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Relationship between plasma *S*-adenosylhomocysteine concentration and glomerular filtration rate in children

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Abstract

S-Adenosylhomocysteine (SAH) is the metabolic precursor of all the homocysteine (Hcy) produced in the body. It is formed by the enzyme SAH hydrolase in a reversible reaction. In a previous study we have shown that plasma SAH is a more sensitive indicator of the risk for cardiovascular disease, and in a second study involving patients with renal disease, we also showed that it is a more sensitive indicator of renal insufficiency than plasma Hcy. However, in the latter study, the patients with renal disease were older and had a variety of other diseases such as diabetes and primary hypertension, which are associated with vascular disease and which could reduce renal function by involvement of the kidneys. Our objective was to rule out these complicating factors as the cause of the elevated SAH in renal disease and determine whether renal insufficiency alone was the cause of the elevated SAH. We therefore measured SAH, Hcy, folate, and vitamin B_{12} in 23 patients between the ages of 1 and 18 years with a wide range of renal function, but who had none of these complicating factors. Glomerular filtration rate (GFR) was calculated using serum creatinine according to the Schwartz formula. None of the children were deficient in folate or vitamin B_{12}. After adjusting for age, folate, and vitamin B_{12}, there was a modest and insignificant decrease of 0.033 μmol/L of Hcy associated with an increase of 1 mL/min of GFR (95% confidence interval, -0.066 to 0.0002). However, there was a strong and statistically significant association between log(SAH) and log(GFR): $P < .0005$, $R^2 = 0.76$. This result suggests that plasma SAH rather than Hcy is the metabolite primarily affected in renal disease. We suggest that plasma Hcy elevations that have been linked to vascular disease may be due to elevated SAH resulting from renal insufficiency.

1. Introduction

Hyperhomocysteinemia has been recognized as a risk factor for vascular disease in a large number of studies. This was first suggested by Wilcken and Wilcken [1] in 1976. Total plasma homocysteine (Hcy) was shown to be an independent risk factor for vascular disease by Clarke et al [2] in 1991. Many subsequent studies have confirmed these results. A review published in 1995 of 27 studies relating Hcy to arteriosclerotic vascular disease concluded that 10% of the population's risk of coronary heart disease could be attributable to elevated plasma Hcy [3]. A more recent meta-analysis by the Homocysteine Studies Collaboration surveyed articles from 1966 through 1998 and included data from 30 prospective or retrospective studies [4]. There was a stronger association in retrospective studies than in prospective studies, and after adjustment for other risk factors, it was concluded that a decrease of total plasma Hcy of 3 μmol/L was associated with an 11% decreased risk of ischemic heart disease and a 19% lower risk of stroke. Another meta-analysis investigated the effect of mutations in the methylenetetrahydrofolate reductase gene on Hcy concentrations and various forms of vascular disease in 72 studies [5]. A common mutation in this gene (about 10% of the population) results in higher Hcy values. They found that these genetic studies together with prospective studies gave similar results, that is, highly significant elevations of

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plasma Hcy in patients with a variety of vascular diseases. These results indicated to them that the association of Hcy with cardiovascular disease (CVD) is causal. There is also evidence that elevated plasma Hcy is a function of the plasma levels of folate, vitamin B_{12}, and vitamin B_6 because they are required in the metabolic disposition of Hcy [6,7]. In the absence of vitamin B_{12} deficiency, elevated concentrations of Hcy are most responsive to folate, and supplemental folic acid can return them to normal [8].

Adults with renal disease have a very high incidence of vascular disease and have high levels of plasma Hcy [9]. Almost all patients with chronic renal failure have Hcy levels that are 3 to 4 times normal [10]. Unlike other patients with hyperhomocysteinemia, administration of supplemental folic acid to patients with renal disease lowers, but does not normalize, plasma Hcy levels [9,11]. In many of the studies relating hyperhomocysteinemia to vascular disease, elevated Hcy was associated with increased plasma creatinine levels [12-18].

In an earlier study, we compared the relative risk of CVD with plasma total Hcy and plasma *S*-adenosylhomocysteine (SAH) in a small number of patients and controls [19]. SAH is the metabolic precursor of Hcy and is formed from SAH by the enzyme SAH hydrolase, an enzyme that is reversible with the equilibrium favoring SAH formation. We found a significant association of CVD with SAH but not with Hcy. We also found a significant association of both Hcy and SAH with serum creatinine [19]. A study in adults with chronic renal disease showed inverse correlations between glomerular filtration rate (GFR), an indicator of renal function, and both SAH and Hcy [20]. That total plasma Hcy is related to renal function has been noted previously [12,21]. Hyperhomocysteinemia was also shown to be more frequent in children with chronic renal failure (66%) than in control children (5%) [22].

A problem with evaluating the significance of elevated plasma Hcy with regard to CVD or renal disease is that there are confounding variables such as dietary factors related to folate, vitamin B_{12}, and vitamin B_6 status. Diseases, such as hypertension and diabetes, and lifestyle parameters, such as smoking [23] and coffee consumption [13], have been shown to increase the risk of vascular disease associated with hyperhomocysteinemia. An ideal population to evaluate both plasma Hcy and plasma SAH concentrations as indicators of renal disease would be one with varying degrees of renal disease, but with none of these other factors associated with increased plasma total Hcy. However, it is extremely difficult to find adult subjects who meet these criteria.

In this study, we sought to determine the relationship between severity of renal disease and plasma Hcy and SAH levels. This task is complicated by the fact that most adult patients with renal disease may also have CVD, hypertension, and/or diabetes that confounds the relation between GFR and these metabolites. For this study, we chose children seen in the pediatric renal clinic at Vanderbilt Medical Center with varying degrees of renal disease, including some who have GFR values in the reference range. All were free of these confounding disorders. This patient population allowed us to elucidate the relationship among Hcy, SAH, and GFR in patients with only decreased renal function.

2. Subjects and methods

2.1. Patient population

Consecutive patients in the Pediatric Nephrology Clinic at the Vanderbilt University Medical Center were enrolled if they were 2 to 18 years and did not have diabetes, primary hypertension, or treatment with dialysis or transplantation. All had scheduled phlebotomies as part of their care. The study was approved by the Vanderbilt University Institutional Review Board, and informed consent was obtained from the parent or a guardian with assent by children if older than 13 years. No control group without renal disease was selected because this study was designed to examine the relationship between Hcy and GFR and between SAH and GFR in the reference range and at different degrees of renal disease.

2.2. Analytical methods

Nonfasting blood was collected in EDTA tubes at the time of scheduled routine phlebotomy associated with the clinic visit. The plasma was kept on ice and separated by centrifugation at 400*g* for 10 minutes within 1 hour of collection. There is no leakage of Hcy from the red cells during this period [24]. Plasma Hcy was measured by the Abbott IMx fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL). This has been shown to be equivalent to the traditional high-performance liquid chromatography assay for Hcy [25]. Plasma *S*-adenosylmethionine (SAM) and SAH were measured by the method of Capdevila and Wagner [26]. Plasma vitamin B_{12} levels were measured by the Abbott IMx microparticle intrinsic factor assay, and plasma folate was measured by the Abbott IMx ion-capture method using folate-binding protein. Creatinine was measured in the Vanderbilt clinical laboratory by a method based on the Jaffe reaction. Height and serum creatinine (S_{cr}) were used to estimate GFR normalized to body surface area, according to the Schwartz formula ($GFR = k \times height/S_{cr}$), where k is a proportionality constant appropriate for age and sex [27].

2.3. Statistical methods

Simple and multiple regression analyses were used to assess the relationship among plasma Hcy, plasma SAH, and GFR. These analyses were adjusted for age, folate, and vitamin B_{12} by including these covariates in our models. We performed regression analysis of log(SAH) plotted against log(GFR) because the relationship between these transformed variables appeared to be linear. This regression was transformed back to the linear scale. Ninety-five percent

confidence bands for these curves were derived using standard techniques [28]. These analyses were done and figures drawn using the Stata software package [29].

3. Results

Table 1 shows the patients' ages, diagnoses, estimated GFRs, and plasma levels of Hcy, SAM, SAH, the ratio of folate, and vitamin B_{12}. None of the patients had primary hypertension, but 8 of the patients were receiving antihypertensive medications at the time of the study; the blood pressure was well controlled in each patient, and all had normal blood pressure on the day of the evaluation. We examined the relationship between renal function (GFR) and both plasma Hcy and plasma SAH. Fig. 1 shows that there is a weak inverse linear correlation between GFR and plasma Hcy for the 23 subjects with varying degrees of renal disease (R^2 = 0.09 with a slope of −0.0377; 95% confidence interval [CI], −0.09 to 0.015). Adjusting this analysis for age, folate, and B_{12} gave very similar results. We measured both plasma folate and vitamin B_{12} in all our patients, and none was found to be deficient in either vitamin. The relationship between plasma SAH and GFR was much stronger (Fig. 2). There was a strong linear association between log(SAH) and log(GFR) The slope coefficient on the log-log scale was −0.976, which is

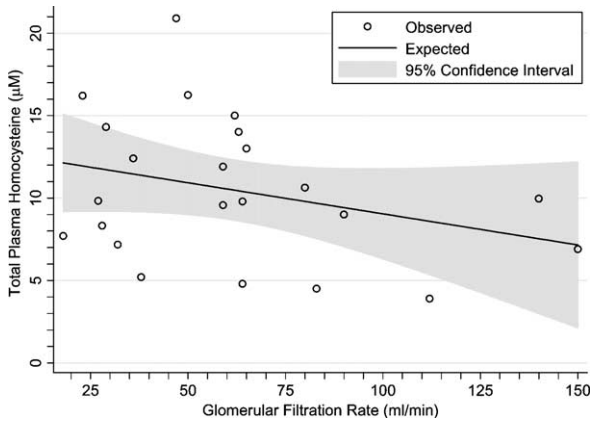

Fig. 1. Homocysteine vs GFR. The shaded area represents the 95% CI.

significantly different from 0 ($P < .0005$; 95% CI, −1.3 to −0.64). Converting this regression back to the linear scale gave the relationship between SAH and GFR to be

$$SAH = 1967 \times (GFR)^{-0.976}.$$

This curve is typical for a metabolite that is primarily filtered by the kidney, and the metabolite concentrations in the plasma increase in an exponential manner as renal function decreases, for example, serum creatinine [30]. When the data were adjusted for differences in age, folate,

Table 1
Characteristics of the patient population

Patient	Age (y)	Diagnosis	GFR (mL/min)	SAM (nmol/L)	SAH[a] (nmol/L)	SAM/SAH	Hcy[a] (μmol/L)	Vitamin B_{12}[a] (pmol/L)	Folate[a] (ng/mL)
01	14	Chronic glomerulonephritis	140	308	27	11.4	10.0	234	10.1
02	4	Nephrotic syndrome	112	108	16	7.2	3.9	992	17.0
03	16	Renal dysplasia	80	579	15	38.6	10.6	313	9.5
04	16	Hydronephrosis	63	90	37	2.4	14.0	470	10.0
05	12	Interstitial nephritis	59	134	25	5.4	9.6	619	12.5
06	17	Focal segmental glomerulosclerosis	64	183	52	3.5	9.8	634	8.4
07	3	Renal dysplasia	28	285	94	3.0	8.3	753	11.1
08	7	Renal dysplasia	18	494	149	3.3	7.7	1480	17.0
09	16	Focal segmental glomerulosclerosis	59	218	63	3.5	11.9	625	7.6
10	15	Obstructive uropathy	65	393	70	5.6	13.0	223	8.6
11	6	Resolved renal tubular acidosis	150	117	20	5.9	6.9	478	10.6
12	15	Hemolytic uremic syndrome	62	117	38	3.1	14.9	393	6.1
13	14	Focal segmental glomerulosclerosis	29	137	82	1.7	14.3	595	6.8
14	1	Renal dysplasia	64	148	21	7.1	4.8	1889	20.0
15	18	Chronic glomerulonephritis	36	119	46	2.6	12.4	2000	18.2
16	5	Obstructive uropathy	90	279	22	12.7	9.0	604	14.5
17	15	Focal segmental glomerulosclerosis	23	163	92	1.8	16.2	428	15.5
18	4	Renal dysplasia	38	163	36	4.7	5.2	1326	17.8
19	17	Obstructive uropathy	47	119	43	2.8	20.9	630	7.7
20	5	Renal dysplasia	32	235	63	3.7	7.2	760	15.6
21	11	Renal dysplasia	27	273	131	2.1	9.8	493	7.9
22	15	Recurrent pyelonephritis	50	71	17	4.2	16.2	345	5.8
23	3	Neonatal ischemia	83	88	21	4.2	4.5	1171	17.1

[a] All metabolite concentrations were measured in plasma.

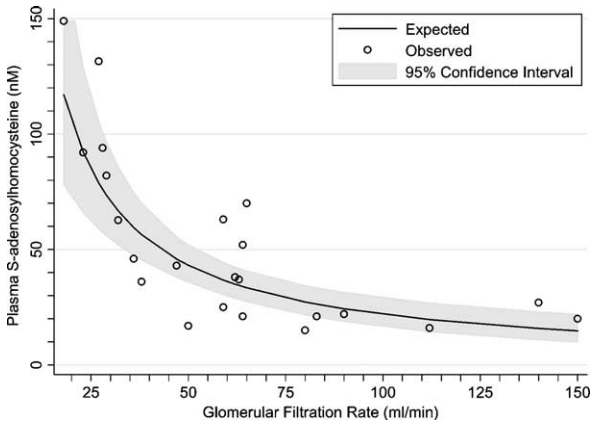

Fig. 2. SAH vs GFR. The shaded area represents the 95% CI. We used linear regression to model log(SAH) concentrations as a function of GFR. Converting both sides of this regression equation to exponential equivalents gives SAH = α GFR$^{\beta}$, which is the plotted curve.

and vitamin B_{12} status, there was no statistical difference in the results for Hcy vs GFR or log(SAH) vs log(GFR).

4. Discussion

SAH is the precursor of all the Hcy produced in the body. Unlike elevated plasma Hcy, for which there is no generally accepted mechanism for the pathophysiology of vascular disease, SAH is a potent end-product inhibitor of most methyltransferase reactions [31]. Red-cell SAH levels were found to be significantly elevated in patients with chronic renal failure, and it was suggested that elevated red-cell SAH was responsible for decreased methyl esterification of membrane proteins [32]. These altered methylations were attributed to elevated SAH resulting from elevated Hcy, although plasma SAH was not measured.

It is very difficult to measure the impact of renal disease on plasma total Hcy in an adult population because of other factors that develop with age and may affect Hcy levels. From 1000 patients with renal insufficiency, Arnadottir et al [33] selected for studies 77 patients who were free of diabetes or other endocrine disease, obesity, liver disease, cancer, active inflammatory disease, recent infection, abnormalities of fluid balance, moderate-severe proteinuria, and treatment with steroids, cyclosporin, or vitamin B_{12}. However, among these 77 patients, individuals with hypertension or CVD were not excluded. In these patients, Hcy was inversely correlated with GFR, serum creatinine, and red-cell folate.

In view of the of the problems associated with vascular disease in adults, we decided to measure plasma SAH and plasma Hcy in children. Merouani et al [22] carried out a study involving Hcy in children with chronic renal failure, but only examined the relationship between total plasma Hcy and age, vitamin B_{12}, and folate. They found that Hcy was positively correlated with age and negatively correlated with vitamin B_{12} and folate in patients. In our pediatric population, the levels of plasma folate are all within normal limits, almost certainly because of the fortification of grain products with folic acid [8]. As might be expected in a group of pediatric patients without gastrointestinal or nutritional disease, the plasma levels of vitamin B_{12} were all within normal limits. Thus, we studied renal disease in a population with normal folate and vitamin B_{12} status who did not have other factors known to influence plasma Hcy, including primary hypertension, diabetes, and CVD. In these patients without other confounding factors, our data show that SAH was highly correlated with the GFR, whereas Hcy was not.

The role of the kidney in the metabolism of methionine and Hcy has been the subject of much recent investigation [9]. Contrary to the situation in rat kidney, there appears to be no net extraction of Hcy across the normal human kidney [34]. A stable-isotope study was conducted [34] comparing the routes of methionine metabolism in healthy subjects and patients with end-stage renal disease. The results showed impaired metabolic clearance of Hcy by both trans-sulfuration to cystathionine and remethylation to methionine [35,36]. They also measured remethylation and transmethylation flux rates, which showed inverse correlations between these fluxes and the concentration of SAH in the red cells. Although the authors did not emphasize it, these inverse relationships also extended to plasma SAH levels, indicating considerable metabolism of methionine in the kidney.

A surprising observation of our study is the weak inverse relationship between plasma Hcy and GFR despite the many studies that have shown a markedly elevated level of plasma Hcy in patients with renal disease (through the action of the enzyme SAH hydrolase). SAH is the precursor of all the Hcy produced in the body. SAH hydrolase cleaves SAH with the formation of Hcy and adenosine. SAH hydrolase is reversible and the equilibrium favoring SAH formation; however, adenosine deaminase in tissues serves to pull the reaction toward Hcy synthesis. For this reason, it has generally been assumed that elevation of Hcy as a result of decreased remethylation or decreased trans-sulfuration due to enzymatic or nutritional inadequacies would eventually lead to the reversal of SAH hydrolase and the elevation of SAH. We have shown previously that plasma SAH and Hcy levels are correlated in patients with CVD, but only barely so in control subjects (P = .049) [19]. In a previous study [20], we measured, but did not report, a strong correlation between plasma Hcy and SAH in patients with renal disease. There was no correlation between these 2 parameters in control subjects. In the present study, plasma SAH levels were greatly elevated when renal function was diminished but Hcy levels were only slightly elevated (Figs. 1 and 2, respectively). It appears that only when the values for plasma Hcy are very high (as in adult patients with renal disease) is the activity of SAH hydrolase reversed to bring about an increase in plasma SAH values. It is surprising that SAH and Hcy were not similarly elevated. Hcy is not excreted in the urine to any great extent because

most of it is bound to plasma proteins [37]. A similar discrepancy between changes in plasma SAH and Hcy comes from a study by Becker et al [38] in which they show that plasma levels of SAH are not associated with folate, cobalamin, and vitamin B_6 concentrations, although levels of Hcy are. The reason for the seeming lack of coordination between the 2 metabolites may be the entry of SAH and Hcy into the blood. It is possible that SAH and Hcy equilibrate rapidly in tissues. However, if SAH enters the blood more rapidly than Hcy, then the plasma SAH levels would be expected to be relatively elevated compared with plasma Hcy levels.

SAH is a toxic metabolite leading to endothelial dysfunction and vascular disease [39]. In addition, SAH is cleared by the kidney [40]. If reduced kidney function results in higher plasma SAH levels, this would also lead to elevated plasma Hcy. Our data suggest that renal insufficiency by itself, in the absence of folate deficiency, vitamin B_{12} deficiency, diabetes mellitus, and atherosclerotic CVD, can account for elevated levels of plasma SAH and perhaps, by extension, elevated Hcy. Our results suggest that Hcy elevations that have been linked to vascular disease may reflect an elevated Hcy secondary to an elevated SAH resulting from the renal insufficiency associated with vascular disease involving the kidneys.

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References

[1] Wilcken DE, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. J Clin Invest 1976; 57:1079-82.

[2] Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991;324:1149-55.

[3] 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 [see comments]. JAMA 1995;274:1049-57.

[4] Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 2002;288:2015-22.

[5] Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325: 1202-9.

[6] Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. J Lab Clin Med 1989;114:473-501.

[7] Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, et al. Relationship between plasma homocysteine, vitamin status and extracranial carotid-artery stenosis in the Framingham Study population. J Nutr 1996;126(4 Suppl):1258S-65S.

[8] Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations [see comments]. N Engl J Med 1999;340: 1449-54.

[9] van Guldener C, Robinson K. Homocysteine and renal disease. Semin Thromb Hemost 2000;26:313-24.

[10] Suliman ME, Lindholm B, Barany P, Bergstrom J. Hyperhomocysteinemia in chronic renal failure patients: relation to nutritional status and cardiovascular disease. Clin Chem Lab Med 2001;39: 734-8.

[11] De Vriese AS, Verbeke F, Schrijvers BF, Lamiere NH. Is folate a promising agent in the prevention and treatment of cardiovascular disease in patient with kidney failure? Kidney Int 2002;61:1199-209.

[12] Kang SS, Wong PW, Cook HY, Norusis M, Messer JV. Protein-bound homocyst(e)ine. A possible risk factor for coronary artery disease. J Clin Invest 1986;77:1482-6.

[13] Nygard O, Refsum H, Ueland PM, Stensvold I, Nordrehaug JE, Kvale G, et al. Coffee consumption and plasma total homocysteine: the Hordaland Homocysteine Study. Am J Clin Nutr 1997;65: 136-43.

[14] Bortolotto LA, Safar ME, Billaud E, Lacroix C, Asmar R, London GM, et al. Plasma homocysteine, aortic stiffness, and renal function in hypertensive patients. Hypertension 1999;34:837-42.

[15] Ventura P, Panini R, Verlato C, Scarpetta G, Salvioli G. Hyperhomocysteinemia and related factors in 600 hospitalized elderly subjects. Metabolism 2001;50:1466-71.

[16] Jager A, Kostense PJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM, et al. Serum homocysteine levels are associated with the development of (micro)albuminuria: the Hoorn Study. Arterioscler Thromb Vasc Biol 2001;21:74-81.

[17] Henry RM, Kostense PJ, Bos G, Dekker JM, Nijpels G, Heine RJ, et al. Mild renal insufficiency is associated with increased cardiovascular mortality: the Hoorn Study. Kidney Int 2002;62: 1402-7.

[18] Clarke R, Lewington S, Landray M. Homocysteine, renal function, and risk of cardiovascular disease. Kidney Int Suppl 2003;S131-3.

[19] Kerins DM, Koury MJ, Capdevila A, Rana S, Wagner C. Plasma *S*-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine. Am J Clin Nutr 2001;74: 723-9.

[20] Wagner C, Stone WJ, Koury MJ, Dupont WD, Kerins DM. *S*-adenosylhomocysteine is a more sensitive indicator of renal insufficiency than homocysteine. Nutr Res 2004;24:487-94.

[21] Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 2000; 72:324-32.

[22] Merouani A, Lambert M, Delvin EE, Genest Jr J, Robitaille P, Rozen R. Plasma homocysteine concentration in children with chronic renal failure. Pediatr Nephrol 2001;16:805-11.

[23] Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 1997;277: 1775-81.

[24] Stehouwer CD, Gall M-A, Hougaard P, Jacobs C, Parving H-H. Plasma homocysteine concentration predicts mortality in non–insulin-dependent diabetic patients with and without albuminuria. Kidney Int 1999;55:308-14.

[25] Nexo E, Engbaek F, Ueland PM, Westby C, O'Gorman P, Johnston C, et al. Evaluation of novel assays in clinical chemistry: quantification of plasma total homocysteine. Clin Chem 2000;46:1150-6.

[26] Capdevila A, Wagner C. Measurement of plasma *S*-adenosylmethionine and *S*-adenosylhomocysteine as their fluorescent isoindoles. Anal Biochem 1998;26:180-4.

[27] Schwartz GJ, Haycock GB, Edelmann Jr CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976;58:259-63.

[28] Dupont WD. Statistical modeling for biomedical researchers: a simple introduction to the analysis of complex data. Cambridge: Cambridge University Press; 2002.

[29] StataCorp. Stata statistical software: release 8.0. College Station (Tex): Stata Corporation; 2003.

[30] Greger R. Renal handling of the individual solutes of glomerular filtrate. In: Greger R, Windhorst U, editors. Comprehensive human physiology. Berlin: Springer-Verlag; 1996. p. 1540.

[31] Clarke S, Banfield K. *S*-adenosylmethionine–dependent methyltransferases. In: Carmel R, Jacobsen DW, editors. Homocysteine in health and disease. Cambridge (UK): Cambridge University Press; 2001. p. 63-78.

[32] Perna AF, Ingrosso D, Zappia V, Galletti P, Capasso G, De Santo NG. Enzymatic methyl esterification of erythrocyte membrane proteins is impaired in chronic renal failure. Evidence for high levels of the natural inhibitor *S*-adenosylhomocysteine. J Clin Invest 1993; 91:2497-503.

[33] Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysell H. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. Scand J Clin Lab Invest 1996;56:41-6.

[34] 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;54:166-9.

[35] van Guldener C, Kulik W, Berger R, Dijkstra DA, Jakobs C, Reijngoud DJ, et al. Homocysteine and methionine metabolism in ESRD: a stable isotope study. Kidney Int 1999;56:1064-71.

[36] Stam F, vanGuldener C, ter Wee PM, Kulik W, Smith DE, Jakobs C, et al. Homocysteine clearance and methylation flux rates in health and end-stage renal disease: association with *S*-adenosylhomocysteine. Am J Physiol Renal Physiol 2004;287:F215-23.

[37] Jacobsen DW. Homocysteine and vitamins in vascular disease. Clin Chem 1998;44:1833-43.

[38] Becker A, Smulders YM, Teerlink T, Struys EA, de Meer K, Kostense PJ, et al. *S*-adenosylhomocysteine and the ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine are not related to folate, cobalamin and vitamin B6 concentrations. Eur J Clin Invest 2003;33:17-25.

[39] Wang H, Yoshizumi M, Lai K, Tsai JC, Perrella MA, Haber E, et al. Inhibition of growth and p21ras methylation in vascular endothelial cells by homocysteine but not cysteine. J Biol Chem 1997;272:25380-5.

[40] Stabler SP, Allen RH. Quantification of serum and urinary *S*-adenosylmethionine and *S*-adenosylhomocysteine by stable-isotope–dilution liquid chromatography–mass spectrometry. Clin Chem 2004;50:365-72.