

# The Effect of Homocysteine-Lowering Therapy on Vascular Endothelial Function in Chronic Renal Insufficiency

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## A. Purpose and Rationale

Homocysteine is a reactive sulfur-containing amino acid formed as an intermediary in the catabolism of the essential amino acid methionine. It has long been known that patients with certain genetic errors in homocysteine metabolism have very high total homocysteine levels (100 - 450  $\mu\text{mol/L}$ ), and that these patients suffer from premature arterio sclerosis and consequent cardiovascular events (1). The role of mild to moderate homocysteine elevation (15-100  $\mu\text{mol/L}$ ) in the pathogenesis of cardiovascular disease in those without such genetic defects remains to be defined. Several large, prospective observational cohort studies have demonstrated that patients with moderately elevated homocysteine levels have an increased risk of cardiovascular disease compared to controls (2,3). This association has been shown, with some exceptions, to be independent of known cardiovascular risk factors and therefore it has been suggested that elevated homocysteine may be a novel risk factor for cardiovascular disease. Indeed, at the cellular level homocysteine has been implicated in endothelial injury, platelet activation, smooth muscle proliferation, oxidative modification of low-density lipoproteins, and in endothelial-leukocyte interactions (4).

Large-scale clinical trials are now underway to assess the effect of homocysteine lowering on the primary and secondary prevention of cardiovascular events (5). However, their enrollment has begun in a period where widespread folic acid fortification of cereal grains has been undertaken by the governments of the U.S. and Canada with the intention of preventing neural tube defects in the newborn (6,7). This will almost certainly reduce the statistical power of these studies (10) by diminishing the prevalence of mild hyperhomocysteinemia ( $>13 \mu\text{mol/L}$ ) in the study population by 50% according to one population-based analysis (8). Comparing folate supplementation against a background of population-wide dietary supplementation will make achieving significant homocysteine reductions more difficult as well as make less probable a significant difference in outcomes among treatment arms.

Patients with chronic renal insufficiency, end-stage renal disease, and who are recipients of renal transplants compose a population exposed to dietary folic-acid fortification that retain an excess prevalence of hyperhomocysteinemia (9,11). This is a population that is also disproportionately stricken by cardiovascular disease (11). This "high-risk" group clearly has an excess burden of traditional cardiovascular risk factors (i.e. diabetes, hypertension, dyslipidemia), but this only partially explains their excess prevalence of events (12). Elucidation of the roles of renal-specific cardiovascular risk factors including anemia, proteinuria, elevated thrombotic factors and homocysteine is an area of active investigation.

Confounding the research into a causal role for homocysteine and cardiovascular events in chronic renal failure is that serum total homocysteine levels are inversely proportional to glomerular filtration rate (13), perhaps reflecting renal metabolism rather than excretion (13). Elevated homocysteine in this population might be conceived of as a benign epiphenomenon reflecting merely the degree of renal dysfunction rather than an independent risk factor. A large-scale randomized placebo controlled homocysteine lowering trial whose primary endpoint would be cardiovascular events would help resolve this question.

Endothelial dysfunction is the primary process in atherogenesis and both in vitro and in vivo studies have suggested that this may be the mechanism of homocysteine-induced vascular injury (4). Hyperhomocysteinemia has been associated with impaired endothelial-dependent vasodilation (14), which is a prevalent finding in chronic renal insufficiency (12), and a recent study of patients with known CAD randomized to homocysteine lowering therapy for 12 weeks had improvement in this parameter (15).

The study proposed is a double-blind placebo controlled trial to investigate whether folate-based therapy in patients with chronic renal insufficiency can improve endothelial-dependent vasodilation.

## **B. Hypothesis**

Among patients with stable chronic renal insufficiency, folate and B-vitamin based therapy will improve endothelial-dependent vasodilation.

## **C. Study Design and Statistical Analysis**

This is a double-blind, randomized, controlled trial with two arms: intervention (folate 5 mg/d, vitamin B<sub>12</sub> 1 mg/d, vitamin B<sub>6</sub> 250 mg/d) and placebo. Based on the results of a previous such study in patients with established CAD (15), and an expected improvement in flow-mediated dilation by 1.5% (std. dev. ~ 3%), 65 patients in each arm of the study (~ 130 total patients) will be necessary to achieve 80% power testing at  $p=0.05$ . The randomization will be computer assisted and without stratification. No crossover is planned.

Statistical analysis will consist of paired t tests comparing mean change in flow-mediated dilation at 12 weeks vs baseline in both treatment and placebo groups. In the treatment group, change in flow-mediated dilation will be correlated to change in homocysteine, folate, and B<sub>12</sub> levels. Statistical significance will be inferred at a  $p<0.05$ .

## **D. Study Procedure**

Approximately 130 patients will be randomized to either treatment with folate 5 mg/d, vitamin B<sub>12</sub> 1 mg/d, and vitamin B<sub>6</sub> 250 mg/d or matching placebo tablets. At baseline and at follow-up (12 weeks) all patients will undergo phlebotomy to determine fasting plasma homocysteine (free and total), folate, B<sub>12</sub>, hemoglobin A1c, HDL-C, total cholesterol, triglycerides, and creatinine. Standard assay protocols at the CPMC core lab will be used. A baseline assessment, per patient report, of a history of hypertension or current antihypertensive use, diabetes, high cholesterol or current antihyperlipidemic use, family history (1<sup>st</sup> degree relative) of CAD, personal reported history of angina, MI, or stroke, and history of smoking within the past year, will be made. Resting systolic and diastolic blood pressures will also be measured at baseline. At the beginning of the trial, fasting brachial artery flow-mediated dilation (endothelial dependent) and nitroglycerin-induced dilation (endothelium independent) will be measured (described below). This measurement will be repeated in the same fashion at week 12. A twelve-week period was chosen given the proven efficacy of this duration of treatment in several previous investigations of homocysteine lowering (15,16).

### **a. NO Resolution Ultrasound of the Brachial Artery (15,16)**

After discontinuation of vasoactive medications (i.e. antihypertensives, nitrates, sildenafil) for 18 hours, and 8 hours of fasting, high resolution ultrasound of the brachial artery will be performed. Subjects will be studied supine, at ambient temperature (20-23 degrees Q, by a single investigator, blinded to treatment, who will also perform all analyses. This will occur in the CPMC radiology suite. The brachial artery end-diastolic diameter will be calculated as the average of measurements made during four cardiac cycles. Each study consists of a series of four measurements: 1) resting scan after 10 minutes of quiet rest, 2) a pneumatic cuff will be inflated to 3001-nm Hg for 5 minutes, and the second scan will be performed approximately one minute after deflation (endothelial dependent dilatation), 3) fifteen n-minutes of quiet relaxation (recovery phase), followed by a second baseline scan, 4) one 400 microgram nitroglycerin tablet will be administered sublingually, followed after 4 minutes by another scan (endothelial independent dilatation). The results will be expressed as a percentage change from mean resting artery diameter.

The assessment of cholesterol, creatinine, and hemoglobin A<sub>1c</sub> every 12 weeks roughly approximates standard of care for patients with chronic renal insufficiency and likely multiple other cardiovascular risk factors. The overall time anticipated for each subject to complete data collection is two hours (one hour at both baseline and follow-up). The duration of the entire study is 12 weeks.

### **E. Study Drugs**

Oral formulations of folate, vitamins B<sub>6</sub> and B<sub>12</sub> are FDA approved drugs indicated for the treatment of their deficiencies. The optimal means of achieving normalization of homocysteine levels (< 13 μmol/L) through oral folate and B-vitamin supplementation in chronic renal insufficiency has not yet been defined. Most protocols have used 2.5-5.0 mg of folate with or without vitamin B<sub>12</sub> (0.4mg--1mg) and B<sub>6</sub> (50mg--250mg). Patients with hyperhomocysteinemia in the setting of end-stage renal disease are particularly refractory to the effects of high doses of both folate and B-complex vitamins (17). The inability of 5 mg of folate alone to normalize homocysteine levels in predialysis renal failure patients (18) suggests that this condition is likely also associated with a degree of resistance to supplements. A study of folate 2.4 mg/d, vitamin B<sub>6</sub> 50 mg/d, and vitamin B<sub>12</sub> 0.4 mg/d for 12 weeks in renal transplant recipients normalized homocysteine levels (<12 gmol/L) in 50% (17). Presumably, however, these patients were on multiple immunosuppressive medications whose interactions are multiple and are not well defined with respect to folate and B complex vitamins. Given the anticipated refractoriness of the study population to homocysteine lowering therapy, an aggressive regimen consisting of folate 5 mg/d, B<sub>6</sub> 250 mg/d, and B<sub>12</sub> 1.0 mg/d has been chosen.

#### **a. Folate**

The side effects of folate at the doses planned are minimal. The risk of unmasking B<sub>12</sub> deficiency and precipitating neurological disease (i.e., subacute combined degeneration) is low, but real, and patients with B<sub>12</sub> deficiency at baseline will be excluded (see below)

#### **b. Vitamin B<sub>6</sub>**

Side effects at the dose proposed include nausea, (A upset, rarely photosensitivity, and peripheral neuropathy. The peripheral neuropathy of pyridoxine generally occurs at high doses (500mg-1000mg) over prolonged exposure (>1 year), although symptoms at lower doses have been described (19). The symptoms generally resolve with discontinuation (19).

#### **c. Vitamin B<sub>12</sub>**

No common side effects have been reported.

#### **d. Sublingual nitroglycerin**

Side effects include GI upset, headache, lightheadedness, and syncope. These will be minimized by maintaining supine position during the brachial artery measurements, by holding vasoactive medications for the preceding 18 hours, and by excluding those dependent on oral nitrates.

### **F. Study Subjects and Recruitment**

Outpatients will be recruited in the AIM Clinic (General Internal Medicine), and Nephrology Clinic at CPMC as well as among patients of private CPMC internal medicine and nephrology attendings.

#### **a. Inclusion criteria**

age > 18, estimated creatinine clearance 10-60 ml / min (by Cockcroft-Gault formula)

#### **b. Exclusion criteria**

evidence for progressive renal insufficiency over previous three months; previous history of renal replacement therapy; genetic hyperhomocysteinemia syndrome; dependence on daily nitrates; history of hypersensitivity to study medications; presence of A-V fistula or peripheral catheter in upper extremity; B<sub>12</sub> deficiency; transplant recipients (any organ); current use of folate, B-complex supplements, or multivitamins; presence of peripheral neuropathy

### **G. Confidentiality**

All study participants will be assigned a unique identification number and all data will be kept in a secure location accessible only to investigators.

### **H. Study Location**

All interviews and blood draws will be performed in the Nephrology Clinic (Vanderbilt Clinic 240); ultrasound will be performed in the Radiology Suite (Milstein 3),

### **I. Potential Risks and Benefits**

The proposed study drugs are well known and have a benign side effect profile. Most serious is the occurrence of peripheral neuropathy due pyridoxine, as described above, which is generally reversible. The ultrasound-guided assessment of vascular endothelial function also has minor risks. They include discomfort with cuff inflation; lightheadedness and headache with nitroglycerin administration - syncope is not anticipated given the supine position of the patients during the assessment. The risk associated with phlebotomy is small and includes discomfort, mild bleeding, and infection - these will be minimized by the use of experienced technicians.

Patients may or may not benefit in this relatively short duration study. As the primary outcome measure is a surrogate for atherosclerosis, improvement with supplementation may imply a diminished risk of atherosclerosis with prolonged therapy. Whether inexpensive, safe therapy with folate and B vitamins can reduce the excess incidence of cardiovascular disease in the millions of patients with chronic renal insufficiency is of great public health and economic interest to society.

### **J. Compensation**

Patients will receive a stipend of \$50 after the follow-up visit to compensate for their time and to encourage compliance.

### **K. Lay Summary**

#### Study Purpose

Patients with chronic kidney disease are at much higher risk of suffering a heart attack or stroke than the general population. In fact, the leading cause of death, in patients with chronic kidney disease are heart attack and stroke and not the kidney disease primarily. Why patients with chronic kidney disease are at increased risk of such problems is an area of active investigation. Recent attention has focused on the role of a compound the body naturally makes called homocysteine (homo-cis-teen). It has been observed that levels of this compound are higher than normal in patients with chronic kidney disease and that it may be damaging to blood vessels. This damage, over time, can lead to the "clogging" of arteries that causes heart attacks and strokes.

When taken orally, the naturally occurring vitamins folate, B 12 and B6 can effectively lower the level of homocysteine in the blood. What is not known is whether lowering the blood levels of homocysteine will help prevent heart attacks, strokes or death.

The intention of this study is to treat patients with chronic kidney disease with a combination of these vitamins and observe whether their blood vessels expand more efficiently at the end of the treatment. The blood vessels' ability to expand more efficiently suggests that damage to it is being reversed by the study vitamins. This would help provide evidence that all patients with chronic kidney disease should take these vitamins to prolong their lives.

### Study Subjects and Recruitment

Approximately 130 adult subjects will be recruited for this study. They will be referred by their primary care provider or kidney specialist who has been informed about the details of study, including the qualifying criteria. Recruitment will occur over about a 10-12 month period beginning in May 2002.

### Study Procedures

The subjects will be randomly assigned to receive either the vitamin supplements or placebo (inactive tablets that appear like the supplements). Blood will be drawn at the beginning of the study to determine baseline levels of cholesterol, blood sugar, kidney function and homocysteine. Next, blood vessel testing by ultrasound will occur. This consists of measuring the diameter of a major blood vessel in the arm (brachial artery) both at rest and then after inflation of a blood pressure cuff. A repeat resting measurement will be taken and then again after taking a nitroglycerin tablet. These blood tests and procedures will occur once at the beginning of the study and then once at the end of 12 weeks.

It is anticipated that each session will last about 1 hour.

### Issues

This study is short-term and has a very low risk to the participant. No major side effects are anticipated using the vitamins in the dosages planned. Vitamin B6, in high doses for prolonged periods, has been associated with damage to nerves in the feet and hands resulting in numbness or "pins and needles" sensation. This side effect is generally reversible once the vitamin is stopped. Patients who have such nerve damage already are to be excluded from the study.

### Compensation

Study subjects will be compensated \$50 after the completion of the follow-up visit.

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