Total Homocysteine Is Associated With Nephropathy in Non–Insulin-Dependent Diabetes Mellitus

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Non–insulin-dependent diabetes mellitus (NIDDM) and hyperhomocysteinemia are both associated with premature vascular disease. We tested the hypothesis that homocysteine is associated with vascular disease and other diabetic complications in patients with NIDDM. The current investigation is a cross-sectional analysis of baseline variables for participants in the Appropriate Blood Pressure Control in Diabetes (ABCD) Trial. Men and women aged 40 to 74 years with NIDDM and a mean diastolic blood pressure (BP) of 80 mm Hg or higher were eligible. We measured serum levels of total homocysteine (tHcy), cystathionine, and methylmalonic acid (MMA) and correlated these values with clinical and other laboratory measures of the complications of diabetes mellitus in 452 subjects. tHcy was higher in males than in females and correlated with the duration of hypertension and systolic BP. tHcy was significantly correlated with MMA ($r = .35$, $P < .0001$) and cystathionine ($r = .53$, $P < .0001$) levels and inversely correlated with serum B_{12} ($r = -.23$, $P < .0001$) and folate ($r = -.18$, $P < .0001$). It was significantly correlated with serum creatinine ($r = .28$, $P < .0001$ for males and $r = .39$, $P < .0001$ for females) and inversely correlated with creatinine clearance ($r = -.19$, $P < .005$ for males and $r = -.30$, $P < .0001$ for females). tHcy was not increased in subjects with cardiovascular disease or retinopathy, but it was increased in those with neuropathy (10.3 *v* 9.3 μmol/L, $P < .05$) and macroalbuminuria (11.0 *v* 9.2 μmol/L, $P < .005$). Of these subjects, 2.2% met the criteria for vitamin B_{12} deficiency and 1% met the criteria for folate deficiency. We conclude that elevations of tHcy in this population appear to be the result of a combination of vitamin deficiency and decreased renal function and do not appear to be a predictor of cardiovascular disease.

NON–INSULIN-DEPENDENT diabetes mellitus (NIDDM)[1,2] and hyperhomocysteinemia both are associated with premature vascular disease.[3-7] Since hyperhomocysteinemia can often be corrected by vitamin treatment,[8,9] it would be important to know if NIDDM subjects with hyperhomocysteinemia have a greater risk of vascular disease than NIDDM subjects with lower values for total homocysteine (tHcy). Previous studies in insulin-dependent diabetes mellitus (IDDM) have shown that tHcy levels are correlated with the presence of nephropathy[10] but not retinopathy, and that there is no association between plasma tHcy and microangiopathy.[11] The tHcy concentration was shown to be associated with diabetic macroangiopathy but not nephropathy in a population of subjects with NIDDM.[12] Baseline tHcy concentrations were not higher in patients with vascular disease[13] in another study of diabetes (IDDM and NIDDM). Both diabetes and hyperhomocysteinemia appear to be unrelated independent risk factors for vascular disease.[3]

Homocysteine is a sulfhydryl-containing amino acid that is an intermediary in methionine metabolism. Elevated concentrations have been found in subjects who are deficient in folate, vitamin B_{12}, and vitamin B_6 individually and in combination,[14-17] as well as subjects with inborn errors such as homocystinuria (cystathionine β-synthase deficiency) or inborn errors of folate or vitamin B_{12} metabolism.[18] Renal insufficiency is also an important cause of elevated tHcy levels, even in subjects with a modest decrease in the glomerular filtration rate (GFR).[19-22] Previous investigations have shown strong relationships between serum creatinine, calculated creatinine clearance or GFR, and serum tHcy.[23-25] Thus, it seems likely that diabetic patients with an increased risk of nephropathy would also be at risk for hyperhomocysteinemia. Consequently, we measured tHcy and other related metabolites in baseline samples from participants in a large trial, the Appropriate Blood Pressure Control in Diabetes (ABCD) Trial,[26,27] and correlated these values with various baseline characteristics to determine whether tHcy is associated with vascular disease and other complications of diabetes.

SUBJECTS AND METHODS

Patients

The ABCD Trial has been described in detail previously.[26,27] It is a large prospective, randomized double-blind clinical trial designed to test the efficacy of intensive versus moderate antihypertensive control on the outcome of end-organ complications in NIDDM. Men and women aged 40 to 74 years with NIDDM and a mean baseline diastolic blood pressure (BP) of 80 mm Hg or higher were recruited into the trial, with enrollment ending in January 1993. Baseline serum was available for assay of tHcy and other metabolites for 452 of the subjects. Multiple measures of end-organ damage were determined during the "run-in" period of the trial as previously described.[27] The GFR was estimated by 24-hour urine creatinine clearance measurements. The urinary albumin excretion rate was measured from overnight and 24-hour collections at baseline. Retinopathy was staged using criteria established by the Wisconsin Retinal Reading Center.[28] Retinal photographs were taken on-site at the Colorado Prevention Center. The presence and severity of diabetic neuropathy were assessed according to criteria previously described,[29] including a neurologic symptoms score, neurologic disability score, autonomic nervous system testing with heart rate response to deep breathing, and quantitative sensory testing (vibration and temperature). Cardiovascular morbidity was described as including (1) myocardial infarction, documented or silent nonfatal, defined by electrocardiographic criteria; (2) congestive heart failure; (3) coronary artery disease

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(CAD) documented by coronary angiography or prior corrective procedure; (4) stroke; (5) atherosclerotic complications, including aortic dissection, atherosclerotic arterial aneurysm or mesenteric ischemia or infarction; (6) angina, either new onset or progression to unstable angina; (7) exercise-induced myocardial ischemia; (8) serious ventricular arrhythmia; (9) peripheral vascular disease; or (10) transient ischemic attack. Peripheral vascular disease was also analyzed by an ankle-to-brachial BP ratio less than 0.95.

The patients were recruited from mailing lists of diabetic patients from participating Denver hospitals, health maintenance organizations, and the Colorado affiliate of the Diabetes Association. The inclusion and exclusion criteria have been described in detail[27]: some important variables were exclusion of pregnant or lactating females, recent or recurrent substance abusers, subjects with severe peripheral vascular disease with gangrene or imminent amputation, and subjects on hemodialysis or peritoneal dialysis or with a serum creatinine greater than 3 mg/dL.

Serum was sent to a reference laboratory and an aliquot was later delivered to the research laboratory at the University of Colorado Health Sciences Center, where tHcy, cystathionine, and methylmalonic acid (MMA) were assayed by stable-isotope dilution gas chromatography/mass spectrometry as previously described.[30-33] Serum was stored frozen for up to 7 months before analysis. The stable-isotope–labeled homocysteine standard was added prior to sample reduction with dithiothreitol and sample deproteinization, in order to measure total homocysteine (the sum of protein bound and free oxidized and reduced). Elevated levels of MMA are found in most subjects with clinical B_{12} deficiency[16] and thus can be used to distinguish B_{12} deficiency from folate deficiency as a cause of elevated tHcy levels. Cystathionine levels can be increased in both folate and B_{12} deficiency,[32] as well as B_6 deficiency.[17] All of these metabolites can be elevated in renal insufficiency.[32-34]

Serum B_{12} and folate levels were assayed using the Corning Magic kit (Ciba-Corning, Medfield, MA).

Statistics

The Statistical Analysis Software system (SAS Institute, Cary, NC) was used for all statistical analyses. Pearson correlation coefficients were used to describe univariate correlations between tHcy and various clinical and laboratory values and renal-function parameters. ANOVA and *t* tests were used to compare groups for differences in the mean level of tHcy. When the variances were clearly unequal, an adjustment was made to account for the unequal variances. Furthermore, when the values were clearly nonnormally distributed, a suitable data transformation or nonparametric analysis was undertaken. Logistic regression was used to evaluate the effects of a continuous or categorical covariate(s) on a dichotomous outcome. The odds ratio and the corresponding confidence interval were then calculated from the logistic regression parameter estimates. All values are reported as the mean ± SD.

RESULTS

The mean age of the subjects was 58.3 ± 8.0 years (59.0 ± 7.9 for males and 57.9 ± 7.9 for females). Various other clinical and laboratory parameters are shown in Table 1. The mean levels of metabolites and vitamins were all in the normal range. The overall mean tHcy was 9.7 ± 3.8 μmol/L. Males had higher tHcy (10.2 ± 4.2 μmol/L) than females (9.1 ± 3.2 μmol/L, $P = .0015$; Table 2). The mean MMA level was 153 ± 251 nmol/L, cystathionine 243 ± 190 nmol/L, vitamin B_{12} 632 ± 302 pg/mL and serum folate 10.1 ± 7.4 ng/mL. Ten subjects (2.2%) met criteria for vitamin B_{12} deficiency defined as serum B_{12} less than 350 pg/mL and serum MMA greater than 271 nmol/L. The tHcy concentration was elevated in four of 10 B_{12}-deficient subjects, and the mean value was 20.6 ± 16.0 μmol/L, significantly greater than the mean tHcy in the other 442 subjects (9.5 ± 2.7 μmol/L, $P = .0001$). There were five folate-deficient subjects (1.1%), defined as serum folate less than 4 ng/mL and tHcy greater than 13.9 μmol/L with MMA less than 271 nmol/L. The mean tHcy in folate-deficient subjects was 14.7 ± 0.4 μmol/L, which is also higher versus the level in the other 449 subjects (9.7 ± 3.8 μmol/L, $P = .0001$).

Pearson correlation coefficients between tHcy and other clinical and laboratory parameters are also shown in Table 1. For all subjects, the duration of hypertension was modestly correlated with the level of tHcy, as was the systolic BP, but not the diastolic BP. The tHcy level was positively correlated with serum MMA and cystathionine and negatively correlated with serum B_{12} and serum folate levels. tHcy was not correlated with age or duration of diabetes for the whole group. Because the tHcy level was significantly different in males and females, some of the clinical variables were also analyzed separately by gender (Table 2). Creatinine clearance was inversely correlated with tHcy and positively correlated with serum creatinine for both males and females. The body mass index did not correlate with tHcy in males ($r = .00$, $P = .957$) or females ($r = .11$, $P = .14$).

The mean tHcy and other metabolite levels were then compared in subjects with or without complications of diabetes (Table 3). The mean tHcy was only slightly higher in subjects with any type of cardiovascular disease, and the difference was not statistically significant. The subgroup with CAD also had a nonsignificant increase in tHcy. The higher standard deviation for the mean tHcy found in this subgroup was due to only one outlier with CAD who had an extremely high tHcy concentration of 61 μmol/L and low serum folate. However, the mean tHcy was significantly greater in those with any degree of diabetic neuropathy (10.3 ± 4.9 *v* 9.3 ± 2.6 μmol/L, $P < .05$). tHcy was not significantly greater in subjects with retinopathy. The mean MMA level was higher in those with neuropathy (181 ± 369 *v* 131 ± 69 nmol/L, $P < .06$). However, serum B_{12} and folate levels were not significantly lower in subjects with neuropathy. There was a trend in that the mean cystathionine

Table 1. Mean Clinical and Laboratory Values in 452 Subjects With NIDDM

Variable	Mean ± SD	Correlation With tHcy (*r*)*
Age (yr)	58.3 ± 8.0	.08
Duration of diabetes (yr)	9.3 ± 7.1	.05
Duration of hypertension (yr)	10.1 ± 9 8	.12†
Systolic BP (mm Hg)	148 ± 19	.11†
Diastolic BP (mm Hg)	92 ± 9	.05
tHcy (μmol/L)	9.7 ± 3.8	—
MMA (nmol/L)	153 ± 251	.35‡
Cystathionine (nmol/L)	243 ± 190	.53‡
Serum vitamin B_{12} (pg/mL)	632 ± 302	−.23‡
Serum folate (ng/mL)	10.1 ± 7.4	−.18‡

*Pearson correlation coefficient.
†$P < .05$.
‡$P < .0001$.

Table 2. Mean Values and Pearson Correlation Coefficients for tHcy and Parameters of Renal Function in Males and Females

Parameter	Males (n = 274)			Females (n = 178)			*P* for Male *v* Female
	Mean ± SD	*r**	*P*†	Mean ± SD	*r**	*P*†	
tHcy (μmol/L)	10.2 ± 4.2			9.1 ± 3.2			.0015
Serum creatinine (mg/dL)	1.2 ± 0.2	.28	<.0001	1.0 ± 0.2	.39	<.0001	.0001
Creatinine clearance (mL/min)	88 ± 25	−.19	<.005	77 ± 24	−.30	<.0001	.0001
Microalbumin excretion (μg/min)	214 ± 570	.07	NS	129 ± 415	.19	<.05	NS
Glycosylated hemoglobin (%)	11.6 ± 3.2	−.05	NS	12.0 ± 3.4	.02	NS	NS

*Pearson correlation coefficient between tHcy and the listed variable.

†*P* for Pearson correlation coefficient.

was higher in subjects with neuropathy versus those without neuropathy.

The mean tHcy was significantly increased in subjects with macroalbuminuria compared with those with no albuminuria (11.0 ± 4.3 *v* 9.2 ± 2.2 μmol/L, $P < .005$). The mean serum MMA also was increased to 228 ± 567 versus 134 ± 90 nmol/L ($P < .005$) and serum cystathionine increased to 302 ± 274 versus 226 ± 150 nmol/L ($P < .005$) in patients with macroalbuminuria. Mean tHcy levels were higher in subjects in the two lowest GFR quartiles (10.6 ± 4.1 and 10.2 ± 5.6 μmol/L) compared with the third and fourth quartiles (9.3 ± 2.3 and 8.9 ± 2.3 μmol/L, ($P < .05$ for 1 *v* 3 and 4 and 2 *v* 3 and 4). For serum MMA, the value was significantly higher ($P < .05$) in the lowest GFR quartile (203 ± 477 nmol/L) versus the third and fourth quartiles (131 ± 65 and 134 ± 119 nmol/L, respectively, $P < .05$), and cystathionine in the lowest GFR quartile (293 ± 258) was significantly higher ($P < .05$) versus the other quartiles. Mean vitamin B_{12} and folate levels were not different in the four quartiles of GFR. Subjects with overt albuminuria still had a significantly elevated tHcy (10.1 *v* 9.1 μmol/L, $P < .05$), although cystathionine and MMA were no longer elevated after excluding subjects in the lowest quartile of GFR and the 10 B_{12}-deficient patients. The tHcy concentration, vascular disease, and retinopathy were not correlated, but tHcy (8.9 ± 2.2 *v* 9.6 ± 2.5 μmol/L, $P < .05$) and MMA (123 ± 53 *v* 145 ± 117 nmol/L, $P < .05$) were still higher in subjects with neuropathy after excluding folate- and B_{12}-deficient subjects and the lowest quartile of GFR (data not shown). The clinical and laboratory variables were also analyzed in male and female subjects separately, comparing those in the lowest quartile of tHcy with the highest quartile (data not shown). Age, serum creatinine, and diastolic BP were higher in men in the top quartile of serum tHcy (≥11.2 μmol/L), whereas creatinine clearance was lower. In women, the duration of hypertension, serum creatinine, and systolic BP were higher in the top quartile of tHcy (≥10.2 μmol/L), whereas creatinine clearance was lower. There were no other significant differences in comparing the top and bottom quartiles of tHcy. A logistic regression analysis demonstrated that tHcy was an independent predictor of neuropathy with an odds ratio of 1.48 ($P = .063$; Table 4) in subjects without folate or B_{12} deficiency and in the top three quartiles of GFR. However, if subjects in the lowest quartile of GFR were included, tHcy was no longer independently associated with neuropathy. Table 4 also shows an odds ratio for tHcy of 1.67 in predicting albuminuria for the subjects excluding only B_{12}- and folate-deficient subjects. Serum MMA or vitamin B_{12} were not independently correlated with neuropathy or albuminuria in either of these models.

There were 21 (4.8%) and 20 (4.6%) subjects with elevated tHcy or MMA, respectively, who did not meet criteria for B_{12} or folate deficiency because their vitamin levels were above our criteria levels for deficiency. Table 5 shows that the mean serum creatinine was higher (1.45 and 1.30 mg/dL) and creatinine clearance was lower (65 and 72 mL/min) for those with elevated tHcy and MMA, respectively, compared with the rest of the subjects. However, serum B_{12} and folate levels were also lower in subjects with elevated metabolites. These data suggest that the subjects with elevated metabolites may have had a combination of renal insufficiency and relative vitamin inadequacy.

Table 3. Mean tHcy and Other Laboratory Values in the Presence of Complications of Diabetes Mellitus

	Neuropathy		Albuminuria			CVD*		CAD		Retinopathy	
	Absent	Present	Absent	Micro	Macro	Absent	Present	Absent	Present	Absent	Present
No. of subjects	250	198	259	118	75	227	225	350	102	223	218
tHcy (μmol/L)	9.3 ± 2.6	10.3 ± 4.9†	9.2 ± 2.2	10.2 ± 5.7	11.0 ± 4.3‡	9.4 ± 2.9	10.1 ± 4.6	9.5 ± 3.0	10.5 ± 5.7	9.5 ± 4.3	10.0 ± 3.3
MMA (nmol/L)	131 ± 69	181 ± 369§	134 ± 90	149 ± 130	228 ± 567‡	147 ± 124	159 ± 333	155 ± 282	142 ± 118	142 ± 118	164 ± 340
Cystathionine (nmol/L)	229 ± 181	259 ± 201	226 ± 150	242 ± 198	302 ± 274‡	238 ± 184	247 ± 196	238 ± 175	260 ± 233	238 ± 183	249 ± 200
Vitamin B_{12} (pg/mL)	647 ± 332	618 ± 260	644 ± 309	656 ± 320	556 ± 231	633 ± 299	632 ± 306	640 ± 306	606 ± 302	629 ± 328	641 ± 277
Folate (ng/mL)	9.8 ± 7.1	10.5 ± 7.7	10.7 ± 8.2	9.1 ± 6.0	9.5 ± 5.9	9.9 ± 7.4	10.3 ± 7.4	10.0 ± 7.6	10.4 ± 6.6	10.5 ± 7.8	9.8 ± 7.0

Abbreviation: CVD, cardiovascular disease.

*All forms of CVD.

†$P < .05$.

‡Macro is statistically different *v* none ($P < .005$); micro is statistically different *v* none ($P < .05$).

§$P < .06$ (marginally statistically significant).

Table 4. Logistic Regression Analysis for the Presence of Neuropathy and Albuminuria

Parameter	Variable	Odds Ratio	95% CI	P
Neuropathy (n = 319)*	Age (unit = 10 yr)	1.52	1.12-2.06	.0073
	Duration of diabetes (unit = 10 yr)	2.11	1.45-3.07	0001
	tHcy (unit = 4 μmol/L)	1.48	0.98-2.20	.0627
Albuminuria (n = 437)†	Age (unit = 10 yr)	1.34	1.04-1.72	.0216
	Glycosylated hemoglobin (unit = 1%)	1.15	1.08-1.22	.0001
	tHcy (unit = 4 μmol/L)	1.67	1.25-2.22	.0005

Abbreviation: CI, confidence interval.

*Excludes subjects in the lowest quartile of GFR and those with vitamin B_{12} or folate deficiency.

†Excludes only B_{12}- or folate-deficient subjects.

DISCUSSION

We have found that serum tHcy correlated with the presence of neuropathy and several measures of renal function, including creatinine clearance, serum creatinine, duration of hypertension, and the presence of macroalbuminuria in a large study of subjects with NIDDM. However, tHcy did not correlate with the presence of cardiovascular disease, including coronary artery disease, stroke, or peripheral vascular disease. It also did not correlate with the other risk factors for vascular disease such as serum lipids, smoking pack-years, glycosylated hemoglobin, age, or duration of diabetes for the whole group. tHcy did correlate inversely with vitamin B_{12} and folate levels, and correlated directly with two other vitamin B_{12}-related metabolites, MMA, and cystathionine.

There have been a number of previous studies of tHcy in NIDDM. In contrast to our findings, a Japanese study of NIDDM subjects[12] found that tHcy levels were higher in the patients with macroangiopathy, which included cerebral infarction, ischemic heart disease, and peripheral vascular disease. They also found that high levels of tHcy were associated with neuropathy, but not with retinopathy or nephropathy. However, there were major differences between this latter study and the current investigation. In the present cross-sectional study, the NIDDM subjects may have had silent vascular disease, as compared with the Japanese study, which compared nondiabetic controls without vascular disease to subjects with NIDDM. Also, the mean age of the subjects in the Japanese trial was 64 years, which is older than in the current study, and it is not clear if the subjects were consecutive or actually selected for severity of vascular disease.

The basal tHcy levels were not different between normal volunteers and subjects with both vascular disease and diabetes as compared with those with diabetes without vascular disease in another investigation of a mixture of subjects with IDDM and NIDDM.[13] This result is similar to the findings in the present investigation.

In a much younger IDDM population, investigators in Sweden[11] found that subjects with microalbuminuria (5 to 20 mg/L) did not have increased plasma tHcy levels; however, those with frank proteinuria (>200 mg/L) had an increase of almost 5 μmol/L. This finding in IDDM patients is similar to that observed in our NIDDM patients. Similarly, no relationship between retinopathy and tHcy was observed in their IDDM patients, which is also the case for our NIDDM patients. Another recent investigation of IDDM patients found higher albumin excretion rates in those with hyperhomocysteinemia and a higher prevalence of macroangiopathy.[35] The albumin excretion rate was found to be independently correlated with tHcy levels in a recent study of both IDDM and NIDDM, and macroangiopathy was not more prevalent in NIDDM subjects with elevated tHcy, consistent with our findings.[36] A similar relationship between microalbuminuria and tHcy was also found in a cohort of subjects from the general population, so this relationship may be important not only in diabetes.[37]

The relationship we found between tHcy and neuropathy is interesting. It is unlikely that this relationship was due to occult B_{12} deficiency with associated neuropathy symptoms, since serum MMA, the most sensitive measure of B_{12} status,[16] was not independently correlated with the presence of neuropathy. Also, the relationship still held between tHcy and neuropathy when subjects with vitamin B_{12} deficiency were removed from the analysis. The pathophysiology of diabetic neuropathy is an area of intensive investigation, and an important hypothesis is that impaired vascular supply in diabetic patients may have a deleterious effect on neuronal function.[38] There is no simple theory for the cause of the decreased neuronal perfusion, with investigations directed at an increase in vasoconstrictors, defective prostacyclin or nitric oxide synthesis or action, changes in polyol synthesis, abnormalities in the availability of glutathione, and abnormal glycation processes.[38] Elevated homocysteine is considered injurious to vascular endothelium and could contribute to microvascular pathology in NIDDM. The fact that tHcy was strongly correlated with serum levels of vitamin B_{12},

Table 5. Mean Levels of Serum Metabolites and Vitamins After Excluding Subjects With Vitamin B_{12} or Folate Deficiency

Parameter	tHcy >13.9 μmol/L			MMA >271 nmol/L		
	Yes (n = 21)	No (n = 416)	P	Yes (n = 20)	No (n = 417)	P
Serum vitamin B_{12} (pg/mL)	516 ± 109	648 ± 305	.0001	557 ± 199	646 ± 303	.0703
Serum folate (ng/mL)	6.6 ± 3.3	10.4 ± 7.5	.0001	8.1 ± 5.3	10.3 ± 7.5	NS
Serum MMA (nmol/L)	276 ± 224	129 ± 62	0067			
Serum tHcy (μmol/L)				14.0 ± 4.2	9.2 ± 2.3	.0001
Serum creatinine (mg/dL)	1.4 ± 0.4	1.1 ± 0.2	.0006	1.3 ± 0.4	1.1 ± 0.2	.0340
Creatinine clearance (mL/min)	65 ± 27	85 ± 25	0006	72 ± 27	84 ± 25	.0394

NOTE. B_{12} deficiency was defined as serum B_{12} <350 pg/mL and MMA >271 nmol/L. Folate deficiency was defined as serum folate <4 ng/mL and tHcy >13.9 μmol/L and MMA <271 nmol/L.

MMA, cystathionine, and folate in these subjects suggests that vitamin therapy may reduce tHcy levels. Whether this would be beneficial can only be answered by large prospective clinical trials. Combinations of vitamin B_{12}, folate, and pyridoxine would likely have the most efficacy in decreasing tHcy in this patient population.

The finding that tHcy is inversely correlated with creatinine clearance and directly correlated with serum creatinine and the presence of albuminuria confirms previous investigations.[10,11,20-25] tHcy correlated with the duration of diabetes and systolic BP, which also correlated with albuminuria.[26] Thus, tHcy seems to be a sensitive marker of renal damage in these patients with NIDDM. Two other metabolites, MMA and cystathionine, were also increased in subjects with the lowest GFR but were not independently correlated with the presence of overt albuminuria. The latter two metabolites have also been shown to increase in subjects with renal insufficiency, and renal excretion is responsible for at least 50% of the daily clearance.[30,32,34] In contrast, renal excretion is not a major route for elimination of tHcy,[21,39] and it is not known why tHcy levels increase in renal failure.

In the present cross-sectional study, we did not find a significant increase in tHcy levels in subjects with vascular disease. Although there is much enthusiasm for detecting elevated tHcy (either basal or post–methionine load) in subjects with vascular disease,[6] there have been a number of negative studies that failed to confirm a relationship between tHcy and vascular disease.[40-43] Some discrepancies in the results may depend on the folate nutritional status of the study population. The mean tHcy levels in the present investigation (9 to 10 μmol/L) are well below the mean levels of 11 to 16 μmol/L found in some studies.[4,6,24] These lower tHcy levels and the fact that only 1% of the patients met criteria for frank folate deficiency suggest that this population of American subjects with NIDDM may have more adequate folate nutrition than subjects in other studies. Another difference in this investigation is that subjects with moderate to severe renal insufficiency were not included, whereas in some of the previous positive studies of Hcy in vascular disease, such subjects may have been included.

We found that 2.2% of the study population met criteria for untreated vitamin B_{12} deficiency based on the combination of a low serum B_{12} level and an elevation of serum MMA. In addition to these subjects, there were others (4.8%) with elevated MMA and B_{12} levels in the frankly normal range, which may also represent tissue vitamin B_{12} deficiency either alone or combined with a mild degree of renal insufficiency. Serum MMA and tHcy levels were directly correlated, and the mean MMA level in subjects with elevated tHcy was more than twice the level in the rest of the subjects. Therefore, hyperhomocysteinemic subjects should not be routinely treated with folic acid alone without screening for vitamin B_{12} deficiency with simultaneous serum MMA and/or vitamin B_{12} levels. This is particularly important in a population with NIDDM, where the clinical distinction between B_{12}-deficient myelopathy or neuropathy and the neuropathy caused by diabetes may be difficult.

In summary, in this large study of 452 NIDDM patients, tHcy levels did not correlate with vascular disease or retinopathy, but there was a significant correlation with neuropathy and nephropathy. Decreased renal function and vitamin deficiency appeared to be the main contributors to the elevation in serum tHcy in this NIDDM population.

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The University of Colorado and Columbia University own a number of patents that deal with assays for MMA and homocysteine. The University of Colorado has established a company to perform these assays. This company, Metabolite Laboratories Inc, owns a number of patents that deal with reducing MMA and homocysteine levels using various oral combinations of cobalamin, folate, and vitamin B_6.

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