The Relationship Among Homocysteine, Creatinine Clearance, and Albuminuria in Patients With Type 2 Diabetes

LINDA DAVIES, BASC[1]
ERROL G. WILMSHURST, MBBS, MRACP, MD, FRACP[1]
AIDAN MCELDUFF, MBBS, PHD, FRACP[1]
JENNY GUNTON, MBBS, FRACP[1]
PHILLIP CLIFTON-BLIGH, BSC, MBBS, FRACP[1]
GREGORY R. FULCHER, MBBS, MD, FRACP[1]

OBJECTIVE — Although it is accepted that elevated plasma homocysteine (tHcy) levels occur in end-stage renal disease and type 2 diabetes, the changes with milder renal dysfunction (e.g., microalbuminuria) are less clearly established. This study explores the relationship among tHcy, creatinine clearance (Ccr), and albumin excretion rate (AER) in a population with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 260 patients with type 2 diabetes were screened in our outpatient clinic during 10 months. Fasting blood samples were collected, and AER was calculated from an overnight timed urine sample. Ccr was calculated using the Cockroft-Gault formula.

RESULTS — A total of 198 subjects (76%) had normoalbuminuria (<20 μg/min), 50 subjects (19%) had microalbuminuria (20–200 μg/min), and 12 subjects (5%) had macroalbuminuria (≥200 μg/min). Those with microalbuminuria had higher levels of tHcy than those with normoalbuminuria (13.2 ± 7.8 vs. 11.3 ± 4.6 μmol/l, $P < 0.05$). Patients were then subdivided based on low Ccr (<80 ml · min^{-1} · 1.73 m^{-2}) and normal Ccr (≥80 · min^{-1} · 1.73 m^{-2}). None of the patients with macroalbuminuria had normal Ccr. In those with normoalbuminuria, tHcy levels were higher than in those with low Ccr than in those with normal Ccr (12.0 ± 4.6 vs. 10.0 ± 4.4 μmol/l, $P < 0.01$). The same was found for those with microalbuminuria (low Ccr versus normal Ccr: 14.6 ± 9.0 vs. 10.2 ± 2.8 μmol/l, $P < 0.02$). For normal Ccr, tHcy was similar irrespective of AER (normoalbuminuria versus microalbuminuria: 10.0 ± 4.4 vs. 10.2 ± 2.8 μmol/l, NS). For low Ccr, tHcy was higher in those with microalbuminuria versus normoalbuminuria (14.6 ± 9.0 vs. 12.0 ± 4.6 μmol/l, $P = 0.01$). Using multivariate regression, Ccr, but neither AER nor the presence of albuminuria, was an independent predictor of tHcy.

CONCLUSIONS — These data strongly suggest that in patients with type 2 diabetes, the relationship between plasma tHcy and AER is largely due to associated changes in renal function, as defined by Ccr.

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Increased plasma levels of homocysteine (tHcy) were first associated with the presence of arterial disease in the 1960s, when extensive atherosclerosis was described postmortem in individuals with homocysteinuria (1). Since then, elevated tHcy has been associated with atherogenesis in normal subjects (2,3) and in patients with type 2 diabetes (4–6). In both these populations, hyperhomocysteinemia has been established as an independent risk factor for atherothrombotic disease.

It has also been clearly documented that increased levels of homocysteine occur in association with marked degrees of renal dysfunction (7,8). More recently, the association of tHcy and milder renal dysfunction, as defined by albuminuria, has been studied (9–11). For example, in the Hoorn Study (a population-based survey of glucose tolerance and cardiovascular risk factors) (9), higher levels of tHcy were associated with microalbuminuria independent of other determinants, including the presence of type 2 diabetes and serum creatinine (Scr). Similarly, Lanfredini et al. (10) found increased levels of tHcy in patients with type 2 diabetes and microalbuminuria but not in patients with type 1 diabetes who did not have overt nephropathy.

A strong relationship between creatinine clearance (Ccr) and tHcy has been demonstrated by some investigators, both in patients with renal disease and in those with diabetes (12–14). Therefore, when Ccr is considered, tHcy may not be independently associated with albumin excretion rate (AER) (15). Overall, the relationship between AER and tHcy concentrations independent of Ccr remains controversial. For this reason, we performed a large cross-sectional study of patients with type 2 diabetes to further examine these associations.

RESEARCH DESIGN AND METHODS

— A total of 260 patients with type 2 diabetes (145 men and 115 women), aged 65 ± 12 years, with a known duration of diabetes of 11 ± 8 years, were consecutively screened in our clinic. All patients were examined clinically by an endocrinologist. Fasting blood samples were collected and AER was calculated as described previously (16) from a single overnight timed urine collection performed by the patient following written and oral instructions. The accuracy of the collection was determined

From the [1]Department of Diabetes, Endocrinology, and Metabolism, Royal North Shore Hospital, St. Leonards, Australia.

Address correspondence and reprint requests to Greg Fulcher, Department of Diabetes, Endocrinology, and Metabolism, Level 3 Main Block, Royal North Shore Hospital, Pacific Highway, St. Leonards 2065, Australia. E-mail: gfulcher@med.usyd.edu.au.

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Abbreviations: AER, albumin excretion rate; Ccr, creatinine clearance; Scr, serum creatinine; tHcy, homocysteine.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of the study population*

	All patients (n = 260)	tHcy ≤15 mmol/l (n = 212: 81.5%)	tHcy >15 mmol/l (n = 48: 18.5%)	P (normal versus high tHcy)
Male/female	145/115	116/96	29/19	NS
Age (years)	66 ± 11	65 ± 12	68 ± 11	0.055
Duration (years)	11 ± 8	10 ± 8	15 ± 9	<0.001
HbA_{1c} (%)	6.9 ± 1.5	6.9 ± 1.5	6.9 ± 1.4	NS
Plasma tHcy (μmol/l)	10.5 (2.5–58.1)	9.8 (2.5–14.9)	20.2 (15.3–58.1)	<0.001
Total cholesterol (mmol/l)	5.19 ± 1.03	5.21 ± 1.03	5.11 ± 1.02	NS
HDL cholesterol (mmol/l)	1.29 ± 0.37	1.29 ± 0.37	1.30 ± 0.38	NS
Triglycerides (mmol/l)	1.78 ± 1.08	1.81 ± 1.11	1.66 ± 0.95	NS
Scr (mmol/l)	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	<0.001
AER (μg/min)	6 (1–1,339)	6 (1–744)	9 (2–1,339)	NS
Ccr (calculated ml · min^{-1} · 1.73 m^{-2})	69.4 (26.9–167.2)	72.1 (34.4–167.2)	56.3 (26.9–104.4)	<0.001
BMI (kg/m^2)	29 ± 6	29 ± 6	29 ± 5	NS
Systolic blood pressure (mmHg)	140 ± 18	140 ± 18	140 ± 15	NS
Diastolic blood pressure (mmHg)	77 ± 10	78 ± 10	74 ± 10	0.025
Treatment (%): diet / OHA / insulin / OHA + insulin	16/55/22/4	19/56/21/4	7/56/30/7	NS
Antihypertensive medication (% treated)	59.0	59.6	58.3	NS
Angiotensin-converting enzyme inhibitor (%)	31.3	30.8	33.3	NS
Lipid-lowering therapy (%)	41	41.1	41.7	NS
Eye complications (%)	17	15.1	27.3	0.054
Ischemic heart disease (%)	21	19.1	27.7	NS
Cerebrovascular disease (%)	9.4	8.7	12.5	NS
Peripherovascular disease (%)	32	29.8	40.9	NS
Neuropathy (%)	54	52.2	60.0	NS

Data are n, mean (range), or %. OHA, oral hypoglycemic agent.

by direct questioning. Patients were divided according to AER as follows: normoalbuminuria (<20 μg/min), microalbuminuria (20–200 μg/min), or macroalbuminuria (≥200 μg/min).

Ccr (ml · min^{-1} · 1.73 m^{-2}) was calculated according to the Cockcroft and Gault formula (17):

$$\frac{SF \times [140 - \text{age (years)}] \times [\text{weight (kg)} \times 1.73]}{1{,}000 \times \text{Scr} \times \text{BSA}}$$

where SF is 1.23 for men and 1.05 for women; Scr (mmol/l); and body surface area (BSA) = 0.0007184 × height (cm) (0.725) × weight (kg) (0.425).

Homocysteine was measured by high-performance liquid chromatography using fluorescence detection (Bio-Rad, Richmond, CA); the normal range in our laboratory was 5–15 μmol/l.

Statistical analysis

Statistical analyses were performed using SPSS for Windows software (version 9; SPSS, Chicago). Spearman' s rank correlation coefficient was used for univariate analyses. Backward linear regression (continuous dependent variables) or logistic regression (categorical dependent variables) were used for multivariate analyses. Comparisons between groups were made using analysis of variance with the least-significant-differences method for post-hoc analysis. Values not normally distributed were log-transformed before all analyses. Results are mean ± SD or median (range).

RESULTS

— The study population characteristics in total (n = 260) and subdivided by tHcy are presented in Table 1. Patients with an elevated tHcy level tended to be older (68 ± 11 vs. 65 ± 12 years, P = 0.055) and had a longer known duration of diabetes (15 ± 9 vs. 10 ± 8 years, P < 0.001), a higher serum creatinine level (0.11 ± 0.03 vs. 0.09 ± 0.02 mmol/l, P < 0.001), and a lower Ccr (56.3 [26.9–104.4] vs. 72.1 [34.4–167.2] ml · min^{-1} · 1.73 m^{-2}, P < 0.001). AER was similar in both groups (9 [2–1,339] vs. 6 [1–744] μg/min, NS), but there was a trend for patients with increased tHcy to have an increased prevalence of retinopathy (P = 0.055).

The data were subdivided according to AER into normoalbuminuria, microalbuminuria, and macroalbuminuria. Those with microalbuminuria tended to have higher tHcy levels than those with normoalbuminuria (13.2 ± 7.8 vs. 11.3 ± 4.6 μmol/l, P = 0.051) but levels similar to those with macroalbuminuria (13.2 ± 7.8 vs. 13.3 ± 5.9 μmol/l, NS) (Fig. 1).

To explore these relationships further, these groups were subdivided based on low Ccr (<80 ml · min^{-1} · 1.73 m^{-2}) or normal Ccr (≥80 ml · min^{-1} · 1.73 m^{-2}). No patients with macroalbuminuria had a normal Ccr. In patients with

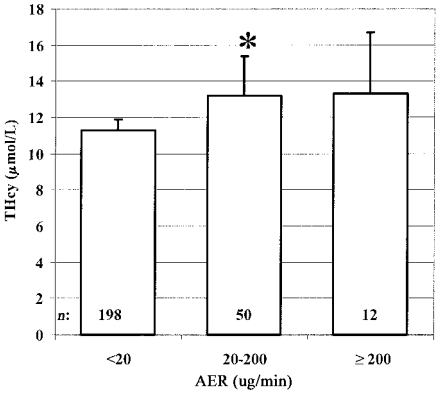

Figure 1—*Relationship between tHcy and AER.* *P < 0.05 (least significant difference; analysis of variance, P = 0.051).

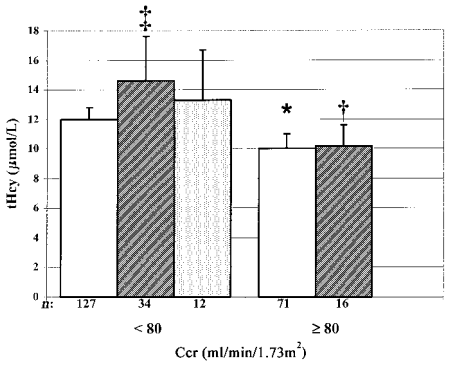

Figure 2—*Relationship between tHcy and AER subdivided according to Ccr.* □, *AER <20 μg/min;* ▨, *AER 20–200 μg/min;* ▩, *AER >200 μg/min.* **P* < 0.05 vs. Ccr <80 ml · min^{-1} · 1.73m^2; †*P* < 0.05 vs. Ccr <80 ml · min^{-1} · 1.73m^2; ‡*P* < 0.05 vs. AER <20 μg/min (same Ccr).

normoalbuminuria, those with low Ccr had higher levels of tHcy than those with normal Ccr (12.0 ± 4.6 vs. 10.0 ± 4.4 μmol/l, $P < 0.01$). Similarly, in patients with microalbuminuria, plasma tHcy levels were higher in those with low Ccr than in those with normal Ccr (14.6 ± 9.0 vs. 10.2 ± 2.8 μmol/l, $P < 0.02$). Plasma tHcy level was similar in patients with normal Ccr, irrespective of AER (normoalbuminuria versus microalbuminuria 10.0 ± 4.4 vs. 10.2 ± 2.8 μmol/l, NS). In the group with low Ccr, tHcy was higher in subjects with microalbuminuria than in those with normoalbuminuria (14.6 ± 9.0 vs. 12.0 ± 4.6 μmol/l, $P = 0.01$) (Fig. 2).

Because of the established association between tHcy and serum levels of B_{12} and folate, the serum levels of B_{12} and folate were compared between all groups. Patients with high tHcy had lower (although normal) levels of B_{12} than those with normal tHcy levels (244 [113–581] vs. 283 [119–852] pmol/l, $P < 0.01$). Folate levels were the same in both groups. Levels of B_{12} and folate were compared between groups of patients with normoalbuminuria, microalbuminuria, and macroalbuminuria and between groups with low and normal glomerular filtration rate. No differences were observed (data not shown).

By univariate analysis, tHcy was strongly correlated with AER (Spearman's coefficient, $P = 0.21$, 0.001), Ccr (−0.40, <0.001), age (0.28, <0.001), known duration of diabetes (0.24, <0.001), Scr (0.49, <0.001), and serum urea (0.36, <0.001), as well as diastolic blood pressure (−0.15, 0.02) and total cholesterol (−0.15, 0.02). However, by multivariate regression with tHcy as the dependent variable, the strongest predictor (negative) of tHcy was Ccr ($P < 0.001$) (Table 2). The presence of albuminuria (as a categorical variable) did not independently predict tHcy, nor did AER (as a continuous variable) when entered into separate analyses.

Table 2—*Multivariate regression analysis with tHcy as the dependent variable (Model $R^2 = 0.38317$)*

Independent predictors	β	*P*
Use of oral contraceptives	10.856	<0.01
Sex	1.744	0.005
Use of metformin	1.459	<0.02
Total cholesterol	−0.656	0.02
BMI	0.225	0.001
Ccr	−0.114	<0.001
Duration	0.088	<0.05

Conversely, when the analyses were performed with AER as the dependent variable, Ccr remained a predictor independent of age, sex, or use of antihypertensive medication (Table 3). Plasma tHcy level did not independently predict either AER or the presence of albuminuria.

CONCLUSIONS — This study is one of the largest to examine the relationship between tHcy and albuminuria in patients with type 2 diabetes and one of the few to relate this association to Ccr. In contrast to published data (9–11), we could not confirm that plasma tHcy was a predictor of the presence of albuminuria or of AER or that either AER or the presence of albuminuria were predictors of tHcy, independent of changes in Ccr.

Of the 260 patients with type 2 diabetes that we screened, 50 (19%) had microalbuminuria, 12 (5%) had macroalbuminuria, and 198 (76%) had normoalbuminuria. Of these, 173 (67%) had evidence of impaired renal function when defined as Ccr <80 ml · min^{-1} · 1.73 m^{-2}, including all patients with macroalbuminuria ($n = 12$), 64% of patients with normoalbuminuria, and 68% of patients with microalbuminuria. Interestingly, these data are very similar to those of the Hoorn Study (9), in which 64% of those with normoalbuminuria and 65% of those with microalbuminuria had renal impairment using the same criteria. This reinforces the concept that for patients with type 2 diabetes, factors other than the development of diabetic nephropathy are important determinants of renal function.

Our finding of a univariate correlation between plasma tHcy levels and AER is in agreement with data published by Lanfredini et al. (10) from a small cohort of patients with type 2 diabetes. In their study, patients with type 2 diabetes had fasting and postmethionine load tHcy levels similar to those in control subjects, despite the correlation with AER. They found no differences between fasting tHcy in patients with microalbuminuria or normoalbuminuria ($P = 0.08$), although postload tHcy was higher in subjects with microalbuminuria ($P = 0.02$) and in subjects with a range of diabetic complications compared with those without ($P = 0.003$). This study differed from ours in a number of ways. Their numbers were small ($n = 33$), and subjects with hypertension or overt renal dysfunction were excluded. Furthermore, Ccr was not included in the analyses. This raises the very real possibility that differences between the groups with respect to Ccr could have confounded the results. Using

Table 3—*Linear regression with log-transformed AER as the dependent variable (Model $R^2 = 0.32667$)*

Independent predictors	β	*P*
Antihypertensive medication	0.869	0.0001
Sex	−0.773	0.0001
Age	−0.420	<0.005
Existing peripheral vascular disease	0.397	0.0505
Use of angiotensin-converting enzyme	−0.384	0.0926
HbA_{1c}	0.301	<0.0001
BMI	0.068	0.001
Ccr	−0.028	0.0001
Pack-years of smoking	0.007	<0.05

baseline data from the ABCD trial, Stabler et al. (18) found that tHcy was positively correlated with Scr and inversely correlated with Ccr and that tHcy was significantly increased in patients with type 2 diabetes and nephropathy but not retinopathy. These findings are similar to those of Hultberg et al. (19) in patients with type 1 diabetes. Conversely, Chico et al. (11) found that AER had the strongest independent association with tHcy. Interestingly, in that study, patients with type 2 diabetes and nephropathy had the highest tHcy concentrations. When groups of patients with varying degrees of renal impairment were compared, it was only in those with Scr >120 umol/l that tHcy was elevated. Using multiple regression analysis, AER, age, and Scr were the independent predictors of tHcy, although it is relevant that Scr (and not Ccr) was included in the model. The omission of Ccr from the analysis thus leaves unanswered the question of the relative importance of AER versus renal function as independent predictors of tHcy. This issue is further addressed in a recent article discussing these results (15). When we included Ccr in our regression model, AER failed to predict plasma tHcy levels consistent with other published data for groups with either type 1 or type 2 diabetes (4,18,19). Furthermore, entering albuminuria as a categorical variable instead of AER did not affect the outcome. These data support the proposition that Ccr is more important as a predictor of tHcy than the presence of albuminuria per se or the AER.

To examine these issues further, we subdivided patients into groups based on both AER and Ccr using the lower limit of normal for Ccr ($80\ ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) as the cut point. In patients with normoalbuminuria, low Ccr was associated with increased tHcy levels. This is consistent with the data of Wollesen et al. (13), who found that the glomerular filtration rate was both an independent determinant of plasma tHcy and rate limiting for the renal clearance of homocysteine in patients with diabetes. Interestingly, in our patients with low Ccr, tHcy levels were higher in those with microalbuminuria than in those with normoalbuminuria ($P = 0.01$). In fact, the presence of a low Ccr was associated with 43% higher tHcy levels in those with microalbuminuria and 20% higher levels in those with normoalbuminuria. It is possible that the increase of homocysteine levels in patients with low Ccr could lead to endothelial dysfunction and, therefore, microalbuminuria in some susceptible individuals. The present study does not allow us to determine whether this hypothesis is correct or which patient characteristics, if any, may underlie these effects.

Although our data are broadly consistent with the published literature, they are somewhat at odds with the results of the Hoorn Study (9), a large population-based cross-sectional study of glucose tolerance and cardiovascular risk factors. This study did not attempt to address the issue of factors that predicted tHcy levels but, conversely, sought to determine whether tHcy predicted an increased risk of microalbuminuria, independent of classical risk factors. A 5-μmol/l increment of tHcy was associated with a 30% increased risk of microalbuminuria. We were unable to confirm these results. In our study, Ccr, HbA_{1c}, sex, use of antihypertensive medications, and BMI were the most significant predictors of AER (Table 3). Additionally, tHcy did not predict the presence of albuminuria. The explanation for the differences in these data are not clear but may lie in the fact that most participants in the Hoorn Study did not have diabetes.

In conclusion, our results make a number of relevant points concerning the relationship between Ccr, AER, and tHcy. First, tHcy is strongly correlated with AER, Scr, and Ccr in patients with type 2 diabetes. Second, homocysteine levels are unaffected by the presence of microalbuminuria when renal function is normal (Ccr $\geq 80\ ml \cdot min^{-1} \cdot 1.73\ m^{-2}$). Third, patients with Ccr $< 80\ ml \cdot min^{-1} \cdot 1.73\ m^{-2}$ have higher tHcy levels than those with normal Ccr, and this is particularly true in the patients with microalbuminuria. Fourth, in multivariate analyses, Ccr, but neither AER nor the presence of albuminuria, is an independent predictor of tHcy.

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