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Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus

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Background. The plasma concentrations of total homocysteine (tHcy) and total cysteine (tCys) are determined by intracellular metabolism and by renal plasma clearance, and we hypothesized that glomerular filtration is a major determinant of plasma tHcy and tCys. We studied the relationships between the glomerular filtration rate (GFR) and plasma tHcy and tCys in populations of diabetic patients with particularly wide ranges of GFR.

Methods. We measured GFR, urine albumin excretion rate (UAER), plasma tHcy, tCys, methionine, vitamin B_{12}, folate, C-peptide, and routine parameters in 50 insulin-dependent diabetes mellitus (IDDM) and 30 non–insulin-dependent diabetes mellitus (NIDDM) patients. All patients underwent intensive insulin treatment and had a serum creatinine concentration below 115 μmol/liter.

Results. Mean plasma tHcy in diabetic patients (0.1 μmol/liter) was lower than in normal persons (11.1 μmol/liter, $P = 0.0014$). Mean plasma tCys in diabetic patients (266.1 μmol/liter) was also lower than in normal persons (281.9 μmol/liter, $P = 0.0005$). Seventy-three percent of the diabetic patients had relative hyperfiltration. Plasma tHcy and tCys were closely and independently associated with GFR, serum folate, and serum B12. However, plasma tHcy was not independently associated with any of the 22 other variables tested, including age, serum creatinine concentration, UAER, total daily insulin dose, and glycemic control.

Conclusions. Glomerular filtration rate is an independent determinant of plasma tHcy and tCys concentrations, and GFR is rate limiting for renal clearance of both homocysteine and cysteine in diabetic patients without overt nephropathy. Declining GFR explains the age-related increase in plasma tHcy, and hyperfiltration explains the lower than normal mean plasma tHcy and tCys concentrations in populations of diabetic patients.

Key words: NIDDM, IDDM, glomerular filtration rate, clearance, age, hyperfiltration, folate, methyl cycle.

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An elevated plasma concentration of the amino acid homocysteine is associated with precocious atherosclerosis in patients with inherited homocystinuria, irrespectively of the site of the responsible enzyme deletion [1, 2]. Experimental studies have shown that high concentrations of homocysteine may cause vascular damage [3], and epidemiological studies published during the last 10 years show that an elevated total homocysteine (tHcy) concentration in plasma is associated with symptoms of cardiovascular disease (CVD) [4–19]. Thus, clinical, experimental, and epidemiological evidence all suggest a link between atherosclerosis and plasma tHcy. Diabetic patients have a twofold to sixfold higher prevalence of atherosclerosis than nondiabetic persons [20–22], but the relationship between plasma tHcy and this excess of atherosclerosis is unknown. In spite of their higher prevalence of CVD compared with nondiabetic persons, the mean plasma tHcy concentration is normal or low in insulin-dependent diabetes mellitus (IDDM) and non–insulin-dependent diabetes mellitus (NIDDM) patients [23–26]. The cause of this low plasma tHcy relative to the high prevalence of CVD in populations of diabetic patients is not known.

The plasma concentration of tHcy is determined by intracellular metabolism of homocysteine within the activated methyl cycle [27], by *trans*-sulfuration to cysteine [28], and by renal clearance [29]. Abnormally low levels of plasma tHcy might be an effect of abnormal homocysteine metabolism in the diabetic organism. However, the plasma concentration of tHcy in diabetic patients might also be an effect of abnormal renal clearance of homocysteine.

In normal kidneys, plasma amino acids are first filtered in glomeruli, then reabsorbed in tubuli, and finally degraded in renal parenchyma. Bostom et al showed that degradation in renal tissue following tubular reabsorption of homocysteine is a major fraction of total plasma tHcy clearance in the rat [30]. The authors suggested that the reduced metabolic capacity of kidney tissue might be

rate limiting for renal tHcy clearance and might cause the elevation of plasma tHcy seen in human end-stage renal disease [31, 32]. However, although a reduced metabolic capacity of kidney tissue may explain the elevated plasma tHcy concentrations in patients with end-stage nephropathy, reduced renal metabolic capacity is unlikely to explain the decreased or normal mean plasma tHcy concentrations of diabetic patients.

The glomerular filtration rate (GFR) of diabetic patients in unselected populations covers a wide range from diabetic hyperfiltration to the hypofiltration of diabetic nephropathy, and the mean GFR of each population depends on the degree and prevalence of these two conditions. If the GFR were rate limiting for plasma tHcy clearance, then prevalent diabetic hyperfiltration would be expected to cause reduced mean tHcy concentrations. Furthermore, plasma tHcy then might be correlated to GFR independently of age, homocysteine metabolism, and glycemic control.

We hypothesized that GFR is rate limiting for plasma tHcy clearance in persons without nephropathy, and we characterized the association between plasma tHcy and GFR in populations of IDDM and NIDDM patients. The patients were under intensive glycemic control. They had a wide range of GFR and had moderate microalbuminuria. Patients with serum creatinine above normal were excluded from the study.

METHODS

Patients

All patients attended the same diabetes referral clinic. They had either IDDM (defined by a fasting serum C-peptide below 0.24 nmol/liter) or NIDDM with secondary failure (defined by a fasting serum C-peptide equal to or above 0.24 nmol/liter and insulin requirements). Inclusion criteria were the need for insulin treatment and a willingness to undergo intensive insulin treatment intended to maintain HbA_{1c} below 6.0% for a minimum of two years.

All patients visited the diabetes clinic at least four times yearly. The insulin treatment of all patients was at least three daily injections of fast-acting insulin at mealtime and two daily injections of intermediate-acting insulin in the morning and late afternoon throughout the study period.

Two hundred and ten patients were referred for the control of insulin treatment during the two-year inclusion period, and 136 of these fulfilled the inclusion criteria. One hundred and nine patients completed the study. Twenty-nine of the 109 patients were excluded because of a serum creatinine concentration above 115 μmol/liter, limiting the study to 80 patients with clinically normal renal function (Table 1).

Plasma samples from 160 healthy men between the ages of 41 and 68 (median 54) and 169 healthy women between the ages of 41 and 69 (median 54) defined the normal ranges for tHcy and tCys.

Protocol

The following laboratory determinations were made at each visit to the clinic: fasting blood glucose, HbA_{1c}, serum total cholesterol, high-density lipoprotein cholesterol, triglycerides, and creatinine, timed overnight urinary albumin excretion rate (UAER), supine blood pressure, and body weight. Blood samples were drawn in the morning before meals and before insulin administration. Results were calculated as the mean of four different measurements within the last year of the observation period. All other tests were done once during the year.

Laboratory determinations

Plasma tHcy and total cysteine (tCys) were measured in one assay using an automated, high-performance liquid chromatography method as previously described [10, 33]. The intra-assay coefficient of variation is less than 3%. The normal ranges (mean ± 2 SD) were 6.1 to 19.9 μmol/liter for tHcy and 209.8 to 342.7 μmol/liter for tCys.

Plasma C-peptide was measured by a modification of the radioimmunoassay described by Heding [34]. Sensitivity of the assay was 0.02 nmol/liter. Intra-assay coefficient of variation was 5.8%, and interassay coefficient of variation was 8.1%. The normal range was 0.24 to 0.64 nmol/liter.

The following laboratory analyses were routine assays at the central clinical laboratory of the hospital, which participates in the Murex Quality Assessment Programs (Murex Biotech Ltd., Dartford, UK).

HbA_{1c} was assessed by high-performance liquid chromatography as described by Jeppsson et al [35]. The upper normal limit for the assay was 5.2%.

Serum vitamin B_{12} and folate were assayed simultaneously in a radioimmunoassay as described by Chen et al [36]. The normal range for serum vitamin B_{12} was 125 to 700 pmol/liter, and for serum folate, it was 5 to 40 nmol/liter.

Urinary albumin concentration was analyzed by an automated nephelometric enzyme-linked immunosorbent assay (Behringwerke AG, Marburg, Germany), which is described by Tuengler et al [37]. The UAER was calculated from an overnight timed urine collection done by the patient following written and oral instructions. Normal range was 4 to 15 μg/min.

The GFR was determined after a single ^{51}CR-EDTA injection as the clearance over a period of four hours and expressed as milliliters per minute per square meter of body surface area (ml/min/m^2). The normal range for

Table 1. Clinical characteristics of diabetic patients with serum creatinine below 115 μmol/liter

Variables	Mean	95% Confidence interval	Minimum	Maximum
Plasma total homocysteine *μmol/liter*	10.1	9.5–10.7	5.4	21.0
Plasma total methionine *μmol/liter*	24.5	22.8–26.2	10.3	47.3
Plasma total cysteine *μmol/liter*	266	257–275	193	380
Serum folate *nmol/liter*	20	18–22	7	55
Serum vitamin B_{12} *pmol/liter*	334	295–372	191	1361
Glomerular filtration rate *ml/min/1.73 m²*	105	99–111	47	165
Urinary albumin excretion rate *μg/min*	33	19–46	4	310
Serum creatinine *μmol/liter*	90	87–93	59	113
Systolic blood pressure *mm Hg*	144	140–148	112	188
Diastolic blood pressure *mm Hg*	82	80–84	58	100
Age *years*	51	47–55	23	85
Duration of diabetes *years*	17	14–20	2	64
HbA_{1c} %	6.3	6.0–6.5	4.4	10.2

20-year-old men and women is 85 to 135 ml/min/1.73 m^2, and for 80-year-old men and women, it is 45 to 95 ml/min/1.73 m^2 [38].

The sagittal abdominal diameter was measured on the recumbent patient as the perpendicular distance between the plane of support and the highest point of the abdomen. A specially constructed gallows was used for all measurements.

Skinfold thickness refers to the sum of the triceps, subscapular, and suprailliac skinfolds measured according to Reinken et al [39]. The skinfold thickness was measured by one observer using a Holtain caliper (Holtain Ltd., Crymych, UK).

Left ventricular hypertrophy was estimated electrocardiographically as the sum of amplitudes of S in lead V1 and of R in lead V5.

Data analysis

Normality of distribution was tested by the Shapiro and Wilks W statistic. For variables with normal distributions, the results were calculated as means with 95% confidence limits. For nonnormally distributed variables, results were calculated as medians with interquartile ranges. All *t*-tests were two tailed. A *P* value of less than 0.05 was defined as significant. A one-way analysis of covariance was used to adjust group means of plasma tHcy and tCys for covariates in stratification for intervals of GFR. A two-way analysis of variance was applied to decide the additive effect (interaction) of two variables on plasma tHcy.

Simple associations between variables were calculated as the Pearson coefficient of correlation (*r*). Two explorative multiple-regression models, stepwise regression, and all possible subsets regression were used to identify the combination of variables with the best predictive value [40]. The following variables or transformations of these were tested both in simple-regression analyses and in the multiple-regression models: age, duration of diabetes, body weight, abdominal sagittal diameter, sum of three skinfold thicknesses, number of cigarettes smoked per day, daily insulin dose, fasting blood sugar, HbA_{1c}, serum C-peptide, total cholesterol, high-density lipoprotein cholesterol, triglyceride, and uric acid, plasma fibrinogen, tHcy, and tCys, serum vitamin B_{12} and folate, UAER, GFR, serum creatinine, electrocardiogram-left ventricular hypertrophy, systolic blood pressure, and diastolic blood pressure.

RESULTS

Clinical characteristics

Clinical characteristics of the 50 IDDM patients and 30 NIDDM patients in the study are listed in Table 1.

The age range for IDDM patients was 22 to 79 years [mean 47, 95% confidence interval (CI), 42 to 52 years] and for NIDDM patients, it was 23 to 85 years (mean 58, 95% CI, 53 to 63 years, $P = 0.0029$). However, the two types of diabetic patients were not different with respect to mean HbA_{1c} ($P = 0.6450$) or total daily insulin dose ($P = 0.3899$).

Homocysteine. The mean tHcy plasma concentration in all diabetic patients was 10.1 μmol/liter (95% CI, 9.5 to 10.7 μmol/liter; Table 1), which was lower than the mean plasma tHcy concentration of 329 normal control persons, which was 11.1 (95% CI, 10.6 to 11.6 μmol/liter, $P = 0.0014$). The mean plasma tHcy concentration in IDDM patients was 9.8 (95% CI, 9.0 to 10.6 μmol/liter), and in NIDDM patients, it was 10.6 μmol/liter (95% CI, 9.6 to 11.5 μmol/liter, $P = 0.2189$). One patient (IDDM) had a plasma tHcy (23 μmol/liter) above the 95th percentile (17.0 μmol/liter) of the 329 normal control persons.

The mean tCys plasma concentration of all diabetic patients was 266 μmol/liter (95% CI, 257 to 275 μmol/liter; Table 1), and in 329 normal control persons, it was 282 μmol/liter (95% CI, 278 to 286 μmol/liter, $P = 0.0005$). The mean plasma tCys concentration in IDDM patients was 261 μmol/liter (95% CI, 250 to 272 μmol/liter), and in NIDDM patients, it was 275 μmol/liter (95% CI, 260 to 290 μmol/liter, $P = 0.1161$).

The mean methionine plasma concentration in IDDM

patients was 24.4 μmol/liter (95% CI, 22.1 to 26.8 μmol/liter), and in NIDDM patients, it was 24.6 μmol/liter (95% CI, 22.1 to 27.1 μmol/liter, $P = 0.9069$).

The mean folate serum concentration in IDDM patients was 21 (95% CI, 19 to 24 nmol/liter), and in NIDDM patients, it was 18 nmol/liter (95% CI, 15 to 20, $P = 0.0963$). The mean vitamin B_{12} serum concentration was 352 (95% CI, 296 to 409 pmol/liter), and in NIDDM patients, it was 303 pmol/liter (95% CI, 110 to 558 pmol/liter, $P = 0.2185$). All patients had folate and serum vitamin B_{12} concentrations above the lower normal limits.

Renal function. All patients had clinically normal renal function, defined as a serum creatinine concentration below 115 μmol/liter and a UAER below 310 μg/min.

The GFR was above 135 ml/min/1.73 m^2 (absolute hyperfiltration) in four IDDM patients and in four NIDDM patients. Thirty-five IDDM patients (70%) and 23 NIDDM patients (77%) had a GFR that was more than 100% of the mean value for nondiabetic persons of the same age and sex (relative hyperfiltration). The mean GFR for IDDM patients was 106 (95% CI, 100 to 111), and for NIDDM patients, it was 103 ml/min/1.73 m^2 (95% CI, 94 to 113 ml/min/1.73 m^2, $P = 0.6232$).

In the 58 diabetic patients who had a higher GFR than the normal mean value for their age and sex, the mean plasma tHcy concentration was 9.4 μmol/liter (95% CI, 8.8 to 10.0 μmol/liter). In the 22 patients with a lower GFR, the mean plasma tHcy concentration was 12.0 μmol/liter (95% CI, 10.7 to 13.3 μmol/liter, $P < 0.0001$).

The UAER distribution was significantly skewed in both IDDM and NIDDM patients with P values for Wilks statistic of less than 0.0001 in both types of diabetes. Seventeen IDDM patients (34%) and nine NIDDM patients (30%) had a UAER between 15 and 310 μg/min (microproteinuria). Median UAER was 8 μg/min in IDDM patients (range 4.3 to 220) and 9 μg/min in NIDDM patients (range 4.4 to 310, Mann–Whitney P value = 0.8180).

Fifty-four patients with a UAER below or equal to 15 μg/min had a mean plasma tHcy concentration of 9.7 μmol/liter (95% CI, 9.0 to 10.4). In the 14 patients with UAER between 15 and 51 μg/min, the mean plasma tHcy concentration was 9.8 μmol/liter (95% CI, 8.6 to 11.0).

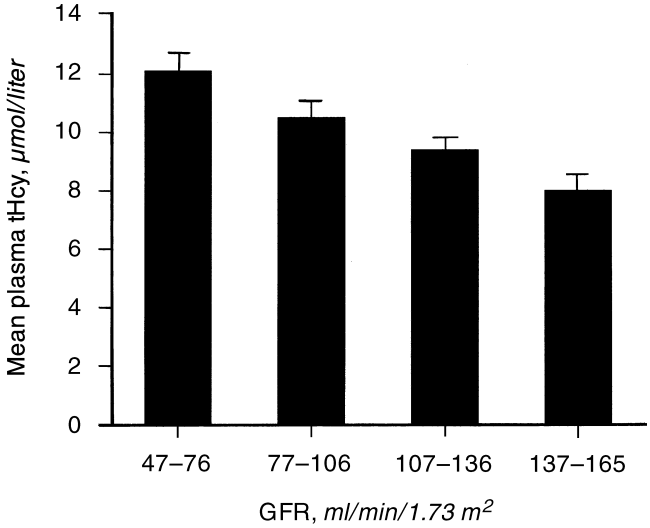

Fig. 1. Means and standard errors of the means for plasma total homocysteine (tHcy) concentration stratified by equal intervals of the glomerular filtration rate (GFR) in 80 diabetic patients without overt nephropathy. The mean plasma tHcy concentrations have been adjusted for serum folate and vitamin B_{12}. The P value for equality of the adjusted means < 0.0001. All differences between group means were significant except for the difference between the two lowest means of tHcy. The number of patients in the four intervals was 13, 27, 32, and 8, respectively.

Bivariate relationships

The plasma tHcy concentration was strongly correlated with GFR, as shown in Figures 1 and 2 and Table 2.

Mean plasma tHcy in 39 patients below age 50 was 9.12 μmol/liter (95% CI, 8.44 to 9.81 μmol/liter) and in 41 patients above age 50, it was 11.0 μmol/liter (95% CI, 10.1 to 11.9 μmol/liter, $P = 0.0016$). The mean plasma tHcy in 32 patients with GFR below 100 ml/min/1.73 m^2 was 11.3 μmol/liter (95% CI, 10.3 to 12.4 μmol/liter) and in 48 patients with GFR above 100 ml/min/1.73 m^2, it was 9.26 μmol/liter (95% CI, 8.61 to 9.91 μmol/liter, $P = 0.0007$).

The GFR was also inversely related with age (partial $r = -0.4977$, $P = 2.6^{-6}$), with serum creatinine (partial $r = -0.5017$, $P = 2.1^{-6}$), and with plasma tCys (partial $r = -0.5298$, $P = 4.3^{-7}$). However, GFR was not related with serum folate (partial $r = -0.0439$, $P = 0.6987$) or with plasma methionine (partial $r = 0.0655$, $P = 0.5638$). In the 58 patients with relative hyperfiltration, GFR remained inversely related to age (partial $r = -0.5297$, $P = 1.9^{-5}$), although other factors than age contributed to GFR in this group of hyperfiltrating diabetic patients.

Age was closely related with plasma tCys (partial $r = 0.6116$, $P = 1.7^{-9}$), but not with serum folate (partial $r = 0.1615$, $P = 0.7099$) or with plasma methionine (partial $r = 0.1615$, $P = 0.1524$).

Multivariate relationships

A one-way analysis of variance between plasma tHcy and GFR with serum folate and vitamin B_{12} as covariates is shown in Figure 1 ($P < 0.0001$). The mean plasma tCys for the same four intervals of GFR was also significantly different, except for the two groups with the highest GFR ($P < 0.0001$). A two-way analysis of variance for plasma tHcy with respect to age and GFR is presented in Table 3. The best possible subset of predictor variables selected from a pool of 25 relevant variables for the

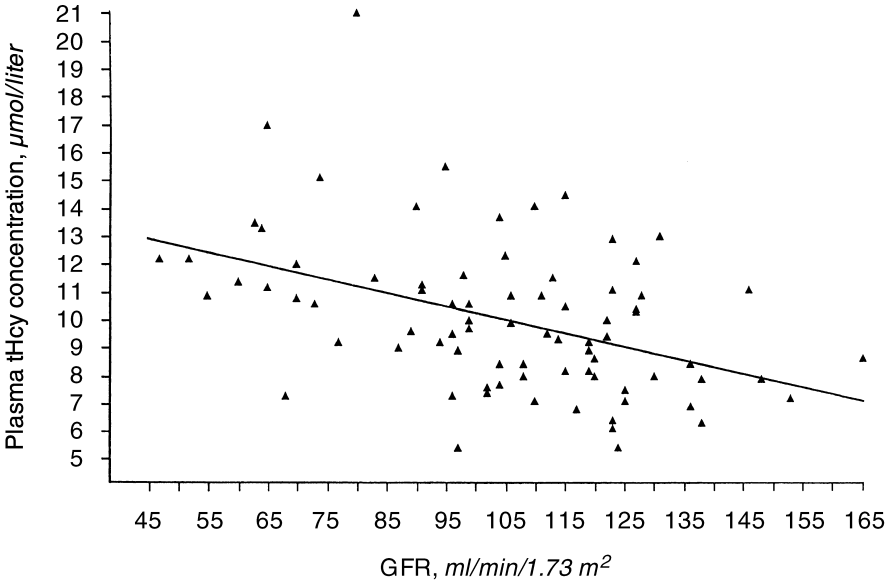

Fig. 2. Scatterplot and simple regression analysis of plasma homocysteine (tHcy) concentrations and glomerular filtration rates in 80 diabetic patients without overt nephropathy (r = 0.4351; $P < 0.0001$).

Table 2. Stratification of clinical characteristics by glomerular filtration rate (GFR) in eighty diabetic patients with serum creatinine below 115 μmol/liter

	GFR *ml/min/1.73 m²*			
Variables	47 to 76 $N = 13$	77 to 106 $N = 27$	107 to 136 $N = 32$	136 to 165 $N = 8$
Plasma total homocysteine *μmol/liter*	12.1 (10.7–13.5)	10.4 (9.3–11.7)	9.4 (8.6–10.3)	8.0 (6.8–9.3)
Plasma total methionine *μmol/liter*	23.5 (19.6–27.4)	23.4 (21.0–25.8)	26.3 (23.0–29.6)	22.5 (16.9–28.1)
Plasma total cysteine *μmol/liter*	304 (284–324)	280 (265–295)	245 (234–256)	243 (230–255)
Serum folate *nmol/liter*	21 (13–29)	20 (17–24)	19 (16–21)	22 (19–26)
Serum vitamin B_{12} *pmol/liter*	400 (211–588)	331 (266–396)	309 (268–351)	332 (259–404)
Urinary albumin excretion rate *μg/min*	81 (17–145)	35 (12–59)	15 (7–24)	14 (5–24)
Serum creatinine *μmol/liter*	99 (93–106)	93 (87–98)	87 (83–91)	79 (71–87)
Systolic blood pressure *mm Hg*	150 (143–158)	150 (142–157)	137 (130–143)	145 (129–161)
Diastolic blood pressure *mm Hg*	80 (74–85)	85 (82–89)	79 (77–82)	84 (76–92)
Age *years*	69 (63–74)	54 (48–60)	44 (39–49)	43 (35–51)
Duration of diabetes *years*	24 (13–34)	20 (15–25)	13 (10–17)	13 (7–19)
HbA_{1c} %	6.2 (5.7–6.7)	6.5 (5.9–7.0)	6.1 (5.8–6.5)	6.4 (5.7–7.1)

Numbers are means and 95% confidence limits.

plasma tHcy concentration of 80 diabetic patients is shown in Table 4. Serum folate and serum vitamin B_{12} contributed to the model independently of each other with tolerance values of 0.8416 and 0.8111, respectively. Most of the predictive effects of age and serum creatinine were contributed to the model through collinearity with plasma tCys and GFR. With plasma tCys and GFR in the model, no further prediction was contributed either by age or serum creatinine alone or in combination. Therefore, GFR contributed more to prediction of variations in plasma tHcy than contributions by age and serum creatinine, and GFR entered the model independently of plasma tCys, serum folate, and serum vitamin B_{12}. The model was underspecified, with an adjusted R^2 of 0.4444, and other contributing variables than those tested in this study remain unidentified.

DISCUSSION

We have shown that GFR is a strong determinant of the plasma tHcy and tCys concentrations, and that diabetic patients with relative hyperfiltration have lower than normal plasma tHcy and tCys. GFR determines the plasma tHcy and tCys concentrations independently of age, serum vitamins, serum creatinine, and UAER. No combinations of these variables fully explain the relationships between GFR and plasma tHcy and tCys.

The GFR was closely and independently associated with plasma tHcy and tCys. The associations were found over the entire range of GFR, showing that a dose–response relationship exists between GFR and plasma tHcy and tCys. Our findings agree with observations of plasma tHcy and tCys in nondiabetic patients published by Arnadottir et al [29]. Homocysteine is ultrafiltrated

Table 3. Two-way analysis of variance of plasma total homocysteine (μmol/liter) for two levels of GFR (ml/min/1.73 m^2) and two levels of age

Age	GFR < 100			GFR > 100		
years	*N*	Mean	95% CI	*N*	Mean	95% CI
<51	7	10.7	9.0–12.4	33	8.8	8.1–9.6
≥51	25	11.5	10.2–12.9	15	10.2	8.8–11.5

P value for GFR effect is 0.0183; *P* value for age effect, 0.0920; and *P* value for interaction effect, 0.6463.

Table 4. Multiple regression analysis with plasma total homocysteine as dependent variable in eighty diabetic patients with serum creatinine below 115 μmol/liter

Independent variables	Partial *r*	*P* value
In model		
Plasma total cysteine	0.4843	0.000005
Serum folate	−0.4577	0.00002
Serum B_{12} vitamin	−0.2519	0.0261
Glomerular filtration rate	−0.2495	0.0286
Not in model		
Serum creatinine	0.0954	0.4125
Age	−0.0099	0.9320

Multiple $R^2 = 0.4686$

in glomeruli, almost completely absorbed in tubuli, and degraded in kidney tissue by *trans*methylation and *trans*-sulfuration in the activated methyl cycle [27]. van Guldener et al recently found that humans with normal kidneys return as much homocysteine to the circulation as is ultrafiltrated in glomeruli [41]. Bostom et al have shown that metabolism in kidney tissue accounts for a major fraction of total renal clearance of plasma homocysteine [30], and suggested that loss of capacity for homocysteine degradation might explain the increase in plasma tHcy seen in end-stage renal disease. With this background, we interpret the close relationship between GFR and the plasma concentration of tHcy in our patients to show that normal kidney tissue compensates for changes in ultrafiltration by regulating the degradation of homocysteine, thus keeping the return of homocysteine to the circulation constant. When GFR and ultrafiltration and tubular absorption of homocysteine are reduced, *trans*-methylation and *trans*-sulfuration are also reduced, and an unchanged amount of homocysteine is returned to the circulation. When GFR is increased, *trans*methylation and *trans*-sulfuration are increased to keep the return of homocysteine to the circulation constant. These compensatory mechanisms in the autoregulated, activated methyl cycle explain the association between GFR and plasma tHcy and tCys in patients with normal renal tissue function. The associations of plasma tHcy and tCys with GFR show that GFR is rate limiting for kidney clearance of plasma homocysteine and cysteine in patients with intact renal parenchyma. Thus, age-dependent reduction of GFR causes increased plasma tHcy, and diabetic hyperfiltration causes decreased plasma tHcy concentration.

Serum folate was negatively associated with plasma tHcy. This association is well known in nondiabetic persons [17, 32, 42] and reflects homocysteine remethylation to methionine in the activated methyl cycle [27]. Plasma tCys was positively related to both serum folate and plasma tHcy, a finding that fully supports the concept of coordinate remethylation and *trans*-sulfuration in the metabolism of homocysteine, first proposed by Selhub and Miller [28]. The strong, positive correlation between tHcy and tCys was independent of GFR, age, serum folate, and plasma methionine. Thus, the rate of homocysteine *trans*-sulfuration and the rate of glomerular filtration were the two major, independent determinants of the plasma concentration of tHcy.

Aging reduces GFR, and in our study, the association of plasma tHcy with age was entirely caused by this effect of age on GFR, because adjustment for GFR eliminated the association between tHcy and age. Thus, declining GFR may explain the age-related increase in plasma tHcy found in both diabetic patients and nondiabetic persons.

The mean plasma tHcy and tCys concentrations in diabetic patients were lower than in our population of normal control persons with the same age range and sex ratio. Despite the exclusion of patients with serum creatinine concentrations above normal, 33% of our diabetic patients, nevertheless, had microalbuminuria. However, microalbuminuria had no effect on the relationship between GFR and plasma tHcy and tCys, a finding that agrees with that of Agardh et al in IDDM patients [23]. Diabetic hyperfiltration was found in 10% of our patients, and 72% had a GFR above the normal mean for their age and sex. Robillon et al also found reduced plasma tHcy in IDDM patients without overt nephropathy [24], whereas Araki et al [25] and Munshi et al [26] found normal plasma tHcy, and Hofman et al [43] found elevated tHcy in studies of diabetic populations that included patients with nephropathy. In populations of diabetic patients, including patients with nephropathy, either renal tissue damage or reduced GFR or both may cause the mean plasma tHcy to rise to normal or above normal concentrations, depending on the prevalence of nephropathy. We consider the low mean plasma tHcy and tCys concentrations of diabetic patients without nephropathy to be an effect of their relative hyperfiltration.

The strong influence of renal clearance on the plasma concentrations of tHcy and tCys implies that associations between plasma tHcy and tCys and other age-related variables must be adjusted for renal function. Serum creatinine has often been used to adjust for this effect of renal function on the relationship between plasma tHcy and age-related CVD [44, 45]. However, the close association

found in our study between serum creatinine and GFR is unique to diabetic patients with hyperfiltration and, thus, was entirely due to the subpopulation of patients with GFR above 105 ml/min/1.73 m^2. In nondiabetic persons without nephropathy, serum creatinine is weakly associated with GFR, particularly in older age, when GFR declines and serum creatinine remains unchanged. Associations between plasma tHcy and CVD must therefore be adjusted for the effect of renal function by GFR rather than by the serum creatinine concentration.

We conclude that GFR is rate limiting for renal clearance of homocysteine and cysteine. GFR is an independent determinant of the plasma concentrations of tHcy and tCys. Relative hyperfiltration is the cause of the lower than normal mean plasma tHcy and tCys concentrations in populations of diabetic patients, and declining GFR causes increasing plasma tHcy as age advances. These findings imply that the reduced mean plasma tHcy level in diabetic patients is not the result of metabolic abnormalities. Other factors than plasma tHcy cause the increased prevalence of atherosclerosis in populations of diabetic patients.

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