*Kidney International, Vol. 52 (1997), pp. 10–20*

PERSPECTIVES IN BASIC SCIENCE

# Hyperhomocysteinemia in end-stage renal disease: Prevalence, etiology, and potential relationship to arteriosclerotic outcomes

**ANDREW G. BOSTOM and LORI LATHROP**

*Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts New England Medical Center, Boston, Massachusetts, USA*

The end-stage renal disease (ESRD) population requiring chronic maintenance dialysis or renal transplantation is growing at an exponential rate, with over 62,000 incident (newly developed ESRD) cases reported per year in the United States [1]. This patient population experiences an excess morbidity and mortality due to arteriosclerotic cardiovascular disease (CVD) outcomes [1–5]. Specifically, event rates for myocardial infarction and stroke are 5- to 10-fold higher in ESRD versus general populations [1–5]. Traditional CVD risk factors, such as smoking, hypertension, glucose intolerance/diabetes, and dyslipidemia [6], despite their widespread prevalence [7], are relatively limited predictors of CVD-specific morbidity and mortality in the ESRD population [7–11]. Wu [10], for example, reported that the two-year cumulative incidence of *de novo* coronary heart disease (CHD) in ESRD patients undergoing chronic peritoneal (18.0%) or hemodialysis (15.0%), exceeded the six-year cumulative incidence (12.7%) of *de novo* CHD among original Framingham Study cohort participants similarly free of baseline CHD, and frequency matched to the dialysis patients for prevalence of the established CVD risk factors.

Recently, controlled evidence [12] has been provided that hyperhomocysteinemia, that is, elevated total plasma levels of the atherothrombotic sulfur amino acid homocysteine [13, 14], occurs more commonly than any of the traditional CVD risk factors in ESRD patients on maintenance dialysis. Initial follow-up data further suggest that the markedly elevated fasting plasma total homocysteine (tHcy) levels found in these dialysis-dependent ESRD patients contributes independently to their excess incidence of fatal and non-fatal CVD outcomes [15]. Confirmation of these prospective findings in more sizable ESRD cohorts is urgently required.

This review will first place hyperhomocysteinemia in the context of the other established amino acid abnormalities characteristic of ESRD. What is known from studies of general and ESRD populations about the prevalence and pathogenesis of hyperhomocysteinemia will then be summarized, followed by discussions of the epidemiological and experimental evidence linking hyperhomocysteinemia to arteriosclerotic outcomes, and an overview of Hcy-lowering therapeutic interventions.

**Key words:** end-stage renal disease, hyperhomocyteinemia, arteriosclerosis, cardiovascular disease risk factor.

Received for publication October 16, 1997
and in revised form November 20, 1996
Accepted for publication November 27, 1996



## AMINO ACID ABNORMALITIES IN ESRD

Detailed, elegant studies of amino acid metabolism (that is, relying upon direct, *in vivo* measurement of arteriovenous amino acid concentration differences across the kidneys, along with simultaneous determination of renal plasma flow, urine flow, and urinary amino acid concentration) have been reported by Brosnan [16] and Tizianello and colleagues [17]. These data have highlighted the major "unexpected" [16] role normally played by the kidneys in amino acid metabolism. As a consequence, significant, predictable changes in plasma amino acid levels accompany chronic renal insufficiency/ESRD-induced alterations of normative renal amino acid metabolism. For example, measurement of arteriovenous differences for free amino acids across normative kidneys reveals that glycine and citrulline are removed, and serine and arginine added to the circulation [16, 17]. Under conditions requiring the excretion of substantial quantities of acid (such as diabetes, high intakes of dietary protein), large amounts of glutamine, the major precursor of urinary ammonia, are taken up [16]. Not surprisingly, the uptake of glycine and citrulline, and the release of serine and arginine are all reduced in chronic renal insufficiency [17]. Furthermore, serine and arginine levels are lower, while the levels of glycine and citrulline tend to be higher, in patients with chronic renal insufficiency or ESRD [18, 19]. Recently, in collaboration with Dr. Brosnan's group, we provided the first *in vivo* demonstration that there is consistent, significant net renal uptake and metabolism of Hcy among post-absorptive state rats [20]. How these findings may be relevant to the B-vitamin refractory hyperhomocysteinemia frequently observed in ESRD will be elaborated in a subsequent section.

## DETERMINANTS OF PLASMA/SERUM HOMOCYSTEINE LEVELS, AND THE PREVALENCE AND ETIOLOGY OF HYPERHOMOCYSTEINEMIA

### General populations

Homocysteine is a non-protein forming, sulfur amino acid whose metabolism is at the junction of two metabolic pathways [21]: remethylation and transsulfuration (Fig. 1). In remethylation, homocysteine acquires a methyl group from N-5-methyltetrahydrofolate (MTHF) or from betaine to form methionine. The reaction with MTHF occurs in all tissues and is vitamin B12-dependent, while the reaction with betaine is confined mainly to

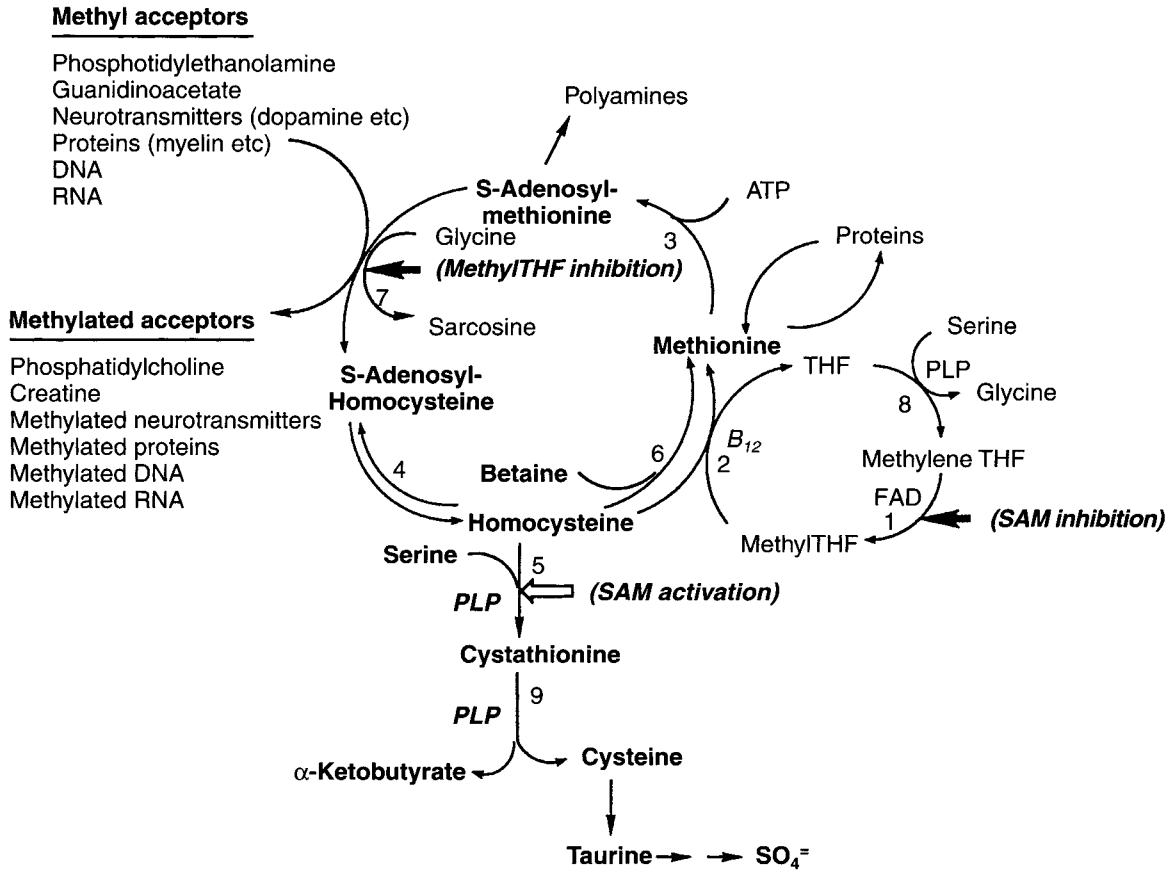


**Fig. 1. Homocysteine metabolism.** Enzyme reactions that are regulated by S-adenosylmethionine (SAM) and 5-methyltetrahydrofolate (Methy-THF) are indicated by large arrows. Open arrows indicate activation, closed arrows indicate inhibition. Enzymes: (1) 5,10 methylenetetrahydrofolate reductase; (2) methionine synthase; (3) S-adenosylmethionine synthase; (4) S-adenosylhomocysteine hydrolase; (5) cystathionine beta synthase; (6) betaine:homocysteine methyltransferase; (7) glycine N-s; (8) serine hydroxymethylase; (9) cystathionase. (Reprinted with permission from: Folic acid (chapt 21), in *Present Knowledge in Nutrition*, edited by SELHUB J, ROSENBURG IH, International Life Sciences Institute, 1996, p 209).

the liver (and kidneys, in humans [22]), and is vitamin B12-independent. A considerable proportion of methionine is then activated by ATP to form S-adenosylmethionine (SAM). SAM serves primarily as a universal methyl donor to a variety of acceptors including guanidinoacetate (for creatine-creatinine synthesis), nucleic acids, neurotransmitters, phospholipids, and hormones. S-adenosylhomocysteine (SAH), the byproduct of these methylation reactions, is subsequently hydrolyzed, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer.

In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal-5′-phosphate (PLP)-containing enzyme, cystathionine beta synthase (CBS). Cystathionine is hydrolyzed by a second PLP-containing enzyme, cystathionase, to form cysteine and alpha-ketobutyrate. Excess cysteine is oxidized to taurine and inorganic sulfates or excreted in the urine. Thus, in addition to the synthesis of cysteine, the transsulfuration pathway effectively catabolizes excess homocysteine which is not required for methyl transfer. Homocysteine is not a normal dietary constituent, so the sole source of homocysteine is methionine. Finally, it is important to understand that valid, modern assays of routinely handled fresh blood samples, and all short- or long-term frozen specimens, measure total homocysteine in plasma or serum [23]. Approximately 70 to 80% of circulating plasma/serum total homocysteine (tHcy) is bound to large proteins (such as albumin [23]), the remainder consisting of a "free" acid-soluble fraction, that is, reduced Hcy (< 1%), homocystine disulfide, and the predominant non protein-bound forms, Hcy-mixed disulfides [23].

Due to the existence of a cellular homocysteine export mechanism, plasma normally contains a small amount of homocysteine averaging 10 μmol/liter [23]. This export mechanism complements the catabolism of homocysteine through transsulfuration; together these mechanisms help maintain low intracellular concentrations of this potentially cytotoxic sulfur amino acid. In hyperhomocysteinemia, plasma homocysteine levels are elevated and, barring renal insufficiency, the occurrence of hyperhomocysteinemia indicates that homocysteine metabolism has in some way been disrupted, causing the export mechanism to dispose into the blood excess homocysteine that has accumulated in the cell. This limits intracellular toxicity, but leaves vascular tissue exposed to the possibly deleterious effects of excess homocysteine.

The more severe cases of hyperhomocysteinemia are due to homozygous defects in genes encoding for enzymes of homocysteine metabolism. In such cases, a defective enzyme involved in either homocysteine remethylation or transsulfuration leads to

large elevations of homocysteine in the blood and urine. The classic form of such a disorder is that caused by homozygosity for a defective gene encoding for cystathionine beta synthase (CBS), a condition in which fasting plasma homocysteine concentrations can be as high as 400 μmol/liter [24]. Homozygous defects of other genes that lead to similar elevations in plasma homocysteine concentration include those encoding for methylenetetrahydrofolate reductase (MTHFR) [25] or for any of the enzymes that participate in the synthesis of methylated vitamin B12 [26]. Recently the cDNA has been isolated, and point mutations described, for the vitamin B12-dependent enzyme methionine synthase (Dr. R. Rozen, personal communication). However, the functional impact on enzyme activity and/or the clinical relevance of these mutations has not been elucidated.

Most cases of hyperhomocysteinemia are moderate in character, and may be due to defects in genes encoding for Hcy-metabolizing enzymes or from deficiencies in vitamins that are involved in Hcy metabolism. Plasma Hcy concentrations in these instances may differ depending on which arm of the two metabolic pathways of homocysteine metabolism is defective. (For full details see [21])

(1.) An impairment in the remethylation pathway, even if it is mild, will lead to a *substantial increase* in plasma homocysteine concentrations under fasting conditions. Such an impairment may be due to defects in the gene encoding for MTHFR or deficiencies of either folate or vitamin B12 [21].

(2.) In contrast, a mild impairment in the transsulfuration pathway will lead, at most, to a very *slight increase* in fasting plasma homocysteine levels. This mild impairment, which may be due to heterozygous defects in the CBS gene or deficiency in vitamin B6 [21], is normally identified by the methionine loading test [21]. As Brattstrom, Israelsson and Hultberg first noted [27], however, it is critical to define the post-methionine loading (PML) response based on the increase in tHcy above fasting levels, to avoid confounding by fasting hyperhomocysteinemia.

Studies conducted in vitamin deficient animal models support this hypothesis. Miller and colleagues have shown that fasting plasma homocysteine concentrations are 10-fold higher in folate deficient rats than in folate supplemented rats, and that these high concentrations of tHcy in plasma are in part due to lack of sufficient S-adenosylmethionine for the activation of the transsulfuration pathway [28]. A second study demonstrated that after methionine loading, the tHcy concentrations increased 35-fold in rats that were vitamin B6 deficient compared to about fourfold in control rats and less than 35% in folate deficient rats [29]. Preliminary data from 274 participants in the Family Heart Study provide human evidence to further support the hypothesis of two distinct forms of hyperhomocysteinemia [30]. For each participant, plasma tHcy concentrations were determined under fasting conditions, and four hours after a methionine load. Using the 90% percentile values to define hyperhomocysteinemic (both fasting and 4-hour PML), it was demonstrated that out of 43 hyperhomocysteinemia individuals, 20 (46%) had fasting hyperhomocysteinemia only, 17 (39.5%) had PML hyperhomocysteinemia only and only 7 (14%) had both fasting and PML hyperhomocysteinemia. Following validation of an abbreviated (that is, 2 hour) protocol [31] these analyses were replicated in 294 individuals who underwent two-hour PML tests, and the results obtained were identical (Dr. A. Bostom, unpublished data).

Both genetic disorders and nutritional status may influence the prevalence of hyperhomocysteinemia, but there are few data to indicate how important these determinants might be in the general population. The independent associations between individual nutrients and non-fasting tHcy concentrations in an established cohort of Americans (the Framingham Study cohort) were first examined by Selhub and colleagues [32]. Approximately two-thirds of all cases of elevated homocysteine levels (based on non-fasting samples) were associated with inadequate status of one or more of the nutritional co-factors/substrates involved in Hcy metabolism, suggesting that the most important cause of hyperhomocysteinemia, from a public health standpoint, may be inadequate nutritional status. More recently, it was shown that nutritional status modifies the relationship between a genetic variant of an enzyme involved in the remethylation pathway of Hcy metabolism, and fasting tHcy levels [33]. This common variant of methylenetetrahydrofolate reductase (MTHFR), which has a lower specific activity and greater sensitivity to heat inactivation (hence the name thermolabile MTHFR), is the result of a C to T transition at nucleotide 677 of the MTHFR gene [34, 35]. The C677T transition results in an alanine (ala) to valine (val) substitution in the expressed enzyme, and has an allele frequency of about 35%, and homozygote frequency of about 12% [33, 35]. A study of 365 participants in the NHLBI Family Heart Study revealed that homozygotes (val-val) for the ala to val substitution had higher geometric mean fasting plasma tHcy levels than (ala-val) heterozygotes or homozygotes (ala-ala) for the normal enzyme when folate status was below the median ($< 6.8$ ng/ml), but no differences were detected between individuals with different genotypes when folate status was at the median or above ($\geq 6.8$ ng/ml). In accord with the hypothesis that remethylation defects have no significant independent effect on PML tHcy levels, there was no difference in the geometric mean PML increase in tHcy levels among the different MTHFR genotype groups [33].

Creatinine [36–38] and albumin [39] are two additional, independent determinants of tHcy levels in general populations, unrelated to B-vitamin status. The generation of s-adenosylhomocysteine from s-adenosylmethionine is coupled to creatine-creatinine synthesis [40], which likely accounts for the direct association observed between creatinine and fasting tHcy levels in persons with normative renal function [36–39]. As noted earlier, 70 to 80% of serum/plasma tHcy is protein-bound, most likely to albumin [23], which may account for the direct relationship observed between albumin and tHcy levels in the general populations [39]. Finally, acute reductions in plasma tHcy documented in patients hospitalized for cerebral infarctions are believed to be linked to parallel decreases in albumin [41].

### ESRD populations

Seventeen independent investigations [42–58] published between 1972 and 1996 have documented markedly increased plasma or serum levels of free, protein-bound, or tHcy among ESRD patients in the pre-dialysis phase, while undergoing maintenance dialysis, and following renal transplantation. Reports by our group were the first studies documenting the prevalence of hyperhomocysteinemia in dialysis-dependent ESRD patients relative to age, sex, and race matched, population-based controls free of clinical renal disease, whose serum creatinine levels were 1.5 mg/dl or less [12, 54]. These data (also, Fig. 2 and Table 1) indicated that hyperhomocysteinemia (fasting tHcy levels $> 13.9$

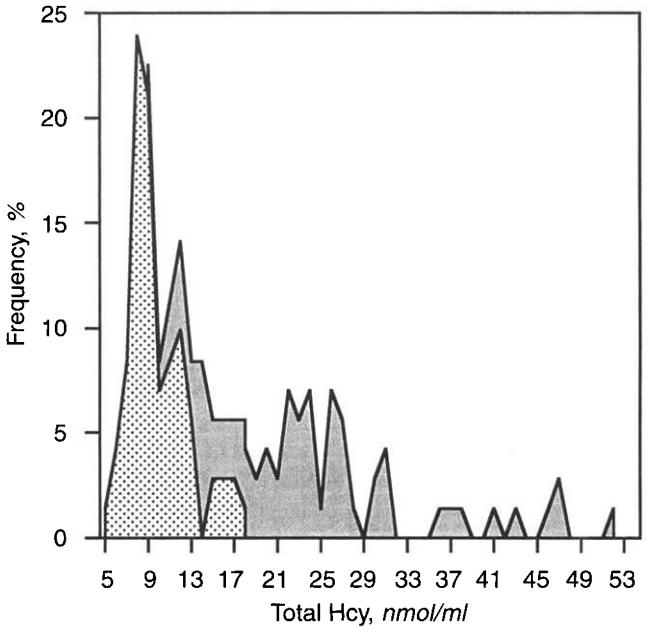


**Fig. 2. Distributions of fasting tHcy levels in ($N = 71$) dialysis-dependent ESRD patients, and ($N = 71$) age, sex, and race matched Framingham Offspring/Omni Study controls free of renal disease, with serum creatinine levels ≤1.5 mg/dl.** Data are from Bostom et al [12]. Symbols are: (▨) control; (■) ESRD.

μmol/liter, the 90th percentile control value) occurred in 83% of the dialysis patients, a 105-fold increased risk (matched prevalence odds) relative to the controls. Three basic questions arise from these findings:

(1.) Are the major established determinants of tHcy levels in general populations equally important in ESRD populations?

(2.) Are there significant determinants of tHcy levels specific to the ESRD population, related, for example, to the etiology and treatment of ESRD?

(3.) Are there unique pertubations of normal tHcy metabolism in ESRD that account for the excess prevalence of hyperhomocysteinemia observed in this patient population?

Dialysate losses of water-soluble vitamins, including the B-vitamins, and restricted overall dietary intake patterns, are the rationale for the clinical practice of providing routine multivitamin supplementation to dialysis-dependent ESRD patients [59]. Additional evidence suggests that uremia may result in increased hydrolysis of plasma PLP, reducing the supply of this coenzyme to peripheral tissues [60, 61]. In the clinical setting, therefore, investigators are confronted with a population who, although at heightened risk for deficiencies in Hcy-metabolizing coenzymes/substrates, is most often receiving routine supplementation with greater than RDA (recommended daily allowance) amounts of these micronutrients (that is, folic acid, B-6, and B-12). Awareness of these opposing influences on plasma B-vitamin status is important when evaluating studies of homocysteinemia in clinical ESRD populations.

Wilcken and Gupta [44] made the initial observation that cysteine-homocysteine mixed disulfide (MDS) levels were directly correlated with serum creatinine levels in $N = 22$ patients with mild to severe renal impairment (that is, serum creatinine 130 to 1160 μmol/liter). Circulating levels of folate, PLP, or B-12 were not determined, but the authors noted that all patients were receiving supplements containing these vitamins, and they concluded it was "unlikely" that the observed increase in cysteine-homocysteine MDS was related to "shortages of these cofactors." In a related study of 27 stable renal transplant recipients, Wilcken and colleagues [46] reported similar findings regarding the association between creatinine and cysteine-homocysteine MDS within a relatively narrower range of serum creatinine, consistent with less impaired renal function (that is, serum creatinine of ~100 to 500 μmol/liter). Short-term (4-weeks) folic acid-based B-vitamin supplementation, given as part of an uncontrolled, open-label subgroup study of 11 arbitrarily-selected renal transplant recipients, reduced fasting cysteine-homocysteine MDS levels by 41%. Wilcken, Gupta and Betts [46] concluded that "relative shortages of folic acid" might have contributed to the "moderately elevated" cysteine-homocysteine MDS levels they observed in these patients. Hultberg, Andersson and Sterner [50] assessed the crude correlations between fasting tHcy and serum B-12, red blood cell folate, serum creatinine, and other routine biochemical parameters in ESRD patients in the pre-dialysis phase ($N = 30$), on maintenance peritoneal dialysis ($N = 17$), and maintenance hemodialysis ($N = 29$). They reported the following significant crude rank-order (Spearman's rho) correlations: serum creatinine in the pre-dialysis stage ESRD patients ($r = +0.70, P < 0.001$), and CAPD patients ($r = +0.49, P < 0.05$); red blood cell folate in the pre-dialysis stage ESRD patients ($r = -0.43, P < 0.05$), and CAPD patients ($r = -0.59, P < 0.05$); serum albumin in the predialysis stage ESRD patients ($r = +0.39, P < 0.05$) and hemodialysis patients ($r = +0.39, P < 0.05$); and serum B-12 in the CAPD patients only ($r = -0.49, P < 0.05$). Using general linear modeling with analysis of covariance, we [62] performed a comprehensive assessment of the potential determinants of fasting plasma tHcy levels in 75 maintenance dialysis patients ($N = 50$ on hemodialysis; $N = 25$ on CAPD). Approximately 95% of these patients were prescribed 1 to 2 mg of folic acid, 10 mg of B-6, and 12 μg of B-12 daily. Fasting tHcy, folate, PLP, B-12, creatinine, glucose, and total and HDL-cholesterol levels were determined, along with measures of dialysis adequacy/residual renal function.

**Table 1.** Total homocysteine (tHcy) and B-vitamin levels in maintenance dialysis patients, renal transplant recipients, and their respective, matched population-based control groups

| | MDP ($N = 71$) | MDP controls ($N = 71$) | $P$ value[a] | RTR ($N = 29$) | RTR controls ($N = 58$) | $P$ value[b] |
|---|---|---|---|---|---|---|
| tHcy *μmol/liter* | | | | | | |
| -fasting | 21.7[c] | 9.6 | <0.001 | 18.1 | 9.8 | <0.001 |
| -PML[d] | — | — | — | 22.0 | 15.2 | 0.001 |
| Folate *ng/ml* | 21.3 | 7.6 | <0.001 | 3.2 | 7.0 | 0.019 |
| PLP *pmol/ml* | 59.0 | 65.9 | 0.474 | 40.3 | 54.4 | 0.073 |
| B-12 *pg/ml* | 632.5 | 421.4 | <0.001 | 426.0 | 367.3 | 0.072 |

Abbreviations are: MDP, maintenance dialysis patients; RTR, renal transplant recipients.

[a] Based on paired $t$-test of geometric means, MDP vs. age, sex, and race matched (1:1) controls free of renal disease

[b] Based on conditional analysis of variance comparisons of geometric means, RTR vs. age and sex matched (2:1) controls free of renal disease

[c] Geometric means

[d] Post-methionine load increase in tHcy above fasting levels (from Bostom et al [12].

Etiology of ESRD, presence of clinical cardiovascular disease (CVD), the traditional CVD risk factors (such as, smoking, hypertension, diabetes/glucose intolerance, dyslipidemia, age, and sex), the C677T transition in the MTHFR gene, and length of time since first initiation of dialysis, were also ascertained. Consistent with findings from the investigation of Hultberg and colleagues [50], we observed unadjusted correlations between fasting plasma tHcy and plasma folate, B-12, and serum creatinine. We also reported the first crude correlation between plasma PLP status, and fasting tHcy levels in this patient population. Multivariable-adjusted analyses, however, revealed that plasma folate status (into the supernormal range) and serum creatinine were the only significant, independent predictors of fasting tHcy levels. Subsequent analyses in which albumin levels were added to these models confirmed that serum albumin was also an independent determinant of fasting tHcy levels (Dr. A. Bostom, unpublished data). Presence of the C677T transition in MTHFR (that is, pooled CT heterozygotes and TT homozygotes, vs. CC wild types) appeared to interact with folate status to increase fasting tHcy levels only in those patients with folate levels below the median [62]. Larger, preliminary analyses including MTHFR genotype data from twice as many subjects ($N = 140$ vs. $N = 68$) suggest that the "true" interaction may be occurring only in MTHFR TT homozygotes who have folate levels below the general population median (< 6.8 ng/ml) as opposed to below the dialysis population median (< 20 to 30 ng/ml; Dr. A. Bostom, unpublished data). We also provided the initial data regarding potential determinants of homocysteinemia unique to the dialysis-dependent ESRD population, such as the cause of ESRD, and the effect of treatment modality or adequacy [62]. There was no evidence that either the etiology of ESRD, the length of time since first initiation of dialysis, dialysis mode (that is, standard 3 times weekly hemodialysis vs. CAPD), or measures of dialysis adequacy (urea reduction ratios) or residual renal function (weekly creatinine clearance), have any significant, independent effect on chronic tHcy levels in maintenance dialysis patients [62]. Finally, although hemodialysis results in a transient reduction of plasma tHcy of ~30% [50, 63, 64], consistent with removal of the nonprotein-bound, dialyzable fraction of Hcy, there is a rapid return to predialysis tHcy levels within 24 hours [50].

Limited data are available on the prevalence and determinants of hyperhomocysteinemia in stable renal transplant recipients [46, 53, 57]. We recently provided more adequately controlled confirmation of these earlier reports [46, 53, 57] describing an increased prevalence of fasting hyperhomocysteinemia in renal transplant recipients (Table 1; **Note added in proof, A**). In addition, our study provided the first documentation of an apparent excess prevalence of PML hyperhomocysteinemia (matched odds ratio 6.9), and combined fasting and PML hyperhomocysteinemia (matched odds ratio 18.0) in renal transplant recipients versus age and sex-matched population-based controls with normative renal function. These data also suggested that suboptimal folate and B-6 status are common in renal transplant recipients (who, unlike maintenance dialysis patients, are *not* routinely supplemented with these vitamins), confirming two reports from the early 1980s [65, 66]. Furthermore, our findings indicated that residual renal function may be a particularly crucial determinant of homocysteinemia in renal transplant recipients, both under fasting conditions, and PML.

Wilcken and Gupta [44] originally suggested that the etiology of hyperhomocysteinemia in ESRD might be due to loss of urinary Hcy excretion, or the adverse effect of uremia on Hcy-metabolizing enzymes. Noting that normal kidneys release approximately 4 g of serine per day into the circulation [16], and ESRD patients have significantly depressed plasma serine levels [18, 19], we hypothesized that relative serine depletion might compromise the initial step of Hcy transsulfuration, that is, the condensation between Hcy and serine catalyzed by CBS [54]. As described below, however, none of these hypotheses have been substantiated in relevant animal model, or human studies.

Stabler and colleagues [67] were the first to demonstrate that urinary excretion of Hcy in healthy humans is trivial, an observation confirmed in subsequent studies of both humans [68, 69] and rats [20]. Furthermore, Hultberg and colleagues [50] reported that ESRD patients (who were not anuric) exhibited a greater urinary Hcy clearance in comparison to normal renal function controls. They reasoned that a decline in the normally avid (as earlier demonstrated by Foreman and colleagues *in vitro* [70]) tubular reabsorption of Hcy, more than offset any decrease in urinary Hcy excretion in these patients due to a reduction in their glomerular filtration rate. Thus, despite greater urinary Hcy clearance [54], ESRD patients maintain a chronic two- to threefold increase in plasma tHcy levels, relative to persons with normative renal function [12; 49–58]. Bocock and Zlotkin [71] found that hepatic CBS activity was normal in uremic rats. In addition, Guttormsen and colleagues [72] reported normative increases in plasma methionine following peroral L-homocysteine loading in subjects with ESRD who were folate and B-12 replete. These combined observations [71, 72] do not support the hypothesis that ESRD has an adverse effect on extrarenal transsulfuration or remethylation of Hcy. Finally, we conducted an investigation that failed to confirm our hypothesis regarding the potential role of depressed serine levels in the etiology of ESRD hyperhomocysteinemia. Supplementation with 3 to 4 g of L-serine per day raised plasma serine levels by almost 40%, but had no effect on fasting tHcy levels in four hyperhomocysteinemic hemodialysis patients whose baseline serine levels were below the 10th percentile distribution of a matched healthy control population free of renal disease [54].

In stark contrast to the aforementioned hypotheses, clear and consistent evidence has been provided supporting the notion that loss of normal renal metabolism plays a crucial role in the B-vitamin refractory [12] hyperhomocysteinemia frequently observed in ESRD. We determined net renal uptake and metabolism of tHcy using an established rat model for measuring arteriovenous amino acid differences across the rat kidney, along with simultaneous measurement of renal plasma flow, urine flow, and urinary tHcy levels [20]. Uniformly higher renal artery versus renal vein plasma tHcy levels were observed. The resulting (positive) renal arteriovenous difference for tHcy was equivalent to 20% (0.92/4.53 nmol/ml) of the mean arterial plasma tHcy level. Calculated mean renal tHcy uptake and metabolism ($8.07 \pm 5.31$ nmol/min) was markedly greater ($P = 0.024$) than mean urinary tHcy excretion ($0.19 \pm 0.10$ nmol/min). These data provided the first *in vivo* confirmation of significant Hcy metabolism, along with minimal urinary excretion, by the normative rat kidney. When extrapolated to humans, we calculated that an equivalent arteriovenous difference for tHcy across the human kidney, at a renal plasma flow of 650 ml/min, subtracting the trivial urinary tHcy excretion, would yield a tHcy uptake and metabolism by normative kidneys of approximately 1 mmol/day. Although

**Table 2.** Comparison of crude and adjusted geometric mean fasting total homocysteine levels (μmol/liter) in maintenance dialysis patients and stable renal transplant recipients

| | MDP ($N$ = 73) | RTR ($N$ = 29) | % Difference | $P$ value[a] |
|---|---|---|---|---|
| Unadjusted | 21.6 | 18.1 | 16.2% | 0.07 |
| Adjusted for folate | 22.5 | 16.4 | 27.1% | <0.001 |
| Adjusted for folate, PLP, B-12, albumin, age, sex, smoking, and CVD | 22.2 | 17.0 | 23.4% | 0.008 |

Abbreviations are: MDP, maintenance dialysis patients; RTR, renal transplant recipients; CVD, cardiovascular disease.

[a] Derived from between group comparisons of geometric means by unpaired $t$-test (unadjusted), or general linear modeling with analysis of covariance (folate, and multivariable-adjusted)

(from Bostom et al [62], and **Note added in proof, B**)

**Table 3.** Relative risk estimates (hazards ratios) for incident CVD associated with plasma tHcy levels ≥27 μmol/liter in dialysis-dependent ESRD patients [15]

| | First incident non-fatal or fatal CVD (16 Events) | Fatal CVD Only (9 Events) |
|---|---|---|
| | Hazards ratio (95% CI) | |
| Unadjusted | 3.6 (1.3–9.6) | 7.1 (1.8–28.4) |
| Adjusted for: | | |
| Age | 3.5 (1.3–9.5) | 6.7 (1.6–26.8) |
| Sex | 3.9 (1.4–10.4) | 7.2 (1.8–29.3) |
| Age and sex | 3.9 (1.4–10.5) | 7.1 (1.7–29.2) |
| Race | 3.7 (1.4–9.8) | 7.1 (1.8–28.5) |
| Total cholesterol | 3.1 (1.1–8.7) | 6.4 (1.6–26.5) |
| Total/HDL cholesterol | 3.5 (1.3–9.3) | 7.0 (1.7–27.9) |
| Total/HDL cholesterol > 6 | 3.6 (1.3–9.6) | 7.1 (1.8–28.3) |
| Glucose intolerance | 4.2 (1.6–11.5) | 7.6 (1.9–30.6) |
| Diabetes | 4.4 (1.6–12.0) | 7.4 (1.9–29.9) |
| Hypertension | 3.8 (1.4–10.3) | 7.9 (1.9–32.2) |
| Smoking | 3.0 (1.1–8.1) | 7.1 (1.7–29.6) |
| Baseline CVD | 4.4 (1.6–12.2) | 10.3 (2.4–43.2) |
| Albumin | 3.5 (1.3–9.4) | 7.7 (1.9–31.9) |
| Creatinine | 3.7 (1.4–10.0) | 8.1 (2.0–32.6) |
| Dialysis mode | 3.6 (1.3–9.5) | 8.1 (2.0–32.6) |
| Months since dialysis initiation | 3.6 (1.3–9.6) | 7.3 (1.8–29.5) |
| Low residual adequacy or residual renal function | 3.5 (1.3–9.6) | 6.5 (1.6–26.8) |

daily Hcy production is an estimated 15 to 20 mmol/day in humans [40], most of this Hcy is metabolized intracellularly [23], and approximately 1.5 mmol/day or less is normally exported into the plasma compartment [69]. Thus, our calculations suggested that renal uptake and metabolism could account for ~70% (1 mmol/day 184 1.5 mmol/day) of daily tHcy elimination from plasma. Remarkably concordant findings from a study examining plasma tHcy elimination in healthy human subjects, and those with ESRD, were recently presented by Guttormsen and colleagues [72]. Plasma tHcy responses to oral L-homocysteine loading were compared in the ESRD patients and healthy controls. The elimination rate of tHcy from plasma was decreased (that is, the elimination half-life was increased), and the systemic exposure (area under the curve) was markedly greater in the ESRD patients versus the matched, normative renal function controls. Pharmacokinetic modeling revealed an increase in the half-life of elimination from a mean of 3.5 hours in the controls, to 11.0 hours in the ESRD patients. These data are consistent with a 70% reduction in plasma tHcy clearance due to ESRD. Despite *in vitro* evidence of both specific and nonspecific renal tubular transport mechanisms for Hcy [70], and the documented presence of the major remethylation (methionine synthase, betaine:homocysteine methyltransferase) and transsulfuration (CBS) enzymes in human kidney [22; 73–75], the fate of intrarenal Hcy remains unknown. Hopefully, elucidating the specific details of renal Hcy metabolism will receive increased attention in the future. In the interim, additional observations from studies of ESRD patients confirm that the kidneys play a pivotal role in tHcy elimination from plasma. For example, preliminary data suggest that successful renal transplantation subacutely reduces fasting tHcy levels by approximately 33% [76]. In a complementary study (**Note added in proof, B**) we recently demonstrated that partial restoration of renal function following successful renal transplantation results in a chronic reduction in fasting tHcy levels relative to ongoing maintenance dialysis treatment of ESRD patients (Table 2). Geometric mean fasting tHcy levels, adjusted for age, sex, plasma folate, PLP, and B-12, serum albumin, and the presence of cardiovascular disease, were 23.4% lower in $N$ = 29 stable renal transplant recipients, in comparison to $N$ = 73 maintenance dialysis patients (17.0 μmol/liter vs. 22.2 μmol/liter, $P$ = 0.008). Moreover, delayed tHcy elimination after methionine loading has been demonstrated in subjects with ESRD [63], which likely contributes to the mild PML hyperhomocysteinemia observed in both maintenance dialysis patients [50, 63] and stable renal transplant recipients (Dr. A. Bostom, unpublished data). An elegant study by Arnadottir and colleagues [77] examined the association between a direct measure (that is, iohexol clearance) of glomerular filtration rate (GFR), and fasting plasma tHcy levels in a select group of patients ($N$ = 64) with renal function ranging from normal to predialysis ESRD. Plasma tHcy levels were strongly correlated (Spearman's rho = −0.70, $P < 0.001$) with GFR. Significantly increased tHcy levels (+34%, $P < 0.01$) were already apparent in the group with moderately reduced GFR [mean (± standard deviation) 51 ± 10 ml · min$^{-1}$, adjusted for body surface area]. A strong crude correlation ($r$ = +0.69, $P <$ 0.01) between creatinine and fasting tHcy levels was also observed. Multivariable regression analysis, however, revealed that only GFR and red blood cell folate remained independent predictors of tHcy levels in models including creatinine and other potential determinants, such as PLP and B-12 levels. Finally, within the very narrow range characteristic of dialysis-dependent ESRD, we found no correlation between residual renal function (as determined by weekly creatinine clearance in CAPD patients), and fasting tHcy levels [62]. This observation may explain, in part, why increased age, a surrogate for declining glomerular filtration rate [78], and a significant determinant of tHcy levels in general populations [36–39], was not associated with fasting tHcy levels in the dialysis patients.

## HOMOCYSTEINE AND ARTERIOSCLEROSIS: EPIDEMIOLOGICAL EVIDENCE

### General population data

In 1969, the seminal observations of McCully first linked marked hyperhomocysteinemia to precocious arteriosclerotic disease in autopsied children who died from distinct metabolic forms

of homocystinuria [13]. Nearly thirty years later, a burgeoning amount of observational evidence has accumulated indicating that mild to moderate fasting or non-fasting hyperhomocysteinemia (tHcy levels ≥14 μmol/liter to ≤100 μmol/liter [23]) is an independent risk factor for arteriosclerotic outcomes. The recent meta-analysis of Boushey and colleagues [79], primarily representing findings from retrospective studies, suggested each 5 μmol/liter increment in fasting or non-fasting tHcy above 10 μmol/liter is associated with a 60% (in men) to 80% (in women) greater risk for coronary artery disease, and a 50% greater risk for cerebrovascular disease in both men and women. Three prospective cohort studies [80–82] reported subsequent to this meta-analysis confirmed that there is a graded association between non-fasting tHcy levels and incident myocardial infarction [80, 81] or stroke [82], which persists after adjustment for the major established arteriosclerotic risk factors. Finally a large, multicenter European case control study recently confirmed that PML hyperhomocysteinemia confers a risk for prevalent CVD equal in magnitude to, and independent of, fasting hyperhomocysteinemia [83].

### Studies of ESRD populations

Conflicting findings, both across and within studies, have been reported regarding the association between fasting tHcy levels, and the prevalence of clinical arteriosclerosis in ESRD patients [49, 56–58, 62; **Note added in proof, C**]. Two of these investigations [49, 57] included only crude comparisons of mean tHcy levels between groups of ESRD patients with and without CVD. A third study [56] adjusted inadequately for potential confounding by the established CVD risk factors, and only observed a "significant" increase in the tHcy levels among men with CVD. In a study of $N = 75$ maintenance dialysis patients [62], we found no association between fasting tHcy levels and prevalent CVD in either crude ($N = 22$ with CVD, geometric mean tHcy 20.4; $N = 53$ without CVD, geometric mean tHcy = 22.8; $P = 0.297$), or multiple logistic regression analyses adjusted for age, sex, race, total and HDL cholesterol levels, glucose intolerance/diabetes, hypertension, smoking, and dialysis mode (odds ratio per μmol/liter increase in tHcy = 0.99, 95% confidence interval = 0.91 to 1.07). A recent cross sectional report by Robinson and colleagues [58], in contrast, suggested that hyperhomocysteinemia (comparing the upper to lower two tertiles of fasting tHcy) conferred a 2.8-fold increased risk for prevalent arteriosclerotic outcomes in maintenance dialysis patients, which persisted after adjustment for age, sex, hypertension, hypercholesterolemia, and smoking. Finally, in the largest cross-sectional study yet performed, we found no association between non-fasting serum tHcy levels and non-fatal MI prevalence in the CANUSA [3] peritoneal dialysis inception cohort. Baseline tHcy levels did not differ between 79 ESRD patients initiating peritoneal dialysis who had a clinical history of myocardial infarction (MI) versus 366 similar patients free of any history of cardiovascular disease (CVD), that is, congestive heart failure, coronary heart disease, cerebrovascular disease, or lower extremity arterial disease (tHcy MI cases, 26.3 ± 16.3 μmol/L; tHcy no CVD, 27.6 ± 23.3; $P = 0.64$). Crude and mutivariable-adjusted (for age, sex, diabetes/glucose intolerance) odds ratios (with 95% confidence intervals) comparing the highest (≥ 30.3 μmol/liter) to the lower three quartiles (< 30.3 μmol/liter) of tHcy, MI present versus MI/CVD absent, were 0.75 (0.41 to 1.35), and 0.78 (0.42 to 1.42), respectively (**Note added in proof, C**). Intractable survivorship effects resulting from the excess yearly mortality in dialysis-dependent ESRD [1, 3–5], and the failure to establish whether or not arteriosclerotic outcomes antedated the development of ESRD, renders any inference about tHcy-CVD associations suggested by these published cross-sectional studies hazardous [49, 56–58, 62; **Note added in proof, C**]. Such serious methodologic limitations undermine the inherent validity of any cross-sectional study evaluating putative risk factors for CVD in ESRD populations. The potential relationship between hyperhomocysteinemia and arteriosclerotic outcomes in ESRD requires more rigorous validation via prospective observational studies, and ultimately, clinical tHcy-lowering intervention trials.

The association between fasting tHcy levels and CVD incidence was examined in a preliminary, nested case-control study of 42 renal transplant recipients [53]. Post-hoc subgroup analyses indicated that elevated fasting tHcy levels predicted CVD incidence in men, but not women. These findings are discordant with data from much larger studies conducted in general populations, where sex was not an effect modifier of the relationship between Hcy and CVD [79]. The inexplicable issue of effect modification by sex notwithstanding, missing baseline covariate information (for example, regarding the presence/absence of pre-existing CVD), makes it impossible to discern whether the reported association between tHcy levels and incident CVD, even within the subgroup of renal transplant recipient men, was free of important confounding factors [53]. Recently, we reported the results from a prospective study of the relationship between fasting tHcy levels and CVD in 73 dialysis-dependent ESRD patients (Table 3) [15]. After a median follow-up of 17 months, 16 individuals experienced incident non-fatal and/or fatal CVD events. Fasting hyperhomocysteinemia [that is, comparing the upper (tHcy ≥ 27 μmol/liter) to lower three quartiles (tHcy < 27 μmol/liter)] conferred a significantly increased risk for incident CVD of ~sevenfold for fatal events, and ~3.5-fold for pooled non-fatal and fatal events, after adjustment for pre-existing CVD, the established arteriosclerotic risk factors, creatinine and albumin levels, and dialysis adequacy. Further analyses revealed that hyperhomocysteinemia conferred a similar risk for incident CVD in women and men. Prospective studies of large ESRD cohorts will be required to confirm the external validity of these findings.

## HOMOCYSTEINE AND ARTERIOSCLEROSIS: EXPERIMENTAL EVIDENCE

The pathologic mechanisms by which Hcy promotes arteriosclerosis remain unclear. Experimental data support a range of possibilities, including endothelial cell injury [84, 85], enhanced low density lipoprotein oxidation [86], increased thromboxane-mediated platelet aggregation [87], inhibition of cell surface thrombomodulin expression and protein C activation [88], enhancement of lipoprotein (a)-fibrin binding [89], and promotion of smooth muscle cell proliferation [90]. The *in vivo* relevance of findings from such experimental studies, however, has been seriously questioned [91, 92] due to their lack of specificity to Hcy versus other much more abundant plasma thiols, including cysteine, and the use of grossly supraphysiologic concentrations or non-physiologic forms (that is, D-L as opposed to L-) of reduced Hcy. The data of Mansoor and colleagues [93] provide the background appropriate for adequate understanding of the specific criticism regarding grossly supraphysiologic concentrations. These investigators assessed concentrations of reduced Hcy across

the widest possible spectrum of tHcy concentrations. Their data revealed that at tHcy concentrations of up to 100 μmol/liter, levels of reduced Hcy accounted for only 1% (≤1 μmol/L) of plasma tHcy. When tHcy exceeded 100 μmol/liter, reduced Hcy began to rise exponentially, likely due to saturation of plasma protein-binding sites [93]. However, the highest reduced Hcy value they documented was in a subject with homozygous homocystinuria who had a tHcy > 350 μmol/liter, but a reduced Hcy of < 100 μmol/liter [93]. When juxtaposed to the concentrations of reduced Hcy used in experimental studies [84–88, 90], that is, 1000 to 10,000 μmol/liter, the findings of Mansoor and colleagues [93] illustrate the very dubious clinical relevance of these published data. In contrast, physiologic models of mild, dietary-induced hyperhomocysteinemia causing subclinical or frank atherothrombotic sequelae have recently been described in minipigs [94] and cynomolgus monkeys [95]. Follow-up investigations employing these models may elucidate the *in vivo* relevance of the putative pathologic mechanisms cited above [84–90].

## TREATMENT OF HYPERHOMOCYSTEINEMIA

### Populations with normative renal function

The severe hyperhomocysteinemia found in homozygous cystathionine beta synthase (CBS) deficiency has been treated with methionine restriction and supraphysiologic doses of vitamin B-6, vitamin B-12, folate, and betaine [24, 96]. Such treatment lowers Hcy levels, and more importantly, reduces the incidence of atherothrombotic events and mortality in these patients [24, 96]. Management of this severe form of hyperhomocysteinemia (that is, tHcy levels of 100 to 400 μmol/liter) is the paradigm for treatment of the more common mild to intermediate forms of hyperhomocysteinemia. With the exception of methionine restriction, all the major therapeutic approaches to lowering Hcy alluded to earlier have been applied to populations with moderate hyperhomocysteinemia.

Acknowledging the major limitation that few of the micronutrient trials for treatment of fasting hyperhomocysteinemia [97–102], and none of the trials for treatment of PML hyperhomocysteinemia [103–106] were placebo-controlled, some general conclusions can be drawn from these published data:

(1.) Folic acid at 0.65 to 5.0 mg/day can effectively reduce fasting Hcy levels in moderately hyperhomocysteinemic and normohomocysteinemic persons with or without CVD who are free of renal insufficiency, and are not vitamin B-12 deficient.

(2.) Vitamin B-12 (0.4 mg to 2.0 mg/day orally) can effectively normalize fasting Hcy in vitamin B-12—deficient persons who respond only marginally to folic acid supplementation.

(3.) Vitamin B-6 at doses up to 240 to 250 mg/day has no lowering effect on fasting Hcy levels.

(4.) Vitamin B-6 at 20 to 250 mg/day appears to reduce the absolute PML, or PML increase in Hcy levels, by ~15 to 50% in persons with or without CVD who have mild PML hyperhomocysteinemia.

(5.) Even at doses of 250 mg/day vitamin B-6, combined with 5 mg/day folic acid, an effective fasting and/or PML Hcy-lowering response may not be achieved in some individuals until after 6 to 12 weeks of treatment.

### ESRD populations

With a single exception [106], all the published Hcy-lowering intervention studies conducted in ESRD patients were uncontrolled, open label investigations [54, 63, 64, 107–110]. Bearing this important caveat in mind, the following conclusions can be drawn from these studies:

(*a*) Folic acid-based B-vitamin regimens, including folic acid at doses of 5 to 10 mg/day, appear to lower fasting tHcy levels by ~30 to 50%.

(*b*) The addition of folic acid at 15 mg/day (vs. additional placebo) to a baseline regimen including 1 mg/day of folic acid, reduces fasting tHcy levels by ~25 to 30%.

(*c*) Even when given a total dose of 16 mg/day of folic acid, two-thirds of maintenance dialysis patients whose baseline fasting or non-fasting tHcy levels are > 15 to 16 μmol/liter, will continue to maintain tHcy levels at or above this value.

(*d*) There are no data available on the independent effect of B-12 on fasting tHcy levels.

(*e*) In accord with findings from general populations, B-6 at up to 300 mg/day has no apparent effect on fasting tHcy levels. There are no data on the independent effect of B-6 on PML tHcy levels.

(*f*) Neither serine at 3 to 4 g/day, nor betaine at 6 g/day appear to have any effect on fasting tHcy levels.

(*g*) Oral N-acetylcysteine (NAC) at 1.2 g/day subacutely lowers non-fasting pre-hemodialysis tHcy levels by ~16%.

Finally, methionine restriction, as recently advocated [58, 111], would seem to be an inappropriate method to reduce tHcy levels in dialysis-dependent ESRD patients given their tendency toward inadequate dietary protein-energy intake, and the overall catabolic stress they experience [112].

## CONCLUSIONS AND FUTURE CONSIDERATIONS

There is an excess prevalence of B-vitamin refractory hyperhomocysteinemia in ESRD patients relative to age, sex, and race matched, population-based controls free of renal disease. ESRD hyperhomocysteinemia is due primarily to loss of renal Hcy metabolism, which normally accounts for ~70% of daily Hcy elimination from plasma. The details of intrinsic renal Hcy metabolism, however, are essentially unknown, and require elucidation.

Due to intractable methodologic limitations, cross-sectional studies of the association between Hcy levels and prevalent arteriosclerotic disease in ESRD populations are invalid. Recently, the first appropriately controlled, longitudinal evidence was provided that hyperhomocysteinemia may contribute independently to the excess incidence of fatal and non-fatal CVD outcomes among maintenance dialysis patients. Confirmation of these prospective findings in large ESRD cohorts is urgently required.

Current treatment regimens for ESRD hyperhomocysteinemia, which are based upon pharmacologic doses (that is, 5 to 15 mg/day) of folic acid, frequently result in suboptimal lowering of tHcy levels. Other potential therapeutic approaches (such as oral N-acetylcysteine) merit controlled investigation. Ultimately, placebo-controlled clinical trials of effective tHcy-lowering treatments will be required to confirm the hypothesis that hyperhomocysteinemia contributes independently to the excess incidence of arteriosclerotic outcomes in ESRD.

*Reprint requests to Andrew G. Bostom, M.D., M.S., Vitamin Bioavailability Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts New England Medical Center, 711 Washington Street, Boston, Massachusetts 02111, USA.*
*E-mail: bostom_vb@hnrc.tufts.edu*

## NOTES ADDED IN PROOF

A. Bostom AG, Gohh RY, Tsai MY, Hopkins-Garcia BJ, Nadeau MR, Bianchi L, Jacques PF, Rosenberg IH, Selhub J: Excess prevalance of fasting and post-methionine loading hyperhomocysteinemia in stable renal transplant recipients. *Arteriosclerosis Thromb Vasc Biol* (in press)

B. Bostom AG, Shemin D, Gohh RY, Verhoef P, Nadeau MR, Hopkins-Garcia BJ, Bianchi LA, Jacques PF, Selhub J, Dworkin L, Rosenberg IH: Lower fasting total plasma homocysteine levels in stable renal transplant recipients versus chronic maintenance dialysis patients. (abstract) *Can J Cardiol* (in press)

C. Bostom AG, Thorpe K, Beecroft ML, Nadeau MR, Jacques PF, Selhub J, Rosenberg IH, Churchill DN: Lack of association between serum total homocysteine levels and non-fatal myocardial infarction prevalance in a large peritoneal dialysis inception cohort. (abstract) *Can J Cardiol* (in press)

## REFERENCES


1. Excerpts From The United States Renal Data System, United States Renal Data System: Annual Report. *Am J Kid Dis* 26(Suppl 2):S85–S94, 1995
2. Churchill DN: Comparative morbidity among hemodialysis and continuous ambulatory peritoneal dialysis patients. *Kidney Int* 43(Suppl):S16–S22, 1993
3. Canada-USA (CANUSA) Peritoneal Dialysis Study Group: Adequacy of dialysis and nutrition in continuous peritoneal dialysis: Association with clinical outcomes. *J Am Soc Nephrol* 7:198–207, 1996
4. Lemmers MJ, Barry JM: Major role for arterial disease in morbidity and mortality after kidney transplantation in diabetic recipients. *Diabetes Care* 14:295–301, 1991
5. Kasiske BL, Guijarro C, Massy Z, Wiederkehr MR, Ma JZ: Cardiovascular disease after renal transplantation. *J Am Soc Nephrol* 7:158–165, 1996
6. Wilson PWF, Castelli WP, Kannel WB: Coronary risk prediction in adults (the Framingham Heart Study). *Am J Cardiol* 59:91G–94G, 1987
7. Ma KW, Greene EL, Raij L: Cardiovascular risk factors in chronic renal failure and hemodialysis populations. *Am J Kid Dis* 19:505–513, 1992
8. Rostand SG, Kirk KA, Rutsky EA: Relationship of coronary risk factors to hemodialysis-associated ischemic heart disease. *Kidney Int* 22:304–308, 1982
9. Cressman MD, Heyka RJ, Paganini EP, O'Neill J, Skibinski CI, Hoff HF: Lipoprotein (a) is an independent risk factor for cardiovascular disease in hemodialysis patients. *Circulation* 86:475–482, 1992
10. Wu G: Cardiovascular deaths among CAPD patients. *Perit Dial Bull* 4(Suppl):S23–S25, 1983
11. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE: Impact of hypertension on cardiomyopathy, morbidity, and mortality in end-stage renal disease. *Kidney Int* 49:1379–1385, 1996
12. Bostom AG, Shemin D, Lapane KL, Sutherland P, Nadeau MR, Wilson PWF, Yoburn D, Bausserman L, Tofler G, Jacques PF, Selhub J, Rosenberg IH: Hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein (a) excess in maintenance dialysis patients: A matched case-control study. *Atherosclerosis* 125:91–101, 1996
13. McCully KS: Vascular pathology of homocysteinemia: implications for pathogenesis of arteriosclerosis. *Am J Pathol* 56:111–128, 1969
14. Stampfer MJ, Malinow MR, Willet WC, Newcomer LM, Upson B, Ullman D, Tishler PV, Hennekens CH: A prospective study of plasma homocyst(e)inc and risk of myocardial infarction in US physicians. *JAMA* 268:877–881, 1992
15. Bostom AG, Shemin D, Verhoef P, Nadeau MR, Jacques PF, Selhub J, Dworkin L, Rosenberg IH: Elevated fasting total plasma homocysteine levels and cardiovascular disease outcomes in maintenance dialysis patients: A prospective study. *Arteriosclerosis Thromb Vasc Biol* (in press)
16. Brosnan JT: The 1986 Borden Award Lecture. The role of the kidney in amino acid metabolism and nutrition. *Can J Physiol Pharmacol* 65:2355–2362, 1987
17. Tizianello A, De Ferrari G, Garibotto G, Gurreri G, Robaudo C: Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. *J Clin Invest* 65:1162–1173, 1980
18. Flugel-Link RM, Jones MR, Kopple JD: Red cell and plasma amino acid concentrations in renal failure. *J Paren Ent Nutr* 7:450–456, 1983
19. Laidlaw SA, Berg RL, Kopple JD, Naito H, Walker WG, Walser M: Patterns of fasting plasma amino acid levels in chronic renal insufficiency: Results from the feasibility phase of the Modification of Diet in Renal Disease Study. *Am J Kid Dis* 23:504–513, 1994
20. Bostom AG, Brosnan JT, Hall B, Nadeau MR, Selhub J: Net uptake of plasma homocysteine by the rat kidney in vivo. *Atherosclerosis* 116:59–62, 1995
21. Selhub J, Miller JW: The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by s-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr* 55:131–138, 1992
22. McKeever MP, Weir DG, Molloy A, Scott JM: Betaine: Homocysteine methyltransferase: organ distribution in man, pig, and rat and subcellular distribution in the rat. *Clin Sci* 81:551–556, 1991
23. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH: Total homocysteine in plasma or serum: methods and applications. *Clin Chem* 39:1764–1773, 1993
24. Mudd SH, Levy HL, Skovby F: Disorders of transsulfuration, in *Metabolic Basis of Inherited Disease* (6th ed), edited by Scriver CR, Beaudet AL, Sly WS, Valle D, New York, McGraw Hill, Inc., 1989, pp 693–734
25. Rosenblatt DS: Inherited disorders of folate transport and metabolism, in *Metabolic Basis of Inherited Disease* (6th ed), edited by Scriver CR, Beaudet AL, Sly WS, Valle D, New York, McGraw Hill, Inc., 1989, pp 2049–2064
26. Linnell JC, Bhatt HR: Inherited disorders of cobalamin metabolism and their management. *Ballières Clin Haematol* 8:567–601, 1995
27. Brattstrom L, Israelsson B, Hultberg B: Plasma homocysteine and methionine tolerance in early-onset vascular disease. *Haemostasis* 19(Suppl 1):35–44, 1989
28. Miller JW, Nadeau MR, Smith J, Smith D, Selhub J: Folate deficiency-induced homocysteinemia in rats: Disruption of S-adenosylmethionine's coordinate regulation of homocysteine metabolism. *Biochem J* 298:415–419, 1994
29. Miller JW, Nadeau MR, Smith D, Selhub J: Vitamin B-6 deficiency vs. folate deficiency: comparison of responses to methionine loading in rats. *Am J Clin Nutr* 59:1033–1039, 1994
30. Bostom AG, Jacques PF, Nadeau MR, Williams RR, Ellison RC, Selhub J: Post-methionine load hyperhomocysteinemia in persons with normal fasting total plasma homocysteine: initial results from the NHLBI Family Heart Study. *Atherosclerosis* 116:147–151, 1995
31. Bostom AG, Roubenoff R, Dellaripa P, Nadeau MR, Sutherland P, Wilson PW, Jacques PF, Selhub J, Rosenberg IH: Validation of abbreviated oral methionine loading test. *Clin Chem* 41:948–949, 1995
32. Selhub J, Jaccques PF, Wilson PWF, Rush D, Rosenberg IH: Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 270:2693–2697, 1993
33. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R: Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 93:7–9, 1996
34. Goyette P, Sumner JS, Milos R, Duncan MV, Rosenblatt DS, Matthews RG, Rozen R: Human methylenetetrahydrofolate reductase: Isolation of cDNA, mapping, and mutation identification. *Nature Genet* 7:195–200, 1994
35. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nature Genet* 10:111–113, 1995

36. Brattstrom L, Lindgren A, Israelsson B, Andersson A, Hultberg B: Homocysteine and cysteine: Determinants of plasma levels in middle-aged to elderly subjects. *J Intern Med* 236:633–641, 1994
37. Araki A, Sako Y, Fukushima Y, Matsumoto M, Asada T, Kita T: Plasma sulfhydryl amino acids in patients with cerebral infarction and in hypertensive subjects. *Atherosclerosis* 79:139–146, 1989
38. Wu LL, Wu J, Hunt SC, James BC, Vincent GM, Williams RR, Hopkins PN: Plasma homocyst(e)ine as a risk factor for early familial coronary artery disease. *Clin Chem* 40:552–561, 1994
39. Lussier-Cacan, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest JJ Jr: Plasma total homocysteine in healthy subjects: Sex-specific relation with biological traits. *Am J Clin Nutr* 64:587–593, 1996
40. Mudd SH, Poole JR: Labile methyl balances for normal humans on various dietary regimens. *Metabolism* 24:721–735, 1975
41. Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johansson BB: Plasma homocysteine in the acute and convalescent phases after stroke. *Stroke* 26:795–800, 1995
42. Robins AJ, Milewczyk BK, Booth EM, Mallick NP: Plasma amino acid abnormalities in chronic renal failure. *Clin Chim Acta* 42:215–217, 1972
43. Cohen BD, Patel H, Kornhauser RS: Alternate reasons for atherogenesis in uremia. *Proc Dial Transplant Forum* 7:178–180, 1977
44. Wilcken DEL, Gupta VJ: Sulphur-containing amino acids in chronic renal failure with particular reference to cysteine-homocysteine mixed disulfide. *Eur J Clin Invest* 9:301–307, 1979
45. Wilcken DEL, Gupta VJ: Accumulation of sulphur-containing amino acids including cysteine-homocysteine mixed disulfide in patients on hemodialysis. *Clin Sci* 58:427–431, 1980
46. Wilcken DEL, Gupta VJ, Betts AK: Homocysteine in the plasma of renal transplant recipients: Effects of cofactors for methionine metabolism. *Clin Sci* 61:743–749, 1981
47. Kang SS, Wong PWK, Bidani A, Milanez S: Plasma protein-bound homocyst(e)ine in patients requiring chronic hemodialysis. *Clin Sci* 65:335–337, 1983
48. Smolin LA, Laidlaw SA, Kopple JD: Altered plasma free and protein-bound sulfur amino acid levels in patients undrgoing maintenance hemodialysis. *Am J Clin Nutr* 45:737–743, 1987
49. Chauveau P, Chadefaux B, Coudé M, Aupetit J, Hannedouche T, Kamoun P, Jungers P: Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. *Kidney Int* 43:(Suppl 41) S72–S77, 1993
50. Hultberg B, Andersson A, Sterner G: Plasma homocysteine in renal failure. *Clin Nephrol* 40:230–234, 1993
51. Stabler SP, Lindenbaum J, Savage DG, Allen RH: Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. *Blood* 81:3404–3413, 1993
52. Kim SS, Hirose S, Tamura H, Nagasawa R, Tokushima H, Mitarai T, Isoda K: Hyperhomocysteinemia as a possible role for atherosclerosis in CAPD patients. *Adv Perit Dial* 10:282–285, 1994
53. Massy ZA, Chadefaux-Vekemans B, Chevalier A, Bader CA, Drueke TB, Legendre C, Lacour B, Kamoun P, Kreis H: Hyperhomocysteinemia: A significant risk factor for cardiovascular disease in renal transplant recipients. *Nephrol Dial Transplant* 9:1103–1108, 1994
54. Bostom AG, Shemin D, Lapane KL, Miller JW, Sutherland P, Nadeau MR, Seyoum E, Hartman W, Prior R, Wilson WF, Selhub J: Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: A case control study. *Atherosclerosis* 114:93–103, 1995
55. Perna AF, Ingrosso D, De Santo NG, Galletti P, Zappia V: Mechanism of erythrocyte accumulation of methylation inhibitor s-adenosylhomocysteine in uremia. *Kidney Int* 47:247–253, 1995
56. Bachmann J, Tepel M, Raidt H, Riezler R, Graefe U, Langer K, Zidek W: Hyperhomocysteinemia and the risk for vascular disease in hemodialysis patients. *J Am Soc Nephrol* 6:121–125, 1995
57. Arnadottir M, Hultberg B, Vladov V, Nilsson-Ehle P, Thysell H: Hyperhomocysteinemia in cyclosporine-treated renal transplant recipients. *Transplantation* 61:509–512, 1996
58. Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, Vigo P, Mayer EL, Selhub J, Kutner M, Jacobsen DW: Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 94:2743–2748, 1996
59. Descombes E, Hanck AB, Fellay G: Water soluble vitamins in chronic hemodialysis patients and need for supplementation. *Kidney Int* 43:1319–1323, 1993
60. Spannuth CL, Warnock LG, Wagner C, Stone WJ: Increased plasma clearance of pyridoxal 5′-phosphate in vitamin B-6 deficient uremic man. *J Lab Clin Invest* 90:632–637, 1977
61. Wei I-L, Young T-K: Activities of hepatic vitamin B-6 metabolizing enzymes and concentrations of vitamin B-6 vitamers in tissues of chronically azotemic rats. *J Nutr Biochem* 6:494–498, 1995
62. Bostom AG, Shemin D, Lapane KL, Nadeau MR, Sutherland P, Chan J, Rozen R, Yoburn D, Jacques PF, Selhub J, Rosenberg IH: Folate status is the major determinant of fasting total plasma homocysteine levels in maintenance dialysis patients. *Atherosclerosis* 123:193–202, 1996
63. Janssen MJFM, van Guldener C, de Jong GMT, van den Berg M, Stehouwer CDA, Donker AJM: Folic acid treatment of hyperhomocysteinemia in dialysis patients. *Miner Electrol Metab* 22:110–114, 1996
64. Bostom AG, Shemin D, Yoburn D, Fisher DH, Nadeau MR, Selhub J: Brief report: Lack of effect of oral N-acetylcysteine on the acute dialysis-related lowering of total plasma homocysteine in hemodialysis patients. *Atherosclerosis* 120:242–244, 1996
65. Zazgornik J, Druml W, Balcke P, Kopsa H, Marosi L, Neumann E, Schmidt P: Diminished serum folic acid levels in renal transplant recipients. *Clin Nephrol* 18:306–310, 1982
66. Lacour B, Parry C, Drueke T, Touam M, Kreis H, Bailly M, Durand D: Pyridoxal 5′-phosphate deficiency in uremic undialyzed, hemodialyzed, and non-uremic kidney transplant patients. *Clin Chim Acta* 127:205–215, 1983
67. Stabler SP, Marcell PD, Podell ER, Allen RH: Quantitation of total homocysteine, total cysteine, and methionine in normal serum and urine using gas chromatography-mass spectrometry. *Anal Biochem* 162:186–196, 1988
68. Brattstrom L, Lindgren A, Israelsson B, Malinow MR, Norrving B, Upson B, Hamfelt A: Hyperhomocysteinemia in stroke: prevalence, cause, and relationships to type of stroke and stroke risk factors. *Eur J Clin Invest* 22:214–221, 1992
69. Guttormsen AB, Schneede J, Ueland PM, Refsum H: Kinetics of total plasma homocysteine in subjects with hyperhomocysteinemia due to folate or cobalamin deficiency. *Am J Clin Nutr* 63:194–202, 1996
70. Foreman JW, Wald H, Blumberg G, Pepe LM, Segal S: Homocysteine uptake in isolated rat renal cortical tubules. *Metabolism* 31:613–619, 1982
71. Bocock MA, Zlotkin SH: Hepatic sulfur amino acid metabolism in rats with chronic renal failure. *J Nutr* 120:691–699, 1990
72. Guttormsen AB, Ueland PM, Svarstad E, Refsum H: The kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney Int* (in press)
73. Sturman JA, Gaull GE, Niemann WH: Activities of some enzymes involved in homocysteine methylation in brain, liver, and kidney of the developing rhesus monkey. *J Neurochem* 27:425–431, 1976
74. Gaull GE, Von Berg W, Raiha NCR, Sturman JA: Development of methyltransferase activities of human fetal tissues. *Pediatr Res* 7:527–533, 1973
75. Sturman JA, Rassin DK, Gaull GE: Distribution of transsulfuration enzymes in various organs and species. *Int J Biochem* 1:251–253, 1970
76. van Guldener C, Janssen MJFM, Surachno J: Short term effect of kidney transplantation on plasma homocysteine in dialysis patients. (abstract) *Irish J Med Sci* 164:22, 1995
77. 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* 56:41–46, 1996
78. Cockroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16, 1976
79. Boushey CJ, Beresford SAA, Omen GS, Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* 274:1049–1057, 1995
80. Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forde OH,

NORDREHAUG JE: Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 24:704–709, 1995
81. PERRY IJ, REFSUM H, MORRIS RW, EBRAHIM SB, UELAND PM, SHAPER AG: Serum total homocysteine and coronary heart disease in middle-aged British men. *Heart* 75(Suppl 1):P53, 1996
82. PERRY IJ, REFSUM H, MORRIS RW, EBRAHIM SB, UELAND PM, SHAPER AG: Prospective study of serum total homocysteine and risk of stroke in middle-aged British men. *Lancet* 346:1395–1398, 1995
83. GRAHAM IM, DALY LE, REFSUM HM, ROBINSON K, BRATTSTROM LE, UELAND PM, PALMA-REIS RJ, BOERS GHJ, SHEAHAN RG, ISRAELSON B, UITERWAAL CS, MELEADY RM: Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. *JAMA* (in press)
84. HARKER LA, ROSS R, SLICHTER SJ, SCOTT CR: Homocystine-induced arteriosclerosis. The role of endothelial injury and platelet response in its genesis. *J Clin Invest* 58:731–741, 1976
85. WALL RT, HARLAN JM, HARKER LA, STRIKER GE: Homocystine-induced endothelial injury in vitro: A model for the study of vascular injury. *Thromb Res* 18:113–121, 1980
86. HEINECKE JW, ROSEN H, SUZUKI LA, CHAIT A: The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J Biol Chem* 262:10098–10103, 1987
87. DI MINNO G, DAVI G, MARGAGLIONE M, CIRILLO F, GRANDONE E, CIBATTONI G, CATALANO I, STRISCIUGLIO P, ANDRIA G, PATRONO C, MANCINI M: Abnormally high thromboxane biosynthesis in homozygous homocystinuria. Evidence for platelet involvement and probucol sensitive mechanism. *J Clin Invest* 92:1400–1406, 1993
88. LENTZ SR, SADLER JE: Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest* 88:1906–1914, 1991
89. HARPEL PC, CHANG VT, BORTH W: Homocysteine and other sulfhydryl compounds enhance the binding of lipoprotein (a) to fibrin: A potential link between thrombosis, atherogenesis, and sulfhydryl compound metabolism. *Proc Natl Acad Sci USA* 89:10193–10197, 1992
90. TSAI JC, PERRELLA MA, YOSHIZUMI M, HSIEH C-M, HABER E, SCHLEGEL R, LEE M-E: Promotion of vascular smooth muscle cell growth by homocysteine: A link to atherosclerosis. *Proc Natl Acad Sci USA* 91:6369–6373, 1994
91. DUDMAN NP, HICKS C, WANG J, WILCKEN DE: Human arterial endothelial cell detachment in vitro: its promotion by homocysteine and cysteine. *Atherosclerosis* 91:77–83, 1991
92. WANG J, DUDMAN NP, WILCKEN DE: Effects of homocysteine and related compounds on prostacyclin production by cultured human vascular endothelial cells. *Thromb Haemost* 70:1047–1052, 1993
93. MANSOOR MA, UELAND PM, AARSLAND A, SVARDAL AM: Redox status and protein binding of plasma homocysteine and other aminothiols in patients with homocystinuria. *Metabolism* 42:1481–1485, 1993
94. ROLLAND PH, FRIGGI A, BARLAITER A, PIQUET P, LATRILLE V, FAYE MM, ET AL: Hyperhomocyst(e)inemia-induced vascular damage in the minipig. *Circulation* 91:1161–1174, 1995
95. LENTZ SR, SOBEY CG, PIEGORS DJ, BHOPATKAR MY, FARACI FM, MALINOW MR, HEISTAD DD: Vascular dysfunction in monkeys with diet-induced hyperhomocysteinemia. *J Clin Invest* 98:24–29, 1996
96. MUDD SH, SKOVBY F, LEVY HL, PETTIGREW KD, WILCKEN B, PYERITZ RE, ANDRIA G, BOERS GH, BROMBERG IL, CERONE R, FOWLER B, GRÖBE H, SCHMIDT H, SCHWEITZER L: The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 37:1–31, 1985
97. BRATTSTROM LE, ISRAELSSON B, JEPPSSON J-O, HULTBERG B: Folic acid- an innocuous means to reduce plasma homocysteine. *Scand J Clin Lab Invest* 48:215–221, 1988
98. GLUECK CJ, SHAW P, LANG JE, TRACY T, SIEVE-SMITH L, WANG Y: Evidence that homocysteine is an independent risk factor for atherosclerosis in hyperlipidemic patients. *Am J Cardiol* 132–136, 1995
99. UBBINK JB, VERMAAK WJH, VAN DER MERWE A, BECKER PJ: Vitamin B-12, vitamin B-6, folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* 57:47–53, 1993
100. UBBINK JB, VERMAAK WJH, VAN DER MERWE A, BECKER PJ, DELPORT R, POTGEITER HC: Vitamin requirements for the treatment of hyperhomocysteinemia in humans. *J Nutr* 124:1927–1933, 1994
101. LANDGREN F, ISRAELSSON B, LINDGREN A, HULTBERG B, ANDERSSON A, BRATTSTROM L: Plasma homocysteine in acute myocardial infarction: Homocysteine-lowering effect of folic acid. *J Intern Med* 237: 381–388, 1995
102. BRATTSTROM L, ISRAELSSON B, LINDGARDE F, HULTBERG B: Higher total plasma homocysteine in vitamin B12 deficiency than in heterozygosity for homocystinuria due to cystathionine beta synthase deficiency. *Metabolism* 37:175–178, 1988
103. BRATTSTROM L, ISRAELSSON B, NORRVING B, BERQVIST D, THORNE J, HULTBERG B, HAMFELT A: Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease: Effects of pyridoxine and folic acid treatment. *Atherosclerosis* 81:51–60, 1990
104. FRANKEN DG, BOERS GHJ, BLOM HJ, TRIJBELS JMF: Effect of various regimens of vitamin B-6 and folic acid on mild hyperhomocysteinemia in vascular patients. *J Inherit Metab Dis* 17:159–162, 1994
105. UBBINK JB, VAN DER MERWE A, DELPORT R, ALLEN RH, STABLER SP, RIEZLER R, VERMAAK WJH: The effect of a subnormal vitamin B-6 status on homocysteine metabolism. *J Clin Invest* 98:177–184, 1996
106. BOSTOM AG, SHEMIN D, LAPANE KL, HUME AL, YOBURN D, NADEAU MR, BENDICH A, SELHUB J, ROSENBERG IH: High dose B-vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 49:147–152, 1996
107. WILCKEN DEL, DUDMAN NP, TYRRELL PA, ROBERTSON MR: Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: Possible implications for prevention of vascular disease. *Metabolism* 37:697–701, 1988
108. ARNADOTTIR M, BRATTSTROM L, SIMONSEN O, THYSSELL H, HULTBERG B, ANDERSSON A, NILSSON-EHLE P: The effect of high dose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentrations in dialysis patients. *Clin Nephrol* 40: 236–240, 1993
109. CHAUVEAU P, CHADEFAUX B, COUDE M, AUPETIT J, KAMOUN P, JUNGERS P: Long-term folic acid (but not pyridoxine) supplementation lowers elevated plasma homocysteine level in chronic renal failure. *Miner Electrol Metab* 22:106–109, 1996
110. BOSTOM AG, SHEMIN D, NADEAU MR, SHIH VE, STABLER SP, ALLEN RH, SELHUB J: Short term betaine therapy fails to lower elevated fasting total plasma homocysteine levels in hemodialysis patients maintained on chronic folic acid supplementation. *Atherosclerosis* 113:129–132, 1995
111. DENNIS VW, ROBINSON K: Homocysteinemia and vascular disease in end-stage renal disease. *Kidney Int* 50(Suppl 57):S11–S17, 1996
112. BERGSTRÖM J: Nutrition and mortality in hemodialysis. *J Am Soc Nephrol* 6:1329–1341, 1995