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Plasma homocysteine and its association with carotid intimal-medial wall thickness and prevalent coronary heart disease: NHLBI Family Heart Study

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Abstract

Mildly elevated plasma total homocysteine (tHcy) levels have been associated with increased risk of coronary heart disease (CHD). Carotid artery intimal-medial wall thickening is a predictor of cardiovascular disease and has been previously shown to be positively associated with plasma tHcy in studies of asymptomatic subjects. In the current study we examined 1467 subjects with regard to their fasting plasma tHcy levels and intimal-medial wall thickness as measured by B-mode ultrasound and early onset CHD. The results showed that there is a significant positive association between plasma tHcy levels and carotid-artery wall thickness in participants 55 years or older even after the tHcy levels are adjusted for age, smoking and anti-hypertensive medication. The direction and magnitude of the relationship is similar although the result was not statistically significant in younger participants (<55 years). Early onset CHD at any age was not significantly different across the tHcy quintiles. The lack of an association of tHcy and CHD in the presence of a positive association with intimal-medial wall thickening may be a reflection of increased statistical power of quantitative versus qualitative traits. We conclude that the present finding of a positive association between tHcy and intimal-medial wall thickness strengthens the in vitro finding of the stimulating effect of homocysteine on vascular smooth muscle cell growth. Vascular smooth muscle cell proliferation may be an important mechanism through which mildly elevated plasma tHcy promotes atherosclerosis. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Over the past decade, a large number of case-control and cross-sectional studies [1–3] and several prospective studies [4–6] have all demonstrated an association between mildly elevated plasma total homocysteine (tHcy) and coronary and peripheral vascular disease. A meta-analysis showed that each 5 μmol/l increment in tHcy is associated with a 60% increase in men and 80% increase in women in coronary heart disease (CHD) [7]. Also, a recent report indicates that plasma tHcy concentration is a strong predictor of mortality in patients with angiographically confirmed CHD [8]. Thus, hyperhomocysteinemia is now becoming increasingly accepted as an important risk factor or risk marker for atherosclerotic occlusive vascular disease.

Despite the compelling evidence, some controversies remain. For example, several recent prospective studies did not show a statistically significant association between plasma tHcy and CHD incidence [9–11]. Moreover, the mechanism by which mildly elevated plasma tHcy causes increased atherogenicity is not clearly

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understood. The NHLBI Family Heart Study (FHS) was established to identify and evaluate non-genetic and genetic determinants of CHD, preclinical atherosclerosis, and cardiovascular disease risk factors in individuals and families [12]. In the current report, we describe the association between fasting plasma tHcy and carotid intimal-medial wall thickness and early onset CHD in selected participants of the FHS.

2. Methods

2.1. Study population

FHS recruited individuals and families from three existing population-based studies, the Framingham Heart Study, the Utah Family Tree Study, and the Atherosclerosis Risk in Communities (ARIC) Study (Minneapolis, Forsyth County cohorts). Participants were recruited and examined in two phases. In phase I, conducted in 1993–1995, randomly selected participants were sent questionnaires regarding the family history of CHD and related conditions on their parents, children, and siblings. Of 4679 individuals contacted, responses were obtained from 3150 (67%); their other family members were then contacted, and self-reported health data were obtained from a total of 22 908 individuals (86% of those contacted). Based on the validation of the CHD events reported in phase I, random families (n = 541) and high risk families (determined by a calculated family risk score greater than expected and at least two validated CHD events in the family, n = 610) participated in phase II at the four sites combined. All family members over 25 years of age were invited to participate in phase II, including parents, siblings, and children; a total of 5348 participants were examined. The phase II examination included multiple interviews and physiologic measurements, as previously described [12].

For the purpose of efficiency, a subset of the total sample was selected for special assays and genotyping. The first series included sibships in which at least two of the siblings were CHD affected full sibs (n = 243 sibs). The second series was defined on the basis of the carotid artery intimal-medial thickness (described in detail below), in which at least one sibling was above the 90th percentile of the distribution of intimal-medial thickness in the phase II population (n = 231 sibs); the 90th percentile was selected based on prior experience in the ARIC study. The third series was derived from a computed risk score of CHD (i.e. the Individual Risk Score, IRS) based on measured cardiovascular risk factors and sibships in which at least one of the siblings was above the age–sex specific 80th percentile of the IRS (n = 1090 sibs). The IRS was estimated for each phase II individual using sex-specific proportional hazards models to predict age-of-onset of CHD; the variables included carotid artery intimal-medial thickness, LDL and HDL cholesterol, Lp(a), body mass index, blood pressure, hypertension, diabetes, the residual Family Risk Score (i.e. an estimate of familial risk not accounted for by any particular risk factor domain). The final series included 200 randomly selected, unrelated subjects. These randomly selected subjects were recruited for case-control comparison. In comparisons involving continuous variables such as the intimal-medial wall thickness, inclusion of these individuals provides a full range of the distribution. Since several participants were reflected in more than one of the above groups, the final sample size was 1467.

2.2. Physiologic measurements

Sitting brachial artery blood pressure measurements were taken after a 5-min rest with a Hawksley random zero sphygomanometer by trained and certified technicians; the average of the last two of three blood pressure measurements was used in analysis. Participants wore scrub suits while height and weight were measured; body mass index was calculated as weight (kg) over height (cm). Smoking status was based on self-report. The FHS Lipid Laboratory, which is certified by the Center for Disease Control, measured plasma cholesterol and triglyceride, and HDL cholesterol using enzymatic methods. LDL cholesterol was calculated using the Friedewald equation [13].

2.3. Assay of plasma tHcy

A fasting heparinized blood sample was drawn and plasma was separated within 30 min for the measurement of baseline tHcy concentration. Total homocysteine was analyzed by the reversed-phase HPLC method of Ubbink et al. [14] with minor modifications. Plasma samples containing cysteamine as an internal standard were reduced with tri-n-butylphosphine, deproteinized with trichloroacetic acid, and the resulting serum thiols were derivatized with SBD-F (ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate), a thio-specific fluorogenic probe. The derivatized samples were chromatographed on a Supelcosil LC-18-DB column (Bellefonte, PA) and fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm using a GTI/Spectro-Vision FD-100 fluorescence detector (GTI, Concord, MA). Quantitation was achieved by measuring the peak height of the thiol containing derivative relative to the internal standard peak height and comparing it to a standard line of known homocysteine concentration. The CV of the method was 4.30% [15].
2.4. Ultrasound measurements of carotid artery intima-media thickness

Carotid B-mode real-time ultrasound examinations were performed by trained and certified technicians with a Biosound 2000IIa using a common scanning protocol at each of the field centers. Readings were performed at a central ultrasound reading center by trained and certified readers. Measurements of intimal-medial wall thickness were derived in the far wall of three segments of the right and left extracranial carotid arteries: (1) the common carotid artery (1 cm proximal to the dilatation of the carotid bulb); (2) the bifurcation (the 1 cm segment proximal to the flow divider); and (3) the internal carotid artery (the 1 cm segment in the internal branch distal to the flow divider). Eleven contiguous measurements (1 mm each) were attempted in each of the six segments. The overall average value of the mean wall thickness at the six segments combined was calculated and used in analysis.

2.5. Early onset CHD

All CHD events were validated using medical records and/or death certificates. A CHD event was defined by myocardial infarction or revascularization procedure (angioplasty or coronary artery bypass grafting). Early onset CHD was defined to be before age 55 years in men or before 65 years in women; the total CHD definition ignored age-of-onset.

2.6. Statistical methods

Descriptive statistics were calculated for all study variables. To evaluate the association of plasma tHcy with intima-media thickness, an analysis of covariance was performed. Logistic regression was used to evaluate the relationship between tHcy and early onset CHD. Additional models were subsequently developed to further adjust for factors associated with IMT/CHD and possibly to tHcy (age, smoking, systolic blood pressure, hypertension medication, and current smoking). Significant interactions between age and tHcy with intima-media thickness and tHcy with early onset CHD were found; descriptive characteristics, the intima-media models, and the CHD models were calculated separately for <55 years and ≥55 years of age (55 years was selected based on the sample mean age). No other interactions between tHcy and conventional risk factors were detected. All analyses were conducted using the Statistical Analysis System (SAS) version 6.6 [16].

3. Results

Table 1 gives the descriptive characteristics for the study population. The association between plasma tHcy and demographic, anthropomorphic, biochemical, and physiologic variables is described in Table 2(a) for participants <55 years of age and in Table 2(b) for those ≥55 years of age. In the younger group, tHcy levels were significantly and positively associated with LDL cholesterol, systolic blood pressure, male sex, and current cigarette smoking; age, fibrinogen, and antihypertensive medication use were not associated with tHcy levels. In contrast, in the older age group, age, fibrinogen, antihypertensive medication, male sex and current cigarette smoking were positively and significantly associated with tHcy; while LDL cholesterol and systolic blood pressure were not. HDL cholesterol was inversely related to tHcy in both age groups; however, neither diabetes nor body mass index demonstrated a significant association with tHcy.

Carotid artery intimal-medial wall thickness and S.D. according to plasma tHcy quintiles and age groups are listed in Table 3. In the younger age group, there was a positive but non-significant association between intimal-medial wall thickness and tHcy. In participants ≥55 years of age, intimal-medial wall thickness was significantly greater in the upper two quintiles relative to the three lower quintiles. The older age group also had greater intimal-medial wall thicknesses relative to their younger counterparts.

Table 4 shows the logistic odds ratios for early CHD and total CHD according to quintiles of plasma tHcy concentration. An age by tHcy interaction was not detected for early onset or total CHD. Neither early onset CHD nor total CHD was associated with plasma tHcy levels.
Table 2
Mean and S.D.* or frequency of demographic, anthropomorphic, biochemical and physiologic characteristics according to quintiles of plasma tHcy concentration for participants <55 years of age (a) and ≥55 years of age (b)

<table>
<thead>
<tr>
<th>tHcy (μmol/l)</th>
<th>160</th>
<th>128</th>
<th>128</th>
<th>115</th>
<th>128</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6.70</td>
<td>6.71–8.05</td>
<td>6.70</td>
<td>6.70</td>
<td>8.06–9.36</td>
<td>8.06–9.36</td>
</tr>
<tr>
<td>8.06–9.36</td>
<td>50.8 (16.2)</td>
<td>45.4 (13.3)</td>
<td>47.2 (14.6)</td>
<td>44.8 (13.8)</td>
<td>44.0 (14.8)</td>
</tr>
<tr>
<td>9.37–11.08</td>
<td>11.09</td>
<td>0.748</td>
<td>0.693</td>
<td>0.700</td>
<td>0.690</td>
</tr>
<tr>
<td>≥11.09</td>
<td>0.700</td>
<td>0.700</td>
<td>0.693</td>
<td>0.690</td>
<td>0.686</td>
</tr>
</tbody>
</table>

* S.D. is presented in parentheses.

4. Discussion

The present study showed that concentration of plasma tHcy is positively associated with intimal-medial wall thickness of the carotid artery, as measured by ultrasound in older participants in the NHLBI FHS. Plasma tHcy level is also positively associated with age, sex, smoking and antihypertensive medication. Adjustment for these risk factors, however, did not affect the observed positive finding between intimal-medial wall thickness and plasma tHcy.

A case control study of 287 pairs of participants in the Atherosclerosis Risk in Communities Study (ARIC) also demonstrated a positive association between carotid artery wall thickness and plasma tHcy in asymptomatic adults [3]. In the ARIC study, 57% of the pairs were men and 67% were ≥55 years old. Recently, several epidemiological and clinical studies have confirmed the positive association between elevated tHcy and increased carotid artery wall thickness [17–21]. For example, Bots et al. [17] found in the Rotterdam Study that elevated tHcy is associated with increased intimal-medial wall thickness in subjects aged 55–74 [17]. Similarly, McQuillan et al. [18] confirmed this association in 1111 subjects with a mean age of 52 years. Vuolilainen et al. [19], in their study of 513 men and women aged 45–69, found a positive association between elevated tHcy and increased carotid artery wall thickness in men but not in women. Carotid intimal-medial wall thickness increases with age as well as with several major risk factors for atherosclerosis, such as serum cholesterol, cigarette smoking and blood pressure [22], and is generally considered to be a reliable measure of subclin-
Table 4
Logistic odds ratios for early CHD and total CHD according to quintiles of plasma tHcy concentrationa

<table>
<thead>
<tr>
<th>tHcy (μmol/l)</th>
<th>Age &lt; 55 years</th>
<th></th>
<th></th>
<th>Age ≥ 55 years</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early CHD</td>
<td>Total CHD</td>
<td>Early CHD</td>
<td>Total CHD</td>
<td>Early CHD</td>
<td>Total CHD</td>
</tr>
<tr>
<td></td>
<td>Odds ratio</td>
<td>P-value</td>
<td>Odds ratio</td>
<td>P-value</td>
<td>Odds ratio</td>
<td>P-value</td>
</tr>
<tr>
<td>≥11.09</td>
<td>0.55</td>
<td>0.31</td>
<td>0.66</td>
<td>0.47</td>
<td>1.31</td>
<td>0.43</td>
</tr>
<tr>
<td>9.37–11.08</td>
<td>1.92</td>
<td>0.21</td>
<td>2.03</td>
<td>0.17</td>
<td>1.25</td>
<td>0.52</td>
</tr>
<tr>
<td>8.06–9.36</td>
<td>0.41</td>
<td>0.14</td>
<td>0.42</td>
<td>0.15</td>
<td>0.65</td>
<td>0.26</td>
</tr>
<tr>
<td>6.71–8.05</td>
<td>0.19</td>
<td>0.05</td>
<td>0.19</td>
<td>0.05</td>
<td>1.22</td>
<td>0.57</td>
</tr>
<tr>
<td>≤6.70</td>
<td>1.00</td>
<td>–</td>
<td>1.00</td>
<td>–</td>
<td>1.00</td>
<td>–</td>
</tr>
</tbody>
</table>

a Adjusted for age, systolic blood pressure, antihypertensive medication, current smoking and sex.

In the current study plasma tHcy concentration is not associated with either early onset CHD or total CHD in the overall sample despite a positive association with carotid intimal-medial thickness. This may be related to the design of the study, which includes both CHD cases and non-CHD individuals with elevated cardiovascular risk factors. The lack of an association of tHcy and CHD in the presence of a positive association with intimal-medial wall thickening may reflect enhanced power for the latter as a quantitative trait.

Currently, there is controversy regarding whether homocysteine is a causative risk factor for CHD. While numerous case/control studies consistently showed that patients with myocardial infarction, stroke, and peripheral vascular disease have higher plasma tHcy levels than controls, these results were not confirmed by some of the more recent prospective studies, such as ARIC [11], and Multiple Risk Factor Intervention Trial (MR-FIT) [10]. Our finding of a positive association between tHcy and intimal-medial wall thickness confirmed previous reports [1,3] and supported the hypothesis that homocysteine may be a causative risk factor for CHD. A previous in vitro study had also demonstrated that homocysteine promotes the growth of smooth muscle cells while inhibiting endothelial cell growth [23]. Thus, the results of these studies, when taken together, may be interpreted as supporting the in vitro finding that homocysteine promotes the growth of vascular wall smooth muscle cells, resulting in increased intimal-medial wall thickness. However, the inconsistent findings between plasma tHcy and CHD has led to suggestions that homocysteine is merely a marker of atherosclerotic burden, and increased homocysteine may be the result of the inflammatory process associated with atherosclerosis [24]. Thus, there is now an urgent need to distinguish whether moderately elevated plasma tHcy is a marker of the process of atherosclerosis or is an active participant in enhancing the progress of atherosclerosis and/or thrombosis. Experimental animal model studies and longitudinal human studies where homocysteine is measured serially along with non-invasive means of assessing atherosclerosis such as ultrasound and/or electron beam computerized tomography are needed. Since plasma tHcy has been shown to be a strong predictor of mortality in patients with angiographically confirmed CAD [8], secondary prevention trials, in which vitamin therapies are used to lower tHcy levels, are also needed. If vitamin therapies reduce the risk of CHD and/or mortality, then the hypothesis that a moderately elevated plasma tHcy level is as an independent risk factor for CHD will be strengthened.

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References


