Folic acid supplementation for 3 wk reduces pulse pressure and large artery stiffness independent of MTHFR genotype

Carolyn Williams, Bronwyn A Kingwell, Kevin Burke, Jane McPherson, and Anthony M Dart

ABSTRACT
Background: Folic acid reduces plasma homocysteine and may be an important therapy for preventing cardiovascular disease. A key mechanism may be the reduction of arterial stiffness.

Objective: The effect of folic acid supplementation on blood pressure and large artery stiffness was examined in relation to methyltetrahydrofolate reductase (MTHFR) genotype.

Design: Forty-one asymptomatic men with normal or high-normal ambulatory blood pressure (systolic: >130 to <145 mm Hg; diastolic: >80 to <90 mm Hg) participated. The study had a randomized, placebo-controlled, double-blind, crossover design that incorporated 3-wk treatments with 5 mg folic acid/d or matching placebo; each treatment was separated by a 4-wk washout phase.

Results: Folic acid reduced brachial pulse pressure by 4.7 ± 1.6 mm Hg (P < 0.05) without changing mean arterial pressure. Systemic arterial compliance increased by 0.15 ± 0.03 mL/mm Hg (P < 0.05) after folic acid treatment but did not change after placebo treatment. These responses did not significantly correlate with either homocysteine or folate plasma concentrations. MTHFR genotype CC homozygotes (without the 677C→T polymorphism) with normal blood pressure had a larger reduction in homocysteine concentrations in response to folic acid than did T allele carriers. Blood pressure and arterial stiffness responses were independent of MTHFR genotype.

Conclusion: Folic acid is a safe and effective supplement that targets large artery stiffness and may prevent isolated systolic hypertension. Am J Clin Nutr 2005;82:26–31.

KEY WORDS Homocysteine, genetics, MTHFR gene, folate therapy, arterial stiffness, pulse pressure, hypertension

INTRODUCTION

Hyperhomocysteinemia is emerging as an independent predictor of stroke (1), myocardial infarction (2), atherosclerosis (3, 4), systolic hypertension (5), and cardiovascular disease death (6). In a recent meta-analysis, a 5 mmol/L increase in serum homocysteine was associated with an increase in the risk of stroke and ischemic heart disease by >50% and 30%, respectively (7). The mechanisms underlying the relation between elevated homocysteine concentrations and cardiovascular disease risk may partly relate to increased large artery stiffness and impaired endothelium-dependent vasodilation (8–10).

Folate status is one of the most important determinants of plasma homocysteine concentrations (11, 12), and folic acid supplementation significantly and safely improves endothelial dysfunction in patients with coronary artery disease (13, 14). The effects of folic acid supplementation on large artery stiffness have not been investigated. However, interventions that reduce plasma homocysteine concentrations also lower pulse pressure, which implicates a reduction in large artery stiffness as the mechanism (15). Furthermore, hyperhomocysteinaemia induced by folate restriction promotes arterial stiffening (16). Given that large artery stiffness is an independent risk factor for cardiovascular disease (17–19), the effect of folic acid supplementation on the relation between folate status, homocysteine concentrations, and large artery stiffness is of great clinical relevance.

In addition to the role of dietary intake of substrates and vitamin cofactors in homocysteine metabolism, the methyltetrahydrofolate reductase (MTHFR) gene regulates folate-dependent remethylation of homocysteine to methionine. The 677C→T substitution polymorphism within this gene causes thermolability and reduced activity of the enzyme. It has been suggested that this mutation accelerates the onset of coronary artery disease in patients with familial hypercholesterolemia or a previous myocardial infarction (20, 21).

Folic acid supplements are well tolerated and may improve vascular function both in healthy individuals and in those at elevated risk of cardiovascular disease (15, 22, 23). Our study examined the effect of folic acid supplementation on blood pressure and large artery stiffness in healthy individuals and in patients with early stage systolic hypertension. We elected to study young individuals with normal or mildly elevated blood pressure on the premise that they would be more responsive to short-term folic acid treatment than would older individuals with irreversibly stiffened large arteries (24). In addition, a beneficial effect in these groups may have implications for the prevention of isolated systolic hypertension. All variables were assessed in relation to the MTHFR 677C→T genotype with the hypothesis that the T allele would be associated with reduced folate responses.

SUBJECTS AND METHODS

Subjects

Twenty normotensive participants (ambulatory blood pressure ≤130/80 mm Hg) and 21 participants with high-normal blood pressure and large artery stiffness independent of MTHFR genotype were recruited. All variables were assessed in relation to the MTHFR 677C→T genotype with the hypothesis that the T allele would be associated with reduced folate responses.

1 From the Baker Heart Research Institute, Melbourne, Australia.
2 Supported by grants from the National Health and Medical Research Council of Australia.
3 Reprints not available. Address correspondence to AM Dart, Baker Heart Research Institute, PO Box 6492, St Kilda Road Central, Melbourne, Victoria 8008, Australia. E-mail: a.dart@alfred.org.au.

Received January 5, 2005.
Accepted for publication February 22, 2005.
blood pressure (ambulatory blood pressure: >130 to <145 mm Hg (systolic) and >80 to <90 mm Hg (diastolic)) were recruited. The participants were all disease-free and were categorized by their 24-h ambulatory blood pressure rather than by their “office” blood pressure, which is obtained by taking the average of 3 blood pressure measurements taken 3 min apart after 5–10 min rest in a supine position. We had several reasons for measuring 24-h ambulatory blood pressure: 1) it correlates better with end organ damage, 2) it predicts cardiovascular disease risk better, and 3) it is more reproducible and independent of the “white coat” effect (25). All participants were men, were aged 20–40 y, were nonsmokers, were nonusers of medication, were healthy (other than high blood pressure in some), and had no history of cardiovascular disease, diabetes, or liver disease. Two participants were taking medication for high blood pressure, which was discontinued 2 wk before the study began. The study was approved by the Alfred Hospital ethics committee. Each participant gave written informed consent before commencing the study.

Protocol

A randomized, placebo-controlled, double-blind, crossover design was used. The participants were randomly assigned to a 3-wk treatment with either 5 mg folic acid/d or matching placebo. After the first 3-wk phase, the participants entered a washout phase for 4 wk and were then crossed over to the alternate study arm to complete the final 3-wk intervention phase. A daily dose of 5 mg folic acid/d is the conventional clinical dose (26–28) that effectively lowers plasma homocysteine after 3 wk (29) and improves endothelial function after 4 wk (22), with such effects abolished after a 4-wk washout phase (23). The intervention duration of 3 wk was chosen to maximize participant adherence to the protocol. The participants fasted 10–12 h overnight before the 4 study visits and, on arrival, rested in a supine position in a temperature-controlled laboratory. After a period of 5 min, blood pressure was measured followed by measurements of arterial stiffness. Blood samples were collected by venipuncture immediately after the arterial stiffness measurements.

Supine brachial blood pressure and heart rate were determined with an automated oscillometric blood pressure monitor (Dinamap Vital Signs Monitor 1846SX; Critikon, Tampa, FL) after 5 min of quiet rest (30). The mean of 10 measurements made at 3-min intervals was recorded.

Folate intake

All participants kept a 5-d food diary and recorded all food and beverages consumed at the start of the study. The participants were requested to maintain their routine dietary pattern, to cease taking vitamin supplements, and to avoid binging on any particular foods or beverages, especially alcohol. Data were analyzed for nutrient content, including folate, with the FoodWorks AUSTNUT database (version 2; Xyris Software Pty Ltd, Highgate Hill, Australia).

Arterial stiffness

Large artery stiffness was globally assessed through measurements of systemic arterial compliance (SAC) and regionally assessed through measurements of pulse wave velocity (PWV). SAC measures the change in volume for a given change in contained pressure and is determined by the interaction of arterial mechanical properties and vessel geometry. PWV is inversely related to distensibility and estimates the underlying mechanical properties of the artery being assessed. PWV is thus a geometry-independent measure of arterial stiffness.

SAC was determined noninvasively with the use of a 2-element Windkessel model of the arterial system as described previously (31–33). The method involves simultaneous measurements of the ascending aortic blood flow and of the driving pressure in the ascending aorta to derive compliance over the entire arterial tree. Ascending aortic blood flow velocity was measured with a 3.5-MHz transducer (Multi-Dopplex MD1, Huntleigh Technology, Luton, United Kingdom) placed at the suprasternal notch. The product of aortic flow velocity and left ventricular outflow tract area, measured by 2-dimensional echocardiography (Sonos 1500; Hewlett-Packard, Andover, MA), was used to calculate aortic volumetric flow. Aortic root driving pressure was estimated by applanation tonometry of the right carotid artery with a Millar Micro-Tip pressure transducer (SPT-301; Millar Instruments, Houston, TX). Mean and diastolic brachial artery blood pressures were simultaneously measured (Dinamap Vital Signs Monitor 1846SX; Critikon) and used to calibrate the carotid arterial pressure contour. This method has been validated against invasive pressure recordings (34, 35).

PWV was measured centrally (between the right carotid and femoral arteries) and peripherally (between the right femoral and dorsal pedis arteries) by simultaneous applanation tonometry (SPT-301; Millar Instruments) as described previously (36).

Biochemical analysis

Plasma total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols, and glucose were measured with a bench-top analyzer (Cholestech LDX; Cholestech Corp, Hayward, CA). Samples for homocysteine analysis were placed immediately on ice, centrifuged at 1500 × g for 10 min at 4 °C, and frozen at −80 °C within 2 h of collection. Plasma homocysteine concentrations were measured with an Abbott Axsym immunochemistry analyzer assay with fluorescence polarization immunoassay technology (Axis Biochemicals ASA, Oslo, Norway). The normal homocysteine range is 5–15 μmol/L.

MTHFR genotyping

DNA was isolated with the PureGene DNA purification system (Gentra Systems, Plymouth, MN). The 677C→T polymorphism was detected by polymerase chain reaction as described by Frooss et al (37).

Statistics

All data are presented for the total population (ie, normal and high-normal blood pressure combined). Baseline data are presented as means ± SDs. Intervention data are presented as means ± SEs of the difference (SED). The effects of folic acid or placebo intervention were compared with repeated-measures analysis of variance, with variable change as the dependent factor and the number of intervention, genotype, and blood pressure group (normal or high-normal) as independent factors. Proportional data were analyzed by Fisher’s exact test. All analyses were done by using SPSS (version 11.5; SPSS Inc, Chicago, IL).

RESULTS

Baseline data are presented in Table 1 and Table 2. All participants completed the study and no adverse effects were
TABLE 1
Baseline characteristics of the patients1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85 ± 17</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179 ± 8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.90 ± 0.03</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>Plasma triacylglycerol (mmol/L)</td>
<td>1.4 ± 1.0</td>
</tr>
<tr>
<td>Plasma HDL (mmol/L)</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Plasma LDL (mmol/L)</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Plasma Hcy (µmol/L)</td>
<td>8.8 ± 1.9</td>
</tr>
<tr>
<td>Total folate intake (mg/d)</td>
<td>316 ± 161</td>
</tr>
<tr>
<td>Plasma folate (nmol/L)</td>
<td>25 ± 7</td>
</tr>
<tr>
<td>RBC folate (nmol/L)</td>
<td>1034 ± 287</td>
</tr>
<tr>
<td>Plasma vitamin B-12 (pmol/L)</td>
<td>281 ± 106</td>
</tr>
<tr>
<td>Plasma creatinine (mol/L)</td>
<td>0.08 ± 0.01</td>
</tr>
</tbody>
</table>

1 All values are mean ± SD. Hcy, homocysteine; RBC, red blood cell.

reported. There were no significant effects due to the order of intervention for any of the reported variables. There was no significant change in plasma creatinine or vitamin B-12 concentrations in response to folic acid treatment (data not shown).

The MTHFR 677C→T genotype frequencies were as follows: 56.0% CC, 31.7% CT, and 12.3% TT; these frequencies did not differ from the Hardy-Weinberg equilibrium. Because of the low frequency of the T allele, CC homozygotes were compared with T allele carriers (CT and TT) in subsequent analyses.

Folate and homocysteine concentrations

The 3-wk supplementation with folic acid significantly increased plasma folate and red blood cell folate concentrations and reduced plasma homocysteine concentrations compared with placebo (Figure 1). In those participants with a normal blood pressure, the CC genotype group had a significantly greater homocysteine response to folic acid supplementation than did the T allele carriers (−1.46 ± 0.29 µmol/L for CC homozygotes compared with −0.15 ± 0.33 µmol/L for T allele carriers; P < 0.01). However, differences between the CC homozygotes and the T allele carriers were not significant in those with high-normal blood pressure. Regardless of blood pressure

TABLE 2
Baseline blood pressures and arterial stiffness measurements1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial SBP (mm Hg)</td>
<td>131 ± 16</td>
</tr>
<tr>
<td>Brachial DBP (mm Hg)</td>
<td>74 ± 10</td>
</tr>
<tr>
<td>Brachial MAP (mm Hg)</td>
<td>95 ± 12</td>
</tr>
<tr>
<td>Brachial PP (mm Hg)</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>SAC (mL/mm Hg)</td>
<td>0.99 ± 0.4</td>
</tr>
<tr>
<td>Aortic PWV</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>Peripheral PWV</td>
<td>10.6 ± 1.5</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>62 ± 8</td>
</tr>
</tbody>
</table>

1 All values are mean ± SD of 10 consecutive measurements at baseline. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; PP, pulse pressure; HR, heart rate; SAC, systemic arterial compliance; PWV, pulse wave velocity.

FIGURE 1. Mean (±SD) change in plasma folate, red blood cell (RBC) folate, and plasma homocysteine (Hcy) in the placebo- and folic acid–treated groups. **Significantly different from placebo: *P < 0.05, **P < 0.01, ***P < 0.001.

group, plasma homocysteine concentrations were reduced >1 µmol/L in 59% of CC homozygotes compared with 22% of T allele carriers (P < 0.05). There was no significant difference in the serum or red blood cell folate response to folic acid supplementation between genotype groups.

Blood pressure

Mean and diastolic blood pressures were unaffected by folic acid supplementation (changes in mean pressure were −0.8 ± 1.3 and −1.6 ± 1.0 mm Hg for placebo and folate, respectively; respective changes in diastolic pressure were −0.3 ± 1.1 and 0.0 ± 1.0 mm Hg). Decreases in brachial systolic blood pressure (−3.4 ± 1.0 compared with −0.3 ± 1.3 mm Hg) and central systolic blood pressure (−1.6 ± 1.9 compared with −0.3 ± 2.3 mm Hg) were greater during folic acid treatment than during placebo treatment, but the differences were not statistically significant. In contrast, both brachial and central pulse pressures were significantly reduced by folic acid compared with placebo intervention (Figure 2). Interactions between the response to folic acid supplementation and genotype or blood pressure group were not statistically significant.

Arterial stiffness

SAC increased significantly in response to folic acid supplementation compared with placebo (Figure 2). This response was unaffected by genotype or blood pressure group.
Changes in PWV were not significantly different between the folic acid and placebo intervention phases (−0.09 ± 0.21 and 0.19 ± 0.25 m/s peripheral PWV for folic acid and placebo treatments, respectively; −0.10 ± 0.11 and 0.09 ± 0.10 m/s central PWV for folic acid and placebo treatments, respectively). Interactions between the response to folic acid supplementation and genotype or blood pressure group were not statistically significant.

**DISCUSSION**

The major finding of this study was that short-term dietary supplementation with folic acid reduced pulse pressure in young men with normal or mildly elevated systolic blood pressure. Pulse pressure decreased in the absence of any change in mean arterial pressure and in conjunction with a decrease in arterial stiffness. The effect of folic acid supplementation on pulse pressure is thus likely to be secondary to a reduction in arterial stiffness. There was no significant relation between changes in plasma homocysteine concentrations and changes in blood pressure or arterial stiffness. Although the decrease in homocysteine concentration in response to folic acid supplementation was greater in CC homozygotes of the MTHFR 677C→T mutation than in T allele carriers, blood pressure and arterial stiffness responses were similar in both genotype groupings. These data indicate that, regardless of MTHFR genotype, folic acid supplementation may be effective in the prevention of elevated pulse pressure, a condition that is secondary to elevated arterial stiffness.

A previous study reported an association between plasma homocysteine concentrations, systolic blood pressure, and indexes of arterial stiffness (5), but the effects of folic acid on arterial stiffness have not been studied. Interestingly, although folic acid supplementation increased SAC, both central and peripheral PWV were unchanged. This disparity most likely relates to the fact that these measures assess different arterial territories. The carotid to femoral PWV omits the most proximal and elastic portion of the aorta. The absence of a folic acid–mediated effect on this variable suggests that the changes in arterial stiffness occurred primarily in the proximal aorta. The distal PWV data indicate that folic acid supplementation does not influence the stiffness of smaller conduit arteries.

**Mechanisms**

Responses to folic acid were confined to effects on pulse pressures; no effects on mean or diastolic pressures were observed. These findings are consistent with the hypothesis that homocysteine reduces large artery stiffness. The absence of an effect on mean and diastolic pressure makes it less likely that the observed changes in arterial stiffness are themselves secondary to blood pressure changes. The folic acid–induced reduction in arterial stiffness is likely related to multiple homocysteine-dependent and -independent mechanisms. Rapid changes in nitric oxide–mediated endothelial function were observed after supplementation with folic acid but before plasma homocysteine changes were detected (38), which implies a role for plasma homocysteine-independent mechanisms. Such effects may be mediated by the antioxidant properties of folic acid (8, 39) and are likely to mediate a rapid reduction in arterial stiffness through a reduction in the catabolism of nitric oxide and an enhancement of endothelial-dependent vasodilation (40). Nestel et al (41) reported rapid increases in arterial stiffness within 2.5 h of a methionine load before homocysteine concentrations changed. Another study suggests that methionine loading may mediate such detrimental effects on arterial stiffness through an impairment of the nitric oxide system (42). Changes in plasma homocysteine concentrations may also influence endothelial-mediated effects on arterial stiffness. Scholze et al (9) reported improvements in endothelial function and a reduction in pulse pressure in patients with end-stage renal failure after intravenous acetylcysteine reduced homocysteine concentrations during hemodialysis. Such effects may relate to a reduction in oxidative stress and inflammatory factors that are associated with elevated plasma homocysteine (9).

Although effects on endothelial function may rapidly modulate arterial stiffness, inhibition of the destructive effects of homocysteine on the extracellular matrix may mediate long-term structural benefits. Homocysteine increases the expression of elastolytic matrix metalloproteinases, including MMP-2 and MMP-9 (43, 44). This mechanism may explain the elastinolytic erosion of the arterial wall in minipigs after methionine-induced hyperhomocysteinemia (10). Patients with hyperhomocysteinemia released more MMP-9 from peripheral blood mononuclear cells in response to oxidized LDL cholesterol stimulation than did healthy control subjects (45). Folic acid supplementation may thus inhibit the effects of homocysteine-induced extracellular matrix elastolysis and thereby reduce arterial stiffness.
MTHFR genotype

Consistent with a previous study (46), T allele carriers of the 677C→T polymorphism had a smaller reduction in homocysteine concentrations in response to folic acid supplementation than did CC homozygotes. Other investigators suggest that T allele carriers have lower baseline plasma folate concentrations, have higher baseline plasma homocysteine concentrations, and are more sensitive to the homocysteine-lowering effect of folic acid supplementation than are CC homozygotes (47–49). In the present study, there was no difference in baseline plasma homocysteine or plasma folate concentrations between genotype groupings. Given this fact and the lower MTHFR enzyme activity in T allele carriers, the smaller homocysteine response in this group was expected. Despite this difference, arterial compliance increased in response to folic acid supplementation independent of genotype. These data are consistent with the hypothesis that folic acid mediates its arterial and hemodynamic effects partly independent of MTHFR activity.

Clinical relevance

Both arterial stiffness and pulse pressure have been positively related to cardiovascular and, in particular, coronary outcome (17–19, 50). The underlying mechanism likely relates to the mismatch in cardiac blood supply and demand when the heart ejects into a stiff circulation. This effect is mediated by increased cardiac afterload, secondary to an elevation in systolic pressure and a reduction in coronary perfusion as a consequence of lower diastolic pressure (51–53). In patients with coronary artery disease who have stiffer aortas, this translates to a reduction in myocardial ischemic threshold (54). Interventions that reduce arterial stiffness may thus have efficacy not only in reducing systolic and pulse pressure but also in raising the ischemic threshold. Currently available drugs do not specifically target the large arteries. Our data suggest that folic acid supplementation represents a simple and safe strategy to reduce or even prevent age-related arterial stiffening and pulse pressure elevation. Given that ≈20% of middle-aged men and women in Australia and ≈22% in the United States consume less than the recommended daily intake of folate, supplementation with folic acid or fortification of food with B vitamins could have beneficial effects (28, 55).

The results from the 5-d dietary intake analysis indicate that the average daily intake of folate in our subject group was above that recommended by the National Health and Medical Research Council of Australia; however, 21% of participants had an intake of folate that was less than the recommended intake. These data imply that the benefits of folic acid supplementation may not be limited to persons with an inadequate dietary intake of folate.

In summary, short-term folic acid treatment reduces pulse pressure and arterial stiffness in young men. This effect was independent of MTHFR genotype. Our data indicate that folic acid is a safe and effective supplement that targets large artery stiffness and may reduce isolated systolic hypertension.

KB and JM provided expert technical assistance during this project. CW, BAK, and AMD were involved in the study conception, design, and analysis and in the writing of the manuscript. None of the authors had any conflicts of interest.

REFERENCES

24. Ferrier KE, Waddell TK, Gatziada CD, Cameron JD, Dart AM, Kingwell


