Hyperhomocysteinaemia in young adults is not associated with impaired endothelial function

Colm G. HANRATTY*, Daniel F. MCAULEY*, Lawrence T. McGrath*, Ian S. Young† and G. Dennis JOHNSTON*

*Department of Therapeutics and Pharmacology, The Queen's University of Belfast, The Whitla Medical Building, 97 Lisburn Rd, Belfast BT9 7BL, Northern Ireland, U.K., and †Department of Clinical Biochemistry, The Queen's University of Belfast, The Royal Victoria Hospital, Belfast BT12 6BA, Northern Ireland, U.K.

A B S T R A C T

A mild to moderate elevation of the total homocysteine concentration (tHcy) is now recognized as a risk factor for vascular disease. It is also associated with endothelial dysfunction in middle-aged and elderly individuals without overt atherosclerotic vascular disease. This is important, as endothelial dysfunction is a well recognized early and potentially reversible marker of the atherosclerotic process. We investigated whether mild hyperhomocysteinaemia was associated with endothelial dysfunction in otherwise healthy young males. We compared endothelial function, by measuring forearm blood flow, in 17 males with mild hyperhomocysteinaemia (defined as tHcy > 10 μmol/l) and 14 controls with low tHcy (defined as < 5 μmol/l). Forearm blood flow was measured in response to the intra-arterial infusion of acetylcholine (endothelial-dependent response) or sodium nitroprusside (endothelial-independent response). Responses to the vasoactive substances were expressed as the area under the curve of the change in forearm blood flow from baseline. Data are given as mean (95% confidence interval). The two groups were well matched for age, body mass index, pulse rate and blood pressure. tHcy was significantly different between the groups [12.3 (10.4–14.2) μmol/l compared with 4.9 (4.6–5.1) μmol/l; P < 0.001]. Concentrations of vitamin B12 and folate were significantly higher in the control group. There was no difference in basal forearm blood flow between the group with mild hyperhomocysteinaemia and the controls, and both the endothelial-dependent [37.5 (26.2–38.8) and 35.3 (26.1–44.4) arbitrary units respectively] and -independent [26.1 (22.2–29.9) and 25.9 (21.0–30.8) units respectively] responses were not significantly different between the groups. Thus the present study demonstrates that, in healthy adults, mild elevation of tHcy was not associated with impaired endothelial-dependent vasodilation. These data suggest an age effect with regard to homocysteine and endothelial dysfunction. The development of vascular disease in individuals with hyperhomocysteinaemia may only result with higher concentrations or after prolonged exposure.

INTRODUCTION

Hyperhomocysteinaemia is known to be an independent risk factor for the development of atherosclerotic vascular disease [1,2]. There are several possible mechanisms by which an elevated total homocysteine concentration (tHcy) is thought to promote vascular disease, but increasing evidence suggests that homocysteine induces endothelial damage. In vitro work suggests that homocysteine damages the endothelium [3], with altered nitric oxide bioavailability [4]. In primates, dietary manipulation of tHcy resulted in

Key words: endothelial function, homocysteine, plethysmography
Abbreviations: AUC, area under the curve; FBF, forearm blood flow; tHcy, total homocysteine concentration.
Correspondence: Dr Colm G. Hanratty (e-mail c.hanratty@net.ntl.com).
impaired endothelial function and vessel changes that were in keeping with the development of atherosclerosis [5]. *In vivo*, chronic hyperhomocysteinaemia in elderly [6] and middle-aged [7] subjects was associated with impaired endothelial-dependent vasodilation without overt evidence of atherosclerosis. This is important, as endothelial dysfunction is an early [8], and reversible [9], indicator of the atherosclerotic process.

There are no reports assessing endothelial function in younger individuals. We investigated whether mild hyperhomocysteinaemia in young, healthy individuals was associated with impaired endothelial function. Such information may be important in defining risk in otherwise healthy subjects. Accordingly, we measured forearm blood flow (FBF) in response to endothelial-dependent and -independent vasodilation in subjects with and without hyperhomocysteinaemia.

**METHODS**

**Subjects**

Over 400 non-smoking male individuals aged between 18 and 35 years were screened; they had responded to advertisements in the university campus and local papers. Each subject underwent a medical history, examination, ECG and routine laboratory tests, including fasting tHcy and total cholesterol concentration. The subjects were recruited from this population. A total of 17 subjects with hyperhomocysteinaemia (tHcy > 10 μmol/l) were compared with 14 control subjects (tHcy < 5 μmol/l). There was no additional selection process, and all eligible subjects were enrolled. A maximum of 3 months elapsed between screening and study dates. The subjects were all healthy non-smoking males with no other risk factors for the development of atherosclerosis, and were not taking any current medication, specifically vitamin supplements. This study was approved by the ethics committee of The Queen’s University, Belfast. All subjects gave written informed consent for all procedures.

**Design**

The subjects fasted overnight and abstained from alcohol- and caffeine-containing products for 12 h before the study. A physical examination, including measurement of height, weight, pulse and supine resting blood pressure, was performed. Venous blood was sampled for fasting plasma tHcy and cholesterol, vitamin B₁₂ and folate levels.

**FBF measurements**

Studies took place in a temperature-controlled room (24–26 °C). A 27-gauge needle was inserted under local anaesthetic into the non-dominant brachial artery to allow local intra-arterial drug infusion. The brachial artery was cannulated 30 min before the first recording to allow the area to normalize. With the subject supine and the arms resting on a support slightly above the level of the heart, FBF was measured by strain-gauge venous-occlusion plethysmography. A mercury-in-silastic strain gauge was coupled to an electronically calibrated plethysmograph (model SPG16; Medasonics, Newark, CA, U.S.A.). The voltage output was transferred to a Macintosh personal computer (Performa 630; Apple Computer Inc.) with a MacLab analogue-to-digital converter and CHART software (version 3.4.3) (AD Instruments, Hastings, E. Sussex, U.K.). The mean of five consecutive FBF measurements was taken for statistical evaluation. FBF was expressed in units of ml·min⁻¹·100 ml⁻¹ forearm volume.

Following a rest period of at least 30 min, during which 0.9% saline was infused, basal FBF was measured. Sodium nitroprusside was infused intra-arterially in four incremental doses (3, 6, 9 and 12 nmol·min⁻¹), each for 3 min, to assess endothelium-independent vasodilation, with FBF measured in the final 1 min of each infusion. After a washout period of at least 20 min, basal FBF was again measured. Acetylcholine was then infused intra-arterially into the experimental forearm in four incremental doses (60, 120, 180 and 240 nmol·min⁻¹), each for 3 min, to assess endothelium-dependent vasodilation, with FBF again measured in the final 1 min of each infusion. All infusions were administered at a rate of 1 ml/min using a constant-rate infusor. The doses used have been shown previously to have no systemic effects on heart rate, blood pressure or FBF [10]. FBF was measured in both arms, using the non-cannulated arm as a control to demonstrate that local administration of vasoactive drugs did not have a systemic action.

**Plasma tHcy**

Plasma was separated by centrifugation at 950 g for 10 min at 4 °C, within 20 min of venepuncture, and stored at −70 °C until analysis. Plasma tHcy was measured according to the method of Ubbink et al. [11] using HPLC with fluorescence detection. At a plasma tHcy of 7.87 μmol/l, the inter- and intra-assay coefficients of variation were 6.8% and 2.6% respectively.

**Statistical analysis**

The clinical and biochemical characteristics of the patient and control groups were compared by use of independent-samples t-tests.

Basal FBF values were compared by use of an independent-sample t-test. The responses to the vasoactive substances were expressed as the measure of area under the curve (AUC) of the change in FBF from
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base time, expressed in arbitrary units. This avoids the need to make multiple comparisons [12]. Results were
analysed using an independent-sample t-test.

Data are expressed in the form: mean (95% confidence interval). Differences were considered significant at a
d value of \( P < 0.05 \).

RESULTS

Subjects were aged 20–31 years. The two groups were
well matched for basal characteristics, including age,
height, weight, body mass index, blood pressure and total
cholesterol (Table 1). The fasting tHcy values were 12.3
(10.4–14.2) and 4.9 (4.6–5.1) \( \mu \text{mol/l} \) (\( P < 0.001 \)) (Table 1)
in the hyperhomocysteinaemia and control groups re-
spectively. Both vitamin \( B_12 \) and folate concentrations
were significantly higher in the control group compared
with the hyperhomocysteinaemia group [614 (521–707)
and 415 (333–497) \( \mu \text{mol/l} \) respectively (\( P < 0.001 \)) for vitam
(109) and 9.2 (7.7–10.7) and 6.9 (5.6–8.2) nmol/l
respectively (\( P < 0.01 \)) for folate] (Table 1).

Basal FBF did not differ significantly between the
hyperhomocysteinaemia and control groups [2.96
(2.54–3.38) and 2.86 (1.75–3.97) ml/min/100 ml re-
spectively; \( P = 0.9 \)] (Table 2). FBF in the control arm did
not change in response to infusion of any study drug,
thus confirming that the drug effects were confined to the
experimental forearm (results not shown).

The endothelial-independent responses (Figure 1) were
similar in the hyperhomocysteinaemia and control
groups [26.1 (22.2–29.9) and 25.9 (21.0–30.8) arbitrary
units respectively; \( P = 0.96 \)] (Table 2).

| Table 1 | Subject characteristics
Data are mean (95% confidence interval), except for age, which is given as mean (range). Significance of differences:

<table>
<thead>
<tr>
<th>Variable</th>
<th>tHcy &lt; 5 ( \mu \text{mol/l} ) (( n = 14 ))</th>
<th>tHcy &gt; 10 ( \mu \text{mol/l} ) (( n = 17 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (( \mu \text{mol/l} ))</td>
<td>4.9 (4.6–5.1)</td>
<td>12.3 (10.4–14.2)*</td>
</tr>
<tr>
<td>Serum folate (nmol/l)</td>
<td>9.2 (7.7–10.7)</td>
<td>6.9 (5.6–8.3)**</td>
</tr>
<tr>
<td>Serum vitamin ( B_12 ) (( \mu \text{mol/l} ))</td>
<td>614 (521–707)</td>
<td>415 (333–497)*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24.3 (20–30)</td>
<td>25.2 (20–31)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 (1.69–1.77)</td>
<td>1.73 (1.68–1.77)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.8 (70.7–80.9)</td>
<td>77.9 (73.2–82.7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.3 (24.0–26.6)</td>
<td>26.1 (25.0–26.2)</td>
</tr>
<tr>
<td>Pulse (beats/min)</td>
<td>62 (57–67)</td>
<td>63 (59–67)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122 (114–128)</td>
<td>120 (116–127)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74 (70–80)</td>
<td>72 (70–78)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.3 (4.0–4.6)</td>
<td>4.6 (4.2–4.9)</td>
</tr>
</tbody>
</table>

| Table 2 | tHcy, basal FBF and AUC for endothelial-independent and -dependent responses
Data are given as mean (95% confidence interval).

<table>
<thead>
<tr>
<th>Variable</th>
<th>tHcy &lt; 5 ( \mu \text{mol/l} ) (( n = 14 ))</th>
<th>tHcy &gt; 10 ( \mu \text{mol/l} ) (( n = 17 ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal FBF (ml/min/100 ml)</td>
<td>2.86 (1.75–3.97)</td>
<td>2.96 (2.54–3.38)</td>
<td>0.9</td>
</tr>
<tr>
<td>AUC for change in FBF (arbitrary units)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial-independent</td>
<td>25.9 (21.0–30.8)</td>
<td>26.1 (22.2–29.9)</td>
<td>0.96</td>
</tr>
<tr>
<td>Endothelial-dependent</td>
<td>35.3 (26.1–44.4)</td>
<td>37.5 (26.2–38.8)</td>
<td>0.8</td>
</tr>
</tbody>
</table>
DISCUSSION

A mild to moderate elevation in tHcy is now well recognized as an independent risk factor for the development of vascular disease [1,2]. This may involve mechanisms that damage the endothelium [5,13]. Endothelial dysfunction is regarded as an early key [8] and reversible [9] event in the development of atherosclerosis. A chronic moderate elevation in tHcy is associated with impaired endothelial-dependent vasodilation in elderly [6] and middle-aged [7] individuals without overt evidence of atherosclerosis. However, we could not demonstrate any evidence of impaired endothelial-dependent dilation in a group of young healthy individuals with mildly increased tHcy.

We defined elevated tHcy as greater than 10 µmol/l for several reasons. The American Heart Association scientific advisory panel [14] suggested that therapy should be implemented in selected individuals with a tHcy of greater than 10 µmol/l. Patients with proven coronary disease and a tHcy of 10 µmol/l are known to have an increased incidence of death, with an odds ratio of 1.92 (0.73–5.09) [15]. Also, in primates, dietary manipulation of tHcy to this concentration has resulted in evidence of altered vasomotor function [5].

In vitro work has suggested homocysteine damages cultured endothelial cells [3]. Manipulation of tHcy in primates resulted in patchy desquamation of the vascular endothelium and decreased platelet survival time [16], as well as endothelial dysfunction [5].

In vivo there have been some positive studies [6,7] and one negative study [17] regarding impaired endothelial-dependent dilation associated with mildly elevated tHcy. There is also a report of impaired endothelial-dependent dilation in children with homocystinuria and a marked elevation of tHcy [18]. The negative study was in a group of postmenopausal women (mean age 54.8 ± 3.5 years); there was no relationship between tHcy and impairment of endothelial-dependent vasodilation [17]. In the two positive studies, the subjects were significantly older than our population, with a mean age of 71 years [6] and 53 years [7]. Subjects in our study had a mean age of 24 years. It may be that there are age-related effects with regard to homocysteine and endothelial function, and that higher concentrations and/or prolonged exposure is required in order for endothelial dysfunction to occur. Chao et al. [19] demonstrated impaired endothelial-dependent dilation following oral methionine in middle-aged, but not younger, subjects, and the authors suggested that age-related effects were involved.

Interpretation of the data from the study by Woo et al. [7] is difficult. There were differences in the brachial artery diameter at baseline between the groups, and the subsequent changes were small. When the absolute changes were estimated, the group with apparent ‘endothelial dysfunction’ in fact had a greater post-ischaemic brachial artery diameter than the control group. Also, the authors have recently written to correct their initial homocysteine results [20]. They suggest that errors in sample preparation and analysis led to inaccurate estimation of tHcy, and when they recalled and repeated their measurements the tHcy fell quite dramatically (from 34.8 ± 8.5 to 10.5 ± 3.0 µmol/l in the group with ‘high’ tHcy). Thus conclusions based on that study must be guarded.

In children with homocystinuria due to homozygous cystathionine β-synthase deficiency, Celermajer et al. [18] demonstrated impaired endothelial-dependent dilation compared with age-matched controls. The mean tHcy in the children was 63 µmol/l (mean age 9 years). Relatives of these children that were heterozygous for this deficiency were then compared with age-matched controls (mean age 40 years). There was no difference in endothelial-dependent dilation in the two groups. The literature regarding subjects heterozygous for cystathionine β-synthase deficiency is unclear, with some reports suggesting that they have significantly reduced enzyme activity [1] and mildly increased tHcy [21]. Interestingly, in the paper by Celermajer et al. [18], the actual tHcy in each group was not reported, and we are unsure if there was a difference between the groups.

Bellamy et al. [22] found that 6 weeks of folate...
supplementation (5 mg) improved endothelial-dependent dilation in 18 healthy subjects with elevated tHcy (mean 12.1 ± 3.6 μmol/l). The authors concluded that folate supplementation lowers plasma tHcy in adults with mild hyperhomocysteinaemia and reverses endothelial dysfunction. The authors assumed that the subjects had impaired endothelial function on the basis of previous studies. However, they did not compare subjects with low tHcy, and failed to demonstrate impaired endothelial-dependent dilation at baseline. In addition, folate is suggested to improve endothelial-dependent dilation in patients with hypercholesterolaemia [23], and it may have beneficial properties unrelated to its effect in decreasing tHcy. It is therefore possible that these data are due to folate therapy rather than reversal of impaired endothelial-dependent vasodilation. We were unable to demonstrate any evidence of impaired endothelial-dependent vasodilation in similar subjects with a similar tHcy.

While there were significant differences in folate and vitamin B12 concentrations between the two groups, this probably reflects high normal levels in the group with low tHcy rather than low levels in the other group. These concentrations are comparable with other data for this age group [19,22,24]. There are several possible explanations for our negative result. First, there may be age-related effects of homocysteine on endothelial function. The subjects we studied were younger than in the other reports. Furthermore, this concentration of tHcy may not be sufficiently high to induce endothelial damage, especially in this age group with normal folate and vitamin B12 concentrations. It is possible that concomitant factors, higher concentrations or more prolonged exposure to elevated tHcy are required before impaired endothelial function occurs. Another possible factor is that we measured FBF by venous-occlusion plethysmography in response to infusion of vasoactive substances to assess endothelial function; this technique assesses resistance vessel function. In the papers mentioned above, brachial artery diameter was measured in response to hyperaemic flow using an ultrasound technique, which assesses conduit vessel function. The differences in technique may account for the different results. In conclusion, in this group of young healthy subjects, mild hyperhomocysteinaemia was not associated with abnormal endothelial-dependent vasodilation.

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REFERENCES


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