridoxal 5′-phosphate *ng/mL*	−0.19[a]
Percent body fat%	−0.20[a]
Body mass index *kg/m²*	−0.08[a]
Triceps skinfold thickness *mm*	−0.17[a]
Daily protein intake *g/kg/day*	−0.12[a]
Albumin *g/dL*	0.18[a]
Serum creatinine *mg/dL*	0.48[a]

[a] $P < 0.05$.

Table 3. Multivariable regression for independent determinants of homocysteine (tHcy) levels at baseline

Change in predictor	Change in tHcy levels (95% confidence intervals)	*P* value
Doubling of folate *ng/mL*	↓ 12% (11% to 13%)	<0.001
Doubling of vitamin B_{12} *pg/mL*	↓ 14% (12% to 17%)	<0.001
10 mL/min/1.73 m^2 increase in GFR	↓ 18% (17% to 19%)	<0.001
0.1 g/dL increase in albumin	↑ 2% (2% to 3%)	<0.001
10 years increase in age	↑ 3% (2% to 4%)	0.01
Women versus men	↑ 10% (9% to 12%)	<0.001
White race versus nonwhite	↑ 6% (3% to9%)	0.03

GFR is glomerular filtration rate. Natural log transformed values were used for tHcy, vitamin B_{12}, and folate; other variables in the model, that did not reach statistical significance, included smoking status, etiology of kidney disease, and pyridoxal 5′-phosphate.

Baseline tHcy was negatively correlated with baseline values of folate, vitamin B_{12}, PLP, percent body fat, body mass index, triceps skinfold thickness, daily protein intake, and positively correlated with creatinine and albumin (Table 2). There was no association of baseline tHcy with plasma levels of methionine.

Determinants of tHcy at baseline

Independent determinants of tHcy at baseline were identified in multivariable regression analysis. Folate, vitamin B_{12}, and GFR were the primary determinants of tHcy levels at baseline. Higher serum albumin level, older age, female gender, and white race were also associated with higher levels of tHcy (Table 3). Etiology of kidney disease, smoking status, daily protein intake, and PLP did not reach statistical significance. The regression was repeated after inclusion of serum creatinine. There was a significant relationship between tHcy and creatinine (regression coefficient ± standard error = 0.06 ± 0.02) ($P = 0.02$).

Analyses using 1-year follow-up tHcy levels

Frozen samples were available for measurement at 1-year follow-up in 678 participants. There was no difference in baseline level of tHcy ($P = 0.43$) and GFR ($P = 0.09$) between the 678 participants with follow-up tHcy levels (median tHcy = 16.8 μmol/L, mean ± standard deviation GFR = 33 ± 12 mL/min/1.73 m^2) and the 126 patients who did not have 1-year tHcy levels (tHcy = 16.9 μmol/L, GFR = 31 ± 13 mL/min/1.73 m^2). There were no differences between the groups with regard to age, race, history of diabetes, or etiology of kidney disease; however, there were more men in the group with missing follow up tHcy levels (data not shown). Mean ± standard deviation and median (IQR) of tHcy at 1 year was 15.3 ± 6.2 and 14.4 (6.1) μmol/L in the entire cohort.

Table 4. Multivariable regression for independent determinants of homocysteine (tHcy) levels after 1 year of vitamin supplementation

Change in predictor	Change in tHcy levels (95% confidence intervals)	*P* value
Doubling of folate ng/mL	↓ 7% (6% to 9%)	<0.001
Doubling of vitamin B_{12} ng/mL	↓ 10% (7% to 12%)	<0.001
10 mL/min/1.73 m^2 increase in GFR	↓ 10% (9% to 11%)	<0.001
0.1 g/dL increase in albumin	↑ 2% (1% to 2%)	<0.001
10 years increase in age	↑ 5% (4% to 6%)	<0.001
Women versus men	↑ 7% (5% to 9%)	<0.01
White race versus nonwhite	↑ 7% (4% to 10%)	0.02

GFR is glomerular filtration rate. Natural log transformed values were used for tHcy at 1 year, vitamin B_{12}, and folate; other variables in the model, that did not reach statistical significance, included smoking status, etiology of kidney disease, pyridoxal 5′-phosphate, and randomization to different diets and blood pressure goals.

Determinants of tHcy levels at 1 year

In multivariable analysis examining determinants of tHcy at 1 year (Table 4), folate, vitamin B_{12}, and GFR remained important determinants in vitamin-supplemented patients. Other determinants included albumin, gender, race, and age. Randomization to different protein diets was not an independent determinant of tHcy at 1 year. The regression was repeated using estimated protein intake at 1 year rather than randomization assignment to protein diet. Level of dietary protein intake was not a significant predictor of tHcy levels at one year (regression coefficient, 95% CI = −0.05, −0.13 to 0.03) ($P = 0.24$).

In a mixed effects model comparing the effects of GFR, folic acid, vitamins B_{12}, B_6, and albumin on tHcy levels at baseline versus that at 1 year, there was no difference in the regression coefficients for any of these factors except folic acid which appeared to have less of an effect on tHcy levels at 1 year (regression coefficient ± standard error = −0.09 ± 0.02) ($P < 0.01$).

Effect of vitamin supplementation on tHcy levels at 1 year

The prevalence of hyperhomocysteinemia, defined as tHcy >15 μmol/L, was compared at baseline and 1 year in the 678 patients with frozen samples available at both

time points. The prevalence of hyperhomocysteinemia decreased significantly from baseline in both study A and B ($P < 0.001$ for both comparisons). Of the patients with hyperhomocysteinemia at baseline and with tHcy levels available at 1 year ($N = 434$), 49% (130/268) reduced their tHcy levels to the normal range in study A and 24% (39/166) in study B. An additional 29/213 patients in study A and 8/31 patients in study B, who had normal tHcy levels at baseline, developed hyperhomocysteinemia at 1 year.

Effect of different protein diets on tHcy levels at 1 year

Baseline and 1-year levels of tHcy and known determinants, stratified by study and dietary protein assignment, are presented in Table 5. In an ANOVA procedure, percent reductions of tHcy from baseline to 1 year were compared between the different diet groups in each study, controlling for blood pressure assignment. The percent reduction in geometric mean of tHcy was similar between the usual (17%) and low (17%) protein groups in study A ($P = 0.98$). However, there was a trend in the very low protein group in study B (21%) toward a larger percent decrease in tHcy levels than the low protein (13%) group ($P = 0.05$). In a logistic regression procedure accounting for blood pressure assignment and baseline tHcy levels, there was no significant association between randomization assignment to low or usual protein diet and odds of hyperhomocysteinemia at 1 year in either study A [odds ratio (OR) 95% CI = 1.02, 0.66 to 1.55) ($P = 0.94$); however, in study B there was a trend toward lower odds of hyperhomocysteinemia with the very low protein diet compared to the low protein diet (OR 95% CI = 0.56, 0.28 to 1.12) ($P = 0.10$).

Table 5. Levels of homocysteine (tHcy) and selected variables at baseline and 1 year, stratified by study and protein diet

	Study A				Study B			
	Usual protein diet		Low protein diet		Low protein diet		Very low protein diet	
	Baseline $N = 282$	1 Year $N = 239$	Baseline $N = 277$	1 Year $N = 242$	Baseline $N = 120$	1 Year $N = 94$	Baseline $N = 125$	1 Year $N = 103$
tHcy >15 μmol/L%	55	34	56	35	82	66	87	71
tHcy *μmol/L*[a]	15.9 (13.1–19.2)	13.2 (11.0–16.1)	16.0 (13.0–20.0)	13.3 (11.0–16.1)	20.4 (16.4–23.9)	17.7 (14.6–20.9)	22.5 (16.7–28.3)	17.8 (14.2–21.1)
Folate *ng/mL*[b]	8.5 (8.8)	51.3 (38.6)	8.7 (10.4)	49.5 (40.9)	7.5 (6.4)	56.8 (41.1)	8.4 (8.1)	66.9 (41.3)
Vitamin B_{12} *pg/mL*[b]	408.9 (190.9)	453.2 (222.5)	415.4 (208.7)	515.9 (263.3)	437.5 (251.2)	489.4 (237.0)	442.8 (205.8)	516.7 (243.1)
Protein intake *g/kg/day*[c]	1.10 ± 0.37	1.12 ± 0.22[d]	1.07 ± 0.29	0.75 ± 0.16[d]	0.94 ± 0.28	0.69 ± 0.11[d]	0.91 ± 0.27	0.65 ± 0.13[d]
GFR *mL/min/1.73 m*^{2}[c]	39.4 ± 9.0	39.4 (15.4)	38.1 ± 8.8	36.8 (14.9)	18.3 ± 3.6	18.6 ± 3.7	18.8 ± 3.2	19.0 ± 3.1

[a] Geometric mean and 95% confidence intervals.
[b] Median (interquartile range).
[c] Mean ± standard deviation.
[d] Total protein intake estimated from urine urea nitrogen.

DISCUSSION

In the present study, with its large cohort of patients with reduced kidney function, we found a high prevalence of hyperhomocysteinemia during the earlier stages of kidney disease. Supplementation with 1 mg of folic acid, 10 mg PLP, and 6 μg of vitamin B_{12} reduced tHcy levels to within the normal range in half the participants with moderate reductions in GFR (equivalent to CKD stage 3) and a quarter of the participants with more severe reductions in GFR (equivalent to CKD stage 4). Level of protein in the diet did not appear to have a major effect on tHcy levels.

Prevalence and determinants of tHcy levels at baseline

In the MDRD Study population, over half the patients in study A and more than three fourths of the patients in study B had hyperhomocysteinemia at baseline. This is consistent with a reported prevalence of close to 90%

among dialysis patients and those with advanced kidney disease prior to initiation of dialysis [11, 25, 26].

Consistent with studies in the general population and in dialysis patients, in the MDRD Study levels of folate, vitamin B_{12}, and GFR showed the strongest association with baseline tHcy levels [1, 13, 14, 27–29]. Other determinants included albumin, age, gender, and race. It has been suggested that low tHcy levels may be a marker for poor nutritional status and decreased protein intake in patients with kidney failure, and that the relationship between tHcy and cardiovascular disease outcomes may be confounded by these associations [10–12, 30]. In the MDRD Study cohort of patients with CKD stages 3 to 4, tHcy levels were inversely related to body mass index, percent body fat, triceps and biceps skinfold thickness, and protein intake. However, as in dialysis patients [10, 11], tHcy was positively related to serum albumin and creatinine in our sample. Although the positive association of albumin and creatinine with tHcy may be due to a nutritional relationship, it may also be due to the fact that tHcy is bound to albumin [31], and the metabolic pathways for synthesis of creatinine and tHcy are interrelated. That is, the formation of adenosyl-homocysteine from S-adenosyl methionine is coupled with creatinine synthesis [24]**.** In sum, these data suggest that in this group of relatively healthy patients in the earlier stages of CKD, there is insufficient evidence that low tHcy levels are a marker of poor nutritional status, as they may be in patients with kidney failure.

Determinants of tHcy levels at 1 year

In our study population, after 1 year of vitamin supplementation, the effect of folic acid levels diminished while GFR remained a major determinant of tHcy levels. These findings are consistent with the experience of patients with stable coronary artery disease in which following fortification of cereal grain flour products with folic acid, serum creatinine, and vitamin B_{12} supplanted folate as the major determinants of tHcy levels [32].

Effect of vitamin supplementation on tHcy levels at 1 year

In the MDRD Study, repletion with 1 mg of folic acid reduced tHcy levels significantly in both study A and study B. Despite significant reductions in tHcy levels in both groups, more patients in study A normalized their tHcy levels compared to study B. The latter may be due to higher baseline levels of tHcy in study B, but is also consistent with studies examining the effect of folate and B vitamin supplementation in patients with kidney failure which demonstrate that while many patients responded to therapy, tHcy levels in some were refractory to even high doses of folate [25, 33, 34]. It is also consistent with two other studies in patients with advanced kidney disease prior to initiation of dialysis [12, 25, 26]. Our data suggest that vitamin supplementation reduces levels of tHcy in some but not all patients with moderate and severe reductions in GFR. We acknowledge however, that the vitamin doses used in the MDRD Study were significantly lower than those used in some studies of patients with kidney failure [15], and that had larger doses been used, further reductions in tHcy may have been achieved. We also cannot rule out that other unknown factors were partially responsible for the reduction in tHcy.

Effect of different protein diets on tHcy levels

Dietary protein intake can modulate tHcy levels in different ways. First, 25% of circulating tHcy is free and unbound to protein and it is this form of homocysteine that is filtered by the glomerulus and available to metabolic pathways within the kidney [31]. Data from a study by Guttormsen et al [35] suggested that a protein-rich meal acutely increased levels of free tHcy, with slower increases in total tHcy thus altering the ratio of free to bound tHcy [35]. Thus elevation in free tHcy with dietary protein can result in increased plasma clearance secondary to increased filtration and uptake into the kidney as well as at extra renal sites [3]. Second, methionine is the principal dietary source of tHcy; therefore, increased intake of methionine secondary to increased dietary protein may result in elevated tHcy levels.

Several studies have investigated the role of the first pathway (i.e., increased protein intake leading to increased catabolism of tHcy) in the general population. One study found a negative correlation between dietary protein intake and tHcy levels in an elderly population [16]. Conversely a study of healthy men did not note any effect of varying dietary methionine intake on tHcy levels [36]. Similarly, data from the Atherosclerosis Risk in Communities Study and a randomized controlled trial in overweight subjects did not note any association between high methionine or high protein diet and tHcy levels [17, 37]. In the dialysis population, the second pathway (i.e., increased dietary protein and methionine intake leading to increased Hcy levels) may be more important due to the absence of glomerular filtration. In support of this hypothesis, two studies in the dialysis population showed a positive correlation between tHcy levels and the normalized protein equivalent of total nitrogen appearance, a measure of dietary protein intake [10, 11].

In the MDRD Study, in univariate analysis, there was a significant negative correlation between baseline tHcy and dietary protein intake, but this relationship did not persist in multivariate analysis. In addition, there was no statistically significant association between protein diet and tHcy, although we acknowledge that we may have had limited power to detect relationships in study B.

Study strengths and limitations

There were several limitations to our study. Changes in level of tHcy was not a primary outcome of the dietary intervention in the MDRD Study; therefore, as mentioned above, we may be underpowered to detect significant relationships. We measured total tHcy levels; thus, it is possible that we have missed a differential effect of level of dietary protein on the ratio of free versus bound tHcy.

As described in a previous publication [21], a 2-hour delay in sample processing may in theory result in a systematic measurement error resulting in elevated tHcy levels due to release of tHcy from red blood cells. However, studies specifically assessing changes at 2 hours found relatively small increases in tHcy levels [38, 39]. Furthermore, as detailed in our discussion, the range of tHcy levels, prevalence of hyperhomocysteinemia, and magnitude of associations with known variables such as folic acid and GFR in our sample are very similar to those reported in the literature. We acknowledge, however, that measurement errors if they exist may lead to an underestimation of the relationships examined in this study.

The results of this study are generalizable to a relatively healthy, nondiabetic predominantly white CKD population. The strengths include a large, well-characterized cohort with a wide range of kidney function. The low prevalence of comorbid conditions reduces the potential for confounding. Furthermore, the use of randomized groups and intent-to-treat analysis to evaluate the effect of protein diet on tHcy also reduces potential bias.

CONCLUSION

There is a high prevalence of hyperhomocysteinemia in patients with CKD, which is partly amenable to correction by relatively low doses of vitamin supplementation. Kidney function is a major determinant of tHcy levels in vitamin-deficient and vitamin-repleted patients. There is insufficient evidence that low tHcy is a marker of poor nutritional status in the MDRD Study cohort and level of dietary protein intake does not appear to have a major effect on tHcy levels in this patient population.

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