

Two Cardiovascular Risk Factors in One? Homocysteine and Its Relation to Glomerular Filtration Rate

A Meta-Analysis of 41 Studies with 27,000 Participants

Jan T. Kielstein^{a, d} Shelley R. Salpeter^b Nicholas S. Buckley^c John P. Cooke^a
Danilo Fliser^d

Departments of ^aCardiovascular Medicine and ^bMedicine, Stanford University School of Medicine, Stanford, Calif., and ^cCalifornia Institute of Technology, Pasadena, Calif., USA; ^dDepartment of Internal Medicine, Division of Nephrology, Medical School, Hannover, Germany

Key Words

Kidney · Meta-analysis · Risk factors · Cockcroft-Gault equation · Modification of Diet in Renal Disease formula · Glomerular filtration rate · Homocysteine

Abstract

Background: Hyperhomocysteinemia is thought to be an independent risk factor for cardiovascular disease, but the association between renal dysfunction and homocysteine may not have been fully taken into account. We performed a meta-analysis of studies that report correlations between glomerular filtration rate (GFR) and homocysteine plasma levels. **Methods:** Using a prespecified research strategy, we identified 41 studies involving 26,617 participants that reported Pearson or Spearman correlation coefficients for the association between 1/GFR and homocysteine. The summary correlation coefficients with 95% CI were obtained by pooling the logarithmic Z values derived from the individual trial correlation coefficients. Subgroup analysis was performed to compare results for measured GFR using clearance methods and various estimates of GFR. **Results:** The pooled correlation coefficient between homocysteine and

1/GFR was 0.37 (CI 0.32–0.40, $p < 0.0001$). The correlation coefficient based on various estimates of GFR was 0.33 (CI 0.29–0.38, $p < 0.0001$), and for measured GFR it was 0.45 (CI 0.39–0.51, $p < 0.0001$). The correlation coefficient was higher when GFR was measured using clearance methods compared with various estimates GFR (1.36 [CI 1.13–1.65], $p = 0.0014$). **Conclusions:** Homocysteine plasma levels significantly depend on renal function. This correlation is even more robust when GFR is measured using clearance methods. Therefore, in order to assess whether homocysteine is an independent cardiovascular risk factor, accurate adjustments for renal dysfunction are essential.

Copyright © 2008 S. Karger AG, Basel

Introduction

In 1969, McCully [1] published his seminal paper in the *American Journal of Pathology* in which he proposed a connection between hyperhomocysteinemia and car-

J.T. Kielstein and S.R. Salpeter contributed equally to this work.

diovascular disease. His hypothesis was based on autopsy findings of atherosclerotic plaque in 2 children with homocysteinuria due to inborn enzyme defects. The very high level of plasma homocysteine observed in patients with inborn enzyme defects is likely to play a pathophysiological role in the accelerated vascular disease observed in these patients. However, the significance of modest elevations of plasma homocysteine observed in the general population is less clear. To be sure, there are well-known associations between cardiovascular disease and plasma homocysteine levels [2], as well as a correlation of homocysteine with future cardiovascular events [3]. However, recent large randomized trials revealed no reduction in cardiovascular events or death when homocysteine levels were reduced by up to 30% [4] using a combination of folate and B vitamins [4–6]. Several reasons have been proposed for these disappointing results [7]. One explanation is that in the general population, homocysteine may be merely a marker for the severity of kidney disease, and not an independent cardiovascular risk factor [8].

Studies have reported a strong inverse relationship between glomerular filtration rate (GFR) and plasma homocysteine concentrations [9–11]. Furthermore, renal insufficiency is a recognized independent risk factor for cardiovascular disease [12]. We propose that homocysteine is a marker of renal function and that its predictive value for cardiovascular events reflects its association with impaired renal function. Some of the prior studies demonstrating that homocysteine was an independent risk factor for cardiovascular events did not adjust for renal function at all [2, 13]. Other studies may have inadequately adjusted for the confounding effects of kidney dysfunction if serum creatinine was used, as this is inferior to measured GFR using clearance methods in accurately assessing renal function [3].

Methods

We comprehensively searched Medline and the Cochrane Library from 1970 through March 2007 using the following medical subject headings: glomerular filtration rate and homocysteine. We supplemented the search by scanning references of selected articles identified by this search. We did not restrict the language of publication. We included cross-sectional studies as well a baseline data from trials using pharmacological interventions that evaluated homocysteine and provided correlation coefficients (Pearson or Spearman) to various estimates of GFR using calculations (e.g. Cockcroft-Gault equation, Modification of Diet in Renal Disease formula, Schwartz formula in children, and creatinine clearance) or measured GFR (e.g. inulin and ethylene-diamine-tetra-acetic acid (EDTA) clearance, and radioisotope methods). Attempts were made to contact investigators for addi-

tional information. The correlation coefficient for three studies was obtained by personal communication [14–16].

We excluded studies that reported data on homocysteine and GFR without analyzing the correlation between the two values, as well as studies that only provided regression or partial regression analysis. Studies in which renal function was evaluated by measuring cystatin C were also excluded as this parameter is currently not recommended by any guidelines.

The Pearson or Spearman correlation coefficients were recorded from each study, for the values of homocysteine and 1/GFR. The summary correlation coefficients with 95% CI were obtained by pooling the logarithmic Z values derived from the individual trial correlation coefficients. To test for interstudy heterogeneity, the χ^2 value was calculated for the assumption of homogeneity. The results were pooled using the random-effects method, because evidence for potential interstudy heterogeneity was found. In addition, the results from the fixed-effects method were compared with those from the random-effects method [17].

Subgroup analysis was performed to compare results for GFR that were estimated using calculation methods or measured using clearance methods. The results of the two subgroups were compared with each other using the test for interaction [18].

Results

The flow diagram for the trials search is presented in figure 1. We identified 41 studies involving a total of 26,617 patients (table 1). Uniformly, the studies included in the analysis revealed statistically significant correlations between homocysteine and renal function (fig. 2). The pooled correlation coefficient between homocysteine and 1/GFR was 0.37 (CI 0.32–0.40, $p < 0.0001$).

Evidence for significant interstudy heterogeneity was noted for the analysis ($p < 0.005$). The results were similar when the random effects method was used (0.29 [CI 0.28–0.30], $p < 0.0001$).

In subgroup analysis, the correlation coefficient based on various estimates of GFR was 0.33 (CI 0.29–0.38, $p < 0.0001$), and for measured GFR it was 0.45 (CI 0.39–0.51, $p < 0.0001$). The correlation coefficient was higher when GFR was measured using clearance methods compared with various estimates of GFR (1.36 [CI 1.13–1.65], $p = 0.0014$). Evidence for interstudy heterogeneity was present for the analysis of calculation estimates ($p < 0.005$) and for clearance measurements ($p = 0.03$). When using the fixed-effects method of analysis, the correlation coefficient for calculations was 0.28 (CI 0.17–0.29, $p < 0.0001$) and for clearance measurements it was 0.44 (CI 0.40–0.48, $p < 0.0001$).

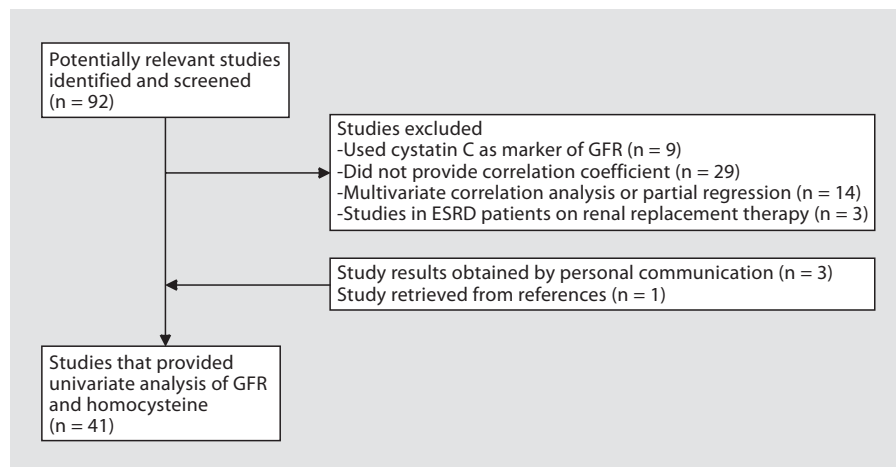


Fig. 1. Study flow diagram.

Discussion

Homocysteine and Cardiovascular Disease

Homocysteine is a sulfur amino acid whose metabolism stands at the intersection of two pathways: remethylation to methionine, which requires folate and vitamin B₁₂ (or betaine in an alternative reaction); and transsulfuration to cystathionine, which requires pyridoxal-5'-phosphate. The two pathways are coordinated by S-adenosylmethionine, which acts as an allosteric inhibitor of the methylenetetrahydrofolate reductase reaction and as an activator of cystathionine beta-synthase [7]. A meta-analysis of 27 retrospective, cross-sectional and prospective trials came to the conclusion that a 5- μ mol/l increase in homocysteine levels elevates coronary artery disease risk by as much as cholesterol increases of 0.5 mmol/l (20 mg/dl) [19]. Other prospective trials further supported the concept by showing that homocysteine was an independent strong predictor of subsequent cardiovascular and cerebrovascular events [2, 3, 20–25]. Despite these strong epidemiological data, homocysteine has recently failed the ultimate test as a cardiovascular risk factor – substantial lowering of homocysteine did not translate into improved outcomes in patients with known cardiovascular disease [4–6]. One potential reason for these disappointing results in secondary prevention is that homocysteine is not an independent cardiovascular risk factor, but may be simply a marker for impaired renal function [8].

Many of the recent studies suggesting that homocysteine was an independent risk factor for cardiovascular events did not adjust for renal function at all [2, 26]. Others adjusted only for creatinine, a notoriously unreliable

marker of renal function [22, 25, 27]. However, the significant inverse correlation between GFR and total plasma homocysteine levels shown in our analysis could explain, at least in part, the predictive value of homocysteine for cardiovascular events. Impaired renal function, which causes an increase in homocysteine levels, has been consistently shown to be a major independent cardiovascular risk factor [28, 29].

Further evidence for homocysteine not being an independent risk factor for cardiovascular events comes from the study by Menon et al. [30] which demonstrated that hyperhomocysteinemia was not a risk factor for all-cause or cardiovascular mortality in the Modification of Diet in Renal Disease Study, when adjustments for GFR were made using ¹²⁵I-iothalamate clearance. The authors therefore suggested that prior studies demonstrating an association between homocysteine and the risk of cardiovascular disease may have inadequately adjusted for the confounding effects of kidney function [30].

Overlap of Hyperhomocysteinemia and Chronic Kidney Disease

The increase in levels of total homocysteine in renal insufficiency has been consistently demonstrated over the past 30 years [8, 10, 11, 31]. In addition to the 41 studies included in our meta-analysis, a few other studies used multivariate regression or partial regression analysis to produce adjusted correlation coefficients [32–37], which showed that homocysteine is independently correlated with renal function after correction for variables such as age, sex, diet, systolic blood pressure and plasma uric acid levels. Moreover, studies in which renal function was evaluated by measuring serum cystatin C con-

Table 1. Included studies

Study, year [reference]	Patient population	GFR method	Homocysteine method	n	Females %	r	p value
Abdella et al. 2000 [47]	type 2 diabetes	CrCl	IMx	283	59	0.46	<0.0001
Aksoy et al. 2006 [9]	heart failure	CrCl	HPLC	62	55	0.65	<0.001
Ambrosch et al. 2001 [48]	type 2 diabetes	CrCl	HPLC	65	55	0.29	<0.05
Arnadottir et al. 2001 [49]	chronic kidney disease	iohexol clearance	HPLC	22	0	0.42	<0.05
Arnadottir et al. 2001 [50]	renal transplant	iohexol and EDTA	HPLC	120	not stated	0.52	<0.0001
Arnadottir et al. 1996 [10]	chronic kidney disease	iohexol clearance	HPLC	77	44	0.70	<0.0001
Arnadottir et al. 1998 [51]	renal transplant	CrCl	HPLC	55	54	0.42	<0.01
Bostom et al. 2001 [52]	chronic kidney disease	iohexol clearance	HPLC	109	29	0.43	<0.001
Cossu et al. 2001 [53]	renal transplant	Cockcroft-Gault	CE-LIF	108	48	0.49	<0.01
Darius et al. 2003 [14]	peripheral arterial disease	MDRD	HPLC	6,888	58	0.15	<0.001
Dinleyici et al. 2006 [54]	children with type 1 diabetes and control group	Schwartz formula	ELISA	55	44	0.32	<0.05
Francis et al. 2004 [15]	NHANES study 1991–1994	MDRD	GPLC	3,387	54	0.30	<0.0001
Gobulev et al. 2005 [55]	chronic kidney disease	not stated	HPLC	125	not stated	0.43	<0.0001
Guallar et al. 2006 [16]	NHANES study	MDRD	IMx	4,447	not stated	0.36	<0.001
Kielstein et al. 2002 [11]	chronic kidney disease	inulin clearance	IMx	44	7	0.73	<0.0001
Korandji et al. 2007 [56]	acute myocardial infarction	MDRD	chemi-luminescence	138	20	0.27	0.002
Krmar et al. 2001 [57]	pediatric renal transplant	Schwartz formula	IMx	38	26	0.47	<0.01
Lin et al. 2006 [58]	hypertension with and without chronic renal insufficiency	Cockcroft-Gault	IMx	137	50	0.45	<0.001
Kryznakowa et al. 2006 [59]	type 2 diabetes	MDRD	IMx	136	40	0.41	<0.0001
Marouf et al. 2006 [60]	acute myocardial infarction and control group	MDRD	IMx	210/167	30	0.37	<0.0001
Marouf et al. 2006 [61]	thrombembolic disease and control group	MDRD	IMx	201/166	52	0.34	<0.0001
Marucci et al. 2000 [62]	renal transplant patients	not stated	HPLC	63	46	0.20	<0.05
Marucci et al. 2001 [63]	renal transplant and control group	not stated	HPLC	70/66	43	0.20	<0.05
Meinitzer et al. 2007 [64]	coronary artery disease	MDRD	not stated	3,238	43	0.39	<0.001
Ndrepepa et al. 2006 [65]	coronary artery disease with and without diabetes	Cockcroft-Gault	IMx	506 1,614	34 26	0.36 0.29	<0.001 <0.001
Nerbass et al. 2006 [66]	chronic kidney disease	CrCl	HPLC	66	30	0.29	0.11
Okumura et al. 2003 [67]	type 2 diabetes and control group	CrCl	HPLC	103	46	0.49	<0.0001
Parson et al. 2002 [68]	chronic kidney disease	EDTA	HPLC	197	28	0.39	<0.0001
Pizzolo et al. 2006 [69]	coronary artery disease	MDRD	HPLC	180	19	0.29	<0.001
Preston et al. 2005 [70]	chronic kidney disease	EDTA	HPLC	114	28	0.27	0.004
Ravani et al. 2005 [71]	chronic kidney disease	MDRD	IMx	131	37	0.34	<0.001
Samuelsson et al. 1999 [72]	chronic kidney disease	EDTA	HPLC	63	22	0.32	<0.01
Sarnak et al. 2002 [73]	chronic kidney disease	¹²⁵ I-iothalamate	HPLC	804	40	0.44	<0.001
Serafinowicz et al. 2000 [74]	renal transplant	iopromide	IMx	71	45	0.45	<0.05
Soedamah-Muthu et al. 2005 [75]	type 1 diabetes	Cockcroft-Gault	IMx	533	52	0.23	<0.0001
Suliman et al. 2006 [76]	stage 5 chronic kidney disease, pre-hemodialysis	mean of CrCl and urea clearance	HPLC	317	38	0.12	<0.05
Veldman et al. 2005 [77]	type 1 diabetes and control group	inulin clearance	not stated	92 44	41 48	0.43 0.39	0.005 0.01
Wang et al. 2006 [78]	coronary artery disease	Cockcroft-Gault	not stated	145	39	0.22	<0.01
Widiana et al. 2004 [79]	chronic kidney disease	Cockcroft-Gault	IMx	26	31	0.39	0.04
Wollesen et al. 1999 [80]	type 1 and 2 diabetes	EDTA	HPLC	80	not stated	0.44	<0.0001
Zebrack et al. 2003 [81]	coronary artery disease	MDRD	not stated	1,484	33	0.18	<0.001

CE-LIF = Capillary electrophoresis with laser-induced fluorescence; CrCl = creatinine clearance; EDTA = ethylenediamine tetraacetic acid; ELISA = enzyme-linked immunosorbent assay; IMx = fluorescence polarization immunoassay; HPLC = high-performance liquid chromatography; MDRD = modification of diet in renal disease formula.



Fig. 2. Correlation coefficients for homocysteine and 1/GFR. Meta-analysis results. The studies are in alphabetical order. The studies by Ndrepepa et al. [65] and Veldman et al. [77] have been subdivided into controls and patients.

centration, a new marker of GFR that has been shown to be more accurate than creatinine, consistently showed a strong association between homocysteine and renal function [38, 39]. Of note, our meta-analysis shows that measured GFR using clearance methods provide a stronger correlation between GFR and homocysteine.

Despite the strong association between homocysteine and renal function, the exact role of the kidney in the elimination of homocysteine is still poorly understood. In a study investigating the arteriovenous difference in homocysteine in the kidney of individuals with normal renal function, no significant renal extraction of homocysteine could be detected [40]. It is also known that only the non-protein-bound fraction of homocysteine is freely filtered at the glomerulus; however, this fraction is highly variable among species (20% in humans vs. 70% in rats). While these facts speak against an important role for healthy kidney in homocysteine plasma clearance, the homocysteine clearance of uremic patients is reduced by 70% compared to healthy controls [41]. Collectively, the changes of plasma homocysteine in the state of decreased renal function can be ascribed to a decrease of both intra- and extrarenal homocysteine clearance, with the latter being attributable to retained solutes and/or kidney disease-related modifications of plasma environment interfering with the systemic homocysteine metabolism [42].

The conclusion of our study does by no means try to defer the results of many preclinical and clinical studies investigating the complex role of homocysteine in the pathophysiology of atherosclerosis [43], it rather sheds light on an important yet neglected role of renal dysfunction in this process. Therefore, it is important to note that homocysteine might still be a modest independent marker for cardiovascular events, even after correcting for renal function, as has been suggested by an earlier meta-analysis of observational studies [20]. Studies have demonstrated the importance of genetic alterations in metabolism and deficiencies of B vitamins and folate on elevated homocysteine levels, which is associated with an increase in cardiovascular events [44]. However, patients with hyperhomocysteinemia have been shown to exhibit elevated cystatin C levels as a sign of renal dysfunction, despite normal creatinine levels [45].

Strengths and Limitations of the Analysis

The strengths and potential limitations of the present study merit careful consideration. One of the main strengths of our analysis is the large number of studies and subjects included. The diversity of underlying diseases and age groups of those studies in all of which the

correlation of GFR and homocysteine was present underlines the robustness of this relationship. There are, however, possible limitations of our study. Our analysis reflects the limitations of included studies concerning the accuracy of homocysteine and creatinine measurements. Moreover, our analysis was performed by omitting important homocysteine determinants such as folate/vitamin plasma levels and MTHFR gene polymorphism. These factors might significantly alter the Hcy/GFR correlation we found in this meta-analysis.

Conclusion and Perspectives

Pooled data from 41 trials and 27,000 patients show that plasma homocysteine levels are significantly inversely correlated with estimates of GFR. This inverse correlation is even more robust when GFR is measured using clearance methods. It is possible that homocysteine may not be an independent cardiovascular risk factor when accurate adjustments for renal dysfunction are made. As an immediate consequence, adequate estimates or measurement of GFR should be an integral part in the design and interpretation of all clinical studies as well as in laboratory studies of patients at risk for cardiovascular events, as recently suggested by a science advisory from the American Heart Association Kidney and Cardiovascular Disease Council [46]. In addition, in patients with hyperhomocysteinemia, renal dysfunction should be assessed with appropriate methods.

Acknowledgements

The authors thank Edwin E. Salpeter, PhD for his statistical expertise. This work was supported by a grant from the DFG (Deutsche Forschungsgemeinschaft; Ki 8591/-1) to Jan T. Kielstein.

References

- 1 McCully KS: Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1969;56: 111-128.
- 2 Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH: Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995;332:286-291.
- 3 Schnabel R, Lackner KJ, Rupprecht HJ, Espinola-Klein C, Torzewski M, Lubos E, Bickel C, Cambien F, Tiret L, Munzel T, Blankenberg S: Glutathione peroxidase-1 and homocysteine for cardiovascular risk prediction:

- results from the AtheroGene study. *J Am Coll Cardiol* 2005;45:1631–1637.
- 4 Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K: Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006;354:1578–1588.
 - 5 Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, McQueen MJ, Probstfield J, Fodor G, Held C, Genest J Jr: Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567–1577.
 - 6 Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH, Stampfer M: Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* 2004;291:565–575.
 - 7 Loscalzo J: Homocysteine trials: clear outcomes for complex reasons. *N Engl J Med* 2006;354:1629–1632.
 - 8 Brattstrom L, Wilcken DE: Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000;72:315–323.
 - 9 Aksoy N, Aksoy M, Cakmak M, Serdar GH, Davutoglu V, Soyuncu S, Meram I: Increased homocysteine in heart failure: a result of renal impairment? *Clin Chem Lab Med* 2006;44:1324–1329.
 - 10 Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysel H: The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest* 1996;56:41–46.
 - 11 Kielstein JT, Boger RH, Bode-Boger SM, Frolich JC, Haller H, Ritz E, Fliser D: Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. *J Am Soc Nephrol* 2002;13:170–176.
 - 12 Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culeton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW: Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003;108:2154–2169.
 - 13 Zee RY, Mora S, Cheng S, Erlich HA, Lindpaintner K, Rifai N, Buring JE, Ridker PM: Homocysteine, 5,10-methylenetetrahydrofolate reductase 677C>T polymorphism, nutrient intake, and incident cardiovascular disease in 24,968 initially healthy women. *Clin Chem* 2007;53:845–851.
 - 14 Darius H, Pittrow D, Haberl R, Trampisch HJ, Schuster A, Lange S, Tepohl HG, Allenberg JR, Diehm C: Are elevated homocysteine plasma levels related to peripheral arterial disease? Results from a cross-sectional study of 6,880 primary care patients. *Eur J Clin Invest* 2003;33:751–757.
 - 15 Francis ME, Eggers PW, Hostetter TH, Briggs JP: Association between serum homocysteine and markers of impaired kidney function in adults in the United States. *Kidney Int* 2004;66:303–312.
 - 16 Guallar E, Silbergeld EK, Navas-Acien A, Malhotra S, Astor BC, Sharrett AR, Schwartz BS: Confounding of the relation between homocysteine and peripheral arterial disease by lead, cadmium, and renal function. *Am J Epidemiol* 2006;163:700–708.
 - 17 DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–188.
 - 18 Altman DG, Bland JM: Interaction revisited: the difference between two estimates. *BMJ* 2003;326:219.
 - 19 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA* 1995;274:1049–1057.
 - 20 Homocysteine Studies Collaboration: Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002;288:2015–2022.
 - 21 Hoogeveen EK, Kostense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD: Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes: 5-year follow-up of the Hoorn Study. *Circulation* 2000;101:1506–1511.
 - 22 Iso H, Moriyama Y, Sato S, Kitamura A, Tanigawa T, Yamagishi K, Imano H, Ohira T, Okamura T, Naito Y, Shimamoto T: Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. *Circulation* 2004;109:2766–2772.
 - 23 Ridker PM, Shih J, Cook TJ, Clearfield M, Downs JR, Pradhan AD, Weis SE, Gotto AM Jr: Plasma homocysteine concentration, statin therapy, and the risk of first acute coronary events. *Circulation* 2002;105:1776–1779.
 - 24 Vollset SE, Refsum H, Tverdal A, Nygard O, Nordrehaug JE, Tell GS, Ueland PM: Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study. *Am J Clin Nutr* 2001;74:130–136.
 - 25 Zylberstein DE, Bengtsson C, Bjorkelund C, Landaas S, Sundh V, Thelle D, Lissner L: Serum homocysteine in relation to mortality and morbidity from coronary heart disease: a 24-year follow-up of the population study of women in Gothenburg. *Circulation* 2004;109:601–606.
 - 26 Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL, Davis CE: Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 1998;98:204–210.
 - 27 Kark JD, Selhub J, Adler B, Gofin J, Abramson JH, Friedman G, Rosenberg IH: Nonfasting plasma total homocysteine level and mortality in middle-aged and elderly men and women in Jerusalem. *Ann Intern Med* 1999;131:321–330.
 - 28 Anavekar NS, McMurray JJ, Velazquez EJ, Solomon SD, Kober L, Rouleau JL, White HD, Nordlander R, Maggioni A, Dickstein K, Zelenkofske S, Leimberger JD, Califf RM, Pfeffer MA: Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med* 2004;351:1285–1295.
 - 29 Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004;351:1296–1305.
 - 30 Menon V, Sarnak MJ, Greene T, Wang X, Pereira AA, Beck GJ, Kusek JW, Selhub J, Collins AJ, Levey AS, Shlipak MG: Relationship between homocysteine and mortality in chronic kidney disease. *Circulation* 2006;113:1572–1577.
 - 31 Wilcken DE, Gupta VJ: Sulphur containing amino acids in chronic renal failure with particular reference to homocysteine and cysteine-homocysteine mixed disulphide. *Eur J Clin Invest* 1979;9:301–307.
 - 32 Canepa A, Carrea A, Caridi G, Dertenio L, Minniti G, Cerone R, Canini S, Calevo MG, Perfumo F: Homocysteine, folate, vitamin B₁₂ levels, and C677T MTHFR mutation in children with renal failure. *Pediatr Nephrol* 2003;18:225–229.
 - 33 Diakoumopoulou E, Tentolouris N, Kirlaki E, Perrea D, Kitsou E, Psallas M, Douglarakis D, Katsilambros N: Plasma homocysteine levels in patients with type 2 diabetes in a Mediterranean population: relation with nutritional and other factors. *Nutr Metab Cardiovasc Dis* 2005;15:109–117.
 - 34 Flicker LA, Vasikaran SD, Thomas J, Acres JG, Norman PE, Jamrozik K, Lautenschlager NT, Leedman PJ, Almeida OP: Homocysteine and vitamin status in older people in Perth. *Med J Aust* 2004;180:539–540.
 - 35 Jabs K, Koury MJ, Dupont WD, Wagner C: Relationship between plasma S-adenosylhomocysteine concentration and glomerular filtration rate in children. *Metabolism* 2006;55:252–257.
 - 36 Loffredo L, Violi F, Fimognari FL, Cangemi R, Sbrighi PS, Sampietro F, Mazzola G, Di Lecce VN, D'Angelo A: The association between hyperhomocysteinemia and ischemic stroke in patients with non-valvular atrial fibrillation. *Haematologica* 2005;90:1205–1211.
 - 37 Schafer SA, Mussig K, Stefan N, Haring HU, Fritsche A, Balletshofer BM: Plasma homocysteine concentrations in young individuals at increased risk of type 2 diabetes are associated with subtle differences in glomerular filtration rate but not with insulin resistance. *Exp Clin Endocrinol Diabetes* 2006;114:306–309.

- 38 Ozmen B, Ozmen D, Turgan N, Habif S, Mutaf I, Bayindir O: Association between homocysteinemia and renal function in patients with type 2 diabetes mellitus. *Ann Clin Lab Sci* 2002;32:279–286.
- 39 Bostom AG, Bausserman L, Jacques PF, Li-angaudas G, Selhub J, Rosenberg IH: Cystatin C as a determinant of fasting plasma total homocysteine levels in coronary artery disease patients with normal serum creatinine. *Arterioscler Thromb Vasc Biol* 1999;19:2241–2244.
- 40 van Guldener C, Donker AJ, Jakobs C, Teerlink T, de Meer K, Stehouwer CD: No net renal extraction of homocysteine in fasting humans. *Kidney Int* 1998;54:166–169.
- 41 Guttormsen AB, Ueland PM, Svarstad E, Refsum H: Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney Int* 1997;52:495–502.
- 42 Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH: The kidney and homocysteine metabolism. *J Am Soc Nephrol* 2001;12:2181–2189.
- 43 Ueland PM, Clarke R: Homocysteine and cardiovascular risk: considering the evidence in the context of study design, folate fortification, and statistical power. *Clin Chem* 2007;53:807–809.
- 44 Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG: MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. *JAMA* 2002;288:2023–2031.
- 45 Wilcken DE, Wang J, Sim AS, Green K, Wilcken B: Asymmetric dimethylarginine in homocystinuria due to cystathionine beta-synthase deficiency: relevance of renal function. *J Inherit Metab Dis* 2006;29:30–37.
- 46 Brosius FC III, Hostetter TH, Kelepouris E, Mitsnefes MM, Moe SM, Moore MA, Penathur S, Smith GL, Wilson PW: Detection of chronic kidney disease in patients with or at increased risk of cardiovascular disease: a science advisory from the American Heart Association Kidney and Cardiovascular Disease Council; the Councils on High Blood Pressure Research, Cardiovascular Disease in the Young, and Epidemiology and Prevention; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: developed in collaboration with the National Kidney Foundation. *Circulation* 2006;114:1083–1087.
- 47 Abdella N, Mojiminiyi OA, Akanji AO: Homocysteine and endogenous markers of renal function in type 2 diabetic patients without coronary heart disease. *Diabetes Res Clin Pract* 2000;50:177–185.
- 48 Ambrosch A, Dierkes J, Lobmann R, Kuhne W, Konig W, Luley C, Lehnert H: Relation between homocysteinaemia and diabetic neuropathy in patients with type 2 diabetes mellitus. *Diabet Med* 2001;18:185–192.
- 49 Arnadottir M, Hultberg B, Berg AL: Plasma total homocysteine concentration in nephrotic patients with idiopathic membranous nephropathy. *Nephrol Dial Transplant* 2001;16:45–47.
- 50 Arnadottir M, Hultberg B, Vladov V, Nilsson-Ehle P, Thysel H: Hyperhomocysteinemia in cyclosporine-treated renal transplant recipients. *Transplantation* 1996;61:509–512.
- 51 Arnadottir M, Hultberg B, Wahlberg J, Fellstrom B, Dimeny E: Serum total homocysteine concentration before and after renal transplantation. *Kidney Int* 1998;54:1380–1384.
- 52 Bostom AG, Kronenberg F, Jacques PF, Kuen E, Ritz E, Konig P, Kraatz G, Lhotta K, Mann JF, Muller GA, Neyer U, Riegel W, Schwenger V, Riegler P, Selhub J: Proteinuria and plasma total homocysteine levels in chronic renal disease patients with a normal range serum creatinine: critical impact of true glomerular filtration rate. *Atherosclerosis* 2001;159:219–223.
- 53 Cossu M, Carru C, Pes GM, Satta R, Mura A, Errigo A, Sanna S, Naitana A, Deiana L, Sorba G, Pinna GG: Plasma homocysteine levels and C677T MTHFR gene polymorphism in stable renal graft recipients. *Transplant Proc* 2001;33:1156–1158.
- 54 Dinleyici EC, Kirel B, Alatas O, Muslumanoğlu H, Kilic Z, Dogruel N: Plasma total homocysteine levels in children with type 1 diabetes: relationship with vitamin status, methylene tetrahydrofolate reductase genotype, disease parameters and coronary risk factors. *J Trop Pediatr* 2006;52:260–266.
- 55 Golubev RV, Blashko EL, Dobronravov VA, Zhloba AA, Smirnov AV: Elevated plasma homocysteine and glutathione level in patients with renal failure. *Biomed Khim* 2005;51:549–551.
- 56 Korandji C, Zeller M, Guillard JC, Vergely C, Sicard P, Duvillard L, Gamber P, Moreau D, Cottin Y, Rochette L: Asymmetric dimethylarginine (ADMA) and hyperhomocysteinemia in patients with acute myocardial infarction. *Clin Biochem* 2007;40:66–72.
- 57 Krmar RT, Ferraris JR, Ramirez JA, Galarza CR, Waisman G, Janson JJ, Llapur CJ, Sorroche P, Legal S, Camera MI: Hyperhomocysteinemia in stable pediatric, adolescents, and young adult renal transplant recipients. *Transplantation* 2001;71:1748–1751.
- 58 Lin YH, Pao KY, Wu VC, Lin YL, Chien YF, Hung CS, Chen YJ, Liu CP, Tsai IJ, Gau CS, Wu KD, Hwang JJ: The influence of estimated creatinine clearance on plasma homocysteine in hypertensive patients with normal serum creatinine. *Clin Biochem* 2007;40:230–234. E-pub 2006.
- 59 Krzyzanowska K, Mittermayer F, Krugluger W, Schnack C, Hofer M, Wolzt M, Schernthaner G: Asymmetric dimethylarginine is associated with macrovascular disease and total homocysteine in patients with type 2 diabetes. *Atherosclerosis* 2006;189:236–240.
- 60 Marouf R, Zubaid M, Mojiminiyi OA, Qurtom M, Abdella NA, Al Wazzan H, Al Humood S: Determinants of plasma homocysteine in relation to hematological and biochemical variables in patients with acute myocardial infarction. *South Med J* 2006;99:811–816.
- 61 Marouf R, Mojiminiyi O, Qurtom M, Abdella N, Al Wazzan H, Al Humood S, Al Mazeedy M: Plasma homocysteine and hematological factors in patients with venous thromboembolic diseases in Kuwait. *Acta Haematol* 2006;117:98–105.
- 62 Marcucci R, Zanazzi M, Bertoni E, Brunelli T, Fedi S, Evangelisti L, Pepe G, Rogolino A, Prisco D, Abbate R, Gensini GF, Salvadori M: Risk factors for cardiovascular disease in renal transplant recipients: new insights. *Transpl Int* 2000;13(suppl 1):S419–S424.
- 63 Marcucci R, Fedi S, Brunelli T, Pepe G, Prisco D, Rosati A, Zanazzi M, Bertoni E, Abbate R, Salvadori M: High cysteine levels in renal transplant recipients: relationship with hyperhomocysteinemia and 5,10-MTHFR polymorphism. *Transplantation* 2001;71:746–751.
- 64 Meinitzer A, Seelhorst U, Wellnitz B, Halwachs-Baumann G, Boehm BO, Winkelmann BR, Marz W: Asymmetrical dimethylarginine independently predicts total and cardiovascular mortality in individuals with angiographic coronary artery disease (The Ludwigshafen Risk and Cardiovascular Health Study). *Clin Chem* 2006;53:273–278.
- 65 Ndrepepa G, Kastrati A, Braun S, Koch W, Kolling K, Mehilli J, Schomig A: Circulating homocysteine levels in patients with type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis* 2008;18:66–73.
- 66 Nerbass FB, Draibe SA, Feiten SF, Chiarello PG, Vannucchi H, Cuppari L: Homocysteine and its determinants in nondialyzed chronic kidney disease patients. *J Am Diet Assoc* 2006;106:267–270.
- 67 Okumura K, Aso Y: High plasma homocysteine concentrations are associated with plasma concentrations of thrombomodulin in patients with type 2 diabetes and link diabetic nephropathy to macroangiopathy. *Metabolism* 2003;52:1517–1522.
- 68 Parsons DS, Reaveley DA, Pavitt DV, Brown EA: Relationship of renal function to homocysteine and lipoprotein(a) levels: the frequency of the combination of both risk factors in chronic renal impairment. *Am J Kidney Dis* 2002;40:916–923.
- 69 Pizzolo F, Friso S, Olivieri O, Martinelli N, Bozzini C, Guarini P, Trabetti E, Faccini G, Corrocher R, Giirelli D: Homocysteine, traditional risk factors and impaired renal function in coronary artery disease. *Eur J Clin Invest* 2006;36:698–704.
- 70 Preston E, Ellis MR, Kulinskaya E, Davies AH, Brown EA: Association between carotid artery intima-media thickness and cardiovascular risk factors in CKD. *Am J Kidney Dis* 2005;46:856–862.

- 71 Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C: Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol* 2005;16:2449–2455.
- 72 Samuelsson O, Lee DM, Attman PO, Knight-Gibson C, Mullen JK, Larsson R, Mulec H, Weiss L, Alaupovic P: The plasma levels of homocysteine are elevated in moderate renal insufficiency but do not predict the rate of progression. *Nephron* 1999;82:306–311.
- 73 Sarnak MJ, Wang SR, Beck GJ, Kusek JW, Selhub J, Greene T, Levey AS: Homocysteine, cysteine, and B vitamins as predictors of kidney disease progression. *Am J Kidney Dis* 2002;40:932–939.
- 74 Serafinowicz A, Kukula K, Cieciora T, Shaibani B, Baczkowska T, Sojn J, Sadowska A, Nowacka-Cieciora E, Lewandowska D, Rell K, Durlik M, Lao M: Homocysteine and lipid peroxidation products: important atherosclerosis risk factors in renal allograft recipients? *Transplant Proc* 2000;32:1367–1368.
- 75 Soedamah-Muthu SS, Chaturvedi N, Teerlink T, Idzior-Walus B, Fuller JH, Stehouwer CD: Plasma homocysteine and microvascular and macrovascular complications in type 1 diabetes: a cross-sectional nested case-control study. *J Intern Med* 2005;258:450–459.
- 76 Suliman M, Stenvinkel P, Qureshi AR, Kallantar-Zadeh K, Barany P, Heimbürger O, Vonesh EF, Lindholm B: The reverse epidemiology of plasma total homocysteine as a mortality risk factor is related to the impact of wasting and inflammation. *Nephrol Dial Transplant* 2007;22:209–217.
- 77 Veldman BA, Vervoort G, Blom H, Smits P: Reduced plasma total homocysteine concentrations in type 1 diabetes mellitus is determined by increased renal clearance. *Diabet Med* 2005;22:301–305.
- 78 Wang J, Sim AS, Wang XL, Salonikas C, Naidoo D, Wilcken DE: Relations between plasma asymmetric dimethylarginine (ADMA) and risk factors for coronary disease. *Atherosclerosis* 2006;184:383–388.
- 79 Widiana IG, Suwitra K: Relationship between creatinine clearance and plasma homocysteine levels in predialytic chronic renal failure patients. *Acta Med Indones* 2004;36:15–18.
- 80 Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C: Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int* 1999;55:1028–1035.
- 81 Zebrack JS, Anderson JL, Beddhu S, Horne BD, Bair TL, Cheung A, Muhlestein JB: Do associations with C-reactive protein and extent of coronary artery disease account for the increased cardiovascular risk of renal insufficiency? *J Am Coll Cardiol* 2003;42:57–63.