Homocysteine Impairs Coronary Microvascular Dilator Function in Humans

Ahmed Tawakol, MD,* Marc A. Forgione, MD,§ Markus Stuehlinger, MD,|| Nathaniel M. Alpert, MD,† John P. Cooke, MD, PhID, FACC,¶ Joseph Loscalzo, MD, PhID, FACC,§ Alan J. Fischman, MD, PhID,† Mark A. Creager, MD, FACC,‡ Henry Gewirtz, MD*

Boston, Massachusetts; and Stanford, California

OBJECTIVES
We sought to use positron emission tomography (PET) to test the hypothesis that hyperhomocysteinemia adversely affects coronary microvascular dilator function.

BACKGROUND
Hyperhomocysteinemia is associated with abnormal endothelium-dependent vasodilation in peripheral human arteries. However, its effect on the coronary circulation is not known.

METHODS
Eighteen healthy humans, age 24 to 56 years, were enrolled in a double-blind, crossover trial. Basal and adenosine-stimulated myocardial blood flow (MBF) was determined by PET: after ingestion of placebo and after methionine-induced hyperhomocysteinemia. Further, brachial ultrasonography was used to assess flow-mediated vasodilation. Additionally, to assess the role of nitric oxide (NO) in adenosine-mediated vasodilation, the MBF response to adenosine was measured in the presence and absence of the NO synthase antagonist N\textsuperscript{\text{tris}}-monomethyl-L-arginine (L-NMMA) (0.3 mg/kg/min intravenously).

RESULTS
Hyperhomocysteinemia resulted in a reduction in the MBF dose-response curve to adenosine (p < 0.05). This was most apparent with low dose adenosine, where MBF augmentation was significantly blunted during hyperhomocysteinemia (1.06 ± 1.00 ml/min/g vs. 0.58 ± 0.78 ml/min/g, placebo vs. methionine, p < 0.05). Similarly, flow-mediated brachial artery vasodilation was impaired during hyperhomocysteinemia (4.4 ± 2.6% vs. 2.6 ± 2.3%, placebo vs. methionine, p < 0.05). In a separate series of experiments, MBF during adenosine was reduced in the presence of L-NMMA (p < 0.05 analysis of variance). This was most apparent at the low dose of adenosine, where MBF response to adenosine was blunted in the presence of L-NMMA (2.08 ± 1.34 ml/min/g vs. 1.48 ± 1.32 ml/min/g, placebo vs. L-NMMA, p < 0.05).

CONCLUSIONS
The data, therefore, support the hypothesis that acute hyperhomocysteinemia impairs microvascular dilation in the human coronary circulation as a result of reduced NO bioavailability. (J Am Coll Cardiol 2002;40:1051–8) © 2002 by the American College of Cardiology Foundation

Prospective and case-controlled studies have demonstrated that elevations in plasma homocysteine levels are associated with an increased risk of cardiovascular diseases, including myocardial infarction (1–5). This may be an issue of significant clinical relevance, since elevations in plasma homocysteine are common, affecting approximately 10% of the general population and over 40% of individuals with coronary artery disease (2–5).

We previously demonstrated impaired flow-mediated vasodilation of the brachial artery in chronically hyperhomocysteinemic, but otherwise healthy humans; an observation that suggests nitric oxide (NO) bioavailability is decreased in those individuals (6). Others have since shown that flow-mediated vasodilation is acutely impaired after the induction of acute hyperhomocysteinemia with methionine (7–9). It is not known whether these homocysteine-associated, peripheral arterial blood flow abnormalities are relevant to the human coronary circulation. Therefore, the present study was designed to test the hypothesis that acute hyperhomocysteinemia impairs the vasodilator function of the coronary microcirculation. We employed positron emission tomography (PET) to assess the coronary microvascular dilator response to low and high dose adenosine in healthy humans in whom we induced transient hyperhomocysteinemia via oral "loading" with the essential amino acid, methionine. Furthermore, to demonstrate that adenosine-induced coronary microvascular dilation is in part mediated by NO, a separate series of experiments was conducted during which the myocardial blood flow (MBF) response to adenosine 70 and 140 μg/kg/min was measured in the presence and absence of the systemic infusion of the nitric oxide synthase (NOS) antagonist, N\textsuperscript{\text{tris}}-monomethyl-L-arginine (L-NMMA) (0.3 mg/kg/min intravenously).
arginine (L-NMMA) (0.3 mg/kg/min). Additionally, brachial ultrasonography was used to measure flow-mediated vasodilation in order to assess the relevance of peripheral vascular dilator function to the coronary microcirculation in the same patient population.

**METHODS**

**Patient population.** A total of 18 healthy subjects (13 males) age 40 ± 10 years (mean ± SD) were recruited from the greater Boston area. Subjects were entered into one or more of three substudies that: 1) assessed the effect of transient hyperhomocysteinemia on coronary microvascular dilation (n = 15 subjects); 2) assessed the contribution of NO to adenosine mediated coronary vasodilation (n = 12 subjects); or 3) assessed the effect of transient hyperhomocysteinemia on flow mediated dilation of the brachial artery (n = 12 subjects). Because of the demanding nature of the study, only eight subjects participated in all three protocols. Exclusion criteria included the following: smoking; systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg; serum cholesterol level above the 75th percentile for age and gender; history of vascular disease; diabetes; family history of premature coronary artery disease; or any clinical manifestation coronary artery disease, peripheral artery disease, or carotid artery disease. The study protocol was approved by the Joint Human Research Committee of the Massachusetts General Hospital and Brigham and Women's Hospital, and informed consent was obtained from each subject.

**Protocol 1: homocysteine and coronary microvascular dilation.** The effect of transient hyperhomocysteinemia on coronary microvascular dilation was assessed in 15 subjects (age 37 ± 3 years, 10 male). Using a double-blinded, placebo-controlled crossover design, methionine powder (100 mg/kg) (Ajinomoto Pharmaceuticals) versus a similar appearing placebo (dextrose) was mixed in juice and administered orally 8 to 10 h prior to blood flow measurements. Such “methionine loading” produces transient hyperhomocysteinemia by metabolically stressing homocysteine metabolism and has been used extensively and safely (10).

**MBF measurements.** Basal and adenosine-stimulated MBF was assessed with PET 8 to 10 h after ingestion of methionine versus placebo. Positron emission tomography measurements of MBF are highly reproducible and correlate well with MBF determined using radiolabeled microspheres (11–13). We and others have shown that one standard deviation for blood flow measurements in the same patient under comparable circumstances is 0.1 to 0.2 ml/min/g (14,15). Furthermore, this technique has been used by our group to demonstrate improvement in coronary microvascular dilation response after lipid lowering therapy (15), (a treatment that improves NO bioavailability).

**Adenosine dose response curve.** Positron emission tomographic imaging is performed on a whole body tomograph (GE Medical Systems Scanditronix PC4096) in subjects after an overnight fast using a previously described protocol (15,16). Briefly, images are acquired in 15 contiguous sections simultaneously with center-to-center separation of 6.5 mm. First, a 10-min transmission scan is performed to correct the emission data for attenuation. Next, three dynamic tomographic acquisitions are performed: at rest, and during two separate intravenous infusions of adenosine (70 and 140 μg/kg/min, from 2 min before to 4 min after initiation of image acquisition). Approximately 25 mCi of $^{13}$N-ammonia is administered for each acquisition, with imaging begun just prior to injection. Data are collected for the first 3 min at 6 s/frame and then at 2 min/frame for 6 min. After each image acquisition, radioactivity is allowed to decay for approximately 30 to 40 min, at which time the count rate seen by the scanner is approximately 7500/s and is effectively overwhelmed by the next dose. The patient’s heart rate (HR) and electrocardiogram is monitored continuously during and following PET blood flow measurements and arterial pressure determined by cuff at 1 to 2 min intervals. An interval of at least one week was allowed between each study visit.

**PET image analysis.** Attenuation-corrected $^{13}$N-ammonia images are reconstructed with a conventional filtered back projection algorithm as 128 × 128 pixel images in the transverse plane normal to the long axis of the body. Filtering of the projection data is performed with a Hanning filter to yield output resolution of 7.8 mm full width at half maximum. The $^{13}$N-ammonia scans (n = 3), for each patient corresponding to the last 6 min of data acquisition, are summed to permit placement of a region of interest over the left ventricular cavity. The region of interest is used to generate the arterial input function for the tracer kinetic model by which regional myocardial blood flow is determined. A computer program developed at our institution is used in conjunction with the dynamic data to generate parametric (K1) images. The images obtained provide a pixel-by-pixel representation of K1 and are used for analysis of regional MBF.

Three short axis rings corresponding to the proximal, middle and distal thirds of the left ventricle were constructed for each K1 scan as described previously (15).

**Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ADMA</td>
<td>asymmetric dimethylarginine</td>
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<tr>
<td>G</td>
<td>myocardial conductance</td>
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<tr>
<td>HR</td>
<td>heart rate</td>
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<tr>
<td>L-NMMA</td>
<td>Nω-monomethyl-L-arginine</td>
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<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
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<tr>
<td>MFB</td>
<td>myocardial blood flow</td>
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<tr>
<td>METH</td>
<td>methionine-induced hyperhomocysteinemia</td>
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<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>RPP</td>
<td>rate-pressure product</td>
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<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
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MBF = myocardial blood flow; PET = positron emission tomography; RPP = rate-pressure product; SBP = systolic blood pressure.
Briefly, circular regions of interest (~8.5 mm radius) were placed over each ring at standard areas of interest: interseptum, mid-septum, anteroseptum, anterior, anterolateral, lateral, posterolateral, and inferior zones. Myocardial blood flow was computed from values of K1 and averaged over all regions to obtain a single value blood flow used each experimental condition. Myocardial conductance (G) was computed by dividing myocardial blood flow by mean arterial pressure. Mean arterial pressure (MAP) was computed as follows: MAP = diastolic arterial pressure + (0.333 × pulse pressure). Rate-pressure product (RPP) is calculated: RPP = HR × SBP.

Protocol 2: assessment of the contribution of NO to adenosine mediated coronary vasodilation. The contribution of NO in adenosine-mediated vasodilation was assessed in a subset of 12 subjects (8 males, age 41 ± 2.7 years). Eight of those subjects had previously participated in the first protocol. Subjects were entered in a placebo-controlled crossover experiment, during which the NOS antagonist, L-NMMA (Climinalpha, Switzerland) versus normal saline was infused before MBF measurements (at rest and during the infusion of adenosine at 70 and 140 µg/kg/min). The L-NMMA was infused intravenously at 0.3 mg/kg/min for 16 min (10 min before and 6 min during) each of the three measurements of MBF. The total cumulative dose of L-NMMA administered to each subject was therefore approximately 14.5 mg/kg (0.3 mg/kg/min × 16 min × 3 infusions). The dose of L-NMMA was chosen based on prior human experiments (17). Resting and adenosine-stimulated blood flow measurements were obtained using PET as described previously.

Protocol 3: homocysteine and brachial artery flow-mediated vasodilation. Twelve subjects who underwent PET imaging after methionine loading, agreed to undergo assessment of peripheral vascular function after ingesting methionine versus placebo. Eight to ten hours after methionine ingestion, flow-mediated vasodilation was assessed by high-resolution ultrasonography, a technique that has been described previously and validated (16,18). Longitudinal images of the brachial artery were obtained 10 to 80 mm proximal to the antecubital fossa by use of a Toshiba model 270 SSA scanner (Tochigi-ken, Japan) equipped with a 7.5-MHz high-resolution linear-array transducer. Flow-mediated vasodilation was assessed by measuring the percent change from baseline of brachial artery diameter during reactive hyperemia. Flow-mediated vasodilation of peripheral conduit vessels is largely an endothelium-dependent process, mediated by nitric oxide and inhibited by L-NMMA (18). Furthermore, flow-mediated vasodilation of peripheral conduit vessels correlates with acetylcholine-stimulated, NO-mediated dilation of epicardial coronary arteries in humans (16). To stimulate flow-mediated vasodilation, a pneumatic cuff was placed on the forearm and inflated to suprasystolic pressures for 5 min. Thereafter, the brachial artery was imaged after cuff deflation, and images were acquired for analysis at 1 min after cuff deflation. Time-averaged flow velocity integral was recorded. In nine subjects, brachial artery scanning was repeated after 0.4 mg sublingual nitroglycerin (10 min after flow-mediated vasodilation) to assess endothelium-independent vasodilation. The artery was scanned for an additional 3 min, at which time images were once again acquired for digitization and measurement. Blood pressure and HR were monitored during this procedure.

An investigator blinded to the subjects’ study drug order analyzed the end-diastolic images for each condition (baseline, reactive hyperemia, repeat baseline, nitroglycerin), using software (Information Integrity) that searched for the shortest distance between the points on the arterial wall. Arterial diameter was measured from the intima-media interface of the posterior wall to the media-adventitia interface of the anterior wall. The average of three measurements was used for each determination of brachial artery diameter.

Biochemical measurements. Blood samples were obtained at the time of blood flow measurements. Samples were promptly spun, separated, frozen and stored at −70°C. Measurement of homocysteine and methionine were performed at the Mayo Clinic Laboratories (Rochester, Minnesota) using previously described methods (19). Plasma isoprostane concentration, an indicator of lipid peroxidation (20), was measured using previously described methods (20). Plasma concentrations of asymmetric dimethylarginine (ADMA) were measured by high-performance liquid chromatography using modifications of previously described methods (21).

Statistical analysis. Data are expressed as mean ± SD. The significance of changes in group mean values of MBF and MBF ratio were assessed with factorial analysis of variance (ANOVA) and Fisher’s protected least significant difference test (Statview v4.0, Abacus Concepts). Wilcoxon Signed Rank tests were used to evaluate the significance of difference in brachial artery vasodilation, hemodynamics, and biochemical variables. Linear regression analysis was used to assess the relationship between group mean values of G and adenosine dose before and after methionine. A paired t test was also employed to assess the significance of the change in percent change (from baseline) in MBF at adenosine 70 (placebo vs. methionine). Log10 transformation of the blood flow data was applied to reduce statistical noise. Values of p < 0.05 were considered significant.

RESULTS

Biochemical changes. The mean fasting plasma homocysteine concentrations were 8.3 ± 2.7 µmol/l after placebo and 27.1 ± 11.2 µmol/l after methionine (p < 0.001). The mean fasting plasma methionine concentrations were 25 ± 4 and 335 ± 215 µl after placebo and methionine, respectively (p < 0.001). Plasma isoprostane concentrations did not significantly change after methionine versus placebo (37.1 ± 9.3 vs. 56.6 ± 15.9 pg/ml, respectively, p = NS).
ADMA did not change after methionine versus placebo (1.1 ± 0.2 vs. 1.1 ± 0.4 μl, respectively, p = NS).

**Dynamics during PET.** During the placebo condition, at rest, MBF and G did not differ significantly between control and methionine conditions. During methionine-induced hyperhomocysteinemia (METH), the MBF response to adenosine was reduced versus placebo (all p < 0.05, Fig. 1A). As such, during hyperhomocysteinemia, there was a 45% reduction in MBF augmentation with the 70 μg/kg/min dose of adenosine (vs. placebo). Furthermore, flow augmentation to low dose adenosine was inversely related to the concentration of plasma homocysteine (r = −0.38; p < 0.05). In contrast, there was no significant association between the MBF response to adenosine and the concentration of methionine.

Conductance changes paralleled those of MBF. Thus, there was a consistent, highly significant (p < 0.001) dose dependent, increase in conductance with each dose of adenosine under control conditions. In contrast, after methionine, the increase in conductance at intermediate adenosine loading was blunted and failed to reach statistical significance.

**The effect of NOS inhibition on resting and adenosine-augmented MBF.** During L-NMMA, no symptoms or electrocardiographic signs of ischemia were observed in any subject. The hemodynamic effects of L-NMMA were evident by an increase in SBP and MAP and decline in HR versus placebo (all p < 0.05) (Table 2). The RPP did not change versus placebo.

At rest, NO inhibition (with L-NMMA) did not affect MBF. However, L-NMMA reduced the MBF response to METH (1.06 ± 0.10 vs. 0.58 ± 0.78 placebo vs. methionine, p < 0.05, Fig. 1B). As such, during hyperhomocysteinemia, there was a 45% reduction in MBF augmentation with the 70 μg/kg/min dose of adenosine (vs. placebo).

**MBF and G.** At rest, MBF and G did not differ significantly between control and methionine conditions. In response to adenosine, there was a dose dependent, graded increase over baseline in MBF after placebo and methionine loading. The changes in flow from baseline to intermediate dose and from intermediate to high dose were all statistically significant for both placebo and methionine (Table 1).

During methionine-induced hyperhomocysteinemia (METH), the MBF response to adenosine was reduced compared with placebo (p < 0.05, ANOVA, Fig. 1A). Furthermore, MBF augmentation (adenosine MBF – baseline MBF) to low dose adenosine was blunted during METH (1.06 ± 0.10 vs. 0.58 ± 0.78 placebo vs. methionine, p < 0.05, Fig. 1B). As such, during hyperhomocysteinemia, there was a 45% reduction in MBF augmentation with the 70 μg/kg/min dose of adenosine (vs. placebo). Furthermore, flow augmentation to low dose adenosine was inversely related to the concentration of plasma homocysteine (r = −0.38; p < 0.05). In contrast, there was no significant association between the MBF response to adenosine and the concentration of methionine.

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At rest, NO inhibition (with L-NMMA) did not affect MBF. However, L-NMMA reduced the MBF response to

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**Table 1.** Effect of Methionine on Myocardial Blood Flow and Conductance (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ado 70</th>
<th>Ado 140</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBF (ml/min/g)</td>
<td>0.70 ± 0.19</td>
<td>1.75 ± 1.13†</td>
<td>3.51 ± 0.84‡</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.76 ± 0.36</td>
<td>1.34 ± 0.26</td>
<td>3.76 ± 1.00‡</td>
</tr>
<tr>
<td>Ratio (METH:Placebo)</td>
<td>1.09 ± 0.28</td>
<td>0.86 ± 0.48*</td>
<td>1.11 ± 0.33</td>
</tr>
<tr>
<td>G (ml/min/g/mm Hg)</td>
<td>8.30 ± 2.53</td>
<td>21.79 ± 15.29‡</td>
<td>45.69 ± 13.97‡</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.94 ± 3.52</td>
<td>15.97 ± 12.04</td>
<td>47.23 ± 12.60‡</td>
</tr>
<tr>
<td>Ratio (METH:Placebo)</td>
<td>1.11 ± 0.07</td>
<td>0.87 ± 0.13*</td>
<td>1.11 ± 0.10</td>
</tr>
</tbody>
</table>

* † ‡ p < 0.05 vs. baseline and Ado 140; † p < 0.05 vs. baseline; ‡ p < 0.005 vs. baseline.

Ado 70 = adenosine 70 μg/kg/min; Ado 140 = adenosine 140 μg/kg/min; G = myocardial conductance; MBF = myocardial blood flow; METH = methionine condition.

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**Figure 1.** (A) Effect of acute hyperhomocysteinemia on the myocardial blood flow (MBF) response to adenosine. This figure depicts MBF at rest and during infusion of low and high dose adenosine, after placebo and during methionine-induced hyperhomocysteinemia (METH). The adenosine dose response curve is significantly impaired during methionine vs. placebo (p < 0.05, ANOVA). (B) Acute hyperhomocysteinemia impairs the coronary microvascular dilator response to adenosine. This figure depicts the MBF response to the low and high doses of adenosine. The coronary microvascular dilator response to low dose adenosine was significantly blunted during METH (p < 0.05). In contrast, the microvascular dilator response to high dose adenosine was unaffected.
Table 2. Effect of l-NMMA on Myocardial Blood Flow and Hemodynamic Variables (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ado 70</th>
<th>Ado 140</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBF</td>
<td>0.67 ± 0.20</td>
<td>2.08 ± 1.34†</td>
<td>3.65 ± 0.65†</td>
</tr>
<tr>
<td>G</td>
<td>7.72 ± 2.39</td>
<td>25.51 ± 17.42†</td>
<td>47.72 ± 12.12‡</td>
</tr>
<tr>
<td>HR</td>
<td>60 ± 9*</td>
<td>68 ± 14*</td>
<td>90 ± 19*</td>
</tr>
<tr>
<td>MAP</td>
<td>87 ± 42*</td>
<td>84 ± 8*</td>
<td>78 ± 10*</td>
</tr>
<tr>
<td>RPP</td>
<td>6,926 ± 1,331</td>
<td>7,811 ± 1,789</td>
<td>9,848 ± 2,445</td>
</tr>
<tr>
<td>l-NMMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBF</td>
<td>0.63 ± 0.23</td>
<td>1.48 ± 1.32‡</td>
<td>3.36 ± 0.83†</td>
</tr>
<tr>
<td>G</td>
<td>6.69 ± 2.36</td>
<td>16.07 ± 15.30†</td>
<td>35.60 ± 9.81†</td>
</tr>
<tr>
<td>HR</td>
<td>53 ± 10</td>
<td>54 ± 17</td>
<td>68 ± 24</td>
</tr>
<tr>
<td>MAP</td>
<td>94 ± 11</td>
<td>93 ± 10</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>RPP</td>
<td>6,607 ± 1,394</td>
<td>6,676 ± 2,157</td>
<td>8,344 ± 2,487</td>
</tr>
</tbody>
</table>

*p < 0.05 (l-NMMA vs. placebo); †p < 0.005 vs. baseline.

Ado 70 = adenosine 70 µg/kg/min; Ado 140 = adenosine 140 µg/kg/min; G = myocardial conductance, (ml/min/g/mmHg); HR = heart rate (beats/min); l-NMMA = N⁶-monomethyl-l-arginine; MBF = myocardial blood flow, (ml/min/g); RPP = rate pressure product (SAP × HR, expressed as mm Hg × beats/min); SAP, DAP, and MAP = systolic, diastolic, and mean arterial pressure, respectively (mm Hg).

Adenosine (p < 0.05 ANOVA) (Fig. 2). This is attributed to l-NMMA’s effect on MBF during infusion of the adenosine 70 dose (2.07 ± 1.33 vs. 1.48 ± 1.32 ml/g/min, placebo vs. l-NMMA, p < 0.05) (Fig. 2A). In contrast to that, l-NMMA did not significantly reduce the MBF response to the adenosine 140 dose (3.65 ± 0.65 vs. 3.36 ± 0.83 ml/g/min, placebo vs. l-NMMA, p = NS). However, l-NMMA reduced conductance during both doses of adenosine (25.51 ± 17.42 vs. 16.07 ± 15.30, and 47.72 ± 12.12 vs. 35.60 ± 9.81 ml/min/g/mm Hg for placebo vs. l-NMMA, adenosine 70 and 140, respectively, p < 0.05) (Fig. 2B).

Peripheral vascular function. The time-averaged flow velocity integral during peak reactive hyperemia, a measure of the stimulus for flow-mediated vasodilation, was the same for both groups (36.2 ± 8.7 vs. 36.5 ± 4.2 cm/s, placebo vs. methionine, respectively; p = NS). Flow-mediated, endothelium-dependent vasodilation was significantly decreased during hyperhomocysteinemia compared with the control state (4.4 ± 2.6% vs. 2.6 ± 2.3%, placebo vs. methionine, p < 0.05), demonstrating that the bioavailability of NO is decreased during acute hyperhomocysteinemia. However, endothelium-independent vasodilation to sublingual nitroglycerin was preserved (20.0 ± 6.1% vs. 20.2 ± 5.1%, placebo vs. methionine; p = NS), demonstrating normal vasodilation in response to the administration of a NO donor. Furthermore, flow-mediated vasodilation correlated with plasma homocysteine concentration (r = –0.50, p < 0.05).

![Figure 2](image-url)

**Figure 2.** (A) Effect of nitric oxide inhibition on the myocardial blood flow (MBF) response to adenosine. This figure depicts the MBF at rest and during low and high dose adenosine infusions, after placebo and during infusion of the nitric oxide synthase inhibitor, N⁶-monomethyl-l-arginine (l-NMMA). The adenosine dose response curve is significantly impaired during l-NMMA vs. placebo (p < 0.05, analysis of variance [ANOVA]). While nitric oxide inhibition significantly reduced MBF to low-dose adenosine, the MBF response to high dose adenosine was unaffected. (B) Effect of nitric oxide inhibition on the myocardial conductance response to adenosine. This figure depicts the myocardial conductance (G) at rest and during low and high dose adenosine infusions, after placebo and during infusion of the nitric oxide synthase inhibitor, l-NMMA. The adenosine dose response curve is significantly impaired during l-NMMA versus placebo (p < 0.05, ANOVA). Nitric oxide inhibition significantly reduced conductance to both low- and high-dose adenosine.
DISCUSSION

The important new findings in this study are: 1) that transient elevations in plasma homocysteine (to concentrations that are commonly encountered in clinical practice) are associated with acute impairment of the myocardial vasodilator response to adenosine in healthy humans and, 2) that the myocardial vasodilator response to adenosine is in part mediated by NO release. Furthermore, the finding of homocysteine-associated impairment in coronary microvascular endothelium-dependent vasodilation is paralleled by similar impairments in the peripheral circulation within the same subjects. As such, this study extends the previous findings of homocysteine-associated circulatory abnormalities, for the first time, to the human coronary circulation.

Role of NO in adenosine-induced vasodilation. Data from studies of the human peripheral circulation and from animal models of the coronary circulation suggest that NO release contributes to the vasodilator response to adenosine, (22–25). In the present study, we demonstrate that the coronary microvascular dilator response to adenosine is reduced during co-administration of the NOS antagonist, L-NMMA, (Figs. 2A and 2B, Table 2). As such, the data support the hypothesis that adenosine’s effect on microvascular dilation is at least in part dependent on NO bioavailability.

The results of the present study are similar to those recently reported by Buus et al. (25) in which NOS inhibition with N^G^-nitro-arginine methyl ester reduced the hyperemic response to adenosine by 21% from 1.90 to 1.50 ml/gm/min, p < 0.05. In our study, we report that L-NMMA reduced the hyperemic response to adenosine by 29% (from 2.07 to 1.49 ml/gm/min, p < 0.05). Hence, at comparable levels of hyperemic blood flow, the effects of NOS inhibition were similar.

In the present study, L-NMMA infusion significantly attenuated the hyperemic response to adenosine 70, but not to adenosine 140 (where the 8% reduction in myocardial blood flow failed to reach statistical significance). However, this does not mean that L-NMMA affects adenosine-induced vasodilation only at low dose. Indeed, NOS inhibition blunted G by 38% and 25% during adenosine 70 and 140, respectively (p < 0.05 for each). The fact that NOS inhibition had a more marked effect on coronary dilation during low versus high dose adenosine is consistent with the data by Hein et al. (22), which demonstrated in an in vitro porcine coronary artery model, that NO contributes relatively more to adenosine mediated vasodilation at lower doses of the nucleoside. Since only 20 to 30% of the hyperemic response to adenosine is blunted by NO antagonism in vivo, it is evident the majority of the response is NO-independent, particularly at higher dose where direct, smooth muscle relaxation predominates.

Mechanism by which homocysteine impairs coronary microvascular dilation. It has been previously shown, in the human peripheral circulation, that NO bioavailability is decreased in subjects with chronic hyperhomocysteinemia (6) as well as in acute METH (8,9). In the present study, we observed a reduction in the coronary microvascular dilator response to adenosine during METH (Figs. 1 and 2). As such the data argue in favor of an adverse effect of hyperhomocysteinemia on NO-mediated vasodilation of the coronary microcirculation. Moreover, our observation, within the same subjects, that NO-mediated vasodilation of the brachial artery is impaired during hyperhomocysteinemia supports the assertion that a systemic reduction in bioavailable NO exists after methionine loading. Furthermore, data obtained in the present study, as noted above, demonstrate that the coronary microvascular response to adenosine is NO-dependent especially at lower doses of the drug. Accordingly, the homocysteine-induced impairment of coronary microvascular dilator response to low-dose adenosine very likely reflects reduction of NO bioavailability.

Mechanism by which homocysteine reduces NO bioavailability. The mechanism by which hyperhomocysteinemia impairs NO bioavailability is not fully understood. A plausible mechanism appears to be related to the increased NO destruction as a result of enhanced production of reactive oxygen species (8,9,26). However, in the present study, we observed no significant change in iPF_2α-II (a plasma marker of oxidant stress), during methionine-induced hyperhomocysteinemia. A number of potential explanations exist for the observed lack of increase in iPF_2α-II. First, the measurement might not adequately reflect the intracellular redox status; there may be a lag between changes in intra- and extracellular redox potential. Second, the relatively short duration of hyperhomocysteinemia (<10 h) might not have been long enough to lead to an increase in the oxidized lipid byproducts that are measured by the assay. Furthermore, extracellular redox status might have been affected by the presence of high concentrations of methionine, which can itself function as a reducing agent (27).

Another putative mechanism by which hyperhomocysteinemia may decrease NO bioavailability is by enhancing the production of ADMA (which in turn inhibits production of NO) (21,28). In the present study, however, we did not detect an increase in ADMA during transient hyperhomocysteinemia in healthy subjects with normal fasting homocysteine concentrations. This may reflect a difference between subacute METH and naturally occurring chronic hyperhomocysteinemia. The precise mechanism, by which hyperhomocysteinemia initiates oxidant stress and reduces NO bioavailability in the coronary and peripheral circulations cannot be stated based on data acquired in the present study and therefore requires additional investigation.

Study limitations. While redistribution of coronary microvascular resistance (29) theoretically could make it appear as if a blunted microvascular response to adenosine in presence of hyperhomocysteinemia reflected an endothelium-dependent effect, when in fact it did not, this seems unlikely.
There is abundant evidence from experiments in the peripheral circulation that hyperhomocysteinemia impairs microvascular dilator responses by an NO dependent mechanism (6,8,9). Moreover, if hyperhomocysteinemia were capable of causing redistribution of microvascular resistance by an NO-independent mechanism, then we would anticipate that the forearm blood flow responses to NO-independent dilators such as sodium nitroprusside and nitroglycerin would be impaired in an analogous manner to that observed for adenosine in the canine study of Jones et al. (29). In fact, however, dilator responses to these drugs (that is sodium nitroprusside and nitroglycerin) in the peripheral circulation are not influenced by hyperhomocysteinemia (6,8,9). Accordingly, we think it unlikely in the present study that blunted coronary microvascular response to low dose adenosine in the presence of hyperhomocysteinemia reflects nonspecific redistribution of coronary microvascular resistance.

Clinical implications. This study’s finding that METH is associated with impaired NO-mediated coronary microvascular dilator function has important clinical implications. First, it is known that impaired endothelium-dependent vasodilation of coronary resistance vessels is associated with exercise-induced myocardial ischemia (30,31). As such, this study’s finding of impaired coronary microvascular dilator response suggests a mechanism by which hyperhomocysteinemia may cause myocardial ischemia in patients with coronary atherosclerosis. Second, it is known that plasma homocysteine concentrations may fluctuate up to 25% throughout the day, in response to variations in protein intake (32). As such, this study raises the possibility that the coronary microvascular dilator response (and hence susceptibility to ischemia) may fluctuate throughout the day, as the homocysteine concentrations fluctuate. Moreover, the reduced nitric oxide bioavailability that results from hyperhomocysteinemia might have effects that extend beyond the symptoms that result from impaired vasodilation. This is due to the fact that NO plays many important roles that are relevant to the pathogenesis and pathophysiology of atherosclerosis, such as: conferring an important antithrombotic property on the endothelial surface (by inhibiting the adhesion, activation, and aggregation of platelets), reducing the adhesion and emigration of leukocytes into the vessel wall, and limiting the growth of vascular smooth muscle cells (33). It follows, then, that hyperhomocysteinemia, by decreasing nitric oxide bioavailability, may initiate as well as accelerate the development of obstructive coronary disease and may also aggravate the symptoms that result from it.

Conclusions. Acute hyperhomocysteinemia induced by methionine impairs coronary microvascular dilator capacity by decreasing bioavailable NO, as evidenced by blunted flow response to low dose adenosine. This extends the observation of homocysteine-associated blood flow abnormalities to the coronary circulation, where the most devastating consequences of hyperhomocysteinemia are thought to occur. These findings underscore the need to determine if lowering homocysteine with inexpensive and well-tolerated B-vitamins could impart clinical benefits to patients with elevated homocysteine concentrations.

Reprint requests and correspondence: Dr. Ahmed Tawakol, Cardiac Unit/Vincent Burnham 3, Massachusetts General Hospital, Boston, Massachusetts 02114. E-mail: atawakol@partners.org.

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