



Proteinuria and plasma total homocysteine levels in chronic renal disease patients with a normal range serum creatinine: critical impact of true glomerular filtration rate

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Abstract

Conflicting data have been reported concerning the independent association between proteinuria and plasma total homocysteine (*t*Hcy) levels, particularly among chronic renal disease (CRD) patients with a normal range serum creatinine. Studies of this potential relationship have been limited by failure to assess true GFR, failure to assess proteinuria in a quantitative manner, or arbitrary restriction of the range of proteinuria examined. We examined the potential independent relationship between plasma *t*Hcy levels and a wide range of quantitatively determined proteinuria (i.e., 0.000–8.340 g/day), among 109 CRD patients with a normal range serum creatinine (range; 0.8–1.5 mg/dl; median = 1.2 mg/dl). Glomerular filtration rate (GFR) was directly assessed by iohexol clearance, and plasma status of folate, pyridoxal 5'-phosphate, and B12, along with serum albumin, were also determined. Linear modeling with ANCOVA revealed that proteinuria was not independently associated with *t*Hcy levels (partial $R = 0.127$; $P = 0.201$), after adjustment for potential confounding by GFR (partial $R = 0.408$; $P < 0.001$), age, sex, plasma B-vitamin status, and serum albumin. Moreover, descending across quartiles (Q) [from Q4 to Q1] of GFR, ANCOVA-adjusted (i.e., for age, sex, and folate status) geometric mean *t*Hcy levels ($\mu\text{mol/l}$) were significantly increased: *t*Hcy Q4 GFR = 9.6; *t*Hcy Q3 GFR = 10.5; *t*Hcy Q2 GFR = 11.9; *t*Hcy Q4 GFR = 14.5; $P < 0.001$ for overall Q difference. We conclude that across a broad spectrum of quantitatively determined proteinuria, after adjustment for true GFR, in particular, there is no independent relationship between proteinuria and *t*Hcy levels among CRD patients with a normal range serum creatinine. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Hyperhomocysteinemia; Renal function

1. Introduction

Proteinuria [1–3], across the spectrum from microalbuminuria [1] to frank nephrosis [3] is associated with an increased risk for the development of arteriosclerotic

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cardiovascular disease outcomes. Recently, conflicting data [4–8] have been reported concerning the independent association between proteinuria and mildly elevated total (*t*) plasma concentrations of the putatively atherothrombotic [9] sulfur amino acid homocysteine (Hcy), particularly among patients with a normal range serum creatinine. The prior investigations [4–8] of this potential association have all been limited by one or more of the following: (i) failure to assess true glomerular filtration rate (GFR) [5–7], which is a major independent determinant of plasma *t*Hcy levels [4,8]; (ii) failure to assess proteinuria in a quantitative manner [6]; or (iii) arbitrary restriction of the range of proteinuria examined [4,6,8].

Accordingly, we evaluated the potential independent relationship between plasma *t*Hcy levels and a wide range of quantitatively determined proteinuria among 109 patients with chronic renal disease (CRD). All the subjects studied had a direct GFR determination, and a serum creatinine of 1.5 mg/dl or less.

2. Methods

A detailed description of the CRD cohort examined for this report has been provided elsewhere [10]. In brief, patients were recruited during 1997 from eight nephrology departments in Germany, Austria and South Tyrol with nearly two thirds of the patients from two departments. Caucasian patients aged 19–65 years, who had visited the outpatient department at least once during the preceding year, were included. Exclusion criteria were diabetes mellitus, malignancy, liver, thyroid or infectious disease at the time of recruitment, organ transplantation, allergy against ionic contrast media, and pregnancy. After a priori exclusion of those with a serum creatinine > 1.5 mg/dl, we investigated 109 subjects who had the following primary CRD diagnoses: glomerulonephritis, $n = 64$ (58.7%); polycystic kidney disease, $n = 9$ (8.3%); pyelonephritis, $n = 6$ (5.5%); other, $n = 19$ (17.4%); and unknown, $n = 11$ (10.1%).

Serum and ethylenediaminetetra-acetic acid (EDTA) plasma were promptly separated from whole blood collected after an overnight 12-h fast, and frozen at -80°C , before analysis. Twenty four-hour urine samples for the determination of proteinuria were provided by patients carefully instructed in proper collection methods. Serum albumin was measured by bromocrescol green method using a kit from Boehringer Mannheim (Mannheim, Germany). Serum creatinine was determined by the Jaffe method adapted for autoanalyzers. Depending on the serum creatinine level, two to three blood samples were collected for the determination of true GFR by plasma iothexol clearance (ml/

min/1.73 m²), as described by Gaspari and colleagues [11]. Plasma *t*Hcy levels were determined by high-performance liquid chromatography with fluorescence detection [12], plasma pyridoxal 5'-phosphate (PLP) levels were measured by radioenzymatic (tyrosine decarboxylase) assay [13], and plasma folate and B12 levels were ascertained by radioassay (BioRad Quantaphase II, Hercules, CA). Total urinary protein from the 24-h urine collections was determined by ultrafiltration and the Biuret reaction (four centers), the pyrogallol-red (two centers) or Coomassie-blue dye-binding (one center) techniques, adapted for autoanalyzers, and an automated nephelometric method (one center). Between and within-run coefficients of variation (CVs) for all key analytes (i.e., *t*Hcy, B-vitamin, iothexol, and urinary protein) were consistent with data reported elsewhere by our group [10,14], and others [11,15–17]. Specifically, CVs for all assays were between 5–10%, with the exception of the PLP assays, whose CVs were between 10–15%.

All skewed variables were appropriately transformed. Descriptive statistics included means (with standard deviations), geometric means (with 25th–75th percentile ranges), and frequencies (with percentages). Unadjusted between groups comparisons were performed by unpaired *t*-test, and unadjusted correlations between continuous variables were assessed in a Pearson correlation matrix. Stepwise general linear modeling with analysis of covariance (ANCOVA) was then performed to determine the independent association between potential predictor covariables (i.e., proteinuria, GFR, age, sex, folate, B12, PLP, or albumin), and fasting *t*Hcy levels. Reported *P*-values were based on two-tailed calculations, and all statistical analyses were performed using SYSTAT (version 7.0.1) software.

3. Results

Basic descriptive data are presented in Table 1. Patients demonstrated a very broad range of 24-h proteinuria, from 0.000 to 8.340 g/day. Despite the arbitrary restriction of creatinine levels to 1.5 mg/dl or less, true GFR by iothexol clearance ranged widely, from 18 to 205 ml/min/m². As depicted in Table 2, unadjusted correlations ($P < 0.100$) were observed between *t*Hcy levels and GFR (-0.427 ; $P < 0.001$), age ($+0.268$; $P = 0.005$), and plasma folate (-0.296 ; $P = 0.002$), but not 24-h proteinuria ($+0.133$; $P = 0.174$). Unadjusted geometric mean *t*Hcy levels were higher in men ($n = 77$; 12.1 $\mu\text{mol/l}$) versus women ($n = 32$; 10.2 $\mu\text{mol/l}$; $P = 0.027$ by unpaired *t*-test). The weak, unadjusted association between proteinuria and plasma *t*Hcy levels was further reduced by stepwise general linear modeling with ANCOVA, upon controlling for GFR, in particular, and the other potential independent determinants

Table 1
Patient characteristics

<i>n</i>	109
Age (years)	43 ^a (13), [18–64] ^b
Sex, no. (% men)	77 (70.6%)
Creatinine (mg/dl)	1.2 ^c [1.0–1.4] ^d
Total homocysteine (μmol/l)	11.5 ^c [9.3–13.6] ^d
GFR (ml/min/1.73 m ²)	105 ^c [91–138] ^d ; {18–205} ^e
Proteinuria (g/24-h)	0.471 ^c [0.157–1.270] ^d ; {0.000–8.340} ^e
Folate (ng/ml)	3.8 ^c [2.8–4.9] ^d
B12 (pg/ml)	375 ^c [298–471] ^d
Pyridoxal 5'-phosphate (nmol/ml)	56.5 ^c [40.6–73.3] ^d
Albumin (mg/dl)	4.5 ^c [4.3–4.9] ^d

^a Mean (standard deviation).

^b [Complete range].

^c Geometric mean.

^d [25th to 75th percentile range].

^e {complete range}.

of *t*Hcy levels (partial *R* for proteinuria = 0.127; *P* = 0.201). In contrast, stepwise general linear modeling with ANCOVA confirmed that GFR itself was strongly and independently associated with *t*Hcy levels (partial *R* = −0.408; *P* < 0.001). Moreover, across descending quartiles of GFR (see Fig. 1), ANCOVA-adjusted (i.e., for age, sex, and plasma folate) geometric mean *t*Hcy levels were significantly increased. Again, in contrast, ANCOVA-adjusted (i.e., for GFR, age, sex, and plasma folate) geometric mean *t*Hcy levels did *not* differ significantly across quartiles of proteinuria. Further adjustment for serum albumin did not substantially strengthen the relationship between quartile of proteinuria and plasma *t*Hcy (*P* = 0.407 for overall between quartile differences; Scheffe test of largest be-

tween groups post-hoc comparison, i.e., Q4 vs. Q2, *P* = 0.451).

4. Discussion

Our findings represent the initial controlled observation that upon adjustment for true GFR, in particular, proteinuria, across the full spectrum from none to frank nephrosis, is not independently associated with increased *t*Hcy levels among CRD patients with a normal range serum creatinine. Two prior reports [4,8] which also included direct GFR assessment, similarly indicated that at least within an arbitrarily truncated range, quantitatively [8] or semi-quantitatively [4] determined proteinuria was not independently associated with fasting *t*Hcy levels in patients with overt non-diabetic CRD, or diabetes. However, the very narrow range of proteinuria examined in both these studies [4,8] may have introduced an important null bias that was avoided in our investigation. Chico and colleagues [5] and Hoogeveen and colleagues [6], in contrast, have reported that proteinuria, as either a continuous or categorical variable, was independently associated with *t*Hcy levels in both diabetic and non-diabetic individuals. Neither of these analyses [5,6] controlled for true GFR, however, and Hoogeveen and colleagues [6] further failed to control for plasma status of folate, B12, or PLP. More recently, Smulders and colleagues [7] found no association between *t*Hcy levels and proteinuria in an unadjusted analysis comparing 'normoalbuminuric' (< 30 mg/24 h), microalbuminuric (30–300 mg/24 h), and macroalbuminuric (> 300 mg/24 h) subjects with Type II diabetes. Potential confounders biasing toward or away from a true association between

Table 2
Spearman correlation matrix (*P*-value) of key (log transformed) continuous variables

	<i>t</i> Hcy	GFR	Age ^a	Folate	B12	PLP	Proteinuria	Albumin
<i>t</i> Hcy	–							
GFR	−0.427 (<0.001)	–						
Age	+0.268 (0.005)	−0.279 (0.004)	–					
Folate	−0.296 (0.002)	−0.051 (0.602)	+0.051 (0.604)	–				
B12	−0.095 (0.332)	+0.079 (0.418)	+0.066 (0.498)	+0.090 (0.355)	–			
PLP ^b	−0.072 (0.462)	+0.136 (0.164)	−0.101 (0.302)	+0.029 (0.770)	+0.123 (0.206)	–		
Proteinuria ^c	+0.133 (0.174)	−0.184 (0.058)	+0.101 (0.300)	+0.137 (0.160)	−0.028 (0.777)	−0.195 (0.044)	–	
Albumin	−0.147 (0.132)	+0.228 (0.018)	−0.262 (0.006)	+0.114 (0.244)	+0.138 (0.175)	+0.381 (<0.001)	−0.445 (<0.001)	–

^a Untransformed.

^b PLP, Pyridoxal 5'-phosphate.

^c Proteinuria = 24-h proteinuria.

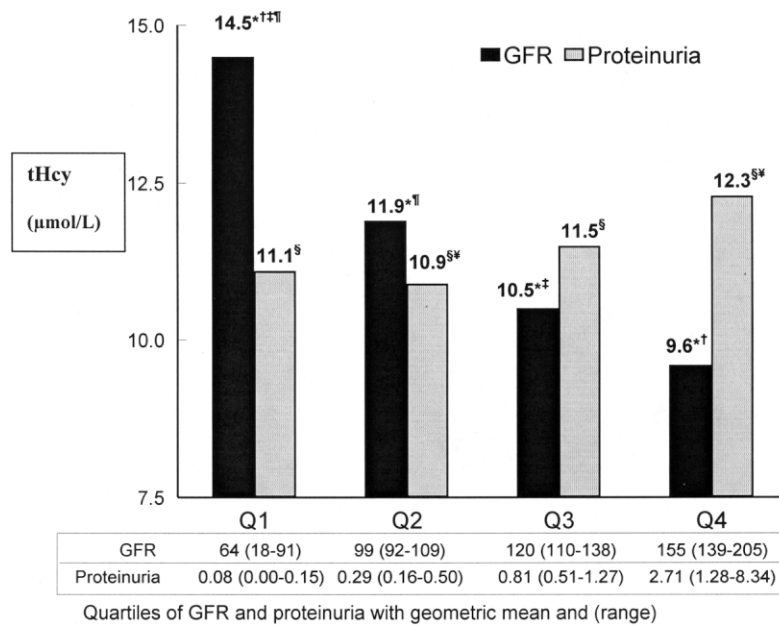


Fig. 1. Comparison of ANCOVA-adjusted geometric mean *t*Hcy levels across quartiles of GFR and proteinuria. *GFR*: from ANCOVA adjusted for age, sex, and plasma folate, * $P < 0.001$ for overall between quartile geometric mean *t*Hcy differences; Scheffe test of post-hoc comparisons, † $P < 0.001$ for Q1 vs. Q4; ‡ $P = 0.002$ for Q1 vs. Q3; § $P = 0.045$ for Q1 vs. Q2. *Proteinuria*: from ANCOVA adjusted for GFR, age, sex, and plasma folate, § $P = 0.453$ for overall between quartile geometric mean *t*Hcy differences; Scheffe test of post-hoc comparisons, ¥ $P = 0.500$ for Q4 vs. Q2 comparison.

proteinuria and *t*Hcy levels in this report, were not evaluated [7].

Persistent mild to moderate hyperhomocysteinemia is characteristic of patients with CRD [14]. Impaired homocysteine metabolism in CRD could result from losses of normal intrarenal homocysteine metabolism, the adverse effect of even subclinical uremia on extrarenal homocysteine metabolism, or combined intra- and extrarenal defects [18]. Despite *in vitro* studies demonstrating renal tubular metabolism of homocysteine [19], and rat model evidence of significant *in vivo* renal homocysteine metabolism [20–22], non-significant *mean* human renal arteriovenous differences for (total and non-protein bound) homocysteine were recently reported [23]. These findings [23] have rekindled a search for ‘uremia-induced’ extrarenal [24] (presumptively, hepatic) defects in homocysteine metabolism. It should be noted, however, that mild decrements in GFR, encompassing clearly non-uremic ranges of GFR, determined either by direct measurement [4,8], or using a sensitive surrogate like cystatin C [25–27], are strongly and independently associated with (linear) increases in fasting *t*Hcy levels. Our confirmation of this robust, independent association between true GFR and *t*Hcy levels among non-uremic patients with at worst, mild to moderate renal impairment, might again suggest that intrarenal homocysteine metabolism is a major determinant of homocysteinemia. These data, however, cannot rule out

the possibility that subtle extrarenal defects in homocysteine metabolism that may accompany even such relatively moderate reductions in renal function, could account for the resulting increases in *t*Hcy levels.

Detection of mild to severe proteinuria [1–3], particularly among individuals with either known diabetes or non-diabetic CRD, identifies those at clearly elevated risk for arteriosclerotic outcomes. Our current findings with regard to *t*Hcy levels, as well as an earlier report from this CRD cohort on plasma lipoprotein (a) levels [10], highlight the importance of true GFR as an independent determinant of these putatively atherothrombotic risk factors [9,28]. Accordingly, true GFR determination may further stratify cardiovascular disease risk within a broad range of proteinuria. Preliminary evidence in support of this hypothesis has recently been provided by the EDIC Investigators [29], Taniwaki and colleagues [30], and Wirta and colleagues [31]. In EDIC, true GFR, but not microalbuminuria, was independently associated with extracranial carotid artery intimal–medial wall thickness among Type 1 diabetics [29]. Similarly, separate analyses of Type 2 diabetic cohorts from Japan [30] and Finland [31], have revealed that minor decrements in true GFR, but not microalbuminuria, were independently associated with extracranial carotid artery intimal–medial wall thickness [30], and CVD mortality [31], respectively.

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