Endothelial dysfunction in patients with recent myocardial infarction and hyperhomocysteinaemia: effects of vitamin supplementation

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ABSTRACT

Hyperhomocysteinaemia is a prothrombotic condition that may cause oxidative endothelial injury and impair endogenous fibrinolysis. Vitamin supplementation enhances endothelial function in hyperhomocysteinaemic patients, but responses in patients with co-existing coronary artery disease have been variable. It is also unknown whether hyperhomocysteinaemia is associated with reduced fibrinolytic responses in patients with coronary artery disease. The study aims were to test the hypothesis that patients with recent myocardial infarction and hyperhomocysteinaemia have impaired endothelium-dependent vasomotion and fibrinolysis that is rectified by vitamin supplementation. From a cohort of 120 patients admitted with acute myocardial infarction, 18 patients were recruited from the upper (n = 9) and lower (n = 9) plasma homocysteine quartiles into a randomized double-blind placebo-controlled crossover trial. Following a 4-week course of placebo or folate/cyanocobalamin/pyridoxine supplements, FBF (forearm blood flow) was measured using venous occlusion plethysmography during intra-arterial substance P (4–16 pmol/min), acetylcholine (5–20 µg/min) and sodium nitroprusside (2–8 µg/min) infusions. All vasodilators caused dose-dependent increases in infused FBF (P < 0.05). Patients in the upper homocysteine quartile (16.8 ± 2.9 compared with 7.9 ± 0.7 µmol/l; P = 0.003) had reduced vasodilatation to acetylcholine (P = 0.01) and substance P (P < 0.05), but not sodium nitroprusside. There were no differences in substance P-induced tissue plasminogen activator release. Vitamin supplementation increased serum folate and vitamin B12 concentrations (P < 0.05), but did not significantly lower homocysteine, or affect FBF or fibrinolytic responses. In patients with recent myocardial infarction, hyperhomocysteinaemia is associated with impaired endothelium-dependent vasodilatation, but no alteration in the acute fibrinolytic capacity. This endothelial vasomotor dysfunction is unaltered by vitamin supplementation.

INTRODUCTION

Several prospective and case-control studies have shown that elevated plasma homocysteine concentrations are an independent risk factor for the development of atherothrombotic vascular disease as well as a prognostic marker in ischaemic heart disease [1–3]. Plasma homocysteine concentrations are consistently higher in patients with premature peripheral and cerebrovascular diseases [4], and almost a third of patients with premature coronary

Key words: endothelial dysfunction, fibrinolysis, homocysteine, myocardial infarction, vasodilatation, vitamin.
Abbreviations: CI, confidence interval; FBF, forearm blood flow; NS, not significant; PAI-1, plasminogen activator inhibitor type 1; t-PA, tissue plasminogen activator.
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artery disease are found to have hyperhomocysteinaemia [1]. In addition, apparently healthy men with plasma homocysteine concentrations 12% above the upper limit of normal have a 3-fold increased risk of acute myocardial infarction [2], and in patients with ischaemic heart disease there is an increased mortality associated with plasma concentrations greater than 9 µmol/l [5].

The vascular endothelium plays a central role in the control of blood flow, haemostasis and endogenous fibrinolysis, and endothelial dysfunction independently predicts cardiovascular events [6,7]. Although the mechanism of vascular damage is unclear, homocysteine may promote atherogenesis through oxidative endothelial injury that is mediated by cytotoxic reactive oxygen species [8–10]. Indeed, acute and chronic hyperhomocysteinaemia are associated with impaired endothelium-dependent flow-mediated dilatation of the brachial artery [10–12].

Hyperhomocysteinaemia is a prothrombotic state [3]. We [13] and others [14] have shown previously that hyperhomocysteinaemia induced by oral methionine loading is associated with alterations in endogenous fibrinolysis in healthy subjects and patients with premature vascular disease. However, the influence of chronic hyperhomocysteinaemia on the acute fibrinolytic capacity is unknown and is the subject of debate [15]. Interestingly, there is an association between plasma homocysteine and t-PA (tissue plasminogen activator) antigen concentrations in stroke patients [16].

Vitamin supplementation with folate, vitamin B₆ and vitamin B₁₂ is safe and may reduce plasma homocysteine concentrations [17]. Although endothelial function is enhanced following treatment with folate in patients with hyperhomocysteinaemia [18,19] and hypercholesterolaemia [20], the response in patients with coronary artery disease has been variable [21–25]. Furthermore, it is unknown whether elevated plasma homocysteine concentrations are associated with reduced resistance vessel vasomotor responses in patients with established coronary artery disease.

The aim of the present study was to test the hypotheses that, in patients with recent myocardial infarction, elevated plasma homocysteine concentrations are associated with impaired endothelium-dependent vasodilatation and endogenous fibrinolytic capacity, and that vitamin supplementation (with folate, vitamin B₆ and vitamin B₁₂) would both lower plasma homocysteine and restore endothelial function.

**METHODS**

**Subject recruitment**

One hundred and twenty patients admitted with an acute myocardial infarction were recruited into the trial. Myocardial infarction was defined as typical ischaemic cardiac pain associated with elevation of cardiac markers (greater than twice the upper limit of normal) and electrocardiographic evidence of myocardial ischaemia. Exclusion criteria were atrial fibrillation on warfarin therapy, impaired renal function (serum creatinine > 120 µmol/l), diabetes mellitus, requirement for folate supplementation or pernicious anaemia. The written informed consent of each subject was obtained before entry into the study. All studies were undertaken with the approval of the local Research Ethics Committee and in accordance with the Declaration of Helsinki (1996).

**Study design**

Fasting plasma homocysteine concentrations were determined in all patients on days 5–7 following acute myocardial infarction [26]. From the upper and lower plasma homocysteine concentration quartiles, nine patients in each quartile were recruited into a randomized double-blind balanced-block placebo-controlled crossover trial at least 4 months after the index event. All patients received two separate 4-week courses of oral sucrose placebo or vitamin supplementation (5 mg of folate/100 µg of cyanocobalamin/10 mg of pyridoxine), and attended at the end of each 4-week treatment period. On each study day, medications were withheld and subjects attended after a 4-h fast and rested recumbent in a quiet temperature-controlled room maintained at 22–25 °C. Strain gauges and cuffs were applied, and the brachial artery of the non-dominant arm was cannulated. After 30 min equilibration with saline infusion, intra-arterial substance P (4–16 pmol/min), acetylcholine (5–20 µg/min) and sodium nitroprusside (2–8 µg/min) were administered in a randomized order for 6–10 min at each dose and separated by 20 min washout periods [27–29]. Venous samples were taken at baseline and during infusion of each substance P dose for determination of t-PA and PAI-1 (plasminogen activator inhibitor type 1). Venous sampling was not performed during sodium nitroprusside or acetylcholine infusion, since they do not affect t-PA or PAI-1 release in this forearm model [13,27,30].

**Intra-arterial administration and drugs**

The brachial artery of the non-dominant arm was cannulated with a 27-standard wire gauge steel needle (Cooper’s Needle Works, Birmingham, U.K.) under 1% lidocaine (Xylocaine; Astra Pharmaceuticals, Kings Langley, Herts., U.K.) local anaesthesia and attached to a 16-gauge epidural catheter (Portex, Hythe, Kent, U.K.). Patency was maintained by infusion of saline (0.9% NaCl) via a MS2000 syringe infusion pump (Graseby Medical, Watford, Herts., U.K.). The total rate of intra-arterial infusions was maintained constant throughout all studies at 1 ml/min. Pharmacological grade substance P (Clinalfa, Läufelfingen, Switzerland), acetylcholine (Cibavision Ophthalmics, Southampton, U.K.) and sodium nitroprusside (David Bull Laboratories, Warwick,
U.K.) were administered following dissolution in saline. All solutions were freshly prepared on the day of study.

**Haemodynamic measurements**

Blood flow was measured in both forearms by venous occlusion plethysmography using mercury-in-silastic strain gauges as described previously [27,28]. During measurement periods, the hands were excluded from the circulation by rapid inflation of the wrist cuffs to 220 mmHg using E20 rapid cuff inflators (DE Hokanson, Bellevue, WA, U.S.A.). Upper arm cuffs were inflated intermittently to 40 mmHg for 8 s in every 10 s to achieve venous occlusion and obtain plethysmographic recordings. Blood pressure was monitored in the non-infused arm at intervals throughout each study with a semi-automated non-invasive oscillometric sphygmomanometer (Takeda UA 751; Takeda Medical, Tokyo, Japan).

**Venous sampling and assays**

Venous cannulae (17-gauge) were inserted into large subcutaneous veins of the antecubital fossae of both arms. Venous blood was withdrawn simultaneously from each arm and collected into acidified buffered citrate (Biopool, Umeå, Sweden) for t-PA assays and trisodium citrate and lithium heparin tubes (Bayer Immuno 1, Sarstedt, Nürnberg, Germany) for PAI-1 and homocysteine assays respectively. Samples were kept on ice before being centrifuged at 2000 g. Platelet-free plasma was decanted and stored at −80 °C before assay. Plasma t-PA and PAI-1 antigens were determined as described previously [27] using ELISA (Coaliza t-PA and Coaliza PAI-1; Chromogenix, Mölndal, Sweden), and plasma t-PA activity was determined using a photometric method (Coatest t-PA; Chromogenix). Plasma homocysteine (Axis, Homocysteine EIA; Axis-Shield, Oslo, Norway) and serum folate and vitamin B12 concentrations (Bayer Immuno 1 automated immunoassay analyser, Bayer, Leverkusen, Germany) were determined using enzyme immunoassay.

**Data analysis and statistics**

Estimated net release of t-PA antigen and activity were defined previously [27] as the product of the infused forearm plasma flow [based on the mean haematocrit (Hct) and the infused FBF (forearm blood flow)] and the concentration difference between the infused ([t-PA]inf) and non-infused ([t-PA]Noninf) arms using the formula:

\[
\text{Estimated net t-PA release} = \text{FBF} \times (1 - \text{Hct}) \times ([\text{t-PA}]_{\text{inf}} - [\text{t-PA}]_{\text{Noninf}})
\]

Data were examined, where appropriate, by ANOVA with repeated measures and two-tailed paired Student’s t test (SAS Institute). All results are expressed as means (S.E.M.) or means [95% CI (confidence interval)]. Statistical significance was taken at the 5% level.

**RESULTS**

**Baseline and biochemical characteristics**

From the upper and lower plasma homocysteine quartiles, 18 patients (nine patients in each quartile) were recruited into the randomized controlled trial. Apart from plasma homocysteine concentrations (\(P < 0.01\), as determined by Student’s t test), which were significantly different by design, there were no significant differences between the baseline clinical characteristics or medical therapies in the two groups of patients (Tables 1 and 2).

All subjects tolerated placebo and vitamin supplementation and no side effects were reported or noted. Serum folate and vitamin B12 concentrations were increased following vitamin supplementation compared with placebo in both patient groups (\(P < 0.001\) and \(P < 0.05\) respectively, as determined by Student’s t test; Table 2). Plasma homocysteine concentrations appeared to be reduced by \(\approx 16\%\) with active treatment in hyperhomocysteinaemic patients, but this was not statistically significant \((P = 0.3\), as determined by Student’s t test; Table 2). Vitamin supplementation had no significant effects on heart rate, blood pressure or basal FBF in either patient group.
**Table 2** Plasma homocysteine, serum folate and vitamin B12 concentrations following vitamin supplementation

<table>
<thead>
<tr>
<th></th>
<th>Patients in the upper quartile</th>
<th>Patients in the lower quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Treatment</td>
<td>Placebo</td>
</tr>
<tr>
<td>Plasma homocysteine</td>
<td>16.8 (2.8)*</td>
<td>14.2 (1.1)*</td>
</tr>
<tr>
<td>Serum folate (µg/l)†‡</td>
<td>8.6 (1.8)</td>
<td>17.7 (1.6)†‡</td>
</tr>
<tr>
<td>Serum vitamin B12 (ng/l)</td>
<td>462 (136)</td>
<td>580 (149)†</td>
</tr>
</tbody>
</table>

‡: Upper limit of serum folate assay was 20 µg/l; 13 patients had concentrations above this after supplementation and were taken as 20 µg/l.

**Endothelium-dependent vasomotion**

Substance P, acetylcholine and sodium nitroprusside caused dose-dependent increases in blood flow in the infused forearm in both patient groups on each study day (P < 0.05, as determined by ANOVA; Figure 1). In comparison with patients in the lower quartile, hyperhomocysteinaemic patients had significantly reduced FBF responses to acetylcholine and substance P (P = 0.01 and P < 0.05 respectively, as determined by ANOVA; Figure 1), but there were no significant differences in the blood flow responses to sodium nitroprusside (P = NS, not significant, as determined by ANOVA).

Neither endothelium-dependent nor endothelium-independent vasodilatation were significantly influenced by vitamin treatment in either patient group (P = NS for all, as determined by ANOVA). For the hyperhomocysteinaemic group, the mean difference for the response to vitamin treatment at the peak dose was 1.1 ml·100 ml⁻¹·min⁻¹ (95% CI, −0.8 to +2.9) for substance P, 0.3 ml·100 ml⁻¹·min⁻¹ (95% CI, −1.3 to +1.8) for acetylcholine, and −0.3 ml·100 ml⁻¹·min⁻¹ (95% CI, −2.8 to +2.1) for sodium nitroprusside. For the lower quartile group, the mean difference for the response to vitamin treatment at the peak dose was 2.0 ml·100 ml⁻¹·min⁻¹ (95% CI, −0.6 to +4.6) for substance P, −0.8 ml·100 ml⁻¹·min⁻¹ (95% CI, −2.9 to +1.2) for acetylcholine, and 1.7 ml·100 ml⁻¹·min⁻¹ (95% CI, −2.8 to +6.2) for sodium nitroprusside.

**Fibrinolytic responses**

Baseline plasma t-PA and PAI-1 antigen and t-PA activity concentrations were similar in both groups (Table 3). Substance P caused dose-dependent increases in plasma t-PA antigen and activity concentrations in the infused forearm of all patients (P < 0.05 for both, as determined by ANOVA; Table 3). The responses were similar in both patient groups and were not influenced by vitamin supplementation (P = NS, as determined by ANOVA). The mean difference of t-PA antigen release for the response to vitamin supplementation at the peak substance P dose was −2.7 ng·100 ml⁻¹·min⁻¹ (95% CI, −15.5 to +10.2; P = NS, as determined by Student’s t test) in the upper quartile group and −7.4 ng·100 ml⁻¹·min⁻¹ (95% CI, −24.3 to +9.5, P = NS, as determined by Student’s t) in the lower quartile group. Plasma PAI-1 concentrations were unaffected by substance P infusion or vitamin treatment (P = NS, as determined by Student’s t test).

**DISCUSSION**

In the present study, we have demonstrated that, in patients with a recent myocardial infarction, elevated plasma homocysteine concentrations are associated with impaired endothelium-dependent vasodilation without affecting acute endogenous t-PA release. However, vitamin supplementation failed to significantly reduce plasma homocysteine concentrations or improve endothelial vasomotor function.

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Table 3  Plasma t-PA and PAI-1 concentrations and release during substance P infusion

Values are means (S.E.M.). ∗P < 0.05 for all responses during substance P infusion, as determined by ANOVA. AUC, area under curve; IU, international units.

<table>
<thead>
<tr>
<th>Substance P (pmol/min)</th>
<th>Placebo</th>
<th>Treatment</th>
<th>Placebo</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infused arm</td>
<td>Non-infused arm</td>
<td>Infused arm</td>
<td>Non-infused arm</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td>Baseline 7.7 (1.2)</td>
<td>8.6 (1.4)</td>
<td>7.5 (1.0)</td>
<td>8.2 (1.4)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.8 (1.5)</td>
<td>8.3 (1.3)</td>
<td>9.2 (1.0)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9.4 (1.5)</td>
<td>8.1 (1.1)</td>
<td>10.3 (1.1)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>12.7 (2.4)*</td>
<td>7.8 (0.9)</td>
<td>11.1 (1.4)*</td>
</tr>
<tr>
<td>Estimated t-PA antigen release (ng · 100 ml⁻¹ · min⁻¹)</td>
<td>Baseline -1.1 (0.7)</td>
<td>-1.2 (1.1)</td>
<td>0.4 (0.7)</td>
<td>-0.3 (0.4)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.0 (3.2)</td>
<td>5.4 (3.4)</td>
<td>3.4 (2.2)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4.7 (4.1)</td>
<td>11.1 (4.2)</td>
<td>9.8 (3.7)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>21.6 (6.7)*</td>
<td>18.9 (5.2)*</td>
<td>24.3 (8.4)*</td>
</tr>
<tr>
<td>Net t-PA antigen release (AUC)</td>
<td>18.9 (9.3)</td>
<td>25.4 (9.0)</td>
<td>24.2 (8.5)</td>
<td>14.9 (4.2)</td>
</tr>
</tbody>
</table>

| t-PA activity (IU/ml) | Baseline 0.9 (0.2) | 0.8 (0.2) | 0.5 (0.1) | 0.6 (0.1) |
|                        | 4        | 1.7 (0.6) | 0.7 (0.2) | 1.5 (0.3) |
|                        | 8        | 1.9 (0.7) | 0.8 (0.3) | 2.3 (0.4) |
|                        | 16       | 3.1 (0.8)* | 1.0 (0.2) | 2.9 (0.5)* |
| Estimated t-PA activity release (IU · 100 ml⁻¹ · min⁻¹) | Baseline 0.0 (0.1) | -0.1 (0.1) | 0.0 (0.1) | 0.1 (0.2) |
|                        | 4        | 2.5 (1.4) | 3.1 (1.3) | 6.0 (2.1) |
|                        | 8        | 3.6 (1.6) | 7.0 (1.7) | 8.4 (3.6) |
|                        | 16       | 9.3 (2.9)* | 11.7 (3.6)* | 16.4 (6.9)* |
| Net t-PA activity release (AUC) | 10.7 (4.2) | 15.9 (4.7) | 21.6 (8.7) | 12.0 (4.9) |

| PAI-1 antigen (ng/ml) | Baseline 47 (5) | 48 (4) | 44 (4) | 48 (5) |
|                        | 16       | 48 (4) | 54 (3) | 45 (4) |

Endothelium-dependent vasodilatation

Previous studies have shown that homocysteine is associated with endothelial dysfunction. Lentz et al. [9] reported that, in monkeys with diet-induced hyperhomocysteinaemia, endothelium-dependent vasodilatation is impaired in carotid artery rings in vitro and hindlimb resistance vessels in vivo. Celermajer et al. [11] documented abnormal endothelium-dependent vasodilatation in children with severe hyperhomocysteinaemia due to homozygous homocystinuria. Impaired endothelium-dependent vasodilatation is detected in healthy subjects with acute hyperhomocysteinaemia induced by oral methionine loading [10,31], as well as in patients with chronic hyperhomocysteinaemia who are free from clinical manifestations of atherosclerotic disease [12,18]. In the present study, we have extended these findings to patients with recent myocardial infarction and demonstrated impaired vasomotor responses in those with elevated plasma homocysteine concentrations.

The vascular endothelium plays a critical role in the control of vascular homoeostasis by regulating vascular tone, platelet activity, coagulation and fibrinolysis, and endothelial dysfunction is believed to be an early step in the pathogenesis and pathophysiology of atherosclerosis. Although patients with coronary artery disease typically demonstrate endothelial dysfunction, there is considerable heterogeneity in the magnitude of impairment in individuals with similar risk factor profiles. This is of particular interest because the extent of coronary as well as peripheral endothelial dysfunction independently predicts the long-term risk of acute cardiovascular events, including sudden cardiac death, myocardial infarction and revascularization procedures [6,7]. Recent prospective data have indicated that, in patients with established coronary artery disease, homocysteine is a significant predictor of mortality independent of other traditional risk factors [5,32]. Our present findings therefore support the role of homocysteine as a secondary risk marker, suggesting that this may be mediated through its effects on endothelial function.

Endogenous fibrinolysis

Although homocysteine impairs endothelial vasomotor function, it does not appear to have a major effect on endothelium-dependent fibrinolytic capacity, as both basal and stimulated release of t-PA or PAI-1 were not significantly different between the two patient groups. Hyperhomocysteinaemia is a prothrombotic condition.
and may interfere with the antithrombotic and fibrinolytic mechanisms of the endothelium and alter endothelial protein secretory pathways. Although endothelial cell-associated t-PA activity is reduced in homocysteine-treated cells [33], our present study failed to detect reduced fibrinolytic activity in vivo. This is consistent with data indicating that homocysteine might perturb the intrinsic fibrinolytic potential by reducing the functional binding site for t-PA without altering the catalytic capability of t-PA or affecting t-PA synthesis and secretion [33].

Effects of vitamin supplementation on plasma homocysteine

Previous studies have shown that treatment with folate and B vitamins can lower plasma homocysteine concentrations to a varying degree. The Homocysteine Lowering ‘Trials’ meta-analysis predicted a 20–30 % reduction in homocysteine in patients with plasma concentrations above 12 µmol/l taking folate, and a further small additional effect with vitamin B₁₂ but not B₆ [17]. However, a more modest 11–14 % reduction is seen in patients with coronary artery disease who consumed fortified breakfast cereals [34,35]. The limited homocysteine lowering seen in our present study was probably related to the relatively mild hyperhomocysteinaemia and normal folate concentrations in our study population as well as the confounding effects of dietary folate fortification.

Effects of vitamin supplementation on endothelial responses

We did not detect an improvement in endothelium-dependent vasodilatation or endogenous fibrinolytic capacity following vitamin supplementation in the present study. Although this may not be surprising in the absence of significant homocysteine reduction, Doshi et al. [22] have suggested that the acute effects of folate on endothelial function may occur by a mechanism independent of homocysteine lowering. Moreover, the evidence of the beneficial effects of vitamin supplementation on endothelial function is conflicting. Chambers et al. [21] and Title et al. [24] demonstrated improved endothelial function in patients with coronary artery disease following folate treatment without or with vitamin B₁₂. However, our present findings are consistent with those of other investigators who failed to detect improved endothelial function in a similar patient population, in healthy siblings of patients with premature atherothrombotic disease or in patients with renal impairment [23,25,36]. These contradictory findings may be related, in part, to the presence of other cardiovascular risk factors such as hypertension or hypercholesterolaemia, which may contribute to endothelial injury [37,38], but would not be expected to respond to folate or B vitamins. The vascular endothelium in patients with established coronary artery disease may have also been subjected to chronic injury and would therefore be less responsive to intervention. Furthermore, although the above studies adopted flow-mediated dilatation as a non-invasive method of assessing conduit artery endothelial function, in the present and other studies [28,39], we have focused on the function of endothelium within resistance vessels. Conduit artery and microvascular endothelial cells have distinct phenotypic differences, and responses to mechanical rather than pharmacological stimuli may also differ, and may contribute to the apparent disparity in the responses.

There is further controversy regarding the effects of vitamin supplementation on cardiovascular outcomes in patients undergoing percutaneous coronary intervention. Contrary to earlier reports that vitamin supplementation may reduce the rate of restenosis and adverse outcomes following coronary artery angioplasty [40,41], Lange et al. [42] have recently demonstrated that folate therapy following coronary stenting may increase the risk of in-stent restenosis. The underlying mechanism for these findings remains uncertain, but it is possible that the proliferative effects of folate may promote the growth of neointimal cells within implanted stents. Therefore more prospective data are needed before any recommendations can be made regarding the use of vitamin supplementation in coronary artery disease.

Study limitations

There are several potential limitations to our present study. First, we studied peripheral vascular function and thus these findings may not be directly applicable to other vascular beds. However, endothelial dysfunction is often a generalized process, and we have shown previously consistent vasomotor and endogenous fibrinolytic responses between the forearm and coronary circulation [27,39]. Secondly, the failure to improve endothelial function may be related to inadequate treatment duration, although studies have suggested that a 4–6-week treatment with folate can improve endothelial function [20,22]. Thirdly, the size of the study was small and, although it was powered to detect a 15–20 % difference in t-PA release or forearm vasodilatation, it is possible that a smaller effect may have been missed. Fourthly, we used a placebo-controlled crossover design and there was the possibility of a carry-over effect of the vitamin therapy on endothelial function during placebo administration, despite a significant difference in the serum concentrations of folate and vitamin B₁₂. Finally, we cannot rule out that homocysteine may be a marker of vascular injury rather than a mediator of endothelial dysfunction, although experimental data support the direct role of homocysteine in causing endothelial damage [9,33].

In conclusion, we have demonstrated that endothelium-dependent vasodilatation, but not endogenous
fibronolysis, is impaired in patients with recent myocardial infarction and elevated plasma homocysteine, and that this endothelial vasomotor dysfunction is not rectified by vitamin supplementation. These results provide further evidence for the role of homocysteine in vascular damage, but do not support the hypothesis that vitamin supplementation improves endothelial function in patients with established coronary artery disease.

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