Does Oral Folic Acid Lower Total Homocysteine Levels and Improve Endothelial Function in Children With Chronic Renal Failure?

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Background—Accelerated vascular disease is common in chronic renal failure (CRF) and accounts for significant mortality and morbidity. Elevated homocysteine levels may contribute by an effect on endothelial function.

Methods and Results—We performed a double-blind placebo-controlled randomized crossover trial of folic acid at 5 mg/m² in 25 normotensive children 12±3 (7 to 17) years of age with CRF (glomerular filtration rate 26.8±13.2 mL/min per 1.73 m²) of noninflammatory etiology. Each subject underwent two 8-week periods of folic acid and placebo separated by an 8-week washout period. The effect of folic acid on homocysteine levels, LDL oxidation, and both endothelial-dependent and -independent vascular function were measured. After oral folic acid, serum folate levels rose from 11.7±4.25 to 635±519 µg/L (P=0.001), red cell folate levels rose from 364±195 to 2891±2623 µg/L (P<0.001), and total homocysteine levels fell from 10.28±4.16 to 8.62±2.32 µmol/L (P=0.03). In addition, there was a significant improvement in flow-mediated dilatation (FMD) (endothelial-dependent dilatation) from 7.21±2.8% to 8.47±3.01% (P=0.036) with no change in response to glyceryl trinitrate (endothelial-independent dilatation). There was no significant change in FMD or glyceryl trinitrate during the placebo phase. There was, however, no significant difference in final FMD after placebo or folic acid. Lag times for LDL oxidation were prolonged during the treatment phase (58.4±18.7 to 68.1±25.9 minutes, P=0.01).

Conclusion—Folic acid supplementation in children with CRF may improve endothelial function with an increased resistance of LDL to oxidation. (Circulation. 2002;105:1810-1815.)

Key Words: homocysteine | folic acid | renal failure | endothelium

Premature atherosclerosis is a major cause of morbidity and mortality in adults with chronic renal failure (CRF). This may be due not only to the increased incidence of classic risk factors such as glucose intolerance, hypertension, and dyslipidemia but also to a direct adverse effect of CRF.¹

We have demonstrated endothelial dysfunction, a key early event in atherogenesis, in children with CRF without additional classic risk factors or clinical vascular disease.² One possible mechanism for endothelial damage in CRF is the presence of high circulating levels of homocysteine. Homocysteine is a sulfur-containing amino acid formed as an intermediate during the metabolism of methionine, which has been shown in population studies to be an independent risk factor for both vascular disease³,⁴ and myocardial infarction.⁵,⁶ In CRF, homocysteine is also an independent risk factor,⁷ and in dialysis patients, hyperhomocysteinemia is more prevalent than traditional cardiovascular risk factors.⁸ Homocysteine may, therefore, contribute to aggressive “accelerated atherosclerosis” in CRF. In vitro and in vivo studies suggest that homocysteine causes endothelial dysfunction either directly or via intermediate reactions by increasing oxidized LDL levels.⁹ Even modestly elevated homocysteine levels may be particularly damaging in the presence of the atherogenic risk profile of CRF.¹⁰

Folic acid has been shown to lower homocysteine levels in several populations and can improve brachial artery endothelial function.¹¹-¹³ In CRF, there appears to be relative resistance to folic acid, but supplementation in adults with doses of 5 to 15 mg/day can decrease homocysteine levels by as much as 40% to 50%.¹⁴ The impact on endothelial function has, however, been disappointing.¹⁵-¹⁷

We report the use of high-resolution ultrasound to study the effect of folic acid supplementation on homocysteine and vascular function in children with moderate to severe CRF. Children were selected specifically both to reduce the influence of confounding factors and, thus, provide a clinical...
model of uremic influences on the arterial wall and to determine whether early intervention might have greater vascular benefits than those seen in adults.

Methods

Subjects
Twenty-five children (11 girls and 14 boys, mean age 12±3 years [range 7 to 17 years]) with CRF (glomerular filtration rate <50 mL/min per 1.73 m²) were recruited from the outpatient department at Great Ormond Street Hospital for Children. Twenty-four children had congenital structural causes of CRF, and 1 child had an acquired (cortical necrosis) cause of CRF. Sample size was based on an estimated benefit of 2% in flow-mediated dilatation (FMD) with 80% power and a 5% significance level. We excluded children who were smokers, hypertensive, diabetic or nephrotic, or on vasoactive medication or dialysis. No child received folic acid supplementation or vitamins (apart from activated vitamin D) before the study. The clinical and biochemical characteristics of the study group are shown in Table 1. Twenty-three children completed the study. One child was transferred to peritoneal dialysis, and 1 child received a renal transplant.

Assessment of Vascular Function
Endothelial function was determined by recording the dilator response of the brachial artery to increased blood flow generated during reactive hyperemia (FMD). Subjects lay supine in a temperature-controlled laboratory (22°C to 25°C). The brachial artery was scanned in longitudinal section with a 7-MHz linear array transducer and an XP 128/10 (Acuson), magnified using a resolution box function and gated with the R wave of the ECG. End-diastolic images of the vessel were acquired every 3 seconds using data acquisition software (Information Integritiy) throughout the whole study and were stored off-line for later analysis. Arterial diameter over a 1- to 2-cm segment was determined for each image using automatic edge detection software (Information Integritiy). Analysis was performed by an experienced vascular technician blinded to the phase of the study. With pulse-wave Doppler, blood flow was recorded continuously throughout the study and was expressed as the velocity time integral (area under the blood velocity/time curve for a complete cardiac cycle). Baseline recordings of arterial diameter were made for 1 minute before inflation of a blood pressure cuff placed distal to the site of arterial imaging. Recording continued for 5 minutes during cuff inflation to 300 mm Hg and for 4 minutes after deflation. The time point of maximum change in diameter was also recorded. Endothelium-independent dilatation of the brachial artery was assessed by measuring the dilator response to a 25-µg dose of the nitric oxide (NO) donor, glycercyl trinitrate (GTN) given sublingually. This elicited vascular dilatation of the same magnitude as that of the endothelium-dependent flow stimulus. Results are expressed as both percentage and absolute maximum change in vessel diameter.

Laboratory Assays
Full blood count, urea, creatinine, bicarbonate, and electrolytes were measured (Vitros 750, Ortho-Clinical Diagnostics). Fasting lipid analyses were performed for total cholesterol, HDL, and triglycerides with colorimetric assays (Vitros 750, Ortho-Clinical). LDL values were calculated, and LDL subfractions were measured with high-resolution polyacrylamide gel electrophoresis (Quantimetrix), reported as the ratio of less dense to more dense (LDL1 + 2:LDL3 + 4 + 5). LDL lag times were measured by isolating LDL with density-gradient ultracentrifugation and were desalted by gel filtration. Oxidation was promoted with copper, conjugated diene production was monitored, and lag times were generated. Total serum antioxidant activity was measured with a chemiluminescent assay. This is based on a catalyzed oxidation of luminol (chemiluminescent substrate) by hydrogen peroxide, which generates free radicals. The duration of suppression of this reaction by the subject’s serum is a measure of its total antioxidant capacity. This is compared against a standard curve created by a calibrant and provides a rapid, reproducible measure of antioxidant defense in biological fluids. Serum and red cell folate levels were determined with a radioimmunoassay (Abott IMx) with a normal range for serum folate of 2 to 20 µg/L, and for red cell folate of 150 to 650 µg/L. Plasma total (free and bound) homocysteine was measured with a competitive fluorescent polarization immunoassay (normal range 4.4 to 13.7 µmol/L for adults, Abbot IMx).

Analysis
Each subject served as their own control. The data were tested for normality with the Shapiro-Wilks and the modified Kolmogorov-Smirnov tests. The data were analyzed in 2 ways. First, change in FMD (post-treatment value minus pretreatment value) on folic acid or placebo was compared with a paired t test. Second, final FMD after folic acid and after placebo were compared with ANCOVA. All descriptive data are expressed as group mean±SD, and significance is interpreted as P<0.05.

Results
The clinical and biochemical characteristics of the study group are shown in Table 1. Twenty-three children completed the study. One child was transferred to peritoneal dialysis, and 1 child received a renal transplant.

Effect of Folic Acid
There was no effect of folic acid on hemoglobin or renal function (Table 2). At entry to the study, serum folate (13.7±3.8 µg/L) and red cell folate levels (334±202 µg/L) were normal. Folic acid produced a significant increase in both serum folate (11.7±4.25 to 635±519 µg/L, P=0.001) and red cell folate (364±195 to 2891±2623 µg/L, P<0.001) levels during the treatment period.

During placebo, there was no change in serum or red cell folate levels when the placebo phase preceded the folic acid
phase (13.6±4.6 to 10.68±5.76 μg/L, and 348±244 to 351±127 μg/L, P=ns). However, in the children who received placebo after folic acid, the serum folate changed from 20±9.9 to 14.01±6.08 μg/L (P=ns) and the red cell folate changed from 820±517 to 470±185 μg/L (P=0.02) during the placebo phase. These postplacebo levels were higher at the end of the study than at entry, which suggested a carry over effect for red cell folate.

### Homocysteine Levels
Homocysteine levels at entry to the study were greater (9.85±3.57 μmol/L) than published data on normal children (Table 2). There was a significant fall in total homocysteine levels after folic acid (10.28±2.74 μmol/L, P=0.03) but not in the placebo phase (9.02±2.19 to 9.84±2.7 μmol/L, P=0.3).

### Lipid Analysis
Baseline total cholesterol levels were within the normal range (4.74±1.05 mmol/L), and there was no significant change with treatment or placebo. Triglycerides were elevated above the normal range (1.66±0.65 mmol/L) and were unchanged after folic acid or placebo (Table 2). HDL and LDL cholesterol were within the normal range at baseline (1.36±0.36 mmol/L [normal range 0.93 to 1.94] and 2.7±0.8 mmol/L [normal range 1.63 to 3.63], respectively) and did not change significantly with either treatment or placebo.

### Oxidant Stress
Baseline values for LDL lag times were within the normal range (Table 2). There was a significant increase in LDL lag times after folic acid (58.4±18.7 to 68.1±25.9 minutes, P=0.01) compared with placebo (62.8±17.4 to 63.2±13.3 minutes P=0.92), which suggests that folic acid supplementation reduced susceptibility of LDL to oxidation. Ratios of LDL to HDL (25±37 to 24±34, P=ns) remained unchanged during treatment and placebo phases (22±32 to 30±36, P=ns) as did total serum antioxidant activity (204±80 to 208±74 on treatment vs 188±65 to 216±74 μtrolox Eq on placebo, P=ns).

### Effect of Folic Acid on Vasomotor Function
There was no significant change in baseline arterial diameter, baseline arterial flow, or peak reactive hyperemia after folic acid or placebo (Table 3).

### Endothelial-Dependent Dilatation: FMD
A significant improvement in FMD, expressed as percentage and absolute change in vessel diameter (7.21±2.81% to 8.47±3.01%, P=0.036, and 0.217±0.106 cm to 0.252±0.081 cm, P=0.47), was seen after folic acid, which was not seen after placebo (8.20±3.41% to 8.80±4.01%, P=0.44, and 0.244±0.102 cm to 0.276±0.104 cm, P=0.14). There was, however, no statistically significant difference in post-treatment FMD after placebo or folic acid (P=ns). Mean time of maximum dilatation after cuff release was not significantly different before or after treatment phases (placebo: 54±16 seconds, pre-folic acid 59±13 seconds, postplacebo 65±19 seconds, and post-folic acid 66±17 seconds). No carry over or period effect on FMD was detected (P=0.2 and P=0.17, respectively).

### Endothelial-Independent Dilatation: GTN
There was no significant change in response to GTN on either folic acid (12.59±6.5% to 11.58±5.39%, P=0.28, and 0.374±0.136 cm to 0.35±0.129 cm, P=0.4) or placebo (12.93±5.71% to 13.75±6.46%, P=0.32, and 0.390±0.119 cm to 0.404±0.170 cm, P=0.5). There was no significant change in resting heart rate or supine blood pressure after folic acid or placebo.

### Discussion
This study shows that in children with CRF, supplementation with high-dose folic acid for 8 weeks results in reduction in homocysteine levels, decrease in LDL susceptibility to oxidation, and improvement in endothelial function. These encouraging findings contrast with the disappointing effects of folic acid supplementation on vascular function in adults with renal disease.

Increased cardiovascular mortality and morbidity is well recognized among adults with CRF.21 The adverse impact of CRF on cardiovascular mortality and morbidity in the young is, however, even greater, with a 500X higher rate of cardiovascular death than a control population.22 Homocysteine levels are consistently elevated in adults with CRF, and this has been suggested to play a role in the pathogenesis of atherosclerosis, especially in view of its strong association with death from vascular disease in the non-uremic population.4,6,23 A high prevalence of other risk factors exists in CRF, but an independent association has been found between elevated total homocysteine levels and the risk of myocardial infarction.24 The data on homocysteine in children is limited. Elevated total homocysteine levels (12.6±5.2 vs 8.2±3.3 μmol/L, P=0.004) have been reported in CRF children compared with controls.25 Total homocysteine levels at entry to our study (9.85±3.57 μmol/L) were also elevated in comparison to these controls.
Homocysteine levels can be lowered with folic acid. This increases tissue methylation of homocysteine to form methionine in both uremic and non-uremic individuals, even in the presence of normal folate levels. Studies in adults with hyperhomocystinemia and hypercholesterolemia have shown improvement in endothelial function as a consequence of lowering total homocysteine with folic acid. Similar studies in CRF have been disappointing. In patients with CRF and those on dialysis, no improvement in endothelial function has been demonstrated despite significant reductions in homocysteine.

We chose to evaluate children because this allowed us to study the process of atherosclerosis early in its natural history, when it is potentially more responsive to intervention. In addition, the young population provided an opportunity to minimize the unquantifiable impact of lifelong confounding risk factors on endothelial function. We excluded children with CRF secondary to inflammatory diseases, diabetes, and hypertension because these are known to have a major impact on vascular function, even in the absence of renal impairment. We did not preselect our study population on the basis of FMD or clinical severity of disease so that they would be representative of the effect of CRF in young subjects.

The technique of FMD developed by our group is ideally suited to this study. It is noninvasive, reproducible, and well validated as a measure of NO-dependent vasodilatation and, hence, endothelial function in conduit arteries. There is good correlation between endothelial-dependent responses in the coronary and forearm circulations. The impact of a range of interventions on FMD is well reported both by our group and others in both children and adults with cardiovascular risk factors.

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The dose of folic acid in our study produced serum and red cell folate levels higher than in most published clinical intervention studies on CRF patients in the literature, in which endothelial function was the primary endpoint. Variations between 1 mg and 60 mg daily have been used in the renal adult literature with no extra benefit on homocysteine levels conferred by the higher doses. Duration of treatment in adult studies varied from 4 weeks to 52 weeks with the maximum effect on homocysteine seen in the first 2 weeks, and no further lowering occurred despite increasing doses of folic acid.

At the end of the folic acid treatment period, homocysteine levels had fallen significantly. There was a 8-week washout period between the treatment phases. Analysis of serum and red cell folate acid levels showed that the subjects who received placebo after the active phase had a reduction in red cell folate levels. This implies that there was a "carry over" from the active phase and that ideally the washout period...
could have been longer. There was, however, no carry over effect on homocysteine levels.

There was a significant improvement in FMD during the folic acid treatment phase without change in response to GTN, which suggests a beneficial effect of folic acid on endothelial function after 8 weeks of treatment. It should, however, be noted that the final FMD after placebo and active phases were not significantly different. Our findings must, therefore, be interpreted with caution, and a longer-term trial may be warranted.

The mechanism by which homocysteine exerts its toxic affect on the endothelium is thought principally to be due to the generation of free radical species. \(^9\) In experimental hyperhomocysteinemia induced by methionine infusion in volunteers, vitamin C improved endothelial function. \(^33\) In our study, we noted a significant reduction in total homocysteine levels with folic acid in parallel with an increase in LDL resistance to oxidation through measurement of lag times. Total antioxidant activity was also measured, but no significant change was noted; thus, increasing the resistance of LDL oxidation might play an important role in the improvement in endothelial function because oxidized LDL is a potent vascular toxin. Alternatively folate may improve endothelial function via endogenous regeneration of tetrahydrobiopterin, \(^34\) an essential co-factor in NO production, or through a direct antioxidant effect as shown in vitro. \(^34,35\)

The improved ability to support renal function in CRF has increased the importance of prevention and treatment of vascular disease. Children are surviving into adult life with prolonged exposure to uremia, and there is good evidence that vascular disease associated with CRF is aggressive and starts very early. Folic acid is safe, lowers homocysteine, reduces LDL susceptibility to oxidation, and may improve endothelial biology relevant to the development of atherosclerosis. Long-term benefits require further study.

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