Role of Oxidant Stress in Endothelial Dysfunction Produced by Experimental Hyperhomocyst(e)inemia in Humans

Prapti M. Kanani, MD; Christine A. Sinkey, RN; Roger L. Browning, BA; Margaret Allaman, BA; Howard R. Knapp, MD, PhD; William G. Haynes, MBChB, MD

Background—Moderate elevations in plasma homocyst(e)ine concentrations are associated with atherosclerosis and hypertension. We tested the hypothesis that experimental perturbation of homocysteine levels produces resistance and conduit vessel endothelial dysfunction and that this occurs through increased oxidant stress.

Methods and Results—Oral administration of L-methionine (100 mg/kg) was used to induce moderate hyperhomocyst(e)inemia (≈25 μmol/L) in healthy human subjects. Endothelial function of forearm resistance vessels was assessed by use of forearm vasodilatation to brachial artery administration of the endothelium-dependent dilator acetylcholine. Conduit vessel endothelial function was assessed with flow-mediated dilatation of the brachial artery. Forearm resistance vessel dilatation to acetylcholine was significantly impaired 7 hours after methionine (methionine, 477±82%; placebo, 673±110%; P=0.016). Methionine did not alter vasodilatation to nitroprusside and verapamil. Flow-mediated dilatation was significantly impaired 8 hours after methionine loading (0.3±2.7%) compared with placebo (8.2±1.6%, P=0.01). Oral administration of the antioxidant ascorbic acid (2 g) prevented methionine-induced endothelial dysfunction in both conduit and resistance vessels (P=0.03).

Conclusions—Experimentally increasing plasma homocyst(e)ine concentrations by methionine loading rapidly impairs both conduit and resistance vessel endothelial function in healthy humans. Endothelial dysfunction in conduit and resistance vessels may underlie the reported associations between homocysteine and atherosclerosis and hypertension. Increased oxidant stress appears to play a pathophysiological role in the deleterious endothelial effects of homocysteine. (Circulation. 1999;100:1161-1168.)

Key Words: endothelium ■ nitric oxide ■ free radicals ■ acetylcholine ■ antioxidants

Homocystinuria markedly increases plasma homocyst(e)ine concentrations (>100 μmol/L) and is associated with premature thrombosis and atherosclerosis. Even moderate hyperhomocyst(e)inemia (>10 μmol/L) is associated with increased risk of atherosclerosis and hypertension.

The endothelium modulates platelet adhesion, macrophage migration, lipid transport, and mitogenesis. Endothelial vasomotor dysfunction is an important predictor of atherosclerosis and its complications. High concentrations of homocysteine have deleterious effects on the vascular endothelium in animals. Thus, homocysteine-induced endothelial dysfunction is a plausible mechanism for predisposition to vascular disease.

Patients with hyperhomocyst(e)inemia exhibit endothelial dysfunction. In addition, experimental hyperhomocyst(e)inemia induced by oral methionine produces conduit vessel endothelial dysfunction in humans. However, the effects of hyperhomocyst(e)inemia on resistance vessel endothelial function are unclear. This study was designed to test experimentally the hypothesis that experimental hyperhomocyst(e)inemia induced by oral methionine impairs endothelium-dependent vasodilatation in both resistance and conduit vessels.

The pathophysiological mechanisms underlying endothelial dysfunction to methionine loading are unclear. Homocysteine increases oxidant stress in vitro. Therefore, we also examined whether administration of a potent antioxidant, ascorbic acid, could prevent endothelial dysfunction induced by experimental hyperhomocyst(e)inemia.

Methods

Subjects
Forty healthy subjects without risk factors for or clinical evidence of atherosclerosis were recruited by advertisement to participate in 3 separate protocols (Table 1). The studies were conducted after written informed consent was obtained from each subject and with approval of the Institutional Review Board. No subject received vasoactive drugs in the week before the study, and all abstained from alcohol for 24 hours and from caffeine for ≥12 hours before any measurements. Studies were performed in a quiet room maintained at a constant temperature between 22°C and 25°C.

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TABLE 1. Baseline Demographics

<table>
<thead>
<tr>
<th>Protocol A</th>
<th>Protocol B</th>
<th>Protocol C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>32 ± 3</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>8/2</td>
<td>15/5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81 ± 5</td>
<td>76 ± 6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177 ± 2</td>
<td>172 ± 2</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>160 ± 10</td>
<td>160 ± 9</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>90 ± 11</td>
<td>92 ± 7</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
<td>43 ± 4</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>110 ± 23</td>
<td>116 ± 17</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>81 ± 3</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>Serum folate (NR, 3.2–20.1 ng/mL), ng/mL</td>
<td>12.1 ± 1.2</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>Erythrocyte folate, (NR, &gt;160 ng/mL), ng/mL</td>
<td>363 ± 24</td>
<td>325 ± 30</td>
</tr>
<tr>
<td>Plasma B6 (NR, 3.6–18 ng/mL), ng/mL</td>
<td>21.0 ± 4.9</td>
<td>15.3 ± 3.0</td>
</tr>
<tr>
<td>Serum B12 (NR, 250–1100 pg/mL), pg/mL</td>
<td>432 ± 35</td>
<td>429 ± 28</td>
</tr>
<tr>
<td>Plasma homocyst(e)ine, µmol/L</td>
<td>8.4 ± 0.7</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>Plasma methionine, µmol/L</td>
<td>25.0 ± 1.2</td>
<td>21.3 ± 1.8</td>
</tr>
</tbody>
</table>

NR indicates normal range.

Measurements

To study resistance vessel endothelial function, forearm blood flow was measured in both arms with strain-gauge venous occlusion plethysmography (model EC-4, Hokanson Inc) during brachial artery infusion of vasoactive agents.\(^1\) To study conduit vessel endothelial function, flow-mediated dilation of the brachial artery was induced with reactive hyperemia as a stimulus.\(^9\) –\(^12\) The brachial artery was imaged in a longitudinal section above the antecubital fossa by use of a 7.5-MHz linear-array ultrasound Doppler transducer (Toshiba SSA-270). We used the same ultrasonographer for image acquisition, a standard arm support, and identical probe distance from the antecubital fossa (2.5 cm proximal). Measurements were obtained for 20 seconds at baseline. An occluding forearm cuff placed 5 cm below the antecubital fossa was inflated to 50 mm Hg above systolic pressure for 5 minutes and then released to induce reactive hyperemia. Brachial artery diameter and flow velocity were also measured before and for 6 minutes after sublingual administration of nitroglycerin spray (400 µg). Blood pressure was recorded with an automated sphygmomanometer (LifeStat 200, Physio Control).

Laboratory Assays

Plasma homocyst(e)ine assays were performed in the University of Iowa General Clinical Research Center Core Laboratory in a modification of the assay described by Noguchi and Higuchi.\(^16\) Samples were collected in chilled EDTA tubes, and plasma was frozen at \(-70^\circ\)C. Plasma was spiked with known amounts of internal standard (mercaptopropionylglycine) reduced with tri-n-butyl phosphate and then deproteinized with sulfosalicylic acid. The thiol-specific fluorogenic labeling reagent ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate was added to the supernatant; the sample was neutralized with HCl, washed with a 5% tributyl phosphate solution, filtered, and injected onto a reversed-phase high-performance liquid chromatography column. Quantification was by measurement of the emission signal of the analyte at 515 nm (excitation, 385 nm). The coefficient of variation was <2%. Results of this assay are expressed as plasma homocyst(e)ine and represent the sum of free and bound forms of homocysteine, homocystine, and homocysteine-cysteine mixed disulfide. Plasma methionine was measured with an automated amino acid analyzer (Beckman 7300, Beckman Instruments) by use of a lithium physiological ion-exchange column system. Quantification was achieved by postcolumn derivatization with ninhydrin, monitoring UV absorbance with norleucine as internal standard. Plasma B6 and B12 and plasma and red cell folate, as well as cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol, were measured by use of established methodologies.

Study Design

Effect of Experimental Hyperhomocyst(e)inemia on Endothelial Function

This was a randomized, double-blind, crossover study comparing the effects of oral L-methionine and placebo on endothelial function with \(\geq\) 1 week between the 2 study days. Subjects were admitted to the University of Iowa General Clinical Research Center the previous night and studied the next morning after fasting. An antecubital vein of the noninfused arm was canulated for blood sampling. Subjects received oral L-methionine (Ajinomoto) 100 mg/kg dissolved in cranberry juice or cranberry juice alone at about 7 AM.

Ten subjects (protocol A) had vascular function assessed within 3 hours of oral methionine or placebo. Flow-mediated dilation of the brachial artery was assessed 1 and 3 hours after methionine or placebo, with nitroglycerin-induced vasodilation tested at 3 hours. Resistance vessel endothelial function was assessed 1.5 to 3 hours after methionine or placebo. The left brachial artery was canulated under local anesthesia with a 27-gauge steel needle attached to an 18-gauge epidural catheter. Baseline forearm blood flows were obtained during infusion of 0.9% saline (1 mL/min) for 30 minutes. Acetylcarnine (3 to 30 µg/min, lobar) and nitroprusside (1 to 10 µg/min, Elkins-Sinn) were then separately administered into the brachial artery, each dose for 6 minutes, separated by saline infusion to allow flow to return to baseline. The order of intra-arterial drugs was randomized, with individual subjects receiving drugs in the same order on the 2 study days. Forearm blood flow was measured in the last 3 minutes of each dose. Arterial pressure was measured twice at baseline and after each dose. Venous blood for assay of plasma homocyst(e)ine and methionine was obtained before and 1, 2, 3, and 4 hours after L-methionine or placebo administration.

Twenty subjects (protocol B) had assessment of vascular function 6 to 8 hours after L-methionine or placebo administration. A standard breakfast containing 58 mg L-methionine and 5 g fat was served 2 hours after methionine or placebo administration. The brachial artery was canulated at 5.5 hours, and a resistance vessel protocol similar to that of the first group was followed except that verapamil was also infused in 10 subjects (10 to 100 µg/min, SoloPak Labs). Verapamil was always administered last because of its long duration of action. Eleven subjects from this group had conduit vessel endothelial function assessed 8 to 8.5 hours after L-methionine or placebo administration. Venous blood for assay of plasma homocyst(e)ine and methionine was obtained before and 2, 4, 6, and 8 hours after L-methionine or placebo administration.

Effect of Ascorbic Acid on Homocyst(e)ine-Induced Endothelial Dysfunction

This was a 4-phase, randomized, double-blind, crossover study in which 10 subjects received (1) placebo at 7 AM, (2) L-methionine at 7 AM, (3) placebo at 7 AM plus ascorbic acid at 11 AM, and (4) L-methionine at 7 AM plus ascorbic acid at 11 AM, with \(\geq\) 1 week between study phases. Ascorbic acid was administered in a dose of 2 g orally, which is known to increase plasma ascorbate concentrations 2.5-fold, with stable concentrations from 2 to 5 hours after dosing.\(^17\) Venous blood for assay of plasma ascorbate concentrations was obtained before and 4, 6, and 8 hours after L-methionine or placebo administration. The study day timetable was otherwise identical to that of protocol B, with endothelial function being assessed 6 to 9 hours after L-methionine administration (2 to 5 hours after ascorbic acid administration).

Data and Statistical Analyses

All analyses were performed by observers blinded to treatment assignment. Basal blood flow and blood pressure were taken as the average of the 3 baseline recording periods during saline infusion. In addition to absolute blood flows, we calculated percent change from
baseline in the ratio of blood flow between infused and noninfused arms, because this halves variability in blood flow responses to infused agents.15 Forearm vascular resistance was calculated as mean arterial pressure/forearm blood flow.

For conduit vessel endothelial function, brachial artery diameter and blood velocity measurements were analyzed by use of a Toshiba ultrasound machine. Mean Doppler velocity was averaged over 3 consecutive cardiac cycles. Percentage changes in diameter and velocity were calculated 10 seconds; 1 and 2 minutes after induction of reactive hyperemia; and 2, 4, and 6 minutes after nitroglycerin. In 40 studies performed in healthy subjects, dilatation to reactive hyperemia was maximal 2 minutes after cuff deflation (1 minute, 5.6±0.7%; 2 minutes, 6.2±0.6%), so the 2-minute time point is shown in graphs and tables. Maximum dilatation to nitroglycerin occurred 6 minutes after administration (2 minutes, 6.2±0.7%; 4 minutes, 14.1±1.4%; 6 minutes, 14.5±1.5%), so the 6-minute time point is shown in graphs and tables. Our methodology for assessment of conduit vessel flow–mediated dilatation has good within-subject reproducibility, with a correlation coefficient of 0.75 between 10 paired studies and an average coefficient of variability of 13.6% in 3 subjects studied on ≥4 occasions.

Two-way repeated-measures ANOVA was used to compare the effects of methionine and placebo on resistance vessel dilatation to acetylcholine, nitroprusside, and verapamil and conduit vessel dilatation to nitroglycerin and increased flow. All doses of drugs or time points after reactive hyperemia were used in the ANOVA. Tukey’s test was used for post hoc analysis if the ANOVA was positive. Multiple regression was used to assess determinants of endothelial function 1, 3, and 8 hours after administration of methionine and placebo in protocols A and B. Data are expressed as mean±SE; P<0.05 was taken as statistically significant. Data were analyzed with StatView software (Brainpower Inc).

Results

**Effect of Experimental Hyperhomocyst(e)inemia on Endothelial Function**

Baseline blood pressure, lipids, vitamins, and homocysteine levels were normal at baseline (Table 1). Methionine loading

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### TABLE 2. Resistance Vessel Endothelial Function in Protocols A and B

<table>
<thead>
<tr>
<th></th>
<th>Protocol A (2 h After Methionine/Placebo)</th>
<th>Protocol B (7 h After Methionine/Placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td><strong>MAP, mm Hg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>91±3</td>
<td>91±3</td>
</tr>
<tr>
<td>Methionine</td>
<td>91±3</td>
<td>92±2</td>
</tr>
<tr>
<td><strong>HR, bpm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>57±3</td>
<td>56±4</td>
</tr>
<tr>
<td>Methionine</td>
<td>59±3</td>
<td>59±3</td>
</tr>
<tr>
<td><strong>FBRInuarted, mL·100 mL⁻¹·min⁻¹</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>3.1±0.3</td>
<td>13.5±1.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.9±0.3</td>
<td>12.5±1.6</td>
</tr>
<tr>
<td><strong>FBRInuested, mL·100 mL⁻¹·min⁻¹</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>2.4±0.2</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.6±0.2</td>
<td>3.1±0.4</td>
</tr>
<tr>
<td><strong>Change in (infused/noninfused) ratio, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>364±83</td>
<td>300±38</td>
</tr>
<tr>
<td>Methionine</td>
<td>312±67</td>
<td>327±43</td>
</tr>
<tr>
<td><strong>FVRInuarted, AU</strong></td>
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<td></td>
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<tr>
<td>Placebo</td>
<td>32±4</td>
<td>8±1</td>
</tr>
<tr>
<td>Methionine</td>
<td>35±4</td>
<td>10±2</td>
</tr>
<tr>
<td><strong>FVRInuested, AU</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>43±5</td>
<td>39±6</td>
</tr>
<tr>
<td>Methionine</td>
<td>38±4</td>
<td>34±5</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; HR, heart rate; FBF, forearm blood flow; and FVR, forearm vascular resistance. Data obtained during infusion of saline, acetylcholine (30 μg/min), nitroprusside (10 μg/min), and verapamil (100 μg/min).

*P<0.05 vs placebo.
slowly increased plasma homocyst(e)ine concentrations by 4-fold to a maximum of 28±2 μmol/L at 8 hours (P<0.001; Figure 1). Plasma homocyst(e)ine concentrations did not change after placebo administration. Methionine concentrations increased more substantially (~30-fold) and rapidly, peaking at 2 hours and then decreasing by ~50% by 8 hours (P<0.001; Figure 1). Blood pressure, heart rate, and forearm blood flow did not differ between study days (Table 2).

Brachial artery infusion of vasodilator agents did not alter blood flow in the noninfused arm, confirming that drug effects were confined to the infused arm (Table 2). Forearm vasodilatation to acetylcholine was nonsignificantly decreased 2 hours after methionine loading (P=0.31; Table 2 and Figure 2A), when plasma methionine concentrations were maximal. However, acetylcholine-induced dilatation was significantly impaired 7 hours after methionine loading (P=0.016; Table 2 and Figure 2A). Nitroprusside and verapamil responses did not differ between the methionine and placebo days (Table 2 and Figure 2B). On multiple regression analysis, plasma homocyst(e)ine was a significant predictor of resistance vessel endothelial function (R=0.375, P=0.04; Figure 3A). Plasma methionine (P=0.59), age (P=0.65), sex (P=0.14), and arterial pressure (P=0.54) were not predictive of resistance vessel endothelial function.

Baseline brachial artery diameter and flow velocity were no different between methionine and placebo study days (Table 3). Methionine administration progressively decreased flow-mediated dilatation, which closely paralleled plasma homocyst(e)ine but not plasma methionine concentration (Figures 1 and 4). Flow-mediated vasodilatation was not impaired 1 hour after methionine loading (P=0.12) but was significantly reduced at 3 hours (P<0.0001) and abolished 8 hours after methionine loading (Figure 4). The increase in flow velocity during reactive hyperemia and brachial artery dilatation to nitroglycerin was similar on methionine and placebo days (Table 3). On multiple regression analysis, plasma homocyst(e)ine was a significant predictor of conduit vessel endothelial function (R=0.492, P=0.0014; Figure 3B). Plasma methionine (P=0.71), age

Figure 2. A, Forearm vasodilatation to intra-arterial administration of acetylcholine after oral L-methionine (●) or placebo (○). Left, Dilatation to acetylcholine 2 hours after methionine (protocol A, n=10). Right, Dilatation to acetylcholine 7 hours after methionine (protocol B, n=20). *P<0.05 vs placebo. B, Forearm blood flow responses to intra-arterial administration of nitroprusside after oral L-methionine (●) or placebo (○).

Figure 3. Regression analysis demonstrating linear relationship between plasma homocyst(e)ine and resistance (A) or conduit (B) vessel endothelial function for subjects in protocols A and B.
Effect of Ascorbic Acid on Homocysteine-Induced Endothelial Dysfunction

The increase in plasma homocyst(e)ine produced by methionine administration was not altered by ascorbic acid. Blood pressure, heart rate, and resting forearm hemodynamics did not change (Tables 4 and 5). Plasma ascorbate increased by 3-fold from 2 to 4 hours after administration of ascorbic acid (ie, 6 to 8 hours after methionine or placebo; Table 5). Plasma ascorbate did not change (Tables 4 and 5). Plasma ascorbate increased by 3-fold from 2 to 4 hours after administration of ascorbic  
i.e., 6 to 8 hours after methionine or placebo; Table 5)

Ascorbic acid alone did not alter endothelium-dependent dilatation resistance and conduit vessels (Figure 5). Impairment of resistance vessel dilatation to acetylcholine by methionine (P=0.045 versus placebo) was prevented by administration of ascorbic acid (P=0.03 versus methionine alone; Table 4 and Figure 5). Similarly, methionine-induced impairment of conduit vessel endothelial function (P=0.035 versus placebo; Table 5 and Figure 5) could be completely prevented by coadministration of ascorbic acid (P=0.03 versus methionine alone; Table 5 and Figure 5).

Discussion

In our study, experimental elevation of plasma homocysteine concentrations rapidly impaired both conduit and resistance vessel endothelial function in human subjects. Our study confirms recent reports that methionine loading produces conduit vessel endothelial dysfunction.\textsuperscript{11,12} The effect of experimental hyperhomocyst(e)inemia on resistance vessel endothelial function has been unclear. One preliminary report in a limited number of subjects suggested that methionine loading did not alter forearm vasodilatation to acetylcholine.\textsuperscript{18} We demonstrated in a substantial number of subjects that methionine loading does impair endothelium-dependent vasodilatation in resistance vessels, albeit partially. Importantly, endothelial dysfunction caused by experimental hyperhomocyst(e)inemia was completely prevented by administration of an antioxidant, ascorbic acid. Given the diverse functions of the endothelium, these results may help to explain how moderate hyperhomocyst(e)inemia predisposes to atherosclerosis and hypertension.

Mechanisms

Impaired resistance vessel dilatation to acetylcholine and conduit vessel dilatation to hyperemia were not due to impaired vascular smooth muscle relaxation, because vasodilatation to verapamil and nitroprusside was not altered. Thus, methionine loading probably decreased the generation or activity of vasoactive endothelium-derived mediators.

Impaired endothelium-dependent vasodilatation after methi-
Online loading could reflect decreased nitric oxide activity, increased thromboxane formation, or alteration in other endothelial mediators. Homocysteine increases generation of free radical oxidant species in vitro, possibly through auto-oxidation, inhibition of glutathione peroxidase, or oxidation of LDL. We have demonstrated that administration of ascorbic acid prevents induction of endothelial dysfunction by homocysteine. Vitamin C is a potent antioxidant scavenger of reactive oxygen species and may prevent direct inactivation of nitric oxide by superoxide or increase intracellular reduced glutathione concentrations. It appears likely that experimental hyperhomocyst(e)inemia caused by methionine loading produces endothelial dysfunction through increased oxidant stress.

Potential Study Limitations
Brachial artery atherosclerosis correlates with coronary and carotid atherosclerosis. Forearm endothelial function is impaired in patients with established coronary atherosclerosis. Thus, the brachial circulation appears to be a reasonable surrogate for study of the coronary circulation.

Our model for inducing experimental hyperhomocyst(e)inemia uses oral methionine loading. A nonspecific effect of amino acids on endothelial function appears unlikely,
because high concentrations of amino acids such as l-arginine and n-acetylcysteine do not impair endothelium-dependent relaxation in humans. Plasma methionine concentrations increased by almost 30-fold compared with an increase of only 4-fold in plasma homocysteine (Figure 1). However, endothelial function was not significantly impaired 1 hour after administration of oral methionine, when plasma methionine was already markedly elevated but plasma homocysteine concentrations had fallen by 50% from their peak but plasma homocyst(e)ine was maximal. Nonetheless, we cannot exclude the possibility that impaired endothelial function after methionine loading is due to changes in plasma methionine. Even if this is the case, our results are still clinically relevant, given that many foods are rich in methionine (see below).

**Implications**

We have shown that experimental induction of moderate hyperhomocyst(e)inemia (20 μmol/L) impairs endothelium-dependent vasodilatation in human resistance and conduit vessels. In addition to vascular tone, the endothelium regulates cell adhesion, platelet aggregation, coagulation, lipid transport and oxidation, inflammation, and mitogenesis. Changes in endothelium-dependent dilatation precede structural changes during experimental induction or regression of atherosclerosis in monkeys. Impaired resistance vessel endothelial function may predispose to hypertensive structural remodeling and offer an explanation for the association between homocysteine and systolic hypertension.

The clinical relevance of these data is emphasized by the recent report that plasma homocysteine concentrations >20 μmol/L are associated with a 4-fold increase in total mortality in patients with coronary artery disease. The average fasting homocyst(e)ine concentration in American middle-aged men is 10 μmol/L, with the 95th percentile at 16 μmol/L. In our study, the largest decrease in endothelial function occurred between 1 and 3 hours after administration of methionine (Figure 3), when plasma homocyst(e)ine increased from 11 to 20 μmol/L. Thus, endothelial dysfunction may be induced at plasma homocyst(e)ine concentrations that are applicable to the general population.

Most epidemiological studies have measured fasting rather than postprandial homocyst(e)ine. Methionine is an essential amino acid, present in relatively large amounts in foods rich in animal protein (0.5% to 1% methionine). Many individuals may consume 2 to 3 g methionine daily compared with the 7 to 8 g administered to our subjects. Such individuals could have postprandial homocyst(e)ine concentrations considerably >20 μmol/L, particularly if fasting plasma homocyst(e)ine was >10 μmol/L. Thus, it is possible that meals high in animal protein may lead to repeated episodes of endothelial dysfunction, which may in turn predispose to atherosclerosis or hypertension. In addition, dietary methionine intake from foods rich in animal protein and fat may have confounded previous assessments of the effect of dietary saturated fat intake on cardiovascular disease.

Our results are consistent with the concept that moderate hyperhomocyst(e)inemia predisposes to atherosclerosis and hypertension by causing endothelial dysfunction. These find-
ings emphasize the need for outcome trials testing whether treatment of moderate hyperhomocyst(e)inemia reduces cardiovascular events. Our studies suggest that increased oxidant stress is an important pathophysiological mechanism underlying the deleterious endothelial effects of homocysteine. Antioxidant therapy warrants investigation as an alternative clinical approach to prevention of the atherothrombotic complications of hyperhomocyst(e)inemia, particularly in patients with hyperhomocyst(e)inemia resistant to B-vitamins.29

Acknowledgments
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