Influence of Folic Acid on Postprandial Endothelial Dysfunction

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Abstract—Triglyceride-rich lipoproteins that circulate postprandially are increasingly being recognized as potentially atherogenic. These particles also have been shown to cause endothelial dysfunction. We recently demonstrated that acute parenteral administration of folic acid restores endothelial function in vivo in patients with increased LDL cholesterol levels. In vitro data suggested that this effect could be mediated by a reduction of radical stress. In the present study, therefore, we evaluated the effect of an acute oral fat load on both endothelial function and oxygen radical production. Next, we studied whether 2 weeks of pretreatment with 10 mg folic acid PO could prevent these fat-induced changes. We conducted a prospective, randomized, placebo-controlled study to evaluate the effect of oral folic acid administration (10 mg/d for 2 weeks) on basal endothelial function as well as endothelial function on an acute fat load in 20 healthy volunteers 18 to 33 years old. Endothelial function was assessed as flow-mediated dilatation (FMD). Endothelium-independent dilatation was measured after sublingual nitroglycerin spray. Oxygen radical stress was assessed by measurement of the urinary excretion of the stable radical-damage end product malondialdehyde. During administration of placebo, FMD decreased significantly after an acute oral fat load, with a median from 10.6% (8.3% to 12.2%) to 5.8% (3.0% to 10.2%), P<0.05. During folic acid administration, FMD was unaffected by a fat load, with a median from 9.6% (7.1% to 12.8%) to 9.9% (7.5% to 14.1%), P=NS. The increase in malondialdehyde excretion in the urine after fat loading was also prevented during folic acid administration (absolute increase after an acute fat load during placebo, 0.11±0.1 µmol/L versus folic acid, 0.02±0.1 µmol/L, P<0.05). The response to the endothelium-independent vasodilator nitroglycerin remained unaltered throughout the study. Pretreatment with oral folic acid prevents the lipid-induced decrease in FMD as well as the lipid-induced increase in urinary radical-damage end products. Because these observations were made in healthy volunteers with normal folate and homocysteine levels, it is suggested that a higher folate intake in the general population may have vasculoprotective effects. (Arterioscler Thromb Vasc Biol. 2000;20:185-188.)

Key Words: endothelial function • malondialdehyde • triglyceride-rich lipoproteins • folic acid

Triglyceride-rich lipoproteins have been shown to stimulate atherosclerosis in both humans and animal models.1–3 In recent observations, these lipid particles could also adversely affect the antithrombotic properties of the endothelium, mainly by interfering with the L-arginine–NO pathway.4–6 Even a transient increase in triglyceride-rich lipoproteins may alter vascular functions, such as endothelium-dependent vasodilatation.7

We have recently demonstrated that acute parenteral administration of the active form of folic acid, 5-methyltetrahydrofolate (5-MTHF), restores endothelial function in patients with familial hyperlipidemia characterized by increased LDL cholesterol levels.8 In these patients, decreased NO bioavailability may relate to both decreased production and increased degradation of NO. In in vitro studies, 5-MTHF was able to scavenge oxygen radicals, suggesting that 5-MTHF in vivo may improve NO availability by preventing NO degradation by oxygen radicals.

In the present study, we evaluated the effect of postprandial lipemia on endothelial function, assessed as flow-mediated dilatation (FMD), and radical stress, measured as the excretion of oxidative-damage end products in the urine before and after fat loading. Using a randomized, placebo-controlled crossover study design, we subsequently tested whether 2 weeks of oral pretreatment with folic acid can prevent the lipid-induced changes in endothelial function and/or redox dysregulation. Because the studies were carried out in healthy volunteers with normal folate and homocysteine levels, the observations made in our study may give an indication of the vasculoprotective effect of dietary folate intake in the general population.

Methods

Subjects
Twenty healthy volunteers 18 to 33 years old participated. All individuals were normotensive (systolic blood pressure <160 mm Hg and diastolic blood pressure <90 mm Hg) and had no
history of cardiovascular disease or family history of premature vascular disease. All subjects had normal plasma folate (>6.8 nmol/L) and homocysteine (<15.5 μmol/L) concentrations. Fasting triglyceride and cholesterol concentrations were <2.0 mmol/L and 4.5 mmol/L, respectively. The subjects did not use medication. The study was approved by the Medical Ethics Committee of the University Hospital Utrecht, and written informed consent was obtained from all participants.

Study Design
A randomized, double-blind, placebo-controlled crossover trial was performed comparing 10 mg folic acid PO versus placebo. A dose of 10 mg folic acid was chosen to reach optimal folic acid saturation during a relatively short (2 weeks) protocol. All subjects were randomly assigned to receive either oral folic acid or placebo treatment for 2 weeks, followed by the forearm vasomotion study. After an 8-week washout period, subjects were crossed over to placebo or folic acid, respectively, for another 2 weeks, again followed by the second vasomotion study. The dosage of folic acid was estimated to cause increments in plasma folate similar to those previously observed with acute infusion of 5-MTHF. The vasomotion study consisted of assessment of FMD and nitroglycerin-induced vasodilation (see below) before and after a standard oral fat load. The fat load consisted of 50 g fat (in the form of whipped cream, 40% fat) per square meter body surface.

All subjects refrained from drinking caffeine-containing beverages, smoking, and eating for 12 hours before the vasomotion studies. At each visit, blood samples were drawn for laboratory determinations of triglycerides, total cholesterol, HDL cholesterol, and homocysteine concentrations before and after 4 hours of an oral fat load. At each visit, urine was collected before the oral fat load and 3 to 6 hours after the oral fat load for determination of excretion of the stable oxidative-damage end product malondialdehyde (MDA).

Forearm Vasomotion Study
The ultrasound measurements were performed at the elbow of the right arm, with the subject in the supine position, with a vessel wall–movement system (Wall Track System, Pie Medical), which consists of an ultrasound imager with a 7.5-MHz linear array transducer connected to a data acquisition system and a personal computer. In short, an optimal 2D B-mode image of the brachial artery was obtained. An M-line perpendicular to the vessel was selected. Next, the ultrasound system was switched to M-mode, after which the storage of data was begun. The vessel-movement detector system registered end-diastolic vessel diameter repeatedly during a period of 5 to 6 cardiac cycles. This procedure was performed 3 times. The measurements were averaged to provide a baseline diameter measurement.

By inflation of a blood pressure cuff for 4 minutes at a pressure of 100 mm Hg above the systolic blood pressure, ischemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 3 minutes after cuff release, with measurements at 30-second intervals. The widest lumen diameter was taken as a measure for maximal diameter. After 10 minutes of rest, allowing the artery to return to its baseline diameter, sublingual nitroglycerin spray was administered as an endothelium-independent dilator. Measurements were obtained for another 5 minutes at 1-minute intervals.

FMD and nitroglycerin-induced dilatation (NTG) were expressed as a percentage change relative to baseline diameter.

Laboratory Determinations
High-performance liquid chromatographic analysis was used to measure MDA in urine, as described previously. A good reproducibility of the method was found, with an intrarun coefficient of variation of 3.1% for MDA and a detection limit of 0.2 μmol/L. The measurement of MDA in urine shows an excellent resolution, and the component is easy to identify.

Statistical Analysis
Group values are expressed as mean±SD. Because of failure of the normality test for FMD data and NTG data, FMD and NTG values are expressed as median (25th to 75th percentile), and statistical tests on ranks were used. Differences in FMD at baseline between the treatment periods were tested with a 1-way repeated-measures ANOVA on ranks. Differences in FMD before and after lipid load within 1 treatment session were tested with the Wilcoxon signed rank test. If variance ratios reached statistical significance, differences were analyzed with the Student-Newman-Keuls test for a value of P<0.05.

Lipid changes before and after lipid load and between treatment groups were tested by paired-samples t test with Bonferroni correction.

MDA is presented as mean±SD. Comparisons between groups were made by repeated-measures ANOVA and Student’s t test.

Differences between changes in FMD and MDA before and after lipid load between the placebo and folic acid treatment sessions were tested with 1-way repeated-measures ANOVA.

Correlation testing was performed by linear regression. Differences were considered significant at a value of P<0.05.

Results
The characteristics of the study group before an oral fat load after either placebo or folic acid treatment are given in Table 1. There were no statistically significant differences between baseline parameters during the placebo and folic acid treatment periods, ie, lipid parameters (Table 2), basal arterial diameter, and basal FMD and NTG.

During placebo therapy, fat loading induced an increase in triglyceride levels, whereas the other lipid parameters remained unaltered (Table 2). FMD was impaired after the fat load (Figure). FMD decreased from a median of 10.6% (8.3% to 12.2%) to 5.8% (3.0% to 10.2%), P<0.05, whereas NTG remained unaffected (preprandial NTG, 19.7% [15.6% to 24.9%]; postprandial NTG, 18.0% [13.3% to 21.6%], P=NS). Urinary MDA concentration in the urine (expressed as the ratio of the urinary creatinine), a stable oxygen radical–damage end product, also demonstrated a significant increase on fat loading (baseline MDA, 0.12±0.10 μmol/L; after fat load, 0.22±0.12 μmol/L, P<0.05). Values are summarized in Table 3.

During folic acid therapy, plasma levels of folate increased significantly, from 13.7 nmol/L before to 701.0 nmol/L after treatment (P<0.05). Homocysteine levels decreased during folic

| TABLE 1. Characteristics of the Study Group Before an Oral Fat Load After Placebo and Folic Acid Treatment |
|-------------------------------|-------------------------------|
|                               | Placebo                       | Folic Acid                  |
| Participants, n               | 20                            | 20                          |
| Age, y                        | 23 (3.4)                      | 23 (3.4)                    |
| Male sex, %                   | 50                            | 50                          |
| Smoking, %                    | 20                            | 20                          |
| Body mass index, kg/m²        | 22.8 (2.6)                    | 21.9 (2.7)                  |
| Systolic blood pressure, mm Hg| 135 (14.8)                    | 137 (14.1)                  |
| Diastolic blood pressure, mm Hg| 69 (8.8)                     | 69 (9.2)                    |
| Folate, nmol/L                | 13.7 (3.9)                    | 701 (207.4)*                |
| Homocysteine, μmol/L          | 7.2 (2.1)                     | 5.0 (0.7)*                  |
| MDA, μmol/L                   | 0.12 (0.10)                   | 0.18 (0.12)                 |
| Vessel size, mm               | 4.0 (0.5)                     | 4.1 (0.4)                   |
| FMD, %                        | 10.6 (8.3–12.2)               | 9.6 (7.1–12.8)              |
| NTG, %                        | 19.7 (15.6–24.9)              | 16.8 (10.4–22.8)            |

Values are percentages, means, or medians, with SD or 25th–75th percentile in parentheses. *P<0.05 vs placebo.
acid treatment (Table 1; \(P<0.05\)). Preprandial FMD was comparable to preprandial FMD during placebo, 10.6% (8.3% to 12.2%) versus 9.6% (7.1% to 12.8%) after folic acid treatment. The basal excretion of MDA was also comparable to placebo therapy (0.12±0.10 versus 0.18±0.13 \(\mu\)mol/L, \(P=\text{NS}\), placebo versus folic acid treatment) (Table 3). Whereas the rise in triglyceride levels after fat load was unaffected by folic acid therapy (Table 2), the impairment in FMD was completely prevented (Table 3). Accordingly, the change in FMD (difference between preprandial and postprandial) was significantly different between placebo and folic acid treatment (Figure). The increase in urinary MDA concentration was also annihilated by folic acid therapy (Table 3). Again, the change in MDA (difference between preprandial and postprandial) was significantly different during placebo and folic acid treatment (placebo, 0.11±0.10 versus folic acid, 0.01±0.08, \(P<0.01\)). The response to the endothelium-independent vasodilator nitroglycerin remained unaffected (Table 3).

By using an independent \(t\) test, we tested whether there was any carryover effect of folic acid after an 8-week washout period. There was no significant difference in the sum of responses of subjects receiving placebo followed by folic acid compared with the sum of responses of subjects receiving folic acid followed by placebo. This test was performed for vessel size (\(P=0.87\)), FMD (\(P=0.17\)), NTG (\(P=0.10\)), MDA (\(P=0.22\)), and homocysteine (\(P=0.99\)).

### Discussion

In the present study, we demonstrate that an acute fat load is associated with increased excretion of oxidative end products in the urine of healthy volunteers. Acute fat loading also causes a sharp decrease in FMD. Both increased radical damage and impaired FMD can be prevented by oral pretreatment with folic acid.

Evidence has accumulated that triglyceride-rich lipoproteins play an important role in the atherogenic process. Accordingly, the impaired remnant clearance in, eg, diabetes, familial combined hyperlipidemia, and renal insufficiency has been put forward as an important risk factor for future cardiovascular disease.\(^3,5\) In support of this hypothesis, it has been shown in both in vivo and in vitro studies that remnant lipoproteins are associated with impaired endothelium-derived NO activity,\(^4,6,7\) which is a key to the antiatherosclerotic properties of the endothelium.\(^12\) In the present study, we also demonstrate a consistent decrease in FMD on lipid loading.

Previous studies have shown that NO is the main determinant for FMD.\(^13\) Triglyceride-rich lipoproteins may impair FMD by compromising production of NO as well as increasing degradation of NO by, eg, oxygen radicals.\(^14,15\) Using a

| Table 2. Preprandial and Postprandial Lipid Values During Placebo and Folic Acid Treatment |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Cholesterol, mmol/L | HDL, mmol/L | Triglyceride, mmol/L |
|                                | Placebo | Folic Acid | Placebo | Folic Acid | Placebo | Folic Acid |
| Preprandial                     | 4.4 (0.7) | 4.4 (0.7) | 1.4 (0.3) | 1.5 (0.3) | 1.0 (0.4) | 1.1 (0.5) |
| Postprandial                    | 4.4 (0.7) | 4.5 (0.7) | 1.4 (0.3) | 1.5 (0.2) | 1.8 (0.8)* | 1.8 (0.8)* |

Values are means with SDs in parentheses. *\(P<0.05\) vs preprandial values.
stable isotope technique, we recently demonstrated that (whole-body) NO production was unimpaired in hypercholesterolemic patients. In line with this, in vitro studies have shown that oxidative inactivation of NO is predominant in hyperlipidemia. Our present data extrapolate this concept to the postprandial state in healthy volunteers.

Oral supplementation of folic acid completely prevents the increase in oxygen radical stress and the impairment in FMD after an acute fat load. The mechanism by which folic acid exerts beneficial effects on redox state and endothelial function simultaneously after an acute fat load can be explained in several ways, as follows.

Folic acid may increase NO production by NO synthase. Folic acid has been suggested to increase endogenous regeneration of tetrahydrobiopterin, an essential cofactor for NO synthase. Such an effect of folic acid may result in decreased NO synthase–dependent O₂⁻ formation as well as increased NO production (Reference 18; E.S.G.S., unpublished observations). Alternatively, as folic acid is reduced by the gastrointestinal tract into 5-MTHF, endothelial uptake of 5-MTHF may improve the active oxidant state by donating electrons) may improve the endothelial redox state. In general, folic acid has been shown to possess direct scavenging effects in vitro.

Finally, the well-known homocysteine-lowering effect of folic acid could contribute to improvements of endothelial dysfunction. However, in the present study, this mechanism probably cannot explain the observed effects on endothelial function, because the folic acid–induced decrease in homocysteine concentrations was not associated with changes in baseline FMD.

In conclusion, our data indicate that oral treatment with folic acid restores endothelial dysfunction and abolishes the increase in radical-damage end products induced by triglyceride-rich lipoproteins. In combination, these data imply that folic acid enhances NO bioavailability through inhibition of lipid-induced oxygen radical stress. These data underscore a potential beneficial effect of folic acid supplementation for cardiovascular prevention strategies, especially in patients with an impaired cholesterol remnant clearance, such as in diabetes and familial combined hyperlipidemia. It is also of interest that higher dietary folate intake apparently may also protect healthy humans from daily fat-associated endothelial insults. This may imply that in the general population as well, a higher folate intake may be vasculoprotective.

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References

TABLE 3. Brachial Artery Responses and MDA Concentrations After an Oral Fat Load Preceded by Placebo and Folic Acid Treatment

<table>
<thead>
<tr>
<th>Time, h</th>
<th>FMD, %</th>
<th>NTG, %</th>
<th>MDA, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Placebo 10.6 (8.3–12.2)</td>
<td>Placebo 19.7 (15.6–24.9)</td>
<td>Placebo 0.12 (0.10)</td>
</tr>
<tr>
<td>4</td>
<td>Folic acid 5.8 (3.0–10.2)*</td>
<td>Folic acid 16.9 (15.2–20.8)</td>
<td>Folic acid 0.22 (0.12)†</td>
</tr>
</tbody>
</table>

Values are means or medians with SD or 25th–75th percentile in parentheses.

*P<0.05 vs placebo values.
†P<0.05 vs preprandial values.
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